Jiri Neuzil · Shazib Pervaiz
Simone Fulda *Editors*

Mitochondria: The Anti-cancer Target for the Third Millennium

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Introduction

The onset of the third millennium has witnessed unprecedented progress in molecular medicine. Despite this fact, a number of pathologies remain an overwhelming burden that is yet to be fully understood and, consequently, harnessed. This applies for some of the most widespread and hard-to-manage pathologies, epitomized in particular by neoplastic and neurodegenerative as well as cardiovascular diseases. Therefore, new approaches and new targets are needed to progress in the fight against these debilitating ailments.

During recent years, the resurgence of the importance of mitochondria as a potential molecular target for the treatment of a variety of pathologies has been seen. This is not all that surprising given the fact that mitochondria, the powerhouse of the cells providing the majority of ATP as well as purveyors of cell death, control numerous processes in the cell that touch on practically all aspects of its homeostasis. Naturally, the research on mitochondria in the context of pathologies such as cancer has undergone considerable progress, fuelled initially by the landmark discoveries of Otto Warburg in the first half of the nineteenth century.

That mitochondria are essential for the regulation of cellular bioenergetics and relay the switch to the glycolytic phenotype of malignant cells has been known for some time. However, only recently the molecular mechanisms of these processes have started to be uncovered. Consequently, it has been suggested and supported by a growing number of compelling reports that mitochondria are very promising targets for a number of therapeutic agents. In the context of mitochondria, these agents have been termed 'mitocans', i.e. small compounds that exert their anti-cancer effect by mitochondrial destabilization. These compounds show substantial promise as potential anti-cancer drugs, and more and more research attests to this paradigm.

This book is dedicated to the recent advancement in mitochondria as an intriguing target for human pathologies, focusing, with one notable exception, on cancer. The first chapter by Miriyala et al. discusses the central role of mitochondria in signalling within cancer cells, followed by a chapter by Kögel on BH3 mimetics as highly promising anti-cancer agents. The next chapter deals with complex II as an intriguing target for cancer treatment, put together by Rohlena et al. This complex, one of five mitochondrial complexes of oxidative phosphorylation (OXPHOS), is at

the crossroads of the tricarboxylic acid cycle and OXPHOS, presenting a worthy target to be explored in detail. Several ensuing chapters discuss individual (or group of) agents acting on mitochondria, including celecoxib, artemisin and its derivatives, as well as vitamin E analogues. Follow three subtly more general chapters on more-or-less specific strategies targeting mitochondria by Chen et al., Biasutto et al. and Ježek et al. Obre et al. discuss the plasticity of malignant cells in the context of their bioenergetics and the mitochondrial function. Itahana and Pervaiz then focus on the cross-talk between the 'gatekeeper' of the genome, i.e. p53, and mitochondrial function, a very important paradigm that has recently emerged. Notably, the last chapter of the book has a non-cancer context. It is written by Ting et al. and highlights the role of mitochondria in Friedrich's ataxia, an autosomal recessive inherited disease that causes progressive damage to the nervous system.

The topics of this book were chosen to cover in both rather general as well as more specific manner the various aspects of mitochondria as important, emerging targets for treatment of various human diseases, exemplified here primarily by malignancies. We hope that this book will contribute to the growing interest in mitochondria as intriguing organelles that are yet to be fully harnessed as an invariant target for the therapy of a number of pathologies.

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Chapter 1
Mitochondrion: A Novel Center for Cancer Cell Signaling

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Abstract Genomic alterations of normal host cells contribute to the development of cancer. Recent cancer research studies have shown that the aggressive growth and metastasis of cancer cells depend on normal host cells such as fibroblasts, endothelial, mesenchymal and immune cells. Balance among this cellular crosstalk determines the natural history of the cancer and its response to therapy. This active tumor-host dynamic mutualism is referred to as a tumor microenvironment, which can be a key target for both cancer prevention and therapy. An important common feature of various host cells and cancer cells is the generation of reactive oxygen species (ROS) that contribute to the effect of the microenvironment. Mitochondria play a central role in the regulation of ROS production and removal. A shift in cell redox status toward an oxidizing condition activates mitochondrial retrograde signaling, a communication pathway from mitochondria to the nucleus that leads to activation of adaptive response or cell death. Thus, in addition to generating ATP, mitochondria, the powerhouse of cells, play an important role in cell signaling in life-and-death conditions.

Keywords Mitochondria • Cancer • Cell signaling • Apoptosis • Innate immunity • Adaptive immunity • Electron transport chain • Mitochondrial DNA • Inside-out signaling • Reactive oxygen species • Tumor microenvironment • Retrograde signaling • Nuclear receptors • Transcription factors

1 Introduction

Tumors are complex, organ-like structures. They are comprised not only of malignant cells but also of a multitude of normal cell types, such as adipocytes, stromal and endothelial cells, as well as various immune system cells (for example, macrophages, neutrophils, T cells) and myoepithelial cells that either inhibit tumor growth or promote growth and metastasis of a malignancy (Jones et al. 2003; Barsky and Karlin 2005; Egeblad et al. 2010; Pandey et al. 2010; Sautes-Fridman et al. 2011). All these normal cells are, therefore, potential targets in tumor treatment (Barnas et al. 2010).

Given the importance of oxidative stress in the development and progression of cancer (Gius and Spitz 2006; Waris and Ahsan 2006; Valko et al. 2007; Roszkowski et al. 2011), studying the interactions between malignant and normal cells in the tumor microenvironment may shed light on the mechanisms by which ROS/RNS drive tumor progression. For example, the presence of cancer cells can dramatically alter stromal cell behavior. Using immortalized human fibroblast cells co-cultured with MCF-7 human breast cancer cells, Martinez-Outschoorn et al. discovered that when cancer cells were present, down regulation of caveolin-1 expression in fibroblasts occurred, resulting in a cancer associated fibroblast phenotype, increased myofibroblast marker expression, and constitutive activation of TGFβ/Smad2 signaling (Martinez-Outschoorn et al. 2010b). It has been well established that interaction between human fibroblast cells and MCF-7 cells has induced a caveolin-1 down

regulation-dependent rise in ROS production in fibroblasts, resulting in increased fibroblast DNA damage. In MCF-7 cells, the presence of fibroblasts increased aneuploidy and multi-nucleation. Loss of caveolin-1 in fibroblasts caused a decrease in mitochondrial function and mass, leading to increased autophagy/mitophagy and a metabolic switch to glycolysis, which may provide cancer cells with lactate to drive cancer cell growth. Many of the phenotypes observed in fibroblast cells co-cultured with MCF-7 cells were mimicked by expression of endothelial nitric oxide synthase (eNOS), suggesting that both ROS and NO are involved in cancer cell-induced changes in fibroblast cells (Martinez-Outschoorn et al. 2010a). Tumor-derived hydrogen peroxide acts on neighboring fibroblasts, leading to decreased mitochondrial activity and increased glucose uptake in fibroblasts and decreased glucose uptake and GLUT1 expression in tumor cells. Addition of extracellular catalase to co-cultures abrogated oxidative stress in fibroblasts and stimulated apoptosis in cancer cells, confirming the importance of oxidative stress in the metabolic coupling between fibroblasts and cancer cells (Martinez-Outschoorn et al. 2011). Co-administration of caveolin-1-deficient fibroblasts with the highly aggressive MDA-MB-231 human breast cancer cell line in nude, athymic mice dramatically increased tumor growth, and overexpression of MnSOD in the caveolin-1-deficient fibroblasts attenuated tumor growth (Trimmer et al. 2011).

Not only can stromal cells affect cancer cell metabolism, but stromal cell-derived ROS can also drive the metastatic behavior of cancer cells. NADPH oxidase-4 (Nox4) is a member of a large family of ROS generating enzymes involved in many normal cellular functions and pathological conditions (Bedard and Krause 2007). Tobar et al. discovered that MCF-7 breast cancer cell migration was enhanced when cells were co-cultured with RMF-EG mammary stromal cells, causing Nox4 expression in the stromal cells through a cancer cell-secreted TGFβ-driven mechanism. Knockdown of Nox4 expression abolished co-culture stimulation of MCF-7 migration (Tobar et al. 2010).

Tumor-derived ROS can also affect immune system function at the site of a tumor. Izawa et al. found a positive correlation between the level of hydrogen peroxide in gastric and esophageal tumors and disease progression, and also found an inverse correlation between hydrogen peroxide production and infiltration of CD56dim natural killer (NK) cells. The researchers discovered that CD56dim NK cells were more sensitive to hydrogen peroxide-induced apoptosis than CD56bright NK cells were and that hydrogen peroxide treatment impaired NK cell antibody-dependent cellular cytotoxicity (Izawa et al. 2011). Corzo et al. have reported that the tumor microenvironment induced macrophage differentiation of myeloid-derived suppressor cells, leading to suppression of both antigen-specific and non-specific T-cell activity through a HIF-1α-dependent mechanism (Corzo et al. 2010). These results suggest that the condition of the tumor microenvironment, such as increased hydrogen peroxide level and hypoxia, inhibits normal immune function at the tumor site, giving the tumor a distinct growth advantage that results in faster disease progression. Thus, ROS generation from tumor cells and stromal cells may have an important role in regulating the tumor microenvironment and this role may lead to cancer development and progression.

Under physiological conditions, mitochondria are the major site of ROS production. Mitochondria are unique cellular organelles that contain their own genome and communicate with the nucleus, which enables them to transcribe and translate gene- encoding components of the electron transport chain (ETC), and serve as the powerhouse for both ATP production and novel cell signaling. It has been proposed that mitochondrial signaling is altered in diseased conditions, where mitochondria are frequently found to anchor mutations within their own genome and display altered functional uniqueness leading to increased glycolysis. Manganese superoxide dismutase (MnSOD) located in the mitochondrial matrix is a very important anti-oxidant enzyme that catalyzes the conversion of superoxide radicals ($O_2^{\cdot -}$) to hydrogen peroxide and molecular oxygen in mitochondria (Weisiger and Fridovich 1973), thereby protecting cells against oxidative damage and regulating cellular concentration of $O_2^{\cdot -}$, which is an oxidant and a byproduct of cellular metabolism (Christianson 1997). During the course of normal metabolism, $O_2^{\cdot -}$ is produced from within the respiratory chain of the mitochondria and are the major source of reactive oxygen species (ROS) production. As signaling molecules, intracellular ROS act to oxidize and inhibit MAPK-specific PTPs causing dephosphorylation of kinase enzymes. Activated JNK and p38 may also trigger apoptosis. Intracellular ROS oxidize and inactivate PTEN but fail to dephosphorylate Akt, thereby leading to downstream gene expression changes that result in enhanced proliferation and survival, an effect particularly advantageous to cancer cells. Intracellular ROS cause dissociation of NF-kB, resulting in free translocation to the nucleus or phosphorylation of factors such as c-Jun and p53 and thereby precipitating the translocation to the nucleus that activates target gene expression. In terms of transcriptional regulation, intranuclear ROS affect phosphorylation, oxidation, and DNA binding of transcription factors such as AP-1, NF-kB, p53, and HIF-1α, leading to changes in target gene expression. Increased ROS production by defective cancer cell mitochondria also results in upregulation of the transcription factor Ets-1, a factor that is increasingly associated with aggressive cancers. The main focus of this review is mitochondrial ROS: how they regulate mitochondrial-resident proteins and facilitate retrograde and outside-inside signaling.

Alterations in MnSOD levels have been associated with a number of degenerative diseases, including neurodegenerative disease (Yahara et al. 1991). Phylogenetic distribution and subcellular localization studies have shown that MnSOD is a ubiquitous metalloenzyme found in virtually all aerobic organisms from bacteria to humans, and even anaerobes (Ravindranath and Fridovich 1975). MnSOD is uniformly distributed throughout the cytoplasm in prokaryotic cells (Steinman et al. 1994). MnSOD is a mitochondrial enzyme that is encoded by a nuclear gene and is transported across two mitochondrial membranes to the matrix. In humans, this translocation involves translation of a proenzyme that includes a 24-amino acid N-terminal peptide.

Fridovich et al. (Gregory and Fridovich 1973; Saltzman and Fridovich 1973) have identified initial evidence that MnSOD is necessary for survival in oxygen-metabolizing cells. MnSOD is vital for healthy aerobic life and the lack or complete elimination of MnSOD expression leads to early death in mouse, due to reduced mitochondrial activity. Complete ablation of MnSOD causes dilated

cardiomyopathy and neurodegeneration leading to early postnatal death. Mice exhibited severe oxidative damage to mitochondria and were extremely sensitive to hyperoxia (Li et al. 1995b; Lebovitz et al. 1996; Van Remmen et al. 2003). It has been clearly demonstrated that MnSOD knock-out mice had a 50 % reduction in MnSOD enzyme activity in all tissues, resulting in an age-dependent increase in oxidative DNA damage (8-oxodeoxyguanidine, 8-oxodG) in both nuclear and mitochondrial DNA compared to wild-type mice.

2 Mitochondria in Cancer Development

Hanahan and Weinberg identified six key hallmarks of cancer: (1) sustaining proliferative signaling, (2) evading growth suppressors, (3) resisting cell death, (4) enabling replicative immortality, (5) inducing angiogenesis, (6) activating invasion and metastasis (Hanahan and Weinberg 2000; Hanahan and Weinberg 2011). Galluzzi et al., in their important review, demonstrated the importance of mitochondrial function in the regulation of these six hallmarks (Galluzzi et al. 2010). Many different types of tumors have been demonstrated to have low MnSOD activity (Oberley and Buettner 1979). Overexpression of MnSOD suppressed the tumorigenicity of human melanoma cells, breast cancer cells and glioma cells, suggesting that MnSOD is a tumor suppressor gene in a wide variety of cancers (Church et al. 1993; Li et al. 1995a; Zhong et al. 1997). For example, St. Clair et al. *have* reported that suppression of tumor metastasis by MnSOD was associated with reduced tumorigenicity and elevated fibronectin (Stclair et al. 1997). They speculated that since oxidative stress can inactivate the promoter of the human fibronectin gene containing SP-1 binding sites and antioxidants support the transcription activity of SP-1 proteins, expression of MnSOD provides an antioxidant environment which supports the activity of SP-1, leading to an increased level of fibronectin in MnSOD transfected cells (Stclair et al. 1997). A recent study by St. Clair's group showed that suppression of Sp1 binding to the MnSOD promoter subsequently suppressed MnSOD expression at early stages while reduced p53 activity was responsible for restoration of MnSOD at later stages of tumorigenesis in which cancer cells survived and underwent metastasis (Dhar et al. 2011). Following is a description of various mitochondrial activities/components that are affected by MnSOD and their potential for cancer development.

3 Electron Transport Chain

Complexes I, II, and III are sources and potential victims of the superoxide that they produce. These complexes are susceptible to attack by superoxide because they contain iron-sulfur centers (Ohnishi 1975; Albracht and Subramanian 1977; Albracht 1980; Teintze et al. 1982; Ohnishi 1998; Roessler et al. 2010), as well as

oxidative modification of important amino acids by superoxide and other ROS/RNS. Chen et al. found that on NADH dehydrogenase, a complex I subunit, Cys206 and Tyr177 were susceptible to oxidative modification, resulting in a decrease in electron transport at complex I (Chen et al. 2005). Complex I (Pearce et al. 2001; Yamamoto et al. 2002; Brown and Borutaite 2004; Chinta and Andersen 2011) and complex III (Pearce et al. 2001) were also susceptible to oxidative inactivation by peroxynitrite.

MnSOD, while important for scavenging electron transport chain-derived superoxide, is also vital for the prevention of ROS-mediated inactivation of these complexes. Mitochondria isolated from $MnSOD^{+/-}$ mice have diminished respiratory control ratio (RCR) compared to $MnSOD^{+/+}$ mice, and this effect was greater in complex I substrates glutamate/malate and duroquinol (a complex II substrate). Decreased RCR was tied to a decrease in state III respiration and was due to oxidation of the iron-sulfur center of complex I (Williams et al. 1998). Complete knockout of MnSOD in mice resulted in diminished activity of succinate dehydrogenase (complex II) in heart tissue (Li et al. 1995b). Martin et al. discovered down-regulation of multiple nuclear gene-encoded subunits of all five complexes of oxidative phosphorylation in $MnSOD^{-/-}$ erythroblasts compared to $MnSOD^{+/+}$ cells (Martin et al. 2011). Larosche et al. found a decrease in complex I and complex V activities in $MnSOD^{+/+}$ and $MnSOD^{+/-}$ mice after acute ethanol exposure; however, $MnSOD^{+/+}$ were protected. Ethanol exposure increased iNOS expression in all three genotypes, but complex I and V nitration was only observed in $MnSOD^{+/-}$ mice, demonstrating the importance for MnSOD in modulation of peroxynitrite formation and nitration of susceptible proteins by removal of excess superoxide that contributes to peroxynitrite formation (Larosche et al. 2010).

Work by this laboratory has focused on the off-target effects of cancer chemotherapeutic drugs; in particular, the mechanisms by which Doxorubicin (Dox) induces cardiac and neurological toxicities. A major side effect of Dox is a dose-dependent cardiotoxicity that eventually causes dilated cardiomyopathy and heart failure (Minotti et al. 2004; Simbre II et al. 2005). Mitochondria are a major target of Dox (Sarvazyan 1996). Therefore, altered ROS-scavenging capacity in cardiac tissue can affect Dox-induced cardiac injury (Sun et al. 2001; Kang et al. 2002; Shioji et al. 2002). This laboratory was the first to demonstrate that mitochondrial ROS are essential for Dox-induced cardiac injury (Yen et al. 1996). In non-transgenic mice, Dox treatment resulted in a significant reduction in state III respiration at complexes I and II and diminished RCR, while complex II was only affected in MnSOD overexpressing mice. These results indicate that MnSOD is important for protection of complex I from Dox-mediated inactivation of complex I caused by Dox-induced superoxide production (Yen et al. 1999).

Chemotherapy induced cognitive impairment (CICI), the generalized decline in cognitive function marked by memory loss, diminished concentration, and decreased reaction time, is another side effect of cancer chemotherapy (Tannock et al. 2004; Wefel et al. 2004; Ahles and Saykin 2007; Nelson et al. 2007). Dox treatment resulted in alterations of both structure (Brown et al. 1998; Inagaki et al. 2007) and activity (Silverman et al. 2007) of myriad brain regions.

Oxidative stress is believed to be an important mechanism for the cognitive decline linked to chemotherapy (Joshi et al. 2005; Tangpong et al. 2007). This laboratory discovered a unique mechanism of Dox-induced neurotoxicity involving elevation of TNF-α in serum and different brain regions that resulted in a significant reduction in state III respiration because of diminished complex I activity (Tangpong et al. 2006). Another important mechanism of Dox-induced neurotoxicity is nitration and subsequent inactivation of MnSOD, which was inhibited by knockout of iNOS, implying a role of iNOS in Dox-induced neurotoxicity and MnSOD in protection from Dox (Tangpong et al. 2007). Further, our laboratory was the first to demonstrate that the redox cycling mechanism of Dox treatment oxidized plasma APOA1; and that, in turn, APOA1 enhanced macrophage TNF-α release contributing to potential subsequent TNF-α-mediated toxicity, and the antioxidant, MESNA, could reduce the systemic side effects of Dox (Aluise et al. 2011).

Mitochondrial ROS generated at various components of the electron transport chain are instrumental to cancer development and progression by various mechanisms (Valko et al. 2004; Waris and Ahsan 2006; Hervouet et al. 2007). Using proteomics on isolated mitochondria from renal oncocytomas (RO) and chromophobe renal cell carcinomas (RCC), Yusenko et al. (2010) discovered a downregulation of the NDUFS3 subunit of complex I and an upregulation of subunits COX5A, COX5B, and ATP5H of complexes IV and V in RO, while RCC had a downregulation of ATP5A1 in complex V. Knockdown of apoptosis-inducing factor (AIF), which is important for maintaining complex I activity (Vahsen et al. 2004), in different colon carcinoma cell lines resulted in an inability to form colonies in soft agar and tumor formation in athymic mice, correlating with a decrease in superoxide production and increased sensitivity to apoptosis (Urbano et al. 2005). Mutations in complex I subunits that increase ROS production resulted in increased conversion of fibroblasts to myofibroblasts, leading to increased migration and invasion, as well as to elevated expression of vascular endothelial growth factor-A, hepatocyte growth factor, and stromal cell-derived factor-1. Conditioned media from these mutated fibroblasts increased melanoma cell invasiveness (Taddei et al. 2012). Sharma et al. discovered that in the C8T osteosarcoma cell line, suppression of complex I activity by rotenone or treatment with paraquat (to induce oxidative stress) increased tumorigenicity of the cells through ROS-mediated activation of the AKT pathway. C8T cells have a heteroplasmic mitochondrial DNA mutation in the ND5 gene of complex I, and introduction of a yeast-derived gene for complex I (NDI1) restored complex I activity, diminished mitochondrial ROS production and decreased tumorigenicity of the cells (Sharma 2011). Owens and coworkers found an increase in the expression of two complex III subunits in primary human breast tumor samples: UQCRFS1 and UQCRH. Knockdown of UQCRFS1 in the MCF-7 human breast cancer cell line resulted in decreased matrigel invasion due to decreased mitochondrial membrane potential and reduced ROS production, which coincided with reduced expression of NADPH oxidases 2, 3, 4, and 5 (Owens et al. 2011).

Mitochondrial ROS can also affect HIF activity (Klimova and Chandel 2008). HIF is a transcription factor consisting of a dimer containing a constitutively expressed HIF-1β subunit and an oxygen-regulated HIF-1α subunit (Wang and Semenza 1995;

Jiang et al. 1996). HIF-1 regulated the expression of genes involved in iron metabolism, cell proliferation, angiogenesis, apoptosis, and glucose metabolism (Ke and Costa 2006), and HIF-1α was overexpressed in myriad cancers and various metastases (Zhong et al. 1999). Hypoxia increased complex III-derived ROS, correlating with an increase in HIF-1α protein levels and HIF-1 transcriptional activity, and loss of either mitochondrial DNA or expression of catalase blocked hypoxia-mediated stabilization of HIF-1α (Chandel et al. 2000; Guzy et al. 2005; Patten et al. 2010). However, other studies have demonstrated that HIF-1α stabilization was not dependent on complex III-derived ROS, but was dependent on an intact electron transport chain (Chua et al. 2010).

MnSOD is important for regulation of HIF-1α stabilization and HIF-1 transcriptional activity in a biphasic manner. Loss of MnSOD increased HIF-1α protein levels and transcriptional activity under both normoxia and hypoxia in various cell types, including endothelial cells (Fijalkowska et al. 2010), oral squamous cell carcinoma (Sasabe et al. 2010), and the MCF-7 human breast cancer cell line (Kaewpila et al. 2008). Surprisingly, MnSOD overexpression also led to increased HIF-1α stabilization. In MCF-7 cells with different levels of MnSOD enzyme activity, Wang et al. discovered that HIF-1α protein levels were elevated at both low and high levels of MnSOD activity, while moderate levels of MnSOD activity (three to six-fold above parental cells) did not elevate HIF-1α protein levels. Overexpression of glutathione peroxidase-1 or mitochondria-targeted catalase suppressed hypoxia-induced HIF-1α stabilization in MCF-7 cells with high MnSOD enzyme activity, indicating that MnSOD-derived ROS may be vital for HIF-1α accumulation (Wang et al. 2005).

4 Mitochondrial DNA (mtDNA) Stability

mtDNA is a double-stranded, circular DNA molecule made up of 16,569 base-pairs encoding 37 genes for 22 tRNAs and 2 rRNAs vital for translation of the remaining 13 genes (Anderson et al. 1981): seven subunits of complex I, one subunit of complex III, three subunits of complex IV, and two subunits of complex V (Wallace 2010). All other proteins localized to mitochondria are encoded by nuclear genes, synthesized in the cytosol, and transported to mitochondria (Wallace 2010). mtDNA is organized into nucleoids containing 6–10 individual mtDNA genomes (Iborra et al. 2004; Legros et al. 2004), as well as multiple proteins necessary for mtDNA synthesis and transcription, such as mitochondrial transcription factor A, mitochondrial single-strand DNA binding protein (mtSSB), and DNA polymerase γ (Polγ) (Garrido et al. 2003; Legros et al. 2004; Chen and Butow 2005).

mtDNA was vulnerable to damage caused by agents such as ultraviolet (Takai et al. 2006) and ionizing radiation (Richter et al. 1988) and ROS (Richter et al. 1988; Williams et al. 1998; Takai et al. 2006), with the D-loop region of mtDNA more susceptible to damage than other regions (Mambo et al. 2003). Oxidative damage to mtDNA and decreased mtDNA content have been linked to an age-related

decline in mitochondrial function (Boffoli et al. 1994; Short et al. 2005) and myriad age-related conditions (Tanaka et al. 1996), including Parkinson's disease, diabetes, heart failure (Kang and Hamasaki 2005), and cancer (Brandon et al. 2006; Lu et al. 2009). Cortopassi and Wang discovered that mtDNA encoding different complex I subunits was more susceptible to damage which may be a factor in superoxide production and disease (Cortopassi and Wang 1995). Cells lacking mtDNA (ρ^0) or cells that express mtDNA with a common 4977-bp deletion have much more ROS production than parental cells or cells in which wild-type mtDNA was restored (Indo et al. 2007). A vicious cycle can occur in which mtDNA damage results in altered mitochondrial function, leading to increased ROS production and further mtDNA damage (Birch-Machin and Swalwell 2010).

Many mtDNA mutations have been identified in numerous cancer types that contribute to cancer development through various mechanisms, including increased ROS production, modified activation of signal transduction pathways, and altered susceptibility to apoptosis (Lu et al. 2009; Shen et al. 2010). One important study by Petros et al. found myriad mtDNA mutations in prostate cancer cells, especially in mtDNA-encoded subunits of complex I, compared to non-cancer controls and the general population. Introduction of mutant mtDNA into PC3 prostate cancer cells increased cell growth compared to cells with introduction of wild-type mtDNA (Petros et al. 2005). A study by Ishikawa et al. using P29 and A11 mouse lung carcinoma cell lines with low (P29) and high (A11) metastatic potential demonstrated that cybrid (cytoplasmic hybrid) cell lines containing A11 mtDNA had decreased complex I activity and increased metastatic potential *in vitro* and *in vivo*, regardless of whether the cells had P29 or A11 nuclear DNA. The mtDNA generating the highest increase in metastatic potential contained a mutation in the gene encoding the ND6 subunit of complex I, resulting in increased ROS production. N-acetylcysteine (NAC) treatment suppressed metastatic potential, revealing the importance of mtDNA damage-derived mitochondrial ROS in cancer aggressiveness (Ishikawa et al. 2008). Jandova et al. discovered that cybrid mouse fibroblasts expressing mtDNA from BALB/cJ mice with a somatic mutation in the mitochondrial $tRNA^{Arg}$ gene displayed more ROS production, growth rate, and invasion/migration characteristics compared to control fibroblasts expressing mtDNA from C57BL/6 J mice. The enhanced tumor-like phenotype in the fibroblasts expression of the mutated mtDNA was suppressed by treatment with either vitamin E or NAC, reinforcing the link between mutant mtDNA-derived ROS production and cancer phenotype (Jandova et al. 2012).

Polγ is vulnerable to ROS-mediated inactivation, potentially affecting mtDNA synthesis and repair. Treatment of the catalytic subunit of Polγ with hydrogen peroxide resulted in oxidative modification, reduced DNA binding activity, and a dose- and time-dependent reduction in polymerase activity. Polγ was more susceptible to oxidation than either of the two nuclear DNA polymerases Polα or Polβ (Graziewicz et al. 2002). Given the localization of Polγ in mitochondria, factors that increase mitochondrial production of ROS may increase the likelihood of Polγ inactivation, suggesting a need for protection of Polγ from oxidative stress to preserve mtDNA fidelity.

MnSOD is vital for guarding mtDNA from ROS-induced damage. In *E. coli* K-12 cells, MnSOD associated with DNA (Steinman et al. 1994), and oxidative mtDNA damage increased in mice with age; however, the damage was much greater in $MnSOD^{+/-}$ compared to $MnSOD^{+/+}$ controls (van Remmen et al. 2003). Peripheral blood mononuclear cells from type 2 diabetic patients exhibited greater oxidative mtDNA damage, and MnSOD expression was increased in these cells as an adaptive response to this damage (Garcia-Ramirez et al. 2008). Overexpression of MnSOD or treatment with a MnSOD mimetic inhibited oxidative mtDNA damage resulting from high glucose in bovine retina endothelial cells (Madsen-Bouterse et al. 2010). MnSOD overexpression also protected mtDNA from the damaging effects of UV radiation (Takai et al. 2006) and acute ethanol exposure (Larosche et al. 2010; Mansouri et al. 2010). MnSOD is part of the nucleoid complex and interacts with mtDNA, Polγ, and glutathione peroxidase to protect mtDNA from oxidative stress-induced damage (Kienhofer et al. 2009). This laboratory confirmed the interaction of MnSOD with Polγ and mtDNA, and expanded these findings to demonstrate that MnSOD protected mtDNA from UV-induced damage by preventing nitration and inactivation of Polγ (Bakthavatchalu et al. 2012). Because of the importance of MnSOD as a tumor suppressor and the role of mtDNA mutations in tumorigenesis, MnSOD may suppress cancer development, in part, by protecting mtDNA from the damaging effects of ROS.

5 Mitochondrial Control of Innate and Adaptive Immunity

The assembly of the T cell activation complex by lipid raft formation enables the ROS production that is vital for adaptive immunity (Lu et al. 2007). Peroxynitrite formation in the thymus has been linked to apoptosis of human thymocytes (Moulian et al. 2008), and glutathione depletion was shown to be important for activated T cell death as a result of exposure to tryptophan metabolite 3-hydroxyanthranilic acid (Lee et al. 2010). In *Drosophila*, ROS are essential for hematopoieitic progenitor cell differentiation (Owusu-Ansah and Banerjee 2009). An important study by Case et al. demonstrated the role of MnSOD in adaptive immunity. The researchers found that thymus-specific knockout of MnSOD in mice resulted in increased immunodeficiency and susceptibility to influenza A H1Na infection compared to control mice and that MnSOD knockout mice were rescued by either Tempol or CTPO treatment (Case et al. 2011).

Innate immunity is vital for defending cells attacked by foreign microbes and cellular damage, and is achieved by different pattern recognition receptors (PRRs). These PRRs recognize sundry cell components that indicate either damaged or injured cells, referred to as danger-associated molecular patterns (DAMPs). PRRs also identify assorted components of microbial organisms known as pathogen-associated molecular patterns (PAMPs). PRRs are either membrane bound or cytosolic (Cinel and Opal 2009; Kawai and Akira 2009; Martinon et al. 2009), and activation of these receptors resulted in activation of many transcription factors, such as AP-1,

interferon-regulatory factor (IRF), and NF-κB (Martinon et al. 2009). PRR activation can also lead to inflammasome formation, multiprotein complexes comprised of a sensing protein (NLR), a caspase (such as caspase 1), and an adaptor protein (an apoptosis-associated speck-like protein containing a CARD [ASC]) (Sidiropoulos et al. 2008). Inflammasomes are platforms for inflammatory cytokine activation such as interleukin-(IL-) 1β, as a result of PAMPs or DAMPs in cells (Martinon et al. 2009).

Mitochondria are vital to the commencement of an immune response. Seth et al. discovered a mitochondrial antiviral signaling (MAVS) protein needed for NF-κB and IRF-3-dependent interferon-β (IFN-β) expression as a result of a viral infection. Knockdown of MAVS inhibited, while MAVS overexpression enhanced, IFN-β expression, and mitochondrial localization of MAVS was vital for its function (Seth et al. 2005). Release of mtDNA or formyl peptides resulting from injury activated polymorphonuclear neutrophils, leading to neutrophil-mediated organ injury (Shen et al. 2010). Innate immunity was also activated by mtDNA. Depletion of mtDNA by ethidium bromide treatment (ρ^0 cells) in J774A.1 macrophages inhibited both lipopolysaccharide- and ATP-induced caspase-1 activation and IL-1β secretion. Treatment with DNase I inhibited, while transfection with mtDNA enhanced, inflammasome formation, indicating that mtDNA release into the cytosol is vital for activation of inflammasomes (Nakahira et al. 2011).

ROS are central to the activation of inflammasomes (Martinon 2010). ROS generated by NADPH oxidase (Nox) are involved in Nalp3 inflammasome activation and IL-1β secretion in human macrophages after asbestos exposure. When Nox activity was inhibited by diphenylene iodonium chloride or apocynin treatment or ROS scavenging by N-acetylcysteine or APDC ([2R,4R]-4-aminopyrrolidine-2,4-dicarboxylate), asbestos-induced IL-1β activation was inhibited (Dostert et al. 2008). Later work by Meissner et al. demonstrated that caspase-1 activation and IL-1β can occur in the absence of active Nox (Meissner et al. 2010), implying that other ROS sources may also participate in inflammasome activation.

Recent work from two laboratories demonstrated involvement of mitochondrial ROS in inflammasome activation. Zhou et al., using the THP1 macrophage cell line, discovered that chemical inhibition of different complexes of the electron transport chain increased mitochondrial ROS production and increased IL-1β activation. NLRP3 and ASC, components of the inflammasome, localized to both the endoplasmic reticulum and mitochondria in response to various inflammasome-stimulating agents. The voltage-dependent anion channel (VDAC) is vital for mitochondrial ROS-dependent inflammasome formation, with either knockdown of VDAC1 or VDAC2 expression or overexpression of bcl-2 inhibiting caspase-1 and IL-1β activation (Zhou et al. 2011). Treatment of LPS and ATP-stimulated macrophages with rotenone increased caspase-1 activation and IL-1β secretion, and this effect was inhibited by the antioxidant Mito-TEMPO (Nakahira et al. 2011).

Proinflammatory cytokines can also be activated by mitochondrial ROS independent of inflammasomes. In mouse embryonic fibroblasts expressing different type 1-TNF receptors (TNFR1) linked to tumor necrosis factor receptor-associated periodic syndrome (TRAPS), basal mitochondrial ROS levels were higher than in

wild-type cells, which is important for LPS-stimulated IL-6 and TNF production, but not IL-1β, in the absence of inflammasome formation. Scavenging mitochondrial ROS impeded LPS-dependent cytokine production (Bulua et al. 2011).

Autophagy is also important for inflammasome formation. Knockdown of LC3 (Nakahira et al. 2011) or beclin-1 (Nakahira et al. 2011; Zhou et al. 2011), or inhibition of autophagy by 3-methyladenine (3-MA) (Zhou et al. 2011) increased mitochondrial ROS, caspase-1 activation, and IL-1β secretion. Autophagy inhibition also prevented LPS and ATP-stimulated mtDNA release into the cytosol, which correlated with diminished caspase-1 activation (Nakahira et al. 2011). The results imply a role for autophagy in the removal of damaged mitochondria to prevent activation of inflammasome-dependent innate immunity. Inhibition of autophagy leads to accumulation of damaged mitochondria, increasing basal mitochondrial ROS production and inflammasome formation. Given the importance of inflammation and immunity in cancer formation and progression (Grivennikov et al. 2010), these studies suggest exciting possibilities for the effects of MnSOD in tumorigenesis by both inflammasome-dependent and –independent mechanisms.

6 Apoptosis

Apoptosis is a highly regulated form of cell death targeting an individual cell or small groups of cells for destruction. Apoptosis is characterized by a distinct set of events involving condensation of the cytoplasm and nucleus, resulting in the formation of apoptotic bodies (membrane-bound fragments containing cellular components), followed by phagocytosis of these apoptotic bodies by surrounding healthy cells (Majno and Joris 1995; Afford and Randhawa 2000). Apoptosis is vital for myriad cellular processes, including embryonic development and immune response (Schwartzman and Cidlowski 1993). Apoptosis can occur by two pathways: extrinsic (dependent on an external stimulus for cell death) (Reed 2000) and intrinsic (initiated by some internal stress) (Roy and Nicholson 2000; Basu et al. 2006).

The mitochondrion is an important site for apoptosis (Gulbins et al. 2003; Spierings et al. 2005), which occurs due to some dysfunction of the organelle, including permeabilization of the mitochondrial membrane, changes in mitochondrial membrane potential, and ROS generation (Green and Reed 1998). When mitochondrial dysfunction occurs, different molecules are released from mitochondria that are responsible for initiation and propagation of apoptotic signals. Cytochrome *c*, a vital component of the electron transport chain, is released into the cytoplasm upon some internal stress and interacts with Apaf-1 to form the protein complex known as the apoptosome, which promotes caspase9 activation. Other proteins released from mitochondria include Omi/Htr2 and Smac/DIABLO, which interfere with members of the inhibitor of the apoptosis (IAP) family, as well as apoptosis inducing factor (AIF) and endonuclease G, which translocate to the nucleus and participate in DNA fragmentation (Adams 2003). A recent finding has shown that enhanced intracellular Ca^{2+} and ROS levels were prerequisites for AIF to be cleaved and released from mitochondria (Norberg et al. 2010).

Mitochondrial membrane phospholipids play a pivotal role in modulating oxidative stress and molecular integrity. The most profound phospholipids species is cardiolipin (CL) which is exclusively located in the inner mitochondrial membrane where it is biosynthesized (Schlame et al. 2000). It is well established that CL plays a vital role in mitochondrial bioenergetics, optimizing the activity of key mitochondrial inner membrane proteins involved in oxidative phosphorylation (Houtkooper and Vaz 2008). Structural alterations of CL led to mitochondrial dysfunction in many pathological settings (Chicco and Sparagna 2007) and, due to its high content of unsaturated fatty acids, it is highly prone to peroxidative attack by ROS. CL peroxidation has been shown to play a critical role in several physiopathological situations (Pope et al. 2008). Oxidation/depletion of CL would negatively affect the biochemical function of mitochondrial membranes, leading to cellular dysfunction and eventually cell death (Petrosillo et al. 2003; Ottet al. 2007).

MnSOD has a complex function in apoptosis regulation. Myriad reports demonstrate the importance of MnSOD in protecting normal tissues from the deleterious effects of ionizing radiation (Zwacka et al. 1998; Epperly et al. 1999, 2002, 2003, 2004). MnSOD also regulates exposure to Fe(II), NO-generating agents, amyloid β-peptide (Keller et al. 1998), and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Mohr et al. 2007). MnSOD protects against apoptosis by preventing the release of various mitochondrial effectors that induce apoptosis, including Smac/DIABLO (Mohr et al. 2007) and cytochrome *c* (Epperly et al. 2002).

MnSOD also affects apoptosis induction by modulation of ROS. In FsaII murine fibrosarcoma cells, MnSOD overexpression inhibited apoptosis induced by antimycin and rotenone (electron transport chain inhibitors) by altering mitochondrial ROS levels, leading to decreased caspase 3 activity and poly(ADP-ribose) polymerase cleavage (Kiningham et al. 1999). MnSOD overexpression hampered peroxynitrite production and lipid peroxidation induced by Fe(II), amyloid β-peptide, and NO-generating agents in the PC6 pheochromocytoma cell line (Keller et al. 1998).

Inflammatory cytokine-mediated apoptosis is also altered by MnSOD. MnSOD overexpression protected A375 human melanoma cells and Chinese hamster ovary cells from inflammatory cytokines (Hirose et al. 1993). Mitochondrial ROS production has been shown to be an important mechanism of tumor necrosis factor (TNF)-stimulated apoptosis (Goossens et al. 1995), and stimulation of MnSOD by TNF may be an adaptive response for protection from further TNF exposure (Wong and Goeddel 1988; Wong et al. 1989). As an example of this adaptive response, Mattson et al. found that pretreatment of hippocampal cells with TNF-α provided protection against Fe(II)- and amyloid β-peptide-induced apoptosis by stimulating MnSOD expression (Mattson et al. 1997). This laboratory discovered that the antiestrogen tamoxifen augmented TNF-α-induced MnSOD expression by increasing p50/p65 NF-κB dimer binding to the second intronic enhancer in the MnSOD gene (Daosukho et al. 2002). Further studies by this laboratory demonstrated that increased MnSOD expression was part of the mechanism by which tamoxifen protected cardiac tissue from Dox-induced apoptosis (Daosukho et al. 2005). In human Jurkat T cells, degradation of MnSOD by caspases was vital for Fas receptor-mediated apoptosis due to increased superoxide production (Pardo et al. 2006). Increased steady-state

hydrogen peroxide is one potential mechanism of MnSOD-induced resistance to further TNF-α treatment (Dasgupta et al. 2006).

MnSOD seems to have a context-specific role in cancer. MnSOD expression has been decreased in many types of cancer (Oberley and Buettner 1979), and overexpression of MnSOD has stimulated apoptosis in myriad cancers. Overexpression of MnSOD alone, or in combination with overexpression of TRAIL, increased apoptosis in cancer cells by stimulation of caspase-8 activation, mitochondrial localization of Bax, and release of AIF and cytochrome *c*, leading to caspase-9 and −3 activation. The increase in apoptosis decreased tumor xenograft growth in cells overexpressing either MnSOD or TRAIL, and tumor xenograft growth was completely eliminated with the combination of MnSOD and TRAIL (Zhang et al. 2006). p53-dependent expression of MnSOD and glutathione peroxidase stimulated apoptosis in the TR9-7 Li-Fraumeni syndrome fibroblast cell line (Hussain et al. 2004). MnSOD overexpression sensitized the WEHI7.2 murine thymic lymphoma cells to dexamethasone-induced apoptosis (Jaramillo et al. 2009).

However, various studies have also demonstrated MnSOD-dependent protection of cancer cells from apoptosis. Overexpression of MnSOD in colorectal carcinoma cells inhibited apoptosis by prevention of Smac/DIABLO release (Mohr et al. 2007). Palazzotti found that resistance of HT29 colon carcinoma cells to serum starvation-induced cell death was due, in part, to expression of MnSOD, and overexpression of MnSOD in HeLa cervical carcinoma cells conferred resistance to serum starvation-induced cell death (Palazzotti et al. 1999). In a study by Hai et al., MnSOD expression was increased in nearly half of all esophageal squamous cell carcinomas tested and was linked to apoptosis resistance (Hu et al. 2007). Kahlos et al. discovered that MnSOD expression in both primary mesothelioma tumors and in different mesothelioma cell lines expressing different levels of MNSOD correlated with increased resistance to epirubicin-induced apoptosis (Kahlos et al. 2000). This laboratory demonstrated MnSOD-dependent resistance of the Fsa-II murine fibrosarcoma cell line to rotenone- and antimycin-stimulated apoptosis due to altered mitochondrial ROS levels (Kiningham et al. 1999).

MnSOD has a dual function: it serves as both a tumor suppressor in early stages of cancer development and it provides support for tumor growth at later stages of cancer development. MnSOD is also important in protecting normal tissues from apoptosis induced by various chemotherapy agents and ionizing radiation. Given these roles of MnSOD, the development of adjuvant therapies that increase MnSOD activity and/or expression will enhance the toxic effects of cancer treatments on cancer cells and simultaneously will protect normal tissues by targeting apoptosis pathways in both types of tissue.

7 Tricarboxylic Acid (TCA) Cycle

The TCA cycle (also known as the Krebs cycle) is a fundamental metabolic pathway localized to mitochondria. The TCA cycle provided reducing equivalents as feedstock for the electron transport chain and generated myriad substrates for other

cellular processes (Rotig et al. 1997; Briere et al. 2006). Many studies in multiple model systems have exhibited the role of MnSOD in maintaining the activity of different TCA cycle proteins, such as yeast (Longo et al. 1999), *Drosophila* (Duttaroy et al. 2003), mouse (Williams et al. 1998), and *Arabidopsis thaliana* (Morgan et al. 2008). Microarray analysis of wild-type and $MnSOD^{-/-}$ mouse erythroids showed that complete loss of MnSOD resulted in a significant reduction in expression of nearly all enzymes of the TCA cycle, as well as many of the regulatory proteins involved in this metabolic pathway (Martin et al. 2011). Liver mitochondria from $MnSOD^{+/-}$ mice have significantly reduced aconitase activity, which was rescued by addition of dithiothreitol and iron, suggesting that aconitase inactivation may be due to superoxide-dependent oxidation (Williams et al. 1998). MnSOD overexpression of MnSOD in A549 human lung adenocarcinoma cells prevented hypoxia-reoxygenation-induced aconitase inactivation (Powell and Jackson 2003), as well as inactivation of aconitase by electron transport inhibitors and phenazine methosulfate (a redox cycling agent) (Gardner et al. 1995). Morgan et al. found that knockdown of MnSOD expression in transgenic *Arabidopsis thaliana* resulted in a decrease in the activities of both aconitase and isocitrate dehydrogenase, as well as a diminished TCA cycle flux (Morgan et al. 2008). Complete knockout of MnSOD in mice resulted in significant reduction in aconitase and succinate dehydrogenase activities in heart and other tissues (Li et al. 1995b). Increased expression of MnSOD in A549 human lung carcinoma cells by adenoviral transduction protected the cells from hypoxia reoxygenation-induced reduction in both aconitase and succinate dehydrogenase activities (Powell and Jackson 2003).

Dysregulation of the TCA cycle has been reported in many types of cancer (Wenner et al. 1952; Rotig et al. 1997; Briere et al. 2006; Denkert et al. 2008; Frezza et al. 2011). Much work has focused on three key enzymes in the TCA cycle: isocitrate dehydrogenase, fumarase (also known as fumarate hydratase), and succinate dehydrogenase. Mutations in the genes encoding these TCA cycle enzymes in various cancer cell lines and tissues have been reported. Mutations in codon 132 of isocitrate dehydrogenase (*IDH1*) have been observed in myriad brain tumors (Balss et al. 2008; Ichimura et al. 2009; Watanabe et al. 2009). Codon 132 encodes an arginine in the binding site for isocitrate, and mutation of this codon resulted in complete loss of isocitrate dehydrogenase enzyme activity (Jennings et al. 1997; Soundar et al. 2000). Arg132 and other types of mutations in both *IDH1* and *IDH2* have been identified in acute myeloid leukemia (Chotirat et al. 2012). Various germline mutations in the fumarate hydratase (*FH*) gene, resulting in reduced enzyme activity, have been associated with uterine and cutaneous leiomyomata and renal cell carcinoma (Consortium 2002; Martinez-Mir et al. 2003; Toro et al. 2003), and also have been identified as having a potential role in both bladder and breast cancers (Lehtonen et al. 2006). Sundry mutations in succinate dehydrogenase (complex II) subunits have been identified in patients with leukemia (Baysal 2007), paraganglioma (Baysal et al. 2000), and in individuals with Cowden and Cowden-like syndrome (Ni et al. 2008).

Various mechanisms by which dysregulation of TCA cycle enzyme function may play a role in tumorigenesis include superoxide and hydrogen peroxide production, which can lead to genomic instability and activation of HIF. Different studies have

demonstrated that TCA cycle intermediates, such as succinate (Selak et al. 2005; Koivunen et al. 2007), as well as fumarate, oxaloacetate, and citrate (Koivunen et al. 2007), contributed to HIF-1α protein stability by inhibiting the activity of different HIF prolyl 4-hydroxylases that act as negative regulators of HIF-1α protein stability. It was found that germline mutations in either fumarate hydratase or succinate dehydrogenase linked to cancer development resulted in accumulation of fumarate and succinate that contribute to HIF-1α stabilization (Koivunen et al. 2007). Loss of the B subunit of succinate dehydrogenase (*SDHB*), by either pharmacological or RNA interference methods, resulted in HIF activation through ROS-dependent (Guzy et al. 2008) or -independent mechanisms (Cervera et al. 2008), leading to increased tumorigenic behavior. In a recent study by Owens et al. (2011) using immortalized Chinese hamster lung fibroblasts, stable expression of a mutant for the D subunit of succinate dehydrogenase (*SDHD*) resulted in a 2-fold increase in steady-state levels of superoxide, leading to a 70-fold increase in mutation frequency. Treatment with PEG-conjugated catalase or MnSOD, or MnSOD adenovirus, significantly reduced the mutation frequency in *SDHD* mutant-expressing cells, indicating an important role for both superoxide and hydrogen peroxide in *SDHD* mutant-mediated genomic instability. The studies discussed above suggest a possible mechanism of the tumor suppressive effects of MnSOD: by protecting TCA cycle proteins from ROS-induced deactivation, it potentially prevents the buildup of the TCA cycle intermediates and TCA cycle enzyme-derived ROS that may play a role in the genomic instability and activation of the transcription factors that contribute to tumorigenesis.

8 Mitochondria as an Originator of Inside-Out Signaling

Src is a family of non-receptor tyrosine kinases with nine members: FGR, LCK, HCK, FYN, LYN, BLK, YRK, YES, and c-Src (hereafter referred to as Src). This family of kinases regulates a multitude of cellular functions, including cell proliferation, angiogenesis, cell adhesion, growth factor signaling, and bone remodeling (Aleshin and Finn 2010). Src family members are important for cancer progression, making them attractive targets for anticancer therapy (Summy and Gallick 2003; Johnson and Gallick 2007; Wheeler et al. 2009; Guarino 2010; Zhang and Yu 2011). Src is kept in an inactive state by phosphorylation at Tyr530 (Tyr527 in mice), maintaining Src in an inactive conformation. Dephosphorylation at Tyr530 leads to a more open conformation. Complete activation occurs when Src autophosphorylates at Tyr419 in the kinase domain (Tyr416 in mice) (Aleshin and Finn 2010).

ROS are important in the regulation of Src activity (Sun and Kemble 2009; Giannoni et al. 2010). Nevertheless, whether oxidation of Src causes activation or inactivation of the kinase is debated. One study revealed that oxidation led to Src inactivation. Oxidation of Cys277 within the catalytic domain of Src led to the

formation of an intermolecular disulfide bond between two Src molecules, leading to inactivation (Kemble and Sun 2009). On the other hand, other reports implied that Src oxidation resulted in activation. Using NIH3T3 rat fibroblasts cells, Akhand et al. found that treatment with hydrogen peroxide, as well as the nitric oxide generators sodium nitroprusside and *S*-nitroso-*N*-acetylpencillamine (SNAP), resulted in autophosphorylation of Src at Tyr416 and disulfide bond formation. These modifications resulted in more Src protein aggregation and increased kinase activity (Akhand et al. 1999). Hydrogen peroxide-induced Src activation was reversed by *N*-acetylcysteine (NAC) treatment by decreasing phosphorylation at Tyr419 and diverting Src away from the plasma membrane to acidic endolysosomes in various human cancer cell lines (Krasnowska et al. 2008). A similar deactivation of Src by antioxidants occurred in HeLa cervical carcinoma cells. Src activation by low-power laser irradiation was inhibited in treatment with vitamin C, catalase, or the combination of catalase and superoxide dismutase (Zhang et al. 2008). In NIH3T3 cells, treatment with nordihydroguaiaretic acid (NDGA) inhibited lipoxygenase activity, leading to a decrease in Src oxidation, diminished autophosphorylation of Src at Tyr416, and reduced association between Src and focal adhesion kinase during cell adhesion, providing evidence for the importance of Src oxidation during cell adhesion and anchorage-dependent cell growth (Giannoni et al. 2005).

Several studies have demonstrated the importance of mitochondria in the regulation of Src activity. A-kinase anchor protein 121 (AKAP121) and AKAP84 are localized at the outer membrane of mitochondria. These proteins bind protein tyrosine phosphatase D1 (PTPD1), which is a positive regulator of Src activity. In HEK293 human embryonic kidney cells, transfection of PTPD1 alone resulted in Src-dependent augmentation of EGF receptor signaling. AKAP121 transfection resulted in diminished Src-dependent EGF receptor activation because of AKAP121-dependent recruitment of PTPD1 to mitochondria (Cardone et al. 2004). The ROS-generating enzyme NADPH oxidase-4 (Nox4), which has been identified in mitochondria (Block et al. 2009; Graham et al. 2010), is important for angiotensin II (AngII)-dependent activation of Src and 3-phosphinositide-dependent protein kinase-1 (PDK-1). Knockdown of Nox4 by siRNA or expression of an oxidation-resistant Src mutant (Cys487Ala) prevented AngII-induced activation of PDK-1 (Block et al. 2008).

Mitochondrial ROS are also important for hypoxic activation of Src. Lluis et al. (2007) discovered that hypoxia-induced mitochondrial ROS production activated Src, leading to increased IκB-α phosphorylation and NF-κB activation. Activation of the Src pathway was inhibited by treatment with a combination of the mitochondrial electron transport chain inhibitors rotenone and TTFA, the Src kinase inhibitor PP2, or by expression of a mutant Src in which Cys487 (kinase domain) was mutated to an alanine, suggesting a vital role for mitochondrial ROS in the activation of Src (Lluis et al. 2007). Using primary rat aortic smooth muscle cells and the C2/2 rabbit aortic smooth muscle cell line, Sato et al. found that hypoxia

increased Src phosphorylation, HIF-1α protein levels, and expression of plasminogen activator inhibitor-1 (PAI-1) through mitochondria-generated hydrogen peroxide (Sato et al. 2005).

While Src is found primarily on the plasma membrane, a number of studies have demonstrated mitochondrial localization of Src, where Src can influence mitochondrial function. Salvi et al. identified several Src family members in mitochondria isolated from rat brain (Fyn, Src, and Lyn). The authors found that the phosphorylation state of mitochondrial proteins changed with PP2 treatment, suggesting that various mitochondrial proteins may be targets of Src family kinases (Salvi et al. 2002). Augereau et al. reported similar effects of PP2 on mitochondrial protein phosphorylation, as well as decreased state 3 respiration with PP2 treatment (Augereau et al. 2005). The downstream of kinase-4 (Dok-4) adaptor protein localized to mitochondria and recruited Src to mitochondria, which is vital for TNF-α-stimulated mitochondrial ROS production and activation of NF-κB (Itoh et al. 2005). Hebert-Chatelain et al. discovered that Src regulated oxidative phosphorylation in human cancer cell lines by specifically targeting the NDUFB10 subunit of complex I (Hebert-Chatelain et al. 2012). Src phosphorylated complex IV and regulated its activity in osteoclast function. Loss of Src activity by deletion of the c-Src gene diminished complex IV activity, which was restored by reexpression of c-Src (Miyazaki et al. 2006). Protein tyrosine phosphatase 1B (PTP1B) in mitochondria regulated mitochondrial Src activity and Src-dependent activation of complex IV (Chatelain et al. 2011). AKAP121 was important to recruiting both PKA and Src to mitochondria, where Src can influence mitochondrial function, such as mitochondrial membrane potential and ATP production through phosphorylation of various mitochondrial proteins (Livigni et al. 2006). These results suggest that not only do mitochondria influence Src function, in part, through ROS-mediated mechanisms, but Src can influence mitochondrial function via phosphorylation of various mitochondrial proteins, making Src a key mediator of both outside-in and inside-out signaling at mitochondria.

9 Mitochondrial as a Signaling Generator/Recipient

9.1 Mitochondria in Outside-In Signaling

Transcription factors and nuclear receptors are important in the regulation of mitochondrial function, which they can do by two mechanisms: regulation of nuclear-encoded mitochondrial genes and localization to mitochondria for direct regulation of mtDNA gene expression and function (Leigh-Brown et al. 2010). Many transcription factors and nuclear receptors have been identified in mitochondria, including p53, cAMP (cyclic AMP response element binding protein), Stat3 (signal transducer and activator of transcription 3), PPARγ2 (peroxisome proliferator-activated receptor γ2), and multiple hormone receptors (Gavrilova-Jordan and Price 2007; Lee et al. 2008; Leigh-Brown et al. 2010).

10 Transcription Factors

10.1 Nuclear Receptors

The nuclear receptor superfamily of transcription factors is a large collection of transcription factors that can be broken up into two broad categories: steroid and non-steroid receptors (Evans 1988). These receptors bind a wide range of ligands, such as steroid hormones, retinoic acid, thyroid hormone, and vitamin D_3, to name just a few. In their inactive forms, steroid hormone receptors have been found in the cytoplasm bound to various heat shock proteins. Upon ligand binding, the receptor dissociated from the heat shock proteins and dimerized, followed by nuclear translocation and binding to hormone response elements (McKenna et al. 1999).

10.2 Estrogen Receptor

Several reports indicate the presence of various steroid hormone receptors in mitochondria (Gavrilova-Jordan and Price 2007), including androgen receptor (AR) (Solakidi et al. 2005a, b) and glucocorticoid receptor (GR) (Solakidi et al. 2007). The presence of estrogen receptor (ER) in mitochondria has been reported in a multitude of tissues, such as human lens epithelial cells (Flynn et al. 2008), murine skeletal muscle cells (Milanesi et al. 2008, 2009), and the brain (Arnold and Beyer 2009), in particular in hippocampal cells (Yang et al. 2004; Milner et al. 2005; Yang et al. 2009). Mitochondrial ER appears to have an important effect on mitochondrial homeostasis. Estrogen treatment prevented a decrease in mitochondrial membrane potential caused by hydrogen peroxide in human lens epithelial cells, which was inhibited by siRNA treatment targeted against the ER present in the cells (Flynn et al. 2008). Yang et al. found that knockdown of the β isoform of ER in HT-22 hippocampal cells resulted in a reduction of the resting mitochondrial membrane potential and increased resistance to hydrogen peroxide-induced depolarization (Yang et al. 2009). ERα was detected in the mitochondria of cerebral vasculature, and treatment with 17β-estradiol (E_2) increased the expression of various mitochondrial proteins encoded by both the nuclear genome and mitochondrial genome. E_2 treatment increased the activities of mitochondrial citrate synthase and complex IV, as well as decreased hydrogen peroxide production. These findings suggest that part of the protective function of E_2 in vasculature may be modulation of mitochondrial activity (Stirone et al. 2005).

The mitochondrial localization of estrogen receptors is also important in cancer cells. Solakidi et al. identified ERβ in mitochondria in both the SaOS-2 human osteosarcoma and HepG2 hepatocarcinoma cell lines (Solakidi et al. 2005a, b, 2007). Ivanova et al. found that ERβ was localized primarily at the cytoplasm and mitochondria in H1793 (female) and A549 (male) lung adenocarcinoma cells independent

of E_2 treatment and irrespective of the sex of the patient from whom the cell lines were derived (Ivanova et al. 2010). Knockdown of ERβ in various non-small-cell lung carcinoma cell lines inhibited cell growth and sensitized the cells to cisplatin-, paclitaxel-, and etoposide-induced apoptosis, which occurred independently of estradiol treatment and was rescued by reintroduction of ERβ into these cells (Zhang et al. 2010). Mitochondrial localization of ER is also important in the MCF-7 human breast cancer cell line. Chen et al. discovered the presence of both the α and β isoforms of ER in mitochondria using a variety of biochemical techniques: confocal microscopy, and immunogold-labeling and electron microscopy. ER mitochondrial localization was enhanced by treatment with E_2 in a time- and concentration-dependent manner. The authors later identified an internal mitochondrial localization sequence within ERβ (Chen et al. 2004b).

Mitochondrial localization of ER has various effects on mitochondrial function. Mitochondrial ERβ inhibited apoptosis by interacting directly with the proapoptotic ligand Bad in a ligand-independent manner and exerted its antiapoptotic function by disrupting the interaction between Bad and the antiapoptotic proteins Bcl-2 and Bcl-X_L (Zhang et al. 2010). The presence of ER in mitochondria led to expression of mitochondrial DNA-encoded subunits I and II of cytochrome *c* oxidase (Chen et al. 2004b). Putative estrogen response elements have been identified in mtDNA (Sekeris 1990; Demonacos et al. 1996). Chen et al. used electrophoretic mobility shift assays and surface plasmon resonance to identify binding of mitochondrial extracts containing ER, as well as recombinant ERα and ERβ, to these putative mtDNA estrogen response elements (Chen et al. 2004a).

Estrogen itself can also act on mitochondria. Parkash et al. found that treatment of MCF-7 cells with 17β-estradiol (E_2) increased mitochondrial Ca^{2+} concentration, and this increase occurred after an E_2-induced increase in mitochondrial ROS. This increase in mitochondrial Ca^{2+} occurred through non-genomic mechanisms (Parkash et al. 2006). Using mitochondria isolated from Wistar rat liver, Moreira et al. discovered that the estrogen receptor modulator tamoxifen and estradiol affected mitochondrial function by decreasing respiratory control ratio, transmembrane potential, and depolarization potential. Treatment with E_2 decreased FCCP (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone)-stimulated respiration, state 3 respiration, and respiratory control ratio. Interestingly, the effects of tamoxifen were amplified by the presence of E_2. The estrogen receptor inhibitor ICI 182.780 had no effect on the actions of either tamoxifen or E_2, suggesting that the effects of estrogens on mitochondria can occur in a receptor-independent manner (Moreira et al. 2006).

10.3 Thyroid Hormone Receptor

Thyroid hormone (T3) is essential for the control of numerous cellular functions, in part, by regulating mitochondrial function. This regulation occurred through coordination of both nuclear and mitochondrial gene expression (Sinha et al. 2010;

Weitzel and Iwen 2011). T3 stimulated expression of many subunits of electron transport chain components encoded by nuclear DNA (Nelson et al. 1995; Li et al. 1997). Treatment of HTC rat hepatoma cells increased complex IV activity and expression of several mitochondrial DNA-encoded electron transport chain subunits, including subunit II of complex IV, but did not affect the expression of nuclear DNA-encoded subunits (Van Itallie 1990). Wiesner et al. reported the T3-dependent expression of various subunits of complex IV of the electron transport chain encoded by both nuclear DNA and mitochondrial DNA, demonstrating the coordination of nuclear and mitochondrial genomes by T3 (Wiesner et al. 1992). Wrutniak et al. later identified a 43 kDa truncated form of the T3 receptor (p43) in the matrix of mitochondria which bound to both palandromic T3 response elements and, more interestingly, the DR2 sequence located within the D-loop of mitochondrial DNA (Wrutniak et al. 1995). p43 had a similar affinity toward T3 as the nuclear T3 receptor and increased expression of mitochondrial mRNA and ribosomal RNA in a T3-dependent manner (Casas et al. 1999).

p43 homozygous knockout ($p43^{-/-}$) mice have proven valuable in determining the *in vivo* effects of T3 on mitochondrial function. Blanchet et al. discovered that complete loss of p43 resulted in hypothermia $p43^{-/-}$ mice, compared to wild-type animals, where hypothermia did not occur. Severe dysregulation in insulin secretion was observed in the $p43^{-/-}$ animals, which had significantly higher insulin levels during food withdrawal and lower insulin levels after feeding, compared to wild-type controls. Altered glucose metabolism was linked to a decrease in pancreatic islet density, as well as to decreased activity in complexes I and IV of the electron transport chain (Blanchet et al. 2012). In skeletal muscle, complete loss of p43 resulted in decreased mtDNA replication and electron transport chain activity, correlating with an increase in glycolytic phenotype of different muscle types tested, as well as increased utilization of lipids compared to wild-type animals. $p43^{-/-}$ mice also had significantly increased muscle mass compared to wild-type mice (Pessemesse et al. 2012). While knockout of p43 resulted in muscle hypertrophy, overexpression of p43 caused age-dependent muscle atrophy linked to a decrease in mitochondrial DNA content and an increase in oxidative stress, as measured by lipid and protein oxidation, in spite of an increase in antioxidant enzyme activity (catalase and superoxide dismutase) (Casas et al. 2009).

Altered T3 receptor function has been linked to cancer development (Cheng 2003; Aranda et al. 2009), and overexpression of p43 has been linked to transformation. Using human dermal fibroblast cells, Grandemange et al. found that overexpression of p43 resulted in myriad phenotypic changes indicative of transformation, including a more compact cell morphology, loss of contact inhibition, increased proliferation and anchorage-independent cell growth, and a decreased requirement for serum growth factors compared to control cells. The authors also discovered that overexpression of p43 increased tumor formation in athymic mice compared wild-type cells. Transformation after p43 overexpression was linked to decreased expression of several tumor-suppressing proteins (p53, retinoblastoma, and $p21^{WAF1}$), as well increased amounts of c-Fos and c-Jun proteins, and elevated mitochondrial activity and ROS production (Grandemange et al. 2005).

10.4 Cyclic AMP Response Element Binding Protein (CREB)

A cAMP/PKA/CREB signaling pathway exists in mitochondria and can regulate mitochondrial function through many mechanisms. Using Wistar rats, Cammarota et al. detected the presence of a small amount of CREB in rat brain mitochondria using a combination of subcellular fractionation and immunogold-staining electron microscopy. This mitochondrial CREB was able to be phosphorylated by PKA and had DNA binding activity, as determined by EMSA using oligonucleotides containing a CREB response element (Cammarota et al. 1999). CREB was able to translocate into mitochondria and stimulate expression of various electron transport chain components encoded by mitochondrial DNA (De Rasmo et al. 2009). PKA was also localized to mitochondria, and was responsible for phosphorylation of mitochondrial CREB and stimulation of binding mitochondrial CREB to the D-loop of mitochondrial DNA at CREB response elements after treatment with the antioxidant deferoxamine (Ryu et al. 2005). Acin-Perez et al. discovered the existence of a soluble adenylyl cycle (sac) in HeLa cervical carcinoma mitochondria, which was responsive to metabolically generated carbon dioxide. cAMP generated from sac stimulated mitochondrial PKA activity, resulting in altered phosphorylation of myriad mitochondrial proteins, including different subunits involved in electron transport (Acin-Perez et al. 2009).

10.5 Signal Transducers and Activators of Transcription (STAT)

STATs, like CREB, have also been identified in mitochondria, where they play an important role in regulating mitochondrial function. Fernley et al. identified GRIM-19, a cell death regulatory protein whose expression was stimulated by interferon-β and retinoic acid, as a subunit of complex I of the electron transport chain (Fearnley et al. 2001). GRIM-19 was later discovered to interact specifically with STAT3 and inhibit STAT3 transcriptional activity and target gene expression (Lufei et al. 2003). STAT3 interacts with complex I and is important for maintaining oxidative phosphorylation function. Knockout of STAT3 expression resulted in a significant reduction in state 3 respiration due to decreased activities of complexes I and II, which was restored by reexpression of STAT3. Similar results were observed in cells expressing a mitochondria-targeted STAT3, implying the importance of STAT3 in maintaining mitochondrial function through a transcription-independent mechanism (Wegrzyn et al. 2009).

The presence of STAT3 is necessary for maintenance of electron transport chain function after ischemia-induced cardiac injury. Using transgenic mice with a cardiomyocyte-specific expression of a mitochondria-targeted STAT3 with a mutation in the DNA binding domain (MT-STAT3E), Szczepanek et al. revealed that when STAT3 was present, mitochondria were protected from ischemia-induced injury. Complex I and II basal activities were slightly lower in the MT-STAT3E

expressing mice. Interestingly, complex I-dependent respiration was protected from ischemia-induced damage in MT-STAT3E hearts compared to wild-type controls. The presence of MT-STAT3E also inhibited cytochrome *c* release and ROS production after ischemia (Szczepanek et al. 2011). STAT3 in mitochondria is also vital for the protective effects of ischemic post-conditioning in heart tissue after infarction. Post-conditioning significantly reduced infarct size compared to immediate full reperfusion and correlated with an increase in tyrosine phosphorylated STAT3. Mitochondrial STAT3 also correlated with improved complex I respiration and calcium retention in isolated mitochondria of post-conditioned heart. The protective effects of post-conditioning were abrogated by pretreatment with the JAK/STAT inhibitor AG490, demonstrating the importance of mitochondrial STAT3 in postconditioning-mediated protection from ischemia (Heusch et al. 2011).

Mitochondrial localization of STATs is also important for tumorigenesis. Gough et al. reported the significance of mitochondrial STAT3 in the Ras-dependent oncogenic transformation in MCF10A normal human breast epithelial cells. The authors found that lack of STAT3 inhibited Ras-induced transformation; expression of STAT3 mutants that could not bind DNA or be tyrosine phosphorylated, or were retained in the cytoplasm, still enhanced Ras-mediated transformation. STAT3 in mitochondria was vital for transformation by maintaining oxidative phosphorylation and glycolysis activities characteristic of cancer cells (Gough et al. 2009). STAT5 translocated to mitochondria, and did so in a cytokine-dependent manner in the LSTRA leukemic T cell line. However, STAT1 and STAT3 did not translocate after cytokine treatment. STAT5 associated with the E2 component of the pyruvate dehydrogenase complex, as well as with the D-loop of mitochondrial DNA, suggesting that STAT5 may be important in direct regulation of mitochondrial activity after cytokine treatment in leukemia (Chueh et al. 2010).

10.6 Other Transcription Factors and Nuclear Receptors

Numerous other transcription factors and nuclear receptors have been identified in mitochondria, though their precise functions have not been fully illuminated. Casas et al. found a protein closely related to PPARγ (mt-PPAR) in the matrix of rat liver mitochondria, as well as in other rat tissues tested. mt-PPAR had a much lower molecular weight (45 kDa) compared to its nuclear counterpart (56 kDa) due to a lack of the C-terminal sequence found in the full-length protein. mt-PPAR did bind the DR2 region of the D-loop of mitochondrial DNA (Casas et al. 2000). Various reports suggest the presence of steroid hormone receptors in mitochondria other than ER, including AR (Solakidi et al. 2005a, b) and GR (Scheller et al. 2000; Moutsatsou et al. 2001; Koufali et al. 2003). The presence of GR in mitochondria affected mitochondrial function (Du et al. 2009), and correlated with sensitivity of various cell types to glucocorticoid-induced apoptosis (Sionov et al. 2006a, b; Talaber et al. 2009). Other transcription factors that have translocated to mitochondria include nuclear factor-κB (NF-κB) family members and activator protein-1 (AP-1) (Ogita et al. 2002, 2003), where mitochondrial DNA gene expression is regulated.

11 Conclusions

The ultimate goals of studying cancer are to prevent cancer growth and progression, and to improve cancer therapy. These can be achieved by understanding the communication between mitochondria and nucleus and by targeting the micro-environment where tumor cells and normal stromal cells interface. Mitochondria play myriad roles in the energy metabolism process and disease pathologies make emerging signaling systems appropriate candidates to target for drug development. By understanding these mechanisms, we will be able to accelerate opportunities to take this information through the translational research process into clinical research (Bench-to-Bedside), which will, in turn, eventually impact clinical practice.

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References

Acin-Perez R, Salazar E, Kamenetsky M, Buck J, Levin LR, Manfredi G (2009) Cyclic AMP produced inside mitochondria regulates oxidative phosphorylation. Cell Metab 9:265–276

Adams JM (2003) Ways of dying: multiple pathways to apoptosis. Genes Dev 17:2481–2495

Afford S, Randhawa S (2000) Demystified…Apoptosis. Mol Pathol 53:55–63

Ahles TA, Saykin AJ (2007) Candidate mechanisms for chemotherapy-induced cognitive changes. Nat Rev Cancer 7:192–201

Akhand AA, Pu M, Senga T, Kato M, Suzuki H, Miyata T, Hamaguchi M, Nakashima I (1999) Nitrix oxide controls Src kinase activity through sulfhydryl group modification-mediated Tyr-527-independent and Tyr-416-linked mechanism. J Biol Chem 274:25821–25826

Albracht SPJ (1980) The prosthetic groups in succinate dehydrogenase number and stoichiometry. Biochim Biophys Acta 612:11–28

Albracht SPJ, Subramanian J (1977) The number of Fe atoms in the iron-sulfur centers of the respiratory chain. Biochim Biophys Acta 462:36–48

Aleshin A, Finn RS (2010) SRC: a century of science brought to the clinic. Neoplasia 12:599–607

Aluise CD, Miriyala S, Noel T, Sultana R, Jungsuwadee P, Taylor TJ, Cai J, Pierce WM, Vore M, Moscow JA, St Clair DK, Butterfield DA (2011) 2-Mercaptoethane sulfonate prevents doxorubicin-induced plasma protein oxidation and TNF-α release: implications for the reactive oxygen species-mediated mechanisms of chemobrain. Free Radic Biol Med 50:1630–1638

Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465

Aranda A, Martinez-Iglesias O, Ruiz-Llorente L, Garcia-Carpizo V, Zambrano A (2009) Thyroid receptor: roles in cancer. Trends Endocrinol Metab 20:318–324

Arnold S, Beyer C (2009) Neuroprotection by estrogen in the brain: the mitochondrial compartment as presumed therapeutic target. J Neurochem 110:1–11

Augereau O, Claverol S, Boudes N, Basurko M-J, Bonneu M, Rossignol R, Mazat J-P, Letellier T, Dachary-Prigent J (2005) Identification of tyrosine-phosphorylated proteins of the mitochondrial oxidative phosphorylation machinery. Cell Mol Life Sci 62:1478–1488

Bakthavatchalu V, Dey S, Xu Y, Noel T, Jungsuwadee P, Holley AK, Dhar SK, Batinic-Haberle I, St Clair DK (2012) Manganese superoxide dismutase is a mitochondrial fidelity protein that protects Polg against UV-induced inactivation. Oncogene 31:2129–2139

Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A (2008) Analysis of the *IDH1* codon 132 in brain tumors. Acta Neuropathol 116:597–602

Barnas JL, Simpson-Abelson MR, Yokota SJ, Kelleher RJ, Bankert RB (2010) T cells and stromal fibroblasts in human tumor microenvironments represent potential therapeutic targets. Cancer Microenviron 3:29–47

Barsky SH, Karlin NJ (2005) Myoepithelial cells: autocrine and paracrine suppressors of breast cancer progression. J Mammary Gland Biol Neoplasia 10:249–260

Basu A, Castle VP, Bouziane M, Bhalla K, Haldar S (2006) Crosstalk between extrinsic and intrainsic cell death pathways in pancreatic cancer: synergistic action of estrogen metabolite and ligands of death receptor family. Cancer Res 66:4309–4318

Baysal BE (2007) A recurrent stop-codon mutation in succinate dehydrogenase subunit B gene in normal peripheral blood and childhood T-cell acute leukemia. PLoS One 5:e436

Baysal BE, Ferrell RE, Willet-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PEM, Rubinstein WS, Myers EN, Richard CW III, Cornelisse CJ, Devilee P, Devlin B (2000) Mutations in *SDHD*, a mitochondrial complex II gene, in hereditary paraganglioma. Science 287:848–851

Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87:245–313

Birch-Machin MA, Swalwell H (2010) How mitochondria record the effects of UV exposure and oxidative stress using human skin as a model tissue. Mutagenesis 25:101–107

Blanchet E, Bertrand C, Annicotte JS, Schlernitzauer A, Pessemesse L, Levin J, Fouret G, Feillet-Coudray C, Bonafos B, Fajas L, Cabello G, Wrutniak-Cabello C, Casas F (2012) Mitochondrial T3 receptor p43 regulates insulin secretion and glucose homeostasis. FASEB J 26:40–50

Block K, Eid A, Griendling KK, Lee D-Y, Wittrant Y, Gorin Y (2008) Nox4 NAD(P)H oxidase mediates Src-dependent tyrosine phosphorylation of PDK-1 in response to angiotensin II: role in mesangial cell hypertrophy and fibronectin expression. J Biol Chem 283:24061–24076

Block K, Gorin Y, Abboud HE (2009) Subcellular localization of Nox4 and regulation in diabetes. Proc Natl Acad Sci U S A 106:14385–14390

Boffoli D, Scacco SC, Vergari R, Solarino G, Santacroce G, Papa S (1994) Decline with age of the respiratory chain activity in human skeletal muscle. Biochim Biophys Acta 1226:73–82

Brandon M, Baldi P, Wallace DC (2006) Mitochondrial mutations in cancer. Oncogene 25:4647–4662

Briere J-J, Favier J, Gimenez-Roqueplo A-P, Rustin P (2006) Tricarboxylic acid cycle dysfunction as a cause of human diseases and tumor formation. Am J Physiol Cell Physiol 291:C1114–C1120

Brown GC, Borutaite V (2004) Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols. Biochim Biophys Acta 1658:44–49

Brown MS, Stemmer SM, Simon JH, Stears JC, Jones RB, Cagnoni PJ, Sheeder JL (1998) White matter disease induced by high-dose chemotherapy: longitudinal study with MR imaging and proton spectroscopy. AJNR Am J Neuroradiol 19:217–221

Bulua AC, Simon A, Maddipati R, Pelletier M, Park H, Kim K-Y, Sack MN, Kastner DL, Siegel RM (2011) Mitochondrial reactive oxygen species promote production of proinflammatory cytokines and are elevated in TNFR1-associated periodic syndrome (TRAPS). J Exp Med 208:519–533

Cammarota M, Paratcha G, Bevilaqua LRM, de Stein ML, Lopez M, de Iraldi AP, Izquierdo I, Medina JH (1999) Cyclic AMP responsive element binding protein in brain mitochondria. J Neurochem 72:2272–2277

Cardone L, Carlucci A, Affaitati A, Livigni A, deCristofaro T, Garbi C, Varrone S, Ullrich A, Gottesman ME, Avvedimento EV, Feliciello A (2004) Mitochondrial AKAP121 binds and targets protein tyrosine phosphatase D1, a novel positive regulator of src signaling. Mol Cell Biol 24:4613–4626

Casas F, Rochard P, Rodier A, Cassar-Malek I, Marchal-Victorion S, Wiesner RJ, Cabello G, Wrutniak C (1999) A variant form of the nuclear triiodothyronine receptor c-ErbAa1 plays a direct role in regulation of mitochondrial RNA synthesis. Mol Cell Biol 19:7913–7924

Casas F, Domenjoud L, Rochard P, Hatier R, Rodier A, Daury L, Bianchi A, Kremarik-Bouillaud P, Becuwe P, Keller J-M, Schohn H, Wrutniak-Cabello C, Cabello G, Dauca M (2000) A 45 kDa

protein related to PPARγ2 induced by peroxisome proliferators, is located in the mitochondrial matrix. FEBS Lett 478:4–8

Casas F, Pessemesse L, Grandemange S, Seyer P, Baris O, Gueguen N, Ramonatxo C, Perrin F, Fouret G, Lepourry L, Cabello G, Wrutniak-Cabello C (2009) Overexpression of the mitochondrial T3 receptor induces skeletal muscle atrophy during aging. PLoS One 4:e5631

Case AJ, McGill JL, Tygrett LT, Shirasawa T, Spitz DR, Waldschmidt TJ, Legge KL, Domann FE (2011) Elevated mitochondrial superoxide disrupts normal T cell development, impairing adaptive immune response to an influenza challenge. Free Radic Biol Med 50:448–458

Cervera AM, Apostolova N, Crespo FL, Mata M, McCreath KJ (2008) Cells silenced for *SDHB* expression display characteristic features of the tumor phenotype. Cancer Res 68:4058–4067

Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriquez AM, Schumacker PT (2000) Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia. A mechanism of O_2 sensing. J Biol Chem 275:25130–25138

Chatelain EH, Dupuy J-W, Letellier T, Dachary-Prigent J (2011) Functional impact of PTP1B-mediated Src regulation on oxidative phosphorylation in rat brain mitochondria. Cell Mol Life Sci 68:2603–2613

Chen XJ, Butow RA (2005) The organization and inheritance of the mitochondrial genome. Nat Rev Genet 6:815–825

Chen JQ, Eshete M, Alworth WL, Yager JD (2004a) Binding of MCF-7 cell mitochondrial proteins and recombinant human estrogen receptors a and b to human mitochondrial DNA estrogen response elements. J Cell Biochem 93:358–373

Chen JZ, Delannoy M, Cooke C, Yager JD (2004b) Mitochondrial localization of ERα and ERβ in human MCF7 cells. Am J Physiol Endocrinol Metab 286:E1011–E1022

Chen Y-R, Chen C-L, Zhang L, Green-Church KB, Zweier JL (2005) Superoxide generation from mitochondrial NADH dehydrogenase induces self-inactivation with specific protein radical formation. J Biol Chem 280:37339–37348

Cheng S-Y (2003) Thyroid hormone receptor mutations in cancer. Mol Cell Endocrinol 213:23–30

Chicco AJ, Sparagna GC (2007) Role of cardiolipin alterations in mitochondrial dysfunction and disease. Am J Physiol Cell Physiol 292:C33–C44

Chinta SJ, Andersen JK (2011) Nitrosylation and nitration of mitochondrial complex I in Parkinson's disease. Free Radic Res 45:53–58

Chotirat S, Thongnoppakhun W, Promsuwicha O, Boonthimat C, Auewarakul CU (2012) Molecular alterations of isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) metabolic genes and additional genetic mutations in newly diagnosed acute myeloid leukemia patients. J Hematol Oncol 5:5

Christianson DW (1997) Structural chemistry and biology of manganese metalloenzymes. Prog Biophys Mol Biol 67:217–252

Chua YL, Dufour E, Dassa EP, Rustin P, Jacobs HT, Taylor CT, Hagen T (2010) Stabilization of hypoxia-inducible factor-1α occurs independently of mitochondrial reactive oxygen species production. J Biol Chem 285:31277–31284

Chueh F-Y, Leong K-F, Yu C-L (2010) Mitochondrial translocation of signal transducer and activator of transcription 5 (STAT5) in leukemic T cells and cytokine-stimulated cells. Biochem Biophys Res Commun 402:778–783

Church SL, Grant JW, Ridnour LA, Oberley LW, Swanson PE, Meltzer PS, Trent JM (1993) Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. Proc Natl Acad Sci U S A 90:3113–3117

Cinel I, Opal SM (2009) Molecular biology of inflammation and sepsis: a primer. Crit Care Med 37:291–304

Consortium TML (2002) Germline mutations in *FH* predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nat Genet 30:406–410

Cortopassi G, Wang E (1995) Modelling the effects of age-related mtDNA mutation accumulation; Complex I deficiency, superoxide and cell death. Biochim Biophys Acta 1271:171–176

Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, Cho HI, Celis E, Quiceno DG, Padhya T, McCaffrey TV, McCaffrey JC, Gabrilovich DI (2010) HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. J Exp Med 207:2439–2453

Daosukho C, Kiningham K, Kasarskis EJ, Ittarat W, St Clair DK (2002) Tamoxifen enhancement of TNF-α induced MnSOD expression: modulation of NF-κB dimerization. Oncogene 21:3603–3610

Daosukho C, Ittarat W, S-M L, Sawyer DB, Kiningham K, Lien Y-C, St Clair DK (2005) Induction of manganese superoxide dismutase (MnSOD) mediates cardioprotective effect of tamoxifen (TAM). J Mol Cell Cardiol 39:792–803

Dasgupta J, Subbaram S, Connor KM, Rodriguez AM, Tirosh O, Beckman JS, Jourd'Heuil D, Melendez JA (2006) Manganese superoxide dismutase protects from TNF-α-induced apoptosis by increasing the steady-state production of H_2O_2. Antioxid Redox Signal 8:1295–1305

De Rasmo D, Signorile A, Roca E, Papa S (2009) cAMP response element-binding protein (CREB) is imported into mitochondria and promotes protein synthesis. FEBS J 276: 4325–4333

Demonacos CV, Karayanni N, Hatzoglou E, Tsiriyiotis C, Spandidos DA, Sekeris CE (1996) Mitochondrial genes as sites of primary action of steroid hormones. Steroids 61:226–232

Denkert C, Budczies J, Weichert W, Wohlgemuth G, Scholz M, Kind T, Niesporek S, Noske A, Buckendahl A, Dietel M, Fiehn O (2008) Metabolite profiling of human colon carcinoma-deregulation of TCA cycle and amino acid turnover. Mol Cancer 7:72

Dhar SK, Tangpong J, Chaiswing L, Oberley TD, St Clair DK (2011) Manganese superoxide dismutase is a p53-regulated gene that switches cancers between early and advanced stages. Cancer Res 71:6684–6695

Dostert C, Petrilli V, van Bruggen R, Steele C, Mossman BT, Tschopp J (2008) Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science 320:674–677

Du J, McEwen B, Manji HK (2009) Glucocorticoid receptors modulate mitochondrial function: a novel mechanism for neuroprotection. Commun Integr Biol 2:350–352

Duttaroy A, Paul A, Kundu M, Belton A (2003) A *Sod2* null mutation confers severely reduced adult life span in drosophila. Genetics 165:2295–2299

Egeblad M, Nakasone ES, Werb Z (2010) Tumors as organs: complex tissues that interface with the entire organism. Dev Cell 18:884–901

Epperly MW, Bray JA, Esocobar P, Bigbee WL, Watkins S, Greenberger JS (1999) Overexpression of the human manganese superoxide dismutase (MnSOD) transgene in subclones of murine hematopoietic progenitor cell line 32D cl 3 decreases irradiation-induced apoptosis but does not alter G2/M or G1/S phase cell cycle arrest. Radiat Oncol Investig 7:331–342

Epperly MW, Sikora CA, DeFilippi SJ, Gretton JE, Zhan Q, Kufe DW, Greenberger JS (2002) Manganese superoxide dismutase (SOD2) inhibits radiation-induced apoptosis by stabilization of the mitochondrial membrane. Radiat Res 157:568–577

Epperly MW, Bernarding M, Gretton J, Jefferson M, Nie S, Greenberger JS (2003) Overexpression of the transgene for manganese superoxide dismutase (MnSOD) in 32D cl 3 cells prevents apoptosis induction by TNF-α, IL-3 withdrawal, and ionizing radiation. Exp Hematol 31:465–474

Epperly MW, Carpenter M, Agarwal A, Mitra P, Nie S, Greenberger JS (2004) Intraoral manganese superoxide dismutase-plasmid/liposome (MnSOD-PL) radioprotective gene therapy decreases ionizing irradiation-induced murine mucosal cell cycling and apoptosis. In Vivo 18:401–410

Evans RM (1988) The steroid and thyroid hormone receptor superfamily. Science 240:889–895

Fearnley IM, Carroll J, Shannon RJ, Runswick MJ, Walker JE, Hirst J (2001) GRIM-19, a cell death regulatory gene product, is a subunit of bovine mitochondrial NADH: ubiquinone oxidoreductase (complex I). J Biol Chem 276:28245–38348

Fijalkowska I, Xu W, Comhair SAA, Janocha AJ, Mavrakis LA, Krishnamachary B, Zhen L, Mao T, Richter A, Erzurum SC, Tuder RM (2010) Hypoxia inducible-inducible factor1α regulates the metabolic shift of pulmonary hypertensive endothelial cells. Am J Pathol 176:1130–1138

Flynn JM, Dimitrijevich SD, Younes M, Skliris G, Murphy LC, Cammarata PR (2008) Role of wild-type estrogen receptor-β in mitochondrial cytoprotection of cultured normal male and female human lens epithelial cells. Am J Physiol Endocrinol Metab 295:E637–E647

Frezza C, Pollard PJ, Gottlieb E (2011) Inborn and acquired metabolic defects in cancer. J Mol Med 89:213–220

Galluzzi L, Morselli E, Kepp O, Vitale I, Rigoni A, Vacchelli E, Michaud M, Zischka H, Castedo M, Kroemer G (2010) Mitochondrial gateways to cancer. Mol Aspects Med 31:1–20

Garcia-Ramirez M, Francisco G, Garcia-Arumi E, Hernandez C, Martinez R, Andreu AL, Simo R (2008) Mitochondrial DNA oxidation and manganese superoxide dismutase activity in peripheral blood mononuclear cells fro type 2 diabetic patients. Diabetes Metab 34:117–124

Gardner PR, Raineri I, Epstein LB, White CW (1995) Superoxide radical and iron modulate aconitase activity in mammalian cells. J Biol Chem 270:13399–13405

Garrido N, Griparic L, Jokitalo E, Wartiovaara J, van der Bliek AM, Spelbrink JN (2003) Composition and dynamics of human mitochondrial nucleoids. Mol Biol Cell 14:1583–1596

Gavrilova-Jordan LP, Price TM (2007) Actions of steroids in mitochondria. Semin Reprod Med 25:154–164

Giannoni E, Buricchi F, Raugei G, Ramponi G, Chiarugi P (2005) Intracellular reactive oxygen species activate Src tyrosine kinase during cell adhesion and anchorage-dependent cell growth. Mol Cell Biol 25:6391–6403

Giannoni E, Taddei ML, Chiarugi P (2010) Src redox regulation: again in the front line. Free Radic Biol Med 49:516–527

Gius D, Spitz DR (2006) Redox signaling in cancer biology. Antioxid Redox Signal 8:1249–1252

Goossens V, Grooten J, De Vos K, Fiers W (1995) Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. Proc Natl Acad Sci U S A 92:8115–8119

Gough DJ, Corlett A, Schlessinger K, Wegrzyn J, Larner AC, Levy DE (2009) Mitochondrial STAT3 supports Ras-dependent oncogenic transformation. Science 324:1713–1716

Graham KA, Kulawiec M, Owens KM, Li X, Desouki MM, Chandra D, Singh KK (2010) NADPH oxidase 4 is an oncoprotein localized to mitochondria. Cancer Biol Ther 10:1–9

Grandemange S, Seyer P, Carazo A, Becuwe P, Pessemesse L, Busson M, Marsac C, Roger P, Casas F, Cabello G, Wrutniak-Cabello C (2005) Stimulation of mitochondrial activity by p43 overexpression induces human dermal fibroblast transformation. Cancer Res 65:4282–4291

Graziewicz MA, Day BJ, Copeland WC (2002) The mitochondrial DNA polymerase as a target of oxidative damage. Nucleic Acids Res 30:2817–2824

Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281:1309–1312

Gregory EM, Fridovich I (1973) Oxygen toxicity and the superoxide dismutase. J Bacteriol 114:1193–1197

Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. Cell 140:883–899

Guarino M (2010) Src signaling in cancer invasion. J Cell Physiol 223:14–26

Gulbins E, Dreschers S, Bock J (2003) Role of mitochondria in apoptosis. Exp Physiol 88:85–90

Guzy RD, Hoyos B, Robin E, Chen H, Liu L, Mansfield KD, Simon MC, Hammerling U, Schumacker PT (2005) Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. Cell Metab 1:401–408

Guzy RD, Sharma B, Bell E, Chandel NS, Schumacker PT (2008) Loss of the SdhB, but not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. Mol Cell Biol 28:718–731

Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100:57–70

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674

Hebert-Chatelain E, Jose C, Cortex NG, Dupuy J-W, Rocher C, Dachary-Prigent J, Letellier T (2012) Preservation of NADH ubiquinone-oxidoreductase activity by Src kinase-mediated phosphorylation of NDUFB10. Biochim Biophys Acta 1817:718–725

Hervouet E, Simonnet H, Godinot C (2007) Mitochondria and reactive oxygen species in renal cancer. Biochimie 89:1080–1088

Heusch G, Musiolik J, Gedik N, Skyschally A (2011) Mitochondrial STAT3 activation and cardioprotection by ishcemic postconditioning in pigs with regional myocardial ischemia/reperfusion. Circ Res 109:1302–1308

Hirose K, Longo DI, Oppenheim JJ, Matsushima K (1993) Overexpression of mitochondrial manganese superoxide dismutase promotes the survival of tumor cells exposed to interleukin-1, tumor necrosis factor, selected anticancer drugs, and ionizing radiation. FASEB J 7:361–368

Houtkooper RH, Vaz FM (2008) Cardiolipin, the heart of mitochondrial metabolism. Cell Mol Life Sci 65:2493–2506

Hu H, Luo ML, Du XL, Feng YB, Zhang Y, Shen XM, Xu X, Cai Y, Han YL, Wang MR (2007) Up-regulated manganese superoxide dismutase expression increases apoptosis resistance in human esophageal squamous cell carcinomas. Chin Med J 120:2092–2098

Hussain SP, Amstad P, He P, Robles A, Lupold S, Kaneko I, Ichimiya M, Sengupta S, Mechanic L, Okamura S, Hofseth LJ, Moake M, Nagashima M, Forrester KS, Harris CC (2004) p53-induced up-regulation of MnSOD and GPx but not catalase increases oxidative stress and apoptosis. Cancer Res 64:2350–2356

Iborra FJ, Kimura H, Cook PR (2004) The functional organization of mitochondrial genomes in human cells. BMC Biol 2:9–22

Ichimura K, Pearson DM, Kocialkowski S, Backlund LM, Chan R, Jones DTW, Collins VP (2009) IDH mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro Oncol 11:341–347

Inagaki M, Yoshikawa E, Matsuoka Y, Sugawara Y, Nakano T, Akechi T, Wada N, Imoto S, Murakami K, Uchitomi Y (2007) Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. Cancer 109:146–156

Indo HP, Davidson M, Yen H-C, Suenaga S, Tomita K, Nishii T, Higuchi M, Koga Y, Ozawa T, Majima HJ (2007) Evidence of ROS generation by mitochondria in cells with impaired electron transport chain and mitochondrial DNA damage. Mitochondrion 7:106–118

Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H, Nakada K, Honma Y, Hayashi J-I (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 320:661–664

Itoh S, Lemay S, Osawa M, Che W, Duan Y, Tompkins A, Brookes PS, Sheu S-S, Abe JI (2005) Mitochondrial Dok-4 recruits Src kinase and regulates NF-kB activation in endothelial cells. J Biol Chem 280:26383–26396

Ivanova MM, Mazhawidza W, Dougherty SM, Klinge CM (2010) Sex differences in estrogen receptor subcellular location and activity in lung adenocarcinoma cells. Am J Respir Cell Mol Biol 42:320–330

Izawa S, Kono K, Mimura K, Kawaguchi Y, Watanabe M, Maruyama T, Fujii H (2011) H_2O_2 production within tumor microenvironment inversely correlated with infiltration of CD56(dim) NK cells in gastric and esophageal cancer: possible mechanisms of NK cell dysfunction. Cancer Immunol Immunother 60:1801–1810

Jandova J, Shi M, Norman KG, Stricklin GP, Sligh JE (2012) Somatic alterations in mitochondrial DNA produce changes in cell growth and metabolism supporting a tumorigenic phenotype. Biochim Biophys Acta 1822:293–300

Jaramillo MC, Frye JB, Crapo JD, Briehl MM, Tome ME (2009) Increased manganese superoxide dismutase expression or treatment with manganese porphyrin potentiates dexamethasone-induced apoptosis in lymphoma cells. Cancer Res 69:5450–5457

Jennings GT, Minard KI, McAlister-Henn L (1997) Expression and mutagenesis of mammalian cytosolic NADP +− specific isocitrate dehydrogenase. Biochemistry 36:13743–13747

Jiang B-H, Rue E, Wang GL, Roe R, Semenza GL (1996) Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J Biol Chem 271:17771–17778

Johnson FM, Gallick GE (2007) Src family nonreceptor tyrosine kinases as molecular targets for cancer therapy. Anticancer Agents Med Chem 7:651–659

Jones JL, Shaw JA, Pringle JH, Walker RA (2003) Primary breast myoepithelial cells exert an invasion-suppressor effect on breast cancer cells via paracrine down-regulation of MMP expression in fibroblasts and tumour cells. J Pathol 201:562–572

Joshi G, Sultana R, Tangpong J, Cole MP, St Clair DK, Vore M, Estus S, Butterfield DA (2005) Free radical mediated oxidative stress and toxic side effects in brain induced by the anticancer drug adriamycin: insight into chemobrain. Free Radic Res 29:1147–1154

Kaewpila S, Venkataraman S, Buettner GR, Oberley LW (2008) Manganese superoxide dismutase modulates hypoxia-inducible factor-1a induction via superoxide. Cancer Res 68:2781–2788

Kahlos K, Soini Y, Paakko P, Saily M, Linnainmaa K, Kinnula VL (2000) Proliferation, apoptosis, and manganese superoxide dismutase in malignant mesothelioma. Int J Cancer 88:37–43

Kang D, Hamasaki N (2005) Alterations of mitochondrial DNA in common diseases and disease states: aging, neurodegeneration, heart failure, diabetes, and cancer. Curr Med Chem 12:429–441

Kang YJ, Sun X, Chen Y, Zhou Z (2002) Inhibition of doxorubicin chronic toxicity in catalase-overexpressing transgenic mouse hearts. Chem Res Toxicol 15:1–6

Kawai T, Akira S (2009) The roles of TLRs, RLRs and NLRs in pathogen recognition. Int Immunol 21:317–337

Ke Q, Costa M (2006) Hypoxia-inducible factor-1 (HIF-1). Mol Pharmacol 70:1469–1480

Keller JN, Kindy MS, Holtsberg FW, St Clair DK, Yen H-C, Germeyer A, Steiner SM, Bruce-Keller AJ, Hutchins JB, Mattson MP (1998) Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. J Neurosci 18:687–697

Kemble DJ, Sun G (2009) Direct and specific inactivation of protein tyrosine kinases in the Src and FGFR families by reversible cysteine oxidation. Proc Natl Acad Sci U S A 106:5070–5075

Kienhofer J, Haussler DJF, Ruckelshausen F, Muessig E, Weber K, Pimentel D, Ullrich V, Burkle A, Bachschmid MM (2009) Association of mitochondrial antioxidant enzymes with mitochondrial DNA as integral nucleoid constituents. FASEB J 23:2034–2044

Kiningham KK, Oberley TD, Lin S-M, Mattingly CA, St Clair DK (1999) Overexpression of manganese superoxide dismutase protects against mitochondrial-initiated poly(ADP-ribose) polymerase-mediated cell death. FASEB J 13:1601–1610

Klimova T, Chandel NS (2008) Mitochondrial complex III regulates hypoxic activation of HIF. Cell Death Differ 15:660–666

Koivunen P, Hirsila M, Remes AM, Hassinen IE, Kivirikko KI, Myliyharju J (2007) Inhibition of hypoxia-inducible factor (HIF) hydroxylases by citric acid cycle intermediates: possible links between cell metabolism and stabilization of HIF. J Biol Chem 282:4524–4532

Koufali M-M, Moutsatsou P, Sekeris CE, Breen KC (2003) The dynamic localization of the glucocorticoid receptor in rat C6 glioma cell mitochondria. Mol Cell Endocrinol 209:51–60

Krasnowska EK, Pittaluga E, Brunati AM, Brunelli R, Costa G, De Spirito M, Serafino A, Ursini F, Parasassi T (2008) *N*-acetyl-l-cysteine fosters inactivation and transfer to endolysosomes of c-Src. Free Radic Biol Med 45:1566–1572

Larosche I, Letteron P, Berson A, Fromenty B, Huang T-T, Moreau R, Pessayre D, Mansouri A (2010) Hepatic mitochondrial DNA depletion after an alcohol binge in mice: probable role of peroxynitrite and modulation by manganese superoxide dismutase. J Pharmacol Exp Ther 332:886–897

Lebovitz RM, Zhang H, Vogel H, Cartwright J Jr, Dionne L, Lu N, Huang S, Matzuk MM (1996) Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. Proc Natl Acad Sci U S A 93:9782–9787

Lee J, Sharma S, Kim J, Ferrante RJ, Ryu H (2008) Mitochondrial nuclear receptors and transcription factors: who's minding the cell? J Neurosci Res 86:961–971

Lee S-M, Lee Y-S, Choi J-H, Park S-G, Choi I-W, Joo Y-D, Lee W-S, Lee J-N, Choi I, Seo S-K (2010) Tryptophan metabolite 3-hydroxyanthranilic acid selectively induces activated T cell death via intracellular GSH depletion. Immunol Lett 132:53–60

Legros F, Malka F, Frachon P, Lombes A, Rojo M (2004) Organization and dynamics of human mitochondrial DNA. J Cell Sci 117:2653–2662

Lehtonen HJ, Kiuru M, Ylisaukko-oja SK, Salovaara R, Herva R, Koivisto PA, Vierimaa O, Aittomaki K, Pukkala E, Launonen V, Aaltonen LA (2006) Increased risk of cancer in patients with fumarate hydratase germline mutation. J Med Genet 43:523–526

Leigh-Brown S, Enriquez JA, Odom DT (2010) Nuclear transcription factors in mammalian mitochondria. Genome Biol 11:215

Li JJ, Oberley LW, St Clair DK, Ridnour LA, Oberley TD (1995a) Phenotypic changes induced in human breast cancer cells by overexpression of manganese-containing superoxide dismutase. Oncogene 10:1989–2000

Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH, Wallace DC, Epstein CJ (1995b) Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. Nat Genet 11:376–381

Li R, Luciakova K, Zaid A, Betina S, Fridell E, Nelson BD (1997) Thyroid hormone activates transcription from the promoter regions of some human nuclear-encoded genes of the oxidative phosphorylation system. Mol Cell Endocrinol 128:69–75

Livigni A, Scorziello A, Agnese S, Adornetto A, Carlucci A, Garbi C, Castaldo I, Annunziato L, Avvedimento EV, Feliciello A (2006) Mitochondrial AKAP121 links cAMP and src signaling to oxidative metabolism. Mol Biol Cell 17:263–271

Lluis JM, Buricchi F, Chiarugi P, Morales A, Fernandez-Checa JC (2007) Dual role of mitochondrial reactive oxygen species in hypoxia signaling: activation of nuclear factor-κB via c-SRC- and oxidant-dependent cell death. Cancer Res 67:7368–7377

Longo VD, Liou L-L, Valentine JS, Gralla EB (1999) Mitochondrial superoxide decreases yeast survival in stationary phase. Arch Biochem Biophys 365:131–142

Lu S-P, Feng M-HL, Huang H-L, Huang Y-C, Tsou W-I, Lai M-Z (2007) Reactive oxygen species promote raft formation in T lymphocytes. Free Radic Biol Med 42:936–944

Lu J, Sharma LK, Bai Y (2009) Implications of mitochondrial DNA mutations and mitochondrial dysfunction in tumorigenesis. Cell Res 19:802–815

Lufei C, Ma J, Huang G, Zhang T, Novotny-Diermayr V, Ong CT, Cao X (2003) GRIM-19, a death-regulatory gene product, suppresses Stat3 activity via functional interaction. EMBO J 22:1325–1335

Madsen-Bouterse SA, Zhong Q, Mohammad G, Ho Y-S, Kowluru RA (2010) Oxidative damage of mitochondrial DNA in diabetes and its protection by manganese superoxide dismutase. Free Radic Res 44:313–321

Majno G, Joris I (1995) Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 146:3–15

Mambo E, Gao X, Cohen Y, Guo Z, Talalay P, Sidransky D (2003) Electrophile and oxidant damage of mitochondrial DNA leading to rapid evolution of homplasmic mutations. Proc Natl Acad Sci 100:1838–1843

Mansouri A, Tarhuni A, Larosche I, Reyl-Desmars F, Demeilliers C, Degoul F, Nahon P, Sutton A, Moreau R, Fromenty B, Ressayre D (2010) MnSOD overexpression prevents liver mitochondrial DNA depletion after an alcohol binge but worsens this effect after prolonged alcohol consumption in mice. Dig Dis 28:756–775

Martin FM, Xu X, von Lohneysen K, Gilmartin TJ, Friedman J (2011) SOD2 deficient erythroid cells up-regulate transferrin receptor and down-regulate mitochondrial biogenesis and metabolism. PLoS One 6:e16894

Martinez-Mir A, Glaser B, Chuang GS, Horev L, Waldman A, Engler DE, Gordon D, Spelman LJ, Hatzibougias I, Green J, Christiano AM, Zlotogorski A (2003) Germline fumarate hydratase mutations in families with multiple cutaneous and uterine leiomyomata. J Invest Dermatol 121:741–744

Martinez-Outschoorn UE, Balliet RM, Rivadeneira DB, Chiavarina B, Pavlides S, Wang C, Whitaker-Menezes D, Daumer KM, Lin Z, Witkiewicz AK, Flomenberg N, Howell A, Pestell RG, Knudsen ES, Sotgia F, Lisanti MP (2010a) Oxidative stress in cancer associated fibroblasts

drives tumor-stroma co-evolution: a new paradigm for understanding tumor metabolism, the field effect and genomic instability in cancer cells. Cell Cycle 9:3256–3276

Martinez-Outschoorn UE, Pavlides S, Whitaker-Menezes D, Daumer KM, Milliman JN, Chiavarina B, Migneco G, Witkiewicz AK, Martinez-Cantarin MP, Flomenberg N, Howell A, Pestell RG, Lisanti MP, Sotgia F (2010b) Tumor cells induce the cancer associated fibroblast phenotype via caveolin-1 degradation: implications for breast cancer and DCIS therapy with autophagy inhibitors. Cell Cycle 9:2423–2433

Martinez-Outschoorn UE, Lin Z, Trimmer C, Flomenberg N, Wang C, Pavlides S, Pestell RG, Howell A, Sotgia F, Lisanti MP (2011) Cancer cells metabolically "fertilize" the tumor microenvironment with hydrogen peroxide, driving the Warburg effect: implications for PET imaging of human tumors. Cell Cycle 10:2504–2520

Martinon F (2010) Signaling by ROS drives inflammasome activation. Eur J Immunol 40:616–619

Martinon F, Mayor A, Tschopp J (2009) The inflammasomes: guardians of the body. Annu Rev Immunol 27:229–265

Mattson MP, Goodman Y, Luo H, Fu W, Furukawa K (1997) Activation of NF-κB protects hippocampal neurons against oxidative stress-induced apoptosis: evidence for induction of manganese superoxide dismutase and suppression of peroxynitrite production and protein tyrosine nitration. J Neurosci Res 49:681–697

McKenna NJ, Lanz RB, O'Malley BW (1999) Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 20:321–344

Meissner F, Seger RA, Moshous S, Fischer A, Reichenbach J, Zychlinsky A (2010) Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease. Blood 116:1570–1573

Milanesi L, de Boland AR, Boland R (2008) Expression and localization of estrogen receptor a in the C2C12 murine skeletal muscle cell line. J Cell Biochem 104:1254–1273

Milanesi L, Vasconsuelo A, de Boland AR, Boland R (2009) Expression and subcellular distribution of native estrogen receptor b in murine C2C12 cells and skeletal muscle tissue. Steroids 74:489–497

Milner TA, Ayoola K, Drake CT, Herrick SP, Tabori NE, McEwen BS, Warrier S, Alves SE (2005) Ultrastructural localization of estrogen receptor b immunoreactivity in the rat hippocampal formation. J Comp Neurol 491:81–95

Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L (2004) Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev 56:185–229

Miyazaki T, Tanaka S, Sanjay A, Baron R (2006) The role of c-Src kinase in the regulation of osteoclast function. Mod Rheumatol 16:68–74

Mohr A, Buneker C, Gough RP, Zwacka RM (2007) MnSOD protects colorectal cancer cells from TRAIL-induced apoptosis by inhibition of Smac/DIABLO release. Oncogene 27:763–774

Moreira PI, Custodio J, Moreno A, Oliveira CR, Santos MS (2006) Tamoxifen and estradiol interact with the flavin mononucleotide site of complex I leading to mitochondrial failure. J Biol Chem 281:10143–10152

Morgan MJ, Lehmann M, Schwarzlander M, Baxter CJ, Sienkiewicz-Porzucek A, Williams TCR, Schauer N, Fernie AR, Fricker MD, Ratcliffe RG, Sweetlove LJ, Finkemeier I (2008) Decrease in manganese superoxide dismutase leads to reduced root growth and affects tricarboxylic acid cycle flux and mitochondrial redox homeostasis. Plant Physiol 147:101–114

Moulian N, Truffault F, Gaudry-Talarmain YM, Serraf A, Berrih-Aknin S (2008) In vivo and in vitro apoptosis of human thymocytes are associated with nitrotyrosine formation. Blood 97:3521–3530

Moutsatsou P, Psarra AMG, Tsiapara A, Paraskevakou H, Davaris P, Sekeris CE (2001) Localization of the glucocorticoid receptor in rat brain mitochondria. Arch Biochem Biophys 386:69–78

Nakahira K, Hapsel JA, Rathinam VAK, Lee S-J, Dolinay T, Lam HC, Englert JA, Rabinovitch M, Cernadas M, Kim HP, Fitzgerald KA, Ryter SW, Choi AMK (2011) Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol 8:222–230

Nelson BD, Luciakova K, Li R, Betina S (1995) The role of thyroid hormone and promoter diversity in the regulation of nuclear encoded mitochodnrial proteins. Biochim Biophys Acta 1271:85–91

Nelson CJ, Nandy N, Roth AJ (2007) Chemotherapy and cognitive deficits: mechanisms, findings, and potential interventions. Palliat Support Care 5:273–280

Ni Y, Zbuk KM, Sadler T, Patocs A, Lobo G, Edelman E, Platzer P, Orloff MS, Waite KA, Eng C (2008) Germline mutations and variants in the succinate dehydrogenase genes in Cowden and Cowden-like syndromes. Am J Hum Genet 83:261–268

Norberg E, Gogvadze V, Vakifahmetoglu H, Orrenius S, Zhivotovsky B (2010) Oxidative modification sensitizes mitochondrial apoptosis-inducing factor to calpain-mediated processing. Free Radic Biol Med 48:791–797

Oberley LW, Buettner GR (1979) Role of superoxide dismutase in cancer: a review. Cancer Res 39:1141–1149

Ogita K, Okuda H, Kitano M, Fujinami Y, Ozaki K, Yoneda Y (2002) Localization of activator protein-1 complex with DNA binding activity in mitochondria of murine brain after *in vivo* treatment with kainate. J Neurosci 22:2561–2570

Ogita K, Fujinami Y, Kitano M, Yoneda Y (2003) Transcription factor activator protein-1 expressed by kainate treatment can bind to the non-coding region of mitochondrial genome in murine hippocampus. J Neurosci Res 73:794–802

Ohnishi T (1975) Thermodynamic and EPR characterization of iron-sulfur centers in the NADH-ubiquinone segment of the mitochondrial respiratory chain in pigeon heart. Biochim Biophys Acta 387:475–490

Ohnishi T (1998) Iron-sulfur clusters/semiquinones in complex I. Biochim Biophys Acta 1364:186–206

Ott M, Gogvadze V, Orrenius S, Zhivotovsky B (2007) Mitochondria, oxidative stress and cell death. Apoptosis 12:913–922

Owens KM, Kulawiec M, Desouki MM, Vanniarajan A, Singh KK (2011) Impaired OXPHOS complex III in breast cancer. PLoS One 6:e23846

Owusu-Ansah E, Banerjee U (2009) Reactive oxygen species prime *Drosophila* haematopoietic progenitors for differentiation. Nature 461:537–541

Palazzotti B, Pani G, Colavitti R, de Leo ME, Bedogni B, Borrello S, Galeotti T (1999) Increased growth capacity of cervical-carcinoma cells over-expressing manganous superoxide dismutase. Int J Cancer 82:145–150

Pandey PR, Saidou J, Watabe K (2010) Role of myoepithelial cells in breast tumor progression. Front Biosci 15:226–236

Pardo M, Melendez JA, Tirosh O (2006) Manganese superoxide dismutase inactivation during Fas (CD95)-mediated apoptosis in Jurkat T cells. Free Radic Biol Med 41:1795–1806

Parkash J, Felty Q, Roy D (2006) Estrogen exerts a spatial and temporal influence on reactive oxygen species generation that precedes calcium uptake in high-capacity mitochondria: implications for rapid nongenomic signaling of cell growth. Biochemistry 45:2872–2881

Patten DA, Lafleur VN, Robitaille GA, Chan DA, Giaccia AJ, Richard DE (2010) Hypoxia-inducible factor-1 activation in nonhypoxic conditions: the essential role of mitochondrial-derived reactive oxygen species. Mol Biol Cell 21:3247–3257

Pearce LL, Epperly MW, Greenberger JS, Pitt BR, Peterson J (2001) Identification of respiratory complexes I and III as mitochondrial sites of damage following exposure to ionizing radiation and nitric oxide. Nitric Oxide Biol Chem 5:128–136

Pessemesse L, Schlernitzauer A, Sar C, Levin J, Grandemange S, Seyer P, Favier FB, Kaminski S, Cabello G, Wrutniak-Cabello C, Casas F (2012) Depletion of the p43 mitochondrial T3 receptor in mice affects skeletal muscle development and activity. FASEB J 26:748–756

Petros JA, Baumann AK, Ruiz-Pesini E, Amin MB, Sun CQ, Hall J, Lim S, Issa MM, Flanders WD, Hosseini SH, Marshall FF, Wallace DC (2005) mtDNA mutations increase tumorigenicity in prostate cancer. Proc Natl Acad Sci 102:719–724

Petrosillo G, Ruggiero FM, Paradies G (2003) Role of reactive oxygen species and cardiolipin in the release of cytochrome c from mitochondria. FASEB J 17:2202–2208

Pope S, Land JM, Heales SJ (2008) Oxidative stress and mitochondrial dysfunction in neurodegeneration; cardiolipin a critical target? Biochim Biophys Acta 1777:794–799
Powell CS, Jackson RM (2003) Mitochondrial complex I, aconitase, and succinate dehydrogenase during hypoxia-reoxygenation: modulation of enzyme activities by MnSOD. Am J Physiol Lung Cell Mol Physiol 285:L189–L198
Ravindranath SD, Fridovich I (1975) Isolation and characterization of a manganese-containing superoxide dismutase from yeast. J Biol Chem 250:6107–6112
Reed JC (2000) Mechanisms of apoptosis. Am J Pathol 157:1415–1430
Richter C, Park J-W, Ames BN (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci U S A 85:6465–6467
Roessler MM, King MS, Aj R, Armstrong FA, Harmer J, Hirst J (2010) Direct assignment of EPR spectra to structurally defined iron-sulfur clusters in complex i by double electron-electron resonance. Proc Natl Acad Sci U S A 107:1930–1935
Roszkowski K, Jozwicki W, Blaszczyk P, Mucha-Malecka A, Siomek A (2011) Oxidative damage DNA: 8-oxoGua and 8-oxodG as molecular markers of cancer. Med Sci Monit 17:CR329–CR333
Rotig A, de Lonlay P, Chretien D, Foury F, Koenig M, Sidi D, Munnich A, Rustin P (1997) Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. Nat Genet 17:215–217
Roy S, Nicholson DW (2000) Cross-talk in cell death signaling. J Exp Med 192:F21–F25
Ryu H, Lee J, Impey S, Ratan RR, Ferrante RJ (2005) Antioxidants modulate mitochondrial PKA and increase CREB binding to D-loop DNA of the mitochondrial genome in neurons. Proc Natl Acad Sci U S A 102:13915–13920
Saltzman HA, Fridovich I (1973) Editorial: oxygen toxicity. Introduction to a protective enzyme: superoxide dismutase. Circulation 48:921–923
Salvi M, Brunati AM, Bordin L, La Rocca N, Clari G, Toninello A (2002) Characterization and location of Src-dependent tyrosine phosphorylation in rat brain mitochondria. Biochim Biophys Acta 1589:181–195
Sarvazyan N (1996) Visualization of doxorubicin-induced oxidative stress in isolated cardiac myocytes. Am J Physiol Heart Circ Physiol 271:H2079–H2085
Sasabe E, Yang Z, Ohno S, Yamamoto T (2010) Reactive oxygen species produced by the knockdown of manganese-superoxide dismutase up-regulate hypoxia-inducible factor-1α expression in oral squamous cell carcinoma cells. Free Radic Biol Med 48:1321–1329
Sato H, Sato M, Kanai H, Uchiyama T, Iso T, Ohyama Y, Sakamoto H, Tamura J, Nagai R, Kurabayashi M (2005) Mitochondrial reactive oxygen species and c-Src play a critical role in hypoxic response in vascular smooth muscle cells. Cardiovasc Res 67:714–722
Sautes-Fridman C, Cherfils-Vicini J, Damotte D, Fisson S, Fridman WH, Cremer I, Dieu-Nosjean MC (2011) Tumor microenvironment is multifaceted. Cancer Metastasis Rev 30:13–25
Scheller K, Sekeris CE, Krohne G, Hock R, Hansen IA, Scheer U (2000) Localization of glucocorticoid hormone receptors in mitochondria of human cells. Eur J Cell Biol 79:299–307
Schlame M, Rua D, Greenberg ML (2000) The biosynthesis and functional role of cardiolipin. Prog Lipid Res 39:257–288
Schwartzman RA, Cidlowski JA (1993) Apoptosis: the biochemistry and molecular biology of programmed cell death. Endocr Rev 14:133–151
Sekeris CE (1990) The mitochondrial genome: a possible primary site of action of steroid hormones. In Vivo 4:317–320
Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E (2005) Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-α prolyl hydroxylase. Cancer Cell 7:77–85
Seth RB, Sun L, Ea C-K, Chen ZJ (2005) Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-κB and IRF3. Cell 122:669–682
Sharma LK, Fang H, Liu J, Vartak R, Deng JJ, Bai Y (2011) Mitochondrial respiratory complex I dysfunction promotes tumorigenesis through ROS alteration and AKT activation. Hum Mol Genet 20:4605–4616

Shen L, Fang H, Chen T, He J, Zhang M, Wei X, Xin Y, Jiang Y, Ding Z, Ji J, Lu J, Bai Y (2010) Evaluating mitochondrial DNA in cancer occurrence and development. Ann N Y Acad Sci 1201:26–33

Shioji K, Kishimoto C, Nakamura H, Masutani H, Yuan Z, S-i O, Yodoi J (2002) Overexpression of thioredoxin-1 in transgenic mice attenuates adriamycin-induced cardiotoxicity. Circulation 106:1403–1409

Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005) Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 102:5618–5623

Sidiropoulos PI, Goulielmos G, Voloudakis GK, Petraki E, Boumpas DT (2008) Inflammasomes and rheumatic diseases: evolving concepts. Ann Rheum Dis 67:1382–1389

Silverman DHS, Dy CJ, Castellon SA, Lai J, Pio BS, Abraham L, Waddell K, Petersen L, Phelps ME, Ganz PA (2007) Altered frontocortical, cerebellar, and basal ganglia activity in adjuvant-treated breast cancer survivors 5–10 years after chemotherapy. Breast Cancer Res Treat 103:303–311

Simbre VC II, Duffy SA, Dadlani GH, Miller TL, Lipshultz SE (2005) Cardiotoxicity of cancer chemotherapy. Implications for children. Pediatr Drugs 7:187–202

Sinha RA, Pathak A, Mohan V, Babu S, Pal A, Khare D, Godbole MM (2010) Evidence of a bigenomic regulation of mitochondrial gene expression by thyroid hormone during rat brain development. Biochem Biophys Res Commun 397:548–552

Sionov RV, Cohen O, Kfir S, Zilberman Y, Yefenof E (2006a) Role of mitochondrial glucocorticoid receptor in glucocorticoid-induced apoptosis. J Exp Med 203:189–201

Sionov RV, Kfir S, Zafrir E, Cohen O, Zilberman Y, Yefenof E (2006b) Glucocorticoid-induced apoptosis revisited: a novel role for glucocorticoid receptor translocation to the mitochondria. Cell Cycle 5:1017–1026

Solakidi S, Psarra AMG, Nikolaropoulos S, Sekeris CE (2005a) Estrogen receptors α and β (ERα and ERβ) and androgen receptor (AR) in human sperm: localization of ERβ and AR in mitochondria of the midpiece. Hum Reprod 20:3481–3487

Solakidi S, Psarra AMG, Sekeris CE (2005b) Differential subcellular distribution of estrogen receptor isoforms: localization of ERα in the nucleoli and ERβ in the mitochondria of human osteosarcoma SaOS-2 and hepatocarcinoma HepG2 cell lines. Biochim Biophys Acta 1745:382–392

Solakidi S, Psarra AMG, Sekeris CE (2007) Differential distribution of glucocorticoid and estrogen receptor isoforms: localization of GRβ and ERα in nucleoli and GRα and ERβ in the mitochondria of human osteosarcoma SaOS-2 and hepatocarcinoma HepG2 cell lines. J Musculoskele Neuronal Interact 7:240–245

Soundar S, Danek BL, Colman RF (2000) Identification by mutagenesis of arginines in the substrate binding site of the porcine NADP-dependent isocitrate dehydrogenase. J Biol Chem 275:5606–5612

Spierings D, McStay G, Saleh M, Bender C, Chipuk J, Maurer U, Green DR (2005) Connected to death: the (unexpurgated) mitochondrial pathway of apoptosis. Science 310:66–67

Stclair D, Wan X, Kuroda M, Vichitbandha S, Tsuchida E, Urano M (1997) Suppression of tumor metastasis by manganese superoxide dismutase is associated with reduced tumorigenicity and elevated fibronectin. Oncol Rep 4:753–757

Steinman HM, Weinstein L, Brenowitz M (1994) The manganese superoxide dismutase of Escherichia coli K-12 associates with DNA. J Biol Chem 269:28629–28634

Stirone C, Duckles SP, Krause DN, Procaccio V (2005) Estrogen increases mitochondrial efficiency and reduces oxidative stress in cerebral blood vessels. Mol Pharmacol 68:959–965

Summy JM, Gallick GE (2003) Src family kinases in tumor progression and metastasis. Cancer Metastasis Rev 22:337–358

Sun G, Kemble DJ (2009) To C or not to C: direct and indirect regulation of Src protein tyrosine kinase. Cell Cycle 8:2353–2355

Sun X, Zhou Z, Kang YJ (2001) Attenuation of doxorubicin chronic toxicity in metallothionein-overexpressing transgenic mouse heart. Cancer Res 61:3382–3387

Szczepanek K, Chen Q, Derecka M, Salloum FN, Zhang Q, Szelag M, Cichy J, Kukreja RC, Dulak J, Lesnefsky EJ, Larner AC (2011) Mitochondrial-targeted signal transducer and activator of transcription 3 (STAT3) protects against ischemia-induced changes in the electron transport chain and the generation of reactive oxygen species. J Biol Chem 286:29610–29620

Taddei ML, Giannoni E, Raugei G, Scacco S, Sardanelli AM, Papa S, Chiarugi P (2012) Mitochondrial oxidative stress due to complex I dysfunction promotes fibroblast activation and melanoma cell invasiveness. J Signal Trans 2012:684592

Takai D, Park S-H, Takada Y, Ichinose S, Kitagawa M, Akashi M (2006) UV-irradiation induces oxidative damage to mitochondrial DNA primarily through hydrogen peroxide: analysis of 8-oxodGuo by HPLC. Free Radic Res 40:1138–1148

Talaber G, Boldizsar F, Bartis D, Palinkas L, Szabo M, Berta G, Setalo G Jr, Nemeth P, Berki T (2009) Mitochondrial translocation of the glucocorticoid receptor in double-positive thymocytes correlates with their sensitivity to glucocorticoid-induced apoptosis. Int Immunol 21:1269–1276

Tanaka M, Kovalenko SA, Gong J-S, Borgeld H-JW, Katsumata K, Hayakawa M, Yoneda M, Ozawa T (1996) Accumulation of deletions and point mutations in mitochondrial genome in degenerative diseases. Ann N Y Acad Sci 102–111

Tangpong J, Cole MP, Sultana R, Joshi G, Estus S, Vore M, St Clair W, Ratanachaiyavong S, St Clair DK, Butterfield DA (2006) Adriamycin-induced, TNF-α-mediated central nervous system toxicity. Neurobiol Dis 23:127–139

Tangpong J, Cole MP, Sultana R, Estus S, Vore M, St. Clair W, Ratanachaiyavong S, St Clair DK, Butterfield DA (2007) Adriamycin-mediated nitration of manganese superoxide dismutase in the central nervous system: insight into the mechanism of chemobrain. J Neurochem 100:191–201

Tannock IF, Ahles TA, Ganz PA, van Dam FS (2004) Cognitive impairment associated with chemotherapy for cancer: report of a workshop. J Clin Oncol 22:2233–2239

Teintze M, Slaughter M, Weiss H, Neupert W (1982) Biogenesis of mitochondrial ubiquinol: cytochrome *c* reductase (cytochrome bc_1 complex). Precursor proteins and their transfer into mitochondria. J Biol Chem 257:10364–10371

Tobar N, Guerrero J, Smith PC, Martinez J (2010) NOX4-dependent ROS production by stromal mammary cells modulates epithelial MCF-7 cell migration. Br J Cancer 103:1040–1047

Toro JR, Nickerson ML, Wei M-H, Warren MB, Glenn GM, Turner ML, Stewart L, Duray P, Tourre O, Sharma N, Choyke P, Stratton P, Merino M, Walther MM, Linehan WM, Schmidt LS, Zbar B (2003) Mutations in the *fumarate hydratase* gene cause hereditary leiomyomatosis and renal cell cancer in families in North America. Am J Hum Genet 73:95–106

Trimmer C, Sotgia F, Whitaker-Menezes D, Balliet RM, Eaton G, Martinez-Outschoorn UE, Pavlides S, Howell A, Iozzo RV, Pestell RG, Scherer PE, Capozza F, Lisanti MP (2011) Caveolin-1 and mitochondrial SOD2 (MnSOD) function as tumor suppressors in the stromal microenvironment: a new genetically tractable model for human cancer associated fibroblasts. Cancer Biol Ther 11:383–394

Urbano A, Lakshmanan U, Choo PH, Kwan JC, Ng PY, Guo K, Dhakshinamoorthy S, Porter A (2005) AIF suppresses chemical stress-induced apoptosis and maintains the transformed state of tumor cells. EMBO J 24:2815–2826

Vahsen N, Cande C, Briere J-J, Benit P, Joza N, Larochette N, Mastroberardina PG, Pequignot MO, Casares N, Lazar V, Feraud O, Debili N, Wissing S, Engelhardt S, Madeo F, Piacentini M, Penninger JM, Schagger H, Rustin P, Kroemer G (2004) AIF deficiency compromises oxidative phosphorylation. EMBO J 23:4679–4689

Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J (2004) Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 266:37–56

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84

Van Itallie CM (1990) Thyroid hormone and dexamethasone increase the levels of a messenger ribonucleic acid for a mitochondrially encoded subunit but not for a nuclear-encoded subunit of cytochrome *c* oxidase. Endocrinology 127:55–62

van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, Alderson NL, Baynes JW, Epstein CJ, Huang T-T, Nelson J, Strong R, Richardson A (2003) Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. Physiol Genomics 16:29–37

Wallace DC (2010) Mitochondrial DNA mutations in disease and aging. Environ Mol Mutagen 51:440–450

Wang GL, Semenza GL (1995) Purification and characterization of hypoxia-inducible factor 1. J Biol Chem 270:1230–1237

Wang M, Kirk JS, Venkataraman S, Domann FE, Zhang HJ, Schafer FQ, Flanagan SW, Weydert CJ, Spitz DR, Buettner GR, Oberley LW (2005) Manganese superoxide dismutase suppresses hypoxic induction of hypoxia-inducible factor-1α and vascular endothelial growth factor. Oncogene 24:8154–8166

Waris G, Ahsan H (2006) Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog 5:14–21

Watanabe T, Nobusawa S, Kleihues P, Ohgaki H (2009) *IDH1* mutations are early events in the development of astrocytomas and oligodendrogliomas. Am J Pathol 174:1149–1153

Wefel JS, Lenzi R, Theriault R, Buzdar AU, Cruickshank S, Meyers CA (2004) 'Chemobrain' in breast carcinoma?: a prologue. Cancer 101:466–475

Wegrzyn J, Potla R, Chwae Y-J, Sepuri NBV, Zhang Q, Koeck T, Derecka M, Szczepanek K, Szelag M, Gornicka A, Moh A, Moghaddas S, Chen Q, Bobbili S, Cichy J, Dulak J, Baker DP, Wolfman A, Stuehr D, Hassan MO, Fu X-Y, Avadhani NG, Drake JI, Fawcett P, Lesnefsky EJ, Larner AC (2009) Function of mitochondrial Stat3 in cellular respiration. Science 323:793–797

Weisiger RA, Fridovich I (1973) Superoxide dismutase. Organelle specificity. J Biol Chem 248:3582–3592

Weitzel JM, Iwen KA (2011) Coordination of mitochondrial biogenesis by thyroid hormone. Mol Cell Endocrinol 342:1–7

Wenner CE, Spirtes MA, Weinhouse S (1952) Metabolism of neoplastic tissue II. A survey of enzymes of the citric acid cycle in transplanted tumors. Cancer Res 12:44–49

Wheeler DL, Iida M, Dunn EF (2009) The role of Src in solid tumors. Oncologist 14:667–678

Wiesner RJ, Kurowski TT, Zak R (1992) Regulation by thyroid hormone of nuclear and mitochondrial genes encoding subunits of cytochrome-c oxidase in rat liver and skeletal muscle. Mol Endocrinol 6:1458–1467

Williams MD, Van Remmen H, Conrad CC, Huang T-T, Epstein CJ, Richardson A (1998) Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. J Biol Chem 273:28510–28515

Wong GHW, Goeddel DV (1988) Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective role. Science 242:941–944

Wong GHW, Elwell JH, Oberley LW, Goeddel DV (1989) Manganese superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. Cell 58:923–931

Wrutniak C, Cassar-Malek I, Marchal S, Rascle A, Heusser S, Keller J-M, Flechon J, Dauca M, Samarut J, Ghysdael J, Cabello G (1995) A 43-kDa protein related to c-Erb A a1 is located in the mitochondrial matrix of rat liver. J Biol Chem 270:16347–16354

Yahara O, Hashimoto K, Taniguchi N, Ishikawa M, Sato Y, Yamashita H, Ohno H (1991) Serum manganese-superoxide dismutase in patients with neuromuscular disorders as judged by an ELISA. Res Commun Chem Pathol Pharmacol 72:315–326

Yamamoto T, Maruyama W, Kato Y, Yi H, Shamoto-Nagai M, Tanaka M, Sato Y, Naoi M (2002) Selective nitration of mitochondrial complex I by peroxynitrite: involvement of mitochondria dysfunction and cell death of dopaminergic SH-SY5Y cells. J Neural Transm 109:1–13

Yang S-H, Liu R, Perez EJ, Wen Y, Stevens SM Jr, Valencia T, Brun-Zinkernagel A-M, Prokai L, Will Y, Dykens J, Koulen P, Simpkins JW (2004) Mitochondrial localization of estrogen receptor b. Proc Natl Acad Sci U S A 101:4130–4135

Yang S-H, Sarkar SN, Liu R, Perez EJ, Wang X, Wen Y, Yan L-J, Simpkins JW (2009) Estrogen receptor b as a mitochondrial vulnerability factor. J Biol Chem 284:9540–9548

Yen H-C, Oberley TD, Vichitbandha S, Ho Y-S, St Clair DK (1996) The protective role of manganese superoxide dismutase against adriamycin-induced acute cardiac toxicity in transgenic mice. J Clin Invest 98:1253–1260

Yen H-C, Oberley TD, Gairola CG, Szweda LI, St Clair DK (1999) Manganese superoxide dismutase protects mitochondrial complex I against adriamycin-induced cardiomyopathy in transgenic mice. Arch Biochem Biophys 362:59–66

Yusenko MV, Ruppert T, Kovacs G (2010) Analysis of differentially expressed mitochondrial proteins in chromophobe renal cell carcinomas and renal oncocytomas by 2-D gel electrophoresis. Int J Biol Sci 6:213–224

Zhang S, Yu D (2011) Targeting Src family kinases in anti-cancer therapies: turning promise into triumph. Trends Pharmacol Sci 33:122–128

Zhang Y, Gu J, Zhao L, He L, Qian W, Wang J, Wang Y, Qian Q, Qian C, Liu XY (2006) Complete elimination of colorectal tumor xenograft by combined manganese superoxide dismutase with tumor necrosis factor-related apoptosis inducing ligand gene virotherapy. Cancer Res 66:4291–4298

Zhang J, Xing D, Gao X (2008) Low-power laser irradiation activates Src tyrosine kinase through reactive oxygen species-mediated signaling pathway. J Cell Physiol 217:518–528

Zhang G, Yanamala N, Lathrop KL, Zhang L, Klein-Seetharaman J, Srinivas H (2010) Ligand-independent antiapoptotic function of estrogen receptor-b in lung cancer cells. Mol Endocrinol 24:1737–1747

Zhong W, Oberley LW, Oberley TD, St Clair DK (1997) Suppression of the malignant phenotype of human glioma cells by overexpression of manganese superoxide dismutase. Oncogene 14:481–490

Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW (1999) Overexpression of hypoxia-inducible factor 1α in common human cancers and their metastases. Cancer Res 59:5830–5835

Zhou R, Yazdi AS, Menu P, Tschopp J (2011) A role for mitochondria in NLRP3 inflammasome activation. Nature 469:221–226

Zwacka RM, Dudus L, Epperly MW, Greenberger JS, Engelhardt JF (1998) Redox gene therapy protects human IB-3 lung epithelial cells against ionizing radiation-induced apoptosis. Hum Gene Ther 9:1381–1386

Chapter 2
Exploiting BH3 Mimetics for Cancer Therapy

Donat Kögel

Contents

Abstract In apoptotic cells, the transcriptional induction or posttranslational activation of Bcl-2-homolgy domain-3 (BH3)-only proteins triggers the activation of the pro-apoptotic pore-forming proteins Bax and Bak. All members of this subgroup of the Bcl-2 family share a nine amino acid BH3-domain which binds to a hydrophobic groove of anti-apoptotic Bcl-2 family members that comprises residues of their BH1, BH2 and BH3 domains. These observations led to the development of BH3 mimetics, a class of small-molecule inhibitors targeting the BH3-binding domain of the pro-survival Bcl-2 family members, thereby facilitating/activating Bax/Bak-dependent apoptosis. In addition, BH3 mimetics can displace the pro-autophagic BH3only protein Beclin-1 from a complex with pro-survival Bcl-2 family members to induce autophagy. BH3 mimetics hold great promise for

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the treatment of cancer and currently, a large variety of natural and synthetic BH3 mimetics are characterized in preclinical studies and developed in clinical studies in an aim to exploit their therapeutic potential for the treatment of cancer.

Keywords Bcl-2 family • BH3-only proteins • Programmed cell death • Apoptosis • Autophagy • Cell death resistance • Cancer • Mitochondrial membrane permeabilization • Intrinsic apoptosis pathway • Caspases • Stress signaling • Target specificity

1 Introduction

Apoptosis is an evolutionary conserved process enabling multicellular organisms to eliminate damaged and unwanted cells by executing a cellular suicide program. Defects in apoptotic signalling pathways and overexpression of anti-apoptotic genes play fundamental roles in the development of cancer (Hanahan and Weinberg 2000; Lowe and Lin 2000; Evan and Vousden 2001; Igney and Krammer 2002; Fulda and Debatin 2004). The caspase family of aspartate proteases are central executioners of apoptotic cell death (Alnemri et al. 1996; Nicholson 1999; Degterev et al. 2003; Fischer et al. 2003) and two major caspase-activating pathways predominate. Activation of caspases can occur either after ligation of death ligands to their cell surface receptors (extrinsic pathway) or after the release of pro-apoptotic factors from mitochondria (intrinsic pathway) (Hengartner 2000; Fulda and Debatin 2006). In the intrinsic pathway, activation of the initiator caspase-9 occurs via binding of adaptor protein apoptotic protease activating factor-1 (Apaf-1) to the caspase recruitment domain (CARD) (Garrido et al. 2006). The association of caspase-9 and Apaf-1 and subsequent apoptosome formation is triggered by the pro-apoptotic factor cytochrome c. Release of cytochrome c from mitochondria is therefore the key regulatory step in the mitochondrial apoptosis pathway (Martinou and Green 2001) (Fig. 1).

1.1 Bcl-2 Family Members: Arbiters of Cell Survival

Pro- and anti-apoptotic members of the Bcl-2 family are key regulators of apoptotic and non-apoptotic cell death. The Bcl-2 family proteins can be classified into three subfamilies: (i) the BH3-only proteins which have only one domain in common, the alpha helical BH3 domain; (ii) the Bax-like proteins which contain three such domains (BH1,2,3) and (iii) the Bcl-2-like proteins which contain 4 domains (BH1-4) (Kroemer et al. 2007). Bax and Bax-like protein Bak trigger mitochondrial permeabilisation which is required for the release of pro-apoptotic factors from the mitochondria into the cytosol (Kroemer et al. 2007) (Fig. 2).

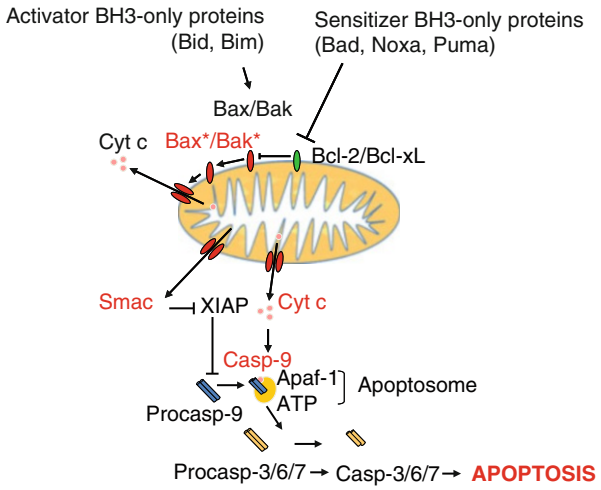

Fig. 1 Bcl-2 family members positively and negatively regulate the activation of the intrinsic apoptosis signalling pathway. In non-apoptotic cells, activation of Bax or Bak is inhibited by direct binding of anti-apoptotic Bcl-2 family members (Bcl-2-like proteins) such as Bcl-2 and Bcl-x_L. Upon apoptosis induction, the transcriptional or posttranslational activation of Bcl-2-homolgy domain-3 (BH3)-only proteins subsequently triggers the activation of Bax and Bak. Activator BH3-only proteins (Bid, Bim) directly bind to and oligomerize Bax and Bak (activated Bax and Bak are denoted as Bax* and Bak*). Sensitizer BH3-only proteins (Bad, Noxa, PUMA) bind to and neutralize the pro-survival Bcl-2 family members, thereby displacing them from Bax and Bak to facilitate MOMP, cytochrome c release and subsequent activation of initiator caspase-9 and downstream effector caspase induction. Abbreviations: *Apaf-1* apoptotic peptidase activating factor 1, *Bax* Bcl-2-associated X protein, *Bak* Bcl-2-antagonist/killer 1, *Bcl-2* B-cell lymphoma 2, *Bcl-w* Bcl2-like-2, *Bcl-x_L* Bcl-x long, *Bid* BH3 interacting domain death agonist, *Cyt c* cytochrome c, *Mcl-1* myeloid cell leukaemia sequence 1, *Procasp* procaspase, *Casp* caspase, *Smac* second mitochondrial activator of caspases, *tBid* truncated Bid, *XIAP* X-linked inhibitor of apoptosis

In non-apoptotic cells, activation of Bax or Bak is inhibited by direct binding of anti-apoptotic Bcl-2 family members (Bcl-2-like proteins) such as Bcl-2 and Bcl-xL (Ranger et al. 2001; Cory and Adams 2002; Adams and Cory 2007; Danial 2007). Both Bcl-2 and Bcl-x_L normally reside in the outer mitochondrial membrane, but to a less extent are also localized to the ER membrane and nuclear envelope, facing the cytosol. Bcl-2 family members can contain a carboxy-terminal hydrophobic transmembrane (TM) domain in addition to up to four Bcl-2 homology domains (BH1-4) corresponding to α-helical regions in the proteins. While homodimerization of Bcl-2 and Bcl-x_L involves a head- to- tail interaction, heterodimerization of Bcl-2/Bcl-x_L with Bax/Bak is performed in tail- to- tail fashion and requires a pocket formed by the BH1, BH2, and BH3 region of Bcl-2/Bcl-x_L as well as a central region in Bax/Bak where the BH3 domain is located. Since the molecular cloning of Bcl-2 by Korsmeyer and colleagues, there has been an ever increasing interest in the role of Bcl-2 in drug resistance and its exploitation as a

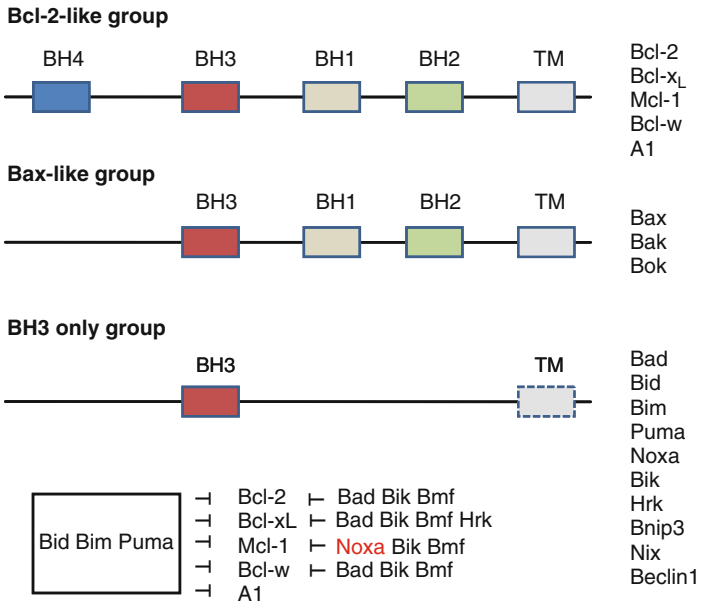

Fig. 2 Pro- and anti-apoptotic members of the Bcl-2 family. The Bcl-2 family proteins can be classified into three subfamilies: (i) the anti-apoptotic Bcl-2-like proteins which contain 4 Bcl-2 homology domains (BH1-4), (ii) the Bax-like proteins which contain three such domains (BH1,2,3) and (iii) the BH3-only proteins which have only the BH3 domain and in some cases a transmembrane (TM) domain, but otherwise share very little structural homology. The BH3 only proteins serve to couple diverse death and stress stimuli to the mitochondrial death program. Activated BH3 only proteins differ in their binding affinities to the pro-survival Bcl-2 proteins, e.g. Noxa which has an especially high affinity to Mcl-1

drug target. In mammalian cells, there are five pro-survival Bcl-2 family members (Bcl-2, Bcl-x_L, Mcl-1, Bcl-w and A1 [Bfl-1]) (Fig. 2) and there is abundant evidence that overexpression of anti-apoptotic Bcl-2 family members constitutes a general hallmark of haematological malignancies and solid tumours. While the role of the four major pro-survival members Bcl-2, Bcl-x_L, Mcl-1 and Bcl-w in tumorigenesis and therapy resistance is well established, the potential role of A1 is only beginning to be unravelled (Vogler 2012).

Upon apoptosis induction, the transcriptional or posttranslational activation of Bcl-2-homolgy domain-3 (BH3)-only proteins subsequently triggers the activation of Bax and Bak. In addition to Bax and Bak, The BH3 domains of BH3 only proteins can also bind to the hydrophobic groove of the anti-apoptotic Bcl-2 family members. All members of the BH3 only subgroup share a nine amino acid BH3-domain, but otherwise possess very little structural homology. Members of this subgroup include Bim, Bid, Bad, PUMA, Noxa, Hrk, and Bmf (Huang and Strasser 2000; Bouillet and Strasser 2002; Puthalakath and Strasser 2002). The role of the individual BH3-only family members is to couple specific upstream stress signals (e.g. DNA damage, ER stress, proteasomal stress) to the intrinsic pathway of apoptosis.

There are two competing models for activation of Bak and Bax by BH3-only proteins (Green 2006). In the direct activation model proposed by Letai et al., BH3-only proteins termed as activators (Bid, Bim) directly bind to and oligomerize Bax and Bak. In the indirect activation model, BH3-only proteins denoted as sensitizers (Noxa, PUMA, Hrk, Bmf) bind to and neutralize the pro-survival Bcl-2 family members, thereby displacing them from Bax and Bak to facilitate MOMP (Fig. 1). Sensitizer BH3 proteins therefore decrease the apoptotic threshold in cells already primed to death.

Induction of apoptosis is a major mechanism by which most chemotherapeutic drugs and radiation kill tumour cells. However, conventional cancer therapies fail to mediate their effects in a target-specific fashion. This chapter focuses on BH3 mimetics, a new class of small molecule inhibitors targeting the BH3-binding domain of the pro-survival Bcl-2 family members (Lessene et al. 2008; Kang and Reynolds 2009). Since pro-survival Bcl-2 family members are known to be overexpressed in a wide variety of human malignancies and since the intrinsic pathway of apoptosis is implicated in the cell death-inducing effects of most chemotherapeutic drugs as well as gamma irradiation, BH3 mimetics are perceived to be highly promising anti-cancer drugs and apoptosis sensitizers. As outlined in detail below, there are several synthetic and natural BH3 mimetics with different binding profiles to the pro-survival Bcl-2 family members (Bcl-2, Bcl-x_L, myeloid cell leukaemia-1 [Mcl-1], Bcl-w [Bcl2-like-2], A1). Most of these BH3 mimetics target Bcl-2 and Bcl-x_L. The highly selective inhibitor ABT-737 and its orally applicable derivative ABT-263 target Bcl-2, Bcl-x_L and Bcl-w with high affinity, but not Mcl-1 (Oltersdorf et al. 2005; van Delft et al. 2006; Chonghaile and Letai 2008). Gossypol and Obatoclax (GX15-070, Geminx) are so-called pan-Bcl-2 inhibitors targeting the four major pro-survival Bcl-2 family members Bcl-2, Bcl-x_L, Mcl-1 and Bcl-w. In contrast to the experimental and clinical progress regarding these various inhibitors targeting the pro-survival Bcl-2 family members and therefore mimicking the function of sensitizing BH3 only proteins, BH3 mimetics that directly activate Bax and Bak are currently in the early stages of preclinical development. One exception to this classification is Obatoclax which may act both as a sensitizer and an activator BH3 mimetic.

Of note, the pro-survival Bcl-2 family members interact with a large number of additional molecules not directly involved in regulation of the intrinsic pathway of apoptosis and these interactions may also be affected by BH3 mimetics. For example, it has been demonstrated that Bcl-2 activates the anti-apoptotic nuclear factor-κB (NF-κB) pathway by a signalling mechanism that involves Raf-1/MEKK-1-mediated activation of IKKβ. In addition, the BH3 binding groove of Bcl-2 was shown to interact with a mitochondrial pool of glutathione (GSH), the major cellular ROS scavenger and this interaction is thought to contribute to the antioxidant function of Bcl-2 (Zimmermann et al. 2007). In line with this hypothesis, BH3 mimetics were shown to disrupt the Bcl-2/GSH interaction in neurons and to suppress the transport of GSH into isolated brain mitochondria (Zimmermann et al. 2007).

In addition to their role in apoptosis, pro-survival Bcl-2 family members are also modulators of autophagy. Autophagy is a form of cellular self-digestion in which cellular constituents are engulfed in double-membrane containing vesicles called autophagosomes (Codogno and Meijer 2005; He and Klionsky 2009). Their

vesicular content is subsequently digested by lysosomal proteases after fusion of autophagosomes with lysosomes (Codogno and Meijer 2005; Kimura et al. 2007). Autophagy is a complex, multistep process which is genetically regulated by the ~30 Atg genes discovered hitherto in mammals. Bcl-2-like proteins can form a complex with the core autophagy regulator Beclin-1 (Atg6) (Pattingre et al. 2005; Pattingre and Levine 2006; Maiuri et al. 2007), and formation/dissociation of this complex plays an important role in modulating autophagy in healthy cells and tumour cells. Interestingly, Beclin-1 is a BH3 protein incapable of inducing apoptosis. The Bcl-like proteins sequester Beclin-1 via binding to its BH3 domain and prevent it from forming a multiprotein complex essential for vesicle nucleation during the early steps of the autophagic process, thereby inhibiting autophagy. Consequently, BH3 mimetics are capable to activate both apoptosis and autophagy (Hetschko et al. 2008). Recently it was also demonstrated that the mitochondrial pool of Bcl-2 can inhibit autophagy by sequestering the pro-autophagic factor AMBRA1 (Pattingre et al. 2005; Strappazzon et al. 2011).

Autophagy is normally involved in regulated turnover of long-lived proteins and damaged organelles, but the net effects of autophagy on cell death are highly contextual and may depend on the extent of autophagy. Autophagy may comprise a primordial pro-survival stress response, e.g. under conditions of nutrient deprivation where it serves to ensure energy balance, but there is also evidence that enforced, prolonged over-activation of autophagy can lead to autophagic cell death (type II cell death), i.e. massive cellular self-digestion via the autophagosomal-lysosomal pathway beyond the point allowing cell survival (Edinger and Thompson 2004; Gozuacik and Kimchi 2004; Codogno and Meijer 2005; Degenhardt et al. 2006; Gozuacik and Kimchi 2007) and it is tempting to speculate that autophagy may possibly act as a backup for apoptosis in apoptosis-deficient cells.

2 BH3 Mimetics Employed in Preclinical Studies

HA14-1 was initially identified in a computer screen based on the structure of Bcl-2 and binds Bcl-2 with an IC_{50} value of ~9 μM as determined in competitive fluorescence polarization assays with a conjugated peptide derived from the BH3 domain of Bak (Wang et al. 2000). HA14-1 does not target the other major anti-apoptotic Bcl-2 family members Bcl-x_L, Mcl-1 and Bcl-w (Table 1). HA14-1 is capable to induce an apoptotic type of cell death in cellular models of various types of cancer, including haematopoietic malignancies, colon cancer, glioblastoma and neuroblastoma and can enhance the cell killing effects of several chemotherapeutic agents including doxorubicin, bortezomib and dexamethasone (Sinicrope et al. 2004; Manero et al. 2006). In line with its proposed action as a Bcl-2 inhibitor, HA14-1 was shown to activate Bax, caspase-9 and downstream effector caspase caspase-3 and its killing effects were reduced, but not completely abrogated in Bax/Bak-deficient cells. One major drawback of HA14-1 is its limited stability, as HA14-1 has been shown to decompose very rapidly in solution (~15 min). Therefore, several HA14-1

Table 1 BH3 mimetics employed in preclinical and clinical studies

	Drug	Target	Stage of development	References
Selective inhibitors	HA14-1	Bcl-2	Preclinical	Kang and Reynolds (2009)
	BH3-Is	Bcl-x_L	Preclinical	Kang and Reynolds (2009)
	YC137	Bcl-2	Preclinical	Bodur and Basaga (2012)
	S1	Bcl-2, Mcl-1		Bodur and Basaga (2012)
	Antimycin A	Bcl-2, Bcl-x_L	Preclinical	Kang and Reynolds (2009)
	ABT-737	Bcl-2, Bcl-x_L, Bcl-w	Preclinical	Lessene et al. (2008)
	ABT-263	Bcl-2, Bcl-x_L, Bcl-w	Phase I/II	Kögel et al. (2010)
Pan-Bcl-2 inhibitors	Gossypol/AT-101	Bcl-2, Bcl-x_L, Bcl-w, Mcl-1	Phase I/II	Kögel et al. (2010)
	TM-1206	Bcl-2, Bcl-x_L, Bcl-w, Mcl-1	Preclinical	Lessene et al. (2008)
	TW-37	Bcl-2, Bcl-x_L, Bcl-w, Mcl-1	Preclinical	Bodur and Basaga (2012)
	Apogossypolone	Bcl-2, Bcl-x_L, Bcl-w, Mcl-1	Preclinical	Bodur and Basaga (2012)
	BI-97C1	Bcl-2, Bcl-x_L, Bcl-w, Mcl-1	Preclinical	Bodur and Basaga (2012)
	Obatoclax	Bcl-2, Bcl-x_L, Bcl-w, Mcl-1	Phase I/II	Kögel et al. (2010)

derivatives have been developed. Tian and colleagues have synthesized sHA14-1, a chemically modified derivative with improved stability (Tian et al. 2008). Another more stable HA14-1 derivate is SV30 (Weyland et al. 2011). SV30-induced cell death was reported to (at least partially) require the mitochondrial pathway of apoptosis (Weyland et al. 2011). A third HA14-1 analogue is EM20-25 (Milanesi et al. 2006). Similar to HA14-1 and sHA14-1, EM20-25 can synergize with other apoptotic stimuli to enhance tumour cell death. However, the killing mechanisms of the three novel derivatives are ill-defined and may also be related to off target effects of the drugs. In line with this notion, sHA14-1 was shown to induce calcium release from the ER prior to activation of the intrinsic apoptosis pathway (Hermanson et al. 2009).

BH3-Inhibitors (BH3-Is) are a series of preclinical small molecule inhibitors that have been identified in a high-throughput screen based on disruption of the interaction of Bak with the BH3 domain of a recombinant glutathione-*S*-transferase (GST)–Bcl-xL fusion protein and they target both Bcl-2 and Bcl-xL (Degterev et al. 2001). They bind to Bcl-2, Bcl-x_L and Mcl-1 in the micromolar range (Table 2), and are capable to activate the intrinsic pathway of apoptosis and to synergize with cancer drugs and radiation. Similar to HA14-1 and its derivatives, BH3-Is however seem to also exert off target effects.

YC137 is a cell-permeable naphthoquinone compound with low micromolar affinity for Bcl-2 (Real et al. 2004). YC137 was reported to selectively induce apoptosis in Bcl-2-overexpressing cells and to sensitize them to DNA damaging agents. Another promising novel BH3 mimetic in preclinical development is S1 that binds both Bcl-2 and Mcl-1 at low nanomolar concentrations to induce the intrinsic apoptotic pathway and exerts significantly lower toxicity in Bax/Bak-deficient cells than in wild type (wt) control cells (Zhang et al. 2011).

Antimycin A is a streptomyces-derived BH3 mimetic and natural inhibitor of the ubiquinone–cytochrome c oxidoreductase complex at the mitochondrial respiration chain. Molecular docking simulations suggest that Antimycin A also binds to the BH3 binding groove of anti-apoptotic Bcl-2 proteins (Tzung et al. 2001). The 2-methoxy-Antimycin A3 derivative may act as a bona fide BH3 mimetic because it lacks the inhibitory function of Antimycin A on mitochondrial respiration. Antimycin A3 also was shown to exert significant *in vivo* anti-tumour activity (Cao et al. 2007).

3 BH3 Mimetics in Clinical Development

3.1 ABT-737 and ABT-263

The most advanced and best-characterized synthetic Bcl-2 inhibitor is the Bad-like BH3 mimetic ABT-737 (Abbott Laboratories) (Tables 1 and 2) which was rationally designed with the aid of structure-activity relationship (sAR) analysis of ligand binding to the hydrophobic groove of BCL-xL by a nuclear magnetic resonance

Table 2 Binding affinities of BH3 mimetics to the major four pro-survival Bcl-2 family members

	K_i (nM)					
Compound	Bcl-2	Bcl-x_L	Mcl-1	Bcl-w	Fluorescence polarization assay	References
ABT-737	<1	<0.5	ND	ND	Bad- and Bax-FITC peptides	Vogler et al. (2009a)
ABT-737	120	64	>20,000	24	Bid-FITC peptide	Vogler et al. (2009a)
ABT-263	<1	<0.5	550	<1		Vogler et al. (2009a)
Gossypol	ND	500	ND	ND	Bad-FITC peptide	Vogler et al. (2009a)
Gossypol	320	480	180	ND	Bid-FAM peptide	Vogler et al. (2009a)
Gossypol	280	3,030	1,750	1,400	Bid-FITC peptide	Vogler et al. (2009a)
Apogossypolone	35	660	25	ND		Vogler et al. (2009a)
BI-97C1	320	310	200	ND	Bak-FITC peptide	Wei et al. (2010)
Obatoclax	1,110	4,690	2,000	7,010	Bid-FITC peptide	Vogler et al. (2009a)
HA14-1	9,000	ND	ND	ND	Bak peptide	Vogler et al. (2009a)
BH3I-1	ND	2,400	ND	ND	Bak-Oregon Green peptide	Vogler et al. (2009a)
BH3I-1	1,140	5,860	2,170	2,330	Bid-FITC peptide	Vogler et al. (2009a)
Antimycin A	2,950	2,700	2,510	4,570	Bid-FITC peptide	Vogler et al. (2009a)
TW-37	290	1,110	260	ND	Bid-FAM peptide	Vogler et al. (2009a)
TW-37	120	1,100	260	ND	Bid-FAM peptide	Vogler et al. (2009a)

Adapted from Vogler et al. (2009a)

(NMR)-based screening approach followed by structure-based drug design (Oltersdorf et al. 2005). A-385358 (compound 73R), a predecessor of ABT-737 derived from this approach, has *K*i values of 0.8 nM against Bcl-xL and 67 nM against Bcl-2 (Shoemaker et al. 2006; Wendt et al. 2006). It also shows cell killing activity in tumour cells overexpressing BCL-XL. A-385358 synergizes with multiple pro-apoptotic cancer drugs *in vitro* but its pro-apoptotic effects were shown to be blunted in the presence of serum. Subsequent studies led to the discovery of ABT-737, a molecule with high affinity for BCL-2 and BCL-xL (IC50 values of <1 nM and <0.5 nM, respectively)(Oltersdorf et al. 2005). These sub-nanomolar affinities of ABT-737 for Bcl-2 and Bcl-xL are significantly lower than for most other BH3 mimetics. Similar to the BH3 domain of Bad, ABT-737 binds to Bcl-2, Bcl-x_L and Bcl-w, but not to Mcl-1 or A1 (Zhai et al. 2006). The pro-apoptotic activity of ABT-737 has been shown to be tightly correlated to the respective expression levels of the pro-survival Bcl-2 family members. In line with the high affinity of ABT-737 for Bcl-2, cancer cells with overexpression of endogenous Bcl-2 are particularly vulnerable to ABT-737-induced apoptosis whereas cells with high expression of Mcl-1 are resistant to the drug.

A major limitation of ABT-737 is that it is not orally bioavailable, but further modifications of ABT-737 have resulted in the development of the orally available derivative ABT-263 (Navtioclax), which retains its high affinity binding to Bcl-2, Bcl-x_L and Bcl-w (Tse et al. 2008) (Table 2). In xenograft models of SCLC, ABT-263 was able to completely eradicate the tumours while it significantly enhanced the efficacy of standard therapies in models of B-cell lymphoma and multiple myeloma (Shoemaker et al. 2008). ABT-263 is currently evaluated in phase I/II trials for SCLC, leukaemia and lymphoma, both as a monotherapy and in combination with other anti-cancer drugs or with monoclonal antibodies (rituximab and erlotinib). Despite the high binding selectivity of ABT-263, its on-target effects are crucial for its safety profile and the maximum tolerated dose of the drug. Because Bcl-x_L plays a pivotal role for cell survival of thrombocytes, the major adverse side effect of ABT-263 observed in the clinic is thrombocytopenia. In a phase I study, monotherapy with ABT-263 was shown to reduce pathologic lymphocytosis, lymphadenopathy, and splenomegaly in patients suffering from relapsed or refractory CLL (Roberts et al. 2012). The therapeutic efficacy of ABT-263 +/− rituximab on relapsed or refractory leukaemia/lymphoma patients is currently investigated in phase II trials. Another phase II multi-centre trial is currently recruiting chemonaive CLL patients to assess the clinical impact of ABT-263 in combination with rituximab in previously untreated patients (www.clinicaltrials.gov).

Structurally the pro-survival Bcl-2 family members can be divided into two categories. Bcl-2, Bcl-x_L and Bcl-w are structurally closely related, whereas the accessibility of the BH3 binding pocket of Mcl-1 and A1 is structurally different from the other three family members. Mcl-1 plays a pivotal role in resistance to the Bcl-2/Bcl-x_L/Bcl-w-specific inhibitors ABT-737 and ABT-263. The pro-apoptotic activity of ABT-737 was shown to negatively correlate with Mcl-1 expression in various cancer models and suppression of Mcl-1 expression was shown to abrogate

ABT-737 resistance, e.g. in acute myeloid leukaemia (AML) cells with high endogenous expression of Mcl-1 (Konopleva et al. 2006). Therefore, the combination of ABT-263 with drugs targeting the expression/stability of Mcl-1 (e.g. sorafenib, maritoclax) may be a feasible approach for future clinical studies. Indeed, one ongoing phase I clinical study in patients with solid tumours addresses the combined effects of ABT-737 and the EGFR receptor erlotinib known to suppress expression of Mcl-1 (Chen et al. 2012). In addition, expression profiling of Bcl-2 family members (Bcl-2, Bcl-x_L, Mcl-1, Bim, Noxa etc.) may help to rationally design future clinical trials with ABT-263 and combinatorial treatments by selecting patients most likely to benefit from the therapy.

3.2 Gossypol and Its Derivatives

The pro-survival Bcl-2 proteins serve partially redundant functions and considerable evidence suggests that – depending on the Bcl-2 expression profile of tumours – inactivation of all Bcl-2-like proteins may significantly enhance the efficiency of Bcl-2-targeted therapy. This may either be achieved by combining a more selective inhibitor such as the Bcl-2/Bcl-xL/Bcl-w inhibitor ABT-737/ABT-263 with another drug interfering with the expression of Mcl-1 or targeting it for degradation, or alternatively with pan-Bcl-2 inhibitors, targeting all four major Bcl-2-like proteins. Gossypol is a natural polyphenolic compound and BH3 mimetic derived from cottonseeds which was initially identified as an antifertility agent in China during the 1950s, and possesses pro-apoptotic effects in various *in vivo* and *in vitro* models (Wolter et al. 2006; Ko et al. 2007; Meng et al. 2008; Paoluzzi et al. 2008). Gossypol acts as a pan-Bcl-2 inhibitor and can inactivate Bcl-2, Bcl-xL, Mcl-1 and Bcl-w (Lessene et al. 2008; Kang and Reynolds 2009) (Tables 1 and 2). There are two enantiomers of Gossypol, (+)-Gossypol and (−)-Gossypol, the latter being more potent as an inhibitor of tumour growth (Lessene et al. 2008). (−)-Gossypol (AT-101, Ascenta) has shown single-agent activity in various types of cancer (Lessene et al. 2008; Kang and Reynolds 2009). In cancer cells with an intact apoptotic machinery, (−)-Gossypol has been reported to induce the intrinsic pathway of apoptosis and apoptotic cell death (Wolter et al. 2006; Balakrishnan et al. 2008; Meng et al. 2008; Paoluzzi et al. 2008). In contrast, cell death triggered by Gossypol largely seems to depend on induction of autophagic cell death in apoptosis-deficient malignant glioma cells and prostate cancer cells (Lian et al. 2010; Voss et al. 2010). (−)-Gossypol has nanomolar affinities to Bcl-2 ($Ki = 320$ nM), Bcl-xL ($Ki = 480$ nM) and Mcl-1 ($Ki = 180$ nM) (Wang et al. 2006).

(−)-Gossypol has good pharmacokinetic properties and appears to exhibit manageable (mainly gastrointestinal) toxicity, and demonstrated single-agent activity in a phase I/II trial in castrate-resistant prostate cancer (Liu et al. 2009). In another study, AT-101 was given in combination with topotecan and a partial response of this combined therapy was observed in patients with relapsed SCLC, although this

study unfortunately did not include a group of patients treated only with topotecan (Heist et al. 2010). Another phase II clinical trial data obtained from advanced and metastatic NSCLC patients revealed no clinical benefit of AT-101 in comparison to standard therapy in regard to overall survival (Ready et al. 2011). The potential clinical impact of AT-101, either as a monotherapy or in combination with other anti-cancer drugs, is currently further investigated in phase I/phase II clinical trials of leukaemia, lymphoma, NSCLC and prostate cancer (www.clinicaltrials.gov). Similar to ABT-737, subjecting patients to expression profiling of Bcl-2 family members may aid the rational design of future clinical trials.

In addition to (−)-Gossypol, a series of Gossypol derivatives are developed in an aim to further improve the therapeutic efficacy and dampen toxic side effects. Structure-based design strategies led to the development of the Gossypol derivative TM-1206 (Tang et al. 2008), which binds to Bcl-2, Bcl-x_L and Mcl-1 proteins with K_i values of 0.11, 0.639 and 0.15 μM, respectively. Employing molecular models of the (−)-Gossypol/Bcl-2 complex allowed the structure-based design of the novel Gossypol analogue and benzenesulfonyl derivative TW-37 (Wang et al. 2006) which binds with submicromolar/low micromolar affinity to Bcl-2 (0.29 μM), Bcl-x_L (1.11 μM), and to Mcl-1 (0.26 μM) (Wang et al. 2006). *In vitro* studies confirmed the on-target effects of TW-37 which disrupts the interaction between Bax and truncated Bid (tBid) with the pro-survival Bcl-2 family members Bcl-2, Bcl-x_L and Mcl-1 (Mohammad et al. 2007). In human endothelial and pancreatic cancer cells, TW-37 interestingly was also demonstrated to inhibit the pro-angiogenic and pro-metastatic activities of Bcl-2 which may be mediated by activation of the NF-κB pathway (Zeitlin et al. 2006; Wang et al. 2008).

Removal of two reactive aldehyde groups that are held responsible for the toxic side effects of (−)-Gossypol observed in clinical studies gave rise to Apogossypolone which retains activity against anti-apoptotic Bcl-2 family proteins in vitro (Arnold et al. 2008). Apogossypolone binds Bcl-2, Bcl-x_L and Mcl-1 with nanomolar affinities (Table 2) and was shown to induce apoptosis either alone or in combination with DNA damaging cancer drugs *in vitro* and *in vivo* and showed in vivo activity in xenograft models (Arnold et al. 2008). In addition to direct inhibition of pro-survival Bcl-2 proteins and similar to (−)-Gossypol, Apogossypolone was suggested to down-regulate Bcl-2, Bcl-x_L and Mcl-1 expression at the protein level. Further attempts to improve the target specificity by molecular docking computer screens led to synthesis of BI-97C1 (Sabutoclax) (Wei et al. 2010), a very promising, optically pure Apogossypol derivative with submicromolar target affinity. BI-97C1 inhibits the binding of BH3 peptides to Bcl-x_L, Bcl-2 and Mcl-1 with IC_{50} values of 0.31, 0.32 and 0.20 μM, respectively (Table 2). The compound also potently inhibits cell growth of human prostate cancer, lung cancer, and lymphoma cell lines with EC_{50} values of 0.13, 0.56, and 0.049 μM, respectively, shows little cytotoxicity in Bax/Bak DKO cells (Wei et al. 2010). Recently, BI-97C1 was also reported to enhance mda-7/IL-24-induced apoptosis of prostate cancer cells and to reduce tumour growth in a xenograft model *in vivo* (Dash et al. 2011). The potential clinical impact of BI-97C1 has not been investigated so far.

3.3 Obatoclax

Obatoclax (GX15-070, discovered by Gemin X, now Cephalon), a polypyrrole Pan-Bcl-2 inhibitor was identified in a high throughput screening of natural compound libraries followed by lead optimization (Shore and Viallet 2005). Obatoclax binds to Bcl-2, Bcl-xL and Mcl-1 with low micromolar affinities (Table 2) and was reported to exert *in vitro* and *in vivo* single agent activity in various types of cancer. Activation and mitochondrial translocation of Bax, mitochondrial depolarization, cytochrome c release, subsequent activation of caspase-9 and caspase-3 were reported to precede apoptosis induced by Obatoclax. Interestingly, Obatoclax was suggested to induce apoptosis by direct activation of Bax in a cell-free system, suggesting that it may act both as a sensitizer BH3 mimetic neutralizing the anti-apoptotic Bcl-2 proteins as well as an activating BH3 mimetic (Smoot et al. 2010). Obatoclax demonstrated single-agent activity in a phase I clinical study in CLL and is currently investigated in phase I/II studies of relapsed or refractory leukaemia, myeloma, lymphoma and SCLC, both as a monotherapy and in combination with other cancer drugs (www.clinicaltrials.gov). Preliminary reports on the outcomes of phase I/II trials appear to be promising. Antineoplastic activity was observed in 2 out of 5 pre-treated advanced non-Hodgkin's lymphoma (NHL) patients. Improved platelet and haemoglobin counts of chronic lymphocytic leukaemia (CLL) and myelofibrosis patients suffering from thrombocytopenia and anaemia were also reported. Although Obatoclax is generally well tolerated, the major observed side effect of the drug appears to be central nervous system (CNS) toxicity including ataxia, euphoria, and confusion.

4 Killing Mechanisms of BH3 Mimetics: The Clinical Perspective

The target specificity of many currently available BH3 mimetics has been put into question recently (van Delft et al. 2006; Vogler et al. 2009b). The combined findings of two comparative studies demonstrated that HA14-1, BH3I-1, antimycin A, chelerythrine, (−)-Gossypol, Apogossypol, Obatoclax, and EM20-25 induced significant cell death in Bax/Bak-deficient MEFs, whereas ABT-737 was shown to be highly dependent on the expression of Bax, Bak and caspase-9 to induce apoptosis (van Delft et al. 2006; Vogler et al. 2009b). These Bax/Bak-independent toxicities indicate that the other inhibitors might not behave solely as BH3 mimetics but might have additional cellular targets implicated in activating cell death. Conversely, it was proposed that ABT-737 may be the only bona fide BH3 mimetic inducing apoptotic cell death in a purely target-specific manner. In the aforementioned studies, Bax/Bak-deficient or Caspase-9-deficient cells were used as an experimental model to analyse the target-specificity of BH3 mimetics. However, the established role of pro-survival Bcl-2 family members in regulation of non-apoptotic forms of cell

death and the interaction of Bcl-2-like proteins with proteins not involved in apoptosis (as outlined below), should be taken into account for the interpretation of these results. In addition, a Bax/Bak-independent, alternative mode of apoptosis activation by the BH3 mimetic Gossypol has been proposed, namely a Gossypol-induced conformational change in Bcl-2 which converges it into a pro-apoptotic molecule activating the mitochondrial pathway of apoptosis (Lei et al. 2006). It is currently unknown whether other Bcl-2 inhibitors are potentially capable to induce a similar change in Bcl-2 conformation.

In addition to their role in apoptosis, all pro-survival Bcl-2 family members are negative endogenous regulators of autophagy. They serve to sequester and inactivate the pro-autophagic BH3 only protein Beclin-1 and consequently, all BH3 mimetics are potential activators of autophagy. Despite its proposed target selectivity, pleiotropic pro-autophagic effects on multiple autophagic signalling pathways have been shown to be induced by ABT-737 recently (Malik et al. 2011). The extent of autophagy induction by BH3 mimetics appears to be highly diverse, however. In this regard, the pan-Bcl-2 inhibitor (−)-Gossypol was shown to be a more potent activator of autophagy than the BH3 mimetics HA14-1 and ABT-737 (Voss et al. 2010). As outlined in the Introduction, autophagy may exert both protective and pro-death effects depending on the respective cellular context. Therefore, autophagy induced by BH3 mimetics may contribute to the cell death observed in apoptosis refractory cells such as glioblastoma cells and Bax/Bak-deficient MEFs. Indeed, in Bax/Bak-deficient cells and glioblastoma cells, autophagy was shown to be required for triggering necroptotic cell death induced by Obatoclax and autophagic cell death triggered by (−)-Gossypol, respectively (Bonapace et al. 2010; Voss et al. 2010). In the case of Obatoclax, the drug was reported to reactivate the sensitivity of multidrug-resistant childhood ALL cells to glucocorticoid treatment and other anti-cancer drugs by inducing an autophagy-dependent necroptotic type of cell death. This type of cell death required the expression of receptor-interacting protein (RIP-1) kinase and cylindromatosis (turban tumour syndrome) (CYLD), both of which are critically involved in necroptosis and was inhibited by knockdown of Beclin-1 (Bonapace et al. 2010). In most tumours and haematological malignancies however, autophagy appears to be a pro-survival stress response and therefore rather limits the therapeutic effects of chemotherapy and radiation. The clinical use of autophagy inhibitors (e.g. chloroquine) together with BH3 mimetics may therefore be a useful strategy to potentiate apoptosis in these apoptosis-proficient malignancies.

The issue of target selectivity may also have important implications for therapy. While highly selective inhibitors offer obvious advantages for mechanistic, experimental studies, at the moment it is still open to debate whether an ultra high target selectivity of Bcl-2 inhibitors actually is an advantage from a purely clinical perspective. On the one hand, an apparent disadvantage of inhibitors with lower selectivity/higher off target effects lies in the increased possibility for non-mechanism based toxicity issues in normal tissue. Despite this notion, the adverse effects of BH3 mimetics with proposed off target mechanisms such as (−)-Gossypol and Obatoclax have been shown to be manageable so far in the clinic. It is even

conceivable that the off target effects (e.g. Bcl-2 independent ROS generation) may actually contribute to the therapeutic efficacy of the drugs and therefore enhance their clinical efficacy. Due to the mechanism-based selection pressure, drug resistance in tumours treated with highly selective inhibitors also may acquire more rapidly. Therefore, highly selective inhibitors may be most successful when applied in combination with other drugs/compounds targeting drug resistance mechanisms such as enhanced Mcl-1 expression in ABT-737-treated malignancies.

5 Outlook

Despite the fact that activation of apoptosis is a major mechanism by which most chemotherapeutic drugs and radiation kill tumour cells, conventional cancer therapy does not allow target-specific intervention in death and survival signalling pathways which appears to be the most reasonable strategy to overcome the intrinsic apoptosis resistance of cancer cells. The ultimate goal for molecular, apoptosis-based therapies is to develop specific drugs selectively targeting various signalling components of pro- and anti-apoptotic pathways in an aim to limit unwanted toxicity in normal tissues and to trigger tumour-selective cell death. BH3 mimetics are an exciting new class of cancer drugs that hold great promise to fulfil these criteria because of their relatively limited toxic side effects in comparison to conventional chemotherapy/radiotherapy. In addition to their anti-tumour activities when used as monotherapy, a vast number of preclinical data suggest BH3 mimetics may be highly useful tools to reduce patient drug loads when applied in synergistic therapies with conventional cancer drugs and radiotherapy. The results of ongoing clinical trials will shed further light on the clinical impact of these novel anti-cancer agents in patients suffering from relapsed, refractory, metastatic or advanced stage cancers. Future trials with chemonaive patients will also allow to scrutinize the clinical potential of BH3 mimetics in early stage/untreated cancer. The rational design of trials with BH3 mimetics may also increase the attention on molecular profiling, such as expression profiling of Bcl-2 family members in an aim to select patients most likely to benefit from the therapy.

Future strategies will aim at designing even more specific Bc-2 inhibitors to further reduce their off target effects in an aim to limit the toxicity of these compounds in normal cells. Very recently, the structure-based design of a novel, highly potent and specific small-molecule inhibitor of Bcl-2/ Bcl-x_L (compound 21) was published. The design of this inhibitor was developed from a novel chemical scaffold and the crystal structures of Bcl-x_L complexed with the Bad BH3 peptide. The novel compound binds to both Bcl-xL and Bcl-2 with $Ki < 1$ nM and inhibited cell growth in two small-cell lung cancer cell lines with IC50 values of 60–90 nM.

Pro-survival Bcl-2 proteins share a functional redundancy in antagonizing cell death, and there is considerable evidence that parallel inhibition of all pro-survival Bcl-2 proteins holds great promise to increase therapeutic efficacy. The pan-Bcl-2 inhibitors (−)-Gossypol and Obatoclax are already under clinical investigation.

Several new compounds are in preclinical development and time will tell if these novel Gossypol derivatives such as the highly promising BI-97C1 will advance to clinical trials. In addition to the currently available published material, ongoing efforts in drug development may yield even better, high affinity pan-Bcl-2 inhibitors in the future. Future clinical studies may also focus on the intelligent design of combined therapies employing BH3 mimetics with more restricted binding profiles and other, more selective drugs, e.g. ABT-263 with agents targeting the expression/stability of Mcl-1.

References

Adams JM, Cory S (2007) The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 26:1324–1337

Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J (1996) Human ICE/CED-3 protease nomenclature. Cell 87:171

Arnold AA, Aboukameel A, Chen J, Yang D, Wang S, Al-Katib A, Mohammad RM (2008) Preclinical studies of Apogossypolone: a new nonpeptidic pan small-molecule inhibitor of Bcl-2, Bcl-XL and Mcl-1 proteins in Follicular Small Cleaved Cell Lymphoma model. Mol Cancer 7:20

Balakrishnan K, Wierda WG, Keating MJ, Gandhi V (2008) Gossypol, a BH3 mimetic, induces apoptosis in chronic lymphocytic leukemia cells. Blood 112:1971–1980

Bodur C, Basaga H (2012) Bcl-2 inhibitors: emerging drugs in cancer therapy. Curr Med Chem 19:1804–1820

Bonapace L, Bornhauser BC, Schmitz M, Cario G, Ziegler U, Niggli FK, Schafer BW, Schrappe M, Stanulla M, Bourquin JP (2010) Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance. J Clin Invest 120:1310–1323

Bouillet P, Strasser A (2002) BH3-only proteins – evolutionarily conserved proapoptotic Bcl-2 family members essential for initiating programmed cell death. J Cell Sci 115:1567–1574

Cao X, Rodarte C, Zhang L, Morgan CD, Littlejohn J, Smythe WR (2007) Bcl2/bcl-xL inhibitor engenders apoptosis and increases chemosensitivity in mesothelioma. Cancer Biol Ther 6:246–252

Chen J, Jin S, Abraham V, Huang X, Liu B, Mitten MJ, Nimmer P, Lin X, Smith M, Shen Y, Shoemaker AR, Tahir SK, Zhang H, Ackler SL, Rosenberg SH, Maecker H, Sampath D, Leverson JD, Tse C, Elmore SW (2012) The Bcl-2/Bcl-X(L)/Bcl-w inhibitor, navitoclax, enhances the activity of chemotherapeutic agents in vitro and in vivo. Mol Cancer Ther 10:2340–2349

Chonghaile TN, Letai A (2008) Mimicking the BH3 domain to kill cancer cells. Oncogene 27(Suppl 1):S149–S157

Codogno P, Meijer AJ (2005) Autophagy and signaling: their role in cell survival and cell death. Cell Death Differ 12(Suppl 2):1509–1518

Cory S, Adams JM (2002) The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer 2:647–656

Danial NN (2007) BCL-2 family proteins: critical checkpoints of apoptotic cell death. Clin Cancer Res 13:7254–7263

Dash R, Azab B, Quinn BA, Shen X, Wang XY, Das SK, Rahmani M, Wei J, Hedvat M, Dent P, Dmitriev IP, Curiel DT, Grant S, Wu B, Stebbins JL, Pellecchia M, Reed JC, Sarkar D, Fisher PB (2011) Apogossypol derivative BI-97C1 (Sabutoclax) targeting Mcl-1 sensitizes prostate cancer cells to mda-7/IL-24-mediated toxicity. Proc Natl Acad Sci U S A 108:8785–8790

Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, Mukherjee C, Shi Y, Gelinas C, Fan Y, Nelson DA, Jin S, White E (2006) Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 10:51–64

Degterev A, Lugovskoy A, Cardone M, Mulley B, Wagner G, Mitchison T, Yuan J (2001) Identification of small-molecule inhibitors of interaction between the BH3 domain and Bcl-xL. Nat Cell Biol 3:173–182

Degterev A, Boyce M, Yuan J (2003) A decade of caspases. Oncogene 22:8543–8567

Edinger AL, Thompson CB (2004) Death by design: apoptosis, necrosis and autophagy. Curr Opin Cell Biol 16:663–669

Evan GI, Vousden KH (2001) Proliferation, cell cycle and apoptosis in cancer. Nature 411:342–348

Fischer U, Janicke RU, Schulze-Osthoff K (2003) Many cuts to ruin: a comprehensive update of caspase substrates. Cell Death Differ 10:76–100

Fulda S, Debatin KM (2004) Targeting apoptosis pathways in cancer therapy. Curr Cancer Drug Targets 4:569–576

Fulda S, Debatin KM (2006) Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene 25:4798–4811

Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G (2006) Mechanisms of cytochrome c release from mitochondria. Cell Death Differ 13:1423–1433

Gozuacik D, Kimchi A (2004) Autophagy as a cell death and tumor suppressor mechanism. Oncogene 23:2891–2906

Gozuacik D, Kimchi A (2007) Autophagy and cell death. Curr Top Dev Biol 78:217–245

Green DR (2006) At the gates of death. Cancer Cell 9:328–330

Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100:57–70

He C, Klionsky DJ (2009) Regulation mechanisms and signaling pathways of autophagy. Annu Rev Genet 43:67–93

Heist RS, Fain J, Chinnasami B, Khan W, Molina JR, Sequist LV, Temel JS, Fidias P, Brainerd V, Leopold L, Lynch TJ (2010) Phase I/II study of AT-101 with topotecan in relapsed and refractory small cell lung cancer. J Thorac Oncol 5:1637–1643

Hengartner MO (2000) The biochemistry of apoptosis. Nature 407:770–776

Hermanson D, Addo SN, Bajer AA, Marchant JS, Das SG, Srinivasan B, Al-Mousa F, Michelangeli F, Thomas DD, Lebien TW, Xing C (2009) Dual mechanisms of sHA 14–1 in inducing cell death through endoplasmic reticulum and mitochondria. Mol Pharmacol 76:667–678

Hetschko H, Voss V, Senft C, Seifert V, Prehn JH, Kögel D (2008) BH3 mimetics reactivate autophagic cell death in anoxia-resistant malignant glioma cells. Neoplasia 10:873–885

Huang DC, Strasser A (2000) BH3-Only proteins-essential initiators of apoptotic cell death. Cell 103:839–842

Igney FH, Krammer PH (2002) Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer 2:277–288

Kang MH, Reynolds CP (2009) Bcl-2 inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy. Clin Cancer Res 15:1126–1132

Kimura S, Noda T, Yoshimori T (2007) Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent-tagged LC3. Autophagy 3:452–460

Ko CH, Shen SC, Yang LY, Lin CW, Chen YC (2007) Gossypol reduction of tumor growth through ROS-dependent mitochondria pathway in human colorectal carcinoma cells. Int J Cancer 121:1670–1679

Kögel D, Fulda S, Mittelbronn M (2010) Therapeutic exploitation of apoptosis and autophagy for glioblastoma. Anticancer Agents Med Chem 10:438–449

Konopleva M, Contractor R, Tsao T, Samudio I, Ruvolo PP, Kitada S, Deng X, Zhai D, Shi YX, Sneed T, Verhaegen M, Soengas M, Ruvolo VR, McQueen T, Schober WD, Watt JC, Jiffar T, Ling X, Marini FC, Harris D, Dietrich M, Estrov Z, McCubrey J, May WS, Reed JC, Andreeff M (2006) Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell 10:375–388

Kroemer G, Galluzzi L, Brenner C (2007) Mitochondrial membrane permeabilization in cell death. Physiol Rev 87:99–163

Lei X, Chen Y, Du G, Yu W, Wang X, Qu H, Xia B, He H, Mao J, Zong W, Liao X, Mehrpour M, Hao X, Chen Q (2006) Gossypol induces Bax/Bak-independent activation of apoptosis and cytochrome c release via a conformational change in Bcl-2. FASEB J 20:2147–2149

Lessene G, Czabotar PE, Colman PM (2008) BCL-2 family antagonists for cancer therapy. Nat Rev Drug Discov 7:989–1000

Lian J, Wu X, He F, Karnak D, Tang W, Meng Y, Xiang D, Ji M, Lawrence TS, Xu L (2010) A natural BH3 mimetic induces autophagy in apoptosis-resistant prostate cancer via modulating Bcl-2-Beclin1 interaction at endoplasmic reticulum. Cell Death Differ 18:60–71

Liu G, Kelly WK, Wilding G, Leopold L, Brill K, Somer B (2009) An open-label, multicenter, phase I/II study of single-agent AT-101 in men with castrate-resistant prostate cancer. Clin Cancer Res 15:3172–3176

Lowe SW, Lin AW (2000) Apoptosis in cancer. Carcinogenesis 21:485–495

Maiuri MC, Le Toumelin G, Criollo A, Rain JC, Gautier F, Juin P, Tasdemir E, Pierron G, Troulinaki K, Tavernarakis N, Hickman JA, Geneste O, Kroemer G (2007) Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1. EMBO J 26:2527–2539

Malik SA, Orhon I, Morselli E, Criollo A, Shen S, Marino G, BenYounes A, Benit P, Rustin P, Maiuri MC, Kroemer G (2011) BH3 mimetics activate multiple pro-autophagic pathways. Oncogene 30:3918–3929

Manero F, Gautier F, Gallenne T, Cauquil N, Gree D, Cartron PF, Geneste O, Gree R, Vallette FM, Juin P (2006) The small organic compound HA14-1 prevents Bcl-2 interaction with Bax to sensitize malignant glioma cells to induction of cell death. Cancer Res 66:2757–2764

Martinou JC, Green DR (2001) Breaking the mitochondrial barrier. Nat Rev Mol Cell Biol 2:63–67

Meng Y, Tang W, Dai Y, Wu X, Liu M, Ji Q, Ji M, Pienta K, Lawrence T, Xu L (2008) Natural BH3 mimetic (−)-gossypol chemosensitizes human prostate cancer via Bcl-xL inhibition accompanied by increase of Puma and Noxa. Mol Cancer Ther 7:2192–2202

Milanesi E, Costantini P, Gambalunga A, Colonna R, Petronilli V, Cabrelle A, Semenzato G, Cesura AM, Pinard E, Bernardi P (2006) The mitochondrial effects of small organic ligands of BCL-2: sensitization of BCL-2-overexpressing cells to apoptosis by a pyrimidine-2,4,6-trione derivative. J Biol Chem 281:10066–10072

Mohammad RM, Goustin AS, Aboukameel A, Chen B, Banerjee S, Wang G, Nikolovska-Coleska Z, Wang S, Al-Katib A (2007) Preclinical studies of TW-37, a new nonpeptidic small-molecule inhibitor of Bcl-2, in diffuse large cell lymphoma xenograft model reveal drug action on both Bcl-2 and Mcl-1. Clin Cancer Res 13:2226–2235

Nicholson DW (1999) Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ 6:1028–1042

Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Bruncko M, Deckwerth TL, Dinges J, Hajduk PJ, Joseph MK, Kitada S, Korsmeyer SJ, Kunzer AR, Letai A, Li C, Mitten MJ, Nettesheim DG, Ng S, Nimmer PM, O'Connor JM, Oleksijew A, Petros AM, Reed JC, Shen W, Tahir SK, Thompson CB, Tomaselli KJ, Wang B, Wendt MD, Zhang H, Fesik SW, Rosenberg SH (2005) An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 435:677–681

Paoluzzi L, Gonen M, Gardner JR, Mastrella J, Yang D, Holmlund J, Sorensen M, Leopold L, Manova K, Marcucci G, Heaney ML, O'Connor OA (2008) Targeting Bcl-2 family members with the BH3 mimetic AT-101 markedly enhances the therapeutic effects of chemotherapeutic agents in in vitro and in vivo models of B-cell lymphoma. Blood 111:5350–5358

Pattingre S, Levine B (2006) Bcl-2 inhibition of autophagy: a new route to cancer? Cancer Res 66:2885–2888

Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, Levine B (2005) Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 122:927–939

Puthalakath H, Strasser A (2002) Keeping killers on a tight leash: transcriptional and post-translational control of the pro-apoptotic activity of BH3-only proteins. Cell Death Differ 9:505–512

Ranger AM, Malynn BA, Korsmeyer SJ (2001) Mouse models of cell death. Nat Genet 28:113–118

Ready N, Karaseva NA, Orlov SV, Luft AV, Popovych O, Holmlund JT, Wood BA, Leopold L (2011) Double-blind, placebo-controlled, randomized phase 2 study of the proapoptotic agent AT-101 plus docetaxel, in second-line non-small cell lung cancer. J Thorac Oncol 6:781–785

Real PJ, Cao Y, Wang R, Nikolovska-Coleska Z, Sanz-Ortiz J, Wang S, Fernandez-Luna JL (2004) Breast cancer cells can evade apoptosis-mediated selective killing by a novel small molecule inhibitor of Bcl-2. Cancer Res 64:7947–7953

Roberts AW, Seymour JF, Brown JR, Wierda WG, Kipps TJ, Khaw SL, Carney DA, He SZ, Huang DC, Xiong H, Cui Y, Busman TA, McKeegan EM, Krivoshik AP, Enschede SH, Humerickhouse R (2012) Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. J Clin Oncol 30:488–496

Shoemaker AR, Oleksijew A, Bauch J, Belli BA, Borre T, Bruncko M, Deckwirth T, Frost DJ, Jarvis K, Joseph MK, Marsh K, McClellan W, Nellans H, Ng S, Nimmer P, O'Connor JM, Oltersdorf T, Qing W, Shen W, Stavropoulos J, Tahir SK, Wang B, Warner R, Zhang H, Fesik SW, Rosenberg SH, Elmore SW (2006) A small-molecule inhibitor of Bcl-XL potentiates the activity of cytotoxic drugs in vitro and in vivo. Cancer Res 66:8731–8739

Shoemaker AR, Mitten MJ, Adickes J, Ackler S, Refici M, Ferguson D, Oleksijew A, O'Connor JM, Wang B, Frost DJ, Bauch J, Marsh K, Tahir SK, Yang X, Tse C, Fesik SW, Rosenberg SH, Elmore SW (2008) Activity of the Bcl-2 family inhibitor ABT-263 in a panel of small cell lung cancer xenograft models. Clin Cancer Res 14:3268–3277

Shore GC, Viallet J (2005) Modulating the bcl-2 family of apoptosis suppressors for potential therapeutic benefit in cancer. Hematol Am Soc Hematol Educ Program 2005:226–230

Sinicrope FA, Penington RC, Tang XM (2004) Tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis is inhibited by Bcl-2 but restored by the small molecule Bcl-2 inhibitor, HA 14–1, in human colon cancer cells. Clin Cancer Res 10:8284–8292

Smoot RL, Blechacz BR, Werneburg NW, Bronk SF, Sinicrope FA, Sirica AE, Gores GJ (2010) A Bax-mediated mechanism for obatoclax-induced apoptosis of cholangiocarcinoma cells. Cancer Res 70:1960–1969

Strappazzon F, Vietri-Rudan M, Campello S, Nazio F, Florenzano F, Fimia GM, Piacentini M, Levine B, Cecconi F (2011) Mitochondrial BCL-2 inhibits AMBRA1-induced autophagy. EMBO J 30:1195–1208

Tang G, Nikolovska-Coleska Z, Qiu S, Yang CY, Guo J, Wang S (2008) Acylpyrogallols as inhibitors of antiapoptotic Bcl-2 proteins. J Med Chem 51:717–720

Tian D, Das SG, Doshi JM, Peng J, Lin J, Xing C (2008) sHA 14–1, a stable and ROS-free antagonist against anti-apoptotic Bcl-2 proteins, bypasses drug resistances and synergizes cancer therapies in human leukemia cell. Cancer Lett 259:198–208

Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, Johnson EF, Marsh KC, Mitten MJ, Nimmer P, Roberts L, Tahir SK, Xiao Y, Yang X, Zhang H, Fesik S, Rosenberg SH, Elmore SW (2008) ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res 68:3421–3428

Tzung SP, Kim KM, Basanez G, Giedt CD, Simon J, Zimmerberg J, Zhang KY, Hockenbery DM (2001) Antimycin A mimics a cell-death-inducing Bcl-2 homology domain 3. Nat Cell Biol 3:183–191

van Delft MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabotar PE, Willis SN, Scott CL, Day CL, Cory S, Adams JM, Roberts AW, Huang DC (2006) The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. Cancer Cell 10:389–399

Vogler M (2012) BCL2A1: the underdog in the BCL2 family. Cell Death Differ 19:67–74

Vogler M, Dinsdale D, Dyer MJ, Cohen GM (2009a) Bcl-2 inhibitors: small molecules with a big impact on cancer therapy. Cell Death Differ 16:360–367

Vogler M, Weber K, Dinsdale D, Schmitz I, Schulze-Osthoff K, Dyer MJ, Cohen GM (2009b) Different forms of cell death induced by putative BCL2 inhibitors. Cell Death Differ 16:1030–1039

Voss V, Senft C, Lang V, Ronellenfitsch MW, Steinbach JP, Seifert V, Kögel D (2010) The pan-bcl-2 inhibitor (−)-gossypol triggers autophagic cell death in malignant glioma. Mol Cancer Res 8:1002–1016

Wang JL, Liu D, Zhang ZJ, Shan S, Han X, Srinivasula SM, Croce CM, Alnemri ES, Huang Z (2000) Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. Proc Natl Acad Sci U S A 97:7124–7129

Wang G, Nikolovska-Coleska Z, Yang CY, Wang R, Tang G, Guo J, Shangary S, Qiu S, Gao W, Yang D, Meagher J, Stuckey J, Krajewski K, Jiang S, Roller PP, Abaan HO, Tomita Y, Wang S (2006) Structure-based design of potent small-molecule inhibitors of anti-apoptotic Bcl-2 proteins. J Med Chem 49:6139–6142

Wang Z, Song W, Aboukameel A, Mohammad M, Wang G, Banerjee S, Kong D, Wang S, Sarkar FH, Mohammad RM (2008) TW-37, a small-molecule inhibitor of Bcl-2, inhibits cell growth and invasion in pancreatic cancer. Int J Cancer 123:958–966

Wei J, Stebbins JL, Kitada S, Dash R, Placzek W, Rega MF, Wu B, Cellitti J, Zhai D, Yang L, Dahl R, Fisher PB, Reed JC, Pellecchia M (2010) BI-97C1, an optically pure Apogossypol derivative as pan-active inhibitor of antiapoptotic B-cell lymphoma/leukemia-2 (Bcl-2) family proteins. J Med Chem 53:4166–4176

Wendt MD, Shen W, Kunzer A, McClellan WJ, Bruncko M, Oost TK, Ding H, Joseph MK, Zhang H, Nimmer PM, Ng SC, Shoemaker AR, Petros AM, Oleksijew A, Marsh K, Bauch J, Oltersdorf T, Belli BA, Martineau D, Fesik SW, Rosenberg SH, Elmore SW (2006) Discovery and structure-activity relationship of antagonists of B-cell lymphoma 2 family proteins with chemopotentiation activity in vitro and in vivo. J Med Chem 49:1165–1181

Weyland M, Manero F, Paillard A, Gree D, Viault G, Jarnet D, Menei P, Juin P, Chourpa I, Benoit JP, Grée R, Garcion E (2011) Mitochondrial targeting by use of lipid nanocapsules loaded with SV30, an analogue of the small-molecule Bcl-2 inhibitor HA14-1. J Control Release 151:74–82

Wolter KG, Wang SJ, Henson BS, Wang S, Griffith KA, Kumar B, Chen J, Carey TE, Bradford CR, D'Silva NJ (2006) (−)-gossypol inhibits growth and promotes apoptosis of human head and neck squamous cell carcinoma in vivo. Neoplasia 8:163–172

Zeitlin BD, Joo E, Dong Z, Warner K, Wang G, Nikolovska-Coleska Z, Wang S, Nor JE (2006) Antiangiogenic effect of TW37, a small-molecule inhibitor of Bcl-2. Cancer Res 66:8698–8706

Zhai D, Jin C, Satterthwait AC, Reed JC (2006) Comparison of chemical inhibitors of antiapoptotic Bcl-2-family proteins. Cell Death Differ 13:1419–1421

Zhang Z, Song T, Zhang T, Gao J, Wu G, An L, Du G (2011) A novel BH3 mimetic S1 potently induces Bax/Bak-dependent apoptosis by targeting both Bcl-2 and Mcl-1. Int J Cancer 128:1724–1735

Zimmermann AK, Loucks FA, Schroeder EK, Bouchard RJ, Tyler KL, Linseman DA (2007) Glutathione binding to the Bcl-2 homology-3 domain groove: a molecular basis for Bcl-2 antioxidant function at mitochondria. J Biol Chem 282:29296–29304

Chapter 3
Regulation of Mitochondrial Function by MicroRNA

Marco Tomasetti, Lan-Feng Dong, and Jiri Neuzil

Contents

Abstract Mitochondria are organelles that are instrumental for the life as well as the death of cell. They have their own genome, present in multiple copies. The mitochondrial genome codes for 13 subunits of mitochondrial complexes, while all other mitochondrial proteins have to be imported from the cytosol, being coded for by the nucleus. Therefore, regulation of the mitochondrial proteome is rather complicated,

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since all complexes have to assemble properly to exert their biological function. A new angle in the regulation of gene expression has been uncovered recently, occurring at the post-transcriptional level and involving transcripts of short 'non-coding' sequences in the nuclear genome, the so called microRNAs (MiRs). These are intriguing molecules that are rather promiscuous, each of them modulating the expression of multiple genes. Recent research indicates their involvement in the mitochondrial function affecting virtually all aspects of the (patho)physiology of the cells. In this paper, we review the role of MiRs in the regulation of the mitochondrial function, largely in the context of human pathologies.

Keywords Mitochondria • Mitochondrial dysfunction • MicroRNA • Cancer • Tricarboxylic acid cycle • Oxidative phosphorylation • Metabolism • Glycolysis • Reactive oxygen species • Mitochondrial DNA • Hypoxia-inducible factor • Insulin receptor substrate-1 • FoxO1 • Akt • Mitophagy

1 Introduction

Mitochondria are primarily responsible for providing energy by a continuous supply of ATP. However, they can efficiently function as death-promoting organelles. In response to changes in the intracellular environment, mitochondria become producers of excessive reactive oxygen species (ROS) and release pro-apoptotic proteins, resulting in disrupted ATP synthesis and activation of cell death pathways (Suen et al. 2008; Kilbride and Prehn 2013). Cell stress stimuli can result in mitochondrial outer membrane (MOM) permeabilisation, after which mitochondria release numerous proteins involved in apoptotic signalling, including cytochrome c, the apoptosis-inducing factor (AIF), endonuclease G, Smac/DIABLO and Omi/HtrA2. Cell fate is determined by signalling involving proteins of the Bcl-2 family, which converge on mitochondria. Mitochondria therefore play a critical role in physiological homeostasis, and their dysfunction is related to a variety of pathological processes and (Galloway and Yoon 2013).

Mitochondria and oxidative stress are the central players, for example in Parkinson's disease pathogenesis. A systemic deficiency in complex I (CI) of the mitochondrial electron transport chain (ETC) is evident in many patients with the pathology (Hauser and Hastings 2013). Changes in the mitochondrial function and ultrastructure have been found in various mental disorders (Gong et al. 2011). Mitochondrial DNA (mtDNA) mutations and deletions, plus excessive ROS production have been shown to contribute to the ageing process and cancer (Chaudhary et al. 2011; Yin et al. 2014; Grzybowska-Szatkowska and Slaska 2012). Dysregulation of the mitochondrial function is a hallmark of cancer cells, and several key genes are closely linked to tumour progression, including succinate dehydrogenase (SDH), fumarate hydratase (FH) and isocitrate dehydrogenase (IDH). Mutations in or loss of the SDH and FH genes are known to be oncogenic (Ishii et al. 2005; Guzy et al. 2008).

It has been reported that the dysfunction of the SDH and FH enzymes is linked to the activation of hypoxia-inducible factor-α (HIF1α), which enhances tumourigenic-related signalling promoting processes like angiogenesis (Selak et al. 2005). Functional CIII of the ETC is required for the hypoxic stabilisation of HIF1α and HIF2α, and that an increase in ROS links this complex to HIFα stabilisation (Mansfield et al. 2005; Guzy et al. 2005).

Mitochondria are dynamic organelles that continually undergo fission and fusion. These opposing processes work in concert to maintain the shape, size, and number of mitochondria and their physiological function. Mitochondrial dynamics has been linked to multiple mitochondrial, including mtDNA stability, respiratory capacity, apoptosis, and the response to cellular stress (Chan 2012). Because of these important processes, mitochondrial fusion and fission are essential in mammalian cells, and even mild defects in mitochondrial dynamics can promote pathological states (Detmer and Chan 2007; Galloway and Yoon 2013). Interestingly, cells have developed a defence mechanism against aberrant cytotoxic mitochondria. This mechanism involves selective sequestration and subsequent degradation of malfunctional mitochondria before they enter the apoptotic process. Induction of mitochondrial autophagy (mitophagy) results in selective cellular clearance of damaged mitochondria. This process is the only known mechanism for mitochondrial turnover. It has been speculated that dysfunction of autophagy may result in the accumulation of malfunctional mitochondria, promoting their abnormal function and oxidative stress.

Mitochondrial dynamics, metabolism and function are tightly regulated by intracellular pathways. The discovery of microRNAs (MiRs) has yielded a new paradigm of post-transcriptional gene regulation with the small non-coding RNAs closely linked to virtually all known fundamental biological pathways. Deregulation of MiRs can contribute to the promotion of human diseases, including cancer, where MiRs can function as either oncogenes or tumour suppressors. MiRs can affect mitochondria by targeting mitochondrial proteins coded for by nuclear genes, and have been also found in mitochondria (Kren et al. 2009; Bian et al. 2010). Conceivably, MiRs may contribute to the mitochondrial dysfunction (Li et al. 2011).

2 Characteristics of MicroRNAs

MiRs are small non-coding RNAs that promote the cleavage or translational repression of specific mRNAs with the cognate site(s) in their 3′-untranslated regions (3′UTRs). In the human genome, MiR sequences can be present either as a part of independent polymerase II transcription units or within annotated ‘host’ genes. Of the 50–80 % of human MiRs that are found in introns, most are preferentially located near their central part (Morlando et al. 2008; Kim and Kim 2007). The biogenesis of MiR is controlled by two RNase-dependent processing steps that convert a long primary transcript into a mature MiR. First, primary MiRs (pri-MiRs) are processed by the Drosha-containing complex, i.e. the RNase III-like enzyme and DGCR8

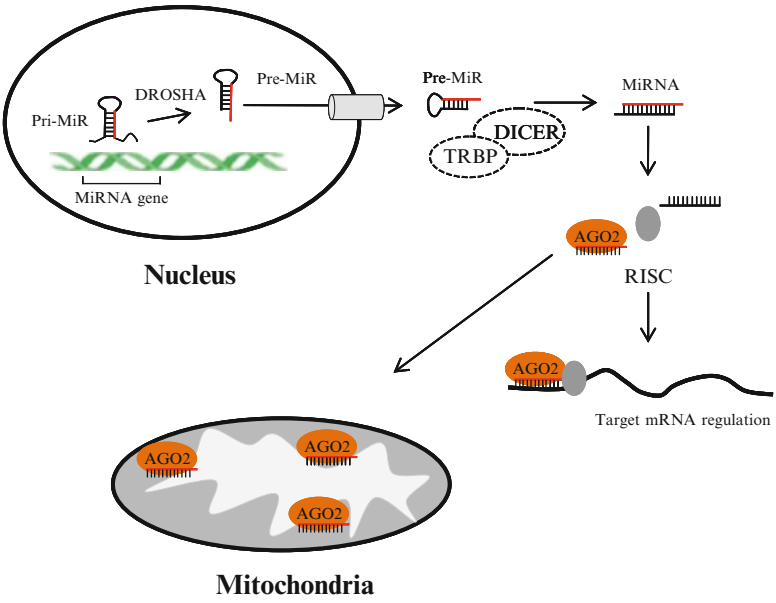

Fig. 1 MiR biogenesis and mitochondrial translocation. The pri-MiR, transcribed from genome by RNA polymerase II, is processed into pre-Mir by RNase III enzyme Dorsha. In cytosol the pre-MiR is cleaved by Dicer to produce mature MiR, which is associated with the RISC assembly to degrade complimentary mRNA. MiRs translocate to various subcellular locations including mitochondria. MiR and AGO2, a core component of the RISC complex, are associated with mitochondria to regulate mitochondrial gene expression

(DiGeorge syndrome critical region gene 8), to stem-loop pre-MiRs that are then further processed by the second RNase, Dicer, to short double-strand duplexes. Eventually, one of the functional strands in the resulting duplex is preserved, forming a functional complex with the RNA-induced silencing complex (RISC) proteins, and acts as a 'guide' strand for specific recognition (Fig. 1). A number of RNA-binding proteins, such as hnRNPs (heterogeneous nuclear ribonucleoproteins) A1, Lin28, Smad proteins and the KSRP protein (KH-type splicing regulatory protein) have been shown to positively or negatively regulate MiR production (reviewed in Siomi and Siomi 2010). Drosha itself can regulate the level of the microprocessor complex by cleaving hairpins in the 3′-UTR and the coding region of the DGCR8 mRNA, whereby destabilising the mature transcript and leading to a decrease in the DGCR8 protein (Kim 2009). This suggests that a balance between the levels of the microprocessor and its regulator proteins is essential for the physiological homeostasis.

MiRs are transcribed within the nucleus and are then extensively processed into the mature form in the cytosol as ~22-bp double-stranded RNAs. Mature MiRs associate with Argonaute (AGO) proteins to form the core of the RISC, which is the basis for RNA interference (RNAi). RNAi occurs upon pairing of one of the two

MiR strands, associated with an AGO protein, with target sites in an mRNA, thereby affecting its stability/translation (for review see Ketting 2011; von Brandenstein et al. 2012). Mammalian cells contain four AGO proteins (AGO1-4), which have been shown to function in translational repression (Fabian et al. 2010), but only AGO2 can catalyse the cleavage of the target transcript (Liu et al. 2004). Furthermore, knock-down and knock-out AGO2 experiments in human cells and in mice, respectively, suggest that this protein has specific functions that may not be complemented by the other three AGO proteins. Initially, mature MiRs and AGO2 were believed to accumulate and function exclusively in the cytosol and/or in unstructured cytosolic foci, such as the processing bodies (P-bodies) and stress granules (Leung et al. 2006; Liu et al. 2005). However, more recent evidence shows that they can also localise to and function within different cellular compartments. To date, MiRs and AGO2 have been found to localise to the nucleus (Hwang et al. 2007; Liao et al. 2010; Ohrt et al. 2008) and to multivesicular bodies (Gibbings et al. 2009). Interestingly, ~90 % of extracellular MiRs are packaged with proteins (i.e. AGO2, HDL, and other RNA-binding proteins) and ~10 % are wrapped in small membranous particles (i.e. exosomes, microvesicles, and apoptotic bodies). It is believed that these extracellular MiRs mediate cell-to-cell communications (Zhuang et al. 2012).

3 MicroRNAs in Mitochondria

The regulation of mitochondrial function is critically determined by proteins encoded by both nuclear and mitochondrial genome. Replication and transcription of mtDNA is initiated from a small non-coding region, the D-loop, and is regulated by nuclear-encoded proteins that are post-translationally imported into mitochondria. The transcription and translation of mtDNA as well as the processing of mitochondrial transcripts requires the involvement of several types of non-coding RNAs, which can be either mitochondrial encoded or transcribed within the nucleus and subsequently localised to mitochondria. Recent studies have reported that there are MiRs that localise to and function in other cellular compartments than the cytosol. Nuclear encoded MiRs have been found to be associated with the MOM (Mercer et al. 2011). This compartment may provide a platform for the assembly of signalling complexes that play an important role in the regulation of transcriptional repression.

It has been reported that MiR-181c, encoded in the nucleus, matures in the cytoplasm, and finally translocates into mitochondria of cardiac myocytes. This MiR can enter and target the mitochondrial genome, ultimately causing re-modelling of CIV and mitochondrial dysfunction (Das et al. 2012). Mitochondria harbour their own genetic system that may be a potential site for MiR-mediated post-transcriptional regulation. For example, MiRs have been identified in mitochondria purified from rat liver (Kren et al. 2009; Bian et al. 2010), and localisation of pre-MiRs and mature MiRs has been demonstrated for mitochondria isolated from human muscles cells (Barrey et al. 2011). Whether mitochondrial MiRs are transported into the mitochondria or are endogenously synthesised remains unknown and should be further

investigated. Several arguments support the MiR import hypothesis. A possible link between mitochondria and RNAi came from co-immunoprecipitation of human AGO2 with mitochondrial $tRNA^{Met}$ (Maniataki and Mourelatos 2005). This suggests that components involved in mitochondrial RISC assembly, in particular AGO2 may be involved in transport of MiRs to mitochondria. In mitochondria, post-transcriptional regulation via MiRs would provide a sensitive and rapid mechanism that will adjust the expression of the mitochondrial genome in relation to the conditions and metabolic demands of the cell (Fig. 1).

A recent study, aimed to investigate the possible link between MiRs and mitochondria in human cells, identified 13 MiRs significantly enriched in mitochondria purified from HeLa cells that have been referred to as mitoMiRs and that are coded for in the nucleus (Bandiera et al. 2011). For example, apart from its role in the cytosol, MiR-494 is likely to have a function in mitochondria due to its localisation to this organelle. Conceivably, hsa-MiR-1974, hsa-MiR-1977 and hsa-MiR-1978 are considered non-canonical MiRs because they map to mitochondrial tRNA and rRNA genes. The actual mitochondrial localisation of certain MiRs implies that small RNA-mediated processes may regulate mitochondrial biogenesis and function. Details of which MiRs modulate the mitochondrial function are rather obscure.

4 MicroRNAs and Mitochondrial Metabolism

A fundamental function of mitochondria is to produce ATP via oxidative phosphorylation (OXPHOS), thereby providing energy for the variety of cellular functions. MiR-15b, MiR-16, MiR-195 and MiR-424 have emerged as regulators of the ATP levels all sharing the same ‘seed’ sequence. Their over-expression down-regulates ATP and affects the mitochondrial integrity. The target of these MiRs is the ADP-ribosylation factor-like 2 (ARL2) mRNA, and knock-down of ARL2 by siRNA also resulted in reduced ATP level and degeneration of mitochondria (Nishi et al. 2010). Conditions that induce up-regulation of MiR-15b as well as several other MiRs that share the same ‘seed’ sequence, such as MiR-195, result in the mitochondrial degeneration and reduction in ATP levels in cardiomyocytes. MiR-338 is another MiR that modulates OXPHOS and the mitochondrial function, since it targets cytochrome c oxidase IV (COX IV) mRNA (Aschrafi et al. 2008). In neurons, MiR-338 over-expression by transfection resulted in decreased COX IV and reduced ATP. In contrast, expression of anti-MiR oligonucleotides increased COX IV levels and improved OXPHOS. It was therefore concluded that over-expression of MiR-338 jeopardises the mitochondrial function.

Mitochondrial glutaminase (GLS) is important for mitochondrial metabolism since it converts glutamine to glutamate that is further catabolised by the tricarboxylic acid (TCA) cycle for the production of ATP. Glutamate also serves as a substrate for glutathione synthesis. Proliferating cells are known to utilise glutamine as a major source of energy, ‘nitrogen’ for biosynthetic pathways and ‘carbon’ for

anabolic processes (Wise et al. 2008; Gao et al. 2009). Recent reports show that transcriptional regulation of the Myc oncogene is coordinated with the expression of genes that promote cells to engage in excessive glutamine catabolism, which exceeds the cellular requirement for protein and nucleotide biosynthesis (Wise et al. 2008). Such Myc-dependent glutaminolysis results in re-programming of the mitochondrial metabolism to depend on glutamine catabolism and to maintain cellular viability and TCA cycle anaplerosis. Concomitantly, this stimulation of mitochondrial glutamine metabolism leads to reduced glucose-derived metabolites to enter the TCA cycle and a decreased contribution of glucose to the mitochondrial-dependent synthesis of phospholipids (Barth et al. 1992). MiR-23a and MiR23b have been demonstrated to target mitochondrial GLS, since they can directly repress GLS levels (Gao et al. 2009). Myc, in turn, up-regulates GLS by suppressing the expression of MiR-23a/b.

Early studies have demonstrated the formation of mitochondrial ROS under hypoxia, and these radicals have been suggested as 'sensors' of oxygen deficiencies (Klimova and Chandel 2008). Cells sense and respond to the shortage of oxygen by activating the transcription factors HIF-1 and HIF-2 to evoke adaptive responses. Mitochondria are at the center of hypoxia sensing and the response relay system. In hypoxic cancer cells, mitochondria re-direct the TCA cycle intermediates to preserve their biosynthetic function. Persistent HIF activation lowers the entry of the mitochondria 'electron-delivering' compounds to reduce the TCA cycle fuelling and β-oxidation, attenuates the expression of the ETC components, limits the mitochondria-associated biosynthetic pathways, and provokes their removal by autophagy (Dehne and Brüne 2014). MiR-210, significantly up-regulated during hypoxic stress in many cell types (Kulshreshtha et al. 2007; Huang et al. 2010; Chan et al. 2012a), has been reported to be involved in repressing mitochondrial respiration and the ensuing down-stream events (Favaro et al. 2010; Chan et al. 2012b). Following exposure to hypoxia, cellular metabolism shifts from OXPHOS to glycolysis (known as the Pasteur effect). MiR-210 contributes to the metabolic shift by down-regulating several steps of mitochondrial metabolism and, in particular, the ETC complexes.

The effect of MiR-210 on the metabolic function is also linked to its direct repression of the iron sulphur cluster assembly proteins ISCU1 and ISCU2. Upon identifying the ISCU1/2 proteins as MiR-210 targets, it has been shown that HIF-expressing tissues *in vivo* feature increased levels of MiR-210, decreased expression of ISCU1/2, and consequently the disruption of the integrity of iron-sulphur clusters. In turn, due to repressing ISCU1/2 during hypoxia, MiR-210 decreases the activity of iron-sulphur enzymes that control the mitochondrial metabolism, including CI and aconitase. Consequently, MiR-210 represses mitochondrial respiration, which, in the presence of normal oxygen, leads to decreased ATP levels. In contrast, during hypoxia, miR-210 appears to increase ATP levels (Chan et al. 2012b) and optimise energy production in the hypoxic cell via the Pasteur effect (Semenza 2007). The repressive effect of MiR-210 on the ETC also impacts on mitochondrial ROS production, a consequence of electron leakage. Indeed, MiR-210 expression increases oxidative stress under normoxic conditions

and this is, in part, mediated by ISCU (Favaro et al. 2010; Chan et al. 2012b). However, conflicting results have been reported for hypoxia. In cancer cell lines, miR-210 alleviated the hypoxia-induced ROS formation. On the other hand, hypoxia exposure did not induce significant changes in ROS production in normal endothelial cells, which increased when miR-210 was inhibited. This discrepancy underlies the differences between normal versus cancer cells.

Other relevant mitochondrial targets of MiR-210 targets that have been reported to be the NADH dehydrogenase (ubiquinone) 1-α sub-complex 4, a subunit of CI (Giannakakis et al. 2008), and SDHD, a subunit of CII (Puisségur et al. 2011). An additional target is glycerol-3-phosphate dehydrogenase (GPD), the catalyst of the glycerol phosphate shuttle, which transfers electrons from cytoplasmic NADH to the ETC. Collectively, MiR-210 suppresses mitochondrial respiration and enhances glycolysis, which leads to a decrease in the ATP level.

More recently, MiR-126 has been found to induce a metabolic shift in tumours toward a more glycolytic phenotype (Tomasetti et al. 2014). MiR-126 suppresses the mitochondrial respiratory activity in malignant mesothelioma (MM) cells and stimulates glycolysis in response to the inhibition of mitochondrial oxygen consumption, indicative of a compensatory process (Fig. 2a–c). This process is coupled to the glycolytically derived pyruvate that enters a truncated TCA cycle, where citrate is preferentially exported to the cytosol via the tricarboxylate transporter (Baggetto 1992). Once in the cytosol, citrate is cleaved by ATP citrate lyase (ACL) to produce cytosolic acetyl-CoA used for endogenous synthesis of fatty acids, cholesterol, and isoprenoids, as well as acetylation reactions that modify proteins. It has been postulated that MiR-126 affects the mitochondrial citrate metabolism by inhibiting the Akt pathway to restore the TCA cycle for the synthesis of ATP. Thus, this mechanism favours glucose oxidation to produce cellular energy rather than converting it into other macromolecules for cellular biosynthesis (Fig. 2d).

5 Mitochondrial Dysfunction and MicroRNAs

There is considerable evidence for mitochondrial dysfunction in association with insulin resistance, obesity, diabetes and cancer (Ren et al. 2010; Rabøl et al. 2009; Garcia-Roves 2011; Kim et al. 2008; Dang 2012). Mitochondrial dysfunction has been shown to compromise insulin signalling via serine phosphorylation of the insulin receptor substrate-1 (IRS-1) (Morino et al. 2005). The contribution of mitochondrial dysfunction to the impairment in insulin metabolic signalling has been also suggested by results of gene array analysis showing that the repression of genes regulating mitochondrial ATP production is associated with insulin resistance and type 2 diabetes mellitus. Moreover, reduction in the oxidative capacity of the ETC has been manifested in obese, insulin-resistant persons as well as diabetic patients. Genetic and environmental factors, oxidative stress, and alterations in mitochondrial biogenesis can adversely affect the mitochondrial function, leading to insulin resistance and various pathological conditions such as the cardiorenal syndrome and type 2 diabetes.

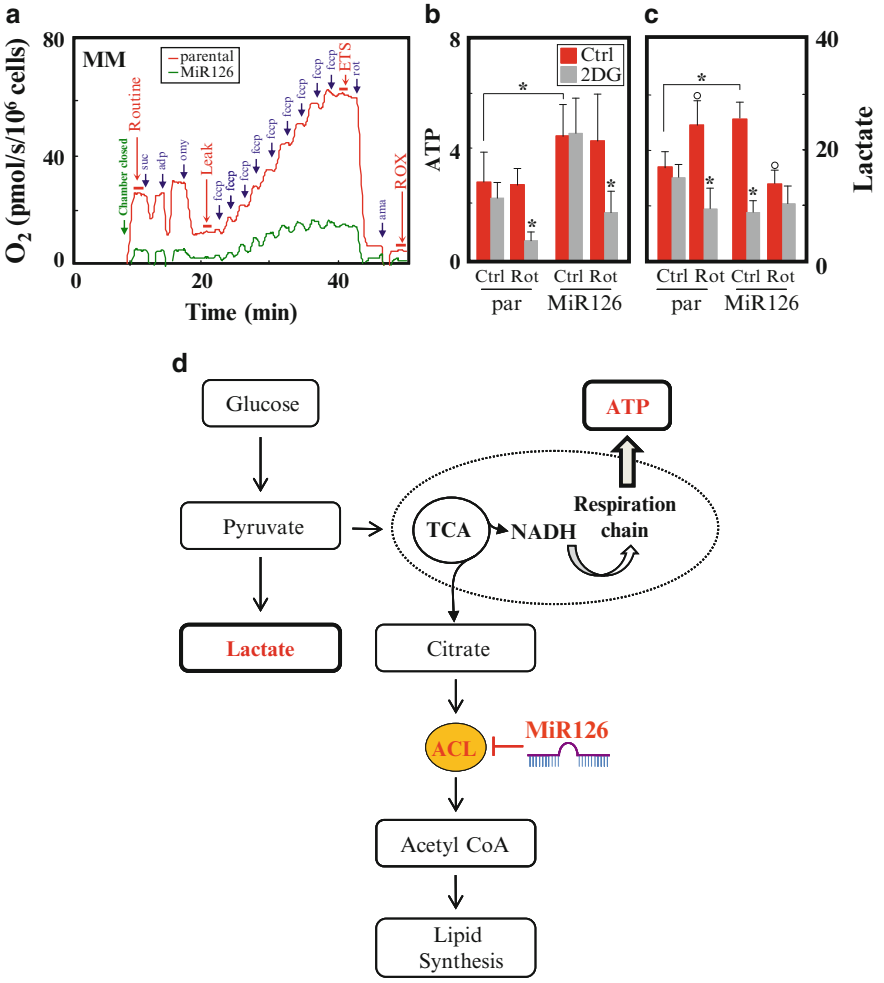

Fig. 2 MiR-126 alters respiration and bioenergetic profile of mesothelioma cancer cells. Parental H28 cells ($H28^{par}$) and their MiR-126-transfected counterparts ($H28^{MiR126}$) were evaluated for mitochondrial respiration (**a**), and ATP (nmol/mg protein) (**b**) and lactate (nmol/mg protein) (**c**) in the presence of rotenone (20 μM) and 2-deoxy glucose (2DG, 5 mM). Ectopic MiR126 was found to suppress mitochondrial respiration and maximise ATP production (via glycolysis). Comparisons among groups were determined by *t*-student test, the symbol '*' indicates significant differences, symbol '°' significance compared to control with $p < 0.05$. (**d**) Scheme of the proposed function of MiR-126. MiR-126 affects the mitochondrial citrate metabolism by inhibiting the ACL to restore the TCA cycle for the synthesis of ATP via OXPHOS

Several MiRs have been implicated in the metabolic homeostasis deregulation, as follows from loss-of-function studies in mice (Näär 2011; Rottiers and Näär 2012). Both MiR-378 and MiR-378* participate in the regulation of mitochondrial metabolism and energy homeostasis in mice via the transcriptional network

controlled by the peroxisome proliferator-activated receptor γ co-activator-1 (PGC-1β) (Carrer et al. 2012). Mice lacking MiR-378 and MiR-378* are resistant to high-fat diet-induced obesity, and exhibited enhanced mitochondrial fatty acid metabolism and elevated oxidative capacity of insulin-target tissues. On the other hand, up-regulation of MiR-378* in cancer cells has been proposed to mediate increased lactate production owing to the shift from oxidative to glycolytic metabolism, which is associated with tumourigenesis (Eichner et al. 2012).

Mitochondrial dysfunction resulting from genetic alteration (mtDNA depletion) or metabolic inhibition (rotenone toxicity) induces insulin resistance in hepatocytes via reduced expression of the IRS-1 protein. Furthermore, it has been found that mitochondrial dysfunction induces the expression of several MiRs thought to target the IRS-1 3′UTR. Of these, MiR-126 is actively involved in the development of insulin resistance as it directly targets IRS-1 (Zhang et al. 2008b; Ryu et al. 2011). MiR-126 acts as a metabolic regulator, its up-regulation being associated with the inhibition of Akt signalling (Yang et al. 2012; Tomasetti et al. 2014).

Activation of the PI3K/Akt pathway is one of the most common events in spontaneous human cancers, and it has been shown to alter metabolism and to promote the flow of precursors into anabolic pathways (Ward and Thompson 2012). Akt activates ACL, promoting the conversion of mitochondria-derived citrate to acetyl-CoA for lipid synthesis (Bauer et al. 2005). Therefore, the re-programming of mitochondrial citrate metabolism is a central aspect of the PI3K/Akt activity (Hatzivassiliou et al. 2005). MiR-126 was found to affect the citrate metabolism by inhibiting the Akt pathway to restore the TCA cycle for ATP synthesis. This mechanism favours glucose oxidation to produce energy rather than convert it into precursors for cellular biosynthesis (Tomasetti et al. 2014).

A key downstream effector of the Akt pathway is the Forkhead box O-class 1 (FOXO1) protein, functionally inactive due to its phosphorylation by activated Akt in a variety of cancers. FOXO1 inactivation favours enhanced cell survival, cell proliferation, and susceptibility to stress, while its activation leads to apoptosis, cell cycle arrest and stress resistance in various tissues (Tothova and Gilliland 2007; Zhang et al. 2011). Recent evidence documents that IRS-1 is an important inhibitor of FOXO1 via its Akt-mediated phosphorylation (Dong et al. 2008; Guo et al. 2009). Ectopic MiR-126 has been found to re-activate FOXO1 via the inhibition of the IRS-1/Akt pathway (Fig. 3a, b). Consistent with this, MiR-126 induced nuclear translocation of FOXO1 in both non-malignant and MM cells, resulting in increased expression of genes involved in the glucose metabolism and mitochondrial function (Tomasetti et al. 2014). MiR-126 induced the expression of phosphoenolpyruvate carboxykinase (PCK1), which is a main control point for the regulation of gluconeogenesis (Fig. 3c). Substrate level phosphorylation occurs in glycolysis, where phosphoenol pyruvate is converted to pyruvate, which then enters the TCA cycle. Under these conditions, increased glycolysis is an important early compensatory mechanism to produce ATP (Fig. 3d). Further, cells expressing MiR-126 feature high levels of mitochondrial superoxide dismutase (SOD2) and catalase (CAT), also regulated by FOXO1 (Tomasetti et al. 2014; Valis et al. 2011). Enhanced ROS production in cancer drives the onset of aerobic glycolysis, with lactate and ketone production promoting mitochondrial biogenesis and anabolic growth of tumour

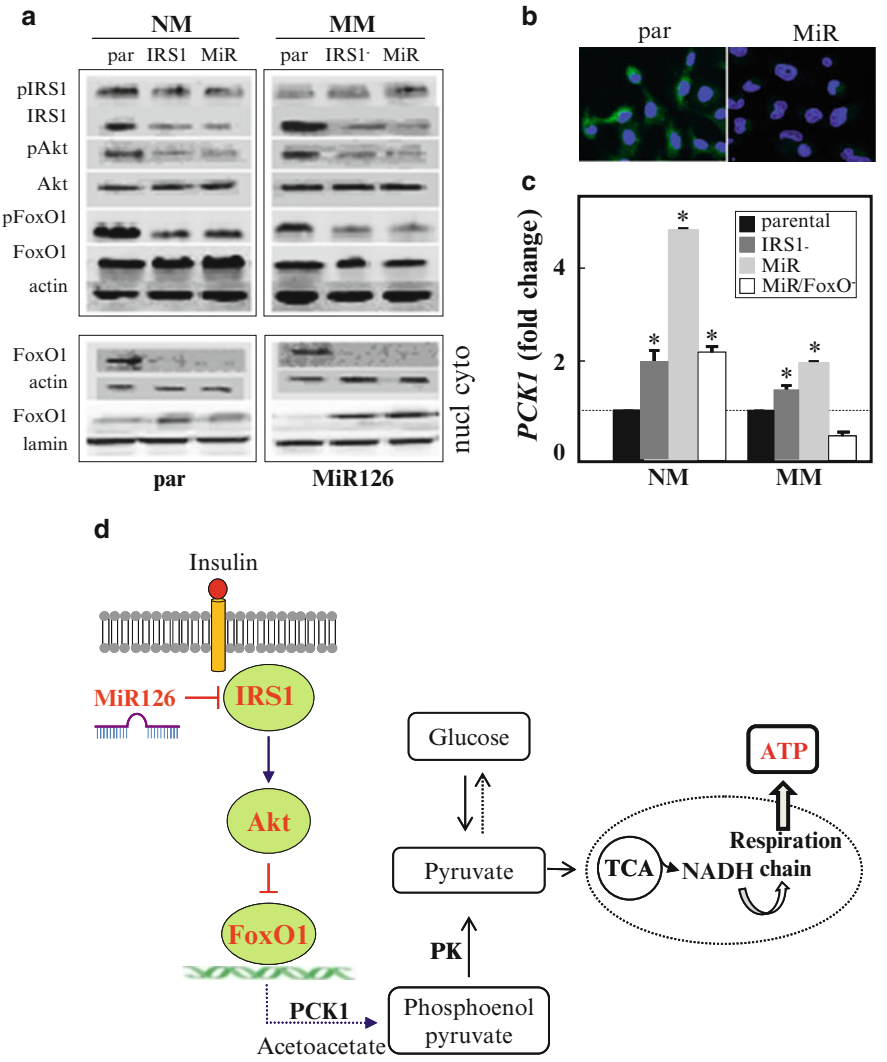

Fig. 3 MiR-126 alters the IRS1 pathway-dependent mitochondrial function and expression of FOXO1-dependent genes. MiR-126 was found to suppress IRS1 with ensuing inhibition of Akt. This causes the failure of FOXO1 sequestration in the cytosol, which results in the increase in the gluconeogenesis gene PCK1 (**a**, **b**) yielding increased pyruvate accompanied by a glycolytic shift (**b**) as represented in the scheme (**d**). *IRS1*$^{-}$ IRS1-silenced, *FOXO1*$^{-}$ FOXO1-silenced. '*' indicates significantly different values with $p < 0.05$. Panel **c** depicts the proposed role of MiR-126

cells. Alleviation of mitochondrial oxidative stress via enhanced expression of antioxidant enzymes targeted to mitochondria was found to be sufficient to lower tumour severity and to considerably reduce the tumour burden, linking MiR-126 to the suppression of the onset and progression of cancer.

An important component of cellular metabolism is the production of ROS (Finkel 2012), which are generated by mitochondria as well as NADPH oxidases (NOX). They can cause damage to membranes and can be mutagenic. Emerging data suggest that conditions of cellular stress can alter the biogenesis of MiRs, the expression of their targets and the activity of MiR-protein complexes. The level of MiR-mediated repression depends not only on the ratio of a particular mRNA target relative to the MiR, but also on the amount of other MiRs present in the transcriptome targeted by the same MiRs. Consistent with this, expression of a number of MiRs is rapidly modulated by stress, as documented for cells exposed to UV radiation (Pothof et al. 2009).

Exposure to H_2O_2 has been reported to alter the MiR expression profile. For example, MiR-126 is induced by oxidative stress exerting a protective function by inducing the expression of antioxidant enzymes (Tomasetti et al. 2014). Overexpression of MiR-145 significantly inhibited H_2O_2-induced cellular apoptosis, ROS production, mitochondrial structure disruption as well as the activation of key signalling proteins in the mitochondrial apoptotic pathway (Li et al. 2012). Haque et al. (2012), demonstrated that a sub-lethal dose of H_2O_2 up-regulated the expression of MiR-30b, which inhibited the expression of CAT. MiR-335 and MiR-34a were found to inhibit the expression of SOD2 and thioredoxin reductase 2 (Txnrd2) by binding to the 3′-UTR of each gene, respectively. Overexpression of MiR-335 and MiR-34a induced premature senescence of young mesangial cells via suppression of SOD2 and Txnrd2 with a concomitant increase in ROS (Bai et al. 2011). Furthermore, MiRNA-320a has been found responsive to oxidative stress and involved in the regulation of glycolysis (Tang et al. 2012). Thus, regulation of MiR processing plays an important role in the response to environmental stress and, consequently, stress-related pathologies.

6 MitoRNAs and Mitochondrial Dynamics

Mitochondrial dynamics and turnover are crucial for cellular homeostasis and differentiation. Mitochondria form dynamic networks that are necessary for the maintenance of the organelle fidelity (Chan 2012; Blomain and McMahon 2012). Mitochondrial biogenesis involves the growth and division of pre-existing mitochondria. It requires the coordination of several distinct processes: (1) asymmetric formation of both the inner and outer mitochondrial membrane; (2) synthesis of mitochondrial proteins; (3) synthesis and import of proteins encoded by the nuclear genome; (4) replication of mtDNA; and (5) mitochondrial fusion and fission. Mitochondrial fission requires the activation of the dynamin-related protein-1 (Drp-1) (Frank et al. 2001), a GTPase that causes the scission of the mitochondrial outer membrane, resulting in the fission of mitochondrial tubules into individual fragments. Drp-1 activity is also linked to cytochrome c release and caspase activation (Tanaka and Youle 2008).

Mitochondria are under constant cycles of fission and fusion. If subjected to stress, they may be removed by autophagy, and their biogenesis might, in turn,

increase to meet the energetic demands. The rates of mitochondrial fission and fusion respond to changes in the metabolism, such that mitochondria become 'more fused' when they are forced to rely on OXPHOS by withdrawing glucose as the carbon source. Fusion is also enhanced by the induction of autophagy, increasing the reliance on OXPHOS via enhanced metabolism of lipids and protein. Alternatively, increased fusion may be necessary to maximise the fidelity of OXPHOS by stimulating the complementation among mitochondria. Autophagy may also evoke a specific stress response called 'stress-induced mitochondrial hyperfusion' or it may inhibit fission to protect mitochondria from autophagic catabolism when their function needs to be preserved (Youle and van der Bliek 2012). These effects are consistent with a model in which mitochondrial dynamics helps maximise the capacity for OXPHOS under conditions of stress.

MiRs have been shown to be involved in the regulation of mitochondrial dynamics. MiR-30 family members have been reported to regulate apoptosis by targeting the mitochondrial fission machinery, inhibiting mitochondrial fission by suppressing the expression of p53 and its downstream target Drp-1 (Li et al. 2010). More recently, MiR-494, whose location is predominantly mitochondrial, has been found to modulate mitochondrial biogenesis by down-regulating the mitochondrial transcriptional factor A (TFAM) and the nuclear transcription factor forkhead box J3 (FOXJ3) during myocyte differentiation and skeletal muscle adaptation to physical exercise (Yamamoto et al. 2012).

The removal of damaged mitochondria by autophagy, a process referred to as mitophagy, is critical for maintaining proper cellular functions. Mitophagy regulates the number of mitochondria to match the metabolic or developmental demands (Kundu et al. 2008), and is also a part of the quality control based on the removal of malfunctional mitochondria (Tolkovsky et al. 2002; Kundu et al. 2008; Okamoto et al. 2009). Malfunctional/damaged mitochondria are removed by autophagy, a process requiring two steps: induction of general autophagy and priming of damaged mitochondria for selective recognition. Recent reports reveal that mitochondrial priming is mediated by the PINK1-Parkin signalling pathway (Parkin-dependent) or by the mitophagic receptors Nix and BNIP3 (Parkin-independent). The induction of canonical autophagy requires ATG proteins and, furthermore, it involves mTOR suppression in response to ROS generated by damaged mitochondria and ATP depletion. Upon mitochondrial membrane depolarisation, PINK1 triggers Parkin translocation from the cytosol to mitochondria where it promotes ubiquitination of the MOM proteins, which may either be degraded by the proteasome or serve as binding partners for the p62 protein. This protein then acts as an adaptor molecule directly interacting with the LC3 protein to recruit autophagosomal membranes to the mitochondria. For Parkin-independent mechanism, damaged mitochondria (particularly under hypoxic condition) increase the expression of the FUNDC1 and Nix proteins, which may in turn recruit autophagosomes to mitochondria by direct interaction with LC3. BNIP3 and Nix (upregulated by oxidative stress and/or hypoxia) can induce mitophagy by triggering mitochondrial depolarisation, which is known to induce mitochondrial removal by autophagy (Mazure and Pouyssegur 2010).

Recent studies have suggested a role for MiRs in autophagy, including MiR-101 (Frankel et al. 2011), MiR-204 (Xiao et al. 2011) and MiR-30a (Zhu et al. 2009).

MiRs target transcripts of autophagy-related proteins, thereby suppressing their function in autophagy with pathological consequences (Frankel and Lund 2012). For example, down-regulation of MiR-34b/c is an early event in Parkinson's disease (Miñones-Moyano et al. 2011). In some tumors, the level of MiR-21 is increased, and it suppresses the expression of PTEN, which regulates the mitophagy-associated PINK1 (Zhang et al. 2010).

Mitophagy has been characterised as an HIF-dependent mechanism (Zhang et al. 2008a). Hypoxic repression of the mitochondrial function by MiR-210 and ISCU1/2 may trigger mitophagy. In addition, MiR-126 has been found to be induced by hypoxia and to have a profound effect on cancer cell morphology. Robust formation of autophagic vacuoles was observed in cancer cells over-expressing MiR-126 as a result of their aberrant bioenergetic status (Tomasetti et al. 2014). Ectopic MiR-126 induces the loss of malignancy and the failure of MM cells to induce tumours; these events were not observed in malignant cells lacking beclin-1, a critical regulator of autophagosome formation. These findings have a clinical context, since autophagy is an important process contributing to tumour suppression.

7 MicroRNAs and Mitochondria-Induced Cell Death

Defective mitochondria can be toxic by generating excessive amounts of ROS, by consuming ATP and by interfering with other metabolic processes. Low levels of damage may be corrected by complementation through mitochondrial fusion. Damaged mitochondria will contaminate other mitochondria if they become part of the mitochondrial network unless they are eliminated by autophagy. High levels of ROS can induce apoptosis by affecting mitochondria. Stimulation of the intrinsic mitochondrial apoptotic pathway by ROS and mitochondrial DNA damage promotes the MOM permeabilisation and cytosolic translocation of cytochrome c, the AIF, or Smac/Diablo that trigger/promote caspase-dependent or -independent apoptotic cascade of reactions (Ryter et al. 2007). In caspase-dependent signalling, cytochrome c forms the apoptosome complex that induces cleavage of the down-stream effector caspases. Additionally, Smac/Diablo antagonises the inhibitory effects of the inhibitor of apoptosis proteins, which promotes caspase activation.

AIF mediates caspase-independent signalling through cytosol-to-nuclear translocation and induction of nuclear chromatin condensation and DNA fragmentation (Susin et al. 1999). The mechanism regulating apoptogenic processing and translocation of mitochondrial AIF into the cytoplasm is complex and not fully understood. Permeabilisation of mitochondria, irrespective of whether it occurs before or after AIF cleavage, is an obligatory event in the AIF-mediated apoptotic signalling. Central to mitochondrial permeabilisation and mitochondrial release of apoptogenic factors is the permeability transition pore (PTP), a megapore spanning the inner and outer mitochondrial membrane composed by cyclophilin, VDAC and the adenine nucleotide translocase (Circu and Aw 2010). Mitochondrial apoptogenic factors can be released through the pores in the outer membrane formed by the pro-apoptotic

Bcl-2 family members. Key members of the anti-apoptotic (e.g. Bcl-2, Bcl-x_L, and Mcl-1) and pro-apoptotic (e.g. Bax, Bak, Bad, Bim, and Bid) Bcl-2 family of proteins are major players in the MOM permeabilisation and apoptotic susceptibility (Kelekar and Thompson 1998).

MiR-15a and the MiR-16-1 induce apoptosis by regulating the mitochondrial function by affecting multiple oncogenic activities including those of Bcl-2 and Mcl-1. Furthermore, MiR-15a promotes mitochondrial dysfunction resulting in cytochrome c release into the cytoplasm and the dissipation of the mitochondrial membrane potential ($\Delta\Psi_{m,i}$) (Gao et al. 2010). MiR-210, MiR-181, and the muscle-specific MiR-1 have been reported to be increased upon apoptotic stimulation and found to be associated with the release of cytochrome c from mitochondria and the $\Delta\Psi_{m,i}$ decrease (Chio et al. 2013; Ouyang et al. 2012; Yu et al. 2008). Recently, several MiRs have been demonstrated to regulate the expression of members of the Bcl-2 family. MiR-195, MiR-24-2 and MiR-365-2 act as negative regulators of Bcl-2 by direct binding to their cognate sites in the 3′-UTR of the human Bcl-2 gene. Over-expression of these MiRs induced dissipation of $\Delta\Psi_{m,i}$ and the release of cytochrome c (Singh and Saini 2012). Furthermore, MiR-135a, considerably down-regulated in malignant gliomas and correlated with the pathological grading of the neoplasia, is capable of inducing mitochondria-dependent apoptosis of malignant gliomas by regulating various genes including STAT6, SMAD5 and BMPR2, as well as affecting the down-stream signalling events (Wu et al. 2012).

Several MiRs inhibit apoptosis by exerting a protective role. MiR-145 protects from the activation of the mitochondrial apoptotic pathways in cardiomyocytes under oxidative stress by direct targeting of the BH3-only proteins such as BNIP3. This protein primarily localising to the MOM functions not only as a sensor of mitochondria to oxidative stress in the cytoplasm, but also as an effector of mitochondria-mediated apoptosis. BNIP3 transduces the apoptotic signals via the activation of pro-apoptotic Bax/Bak proteins, neutralising the anti-apoptotic BH1-4 proteins, and promoting mitochondrial membrane depolarisation by inducing the formation of the detrimental PTP (Li et al. 2012). In addition, ectopic expression of MiR-125b partially restored cell viability and inhibited apoptosis induced by temozolomide and camptothecin (CPT), which are promising chemotherapeutic agent for glioblastomas (Shi et al. 2012; Zeng et al. 2012). It has been demonstrated that CPT induces apoptosis in cancer cells by MiR-125b-mediated mitochondrial pathways via targeting the 3′-UTRs of Bak1, Mcl-1, and p53 (Zeng et al. 2012). These data clearly document the regulatory role of MiRs in apoptosis modulation, both positive and negative.

8 Conclusions

MiRs play a critical role in regulating the mitochondrial function under physiological and pathological conditions. Pre-MiRs and MiRs have been found in human mitochondria, implication their regulatory role in the mitochondrial function and integrity.

Table 1 MicroRNAs involved in mitochondrial function

ATP level	Target	Reference
MiR-15	ARL2	Nishi et al. (2010)
MiR-16	ARL2	Nishi et al. (2010)
MiR-195	ARL2	Nishi et al. (2010)
MiR-338	COX IV	Aschrafi et al. (2008)
MiR-424	ARL2	Nishi et al. (2010)
Mitochondrial metabolism		
MiR-23a/b	GLS	Gao et al. (2009)
MiR-210	ETC components	Favaro et al. (2010), Chan et al. (2012b)
MiR-126	IRS-1	Zhang et al. (2008b), Ryu et al. (2011)
MiR-378	PGC1β	Carrer et al. (2012)
Inhibition of mitochondrial ROS		
MiR-34a	SOD2/Txnrd2	Bai et al. (2011)
MiR-126	CAT/SOD2	Tomasetti et al. (2014)
MiR-145	Bnip3	Li et al. (2012)
MiR-335	SOD2/Txnrd2	Bai et al. (2011)
Mitochondria dynamics		
MiR-30	Mitochondrial fusion	Li et al. (2010)
MiR-494	Mitochondrial biogenesis	Yamamoto et al. (2012)
Mitophagy		
MiR-21	PINK1	Zhang et al. (2010)
MiR-30a		Zhu et al. (2009)
MiR-101		Frankel et al. (2011)
MiR-126		Tomasetti et al. (2014)
MiR-204		Xiao et al. (2011)
Apoptosis		
MiR-1	Cytochrome c release	Yu et al. (2008)
MiR-15a	Bcl-2/Mcl-1	Gao et al. (2010)
MiR-16-1	Bcl-2/Mcl-1	Gao et al. (2010)
MiR-24	Bcl-2	Singh and Saini (2012)
MiR-181	Bcl-2/cytochrome c release	Ouyang et al. (2012)
MiR-195	Bcl-2	Singh and Saini (2012)
MiR-210	Bcl-2/cytochrome c release	Chio et al. (2013)
MiR-365	Bcl-2	Singh and Saini (2012)

ARL2 ADP-ribosylation factor-like 2, *COX IV* cytochrome c oxidase IV, *GLS* mitochondrial glutaminase, *ETC* electron transport chain, *PGC1β* peroxisome proliferator activated receptor-γ co-activator 1, *IRS-1* insulin receptor substrate-1, *Txnrd2* thioredoxin reductase 2, *SOD2* superoxide dismutase, *CAT* catalase

Table 1 gives examples of MiRs that have been detected in mitochondria and, where known, their interacting partner that regulated. As obvious, MiRs modulate a number of diverse processes, including the ATP level, mitochondrial metabolism, modulation of mitochondrial ROS generation, dynamics of the organelles, and mitophagy as well as apoptosis. How MiRs affect the mitochondrial function, in particular when targeted to mitochondria (probably via their association with AGO2 and other,

thus far unknown chaperones) is still obscure. Neither it is clear whether MiRs bind to 3′UTR of mitochondrial transcripts and, ultimately, whether mtDNA codes for mitochondria-specific MiRs. Notwithstanding the limited knowledge, it is clear that MiRs exert, via affecting the mitochondrial function and integrity, a modulatory role in a variety of pathologies, both positive and negative. The translational importance of MiRs, for example, in the context of cancer, can be reconciled with recent findings of unexpected differences in the mutational patterns of the same type of tumours in different patients (Hayden 2008) and even in individual regions of the same tumour (Gerlinger et al. 2012). The unprecedented heterogeneity of tumours makes it rather unlikely to treat cancer on a wider scope with agents that act via a single target or a single signalling pathway. MicroRNAs with their multiple targets affecting mitochondria appear promising vehicles that may be employed for efficient cancer therapy, although whether this approach will be practically applicable is yet to be uncovered. Since mitochondria are implicated critically in a variety of pathologies, ranging from neoplastic diseases through neurodegenerative diseases, to cardiovascular pathologies, their potential use is obvious.

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References

Aschrafi A, Schwechter AD, Mameza MG, Natera-Naranjo O, Gioio AE, Kaplan BB (2008) MicroRNA-338 regulates local cytochrome c oxidase IV mRNA levels and oxidative phosphorylation in the axons of sympathetic neurons. J Neurosci 28:12581–12590

Baggetto LG (1992) Deviant energetic metabolism of glycolytic cancer cells. Biochimie 174:959–974

Bai XY, Ma Y, Ding R, Fu B, Shi S, Chen XM (2011) miR-335 and miR-34a Promote renal senescence by suppressing mitochondrial antioxidative enzymes. J Am Soc Nephrol 22:1252–1261

Bandiera S, Rüberg S, Girard M, Cagnard N, Hanein S, Chrétien D, Munnich A, Lyonnet S, Henrion-Caude A (2011) Nuclear outsourcing of RNA interference components to human mitochondria. PLoS One 6:e20746

Barrey E, Saint-Auret G, Bonnamy B, Damas D, Boyer O, Gidrol X (2011) Pre-microRNA and mature microRNA in human mitochondria. PLoS One 6:e20220

Barth E, Stämmler G, Speiser B, Schaper J (1992) Ultrastructural quantitation of mitochondria and myofilaments in cardiac muscle from 10 different animal species including man. J Mol Cell Cardiol 24:669–681

Bauer DE, Hatzivassiliou G, Zhao F, Andreadis C, Thompson CB (2005) ATP citrate lyase is an important component of cell growth and transformation. Oncogene 24:6314–6322

Bian Z, Li LM, Tang R, Hou DX, Chen X, Zhang CY, Zen K (2010) Identification of mouse liver mitochondria-associated miRNAs and their potential biological functions. Cell Res 20:1076–1078

Blomain ES, McMahon SB (2012) Dynamic regulation of mitochondrial transcription as a mechanism of cellular adaptation. Biochim Biophys Acta 1819:1075–1079

Carrer M, Liu N, Grueter CE, Williams AH, Frisard MI, Hulver MW, Bassel-Duby R, Olson EN (2012) Control of mitochondrial metabolism and systemic energy homeostasis by microRNAs 378 and 378. Proc Natl Acad Sci U S A 109:15330–15335

Chan DC (2012) Fusion and fission: interlinked processes critical for mitochondrial health. Annu Rev Genet 46:265–287

Chan YC, Banerjee J, Choi SY, Sen CK (2012a) miR-210: the master hypoxamir. Microcirculation 19:215–223

Chan SY, Zhang YY, Hemann C, Mahoney CE, Zweier JL, Loscalzo J (2012b) MicroRNA-210 controls mitochondrial metabolism during hypoxia by repressing the iron-sulfur cluster assembly proteins ISCU1/2. Cell Metab 10:273–284

Chaudhary KR, El-Sikhry H, Seubert JM (2011) Mitochondria and the aging heart. J Geriatr Cardiol 8:159–167

Chio CC, Lin JW, Cheng HA, Chiu WT, Wang YH, Wang JJ, Hsing CH, Chen RM (2013) MicroRNA-210 targets antiapoptotic Bcl-2 expression and mediates hypoxia-induced apoptosis of neuroblastoma cells. Arch Toxicol 87(3):459–468

Circu ML, Aw TY (2010) Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med 48:749–762

Dang CV (2012) Links between metabolism and cancer. Genes Dev 26:877–890

Das S, Ferlito M, Kent OA, Fox-Talbot K, Wang R, Liu D, Raghavachari N, Yang Y, Wheelan SJ, Murphy E, Steenbergen C (2012) Nuclear miRNA regulates the mitochondrial genome in the heart. Circ Res 110:1596–1603

Dehne N, Brüne B (2014) Sensors, transmitters, and targets in mitochondrial oxygen shortage-hypoxia-inducible factor relay story. Antioxid Redox Signal 20(2):339–352

Detmer SA, Chan DC (2007) Functions and dysfunctions of mitochondrial dynamics. Nat Rev Mol Cell Biol 8:870–879

Dong XC, Copps KD, Guo S, Li Y, Kollipara R, DePinho RA, White MF (2008) Inactivation of hepatic Foxo1 by insulin signaling is required for adaptive nutrient homeostasis and endocrine growth regulation. Cell Metab 8:65–76

Eichner LJ, Perry MC, Dufour CR, Bertos N, Park M, St Pierre J, Giguere V (2012) MiR-378(*) mediates metabolic shift in breast cancer cells via the PGC-1β/ERRγ transcriptional pathway. Cell Metab 12:352–361

Fabian MR, Sonenberg N, Filipowicz W (2010) Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem 79:351–379

Favaro E, Ramachandran A, McCormick R, Gee H, Blancher C, Crosby M, Devlin C, Blick C, Buffa F, Li JL, Vojnovic B, Pires das Neves R, Glazer P, Iborra F, Ivan M, Ragoussis J, Harris AL (2010) MicroRNA-210 regulates mitochondrial free radical response to hypoxia and Krebs cycle in cancer cells by targeting iron sulfur cluster protein ISCU. PLoS One 5:e10345

Finkel T (2012) Signal transduction by mitochondrial oxidants. J Biol Chem 287:4434–4440

Frank S, Gaume B, Bergmann-Leitner ES, Leitner WW, Robert EG, Catez F, Smith CL, Youle RJ (2001) The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. Dev Cell 1:515–525

Frankel LB, Lund AH (2012) MicroRNA regulation of autophagy. Carcinogenesis 33:2018–2025

Frankel LB, Wen J, Lees M, Høyer-Hansen M, Farkas T, Krogh A, Jäättelä M, Lund AH (2011) microRNA-101 is a potent inhibitor of autophagy. EMBO J 30:4628–4641

Galloway CA, Yoon Y (2013) Mitochondrial morphology in metabolic diseases. Antioxid Redox Signal 19(4):415–430

Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT, Dang CV (2009) c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature 458:762–765

Gao SM, Chen C, Wu J, Tan Y, Yu K, Xing CY, Ye A, Yin L, Jiang L (2010) Synergistic apoptosis induction in leukemic cells by miR-15a/16-1 and arsenic trioxide. Biochem Biophys Res Commun 403:203–208

Garcia-Roves PM (2011) Mitochondrial pathophysiology and type 2 diabetes mellitus. Arch Physiol Biochem 117:177–187

Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, Martinez P, Matthews N, Stewart A, Tarpey P, Varela I, Phillimore B, Begum S, McDonald NQ, Butler A,

Jones D, Raine K, Latimer C, Santos CR, Nohadani M, Eklund AC, Spencer-Dene B, Clark G, Pickering L, Stamp G, Gore M, Szallasi Z, Downward J, Futreal PA, Swanton C (2012) Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 366:883–892

Giannakakis A, Sandaltzopoulos R, Greshock J, Liang S, Huang J, Hasegawa K, Li C, O'Brien-Jenkins A, Katsaros D, Weber BL, Simon C, Coukos G, Zhang L (2008) miR-210 links hypoxia with cell cycle regulation and is deleted in human epithelial ovarian cancer. Cancer Biol Ther 7:255–264

Gibbings DJ, Ciaudo C, Erhardt M, Voinnet O (2009) Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. Nat Cell Biol 11:1143–1149

Gong Y, Chai Y, Ding JH, Sun XL, Hu G (2011) Chronic mild stress damages mitochondrial ultrastructure and function in mouse brain. Neurosci Lett 488:76–80

Grzybowska-Szatkowska L, Slaska B (2012) Mitochondrial DNA and carcinogenesis. Mol Med Rep 6:923–930

Guo S, Copps KD, Dong X, Park S, Cheng Z, Pocai A, Rossetti L, Sajan M, Farese RV, White MF (2009) The Irs1 branch of the insulin signaling cascade plays a dominant role in hepatic nutrient homeostasis. Mol Cell Biol 29:5070–5083

Guzy RD, Hoyos B, Robin E, Chen H, Liu L, Mansfield KD, Simon MC, Hammerling U, Schumacker PT (2005) Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. Cell Metab 1:401–408

Guzy RD, Sharma B, Bell E, Chandel NS, Schumacker PT (2008) Loss of the SdhB, but not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. Mol Cell Biol 28:718–731

Haque R, Chun E, Howell JC, Sengupta T, Chen D, Kim H (2012) MicroRNA-30b-mediated regulation of catalase expression in human ARPE-19 cells. PLoS One 7:e42542

Hatzivassiliou G, Zhao F, Bauer DE, Andreadis C, Shaw AN, Dhanak D, Hingorani SR, Tuveson DA, Thompson CB (2005) ATP citrate lyase inhibition can suppress tumor cell growth. Cancer Cell 8:311–321

Hauser DN, Hastings TG (2013) Mitochondrial dysfunction and oxidative stress in Parkinson's disease and monogenic parkinsonism. Neurobiol Dis 51:35–42

Hayden EC (2008) Cancer complexity slows quest for cure. Nature 455:148

Huang X, Le QT, Giaccia AJ (2010) MiR-210-micromanager of the hypoxia pathway. Trends Mol Med 16:230–237

Hwang HW, Wentzel EA, Mendell JT (2007) A hexanucleotide element directs microRNA nuclear import. Science 315:97–100

Ishii T, Yasuda K, Akatsuka A, Hino O, Hartman PS, Ishii N (2005) A mutation in the SDHC gene of complex II increases oxidative stress, resulting in apoptosis and tumorigenesis. Cancer Res 65:203–209

Kelekar A, Thompson CB (1998) Bcl-2-family proteins: the role of the BH3 domain in apoptosis. Trends Cell Biol 8:324–330

Ketting RF (2011) The many faces of RNAi. Dev Cell 20:148–161

Kilbride SM, Prehn JH (2013) Central roles of apoptotic proteins in mitochondrial function. Oncogene 32(22):2703–2711

Kim VN (2009) Posttranscriptional crossregulation between Drosha and DGCR8. Cell 136:75–84

Kim YK, Kim VN (2007) Processing of intronic microRNAs. EMBO J 26:775–783

Kim J, Wei Y, Sowers JR (2008) Role of mitochondrial dysfunction in insulin resistance. Circ Res 102:101–414

Klimova T, Chandel NS (2008) Mitochondrial complex III regulates hypoxic activation of HIF. Cell Death Differ 15:660–666

Kren BT, Wong PY, Sarver A, Zhang X, Zeng Y, Steer CJ (2009) MicroRNAs identified in highly purified liver-derived mitochondria may play a role in apoptosis. RNA Biol 6:65–72

Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agosto-Perez FJ, Davuluri R, Liu CG, Croce CM, Negrini M, Calin GA, Ivan M (2007) A microRNA signature of hypoxia. Mol Cell Biol 27:1859–1867

Kundu M, Lindsten T, Yang CY, Wu J, Zhao F, Zhang J, Selak MA, Ney PA, Thompson CB (2008) Ulk1 plays a critical role in the autophagic clearance of mitochondria and ribosomes during reticulocyte maturation. Blood 112:1493–1502

Leung AK, Calabrese JM, Sharp PA (2006) Quantitative analysis of Argonaute protein reveals microRNA-dependent localization to stress granules. Proc Natl Acad Sci U S A 103:18125–18130

Li J, Donath S, Li Y, Qin D, Prabhakar BS, Li P (2010) miR-30 regulates mitochondrial fission through targeting p53 and the dynamin-related protein-1 pathway. PLoS Genet 6:e1000795

Li N, Bates DJ, An J, Terry DA, Wang E (2011) Up-regulation of key microRNAs, and inverse down-regulation of their predicted oxidative phosphorylation target genes, during aging in mouse brain. Neurobiol Aging 32:944–955

Li R, Yan G, Li Q, Sun H, Hu Y, Sun J, Xu B (2012) MicroRNA-145 protects cardiomyocytes against hydrogen peroxide-induced apoptosis through targeting the mitochondria apoptotic pathway. PLoS One 7:e44907

Liao JY, Ma LM, Guo YH, Zhang YC, Zhou H, Shao P, Chen YQ, Qu LH (2010) Deep sequencing of human nuclear and cytoplasmic small RNAs reveals an unexpectedly complex subcellular distribution of miRNAs and tRNA 3′ trailers. PLoS One 5:e10563

Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song JJ, Hammond SM, Joshua-Tor L, Hannon GJ (2004) Argonaute2 is the catalytic engine of mammalian RNAi. Science 305:1437–1441

Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R (2005) MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. Nat Cell Biol 7:719–723

Maniataki E, Mourelatos Z (2005) Human mitochondrial $tRNA^{Met}$ is exported to the cytoplasm and associates with the Argonaute 2 protein. RNA 11:849–852

Mansfield KD, Guzy RD, Pan Y, Young RM, Cash TP, Schumacker PT, Simon MC (2005) Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-α activation. Cell Metab 1:393–399

Mazure NM, Pouyssegur J (2010) Hypoxia-induced autophagy: cell death or cell survival? Curr Opin Cell Biol 22:177–180

Mercer TR, Neph S, Dinger ME, Crawford J, Smith MA, Shearwood AM, Haugen E, Bracken CP, Rackham O, Stamatoyannopoulos JA, Filipovska A, Mattick JS (2011) The human mitochondrial transcriptome. Cell 146:645–658

Miñones-Moyano E, Porta S, Escaramís G, Rabionet R, Iraola S, Kagerbauer B, Espinosa-Parrilla Y, Ferrer I, Estivill X, Martí E (2011) MicroRNA profiling of Parkinson's disease brains identifies early downregulation of miR-34b/c which modulate mitochondrial function. Hum Mol Genet 20:3067–3078

Morino K, Petersen KF, Dufour S, Befroy D, Frattini J, Shatzkes N, Neschen S, White MF, Bilz S, Sono S, Pypaert M, Shulman GI (2005) Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. J Clin Invest 115:3587–3593

Morlando M, Ballarino M, Gromak N, Pagano F, Bozzoni I, Proudfoot NJ (2008) Primary microRNA transcripts are processed co-transcriptionally. Nat Struct Mol Biol 15:902–909

Näär AM (2011) MiRs with a sweet tooth. Cell Metab 14:149–150

Nishi H, Ono K, Iwanaga Y, Horie T, Nagao K, Takemura G, Kinoshita M, Kuwabara Y, Mori RT, Hasegawa K, Kita T, Kimura T (2010) MicroRNA-15b modulates cellular ATP levels and degenerates mitochondria via Arl2 in neonatal rat cardiac myocytes. J Biol Chem 285:4920–4930

Ohrt T, Mutze J, Staroske W, Weinmann L, Hock J, Crell K, Meister G, Schwille P (2008) Fluorescence correlation spectroscopy and fluorescence cross-correlation spectroscopy reveal

the cytoplasmic origination of loaded nuclear RISC in vivo in human cells. Nucleic Acids Res 36:6439–6449

Okamoto K, Kondo-Okamoto N, Ohsumi Y (2009) Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. Dev Cell 17:87–97

Ouyang YB, Lu Y, Yue S, Giffard RG (2012) miR-181 targets multiple Bcl-2 family members and influences apoptosis and mitochondrial function in astrocytes. Mitochondrion 12:213–219

Pothof J, Verkaik NS, van Ijcken W, Wiemer EA, Ta VT, van der Horst GT, Jaspers NG, van Gent DC, Hoeijmakers JH, Persengiev SP (2009) MicroRNA-mediated gene silencing modulates the UV-induced DNA-damage response. EMBO J 28:2090–2099

Puisségur MP, Mazure NM, Bertero T, Pradelli L, Grosso S, Robbe-Sermesant K, Maurin T, Lebrigand K, Cardinaud B, Hofman V, Fourre S, Magnone V, Ricci JE, Pouysségur J, Gounon P, Hofman P, Barbry P, Mari B (2011) miR-210 is overexpressed in late stages of lung cancer and mediates mitochondrial alterations associated with modulation of HIF-1 activity. Cell Death Differ 18:465–478

Rabøl R, Højberg PM, Almdal T, Boushel R, Haugaard SB, Madsbad S, Dela F (2009) Effect of hyperglycemia on mitochondrial respiration in type 2 diabetes. J Clin Endocrinol Metab 94:1372–1378

Ren J, Pulakat L, Whaley-Connell A, Sowers JR (2010) Mitochondrial biogenesis in the metabolic syndrome and cardiovascular disease. J Mol Med 88:993–1001

Rottiers V, Näär AM (2012) MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol 13:239–250

Ryter SW, Kim HP, Hoetzel A, Park JW, Nakahira K, Wang X, Choi AM (2007) Mechanisms of cell death in oxidative stress. Antioxid Redox Signal 9:49–89

Ryu HS, Park SY, Ma D, Zhang J, Lee W (2011) The induction of microRNA targeting IRS-1 is involved in the development of insulin resistance under conditions of mitochondrial dysfunction in hepatocytes. PLoS One 6:e17343

Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E (2005) Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-α prolyl hydroxylase. Cancer Cell 7:77–85

Semenza G (2007) Oxygen-dependent regulation of mitochondrial respiration by hypoxia-inducible factor 1. Biochem J 405:1–9

Shi L, Zhang S, Feng K, Wu F, Wan Y, Wang Z, Zhang J, Wang Y, Yan W, Fu Z, You Y (2012) MicroRNA-125b-2 confers human glioblastoma stem cells resistance to temozolomide through the mitochondrial pathway of apoptosis. Int J Oncol 40:119–129

Singh R, Saini N (2012) Downregulation of BCL2 by miRNAs augments drug-induced apoptosis - a combined computational and experimental approach. J Cell Sci 125:1568–1578

Siomi H, Siomi MC (2010) Posttranscriptional regulation of microRNA biogenesis in animals. Mol Cell 38:323–332

Suen DF, Norris KL, Youle RJ (2008) Mitochondrial dynamics and apoptosis. Genes Dev 22:1577–1590

Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G (1999) Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 397:441–446

Tanaka A, Youle RJ (2008) A chemical inhibitor of DRP1 uncouples mitochondrial fission and apoptosis. Mol Cell 29:409–410

Tang H, Lee M, Sharpe O, Salamone L, Noonan EJ, Hoang CD, Levine S, Robinson WH, Shrager JB (2012) Oxidative stress-responsive microRNA-320 regulates glycolysis in diverse biological systems. FASEB J 26:4710–4721

Tolkovsky AM, Xue L, Fletcher GC, Borutaite V (2002) Mitochondrial disappearance from cells: a clue to the role of autophagy in programmed cell death and disease? Biochimie 84:233–240

Tomasetti M, Nocchi L, Staffolani S, Manzella N, Amati M, Goodwin J, Nguyen M, Kluckova K, Bajzikova M, Sobol M, Hozak P, Lettlova S, Truksa J, Lee W, Dong L, Santarelli L, Neuzil J (2014) MicroRNA-126 suppresses mesothelioma malignancy by targeting IRS1 and interfering with mitochondrial function. Antioxid Redox Signal

Tothova Z, Gilliland DG (2007) FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system. Cell Stem Cell 1:140–152

Valis K, Prochazka L, Boura E, Chladova M, Obsil T, Rohlena J, Truksa J, Dong LF, Ralph SJ, Neuzil J (2011) Hippo/Mst1 stimulates transcription of *NOXA* in a FoxO1-dependent manner. Cancer Res 71:946–954

von Brandenstein M, Richter C, Fries JW (2012) MicroRNAs: small but amazing, and their association with endothelin. Life Sci 91:475–489

Ward PS, Thompson CB (2012) Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. Cancer Cell 21:297–308

Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB, Thompson CB (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci U S A 105:18782–18787

Wu S, Lin Y, Xu D, Chen J, Shu M, Zhou Y, Zhu W, Su X, Zhou Y, Qiu P, Yan G (2012) MiR-135a functions as a selective killer of malignant glioma. Oncogene 31:3866–3874

Xiao J, Zhu X, He B, Zhang Y, Kang B, Wang Z, Ni X (2011) MiR-204 regulates cardiomyocyte autophagy induced by ischemia-reperfusion through LC3-II. J Biomed Sci 18:35

Yamamoto H, Morino K, Nishio Y, Ugi S, Yoshizaki T, Kashiwagi A, Maegawa H (2012) MicroRNA-494 regulates mitochondrial biogenesis in skeletal muscle through mitochondrial transcriptional factor A and forkhead box j3. Am J Physiol 303:E1419–E1427

Yang J, Lan H, Huang X, Liu B, Tong Y (2012) MicroRNA-126 inhibits tumor cell growth and its expression level correlates with poor survival in non-small cell lung cancer patients. PLoS One 7:e42978

Yin F, Boveris A, Cadenas E (2014) Mitochondrial energy metabolism and redox signaling in brain aging and neurodegeneration. Antioxid Redox Signal 20(2):353–371

Youle RJ, van der Bliek AM (2012) Mitochondrial fission, fusion, and stress. Science 337:1062–1065

Yu XY, Song YH, Geng YJ, Lin QX, Shan ZX, Lin SG, Li Y (2008) Glucose induces apoptosis of cardiomyocytes via microRNA-1 and IGF-1. Biochem Biophys Res Commun 376:548–552

Zeng CW, Zhang XJ, Lin KY, Ye H, Feng SY, Zhang H, Chen YQ (2012) Camptothecin induces apoptosis in cancer cells via microRNA-125b-mediated mitochondrial pathways. Mol Pharmacol 81:578–586

Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL (2008a) Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem 283:10892–10903

Zhang J, Du YY, Lin YF, Chen YT, Yang L, Wang HJ, Ma D (2008b) The cell growth suppressor, mir-126, targets IRS-1. Biochem Biophys Res Commun 377:136–140

Zhang JG, Wang JJ, Zhao F, Liu Q, Jiang K, Yang GH (2010) MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC). Clin Chim Acta 411:846–852

Zhang X, Tang N, Hadden TJ, Rishi AK (2011) Akt, FoxO and regulation of apoptosis. Biochim Biophys Acta 1813:1978–1986

Zhu H, Wu H, Liu X, Li B, Chen Y, Ren X, Liu CG, Yang JM (2009) Regulation of autophagy by a beclin 1-targeted microRNA, miR-30a, in cancer cells. Autophagy 5:816–823

Zhuang G, Wu X, Jiang Z, Kasman I, Yao J, Guan Y, Oeh J, Modrusan Z, Bais C, Sampath D, Ferrara N (2012) Tumour-secreted miR-9 promotes endothelial cell migration and angiogenesis by activating the JAK-STAT pathway. EMBO J 31:3513–3523

Chapter 4
Mitochondrial Complex II in Cancer

Katarina Kluckova, Jiri Neuzil, and Jakub Rohlena

Contents

A new crucial paper appeared since the time of submission.

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Abstract The mitochondrial connection in cancer has long been suspected, but the first unequivocal genetic link was established only when hereditary paraganglioma-associated mutations were found in genes coding for the subunits of mitochondrial complex II. In this chapter we provide an overview of current knowledge concerning the involvement of complex II in cancer, and discuss molecular mechanisms that may contribute to complex II-mediated tumourigenesis.

Keywords Mitochondria • Cancer • Complex II • Succinate-coenzyme Q oxidoreductase • Metabolism • Oxidative phosphorylation • Succinate • 2-oxoglutarate • Reactive oxygen species • Citric acid cycle • Mitochondrial inner membrane • Apoptosis • Pseudohypoxia • Paraganglioma • Pheochromocytoma

1 Mitochondria and Cancer

Mitochondria play a crucial role in cell survival, providing energy and metabolites for the maintenance and growth of a healthy cell. At the same time, mitochondria function as executors of cell death and upon various stress stimuli or signalling cues initiate apoptosis, a prerequisite for an effective defence against uncontrolled cell proliferation. Given this protean role in both proliferation and cell death, it is perhaps not surprising that mitochondria participate in the development of diseases such as neural degeneration and cancer that are characterised by the deregulation of those two fundamental processes. With respect to cancer, the role for mitochondria in tumourigenesis has been suggested a long time ago, but the identification of the first cancer-associated mitochondrial gene is of a relatively recent date (Baysal et al. 2000). The gene in question is one of the subunits of the mitochondrial respiratory complex II (CII), which together with four other macromolecular protein complexes constitutes the oxidative phosphorylation (OXPHOS) system in the mitochondrial inner membrane (MIM). The investigations into the role of CII in cancer provide a good illustration of the current state of affairs concerning the involvement of mitochondrial and metabolic alterations in cancer development; we know that there is ‘something going on’, but have not yet been able to pinpoint the aspects most relevant for cancer development.

2 Complex II Links Tricarboxylic Acid Cycle and Oxidative Phosphorylation

The function of OXPHOS is critically dependent on the tricarboxylic acid cycle (TCA), a complex system of reactions in the mitochondrial matrix in which pyruvate imported into mitochondria from the glycolytic pathway is degraded, producing reducing equivalents NADH and NADPH, as well as GTP. NADH, which enters OXPHOS via CI, provides electrons that serve to reduce molecular oxygen to water in a multistage process involving the respiratory complexes CI, CIII and CIV. As the electrons travel through OXPHOS via the electron carriers ubiquinone (UbQ) and cytochrome c, CI, III and IV pump protons from the matrix to the inter-membrane space, creating electrostatic and pH gradient which is utilised by CV to produce ATP, the major energy 'currency' of the cell.

Within this general scheme, CII is exceptional for several reasons. First, it directly ties TCA to OXPHOS without the usual NADH intermediary (Fig. 4.1). This is due to the fact that CII possesses two distinct enzymatic activities: it oxidises succinate to fumarate in the TCA which is referred to as the succinate

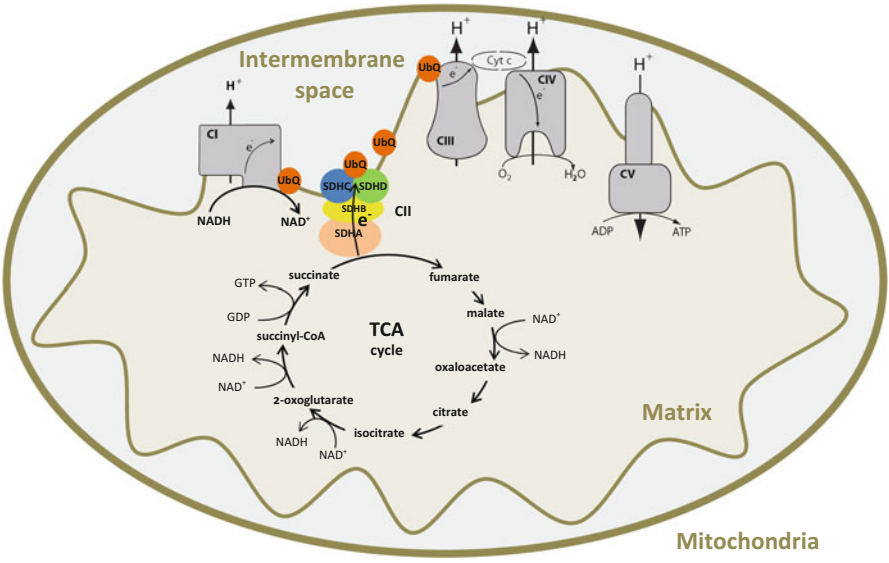

Fig. 4.1 Complex II on the interface between the tricarboxylic acid cycle and oxidative phosphorylation. CII removes electrons from succinate and transfers them via its four protein subunits to ubiquinone, where they are pooled with those originating at CI and further shuttled by CIII, cytochrome c (cyt c) and CIV to molecular oxygen. Unlike other respiratory complexes, CII contributes only indirectly to the proton gradient which is maintained on the inner mitochondrial membrane and allows CV to form ATP

dehydrogenase (SDH) activity (giving CII its alternative name, SDH), and reduces UbQ to ubiquinol, by means of the succinate-coenzyme Q oxidoreductase (SQR) activity, a quintessential OXPHOS reaction. Second, CII, the smallest of respiratory complexes, possesses only four protein subunits, known as SDHA, SDHB, SDHC and SDHD, all of them nuclear-encoded and therefore subject to inheritance pattern typical for nuclear genes. All other OXPHOS complexes contain at least one mitochondrially-encoded gene. Third, unlike other OXPHOS complexes, CII does not truly span the whole MIM, and does not transport protons to the intermembrane space. Instead, electrons originating at CII contribute to the proton pumping activity of CIII and CIV. As mentioned above, the reaction catalysed by CII is complex and requires specific participation of all CII subunits at different stages of the process. The matrix-exposed active site for the succinate to fumarate conversion, the only membrane-associated TCA activity, resides in the SDHA subunit. The catalysis is mediated by the flavine prosthetic group (FAD), and the electrons are subsequently handed over to iron sulfur clusters present in the subunit SDHB. The [2Fe-2S], [4Fe-4S] and [3Fe-4S] clusters channel the electrons to subunits C and D inserted in the MIM, which together form the UbQ-binding sites, where the electrons are released to UbQ and transferred further to CIII. Of the two UbQ-binding sites found in the crystal structure of porcine CII (Sun et al. 2005), only the proximal site (the one closer to the mitochondrial matrix and the [3Fe-4S] cluster) appears to be functional, whereas the distal site may be a non-functional pseudosite (Maklashina and Cecchini 2010). All in all, CII serves a vital function connecting TCA and OXPHOS by providing a direct link unparalleled by any other respiratory complex or TCA enzyme.

3 Role of Complex II in Disease

Being wholly encoded by nuclear DNA, in the field of mitochondrial research CII has been considered as somewhat ‘uninteresting’, ‘housekeeping’ gene. However, in 1995 the first disease-associated mutation was identified in the SDHA subunit of CII in sibling patients suffering from the neurodegenerative Leigh syndrome (Bourgeron et al. 1995), and in 2000 first association with cancer was found for the SDHD subunit (Baysal et al. 2000). This indicated that CII may play an active role in disease development, in particular for neurodegenerative diseases and cancer, and stimulated research into the mechanisms driving these associations. These aspects of CII biology have been summarised recently in comprehensive reviews (Bardella et al. 2011b; Hoekstra and Bayley 2013), and we will provide a short update below.

3.1 Neurodegenerative Diseases and Myopathies

Leigh syndrome is a neurodegenerative disorder resulting from incorrect function of the mitochondrial OXPHOS. Genetically, it is a heterogeneous disease associated with defects in all respiratory complexes, including CV (DiMauro and Schon 2003).

Leigh syndrome-associated CII mutations are found exclusively in the *SDHA* gene and account for only about 2 % of all cases. The mutations are either homozygous, often in siblings of consanguineous parents, or involving two heterozygous mutations that result in a compound loss of the SDHA subunit (Bourgeron et al. 1995; Parfait et al. 2000). The clinical presentation includes bilateral lesions in deep grey matter including rostral spinal cord, the brain stem, thalamus, cerebellum, and the basal ganglia. The peripheral skeletal nervous system is also frequently affected. The clinical neurological symptoms initially consist of hypotonia and psychomotor regression, followed by variable onset of dystonia, involuntary movements, ataxia, and eventually quadriparetic spasticity, accompanied by general symptoms that include failure to thrive and recurrent vomiting, the latter probably related to lactic acidosis (Uziel et al. 2011). Besides Leigh syndrome, *SDHA* mutations can also lead to other degenerative disorders such as cardiomyopathies, and an *SDHB* mutation was reported in a leukodystrophy case (reviewed in Hoekstra and Bayley 2013).

3.2 Cancer

3.2.1 Paraganglioma/Pheochromocytoma Syndrome

The function of CII as a tumour suppressor has come to light when the first germline mutations in *SDHD* (Astuti et al. 2001a; Baysal et al. 2000) and *SDHC* (Niemann and Muller 2000) have been reported in families with hereditary paragangliomas or pheochromocytomas (PGL/PHEO), followed by mutations in *SDHB* (Astuti et al. 2001b). Surprisingly, no disease association between *SDHA* and PGL/PHEO has been found until 2009, when a mutation in a gene now known as *SDH5* or *SDHAF2* and required for flavination of SDHA was reported (Hao et al. 2009), shortly followed by the identification of mutations in *SDHA* itself (Burnichon et al. 2010). Owing to these discoveries, PGL/PHEOs are the best known tumours associated with CII mutations. Initially, 3 PGL/PHEO susceptibility loci were identified and dubbed PGL1-3. As the responsible genes were discovered, PGL1 was ascribed to mutations in *SDHD*, PGL2 to mutations in the assembly factor *SDHAF2* and PGL3 to mutations in *SDHC*. Mutations in *SDHB* were associated with paraganglioma type 4 (Bardella et al. 2011b; Opocher and Schiavi 2011).

PGL/PHEO is the malignancy of the neural crest lineage, and derives from either parasympathetic or sympathetic nervous system. Parasympathetic nervous system usually produces paragangliomas in the head and neck region from carotid bodies, along the vagus nerve, in the jugular foramen and in the middle ear space. These tumours are often referred to as head and neck paragangliomas, and generally do not hypersecrete catecholamines or other hormones. Sympathetic nervous system can produce pheochromocytomas originating from chromaffin cells of the adrenal medulla, or extra-adrenal paragangliomas originating from extra-adrenal sympathetic tissue confined to the abdomen, thorax and pelvis. The sympathetic nervous system-derived tumours typically secrete catecholamines, and extra adrenal paragangliomas have higher risk of metastases (Bardella et al. 2011b; Fishbein and

Nathanson 2012). PGL/PHEO are quite rare, occurring in two to eight people per million, and equally in men and women. At least 25 % are familial and inherited in an autosomal-dominant pattern, which makes PGL/PHEO the cancer most strongly associated with inherited germ line mutations (Fishbein and Nathanson 2012; Kantorovich and Pacak 2010).

Apart from CII-related genes, mutations in *VHL* (von Hippel-Lindau), *NF1* (neurofibromin 1), *RET* (Ret proto-oncogene), *TMEM127* (transmembrane protein 127) and *MAX* (myc-associated factor X) are associated with PGL/PHEO (reviewed in Fishbein and Nathanson 2012). In addition, PGL/PHEO-associated germline mutations were also found in *KIF1Bβ* (kinesin family member 1Bβ) (Schlisio et al. 2008; Yeh et al. 2008) and *EGLN1* (Egl nine homolog 1 in C. elegans, also known as PHD2) (Ladroue et al. 2008). Very recently two paraganglioma cases with mutations in the *EPAS1* gene encoding hypoxia-inducible factor 2α (HIF2α) were reported, and even though these were somatic gain-of-function mutations, the authors speculate that the mutations could have arisen in a post-zygotic event early in embryogenesis (Zhuang et al. 2012).

3.2.2 Other Complex II-Associated Cancers

Even though PGL/PHEO has the strongest association with CII genes, these genes have been found mutated in other types of tumours as well. They include gastrointestinal stromal tumours (GISTs), either sporadic where *SDHB-D* mutations are present in 12 % of patients that are negative for *cKIT* and *PDGFRA* mutations (Janeway et al. 2011), or in the so called Carney-Stratakis syndrome, a genetic predisposition to combined PGL/PHEO and GIST (Carney and Stratakis 2002; McWhinney et al. 2007). In addition, in Cowden and Cowden-like syndromes (CS/CLS), a genetic predisposition to breast, thyroidal and renal cancers, *SDHB-D* mutations were found in 8 % *PTEN* mutation-negative patients and in 6 % *PTEN* mutation-positive patients. The patients positive for *SDHB-D* mutations were also more likely to progress to a full-blown cancer (Ni et al. 2008, 2012). More on SDH mutations and associated cancers can be found in a recent review by Bardella, Pollard and Tomlinson (Bardella et al. 2011b).

4 Mechanism of Complex II-Related Tumourigenesis

Whereas the causal association of CII mutations with PGL/PHEO (and some other cancers) is clear, undisputed and supported by solid genetic evidence, the mechanism underlying the tumourigenicity of CII mutations is still a matter of debate. In contrast to genomic DNA for germ-line mutational analysis, which is relatively easy to obtain, the availability of primary tumour samples for biochemical functional studies is much more limited, given the relatively low frequency of PGL/PHEO and other CII-related tumours and their particular growth characteristics. As a result,

there are no established tumour-derived cell lines, and neither are there suitable animal models to study CII-dependent malignancies (Hoekstra and Bayley 2013).

The overriding feature of CII-related tumours is the diminished expression of CII subunits and reduction in CII activity (Burnichon et al. 2010; Favier et al. 2009; Fliedner et al. 2012; Gimenez-Roqueplo et al. 2001; Janeway et al. 2011). Also characteristic for these tumours, as noted early on, is increased angiogenesis. It was shown already in 2001 that a nonsense mutation in *SDHD* found in three patients resulted in a complete loss of CII activity and activation of hypoxia pathways represented by increased angiogenic markers such as vascular endothelial growth factor (VEGF) and HIF2α (a product of *EPAS1*) (Gimenez-Roqueplo et al. 2001). Importantly, the whole study was performed with tumour samples, and the results were compared to control sporadic PHEO ensuring high relevance. These observations were later extended by the same authors to malignant, apparently sporadic PHEO in a patient with a germline *SDHB* mutation, which led to the loss of CII activity and stimulation of angiogenesis, associated with 'spectacular tumour vascularisation and tumour growth', to borrow the authors' own words (Gimenez-Roqueplo et al. 2002). Furthermore, as individuals living in higher altitudes are more susceptible to PGL/PHEO (Astrom et al. 2003), this suggests that an oxygen-sensing pathway may play a causal role in the development of the disease. Concerning angiogenesis in PGL/PHEO tumours, it is useful to note that transcriptomic analysis classifies PGL/PHEO tumours into two groups: one which includes VHL and CII-derived tumours characterised by angiogenesis/hypoxia profile, and another including RET and NF1-derived tumours which do not show these characteristics (Dahia et al. 2005). Detailed clustering of selected 103 (energy metabolism-related) or 54 (HIF1 or HIF2-regulated) transcripts was used to discriminate between VHL- and CII-related tumours (Burnichon et al. 2010); this suggests that despite some differences, the overall pro-angiogenic signature of VHL and CII PGL/PHEO is rather similar.

4.1 Role of Succinate Accumulation

4.1.1 Induction of Pseudohypoxia

Increased angiogenesis suggests that hypoxia responses may be deregulated in CII-derived tumours. These responses are directed by hypoxia-inducible factors (HIF) 1 and 2, which consist of two protein subunits, the labile α and the stable β subunit, that together initiate a transcriptional programme promoting glycolysis and neovascularisation. The stability of the α subunit of HIF1/2 is mainly governed by the oxygen- and 2-oxoglutarate (2OG)-dependent enzymes called prolyl hydroxylases (PHDs) that hydroxylate two proline residues in the HIF1/2α subunits, providing a signal for proteasomal degradation mediated by VHL (which, as noted above, is also associated with PGL/PHEO). In addition, the factor inhibiting HIF (FIH), another oxygen- and 2OG-dependent hydroxylase, hydroxylates an

asparagine residue in the HIF1/2α, dampening HIF transcriptional activity under normoxia (Keith et al. 2012).

Succinate, the by-product of FIH and PHD hydroxylation reaction is the substrate for CII, and this provided the first connection between CII deficiency and the pro-angiogenic phenotype: cells with SDHD knock-down lost CII activity and accumulated succinate, which resulted in substrate inhibition of PHDs and HIF stabilisation (Selak et al. 2005). The cells thus entered a state referred to as pseudo-hypoxia with hypoxic pathways activated under normal oxygen levels, which could be reverted by the reactivation of PHDs with cell-permeable derivative of the co-substrate 2OG (MacKenzie et al. 2007). In contrast to PHDs, the role of FIH appears minor in this setting, as it is virtually insensitive to succinate inhibition (Hewitson et al. 2007; Koivunen et al. 2007). *In vitro* data published by Selak et al., were replicated in tumour samples where both accumulation of succinate and HIF1α stabilisation were confirmed (Lehtonen et al. 2007; Pollard et al. 2005), providing the rationale for the above mentioned similarity between CII and VHL-derived tumours. In the former, HIFs are stabilised because they cannot bind to VHL due to the lack of hydroxylation, whereas in the latter they are stabilised because VHL itself is missing or non-functional. As HIF transcription factors may promote tumour formation (reviewed in Keith et al. 2012), this was proposed as a possible mechanism of tumourigenesis in both CII and VHL-derived tumours, and succinate accumulation is now considered a standard feature of CII-deficiency.

4.1.2 Epigenetic Changes

HIF-controlling hydroxylases are not the only 2OG-dependent, succinate-producing enzymes present in mammalian cells. In fact, PHDs and FIH are members of a large family of ferrous iron and 2OG-dependent oxygenases, comprising more than 80 individual enzymes with a broad range of physiological roles (McDonough et al. 2010). These include the JmjC domain-containing histone demethylases (JHDM), which modulate the epigenome by removing methyl residues from specific lysines of histones, and TET (ten-eleven translocation) DNA hydroxylases, which demethylate genomic DNA in a multistep process initiated by hydroxylation of 5-methylcytosin. With respect to the JHDMs, soon after the original report of succinate accumulation was published, it was shown in a yeast model of SDHB deficiency that increased succinate inhibits JHDMs as indicated by increased histone H3 lysine 9 methylation, a phenomenon that was recapitulated in mammalian cells by the administration of cell-permeable succinate (Smith et al. 2007). Subsequently, it was demonstrated that the loss of SDHB or SDHD in mammalian cells results in increased histone H3 methylation on several lysine residues, which could be reversed by JMJD3 histone demethylase over-expression. These cells also displayed reduced histone 3 occupancy of *IGFBP7* (insulin-like growth factor-binding protein 7) promoter, a gene previously found up-regulated in SDHB-deficient cells (Cervera et al. 2009). In addition, in PGL samples the chief type 1 cells, the neoplastic component of PGL, stained positive for methylated histone 9.

Concerning TETs, a recent study showed inhibition of both JHDM and TET enzymes upon SDHA/B knock-down, which promoted histone 3 methylation as well as increased 5-hydroxymethylcytosine levels, indicating reduced rate of DNA demethylation, resulting in genome-wide epigenetic changes and increased mRNA expression of *HOXA* genes, whose upregulation was associated with H3 demethylation in leukemia (Xiao et al. 2012). Interestingly, investigation of tumour samples from 55 PGL/PHEO patients identified a strong association of $p16^{INK4A}$ promoter hypermethylation with SDHB mutations. In fact, all SDHB-mutated tumours (no other SDH genes were sequenced) had increased $p16^{INK4A}$ promoter methylation (Kiss et al. 2008), and the hypermethylation phenotype of these tumours applied to other promoters as well (Geli et al. 2008). In addition, the hypermethylation phenotype of SDH-deficient tumours was confirmed in extended and independent sample panels (Killian et al. 2013; Letouze et al. 2013), and it was demonstrated that hypermethylation is tumour-specific (Kiss et al. 2013). We can therefore speculate that this might have happened as a consequence of succinate accumulation following the loss of heterozygosity event inactivating the wild type *SDHB* allele, linking epigenetic changes with the development of PGLs. As both the alteration of the epigenome and HIF stabilisation are driven by elevated succinate levels in CII-deficient cells, it was proposed by Frezza et al. that these two mechanisms might co-operate and the modified epigenome might modulate the transcription of HIF target genes, and in this way bring about the specific pseudohypoxia phenotype associated with tumourigenesis (Frezza et al. 2011a).

4.1.3 Extracellular Succinate

PHG/PHEO tumours (especially those of sympathetic origin) secrete hormones such as catecholamines with striking systemic effects manifesting, among other things, as severe hypertension, cardiac complications, sweating, palpitation and anxiety (Kantorovich and Pacak 2010). Analogically, as CII-derived tumours massively accumulate succinate, a notion was put forward that succinate released by CII-deficient cells could act in an endocrine or paracrine manner and in this way promote certain features of tumourigenesis (Bardella et al. 2011b; Frezza et al. 2011a). It has been shown that succinate binds to a specific receptor known as GPR91 (also referred to as SUCNR1) (Ariza et al. 2012; He et al. 2004). The receptor is most abundantly expressed in kidneys and its engagement leads to hypertension in rodents (He et al. 2004). This is mediated by the stimulation of renin release (Toma et al. 2008), and contributes to hypertension in the diabetic mice. Elevated plasma succinate was detected in patients with CII deficiency in Cowden syndrome (Hobert et al. 2012), but it remains to be seen whether this might contribute to the symptoms typical for PGL/PHEO as well. Besides hypertension, succinate signalling could potentially also stimulate angiogenesis (as shown for the eye) and modulate immunity, both processes with high relevance for cancer development (Rubic et al. 2008; Sapieha et al. 2008). Finally, the finding

that SDHB-suppressed cells up-regulate GPR91 shows that there might be a role for autocrine/paracrine activity of cell-extruded succinate within CII-deficient tumours (Cervera et al. 2008). Accordingly, although speculative, a possibility exists that extracellular succinate might be relevant for the pathogenesis of CII-deficient tumours.

4.2 Role of Oxidative Stress

Whereas succinate accumulation is an undisputed feature of CII-deficient tumours stemming from the absence or reduction of succinate-converting activity, the findings concerning oxidative stress in tumours and cells deficient in CII are less consistent. Even though CII is a *bona fide* source of reactive oxygen species (ROS) (Quinlan et al. 2012), the experimental evidence for increased ROS associated with CII deficiency is mixed. Various investigators found increased (Guo and Lemire 2003; Guzy et al. 2008; Ishii et al. 1998, 2005; Owens et al. 2012; Slane et al. 2006; Smith et al. 2007; Szeto et al. 2007) or normal levels (Cervera et al. 2008; Selak et al. 2005, 2006) of ROS production in cells lacking various CII subunits or containing various CII mutations. The issue is confounded by the fact that various investigators utilise different experimental models (differences in species, techniques of ROS evaluation, acute vs. chronic CII deficiency etc.). For example, the increase of ROS is consistently observed upon CII dysfunction in yeast (Guo and Lemire 2003; Smith et al. 2007; Szeto et al. 2007), whereas for mammalian cells the data is controversial. In primary tumour samples, increased ROS were not detected in SDHB-deficient tumours, however the tumour tissue manifested elevated superoxide dismutase-2 expression, suggesting an adaptation to increased oxidative stress (Fliedner et al. 2012).

It is possible that the production of ROS might differ depending on which of the CII subunits is mutated or missing. In this scenario, the presence of SDHA is required for the electrons to be removed from succinate, but molecular defects down the line in other CII subunits may result in a failure to deliver electrons to ubiquinone and their diversion towards ROS production. However, the experimental evidence is not unequivocal and the issue awaits further clarification.

4.2.1 The Role of ROS in HIF Stabilisation and Induction of Pseudohypoxia

The idea that ROS stabilise HIF in CII-deficient tumours is consistent with the observation that mitochondrial ROS are necessary for HIF stabilisation during hypoxia (Guzy et al. 2005; Chandel et al. 1998, 2000; Klimova and Chandel 2008), and ROS also inhibit PHDs and stabilise HIF1 under normoxia (Gerald et al. 2004). A convincing case was presented in favour of ROS-mediated HIF stabilisation in CII-deficient cells, as pharmacological inhibition of the proximal UbQ (Q_p) site in

SDHC/D, but not that of the SDH active site in SDHA, resulted in ROS production and HIF induction, an effect abrogated by mitochondria-targeted antioxidants. In addition, SDHB knock-down induced ROS and stabilised HIF, which was attenuated by the NP3 inhibitor of the SDH active site, whereas SDHA knock-down did not stabilise HIF (Guzy et al. 2008). In contrast, other investigators found that HIF can be stabilised without ROS formation in SDHB (Cervera et al. 2008) or SDHD (Selak et al. 2005, 2006) knock-down, and they could reverse the HIF stabilisation by the administration of cell-permeable 2OG (MacKenzie et al. 2007). In addition, SDHA-mutated tumours, too, show signs of pseudohypoxia (Burnichon et al. 2010). Finally, in Cowden-like syndrome, the ROS production was increased in the lymphoblastoid cell lines derived from the peripheral blood of SDH- and some PTEN-mutated patients. Despite that, HIF1α stabilisation was associated only with the SDH mutations (Ni et al. 2012).

The regulation of HIF activity by ROS and succinate is a complex process (Qutub and Popel 2008), and it is possible that relative contribution of both factors might differ depending on the cellular context studied and the level of the knock-down achieved. The methodology of ROS measurement might possibly also play a role. Nevertheless, clear discrepancies exist between the reported data, which have yet to be reconciled.

4.2.2 The Role of ROS in Genomic Instability

The story linking oxidative stress to genomic instability in CII-defective settings started with the identification of a *Mev-1* mutant in *C. elegans*, in which a mutation in SDHC led to decreased life-span and increased sensitivity to oxidative stress (Ishii et al. 1998). The mutation, equivalent to the E69 mutation in human SDHC, increased superoxide production, whereas succinate concentrations remained unchanged (Senoo-Matsuda et al. 2001). The nematode mutation was expressed in murine fibroblasts, which resulted in increased superoxide production from mitochondria, in a switch to more tumourigenic phenotype, as evidenced by increased colony and xenograft tumour formation, and mutational frequency in the nuclear *HPRT* gene (Ishii et al. 2005). This also led to induced oxidative stress in yeast (Guo and Lemire 2003). Others showed that Chinese hamster fibroblasts deficient in functional SDHC subunit (due to a premature stop codon) had increased steady state superoxide levels, superoxide dismutase activity and higher aneuploidity indicating chromosomal instability, all features that were corrected by the over-expression of wild-type SDHC (Slane et al. 2006). Similarly, cells expressing a truncated form of SDHD displayed increased ROS levels without much decreased CII activity and higher mutational rate of the *HPRT* gene, which could be reduced by the administration of superoxide dismutase and catalase, and enhanced by the depletion of the glutathione pool (Owens et al. 2012).

In Cowden-like syndrome, SDH mutations are associated with increased ROS levels, lipid peroxidation and DNA damage, which could be diminished by the administration of the lipid-soluble antioxidant α-tocopherol (Ni and Eng 2012). The

relative importance of the direct oxidative damage and indirect effects via modulation of signalling networks is as yet unclear, but at least using cells expressing mutant CII subunit on the wild-type background could help differentiate between the effects of ROS and succinate accumulation, as in these cells the succinate accumulation is expected to be limited.

4.3 Apoptosis Induction and Complex II Deficiency

Resistance to apoptosis is one of the hallmarks of cancer. Functional OXPHOS is required for efficient apoptosis induction (Kwong et al. 2007), and disruption of CI and II in a caspase-dependent manner is an integral feature of mitochondrial apoptosis (Ricci et al. 2003). CII may play a direct role in apoptosis induction. The cells with compromised CII are more apoptosis-resistant (Albayrak et al. 2003), and apoptosis induction by CII-targeting compounds has been described (Dong et al. 2008, 2011; Rohlena et al. 2011a, b). Furthermore, it was shown that CII disintegrates during apoptosis in a pH-dependent manner, leading to the detachment of the A and B subunits and to a failure of electron transfer to UbQ, resulting in ROS formation (Lemarie et al. 2011). It can thus be assumed that this process would be inefficient in CII-deficient tumours, but this has never been shown in primary patient material.

CII deficiency may also contribute to attenuation of the apoptotic responses indirectly. EGLN3 (also known as PHD3), an enzyme from the prolyl hydroxylase family responsible for the stabilisation of HIF in response to succinate accumulation, controls the induction of apoptosis in neuronal progenitor cells in response to growth factor removal (Lee et al. 2005). The apoptotic pathway induced by EGLN3 involves KIF1Bβ, a gene associated with PGL/PHEO (Schlisio et al. 2008; Yeh et al. 2008), suggesting clinical relevance. Reduced sensitivity to apoptosis was also determined in cells derived from Cowden-like patients with CII germ line mutations (Ni et al. 2012). This was possibly connected with reduced p53 protein levels in these cells, related to the increased FAD/NAD ratio stemming from the loss of functional NADH quinone oxidoreductase-1. Accordingly, treatment of control cells with the excess of the FAD precursor riboflavin mimicked the phenotype observed in CII-mutant cells.

4.4 Phenotypic Differences in Complex II Deficiency

One of the interesting features of CII-deficiency are different phenotypes associated with the mutations of individual CII subunits. This is puzzling, as large deletions in any of the subunit should lead to a complete loss of CII, which should give rise to an identical phenotype. In reality, SDHA deficiency is associated predominantly with degenerative diseases and cancer association is week, whereas the loss

of SDHB-D subunits is strongly associated with cancer. PGL/PHEO most often develops in *SDHB* and *SDHD* mutation carriers, whereas cancer associated with *SDHC* mutations is less frequent. *SDHB* mutations often lead to an aggressive, metastatic disease and occur more frequently in sporadic cases, but the penetrance is lower than for *SDHD*. *SDHD* mutations have high penetrance and show strong parent-of-origin effect (the disease develops only when the deficient allele is inherited from the father), but the pathology is frequently non-metastatic. *SDHC*-associated cancers are rare, the penetrance appears to be quite low, and the tumours are usually benign similarly to tumours carrying *SDHD* mutations. The reasons for these differences are not yet clear, but may have biochemical or genetic background.

Chromosomal locations of the genes coding individual CII subunit differ: *SDHA* is on chromosome ch5p15, *SDHB* on ch1p36, *SDHC* on ch1q23, and *SDHD* on ch11q23. Somatic loss of the second allele leading to tumour development may also cause a simultaneous deletion of additional genes, which would differ according to the chromosomal location for each of the subunits. This genetic explanation would reconcile the somewhat puzzling observation of a frequent SDHB loss, probably due to the failure of the CII assembly, in CII-related tumours (van Nederveen et al. 2009). This argues against inter-subunit differences based solely on a biochemical mechanism, such as different ROS formation rates as discussed above. Concerning SDHB, others have shown reduction of its protein level (possibly HIF1α-dependent) even in the absence of CII mutations (Dahia et al. 2005; Janeway et al. 2011), indicating the importance of the tumour suppression role of CII in cancer.

The increased metastatic potential of SDHB-derived PGL/PHEO tumours is a highly complicating feature in the clinic, yet the underlining molecular mechanism remains unknown. Differences among tumours with individual CII subunit deficiencies have not been investigated in this context (possible due to the scarcity of available material), but considerable effort went into the study of SDHB- and VHL-derived tumours, the latter of which are generally metastasis-free. Due to the differences of pseudohypoxia induction in these tumours (the lack of VHL as opposed to succinate/ROS accumulation), attention was given to HIF1α and HIF2α stabilisation, as these two transcription factors may play different roles in tumorigenesis (Keith et al. 2012). The results are still somewhat confusing, as HIF2α was found to be increased more than HIF1α in VHL (Pollard et al. 2006), SDHB (Lopez-Jimenez et al. 2010) or both SDHB and VHL tumours (Favier et al. 2009). Data suggest that VHL tumours may be more glycolytic than SDHB tumours due to p53 down-regulation (Favier et al. 2009), and there is an indication of increased oxidative stress in SDHB tumours as evidenced by higher mitochondrial superoxide dismutase expression (Fliedner et al. 2012). Interestingly, SDHB tumours up-regulate pathways involved in cell-cell interaction and migration (Lopez-Jimenez et al. 2010), also observed for SDHB-silenced cells (Cervera et al. 2008), where compared to controls the SDHB knock-down led to enhanced attachment to various components of the extracellular matrix (Cervera et al. 2008). The elevated adhesiveness may be of high relevance for enhancing metastasis of SDHB tumours, but the exact regulatory mechanism behind this phenotype remains unresolved.

5 Lessons from Other Tricarboxylic Acid Cycle and Oxidative Phosphorylation-Associated Cancers

CII is not the only cancer-associated component of the mitochondrial metabolic machinery. In addition, mutations in fumarate hydratase, (FH) and isocitrate dehydrogenase (IDH) are associated with cancer, and in a number of malignancies the OXPHOS system is compromised due to mitochondrial DNA mutations. Both FH and IDH defects are similar to CII deficiency in that they modulate levels of intracellular metabolites, and some of the findings in those settings, such as the effect on 2OG-dependent enzymes, may be relevant for CII malignancies as well. However, in the clinic there are substantial phenotypic differences between these tumours and although these cancers share similar molecular features, the early stages of their development may have unique initiators (Raimundo et al. 2011). Activation of hypoxia pathways could play an important role in the transformation in CII-associated cancers, but for IDH and FH cancers reduced cellular differentiation was proposed to be the trigger for tumour initiation, pseudohypoxia being only an additional advantage for further tumour progression (Raimundo et al. 2011).

5.1 Fumarate Hydratase-Deficient Tumours

CII (SDH) deficiency might have most in common with defects in FH, an enzyme catalysing the reaction of the TCA cycle placed just after SDH. FH deficiency, which compromises the conversion of fumarate to malate and results in the accumulation of intracellular fumarate, has a strong association with renal cancer. In 2007 Pollard et al. introduced a very useful model to study FH defects in tumour formation. This mouse model bears conditionally inactivated mouse homologue of human *FH* gene (*Fh1*) in the kidney and develops multiple renal cysts (Pollard et al. 2007). Since then, the mouse model and the isolated embryonic fibroblasts provided very valuable data. First, in 2010 it was demonstrated that the pseudohypoxic signature of FH deficient cells is independent of defective mitochondrial energy metabolism, because re-expression of extra-mitochondrial FH was sufficient to restore HIF1α prolyl hydroxylation by reducing the intracellular fumarate levels (O'Flaherty et al. 2010).

Surprisingly, Adam et al. provided evidence that tumourigenesis in *Fh1*-deficient mice is HIF-independent, as combining FH deficiency with that of HIF1α, HIF2α, both HIFα isoforms, or PHD enzymes did not ameliorate renal cyst formation. Instead, the nuclear factor (erythroid-derived 2)-like 2 (NRF2) signalling turned out to be the oncogenic pathway responsible for FH tumourigenesis, which is in agreement with its tumourigenic role in other malignancies (DeNicola et al. 2011; Mitsuishi et al. 2012). NRF2 activity is controlled by the protein inhibitor KEAP1, which is inactivated due to the succinylation of regulatory cysteine residues by accumulated fumarate in FH-deficient cells, resulting in NRF2 stabilisation and

upregulation of NRF2 target genes. One of these genes is haem oxygenase 1 (*HMOX1*) (Adam et al. 2011), whose role in FH-deficient tumours was nicely explained by Frezza et al. (2011b). This study provided an explanation of how cells survive without a functional TCA cycle enzyme by switching to an alternative pathway involving HMOX1, and demonstrated that inhibition of this pathway is synthetically lethal in FH-deficient mouse and human cell lines (Frezza et al. 2011b). The question arises whether the HMOX1 TCA shunt may be also applicable to CII-deficient tumours. KEAP1 inactivation by succinylation is likely restricted only to FH-related malignancies, as no succinylated cysteins were detected in the material from CII-deficient tumours (Bardella et al. 2011a).

The data from FH-deficient mice suggest that HIF stabilisation may not be the sole mechanism of tumourigenesis, as previously discussed for ROS (which also activate NRF2) and epigenetic modifications. The FH story also emphasises the lack of suitable experimental model of CII deficiency. There are no human PGL/PHEO cell lines, and no suitable model of CII deficiency with human or mouse background. The homozygous deletion of *SDHD* in mice is embryonically lethal, and *SDHD* heterozygous animals do not develop PGL/PHEO (Bayley et al. 2009; Piruat et al. 2004). There are no reports of PGL/PHEO in mice over-expressing the *SDHC* Mev1 mutant on the wild-type background either (reviewed in Ishii et al. 2013), and only the animal models of PTEN, retinoblastoma, NF1 or cyclin-dependent kinase inhibitor deficiency develops PGL/PHEO-like symptoms (reviewed in Korpershoek et al. 2012). Accordingly, a proper model of CII deficiency is needed.

5.2 Isocitrate Dehydrogenase-Associated Tumours

Less similar to CII deficiency are defects in IDH enzymes. Whereas the NAD^+-dependent IDH3 catalyses oxidative decarboxylation of isocitrate to 2OG in the TCA and is not associated with cancer, the cytoplasmic IDH1 and mitochondrial IDH2 are NADPH-dependent and catalyse the same reaction in the reversible fashion, and have been found mutated in gliomas, astrocytomas, chondromas and acute myeloid leukemia. Unlike CII which requires a second hit for tumourigenesis and therefore behaves as a tumour suppressor, one allele mutation is sufficient for IDH-driven oncogenesis, and this classifies IDHs as oncogenes. Oncogenic IDH variants catalyse conversion of 2OG to 2-hydroxyglutarate, which surprisingly activates PHD1 and PHD2 to increase the degradation of HIF1α that in this context behaves as a tumour suppressor gene (Koivunen et al. 2012). In addition, the activity of JHDMs is inhibited, resulting in increased histone H3 lysine 9 methylation, a block in cell differentiation and induction of the 'hypermethylator' phenotype (Lu et al. 2012; Turcan et al. 2012). These data suggest that similar mechanisms may modify the same pathways with distinct outcomes, and put a question mark over the role of HIF1α in tumourigenesis. Further investigation of HIF1/2α in the context of CII deficiency is therefore warranted.

5.3 Mitochondrial DNA Mutations and Oncocytic Tumours

As CII participates in both TCA and OXPHOS, parallels can be drawn with other OXPHOS mutations, which predominantly occur in mitochondrial DNA (mtDNA) coding for 13 protein subunits of the OXPHOS CI, CIII, CIV and CV, and also for 22 transfer and 2 ribosomal RNAs. In contrast to CII gene defects, mutations in mtDNA follow the rules of population genetics and can be either homoplasmic or heteroplasmic, *i.e.* present in all or only a fraction of mitochondria of a given cell. The OXPHOS mtDNA mutations are quite frequent in cancer, yet their functional relevance is not always clear. Some of these mutations likely play a causal role in cancer development, as they are enriched in tumour tissue, and may contribute to an aggressive tumour phenotype (Ishikawa et al. 2008; Petros et al. 2005), while many others are functionally neutral. Many of the functionally relevant mtDNA mutations occur in seven mitochondrial genes coding for CI subunits, as these CI genes constitute a large part (about 40 %) of the mitochondrial genome. In this respect the so called oncocytic tumours are of special interest.

These usually benign tumours are epithelium-derived, occur in the kidney or thyroid, parathyroid, salivary and pituitary glands, and are characterised by aberrant hyperplastic mitochondria, where increased biogenesis might be a compensatory effect of OXPHOS dysfunction (reviewed in Gasparre et al. 2011). In these tumours and the derived models, impaired OXPHOS was reported due to defects in CI or a combined defect of CI and CIII (Bonora et al. 2006; Simonnet et al. 2003). Porcelli *et al.* screened 44 pituitary adenomas and 25 head-and-neck tumours (HNT), most of which presented with an oncocytic phenotype, for mtDNA disruptive mutations (Porcelli et al. 2010). The investigation revealed that the oncocytic phenotype was associated with homoplasmic mitochondrial mutations (*i.e.* present in all mitochondria of a cell) responsible for CI impairment. 2OG/succinate ratio was higher than in heteroplasmic and control cells, which resulted in HIF1α destabilisation, as confirmed by the negative HIF1α staining in tumours also negative for the mtDNA-coded MTND6 subunit of CI. The authors explained the observation by accumulation of NADH due to non-functional CI and the consequent activation of 2OG-dependent dehydrogenase, which would result in enhanced prolyl hydroxylation of HIF1α and its proteasome targeting (Porcelli et al. 2010).

An HNT case was also described with the mutated CIV MTCO1 subunit, which also affected CI assembly and led to HIF1α destabilisation (Porcelli et al. 2010). It was suggested that in these tumours oncocytic transformation in fact ameliorates the cancer phenotype and should be separated from oncogenic transformation (Gasparre et al. 2011). Accordingly, the effect of mtDNA mutations might be double-edged; mutations that lead to a complete loss of CI activity might be protective and associated with a benign phenotype, whereas those that only partially decrease CI activity and simultaneously increase ROS formation might be pro-tumourigenic (Iommarini et al. 2013). As of now, this leaves CII the only OXPHOS component unequivocally linked with a genetic predisposition to cancer.

6 Conclusions

Despite considerable progress in understanding various aspects of CII-associated tumourigenesis (summarised in Fig. 4.2), CII-associated tumours remain enigmatic. For example, it is still not clear whether the distinct phenotypic manifestations of individual CII subunit deficiencies relate to a genetic cause, given the varying chromosomal location of genes coding for individual subunits, or to different structural and functional roles each subunit plays in CII assembly and function (reviewed in Iverson et al. 2012). The lack of proper cellular model for CII deficiency in human or murine cell lines as well as clear CII-related phenotypic presentation of (for example) PGL/PHEO in laboratory animals are a hurdle to further progress in CII-related research. The yeast models of CII deficiency are informative, yet possess inherent limitations, such as that a number of tumour-associated mutations are in positions that are not well conserved between humans and yeast (Panizza et al. 2013). In summary, to understand various aspects of CII-related tumourigenesis, better model systems are required to guide the basic research, the results of which could then be verified in the relatively rare human primary tumour samples.

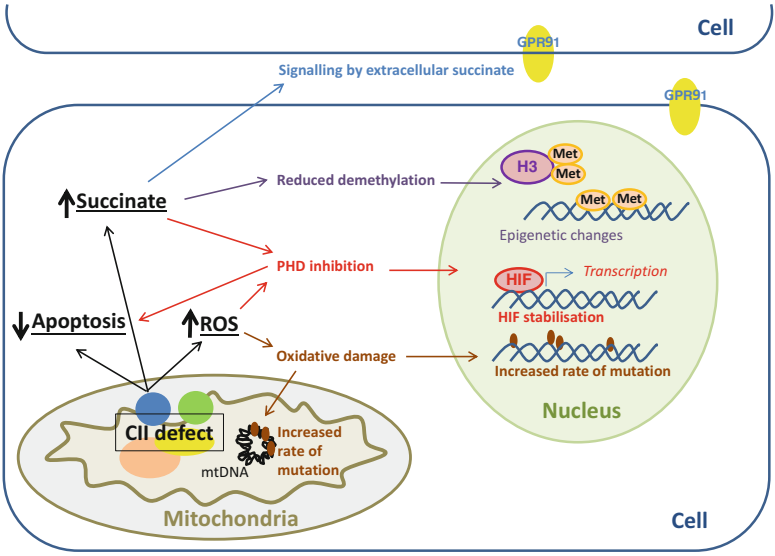

Fig. 4.2 Mechanisms of complex II-associated tumourigenesis. CII defects (such as mutations resulting in the assembly failure or loss of activity) increase intracellular succinate, and possibly produce ROS. Succinate stabilises HIF transcription factors, thereby inducing the transcription of HIF target genes and pseudohypoxic response. Succinate also inhibits other 2OG-dependent enzymes such as histone and DNA demethylases altering epigenetics, and when secreted from cells it may signal via its receptor GPR91. Accumulation of ROS may also stabilise HIF and induce hypoxia, as well as contribute to increased mutational rate and genomic instability. In addition, CII defects may be associated with attenuated apoptosis, either directly due to the lack of functional CII itself, or indirectly via succinate inhibition of PHD3

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References

Adam J, Hatipoglu E, O'Flaherty L, Ternette N, Sahgal N, Lockstone H, Baban D, Nye E, Stamp GW, Wolhuter K, Stevens M, Fischer R, Carmeliet P, Maxwell PH, Pugh CW, Frizzell N, Soga T, Kessler BM, El-Bahrawy M, Ratcliffe PJ, Pollard PJ (2011) Renal cyst formation in Fh1-deficient mice is independent of the Hif/Phd pathway: roles for fumarate in KEAP1 succination and Nrf2 signaling. Cancer Cell 20:524–537

Albayrak T, Scherhammer V, Schoenfeld N, Braziulis E, Mund T, Bauer MK, Scheffler IE, Grimm S (2003) The tumor suppressor cybL, a component of the respiratory chain, mediates apoptosis induction. Mol Biol Cell 14:3082–3096

Ariza AC, Deen PM, Robben JH (2012) The succinate receptor as a novel therapeutic target for oxidative and metabolic stress-related conditions. Front Endocrinol (Lausanne) 3:22

Astrom K, Cohen JE, Willett-Brozick JE, Aston CE, Baysal BE (2003) Altitude is a phenotypic modifier in hereditary paraganglioma type 1: evidence for an oxygen-sensing defect. Hum Genet 113:228–237

Astuti D, Douglas F, Lennard TW, Aligianis IA, Woodward ER, Evans DG, Eng C, Latif F, Maher ER (2001a) Germline SDHD mutation in familial phaeochromocytoma. Lancet 357:1181–1182

Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, Skoldberg F, Husebye ES, Eng C, Maher ER (2001b) Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet 69:49–54

Bardella C, El-Bahrawy M, Frizzell N, Adam J, Ternette N, Hatipoglu E, Howarth K, O'Flaherty L, Roberts I, Turner G, Taylor J, Giaslakiotis K, Macaulay VM, Harris AL, Chandra A, Lehtonen HJ, Launonen V, Aaltonen LA, Pugh CW, Mihai R, Trudgian D, Kessler B, Baynes JW, Ratcliffe PJ, Tomlinson IP, Pollard PJ (2011a) Aberrant succination of proteins in fumarate hydratase-deficient mice and HLRCC patients is a robust biomarker of mutation status. J Pathol 225:4–11

Bardella C, Pollard PJ, Tomlinson I (2011b) SDH mutations in cancer. Biochim Biophys Acta 1807:1432–1443

Bayley JP, van Minderhout I, Hogendoorn PC, Cornelisse CJ, van der Wal A, Prins FA, Teppema L, Dahan A, Devilee P, Taschner PE (2009) Sdhd and SDHD/H19 knockout mice do not develop paraganglioma or pheochromocytoma. PLoS One 4:e7987

Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PE, Rubinstein WS, Myers EN, Richard CW 3rd, Cornelisse CJ, Devilee P, Devlin B (2000) Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science 287:848–851

Bonora E, Porcelli AM, Gasparre G, Biondi A, Ghelli A, Carelli V, Baracca A, Tallini G, Martinuzzi A, Lenaz G, Rugolo M, Romeo G (2006) Defective oxidative phosphorylation in thyroid oncocytic carcinoma is associated with pathogenic mitochondrial DNA mutations affecting complexes I and III. Cancer Res 66:6087–6096

Bourgeron T, Rustin P, Chretien D, Birch-Machin M, Bourgeois M, Viegas-Pequignot E, Munnich A, Rotig A (1995) Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. Nat Genet 11:144–149

Burnichon N, Briere JJ, Libe R, Vescovo L, Riviere J, Tissier F, Jouanno E, Jeunemaitre X, Benit P, Tzagoloff A, Rustin P, Bertherat J, Favier J, Gimenez-Roqueplo AP (2010) SDHA is a tumor suppressor gene causing paraganglioma. Hum Mol Genet 19:3011–3020

Carney JA, Stratakis CA (2002) Familial paraganglioma and gastric stromal sarcoma: a new syndrome distinct from the Carney triad. Am J Med Genet 108:132–139

Cervera AM, Apostolova N, Crespo FL, Mata M, McCreath KJ (2008) Cells silenced for SDHB expression display characteristic features of the tumor phenotype. Cancer Res 68:4058–4067

Cervera AM, Bayley JP, Devilee P, McCreath KJ (2009) Inhibition of succinate dehydrogenase dysregulates histone modification in mammalian cells. Mol Cancer 8:89

Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT (1998) Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. Proc Natl Acad Sci U S A 95:11715–11720

Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, Schumacker PT (2000) Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O2 sensing. J Biol Chem 275:25130–25138

Dahia PL, Ross KN, Wright ME, Hayashida CY, Santagata S, Barontini M, Kung AL, Sanso G, Powers JF, Tischler AS, Hodin R, Heitritter S, Moore F, Dluhy R, Sosa JA, Ocal IT, Benn DE, Marsh DJ, Robinson BG, Schneider K, Garber J, Arum SM, Korbonits M, Grossman A, Pigny P, Toledo SP, Nose V, Li C, Stiles CD (2005) A HIF1alpha regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. PLoS Genet 1:72–80

DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, Mangal D, Yu KH, Yeo CJ, Calhoun ES, Scrimieri F, Winter JM, Hruban RH, Iacobuzio-Donahue C, Kern SE, Blair IA, Tuveson DA (2011) Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature 475:106–109

DiMauro S, Schon EA (2003) Mitochondrial respiratory-chain diseases. N Engl J Med 348:2656–2668

Dong LF, Low P, Dyason J, Wang XF, Prochazka L, Witting PK, Freeman R, Swettenham E, Valis K, Liu J, Zobalova R, Turanek J, Spitz DR, Domann FE, Scheffler IE, Ralph SJ, Neuzil J (2008) α-Tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II. Oncogene 27:4324–4335

Dong LF, Jameson VJ, Tilly D, Prochazka L, Rohlena J, Valis K, Truksa J, Zobalova R, Mahdavian E, Kluckova K, Stantic M, Stursa J, Freeman R, Witting PK, Norberg E, Goodwin J, Salvatore BA, Novotna J, Turanek J, Ledvina M, Hozak P, Zhivotovsky B, Coster MJ, Ralph SJ, Smith RA, Neuzil J (2011) Mitochondrial targeting of alpha-tocopheryl succinate enhances its pro-apoptotic efficacy: a new paradigm for effective cancer therapy. Free Radic Biol Med 50:1546–1555

Favier J, Briere JJ, Burnichon N, Riviere J, Vescovo L, Benit P, Giscos-Douriez I, De Reynies A, Bertherat J, Badoual C, Tissier F, Amar L, Libe R, Plouin PF, Jeunemaitre X, Rustin P, Gimenez-Roqueplo AP (2009) The Warburg effect is genetically determined in inherited pheochromocytomas. PLoS One 4:e7094

Fishbein L, Nathanson KL (2012) Pheochromocytoma and paraganglioma: understanding the complexities of the genetic background. Cancer Genet 205:1–11

Fliedner SM, Kaludercic N, Jiang XS, Hansikova H, Hajkova Z, Sladkova J, Limpuangthip A, Backlund PS, Wesley R, Martiniova L, Jochmanova I, Lendvai NK, Breza J, Yergey AL, Paolocci N, Tischler AS, Zeman J, Porter FD, Lehnert H, Pacak K (2012) Warburg effect's manifestation in aggressive pheochromocytomas and paragangliomas: insights from a mouse cell model applied to human tumor tissue. PLoS One 7:e40949

Frezza C, Pollard PJ, Gottlieb E (2011a) Inborn and acquired metabolic defects in cancer. J Mol Med (Berl) 89:213–220

Frezza C, Zheng L, Folger O, Rajagopalan KN, MacKenzie ED, Jerby L, Micaroni M, Chaneton B, Adam J, Hedley A, Kalna G, Tomlinson IP, Pollard PJ, Watson DG, Deberardinis RJ, Shlomi T, Ruppin E, Gottlieb E (2011b) Haem oxygenase is synthetically lethal with the tumour suppressor fumarate hydratase. Nature 477:225–228

Gasparre G, Romeo G, Rugolo M, Porcelli AM (2011) Learning from oncocytic tumors: why choose inefficient mitochondria? Biochim Biophys Acta 1807:633–642

Geli J, Kiss N, Karimi M, Lee J-J, Bäckdahl M, Ekström TJ, Larsson C (2008) Global and regional CpG methylation in pheochromocytomas and abdominal paragangliomas: association to malignant behavior. Clin Cancer Res 14:2551–2559

Gerald D, Berra E, Frapart YM, Chan DA, Giaccia AJ, Mansuy D, Pouysségur J, Yaniv M, Mechta-Grigoriou F (2004) JunD reduces tumor angiogenesis by protecting cells from oxidative stress. Cell 118:781–794

Gimenez-Roqueplo AP, Favier J, Rustin P, Mourad JJ, Plouin PF, Corvol P, Rotig A, Jeunemaitre X (2001) The R22X mutation of the SDHD gene in hereditary paraganglioma abolishes the enzymatic activity of complex II in the mitochondrial respiratory chain and activates the hypoxia pathway. Am J Hum Genet 69:1186–1197

Gimenez-Roqueplo AP, Favier J, Rustin P, Rieubland C, Kerlan V, Plouin PF, Rotig A, Jeunemaitre X (2002) Functional consequences of a SDHB gene mutation in an apparently sporadic pheochromocytoma. J Clin Endocrinol Metab 87:4771–4774

Guo J, Lemire BD (2003) The ubiquinone-binding site of the Saccharomyces cerevisiae succinate-ubiquinone oxidoreductase is a source of superoxide. J Biol Chem 278:47629–47635

Guzy RD, Hoyos B, Robin E, Chen H, Liu L, Mansfield KD, Simon MC, Hammerling U, Schumacker PT (2005) Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. Cell Metab 1:401–408

Guzy RD, Sharma B, Bell E, Chandel NS, Schumacker PT (2008) Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. Mol Cell Biol 28:718–731

Hao HX, Khalimonchuk O, Schraders M, Dephoure N, Bayley JP, Kunst H, Devilee P, Cremers CW, Schiffman JD, Bentz BG, Gygi SP, Winge DR, Kremer H, Rutter J (2009) SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. Science 325:1139–1142

He W, Miao FJ, Lin DC, Schwandner RT, Wang Z, Gao J, Chen JL, Tian H, Ling L (2004) Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. Nature 429:188–193

Hewitson KS, Lienard BM, McDonough MA, Clifton IJ, Butler D, Soares AS, Oldham NJ, McNeill LA, Schofield CJ (2007) Structural and mechanistic studies on the inhibition of the hypoxia-inducible transcription factor hydroxylases by tricarboxylic acid cycle intermediates. J Biol Chem 282:3293–3301

Hobert JA, Mester JL, Moline J, Eng C (2012) Elevated plasma succinate in PTEN, SDHB, and SDHD mutation-positive individuals. Genet Med 14:616–619

Hoekstra AS, Bayley JP (2013) The role of complex II in disease. Biochim Biophys Acta 1827(5):543–551

Iommarini L, Calvaruso MA, Kurelac I, Gasparre G, Porcelli AM (2013) Complex I impairment in mitochondrial diseases and cancer: parallel roads leading to different outcomes. Int J Biochem Cell Biol 45:47–63

Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, Yanase S, Ayusawa D, Suzuki K (1998) A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature 394:694–697

Ishii T, Yasuda K, Akatsuka A, Hino O, Hartman PS, Ishii N (2005) A mutation in the SDHC gene of complex II increases oxidative stress, resulting in apoptosis and tumorigenesis. Cancer Res 65:203–209

Ishii T, Miyazawa M, Onouchi H, Yasuda K, Hartman PS, Ishii N (2013) Model animals for the study of oxidative stress from complex II. Biochim Biophys Acta (BBA) - Bioenerg 1827(5):588–597

Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H, Nakada K, Honma Y, Hayashi J-I (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 320:661–664

Iverson TM, Maklashina E, Cecchini G (2012) Structural basis for malfunction in complex II. J Biol Chem 287:35430–35438

Janeway KA, Kim SY, Lodish M, Nose V, Rustin P, Gaal J, Dahia PL, Liegl B, Ball ER, Raygada M, Lai AH, Kelly L, Hornick JL, O'Sullivan M, de Krijger RR, Dinjens WN, Demetri GD, Antonescu CR, Fletcher JA, Helman L, Stratakis CA (2011) Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. Proc Natl Acad Sci U S A 108:314–318

Kantorovich V, Pacak K (2010) Pheochromocytoma and paraganglioma. Prog Brain Res 182:343–373

Keith B, Johnson RS, Simon MC (2012) HIF1alpha and HIF2alpha: sibling rivalry in hypoxic tumour growth and progression. Nat Rev Cancer 12:9–22

Killian JK, Kim SY, Miettinen M, Smith C, Merino M, Tsokos M, Quezado M, Smith WI Jr, Jahromi MS, Xekouki P, Szarek E, Walker RL, Lasota J, Raffeld M, Klotzle B, Wang Z, Jones L, Zhu Y, Wang Y, Waterfall JJ, O'Sullivan MJ, Bibikova M, Pacak K, Stratakis C, Janeway KA, Schiffman JD, Fan JB, Helman L, Meltzer PS (2013) Succinate dehydrogenase mutation underlies global epigenomic divergence in gastrointestinal stromal tumor. Cancer Discov 3:648–657

Kiss NB, Geli J, Lundberg F, Avci C, Velazquez-Fernandez D, Hashemi J, Weber G, Hoog A, Ekstrom TJ, Backdahl M, Larsson C (2008) Methylation of the p16INK4A promoter is associated with malignant behavior in abdominal extra-adrenal paragangliomas but not pheochromocytomas. Endocr Relat Cancer 15:609–621

Kiss NB, Muth A, Andreasson A, Juhlin CC, Geli J, Backdahl M, Hoog A, Wangberg B, Nilsson O, Ahlman H, Larsson C (2013) Acquired hypermethylation of the P16INK4A promoter in abdominal paraganglioma: relation to adverse tumor phenotype and predisposing mutation. Endocr Relat Cancer 20(1):65–78

Klimova T, Chandel NS (2008) Mitochondrial complex III regulates hypoxic activation of HIF. Cell Death Differ 15:660–666

Koivunen P, Hirsilä M, Remes AM, Hassinen IE, Kivirikko KI, Myllyharju J (2007) Inhibition of hypoxia-inducible factor (HIF) hydroxylases by citric acid cycle intermediates: possible links between cell metabolism and stabilization of HIF. J Biol Chem 282:4524–4532

Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkissoon S, Losman JA, Joensuu P, Bergmann U, Gross S, Travins J, Weiss S, Looper R, Ligon KL, Verhaak RGW, Yan H, Kaelin WG Jr (2012) Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. Nature 483:484–488

Korpershoek E, Pacak K, Martiniova L (2012) Murine models and cell lines for the investigation of pheochromocytoma: applications for future therapies? Endocr Pathol 23:43–54

Kwong JQ, Henning MS, Starkov AA, Manfredi G (2007) The mitochondrial respiratory chain is a modulator of apoptosis. J Cell Biol 179:1163–1177

Ladroue C, Carcenac R, Leporrier M, Gad S, Le Hello C, Galateau-Salle F, Feunteun J, Pouyssegur J, Richard S, Gardie B (2008) PHD2 mutation and congenital erythrocytosis with paraganglioma. N Engl J Med 359:2685–2692

Lee S, Nakamura E, Yang H, Wei W, Linggi MS, Sajan MP, Farese RV, Freeman RS, Carter BD, Kaelin WG Jr, Schlisio S (2005) Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: developmental culling and cancer. Cancer Cell 8:155–167

Lehtonen HJ, Makinen MJ, Kiuru M, Laiho P, Herva R, van Minderhout I, Hogendoorn PC, Cornelisse C, Devilee P, Launonen V, Aaltonen LA (2007) Increased HIF1 alpha in SDH and FH deficient tumors does not cause microsatellite instability. Int J Cancer 121:1386–1389

Lemarie A, Huc L, Pazarentzos E, Mahul-Mellier AL, Grimm S (2011) Specific disintegration of complex II succinate: ubiquinone oxidoreductase links pH changes to oxidative stress for apoptosis induction. Cell Death Differ 18:338–349

Letouze E, Martinelli C, Loriot C, Burnichon N, Abermil N, Ottolenghi C, Janin M, Menara M, Nguyen AT, Benit P, Buffet A, Marcaillou C, Bertherat J, Amar L, Rustin P, De Reynies A, Gimenez-Roqueplo AP, Favier J (2013) SDH mutations establish a hypermethylator phenotype in paraganglioma. Cancer Cell 23:739–752

Lopez-Jimenez E, Gomez-Lopez G, Leandro-Garcia LJ, Munoz I, Schiavi F, Montero-Conde C, de Cubas AA, Ramires R, Landa I, Leskela S, Maliszewska A, Inglada-Perez L, de la Vega L, Rodriguez-Antona C, Leton R, Bernal C, de Campos JM, Diez-Tascon C, Fraga MF, Boullosa C, Pisano DG, Opocher G, Robledo M, Cascon A (2010) Research resource: transcriptional profiling reveals different pseudohypoxic signatures in SDHB and VHL-related pheochromocytomas. Mol Endocrinol 24:2382–2391

Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, Edwards CR, Khanin R, Figueroa ME, Melnick A, Wellen KE, O'Rourke DM, Berger SL, Chan TA, Levine RL, Mellinghoff IK, Thompson CB (2012) IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature 483:474–478

MacKenzie ED, Selak MA, Tennant DA, Payne LJ, Crosby S, Frederiksen CM, Watson DG, Gottlieb E (2007) Cell-permeating alpha-ketoglutarate derivatives alleviate pseudohypoxia in succinate dehydrogenase-deficient cells. Mol Cell Biol 27:3282–3289

Maklashina E, Cecchini G (2010) The quinone-binding and catalytic site of complex II. Biochim Biophys Acta 1797:1877–1882

McDonough MA, Loenarz C, Chowdhury R, Clifton IJ, Schofield CJ (2010) Structural studies on human 2-oxoglutarate dependent oxygenases. Curr Opin Struct Biol 20:659–672

McWhinney SR, Pasini B, Stratakis CA (2007) Familial gastrointestinal stromal tumors and germline mutations. N Engl J Med 357:1054–1056

Mitsuishi Y, Taguchi K, Kawatani Y, Shibata T, Nukiwa T, Aburatani H, Yamamoto M, Motohashi H (2012) Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. Cancer Cell 22:66–79

Ni Y, Eng C (2012) Vitamin E protects against lipid peroxidation and rescues tumorigenic phenotypes in cowden/cowden-like patient-derived lymphoblast cells with germline SDHx variants. Clin Cancer Res 18:4954–4961

Ni Y, Zbuk KM, Sadler T, Patocs A, Lobo G, Edelman E, Platzer P, Orloff MS, Waite KA, Eng C (2008) Germline mutations and variants in the succinate dehydrogenase genes in Cowden and Cowden-like syndromes. Am J Hum Genet 83:261–268

Ni Y, He X, Chen J, Moline J, Mester J, Orloff MS, Ringel MD, Eng C (2012) Germline SDHx variants modify breast and thyroid cancer risks in Cowden and Cowden-like syndrome via FAD/NAD-dependant destabilization of p53. Hum Mol Genet 21:300–310

Niemann S, Muller U (2000) Mutations in SDHC cause autosomal dominant paraganglioma, type 3. Nat Genet 26:268–270

O'Flaherty L, Adam J, Heather LC, Zhdanov AV, Chung YL, Miranda MX, Croft J, Olpin S, Clarke K, Pugh CW, Griffiths J, Papkovsky D, Ashrafian H, Ratcliffe PJ, Pollard PJ (2010) Dysregulation of hypoxia pathways in fumarate hydratase-deficient cells is independent of defective mitochondrial metabolism. Hum Mol Genet 19:3844–3851

Opocher G, Schiavi F (2011) Functional consequences of succinate dehydrogenase mutations. Endocr Pract 17(Suppl 3):64–71

Owens KM, Aykin-Burns N, Dayal D, Coleman MC, Domann FE, Spitz DR (2012) Genomic instability induced by mutant succinate dehydrogenase subunit D (SDHD) is mediated by O2(−*) and H2O2. Free Radic Biol Med 52:160–166

Panizza E, Ercolino T, Mori L, Rapizzi E, Castellano M, Opocher G, Ferrero I, Neumann HP, Mannelli M, Goffrini P (2013) Yeast model for evaluating the pathogenic significance of SDHB, SDHC and SDHD mutations in PHEO-PGL syndrome. Hum Mol Genet 22(4):804–815

Parfait B, Chretien D, Rotig A, Marsac C, Munnich A, Rustin P (2000) Compound heterozygous mutations in the flavoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome. Hum Genet 106:236–243

Petros JA, Baumann AK, Ruiz-Pesini E, Amin MB, Sun CQ, Hall J, Lim S, Issa MM, Flanders WD, Hosseini SH, Marshall FF, Wallace DC (2005) mtDNA mutations increase tumorigenicity in prostate cancer. Proc Natl Acad Sci U S A 102:719–724

Piruat JI, Pintado CO, Ortega-Saenz P, Roche M, Lopez-Barneo J (2004) The mitochondrial SDHD gene is required for early embryogenesis, and its partial deficiency results in persistent carotid body glomus cell activation with full responsiveness to hypoxia. Mol Cell Biol 24:10933–10940

Pollard PJ, Briere JJ, Alam NA, Barwell J, Barclay E, Wortham NC, Hunt T, Mitchell M, Olpin S, Moat SJ, Hargreaves IP, Heales SJ, Chung YL, Griffiths JR, Dalgleish A, McGrath JA, Gleeson MJ, Hodgson SV, Poulsom R, Rustin P, Tomlinson IP (2005) Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. Hum Mol Genet 14:2231–2239

Pollard PJ, El-Bahrawy M, Poulsom R, Elia G, Killick P, Kelly G, Hunt T, Jeffery R, Seedhar P, Barwell J, Latif F, Gleeson MJ, Hodgson SV, Stamp GW, Tomlinson IP, Maher ER (2006) Expression of HIF-1alpha, HIF-2alpha (EPAS1), and their target genes in paraganglioma and pheochromocytoma with VHL and SDH mutations. J Clin Endocrinol Metab 91:4593–4598

Pollard PJ, Spencer-Dene B, Shukla D, Howarth K, Nye E, El-Bahrawy M, Deheragoda M, Joannou M, McDonald S, Martin A, Igarashi P, Varsani-Brown S, Rosewell I, Poulsom R, Maxwell P, Stamp GW, Tomlinson IP (2007) Targeted inactivation of fh1 causes proliferative renal cyst development and activation of the hypoxia pathway. Cancer Cell 11:311–319

Porcelli AM, Ghelli A, Ceccarelli C, Lang M, Cenacchi G, Capristo M, Pennisi LF, Morra I, Ciccarelli E, Melcarne A, Bartoletti-Stella A, Salfi N, Tallini G, Martinuzzi A, Carelli V, Attimonelli M, Rugolo M, Romeo G, Gasparre G (2010) The genetic and metabolic signature of oncocytic transformation implicates HIF1alpha destabilization. Hum Mol Genet 19:1019–1032

Quinlan CL, Orr AL, Perevoshchikova IV, Treberg JR, Ackrell BA, Brand MD (2012) Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions. J Biol Chem 287:27255–27264

Qutub AA, Popel AS (2008) Reactive oxygen species regulate hypoxia-inducible factor 1α differentially in cancer and ischemia. Mol Cell Biol 28:5106–5119

Raimundo N, Baysal BE, Shadel GS (2011) Revisiting the TCA cycle: signaling to tumor formation. Trends Mol Med 17:641–649

Ricci JE, Gottlieb RA, Green DR (2003) Caspase-mediated loss of mitochondrial function and generation of reactive oxygen species during apoptosis. J Cell Biol 160:65–75

Rohlena J, Dong LF, Kluckova K, Zobalova R, Goodwin J, Tilly D, Stursa J, Pecinova A, Philimonenko A, Hozak P, Banerjee J, Ledvina M, Sen CK, Houstek J, Coster MJ, Neuzil J (2011a) Mitochondrially targeted alpha-tocopheryl succinate is antiangiogenic: potential benefit against tumor angiogenesis but caution against wound healing. Antioxid Redox Signal 15:2923–2935

Rohlena J, Dong LF, Ralph SJ, Neuzil J (2011b) Anticancer drugs targeting the mitochondrial electron transport chain. Antioxid Redox Signal 15:2951–2974

Rubic T, Lametschwandtner G, Jost S, Hinteregger S, Kund J, Carballido-Perrig N, Schwarzler C, Junt T, Voshol H, Meingassner JG, Mao X, Werner G, Rot A, Carballido JM (2008) Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. Nat Immunol 9:1261–1269

Sapieha P, Sirinyan M, Hamel D, Zaniolo K, Joyal JS, Cho JH, Honore JC, Kermorvant-Duchemin E, Varma DR, Tremblay S, Leduc M, Rihakova L, Hardy P, Klein WH, Mu X, Mamer O, Lachapelle P, Di Polo A, Beausejour C, Andelfinger G, Mitchell G, Sennlaub F, Chemtob S (2008) The succinate receptor GPR91 in neurons has a major role in retinal angiogenesis. Nat Med 14:1067–1076

Schlisio S, Kenchappa RS, Vredeveld LC, George RE, Stewart R, Greulich H, Shahriari K, Nguyen NV, Pigny P, Dahia PL, Pomeroy SL, Maris JM, Look AT, Meyerson M, Peeper DS, Carter BD, Kaelin WG Jr (2008) The kinesin KIF1Bbeta acts downstream from EglN3 to induce apoptosis and is a potential 1p36 tumor suppressor. Genes Dev 22:884–893

Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E (2005) Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell 7:77–85

Selak MA, Duran RV, Gottlieb E (2006) Redox stress is not essential for the pseudo-hypoxic phenotype of succinate dehydrogenase deficient cells. Biochim Biophys Acta 1757:567–572

Senoo-Matsuda N, Yasuda K, Tsuda M, Ohkubo T, Yoshimura S, Nakazawa H, Hartman PS, Ishii N (2001) A defect in the cytochrome b large subunit in complex II causes both superoxide anion overproduction and abnormal energy metabolism in Caenorhabditis elegans. J Biol Chem 276:41553–41558

Simonnet H, Demont J, Pfeiffer K, Guenaneche L, Bouvier R, Brandt U, Schagger H, Godinot C (2003) Mitochondrial complex I is deficient in renal oncocytomas. Carcinogenesis 24:1461–1466

Slane BG, Aykin-Burns N, Smith BJ, Kalen AL, Goswami PC, Domann FE, Spitz DR (2006) Mutation of succinate dehydrogenase subunit C results in increased O2.-, oxidative stress, and genomic instability. Cancer Res 66:7615–7620

Smith EH, Janknecht R, Maher LJ 3rd (2007) Succinate inhibition of alpha-ketoglutarate-dependent enzymes in a yeast model of paraganglioma. Hum Mol Genet 16:3136–3148

Sun F, Huo X, Zhai Y, Wang A, Xu J, Su D, Bartlam M, Rao Z (2005) Crystal structure of mitochondrial respiratory membrane protein complex II. Cell 121:1043–1057

Szeto SS, Reinke SN, Sykes BD, Lemire BD (2007) Ubiquinone-binding site mutations in the Saccharomyces cerevisiae succinate dehydrogenase generate superoxide and lead to the accumulation of succinate. J Biol Chem 282:27518–27526

Toma I, Kang JJ, Sipos A, Vargas S, Bansal E, Hanner F, Meer E, Peti-Peterdi J (2008) Succinate receptor GPR91 provides a direct link between high glucose levels and renin release in murine and rabbit kidney. J Clin Invest 118:2526–2534

Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, Campos C, Fabius AWM, Lu C, Ward PS, Thompson CB, Kaufman A, Guryanova O, Levine R, Heguy A, Viale A, Morris LGT, Huse JT, Mellinghoff IK, Chan TA (2012) IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature 483:479–483

Uziel G, Ghezzi D, Zeviani M (2011) Infantile mitochondrial encephalopathy. Semin Fetal Neonatal Med 16:205–215

van Nederveen FH, Gaal J, Favier J, Korpershoek E, Oldenburg RA, de Bruyn EM, Sleddens HF, Derkx P, Riviere J, Dannenberg H, Petri BJ, Komminoth P, Pacak K, Hop WC, Pollard PJ, Mannelli M, Bayley JP, Perren A, Niemann S, Verhofstad AA, de Bruine AP, Maher ER, Tissier F, Meatchi T, Badoual C, Bertherat J, Amar L, Alataki D, Van Marck E, Ferrau F, Francois J, de Herder WW, Peeters MP, van Linge A, Lenders JW, Gimenez-Roqueplo AP, de Krijger RR, Dinjens WN (2009) An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. Lancet Oncol 10:764–771

Xiao M, Yang H, Xu W, Ma S, Lin H, Zhu H, Liu L, Liu Y, Yang C, Xu Y, Zhao S, Ye D, Xiong Y, Guan KL (2012) Inhibition of alpha-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. Genes Dev 26:1326–1338

Yeh IT, Lenci RE, Qin Y, Buddavarapu K, Ligon AH, Leteurtre E, Do Cao C, Cardot-Bauters C, Pigny P, Dahia PL (2008) A germline mutation of the KIF1B beta gene on 1p36 in a family with neural and nonneural tumors. Hum Genet 124:279–285

Zhuang Z, Yang C, Lorenzo F, Merino M, Fojo T, Kebebew E, Popovic V, Stratakis CA, Prchal JT, Pacak K (2012) Somatic HIF2A gain-of-function mutations in paraganglioma with polycythemia. N Engl J Med 367:922–930

Chapter 5
Exploiting Celecoxib in Cancer Therapy

Verena Jendrossek

Contents

Abstract Numerous studies demonstrate that the non-steroidal anti-inflammatory drug (NSAID) Celecoxib is an attractive lead compound for cancer therapy: Epidemiological studies suggest a lower incidence of colonic polyps in patients with the hereditary familiary adenomatous polyposis (FAP) syndrome and a decreased risk for colorectal, skin and other cancers upon continuous uptake of Celecoxib or related compounds. Moreover, preclinical investigations demonstrate promising anti-tumour activity of Celecoxib in a variety of human tumours. Celecoxib not only interferes with tumour initiation and tumour cell growth *in vitro* and *in vivo* but also increases the sensitivity of tumour cells to chemotherapy, radiotherapy, or chemoradiotherapy.

Cell cycle arrest, induction of apoptosis and anti-angiogenic effects contribute to the antineoplastic effects of Celecoxib. This chapter will focus on the molecular

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mechanisms of Celecoxib-induced apoptosis, in particular the ability of Celecoxib to activate the mitochondrial death pathway and to interfere with specific members of the Bcl-2 protein family and the apoptosis regulator Survivin. Moreover, the role of cyclooxygenase-2 (COX-2) in the regulation of Celecoxib-induced apoptosis will be discussed. Finally, some clinical aspects of the use of Celecoxib in cancer therapy will be highlighted.

Keywords Apoptosis • Bcl-2 proteins • Celecoxib • Chemoprevention • Combination therapy • Cyclooxygenase-2 • Cytosolic Ca^{2+} • ER-stress • Intrinsic pathway • Mcl-1 • Nonsteroidal anti-inflammatory drug • Protein kinase B/Akt • SERCA • Survivin • Cancer therapy

1 Introduction

During the last two decades, the implementation of the 'omics' technologies in cancer research has led to an enormous increase in knowledge about cancer biology and treatment resistance, offering numerous novel options for early detection and mechanism-based personalized treatment. These investigations also shed light on the enormous complexity of cancer as a disease as well as the considerable interindividual and intraindividual tumour heterogeneity. They also revealed that a given targeted agent will only be effective in patient subgroups with a specific molecular tumour subtype in which the drug target is deregulated and essential for tumour cell survival.

Celecoxib (Celebrex®, Onsenal®, Pfizer, New York, USA) is a member of the COXIB drug family. COXIBs belong to the most potent specific inhibitors of cyclooxygenase-2 (COX-2), an immediate early response protein that catalyses a critical step in the formation of certain prostaglandins from arachidonic acid. The primary rationale for using COX-2 inhibitors in cancer therapy was based on the observations that COX-2 is up-regulated during early carcinogenesis and that constitutively elevated COX-2 levels in tumour and stromal cells are associated with tumour progression, therapy resistance and poor prognosis (Williams et al. 1999; Gupta et al. 2000; Soslow et al. 2000; Denkert et al. 2004; Brown and DuBois 2005; Ladetto et al. 2005; Jamieson et al. 2011; Thiel et al. 2011; Mohammad et al. 2012). In line with that assumption, Celecoxib-mediated inhibition of COX-2 mostly reduced prostaglandin-mediated tumour growth, tumour neovascularization, and metastasis (Seno et al. 2002; Basu et al. 2005; Dandekar et al. 2005; Wang et al. 2008; Liu et al. 2009; Klenke et al. 2011; Ninomiya et al. 2012; Setia and Sanyal 2012; Xin et al. 2012). However, it turned out that Celecoxib is also effective in tumour cell systems being negative for COX-2 expression (Grosch et al. 2006; Chuang et al. 2008). Moreover, structural derivatives of Celecoxib not inhibiting COX-2 showed potent antineoplastic activity (Waskewich et al. 2002; Grosch et al. 2006; Chuang et al. 2008; Schonthal 2010). Suggested proteins involved in the COX-2-independent antineoplastic actions of Celecoxib and derivatives include 3-phosphoinositide-dependent kinase-1 (PDK-1) and its downstream target protein kinase B (Akt) (Hsu et al. 2000; Arico

et al. 2002; Zhu et al. 2004; Liu et al. 2008), cyclin-dependent kinase inhibitors and cyclins (Ding et al. 2008; Liu et al. 2011; Reed et al. 2011), the signal transducer and activator of transcription 3 (STAT3) (Reed et al. 2011), the anti-apoptotic proteins Survivin, Bcl-2 and Mcl-1 (Pyrko et al. 2006; Rudner et al. 2010; Bosch et al. 2011), the sarcoplasmic/endoplasmic reticulum calcium ATPase SERCA (Johnson et al. 2002), as well as c-myc (Sobolewski et al. 2011) and ß-catenin (Maier et al. 2005; Sakoguchi-Okada et al. 2007; Tuynman et al. 2008; Xia et al. 2010) (for a review see Grosch et al. 2006; Schonthal 2007).

A better understanding of the molecular determinants essential for the antineoplastic action of Celecoxib and of putative resistance mechanisms or compensatory pathways are required to define patient subgroups that may benefit from treatment with Celecoxib and its derivatives alone and in combination with classical cytotoxic chemotherapy, radiotherapy and targeted agents. This book chapter will focus on the molecular details of the pro-apoptotic action of Celecoxib and the relevance of these findings for cancer therapy.

2 Molecular Pathways of Celecoxib-Induced Apoptosis

Apoptosis is an evolutionary conserved death program that is critical for the maintenance of tissue homeostasis and constitutes an important tumour suppressor mechanism. Induction of apoptotic cell death is also a crucial component of the cytotoxic action of chemotherapy and radiotherapy. However, to survive the specific stress conditions within the tumour microenvironment tumour cells acquire genetic or epigenetic alterations that affect the expression or function of critical death promoting proteins or lead to the over-activation of survival signalling pathways thereby acquiring apoptosis resistance (Gatenby and Gillies 2008; Hanahan and Weinberg 2011). Therefore, there is great interest in the development of drugs that specifically induce cell death in apoptosis resistant tumour cells, or that enhance the efficacy of genotoxic therapies e.g. by modulating the cellular apoptosis threshold. Generally, apoptotic cell death is executed via two main pathways, the extrinsic, death receptor-dependent pathway and the intrinsic, mitochondrial pathway. Both pathways lead to the activation of specialized proteases, the caspases that cleave diverse cellular substrates to foster death execution (Timmer and Salvesen 2007) (Fig. 5.1).

There is accumulated evidence from preclinical investigations that Celecoxib and some of its derivatives potently induce apoptotic cell death in tumour cells and endothelial cells (Grosch et al. 2001; Leahy et al. 2002; Song et al. 2002; Jendrossek et al. 2003; Lin et al. 2004a, b; Zhang et al. 2007a, b; Ding et al. 2008; Schiffmann et al. 2008). Up to now, the molecular pathways of Celecoxib-induced apoptosis are still a matter of debate. Most reports demonstrate the activation of classical caspase-dependent apoptosis pathways in response to Celecoxib and its pro-apoptotic derivatives, and only individual reports suggest the involvement of caspase-independent apoptosis pathways (Johnson et al. 2005; Yacoub et al. 2006; Bosch et al. 2011), or alternative modes of cell inactivation such as autophagy (Gao et al. 2008).

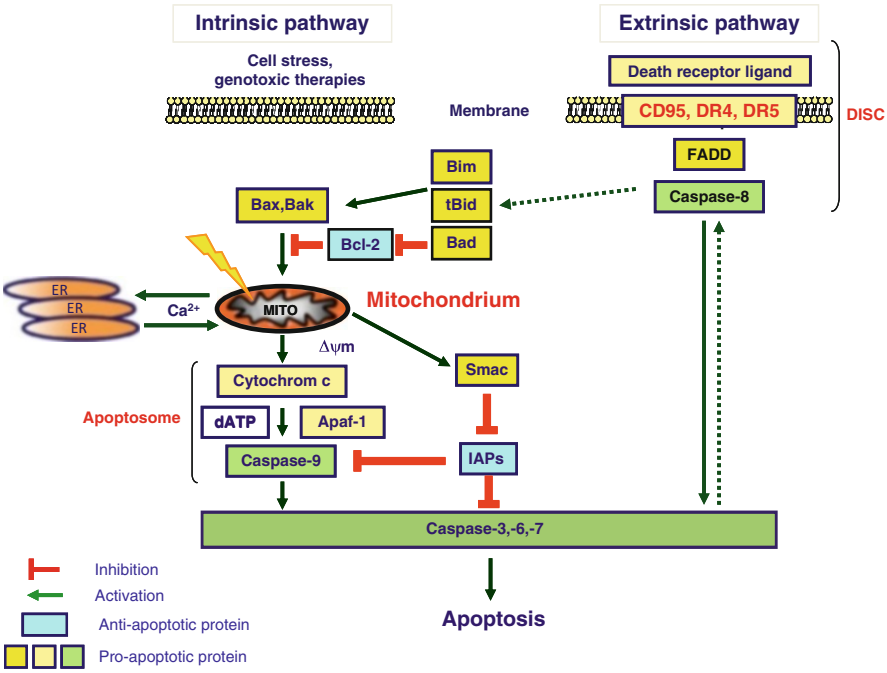

Fig. 5.1 Basic apoptosis signalling pathways. Apoptosis signalling is characterized by the sequential activation of initiator and effector caspases by the extrinsic, death receptor-dependent pathway and the intrinsic, mitochondrial pathway. Both pathways are interconnected at several levels (*dotted arrows*). The extrinsic apoptosis pathway is initiated by binding of so-called death receptor ligands (e.g. CD-95-ligand, Tumour necrosis factor (TNF) related apoptosis inducing ligand, TRAIL) to their receptors (e.g. CD95/Fas/APO-1, DR4/TRAIL-R1 or DR5/TRAIL-R2). Ligand-binding results in the multimerisation of the death receptors followed by the recruitment of the adaptor protein FADD (Fas-associated death domain) and of the initiator caspase procaspase-8 to form the so-called death-inducing signalling complex (DISC) at the cytoplasmic membrane. The induced proximity of the procaspase-8 molecules at the DISC triggers their activation by autoproteolytic cleavage and is a prerequisite for the subsequent proteolytic activation of the effector caspases-3, -6, -7 and cleavage of a multitude of downstream caspase-substrates to provoke apoptotic phenotype and cell death (Timmer and Salvesen 2007). The intrinsic, mitochondrial apoptosis pathway is mainly activated in response to cellular stress. Activation of this pathway involves multiple mitochondrial alterations including the breakdown of the mitochondrial membrane potential and the release of cytochrome c and further pro-apoptotic proteins into the cytosol such as the second mitochondria-derived activator of caspases/direct IAP binding Protein with Low PI (Smac/DIABLO). Calcium (Ca^{2+}) fluxes between the ER and the mitochondria as well as cytosolic Ca^{2+}-levels have been suggested as further important apoptosis regulators (Adams and Cory 2007; Youle and Strasser 2008). Cytosolic cytochrome c binds to Apaf-1 in a dATP-dependent manner to form a cytosolic multiprotein complex called 'apoptosome' that recruits and activates the initiator caspase procaspase-9. Caspase-9 then triggers proteolytic activation of the effector caspase cascade (caspases-3, -6, -7) and finally apoptosis. The intrinsic apoptosis pathway is mainly controlled by members of the Bcl-2 protein family at the level of the mitochondria and the ER (Adams and Cory 2007; Youle and Strasser 2008). The pro-apoptotic multidomain Bcl-2 family members Bax or Bak are critical for the induction of mitochondrial alterations and are therefore considered as central effectors of the intrinsic pathway. While anti-apoptotic Bcl-2 proteins (e.g. Bcl-2, Bcl-xL, Mcl-1 and A1) counteract the activation of Bax and Bak, the pro-apoptotic BH3-only proteins (e.g. Bim, Bad, Puma, NOXA, truncated Bid) promote apoptosis execution either by a direct interaction with

The following paragraphs will highlight current knowledge about the role of the extrinsic and the intrinsic apoptosis pathway as well as the role of the ER stress response in the execution of Celecoxib-induced apoptosis.

2.1 Role of the Extrinsic and Intrinsic Apoptosis Pathway for Celecoxib-Induced Apoptosis

Some early investigations about the molecular mechanisms of Celecoxib-induced apoptosis hint to a role of death receptor activation and the extrinsic pathway in apoptosis induced by Celecoxib and related compounds, at least in non-small cell lung cancer cells (NSCLC) and hepatocellular carcinoma (HCC) cells (Liu et al. 2004; Kern et al. 2006) (Fig. 5.2). In both cell types, Celecoxib-treatment led to the up-regulation of death receptors, activation of the caspase cascade and subsequent apoptosis. Silencing of DR5 or caspase-8 by RNAi (Liu et al. 2004) or overexpression of a dominant negative Fas-Associated Death Domain (FADD) mutant largely reduced Celecoxib-induced apoptosis in these cells (Liu et al. 2004; Kern et al. 2006). Celecoxib down-regulated the expression of cellular FLICE-inhibitory protein (c-FLIP), a negative regulator of the extrinsic apoptosis pathway, and over-expression of c-FLIP inhibited Celecoxib-induced apoptosis (Liu et al. 2006). On a mechanistic basis, Akt-independent inhibition of glycogen synthase kinase-3 by Celecoxib has recently been suggested to be responsible for c-FLIP degradation and apoptosis initiation (Chen et al. 2011).

Nevertheless, most reports including own data argue for a critical role of the intrinsic, mitochondrial pathway in the regulation of Celecoxib-induced apoptosis (Jendrossek et al. 2003; Ding et al. 2005; Kern et al. 2006; Tong et al. 2006; Sinha-Datta et al. 2008; Muller et al. 2008) (Fig. 5.3). This assumption is based on the following findings (i) Celecoxib-induced apoptosis involved breakdown of the mitochondrial membrane potential, release of cytochrome c and activation of caspase-9 (Jendrossek et al. 2003; Ding et al. 2005; Tong et al. 2006); (ii) Celecoxib-induced apoptosis was largely reduced in Apaf-1 deficient fibroblasts and in Jurkat cells expressing a dominant negative caspase-9 (Jendrossek et al. 2003); (iii) loss of FADD or caspase-8, two central effectors of the extrinsic apoptosis pathway, did almost not affect Celecoxib-induced apoptosis in B-cell and T-cell lymphopma cell lines, respectively (Jendrossek et al. 2003); (iv) in Bax-deficient Jurkat cells, the additional loss of Bak or

Fig. 5.1 (continued) Bax or Bak (direct activation model) (Kuwana et al. 2005; Certo et al. 2006), or by thereby releasing the Bax-like effector proteins from their anti-apoptotic counterparts (indirect activation model) (Chen et al. 2005; Willis et al. 2007). Of note, caspase-8 can also function as an effector caspase downstream of caspase-9 to allow amplification of signals originating from the intrinsic apoptosis pathway through cleavage of the pro-apoptotic BH3-only protein Bid to truncated Bid (tBid) (Belka et al. 2000). Caspase-8-mediated cleavage of the Bid also allows the amplification of caspase-signalling via the intrinsic apoptosis pathway in case of weak death receptor-dependent signalling events. Downstream of the DISC and of the apoptosome, inhibitors of apoptosis proteins (IAPs) such as X-IAP or Survivin impair apoptosis execution by inhibiting caspase-function. The anti-apoptotic activity of inhibitors of apoptosis proteins, e.g. Survivin, is suppressed by Smac/DIABLO upon its release from the mitochondrial intermembrane space

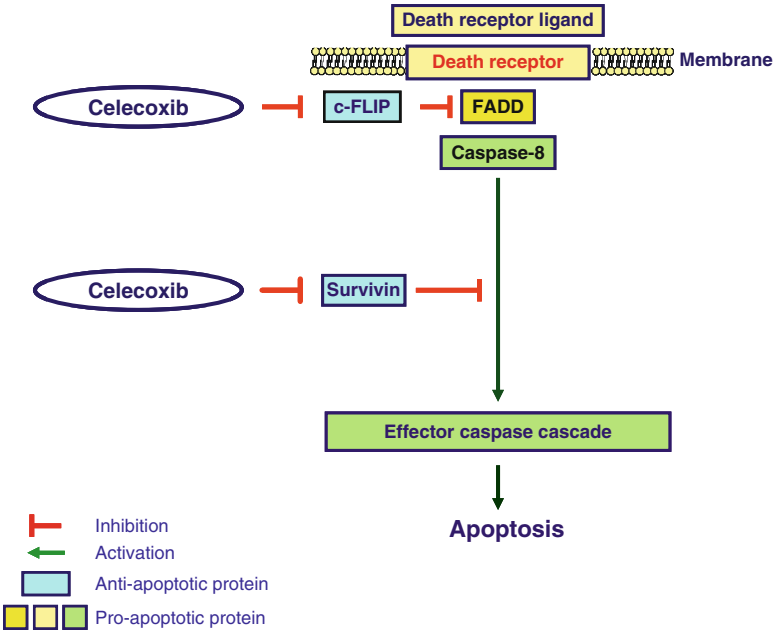

Fig. 5.2 Interaction of Celecoxib with the intrinsic pathway. Celecoxib-treatment triggers activation of the effector caspase cascade via the extrinsic pathway at the level of the DISC by up-regulation of death receptor expression or down-regulation of the expression of c-FLIP (cellular FLICE-inhibitory protein), a negative regulator of caspase-8, respectively. Celecoxib also facilitates apoptosis signalling through the extrinsic pathway by down-regulating Survivin, a member of the inhibitors of apoptosis proteins

siRNA-mediated down-regulation of Bak completely abrogated Celecoxib-induced apoptosis (Muller et al. 2008); (v) Celecoxib interfered with the phosphorylation state of PDK-1 and its downstream target Akt (Hsu et al. 2000; Kucab et al. 2005; Kulp et al. 2004; Wu et al. 2004). Since Akt has a major role in the regulation of the intrinsic pathway, the inhibitory action of Celecoxib on Akt adds another flavour to the effects of Celecoxib and derivatives on the intrinsic apoptosis pathway.

Of note the extrinsic and the intrinsic apoptosis pathway interact at multiple levels. On the one hand, cleavage of the pro-apoptotic BH3-only protein Bid downstream of caspase-8 can co-opt the intrinsic apoptosis pathway to amplify weak death receptor-dependent signalling events. On the other hand, caspase-8 can function as an effector caspase downstream of caspase-9-activation to amplify signals originating from the intrinsic apoptosis pathway (Belka et al. 2000). Finally, through Smac/DIABLO-mediated inhibition of IAPs, activation of the intrinsic apoptosis pathway impacts on the threshold to death receptor-dependent activation of the effector caspase cascade (Fig. 5.1). Therefore, the sensitivity of a given tumour cell to Celecoxib-induced apoptosis will largely depend on its molecular subtype, in particular the integrity of the extrinsic and the intrinsic apoptosis pathways and the expression level of molecules with impact on the death threshold at the level of the death inducing signalling complex (DISC) and the mitochondria, respectively.

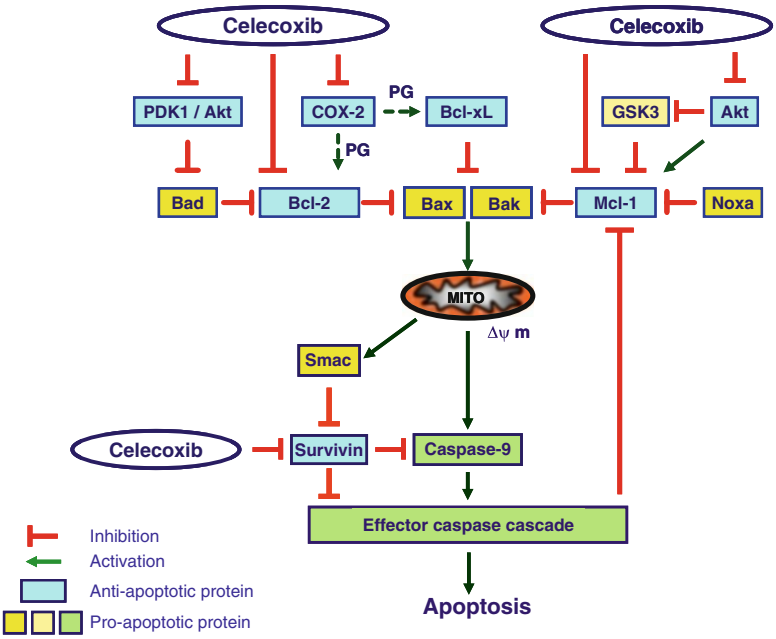

Fig. 5.3 Interaction of Celecoxib with the core mitochondrial pathway. Celecoxib potently induces apoptosis in Bcl-2-overexpressing cells. Celecoxib can down-regulate the expression of anti-apoptotic Bcl-2 and Bcl-xL by inhibition of cyclooxygenase (COX-2)-mediated prostaglandin (PG)-production. Alternatively, Celecoxib can neutralize the anti-apoptotic function of Bcl-2 by triggering an inactivating posttranslational modification of Bcl-2. Neutralization of Bcl-2 may also involve inhibition of the PDK-1/Akt pathway that is responsible for the inhibition of the pro-apoptotic BH3-only protein Bad by phosphorylation. Celecoxib-treatment decreases the levels of anti-apoptotic Mcl-1. This process may include inhibition of the novel synthesis of the short-lived Mcl-1 protein by inhibition of Akt/mammalian target of Rapamycin (mTOR)-mediated protein translation. Moreover, induction of GSK-3ß-activation as a consequence of Akt-inhibition may promote enhanced proteasomal degradation of Mcl-1 downstream of GSK-3ß. Alternatively, Mcl-1-degradation upon Celecoxib-treatment may also involve a secondary caspase-dependent mechanism. Altogether, these effects decrease the death threshold at the mitochondria. In addition, Celecoxib can promote apoptosis execution via the intrinsic pathway by facilitating caspase-activation through down-regulation of Survivin

2.2 Role of the ER-Stress Response

A number of reports suggest that induction of endoplasmic reticulum (ER) stress and the activation of the unfolded protein response (UPR) are major mechanisms for apoptosis initiation by Celecoxib (Tsutsumi et al. 2004; Alloza et al. 2006; Kardosh et al. 2008; Du et al. 2011; Winfield and Payton-Stewart 2012). Similar to Celecoxib, a Celecoxib-derivative without COX-2 inhibitory action called Dimethylcelecoxib (DMC), was also shown to trigger ER-stress; this suggests that the effects of Celecoxib at the ER occur independently from COX-2 inhibition (Pyrko et al. 2007). On a mechanistic basis, inhibition of the Ca^{2+}-ATPase SERCA at the ER by Celecoxib or DMC seem to be critical for the observed effects as it

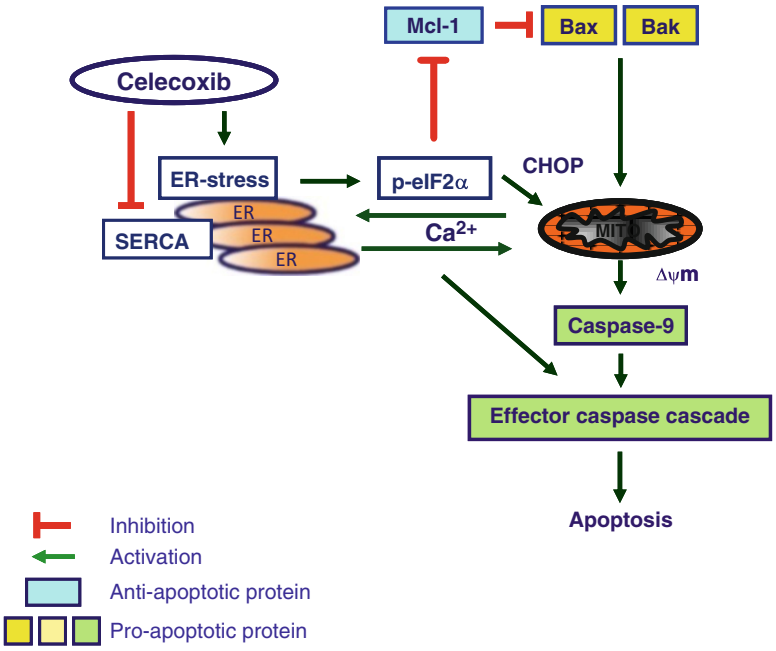

Fig. 5.4 Interaction of Celecoxib with the ER-stress response. Celecoxib-induced apoptosis involves induction of endoplasmic reticulum stress (ER-stress). Celecoxib-mediated inhibition of the Ca^{2+}-ATPase SERCA at the ER results in a rapid increase in intracellular Ca^{2+} and a Ca^{2+}-dependent phosphorylation of the eukaryotic translation initiation factor eIF2α. Phospho-eIF2α (p-eIF2α) can promote cellular protection by transiently inhibiting global protein translation and by stimulating the translation of proteins involved in cellular protection against injury. In case of failure to restore normal ER-function p-eIF2α can promote cell death by up-regulating proteins regulating apoptosis, e.g. the transcription factor c/EBP homologous transcription factor (CHOP). The Celecoxib-increase in the level of intracellular Ca^{2+} may also promote apoptosis by triggering the activation of Ca^{2+}-sensitive proteases, endonucleases and caspase-4

results in a rapid increase in intracellular Ca^{2+} and a Ca^{2+}-dependent phosphorylation of the eukaryotic initiation factor 2-kinase (PERK) and of the eukaryotic translation initiation factor eIF2α (Johnson et al. 2002; Tsutsumi et al. 2004; Pyrko et al. 2007) (Fig. 5.4). Phospho-eIF2α is a bifunctional stress response protein involved in the regulation of the UPR (Rasheva and Domingos 2009; Walter and Ron 2011), it can promote cellular protection by transiently inhibiting global protein translation to conserve cellular resources and by stimulating via activating transcription factor 4 (ATF4) the translation of proteins involved in cellular protection against injury, e.g. the ER chaperone GRP78 (glucose-regulated 78 kDa protein). Alternatively, in case of failure to restore normal ER-function phospho-eIF2α can promote cell death by up-regulating proteins regulating apoptosis, e.g. the transcription factor c/EBP homologous transcription factor (CHOP). There is evidence from multiple reports that Celecoxib-treatment induces an increase in the concentrations of cytosolic Ca^{2+}, accumulation of ATF4, GRP78 and CHOP, a transient inhibition of protein

translation and finally apoptosis (Tsutsumi et al. 2004; Kim et al. 2007; Pyrko et al. 2007). In this scenario, increased concentrations in cytosolic Ca^{2+} may also promote apoptosis by triggering the activation of Ca^{2+}-sensitive proteases, endonucleases, and caspase-4 (Tanaka et al. 2005; Kim et al. 2007; Pyrko et al. 2007; Kardosh et al. 2008). In addition, Celecoxib also inhibits L-type voltage-gated calcium channels in a COX-2-independent manner (Zhang et al. 2007a, b).

3 Role of COX-2 for the Regulation of Celecoxib-Induced Apoptosis

Initial investigations suggested a role of its target protein COX-2 in the regulation of Celecoxib-induced apoptosis. It had been speculated that Celecoxib inhibits COX-2-dependent formation of prostaglandins (PG) and thus PG-mediated up-regulation of anti-apoptotic proteins thereby decreasing the apoptotic threshold (Lin et al. 2001; Altorki et al. 2003; Oltersdorf et al. 2005; Mehar et al. 2008; Bai et al. 2010; Chun and Langenbach 2011) (Fig. 5.2).

Nowadays it is well accepted that Celecoxib is also able to induce apoptosis without an apparent involvement of its target protein COX-2: Numerous reports demonstrated that Celecoxib efficiently induces apoptosis in COX-2 negative cells and that apoptosis sensitivity is rather similar in COX-2-positive and COX-2-negative cell systems (Williams et al. 2000; Grosch et al. 2001; Waskewich et al. 2002; Muller et al. 2008). In line with these findings down-regulation of COX-2 by anti-sense constructs or RNAi did not affect apoptosis sensitivity of prostate cancer cells (Song et al. 2002) corroborating the assumption that sensitivity to Celecoxib-induced apoptosis is independent of the cellular COX-2 expression level. These findings are in good accordance with data showing that Celecoxib-derivatives without COX-2 inhibitory action, e.g. 2,5-dimethylcelecoxib (DMC) or OSU-03012, induce apoptosis in tumour cells with similar or higher potency compared to Celecoxib whereas other members of the COXIB family that inhibit COX-2 with similar or higher potency compared to Celecoxib lack pro-apoptotic activity (Song et al. 2002; Ding et al. 2005; Schonthal 2006; Pyrko et al. 2007; Schiffmann et al. 2008; Chuang et al. 2008). Meanwhile, multiple COX-2-independent targets of Celecoxib with a potential role in cell death regulation have been identified, e.g. SERCA, PDK-1, Akt, the pro-apoptotic proteins Bax and Hrk, and the anti-apoptotic proteins Survivin, Bcl-2 and Mcl-1 (Hsu et al. 2000; Wu et al. 2004; Grosch et al. 2006; Pyrko et al. 2006, 2007; Schonthal 2007; Rudner et al. 2010; Cervello et al. 2011). A recent gene expression profiling study in COX-2-positive and COX-2-negative hepatocellular carcinoma cells demonstrated that Celecoxib-treatment inhibits the NF-κB signalling network in both cell types and thus also independently of COX-2 (Cervello et al. 2011). The comparison of differentially regulated genes in COX-2-positive and COX-2-negative cells also revealed that other cellular processes were regulated in a cell type dependent manner involving COX-2-dependent

effects, e.g. altered expression of genes involved in small molecule, lipid, vitamin and mineral metabolism, and COX-2-independent effects, e.g. altered expression of genes involved in cell signalling (Cervello et al. 2011).

It has recently been reported that DMC, a Celecoxib-derivative without COX-2-inhibitory action, efficiently suppresses the production of PG, particularly PGE2, in different cancer cell lines (Wobst et al. 2008). The same group now demonstrated in HeLa that DMC inhibits PGE2 synthesis by inducing an inhibitory complex composed of histone deacetylase 1 (HDAC-1) and nuclear factor kappa B (NF-κB). This inhibitory complex bound to the EGR1 promoter resulting in down-regulation of EGR-1 expression and transcriptional inhibition of microsomal PGE-synthase-1 (mPGES-1) (Deckmann et al. 2010, 2012). Thus, inhibition of PG-mediated up-regulation of anti-apoptotic proteins may contribute to the pro-apoptotic action of Celecoxib-derivatives without COX-2 inhibitory action.

Altogether, these findings confirm that Celecoxib acts on numerous targets except of COX-2. Nevertheless, although COX-2-expression may not be essential for Celecoxib-induced apoptosis, it may well influence sensitivity of COX-2-expressing cells to apoptosis by up-regulation of proteins like Survivin or anti-apoptotic members of the Bcl-2 protein family that promote apoptosis resistance (Lin et al. 2001; Altorki et al. 2003; Komatsu et al. 2005; Bai et al. 2010) (Figs. 5.2 and 5.3). COX-2-inhibition may be of particular importance for the *in vivo* effects of Celecoxib in COX-2-positive tumours, because COX-2 expression in tumour and stromal cells is known to favour tumour progression and therapy resistance (Ko et al. 2002; Leahy et al. 2002; Seno et al. 2002; Amano et al. 2003; Chang et al. 2004; Oyama et al. 2005).

4 Role of Bcl-2 Proteins for the Activation Celecoxib-Induced Apoptosis at the Mitochondria

As outlined above, Celecoxib-induced apoptosis is mostly executed via the intrinsic apoptosis pathway. Therefore, it was not surprising that Celecoxib-induced apoptosis depends on the expression of Bax or Bak, the two central pro-apoptotic effector proteins of the intrinsic death pathway at the level of the mitochondria (Cianchi et al. 2006; Muller et al. 2008). Tumour cells respond Celecoxib by an increase in the expression of Bax or Bak (Cianchi et al. 2006; Kern et al. 2006; Fantappie et al. 2007; Kim et al. 2007), an increased activation-related conformational change of these proteins (Rudner et al. 2010), or both. Since Bax and Bak can substitute each other, the cellular expression level of Bax and Bak will dictate the cell-type specific dependency of Celecoxib-induced apoptosis from either Bax, Bak, or both (Wei et al. 2001; Hemmati et al. 2006; Reed 2006; Adams and Cory 2007). As an example, in Bax-deficient Jurkat cells, Bak was shown to be responsible for apoptosis-initiation in response to Celecoxib-treatment (Wang et al. 2001; Muller et al. 2008).

A unique characteristic of Celecoxib and its pro-apoptotic derivatives is the fact that overexpression of Bcl-2 does not block apoptosis initiation, suggesting a particular mechanism of drug action (Hsu et al. 2000; Jendrossek et al. 2003; Ding

et al. 2005; Johnson et al. 2005; Sinha-Datta et al. 2008; Muller et al. 2008; Rudner et al. 2010) (Fig. 5.3). Since up-regulated expression of Bcl-2 is a known determinant of chemotherapy and radiotherapy resistance of tumour cells, its Bcl-2-independent action makes Celecoxib an attractive compound for a use in the treatment of Bcl-2 overexpressing tumours.

Importantly, Celecoxib also counteracts the action of the anti-apoptotic Bcl-2 protein Mcl-1 in diverse cancer cells (Kern et al. 2006; Rudner et al. 2010). Celecoxib-induced down-regulation of Mcl-1 seems to include specific drugs effects upstream of caspase activation as well as caspase-dependent Mcl-1 degradation. This assumption is based on the finding that Mcl-1 degradation was observed in both, apoptosis-deficient and apoptosis-proficient Jurkat cells, although to a distinct extent (Rudner et al. 2010). Another regulator of Mcl-1 protein stability is Noxa, a BH-3 only protein that specifically binds to Mcl-1 and A1 but not Bcl-2 or Bcl-xL to neutralize their anti-apoptotic actions (Chen et al. 2005; Willis et al. 2005). Celecoxib-treatment did not increase the expression of Noxa, and Noxa-RNAi failed to alter the Mcl-1 levels in Jurkat cells. But Celecoxib-induced apoptosis in Jurkat cells was significantly reduced when Noxa was down-regulated by using RNAi. Thus, Noxa is most probably needed for the neutralization of Mcl-1 (Rudner et al. 2010). Finally, the levels of the short-lived Mcl-1-protein are controlled by the rates of novel synthesis and proteasomal degradation (Zhong et al. 2005). Mcl-1-expression is induced by growth factors and cytokines at the transcriptional level involving the phosphatidylinositol-3-kinase (PI3K)/Akt pathway, the Janus kinase pathway, and the mitogen activated protein kinase pathway (Kuo et al. 2001; Lin et al. 2001; Craig 2002; Opferman 2006). On the other hand, Mcl-1 levels are controlled at the posttranslational level: In the absence of active Akt, the Akt target protein glycogen synthase kinase-3 (GSK-3) is activated and triggers the Mcl-1 phosphorylation which is a prerequisite for its ubiquitination and proteasomal degradation (Maurer et al. 2006). Celecoxib may decrease cellular Mcl-1 levels by inhibiting novel synthesis via the PI3K/PDK1/Akt pathway or downstream of the ER-stress response (Figs. 5.3 and 5.4). Alternatively, Celecoxib may increase Mcl-1 degradation through activation of GSK-3 downstream of Akt-inhibition. Still, the relative importance of Noxa, Akt/GSK-3 and the ER-stress response for the regulation of Mcl-1 levels in Celecoxib-treated cells remains to be clarified.

Anti-apoptotic Mcl-1 is a critical determinant of cell survival in Jurkat cells (Zhou et al. 1998; Han et al. 2006; Sinha-Datta et al. 2008; Dai et al. 2009). Consequently, the Celecoxib-induced down-regulation of Mcl-1-levels was sufficient to induce Bak-dependent apoptosis in Jurkat cells, an effect that could be mimicked by Mcl-1 RNAi (Rudner et al. 2010). While overexpression of anti-apoptotic Bcl-2 or Bcl-xL protected Jurkat cells from death induced by Mcl-1 RNAi, only overexpression of Bcl-xL but not of the very similar Bcl-2 protected Jurkat cells from Celecoxib-induced mitochondrial alterations and apoptosis although down-regulation of Mcl-1 was still observed (Rudner et al. 2010). Thus, apart from targeting Mcl-1 Celecoxib must initiate signalling events that neutralize the anti-apoptotic action of Bcl-2, execute apoptosis independently of Bcl-2, or both. However, the molecular mechanisms of the Bcl-2-independent pro-apoptotic

actions of Celecoxib are still controversial and may depend on the cell type (Jendrossek et al. 2003; Subhashini et al. 2005; Chen et al. 2007; Reed et al. 2011). It has been hypothesized that Celecoxib compromises the anti-apoptotic action of Bcl-2 by down-regulation (Reed et al. 2011) or by inactivating phosphorylation of the protein (Yamamoto et al. 1999; Del Bello et al. 2001; Wei et al. 2008; Pattingre et al. 2009), by interaction with the nuclear receptor Nur77 (Lin et al. 2004a; Thompson and Winoto 2008; Rudner et al. 2010), or by activation of a specific BH3-only protein that preferentially interacts with Bcl-2 but not with Bcl-xL (Chen et al. 2005; Certo et al. 2006; Rudner et al. 2010). Yet own recent data revealed that only Bcl-xL efficiently sequesters pro-apoptotic Bak upon Celecoxib-treatment when overexpressed in Jurkat cells, whereas anti-apoptotic Bcl-2 failed to form high affinity complexes with pro-apoptotic Bak under similar conditions. The distinct potency of Bcl-2 and Bcl-xL to bind and inactivate Bak provides a sound explanation why only Bcl-xL but not Bcl-2 overcomes the loss of Mcl-1 in Celecoxib-treated Jurkat cells and protects Jurkat cells from Celecoxib-induced apoptosis (Rudner et al. 2011). These findings are in good accordance to earlier observations that the multidomain pro-apoptotic effector protein Bak is mainly kept in check by Mcl-1 and Bcl-xL, whereas Bcl-2 seems to be the predominant anti-apoptotic Bcl-2 protein in the protection from Bax-dependent apoptosis (Cuconati et al. 2003; Willis and Adams 2005; Willis et al. 2007; Zhai et al. 2008) (Fig. 5.3).

The Bcl-2-independent action and the prominent Mcl-1-antagonistic effects of Celecoxib suggest a potential benefit of Celecoxib for the treatment of apoptosis-resistant tumours that overexpress Bcl-2 and Mcl-1, although the relevance of the above-mentioned effects for clinical drug activity remains to be confirmed.

5 Survivin as Target for the Pro-apoptotic Action of Celecoxib

There is accumulated evidence that the inhibitors of apoptosis (IAP) protein family member Survivin is a major determinant of apoptosis resistance in malignant tumours and impairs apoptosis execution by inhibiting the activation of apoptosis-related caspases (Ambrosini et al. 1997; Yamamoto et al. 2008; Kelly et al. 2011) (Fig. 5.1). In addition, Survivin is known to participate in the regulation of mitosis during cell division (Altieri 2008). Preclinical investigations revealed that Celecoxib and its derivatives affect the levels of Survivin-expression in diverse cancer cells (Catalano et al. 2004; Pyrko et al. 2006; Fukada et al. 2007; Kardosh et al. 2007; Sakoguchi-Okada et al. 2007; Zhang et al. 2007a, b; Kim et al. 2010; Reed et al. 2011). Even more important, in some studies the potency of Celecoxib and DMC to inhibit tumour cell growth and to induce apoptosis correlated with their ability to down-modulate Survivin (Kardosh et al. 2005; Ferrario et al. 2011). Importantly, because caspase-activation is important for apoptosis execution upon activation of both, the extrinsic and the intrinsic apoptosis pathway, Survivin modulates apoptosis execution downstream of death receptor activation as well as downstream of the

mitochondria (Figs. 5.2 and 5.3). It is also highly likely that the inhibitory action of Celecoxib and derivatives on Survivin participates in their cytotoxic action as single drug or in combination with genotoxic chemotherapy or radiotherapy (Pyrko et al. 2006; Gaiser et al. 2008; Zhao et al. 2009) (Fig. 5.4). PG constitute important regulators of Survivin-expression; therefore, the down-regulation of Survivin in Celecoxib-treated tumour cells may be linked to drug-induced COX-2-inhibition although COX-2-independent effects have also been suggested (Sakoguchi-Okada et al. 2007; Mehar et al. 2008; Bai et al. 2010; Chun and Langenbach 2011).

6 Clinical Aspects of the Use of Celecoxib in Cancer Therapy

Multiple preclinical investigations demonstrate promising anti-tumour activity of Celecoxib and derivatives in a variety of human tumours including radioresistant glioblastoma-derived $CD133^+$ cells (Williams et al. 2000; Waskewich et al. 2002; El-Rayes et al. 2004; Johnsen et al. 2004; Muller et al. 2008; Zhao et al. 2009; Kim et al. 2010). Celecoxib and derivatives also increased the sensitivity of tumour cells to chemotherapy, radiotherapy, chemoradiotherapy or photodynamic therapy *in vitro* and *in vivo* (Milas et al. 1999; Hida et al. 2000; Trifan et al. 2002; Davis et al. 2004; Zhao et al. 2009; Ferrario et al. 2011; Kaneko et al. 2013).

Moreover, a variety of epidemiological studies reported that Celecoxib and related compounds reduce the number of colonic polyps in patients with the hereditary familiarly adenomatous polyposis (FAP) syndrome and potentially reduce the risk for cancers of the colon, breast, skin, oesophagus, and stomach (Steinbach et al. 2000; Phillips et al. 2002; Arber et al. 2006; Harris et al. 2007; Elmets et al. 2010; Arber et al. 2011) (Table 5.1). On the basis of these observations, Celecoxib had been approved for oral use to reduce the formation of colorectal polyps in patients with FAP as an adjunct to usual care, e.g. endoscopic surveillance and surgery. However, in April 2011, this indication was voluntarily withdrawn from the approved indications for the usage of Celecoxib on the US and European market. It had not been possible to timely prove neither the clinical benefit of its use in FAP patients nor the persistence of potential chemopreventive effects after discontinuation of Celecoxib-treatment, or the efficacy and safety beyond 6 months of treatment. A major reason for that was too slow recruitment of patients to the post-approval confirmatory phase III trial in paediatric patients with FAP 'CHIP' (Study A3191193). But the potential of Celecoxib in the prevention or treatment of advanced human tumours is still under intense investigation in multiple clinical studies (http://clinicaltrial.gov; Table 5.2). Most clinical studies demonstrate that Celecoxib can be safely administered in diverse cancers. However, the various responses point out that the clinical benefit of Celecoxib for individual cancer types warrants further investigation. In particular, it is required to define molecular biomarkers that are suited for the definition of patient subgroups likely to respond to Celecoxib-treatment.

Table 5.1 Selected prevention trials with Celecoxib

Patients	Treatment	Investigation	Results	Reference
Patients with familial adenomatous polyposis	Celecoxib (2×100 or 400 mg/kg daily) or placebo for 6 months	Prevention of colorectal polyps	Daily Celecoxib leads to a significant reduction in the number of colorectal polyps	Steinbach et al. (2000)
Patients with familial adenomatous polyposis	Celecoxib (2×100 or 400 mg/kg daily) or placebo for 6 months	Prevention of colorectal polyps	Patients with clinically significant disease at baseline show a significant reduction in the number of duodenal polyposis	Phillips et al. (2002)
Patients with diagnosed colorectal adenomas after polypectomy	Daily Celecoxib (400 mg/kg) for 1 or 3 years after polypectomy	Prevention of colorectal adenomas	Daily Celecoxib significantly reduced the occurrence of colorectal adenomas within 3 years after polypectomy	Arber et al. (2006)
Patients with Barrett's Oesophagus and low- or high-grade dysplasia	Celecoxib (2×200 mg/kg daily) or placebo for 48 weeks	Prevention of progression of Barrett's dysplasia to cancer	Daily Celecoxib for 48 weeks of treatment does not prevent progression of Barrett's dysplasia to cancer	Heath et al. (2007)
Children with FAP gene mutations	Celecoxib 16 mg/kg/day	Prevention of colorectal polyps	Celecoxib at a dose of 16 mg/kg/day is safe and well tolerated and reduced the number of colorectal polyps	Lynch et al. (2010)
Patients with ductal carcinoma in situ (DCIS)	Exemestane plus placebo or Celecoxib before surgery	Reduction in DCIS	Celecoxib had no beneficial effect on Exemestane-induced inhibition of proliferation in oestrogen receptor-positive DCIS	Bundred et al. (2010)
Patients with actinic keratosis	Celecoxib or Placebo	Prevention of non-melanoma skin cancer	No difference in the incidence of novel actinic keratosis but fewer melanoma skin cancers	Elmets et al. (2010)
Patients with diagnosed colorectal adenomas after polypectomy	3 years on Celecoxib plus 2 years off Celecoxib versus placebo	Prevention of colorectal sporadic adenomatous polyps	*Cumulative measure*: lower incidence of new and advanced adenomas; *Interval measure*: 2 years after cessation of Celecoxib the risk of developing adenomas increases; Side effects: Increased risk of renal/hypertension events and cardiac disorders	Arber et al. (2011)

Table 5.2 Selected clinical trials with Celecoxib

Cancer type	Treatment	Investigation	Results	Reference
Her2/Neu overexpressing metastatic breast cancer	Celecoxib (2×400 mg/kg daily) plus Trastuzumab	Phase II	Celecoxib in combination with Trastuzumab is well tolerated but without a beneficial effect	Dang et al. (2004)
Cancer patients with unresectable brain metastases	Celecoxib (400 mg/kg daily) concomitant to radiation (32 Gy)	Phase I/II	The concurrent treatment regimen was well tolerated. The overall response rate of 66.7 % suggests that radiotherapy plus Celecoxib is safe and a possible active treatment for patients with brain metastases	Cerchietti et al. (2005)
Locally advanced and unresectable non-small cell lung cancer	Celecoxib 5 days before and concomitant to radiation (63 Gy)	Phase I dose escalation study (daily 200–800 mg/kg Celecoxib)	Radiation with concurrent Celecoxib is safe and feasible with encouraging response rates	Liao et al. (2005)
Localized prostate cancer	Celecoxib (2×400 mg/kg daily) plus radiation (70–74 Gy)	Phase I (Celecoxib)	Celecoxib plus radiation is safe and feasible	Ganswindt et al. (2006)
PSA-recurrent prostate cancer after radiotherapy or prostatectomy	Celecoxib (2×400 mg/kg daily) concomitant to chemoradiotherapy	Phase II	Celecoxib can decrease serum PSA levels in patients with biochemical progression after radiotherapy or radical prostatectomy und may thus help to prevent or delay disease progression	Pruthi et al. (2006)

(continued)

Table 5.2 (continued)

Cancer type	Treatment	Investigation	Results	Reference
Locally advanced oesophageal cancer	Celecoxib (200–600 mg/kg daily) plus chemoradiotherapy (5-fluorouracil, cisplatin and radiotherapy with 50 or 50.4 Gy)	Determination of maximum tolerated dose (MTD)	Celecoxib plus chemoradiotherapy is safe and feasible; MTD was not reached	Dawson et al. (2007)
Resectable rectal cancer	Preoperative radiation (45 Gy) plus 5-FU and Celecoxib (2 ×400 mg/kg daily) or placebo	Phase II	The addition of Celecoxib to preoperative chemoradiation is feasible with a trend to an improved pathological response	Debucquoy et al. (2009)
Localized prostate cancer (T1-2 N0 M0)	Pre-surgical Celecoxib versus no drug	Gene expression profiling	Increased expression of genes involved in apoptosis and tumour suppressor function	Sooriakumaran et al. (2009)
Localized prostate cancer (T2b or greater; Gleason sum ≥ 7)	Pre-surgical Celecoxib versus no drug	Phase II; Biomarker evaluation	Measurable tissue levels of Celecoxib were achieved but the treatment had no effect on intermediate biomarkers of prostate carcinogenesis	Antonarakis et al. (2009)
Locoregionally advanced nasopharyngeal carcinoma	Celecoxib (400–800 mg/kg daily) 3 days before and concomitant to radiation (60 or 70–74 Gy)	Phase I (dose escalation study)	Radiation with concurrent Celecoxib is safe with promising response rates	Xue et al. (2011)
Carcinoma of the oesophagus and gastroesophageal junction	Preoperative Celecoxib and concurrent carboplatin and paclitaxel	Phase II	Preoperative Celecoxib with concurrent chemotherapy has a benefit in patients with tumours expressing COX-2 (trends toward improved response to preoperative therapy and improved overall survival) compared with non-expressors	Altorki et al. (2011)

Advanced non small lung cancer (stage IIIB–IV)	Celecoxib (2×400 mg/kg daily) plus palliative chemotherapy	Phase III	The addition of Celecoxib to palliative chemotherapy fails to give a survival benefit but reduced pain and increased quality of life. No cardiovascular events were observed	Koch et al. (2011)
Advanced non-small cell lung cancer (Stage IIIb–IV)	Chemotherapy (5 cycles carboplatin and docetaxel every 3 weeks) alone versus chemotherapy plus Celecoxib (2×400 mg/kg/day)	Phase III (NVALT-4)	Celecoxib does not improve survival in advanced non-small cell lung cancer. COX-2 was neither a prognostic nor a predictive biomarker. No increase in cardiovascular events was observed	Groen et al. (2011)
Hormone sensitive high risk prostate cancer	Hormone therapy alone versus hormone therapy plus Celecoxib (2×400 mg/kg daily; up to 1 year)	Phase II/III (STAMPEDE)	Celecoxib is insufficiently active in patients starting hormone therapy for high-risk prostate cancer. No evidence for differences in the incidence of adverse effects in both groups	James et al. (2012)

The increased risk for life-threatening cardiovascular side effects observed in patients receiving long-term treatment with specific COX-2 inhibitors of the COXIB family has also to be considered when using Celecoxib or its antineoplastic derivatives in cancer prevention or treatment (Fitzgerald 2004; Bresalier et al. 2005; Solomon et al. 2005). These side effects led to the withdrawal of FDA-approved COXIBs Rofecoxib and Valdecoxib from the market in 2004/2005. Luckily, COX-2 inhibition and pro-apoptotic action could be attributed to distinct structural motifs of the Celecoxib molecule so that pro-apoptotic derivatives without effects on COX-2 could be designed (Waskewich et al. 2002; Grosch et al. 2006; Chuang et al. 2008; Schonthal 2010). Since antineoplastic Celecoxib derivatives without COX-2 inhibitory action such as DMC and OSU-03012 have equal or even more pronounced anticancer activity compared to Celecoxib their use may reduce the risk of cardiovascular toxicity in the clinical situation (Zhu et al. 2002; Johnson et al. 2005; Kardosh et al. 2005; Schonthal et al. 2008; Jendrossek 2013). Nevertheless, Celecoxib may still be of value for a cautious use for the treatment of COX-2-expressing tumours as single drug or in combination with classical chemotherapeutic drugs or radiotherapy since COX-2-inhibition also participates in its antineoplastic effects (Ferrandina et al. 2002; Kim et al. 2002; Bundred and Barnes 2005; Ghosh et al. 2010). Fortunately, many clinical studies failed to report about an increase in adverse side effects when combining Celecoxib with chemotherapy or radiotherapy to treat cancer (Table 5.2).

7 Conclusions

The selective COX-2 inhibitor Celecoxib is a potent anti-cancer agent. Although inhibition of COX-2 can contribute to its cytotoxic effects, at least in COX-2-positive tumours, Celecoxib has the unique capacity among the COXIBs to induce apoptotic cell death in tumour cells independently of COX-2. Although Celecoxib-induced apoptosis is mainly executed via the intrinsic, mitochondrial apoptosis pathway, it is not inhibited by overexpression of Bcl-2. Moreover, Celecoxib triggers the down-regulation of the anti-apoptotic proteins Mcl-1 and Survivin. Thus, the molecular demands for the pro-apoptotic action of Celecoxib differ from those of standard genotoxic chemotherapy and radiotherapy offering the opportunity for improving the treatment outcome in patients with apoptosis-resistant tumours. In particular, neoplasms that depend on Bcl-2, Mcl-1, or Survivin for their survival may benefit from a treatment with Celecoxib alone or in combination with chemotherapy, radiotherapy (Fig. 5.5), or agents that target complementary apoptosis pathways. In tumours that are characterized by the additional up-regulation of Bcl-xL, it will be necessary to combine Celecoxib with drugs that counteract Bcl-xL, e.g. the Bad-like BH3-only-mimetics ABT-737 or ABT-263 (Oltersdorf et al. 2005; Huang and Sinicrope 2008).

Of course the success of a treatment with Celecoxib and derivatives will largely depend on the genetic background of the respective tumour cells and their

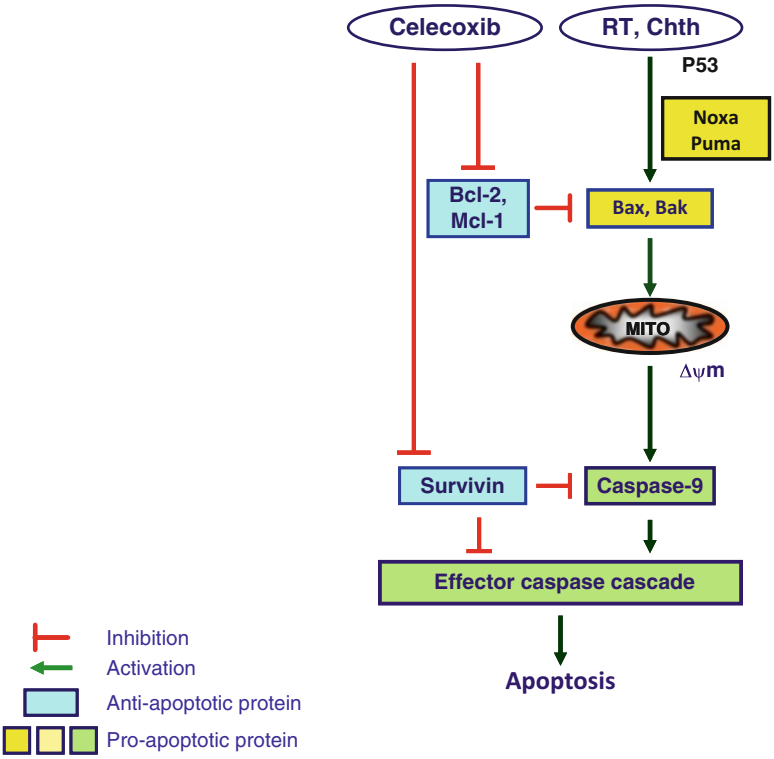

Fig. 5.5 Model of the action of Celecoxib in combination with genotoxic therapy and radiotherapy. Anti-apoptotic Bcl-2 proteins and Survivin mediate resistance to genotoxic chemotherapy (chth) and radiotherapy (RT). Celecoxib counteracts anti-apoptotic Mcl-1, Bcl-2, and Survivin by multiple mechanisms. By lowering the apoptotic threshold in Mcl-1/Bcl-2 overexpressing tumour cells Celecoxib can sensitize tumour cells to apoptosis induction by genotoxic chemotherapy and radiotherapy, that trigger apoptosis via p53-mediated up-regulation of pro-apoptotic BH3-only proteins of the Bcl-2 family and activation of Bax

surrounding stroma. For the definition of patients that may benefit from Celecoxib-treatment it will be of utmost importance that the molecular pathways that are essential for tumour growth and therapy resistance in those patients match the molecular determinants of the antineoplastic action of Celecoxib, eg. up-regulated expression of COX-2, Bcl-2 and Mcl-1 and others. For a pronounced and stable response it will be necessary to additionally target compensatory pathways, resistance-related mechanisms, or both. Moreover, because of potential cardiotoxicity, a strict surveillance and a meticulous monitoring of adverse side effects is highly recommended when using Celecoxib alone or in combination therapies to fight cancer. A safer alternative may be the use of Celecoxib derivatives that lack its COX-2 inhibitory action but induce apoptosis with even higher efficacy such as DMC.

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References

Adams JM, Cory S (2007) The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 26:1324–1337

Alloza I, Baxter A, Chen Q, Matthiesen R, Vandenbroeck K (2006) Celecoxib inhibits interleukin-12 alphabeta and beta2 folding and secretion by a novel COX2-independent mechanism involving chaperones of the endoplasmic reticulum. Mol Pharmacol 69:1579–1587

Altieri DC (2008) New wirings in the survivin networks. Oncogene 27:6276–6284

Altorki NK, Keresztes RS, Port JL, Libby DM, Korst RJ, Flieder DB, Ferrara CA, Yankelevitz DF, Subbaramaiah K, Pasmantier MW, Dannenberg AJ (2003) Celecoxib, a selective cyclo-oxygenase-2 inhibitor, enhances the response to preoperative paclitaxel and carboplatin in early-stage non-small-cell lung cancer. J Clin Oncol 21:2645–2650

Altorki NK, Christos P, Port JL, Lee PC, Mirza F, Spinelli C, Keresztes R, Beneck D, Paul S, Stiles BM, Zhang Y, Schrump DS (2011) Preoperative taxane-based chemotherapy and celecoxib for carcinoma of the esophagus and gastroesophageal junction: results of a phase 2 trial. J Thorac Oncol 6(2011):1121–1127

Amano H, Hayashi I, Endo H, Kitasato H, Yamashina S, Maruyama T, Kobayashi M, Satoh K, Narita M, Sugimoto Y, Murata T, Yoshimura H, Narumiya S, Majima M (2003) Host prostaglandin E(2)-EP3 signaling regulates tumor-associated angiogenesis and tumor growth. J Exp Med 197:221–232

Ambrosini G, Adida C, Altieri DC (1997) A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 3:917–921

Antonarakis ES, Heath EI, Walczak JR, Nelson WG, Fedor H, De Marzo AM, Zahurak ML, Piantadosi S, Dannenberg AJ, Gurganus RT, Baker SD, Parnes HL, DeWeese TL, Partin AW, Carducci MA (2009) Phase II, randomized, placebo-controlled trial of neoadjuvant celecoxib in men with clinically localized prostate cancer: evaluation of drug-specific biomarkers. J Clin Oncol 27(30):4986–4993

Arber N, Eagle CJ, Spicak J, Racz I, Dite P, Hajer J, Zavoral M, Lechuga MJ, Gerletti P, Tang J, Rosenstein RB, Macdonald K, Bhadra P, Fowler R, Wittes J, Zauber AG, Solomon SD, Levin B (2006) Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med 355:885–895

Arber N, Spicak J, Racz I, Zavoral M, Breazna A, Gerletti P, Lechuga MJ, Collins N, Rosenstein RB, Eagle CJ, Levin B (2011) Five-year analysis of the prevention of colorectal sporadic adenomatous polyps trial. Am J Gastroenterol 106:1135–1146

Arico S, Pattingre S, Bauvy C, Gane P, Barbat A, Codogno P, Ogier-Denis E (2002) Celecoxib induces apoptosis by inhibiting 3-phosphoinositide-dependent protein kinase-1 activity in the human colon cancer HT-29 cell line. J Biol Chem 277:27613–27621

Bai XM, Jiang H, Ding JX, Peng T, Ma J, Wang YH, Zhang L, Zhang H, Leng J (2010) Prostaglandin E2 upregulates survivin expression via the EP1 receptor in hepatocellular carcinoma cells. Life Sci 86:214–223

Basu GD, Pathangey LB, Tinder TL, Gendler SJ, Mukherjee P (2005) Mechanisms underlying the growth inhibitory effects of the cyclo-oxygenase-2 inhibitor celecoxib in human breast cancer cells. Breast Cancer Res 7:R422–R435

Belka C, Rudner J, Wesselborg S, Stepczynska A, Marini P, Lepple-Wienhues A, Faltin H, Bamberg M, Budach W, Schulze-Osthoff K (2000) Differential role of caspase-8 and BID activation during radiation- and CD95-induced apoptosis. Oncogene 19:1181–1190

Bosch R, Dieguez-Gonzalez R, Cespedes MV, Parreno M, Pavon MA, Granena A, Sierra J, Mangues R, Casanova I (2011) A novel inhibitor of focal adhesion signaling induces caspase-independent cell death in diffuse large B-cell lymphoma. Blood 118:4411–4420

Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanas A, Konstam MA, Baron JA (2005) Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med 352:1092–1102

Brown JR, DuBois RN (2005) COX-2: a molecular target for colorectal cancer prevention. J Clin Oncol 23:2840–2855

Bundred NJ, Barnes NL (2005) Potential use of COX-2-aromatase inhibitor combinations in breast cancer. Br J Cancer 93:S10–S15

Bundred NJ, Cramer A, Morris J, Renshaw L, Cheung KL, Flint P, Johnson R, Young O, Landberg G, Grassby S, Turner L, Baildam A, Barr L, Dixon JM (2010) Cyclooxygenase-2 inhibition does not improve the reduction in ductal carcinoma in situ proliferation with aromatase inhibitor therapy: results of the ERISAC randomized placebo-controlled trial. Clin Cancer Res 16:1605–1612

Catalano A, Graciotti L, Rinaldi L, Raffaelli G, Rodilossi S, Betta P, Gianni W, Amoroso S, Procopio A (2004) Preclinical evaluation of the nonsteroidal anti-inflammatory agent celecoxib on malignant mesothelioma chemoprevention. Int J Cancer 109:322–328

Cerchietti LC, Bonomi MR, Navigante AH, Castro MA, Cabalar ME, Roth BM (2005) Phase I/II study of selective cyclooxygenase-2 inhibitor celecoxib as a radiation sensitizer in patients with unresectable brain metastases. J Neurooncol 71:73–81

Certo M, Del Gaizo Moore V, Nishino M, Wei G, Korsmeyer S, Armstrong SA, Letai A (2006) Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. Cancer Cell 9:351–365

Cervello M, Bachvarov D, Cusimano A, Sardina F, Azzolina A, Lampiasi N, Giannitrapani L, McCubrey JA, Montalto G (2011) COX-2-dependent and COX-2-independent mode of action of celecoxib in human liver cancer cells. OMICS 15:383–392

Chang SH, Liu CH, Conway R, Han DK, Nithipatikom K, Trifan OC, Lane TF, Hla T (2004) Role of prostaglandin E2-dependent angiogenic switch in cyclooxygenase 2-induced breast cancer progression. Proc Natl Acad Sci U S A 101:591–596

Chen L, Willis SN, Wei A, Smith BJ, Fletcher JI, Hinds MG, Colman PM, Day CL, Adams JM, Huang DC (2005) Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol Cell 17:393–403

Chen JC, Chen Y, Su YH, Tseng SH (2007) Celecoxib increased expression of 14-3-3sigma and induced apoptosis of glioma cells. Anticancer Res 27:2547–2554

Chen S, Cao W, Yue P, Hao C, Khuri FR, Sun SY (2011) Celecoxib promotes c-FLIP degradation through Akt-independent inhibition of GSK3. Cancer Res 71:6270–6281

Chuang HC, Kardosh A, Gaffney KJ, Petasis NA, Schonthal AH (2008) COX-2 inhibition is neither necessary nor sufficient for celecoxib to suppress tumor cell proliferation and focus formation in vitro. Mol Cancer 7:38

Chun KS, Langenbach R (2011) The prostaglandin E2 receptor, EP2, regulates survivin expression via an EGFR/STAT3 pathway in UVB-exposed mouse skin. Mol Carcinog 50:439–448

Cianchi F, Cortesini C, Magnelli L, Fanti E, Papucci L, Schiavone N, Messerini L, Vannacci A, Capaccioli S, Perna F, Lulli M, Fabbroni V, Perigli G, Bechi P, Masini E (2006) Inhibition of 5-lipoxygenase by MK886 augments the antitumor activity of celecoxib in human colon cancer cells. Mol Cancer Ther 5:2716–2726

Craig RW (2002) MCL1 provides a window on the role of the BCL2 family in cell proliferation, differentiation and tumorigenesis. Leukemia 16:444–454

Cuconati A, Mukherjee C, Perez D, White E (2003) DNA damage response and MCL-1 destruction initiate apoptosis in adenovirus-infected cells. Genes Dev 17:2922–2932

Dai H, Meng XW, Lee SH, Schneider PA, Kaufmann SH (2009) Context-dependent Bcl-2/Bak interactions regulate lymphoid cell apoptosis. J Biol Chem 284:18311–18322

Dandekar DS, Lopez M, Carey RI, Lokeshwar BL (2005) Cyclooxygenase-2 inhibitor celecoxib augments chemotherapeutic drug-induced apoptosis by enhancing activation of caspase-3 and -9 in prostate cancer cells. Int J Cancer 115:484–492

Dang CT, Dannenberg AJ, Subbaramaiah K, Dickler MN, Moasser MM, Seidman AD, D'Andrea GM, Theodoulou M, Panageas KS, Norton L, Hudis CA (2004) Phase II study of celecoxib and trastuzumab in metastatic breast cancer patients who have progressed after prior trastuzumab-based treatments. Clin Cancer Res 10:4062–4067

Davis TW, O'Neal JM, Pagel MD, Zweifel BS, Mehta PP, Heuvelman DM, Masferrer JL (2004) Synergy between celecoxib and radiotherapy results from inhibition of cyclooxygenase-2-derived prostaglandin E2, a survival factor for tumor and associated vasculature. Cancer Res 64:279–285

Dawson SJ, Michael M, Biagi J, Foo KF, Jefford M, Ngan SY, Leong T, Hui A, Milner AD, Thomas RJ, Zalcberg JR (2007) A phase I/II trial of celecoxib with chemotherapy and radiotherapy in the treatment of patients with locally advanced oesophageal cancer. Invest New Drugs 25:123–129

Debucquoy A, Roels S, Goethals L, Libbrecht L, Van Cutsem E, Geboes K, Penninckx F, D'Hoore A, McBride WH, Haustermans K (2009) Double blind randomized phase II study with radiation+5-fluorouracil+/-celecoxib for resectable rectal cancer. Radiother Oncol 93:273–278

Deckmann K, Rorsch F, Steri R, Schubert-Zsilavecz M, Geisslinger G, Grosch S (2010) Dimethylcelecoxib inhibits mPGES-1 promoter activity by influencing EGR1 and NF-kappaB. Biochem Pharmacol 80:1365–1372

Deckmann K, Rorsch F, Geisslinger G, Grosch S (2012) Dimethylcelecoxib induces an inhibitory complex consisting of HDAC1/NF-kappaB(p65)RelA leading to transcriptional downregulation of mPGES-1 and EGR1. Cell Signal 24:460–467

Del Bello B, Valentini MA, Zunino F, Comporti M, Maellaro E (2001) Cleavage of Bcl-2 in oxidant- and cisplatin-induced apoptosis of human melanoma cells. Oncogene 20:4591–4595

Denkert C, Winzer KJ, Hauptmann S (2004) Prognostic impact of cyclooxygenase-2 in breast cancer. Clin Breast Cancer 4:428–433

Ding H, Han C, Zhu J, Chen CS, D'Ambrosio SM (2005) Celecoxib derivatives induce apoptosis via the disruption of mitochondrial membrane potential and activation of caspase 9. Int J Cancer 113:803–810

Ding H, Han C, Guo D, Wang D, Duan W, Chen CS, D'Ambrosio SM (2008) Sensitivity to the non-COX inhibiting celecoxib derivative, OSU03012, is p21(WAF1/CIP1) dependent. Int J Cancer 123:2931–2938

Du H, Li W, Wang Y, Chen S, Zhang Y (2011) Celecoxib induces cell apoptosis coupled with up-regulation of the expression of VEGF by a mechanism involving ER stress in human colorectal cancer cells. Oncol Rep 26:495–502

Elmets CA, Viner JL, Pentland AP, Cantrell W, Lin HY, Bailey H, Kang S, Linden KG, Heffernan M, Duvic M, Richmond E, Elewski BE, Umar A, Bell W, Gordon GB (2010) Chemoprevention of nonmelanoma skin cancer with celecoxib: a randomized, double-blind, placebo-controlled trial. J Natl Cancer Inst 102:1835–1844

El-Rayes BF, Ali S, Sarkar FH, Philip PA (2004) Cyclooxygenase-2-dependent and -independent effects of celecoxib in pancreatic cancer cell lines. Mol Cancer Ther 3:1421–1426

Fantappie O, Solazzo M, Lasagna N, Platini F, Tessitore L, Mazzanti R (2007) P-glycoprotein mediates celecoxib-induced apoptosis in multiple drug-resistant cell lines. Cancer Res 67:4915–4923

Ferrandina G, Lauriola L, Distefano MG, Zannoni GF, Gessi M, Legge F, Maggiano N, Mancuso S, Capelli A, Scambia G, Ranelletti FO (2002) Increased cyclooxygenase-2 expression is associated with chemotherapy resistance and poor survival in cervical cancer patients. J Clin Oncol 20:973–981

Ferrario A, Lim S, Xu F, Luna M, Gaffney KJ, Petasis NA, Schonthal AH, Gomer CJ (2011) Enhancement of photodynamic therapy by 2,5-dimethyl celecoxib, a non-cyclooxygenase-2 inhibitor analog of celecoxib. Cancer Lett 304:33–40

Fitzgerald GA (2004) Coxibs and cardiovascular disease. N Engl J Med 351:1709–1711

Fukada K, Takahashi-Yanaga F, Sakoguchi-Okada N, Shiraishi F, Miwa Y, Morimoto S, Sasaguri T (2007) Celecoxib induces apoptosis by inhibiting the expression of survivin in HeLa cells. Biochem Biophys Res Commun 357:1166–1171

Gaiser T, Becker MR, Habel A, Reuss DE, Ehemann V, Rami A, Siegelin MD (2008) TRAIL-mediated apoptosis in malignant glioma cells is augmented by celecoxib through proteasomal degradation of survivin. Neurosci Lett 442:109–113

Ganswindt U, Budach W, Jendrossek V, Bamberg M, Belka C (2006) Combination of celecoxib with percutaneous radiotherapy in patients with localised prostate cancer – a phase I study. Radiat Oncol 1:9

Gao M, Yeh PY, Lu YS, Hsu CH, Chen KF, Lee WC, Feng WC, Chen CS, Kuo ML, Cheng AL (2008) OSU-03012, a novel celecoxib derivative, induces reactive oxygen species-related autophagy in hepatocellular carcinoma. Cancer Res 68:9348–9357

Gatenby RA, Gillies RJ (2008) A microenvironmental model of carcinogenesis. Nat Rev Cancer 8:56–61

Ghosh N, Chaki R, Mandal V, Mandal SC (2010) COX-2 as a target for cancer chemotherapy. Pharmacol Rep 62:233–244

Groen HJ, Sietsma H, Vincent A, Hochstenbag MM, van Putten JW, van den Berg A, Dalesio O, Biesma B, Smit HJ, Termeer A, Hiltermann TJ, van den Borne BE, Schramel FM (2011) Randomized, placebo-controlled phase III study of docetaxel plus carboplatin with celecoxib and cyclooxygenase-2 expression as a biomarker for patients with advanced non-small-cell lung cancer: the NVALT-4 study. J Clin Oncol 29:4320–4326

Grosch S, Tegeder I, Niederberger E, Brautigam L, Geisslinger G (2001) COX-2 independent induction of cell cycle arrest and apoptosis in colon cancer cells by the selective COX-2 inhibitor celecoxib. Faseb J 15:2742–2744

Grosch S, Maier TJ, Schiffmann S, Geisslinger G (2006) Cyclooxygenase-2 (COX-2)-independent anticarcinogenic effects of selective COX-2 inhibitors. J Natl Cancer Inst 98:736–747

Gupta S, Srivastava M, Ahmad N, Bostwick DG, Mukhtar H (2000) Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma. Prostate 42:73–78

Han J, Goldstein LA, Gastman BR, Rabinowich H (2006) Interrelated roles for Mcl-1 and BIM in regulation of TRAIL-mediated mitochondrial apoptosis. J Biol Chem 281:10153–10163

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674

Harris RE, Beebe-Donk J, Alshafie GA (2007) Cancer chemoprevention by cyclooxygenase 2 (COX-2) blockade: results of case control studies. Subcell Biochem 42:193–212

Heath EI, Canto MI, Piantadosi S, Montgomery E, Weinstein WM, Herman JG, Dannenberg AJ, Yang VW, Shar AO, Hawk E, Forastiere AA (2007) Secondary chemoprevention of Barrett's esophagus with celecoxib: results of a randomized trial. J Natl Cancer Inst 99:545–557

Hemmati PG, Guner D, Gillissen B, Wendt J, von Haefen C, Chinnadurai G, Dorken B, Daniel PT (2006) Bak functionally complements for loss of Bax during p14ARF-induced mitochondrial apoptosis in human cancer cells. Oncogene 25:6582–6594

Hida T, Kozaki K, Muramatsu H, Masuda A, Shimizu S, Mitsudomi T, Sugiura T, Ogawa M, Takahashi T (2000) Cyclooxygenase-2 inhibitor induces apoptosis and enhances cytotoxicity of various anticancer agents in non-small cell lung cancer cell lines. Clin Cancer Res 6:2006–2011

Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, Chen CS (2000) The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. J Biol Chem 275:11397–11403

Huang S, Sinicrope FA (2008) BH3 mimetic ABT-737 potentiates TRAIL-mediated apoptotic signaling by unsequestering Bim and Bak in human pancreatic cancer cells. Cancer Res 68:2944–2951

James ND, Sydes MR, Mason MD, Clarke NW, Anderson J, Dearnaley DP, Dwyer J, Jovic G, Ritchie AW, Russell JM, Sanders K, Thalmann GN, Bertelli G, Birtle AJ, O'Sullivan JM, Protheroe A, Sheehan D, Srihari N, Parmar MK (2012) Celecoxib plus hormone therapy versus hormone therapy alone for hormone-sensitive prostate cancer: first results from the STAMPEDE multiarm, multistage, randomised controlled trial. Lancet Oncol 13:549–558

Jamieson NB, Carter CR, McKay CJ, Oien KA (2011) Tissue biomarkers for prognosis in pancreatic ductal adenocarcinoma: a systematic review and meta-analysis. Clin Cancer Res 17:3316–3331

Jendrossek (2013) Targeting apoptosis pathways by Celecoxib in cancer. Cancer Lett 332(2):313–324

Jendrossek V, Handrick R, Belka C (2003) Celecoxib activates a novel mitochondrial apoptosis signaling pathway. FASEB J 17:1547–1549

Johnsen JI, Lindskog M, Ponthan F, Pettersen I, Elfman L, Orrego A, Sveinbjornsson B, Kogner P (2004) Cyclooxygenase-2 is expressed in neuroblastoma, and nonsteroidal anti-inflammatory drugs induce apoptosis and inhibit tumor growth in vivo. Cancer Res 64:7210–7215

Johnson AJ, Hsu AL, Lin HP, Song X, Chen CS (2002) The cyclo-oxygenase-2 inhibitor celecoxib perturbs intracellular calcium by inhibiting endoplasmic reticulum Ca2+-ATPases: a plausible link with its anti-tumour effect and cardiovascular risks. Biochem J 366:831–837

Johnson AJ, Smith LL, Zhu J, Heerema NA, Jefferson S, Mone A, Grever M, Chen CS, Byrd JC (2005) A novel celecoxib derivative, OSU03012, induces cytotoxicity in primary CLL cells and transformed B-cell lymphoma cell line via a caspase- and Bcl-2-independent mechanism. Blood 105:2504–2509

Kaneko et al (2013) Enhanced antitumor effect of lower-dose and longer-term CPT-11 treatment in combination with low-dose celecoxib against neuroblastoma xenografts. Int J Clin Oncol 8(1):116–125

Kardosh A, Soriano N, Liu YT, Uddin J, Petasis NA, Hofman FM, Chen TC, Schonthal AH (2005) Multitarget inhibition of drug-resistant multiple myeloma cell lines by dimethyl-celecoxib (DMC), a non-COX-2 inhibitory analog of celecoxib. Blood 106:4330–4338

Kardosh A, Soriano N, Pyrko P, Liu YT, Jabbour M, Hofman FM, Schonthal AH (2007) Reduced survivin expression and tumor cell survival during chronic hypoxia and further cytotoxic enhancement by the cyclooxygenase-2 inhibitor celecoxib. J Biomed Sci 14:647–662

Kardosh A, Golden EB, Pyrko P, Uddin J, Hofman FM, Chen TC, Louie SG, Petasis NA, Schonthal AH (2008) Aggravated endoplasmic reticulum stress as a basis for enhanced glioblastoma cell killing by bortezomib in combination with celecoxib or its non-coxib analogue, 2,5-dimethyl-celecoxib. Cancer Res 68:843–851

Kelly RJ, Lopez-Chavez A, Citrin D, Janik JE, Morris JC (2011) Impacting tumor cell-fate by targeting the inhibitor of apoptosis protein survivin. Mol Cancer 10:35

Kern MA, Haugg AM, Koch AF, Schilling T, Breuhahn K, Walczak H, Fleischer B, Trautwein C, Michalski C, Schulze-Bergkamen H, Friess H, Stremmel W, Krammer PH, Schirmacher P, Muller M (2006) Cyclooxygenase-2 inhibition induces apoptosis signaling via death receptors and mitochondria in hepatocellular carcinoma. Cancer Res 66:7059–7066

Kim YB, Kim GE, Cho NH, Pyo HR, Shim SJ, Chang SK, Park HC, Suh CO, Park TK, Kim BS (2002) Overexpression of cyclooxygenase-2 is associated with a poor prognosis in patients with squamous cell carcinoma of the uterine cervix treated with radiation and concurrent chemotherapy. Cancer 95:531–539

Kim SH, Hwang CI, Juhnn YS, Lee JH, Park WY, Song YS (2007) GADD153 mediates celecoxib-induced apoptosis in cervical cancer cells. Carcinogenesis 28:223–231

Kim YY, Lee EJ, Kim YK, Kim SM, Park JY, Myoung H, Kim MJ (2010) Anti-cancer effects of celecoxib in head and neck carcinoma. Mol Cells 29:185–194

Klenke FM, Abdollahi A, Bischof M, Gebhard MM, Ewerbeck V, Huber PE, Sckell A (2011) Celecoxib enhances radiation response of secondary bone tumors of a human non-small cell lung cancer via antiangiogenesis in vivo. Strahlenther Onkol 187:45–51

Ko SC, Chapple KS, Hawcroft G, Coletta PL, Markham AF, Hull MA (2002) Paracrine cyclooxygenase-2-mediated signalling by macrophages promotes tumorigenic progression of intestinal epithelial cells. Oncogene 21:7175–7186

Koch A, Bergman B, Holmberg E, Sederholm C, Ek L, Kosieradzki J, Lamberg K, Thaning L, Ydreborg SO, Sorenson S (2011) Effect of celecoxib on survival in patients with advanced non-small cell lung cancer: a double blind randomised clinical phase III trial (CYCLUS study) by the Swedish Lung Cancer Study Group. Eur J Cancer 47:1546–1555

Komatsu K, Buchanan FG, Katkuri S, Morrow JD, Inoue H, Otaka M, Watanabe S, DuBois RN (2005) Oncogenic potential of MEK1 in rat intestinal epithelial cells is mediated via cyclooxygenase-2. Gastroenterology 129:577–590

Kucab JE, Lee C, Chen CS, Zhu J, Gilks CB, Cheang M, Huntsman D, Yorida E, Emerman J, Pollak M, Dunn SE (2005) Celecoxib analogues disrupt Akt signaling, which is commonly activated in primary breast tumours. Breast Cancer Res 7:R796–R807

Kulp SK, Yang YT, Hung CC, Chen KF, Lai JP, Tseng PH, Fowble JW, Ward PJ, Chen CS (2004) 3-phosphoinositide-dependent protein kinase-1/Akt signaling represents a major cyclooxygenase-2-independent target for celecoxib in prostate cancer cells. Cancer Res 64:1444–1451

Kuo ML, Chuang SE, Lin MT, Yang SY (2001) The involvement of PI 3-K/Akt-dependent up-regulation of Mcl-1 in the prevention of apoptosis of Hep3B cells by interleukin-6. Oncogene 20:677–685

Kuwana T, Bouchier-Hayes L, Chipuk JE, Bonzon C, Sullivan BA, Green DR, Newmeyer DD (2005) BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. Mol Cell 17:525–535

Ladetto M, Vallet S, Trojan A, Dell'Aquila M, Monitillo L, Rosato R, Santo L, Drandi D, Bertola A, Falco P, Cavallo F, Ricca I, De Marco F, Mantoan B, Bode-Lesniewska B, Pagliano G, Francese R, Rocci A, Astolfi M, Compagno M, Mariani S, Godio L, Marino L, Ruggeri M, Omede P, Palumbo A, Boccadoro M (2005) Cyclooxygenase-2 (COX-2) is frequently expressed in multiple myeloma and is an independent predictor of poor outcome. Blood 105:4784–4791

Leahy KM, Ornberg RL, Wang Y, Zweifel BS, Koki AT, Masferrer JL (2002) Cyclooxygenase-2 inhibition by celecoxib reduces proliferation and induces apoptosis in angiogenic endothelial cells in vivo. Cancer Res 62:625–631

Liao Z, Komaki R, Milas L, Yuan C, Kies M, Chang JY, Jeter M, Guerrero T, Blumenschien G, Smith CM, Fossella F, Brown B, Cox JD (2005) A phase I clinical trial of thoracic radiotherapy and concurrent celecoxib for patients with unfavorable performance status inoperable/unresectable non-small cell lung cancer. Clin Cancer Res 11:3342–3348

Lin MT, Lee RC, Yang PC, Ho FM, Kuo ML (2001) Cyclooxygenase-2 inducing Mcl-1-dependent survival mechanism in human lung adenocarcinoma CL1.0 cells. Involvement of phosphatidylinositol 3-kinase/Akt pathway. J Biol Chem 276:48997–49002

Lin B, Kolluri SK, Lin F, Liu W, Han YH, Cao X, Dawson MI, Reed JC, Zhang XK (2004a) Conversion of Bcl-2 from protector to killer by interaction with nuclear orphan receptor Nur77/TR3. Cell 116:527–540

Lin HP, Kulp SK, Tseng PH, Yang YT, Yang CC, Chen CS (2004b) Growth inhibitory effects of celecoxib in human umbilical vein endothelial cells are mediated through G1 arrest via multiple signaling mechanisms. Mol Cancer Ther 3:1671–1680

Liu X, Yue P, Zhou Z, Khuri FR, Sun SY (2004) Death receptor regulation and celecoxib-induced apoptosis in human lung cancer cells. J Natl Cancer Inst 96:1769–1780

Liu X, Yue P, Schonthal AH, Khuri FR, Sun SY (2006) Cellular FLICE-inhibitory protein down-regulation contributes to celecoxib-induced apoptosis in human lung cancer cells. Cancer Res 66:11115–11119

Liu B, Shi ZL, Feng J, Tao HM (2008) Celecoxib, a cyclooxygenase-2 inhibitor, induces apoptosis in human osteosarcoma cell line MG-63 via down-regulation of PI3K/Akt. Cell Biol Int 32:494–501

Liu H, Yang Y, Xiao J, Lv Y, Liu Y, Yang H, Zhao L (2009) Inhibition of cyclooxygenase-2 suppresses lymph node metastasis via VEGF-C. Anat Rec (Hoboken) 292:1577–1583

Liu B, Wen JK, Li BH, Fang XM, Wang JJ, Zhang YP, Shi CJ, Zhang DQ, Han M (2011) Celecoxib and acetylbritannilactone interact synergistically to suppress breast cancer cell growth via COX-2-dependent and -independent mechanisms. Cell Death Dis 2:e185

Lynch PM, Ayers GD, Hawk E, Richmond E, Eagle C, Woloj M, Church J, Hasson H, Patterson S, Half E, Burke CA (2010) The safety and efficacy of celecoxib in children with familial adenomatous polyposis. Am J Gastroenterol 105:1437–1443

Maier TJ, Janssen A, Schmidt R, Geisslinger G, Grosch S (2005) Targeting the beta-catenin/APC pathway: a novel mechanism to explain the cyclooxygenase-2-independent anticarcinogenic effects of celecoxib in human colon carcinoma cells. Faseb J 19:1353–1355

Maurer U, Charvet C, Wagman AS, Dejardin E, Green DR (2006) Glycogen synthase kinase-3 regulates mitochondrial outer membrane permeabilization and apoptosis by destabilization of MCL-1. Mol Cell 21:749–760

Mehar A, Macanas-Pirard P, Mizokami A, Takahashi Y, Kass GE, Coley HM (2008) The effects of cyclooxygenase-2 expression in prostate cancer cells: modulation of response to cytotoxic agents. J Pharmacol Exp Ther 324:1181–1187

Milas L, Kishi K, Hunter N, Mason K, Masferrer JL, Tofilon PJ (1999) Enhancement of tumor response to gamma-radiation by an inhibitor of cyclooxygenase-2 enzyme. J Natl Cancer Inst 91:1501–1504

Mohammad MA, Zeeneldin AA, Abd Elmageed ZY, Khalil EH, Mahdy SM, Sharada HM, Sharawy SK, Abdel-Wahab AH (2012) Clinical relevance of cyclooxygenase-2 and matrix metalloproteinases (MMP-2 and MT1-MMP) in human breast cancer tissue. Mol Cell Biochem 366:269–275

Muller AC, Handrick R, Elsaesser SJ, Rudner J, Henke G, Ganswindt U, Belka C, Jendrossek V (2008) Importance of Bak for celecoxib-induced apoptosis. Biochem Pharmacol 76:1082–1096

Ninomiya I, Nagai N, Oyama K, Hayashi H, Tajima H, Kitagawa H, Fushida S, Fujimura T, Ohta T (2012) Antitumor and anti-metastatic effects of cyclooxygenase-2 inhibition by celecoxib on human colorectal carcinoma xenografts in nude mouse rectum. Oncol Rep 28:777–784

Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Bruncko M, Deckwerth TL, Dinges J, Hajduk PJ, Joseph MK, Kitada S, Korsmeyer SJ, Kunzer AR, Letai A, Li C, Mitten MJ, Nettesheim DG, Ng S, Nimmer PM, O'Connor JM, Oleksijew A, Petros AM, Reed JC, Shen W, Tahir SK, Thompson CB, Tomaselli KJ, Wang B, Wendt MD, Zhang H, Fesik SW, Rosenberg SH (2005) An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 435:677–681

Opferman JT (2006) Unraveling MCL-1 degradation. Cell Death Differ 13:1260–1262

Oyama K, Fujimura T, Ninomiya I, Miyashita T, Kinami S, Fushida S, Ohta T, Koichi M (2005) A COX-2 inhibitor prevents the esophageal inflammation-metaplasia-adenocarcinoma sequence in rats. Carcinogenesis 26:565–570

Pattingre S, Bauvy C, Carpentier S, Levade T, Levine B, Codogno P (2009) Role of JNK1-dependent Bcl-2 phosphorylation in ceramide-induced macroautophagy. J Biol Chem 284:2719–2728

Phillips RK, Wallace MH, Lynch PM, Hawk E, Gordon GB, Saunders BP, Wakabayashi N, Shen Y, Zimmerman S, Godio L, Rodrigues-Bigas M, Su LK, Sherman J, Kelloff G, Levin B, Steinbach G (2002) A randomised, double blind, placebo controlled study of celecoxib, a selective cyclooxygenase 2 inhibitor, on duodenal polyposis in familial adenomatous polyposis. Gut 50:857–860

Pruthi RS, Derksen JE, Moore D, Carson CC, Grigson G, Watkins C, Wallen E (2006) Phase II trial of celecoxib in prostate-specific antigen recurrent prostate cancer after definitive radiation therapy or radical prostatectomy. Clin Cancer Res 12:2172–2177

Pyrko P, Soriano N, Kardosh A, Liu YT, Uddin J, Petasis NA, Hofman FM, Chen CS, Chen TC, Schonthal AH (2006) Downregulation of survivin expression and concomitant induction of apoptosis by celecoxib and its non-cyclooxygenase-2-inhibitory analog, dimethyl-celecoxib (DMC), in tumor cells in vitro and in vivo. Mol Cancer 5:19

Pyrko P, Kardosh A, Liu YT, Soriano N, Xiong W, Chow RH, Uddin J, Petasis NA, Mircheff AK, Farley RA, Louie SG, Chen TC, Schonthal AH (2007) Calcium-activated endoplasmic reticulum stress as a major component of tumor cell death induced by 2,5-dimethyl-celecoxib, a non-coxib analogue of celecoxib. Mol Cancer Ther 6:1262–1275

Rasheva VI, Domingos PM (2009) Cellular responses to endoplasmic reticulum stress and apoptosis. Apoptosis 14:996–1007

Reed JC (2006) Proapoptotic multidomain Bcl-2/Bax-family proteins: mechanisms, physiological roles, and therapeutic opportunities. Cell Death Differ 13:1378–1386

Reed S, Li H, Li C, Lin J (2011) Celecoxib inhibits STAT3 phosphorylation and suppresses cell migration and colony forming ability in rhabdomyosarcoma cells. Biochem Biophys Res Commun 407:450–455

Rudner J, Elsaesser SJ, Muller AC, Belka C, Jendrossek V (2010) Differential effects of anti-apoptotic Bcl-2 family members Mcl-1, Bcl-2, and Bcl-xL on celecoxib-induced apoptosis. Biochem Pharmacol 79:10–20

Rudner J, Elsaesser SJ, Jendrossek V, Huber SM (2011) Anti-apoptotic Bcl-2 fails to form efficient complexes with pro-apoptotic Bak to protect from Celecoxib-induced apoptosis. Biochem Pharmacol 81:32–42

Sakoguchi-Okada N, Takahashi-Yanaga F, Fukada K, Shiraishi F, Taba Y, Miwa Y, Morimoto S, Iida M, Sasaguri T (2007) Celecoxib inhibits the expression of survivin via the suppression of promoter activity in human colon cancer cells. Biochem Pharmacol 73:1318–1329

Schiffmann S, Maier TJ, Wobst I, Janssen A, Corban-Wilhelm H, Angioni C, Geisslinger G, Grosch S (2008) The anti-proliferative potency of celecoxib is not a class effect of coxibs. Biochem Pharmacol 76:179–187

Schonthal AH (2006) Antitumor properties of dimethyl-celecoxib, a derivative of celecoxib that does not inhibit cyclooxygenase-2: implications for glioma therapy. Neurosurg Focus 20:E21

Schonthal AH (2007) Direct non-cyclooxygenase-2 targets of celecoxib and their potential relevance for cancer therapy. Br J Cancer 97:1465–1468

Schonthal AH (2010) Exploiting cyclooxygenase-(in)dependent properties of COX-2 inhibitors for malignant glioma therapy. Anticancer Agents Med Chem 10:450–461

Schonthal AH, Chen TC, Hofman FM, Louie SG, Petasis NA (2008) Celecoxib analogs that lack COX-2 inhibitory function: preclinical development of novel anticancer drugs. Expert Opin Investig Drugs 17:197–208

Seno H, Oshima M, Ishikawa TO, Oshima H, Takaku K, Chiba T, Narumiya S, Taketo MM (2002) Cyclooxygenase 2- and prostaglandin E(2) receptor EP(2)-dependent angiogenesis in Apc(Delta716) mouse intestinal polyps. Cancer Res 62:506–511

Setia S, Sanyal SN (2012) Downregulation of NF-kappaB and PCNA in the regulatory pathways of apoptosis by cyclooxygenase-2 inhibitors in experimental lung cancer. Mol Cell Biochem 369:75–86

Sinha-Datta U, Taylor JM, Brown M, Nicot C (2008) Celecoxib disrupts the canonical apoptotic network in HTLV-I cells through activation of Bax and inhibition of PKB/Akt. Apoptosis 13:33–40

Sobolewski C, Cerella C, Dicato M, Diederich M (2011) Cox-2 inhibitors induce early c-Myc downregulation and lead to expression of differentiation markers in leukemia cells. Cell Cycle 10:2978–2993

Solomon SD, McMurray JJ, Pfeffer MA, Wittes J, Fowler R, Finn P, Anderson WF, Zauber A, Hawk E, Bertagnolli M (2005) Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. N Engl J Med 352:1071–1080

Song X, Lin HP, Johnson AJ, Tseng PH, Yang YT, Kulp SK, Chen CS (2002) Cyclooxygenase-2, player or spectator in cyclooxygenase-2 inhibitor-induced apoptosis in prostate cancer cells. J Natl Cancer Inst 94:585–591

Sooriakumaran P, Macanas-Pirard P, Bucca G, Henderson A, Langley SE, Laing RW, Smith CP, Laing EE, Coley HM (2009) A gene expression profiling approach assessing celecoxib in a randomized controlled trial in prostate cancer. Cancer Genomics Proteomics 6:93–99

Soslow RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, Koki AT (2000) COX-2 is expressed in human pulmonary, colonic, and mammary tumors. Cancer 89:2637–2645

Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK, Levin B (2000) The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 342:1946–1952

Subhashini J, Mahipal SV, Reddanna P (2005) Anti-proliferative and apoptotic effects of celecoxib on human chronic myeloid leukemia in vitro. Cancer Lett 224:31–43

Tanaka K, Tomisato W, Hoshino T, Ishihara T, Namba T, Aburaya M, Katsu T, Suzuki K, Tsutsumi S, Mizushima T (2005) Involvement of intracellular Ca2+ levels in nonsteroidal anti-inflammatory drug-induced apoptosis. J Biol Chem 280:31059–31067

Thiel A, Mrena J, Ristimaki A (2011) Cyclooxygenase-2 and gastric cancer. Cancer Metastasis Rev 30:387–395

Thompson J, Winoto A (2008) During negative selection, Nur77 family proteins translocate to mitochondria where they associate with Bcl-2 and expose its proapoptotic BH3 domain. J Exp Med 205:1029–1036

Timmer JC, Salvesen GS (2007) Caspase substrates. Cell Death Differ 14:66–72

Tong Z, Wu X, Chen CS, Kehrer JP (2006) Cytotoxicity of a non-cyclooxygenase-2 inhibitory derivative of celecoxib in non-small-cell lung cancer A549 cells. Lung Cancer 52:117–124

Trifan OC, Durham WF, Salazar VS, Horton J, Levine BD, Zweifel BS, Davis TW, Masferrer JL (2002) Cyclooxygenase-2 inhibition with celecoxib enhances antitumor efficacy and reduces diarrhea side effect of CPT-11. Cancer Res 62:5778–5784

Tsutsumi S, Gotoh T, Tomisato W, Mima S, Hoshino T, Hwang HJ, Takenaka H, Tsuchiya T, Mori M, Mizushima T (2004) Endoplasmic reticulum stress response is involved in nonsteroidal anti-inflammatory drug-induced apoptosis. Cell Death Differ 11:1009–1016

Tuynman JB, Vermeulen L, Boon EM, Kemper K, Zwinderman AH, Peppelenbosch MP, Richel DJ (2008) Cyclooxygenase-2 inhibition inhibits c-Met kinase activity and Wnt activity in colon cancer. Cancer Res 68:1213–1220

Walter P, Ron D (2011) The unfolded protein response: from stress pathway to homeostatic regulation. Science 334:1081–1086

Wang GQ, Gastman BR, Wieckowski E, Goldstein LA, Gambotto A, Kim TH, Fang B, Rabinovitz A, Yin XM, Rabinowich H (2001) A role for mitochondrial Bak in apoptotic response to anticancer drugs. J Biol Chem 276:34307–34317

Wang L, Chen W, Xie X, He Y, Bai X (2008) Celecoxib inhibits tumor growth and angiogenesis in an orthotopic implantation tumor model of human colon cancer. Exp Oncol 30:42–51

Waskewich C, Blumenthal RD, Li H, Stein R, Goldenberg DM, Burton J (2002) Celecoxib exhibits the greatest potency amongst cyclooxygenase (COX) inhibitors for growth inhibition of COX-2-negative hematopoietic and epithelial cell lines. Cancer Res 62:2029–2033

Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB, Korsmeyer SJ (2001) Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 292:727–730

Wei Y, Pattingre S, Sinha S, Bassik M, Levine B (2008) JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. Mol Cell 30:678–688

Williams CS, Mann M, DuBois RN (1999) The role of cyclooxygenases in inflammation, cancer, and development. Oncogene 18:7908–7916

Williams CS, Watson AJ, Sheng H, Helou R, Shao J, DuBois RN (2000) Celecoxib prevents tumor growth in vivo without toxicity to normal gut: lack of correlation between in vitro and in vivo models. Cancer Res 60:6045–6051

Willis SN, Adams JM (2005) Life in the balance: how BH3-only proteins induce apoptosis. Curr Opin Cell Biol 17:617–625

Willis SN, Chen L, Dewson G, Wei A, Naik E, Fletcher JI, Adams JM, Huang DC (2005) Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. Genes Dev 19:1294–1305

Willis SN, Fletcher JI, Kaufmann T, van Delft MF, Chen L, Czabotar PE, Ierino H, Lee EF, Fairlie WD, Bouillet P, Strasser A, Kluck RM, Adams JM, Huang DC (2007) Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. Science 315:856–859

Winfield LL, Payton-Stewart F (2012) Celecoxib and Bcl-2: emerging possibilities for anticancer drug design. Future Med Chem 4:361–383

Wobst I, Schiffmann S, Birod K, Maier TJ, Schmidt R, Angioni C, Geisslinger G, Grosch S (2008) Dimethylcelecoxib inhibits prostaglandin E2 production. Biochem Pharmacol 76:62–69

Wu T, Leng J, Han C, Demetris AJ (2004) The cyclooxygenase-2 inhibitor celecoxib blocks phosphorylation of Akt and induces apoptosis in human cholangiocarcinoma cells. Mol Cancer Ther 3:299–307

Xia JJ, Pei LB, Zhuang JP, Ji Y, Xu GP, Zhang ZP, Li N, Yan JL (2010) Celecoxib inhibits beta-catenin-dependent survival of the human osteosarcoma MG-63 cell line. J Int Med Res 38:1294–1304

Xin X, Majumder M, Girish GV, Mohindra V, Maruyama T, Lala PK (2012) Targeting COX-2 and EP4 to control tumor growth, angiogenesis, lymphangiogenesis and metastasis to the lungs and lymph nodes in a breast cancer model. Lab Invest 92:1115–1128

Xue WP, Bai SM, Luo M, Bi ZF, Liu YM, Wu SK (2011) Phase I clinical trial of nasopharyngeal radiotherapy and concurrent celecoxib for patients with locoregionally advanced nasopharyngeal carcinoma. Oral Oncol 47:753–757

Yacoub A, Park MA, Hanna D, Hong Y, Mitchell C, Pandya AP, Harada H, Powis G, Chen CS, Koumenis C, Grant S, Dent P (2006) OSU-03012 promotes caspase-independent but PERK-, cathepsin B-, BID-, and AIF-dependent killing of transformed cells. Mol Pharmacol 70:589–603

Yamamoto K, Ichijo H, Korsmeyer SJ (1999) BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. Mol Cell Biol 19:8469–8478

Yamamoto H, Ngan CY, Monden M (2008) Cancer cells survive with survivin. Cancer Sci 99:1709–1714

Youle RJ, Strasser A (2008) The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol 9:47–59

Zhai D, Jin C, Huang Z, Satterthwait AC, Reed JC (2008) Differential regulation of Bax and Bak by anti-apoptotic Bcl-2 family proteins Bcl-B and Mcl-1. J Biol Chem 283:9580–9586

Zhang S, Suvannasankha A, Crean CD, White VL, Johnson A, Chen CS, Farag SS (2007a) OSU-03012, a novel celecoxib derivative, is cytotoxic to myeloma cells and acts through multiple mechanisms. Clin Cancer Res 13:4750–4758

Zhang Y, Tao J, Huang H, Ding G, Cheng Y, Sun W (2007b) Effects of celecoxib on voltage-gated calcium channel currents in rat pheochromocytoma (PC12) cells. Pharmacol Res 56:267–274

Zhao S, Cai J, Bian H, Gui L, Zhao F (2009) Synergistic inhibition effect of tumor growth by using celecoxib in combination with oxaliplatin. Cancer Invest 27:636–640

Zhong Q, Gao W, Du F, Wang X (2005) Mule/ARF-BP1, a BH3-only E3 ubiquitin ligase, catalyzes the polyubiquitination of Mcl-1 and regulates apoptosis. Cell 121:1085–1095

Zhou P, Qian L, Bieszczad CK, Noelle R, Binder M, Levy NB, Craig RW (1998) Mcl-1 in transgenic mice promotes survival in a spectrum of hematopoietic cell types and immortalization in the myeloid lineage. Blood 92:3226–3239

Zhu J, Song X, Lin HP, Young DC, Yan S, Marquez VE, Chen CS (2002) Using cyclooxygenase-2 inhibitors as molecular platforms to develop a new class of apoptosis-inducing agents. J Natl Cancer Inst 94:1745–1757

Zhu J, Huang JW, Tseng PH, Yang YT, Fowble J, Shiau CW, Shaw YJ, Kulp SK, Chen CS (2004) From the cyclooxygenase-2 inhibitor celecoxib to a novel class of 3-phosphoinositide-dependent protein kinase-1 inhibitors. Cancer Res 64:4309–4318

Chapter 6
Activation of Mitochondria-Driven Pathways by Artemisinin and Its Derivatives

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Contents

Abstract Mitochondria have recently emerged as promising agents for cancer therapy. Of particular interest and potential clinical relevance are agents that target these organelles, promoting cell death. There are literally thousands of compounds that act on mitochondria and destabilise them. Of these, naturally occurring compounds are particularly interesting, since they are often more 'biocompatible'; besides, natural compounds can be lead drugs for the design of novel and more efficient anti-cancer agents. In this paper, we focus on the natural product artemisin and its semisynthetic derivatives, and document the molecular mechanism of their activity and their potential use as clinically relevant anti-cancer agents.

Keywords Antioxidant tress response • Reactive oxygen species • Sesquiterpene

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1 Introduction

More than a decade ago, we initiated a research program on the molecular pharmacology and pharmacogenomics of natural products derived from traditional Chinese medicine (Efferth et al. 1996). This project turned out to be a fertile ground for the identification and characterization of compounds with activity against tumour cells and viruses. Plant extracts with growth-inhibitory activity against tumour cells have been fractionated by chromatographic techniques. We have isolated the bioactive compounds and elucidated the chemical structures by nuclear magnetic resonance and mass spectrometry (Efferth et al. 2008a).

Among a plethora of different compounds, we focused on artemisinin, the active principle of *Artemisia annua* L. and its semi-synthetic derivative, artesunate, towards cancer cells *in vitro* and *in vivo* (Efferth et al. 2001, 2003b; Dell'Eva et al. 2004). Our data indicated profound also against various viruses, *Trypanosoma*, and even plant crown gall tumours (Efferth et al. 2002a, 2008b).

To elucidate the molecular mode of action of artesunate towards tumour cells, we applied molecular biological and pharmacogenomic approaches *in vitro* and *in vivo* (Efferth et al. 2002b, 2003b; Dell'Eva et al. 2004). Different signalling pathways were affected by artesunate treatment, *e.g.*, antioxidant stress response, inhibition of nitric oxide generation, inhibition of multidrug-resistance conferring membrane transporters, inhibition of proliferation and cell cycle regulators, inhibition of cancer metastasis, induction of apoptosis, inhibition of tumour angiogenesis, interference with EGFR-related signal transduction pathways (Efferth 2005, 2006, 2007; Anfosso et al. 2006; Efferth et al. 2007; Konkimalla et al. 2008, 2009; Bachmeier et al. 2011). Artesunate (as most natural products) attack several rather than single targets to kill cancer cells, *e.g.* it alkylates cell cycle related proteins and it induces oxidative DNA damage (Li et al. 2008; Berdelle et al. 2011).

Despite its multi-target specificity, its toxicity to normal tissues is astonishing low and few side effects appear as shown in meta-analysis of several thousand malaria patients treated with artesunate-type compounds (Ribeiro and Olliaro 1998; Adjuik et al. 2004; Efferth and Kaina 2010). By chemical derivatisation, we synthesized novel artesunate-type compounds with improved efficacy to kill tumour cells (Horwedel et al. 2010).

Artesunate was active to inhibit the growth of human xenograft tumours transplanted to nude mice. Inhibition of angiogenesis was found to be a mechanism relevant in the *in vivo* situation. The successful compassionate use of artesunate for treatment of two uveal melanoma patients and a pilot study with 10 cervical carcinoma patients encourage controlled randomized clinical studies in the future (Berger et al. 2005; Jansen et al. 2011).

Despite the multi-factorial mode of action of artesunate, the generation of ROS, the induction of the mitochondrial pathway of apoptosis appear as prominent modes of action. Therefore, we hypothesized that targeting mitochondrial functions of cancer cells by this class of compounds is of special relevance to explain their activity. In the present chapter, we give an overview of the available data supporting this point of view.

2 Dual Role of Mitochondria for Cell Death and Survival

Mitochondria are crucial cellular organelles making them to exquisite targets for cancer drug development (Zhang et al. 2011). They are structurally and functionally different in cancer cells as compared to normal cells (Fulda et al. 2010; Galluzzi et al. 2010) and they are gateways to both lethal functions triggering cell death and vital functions promoting cell survival.

Among the lethal functions of mitochondria is the regulation of the intrinsic pathway of apoptosis. In a death receptor-independent fashion, mitochondria can activate apoptosis. Disruption of the mitochondrial membrane potential leads to the release of cytochrome C and Smac/DIABLO protein, which further down-stream activates caspases ultimately leading to nuclear fragmentation and cell death (Igney and Krammer 2002). This process is controlled by the balance of pro- and anti-apoptotic proteins of the Bcl-2 family which are located in the mitochondrial membrane. Small molecules bearing the potential to specifically attack intrinsic apoptosis have been described, *e.g.* inhibitors of IAP (Hunter et al. 2007; Varfolomeev and Vucic 2011) or Bcl-2 family members (Goldsmith and Hogarty 2009; Vogler et al. 2009).

Another process which is linked to mitochondrial function is autophagy. ROS and reactive nitrogen species (RNS) are generated by incomplete oxygen reduction during oxidative metabolism. Aerobic respiration leads to ROS formation in the mitochondrial electron transport chain (Murphy 2009; Lee et al. 2012). Whereas low levels of mitochondrial ROS are involved in signalling processes, high ROS amounts damage proteins and contribute to autophagy by degradation of damaged proteins in the lysosomes. Autophagy is involved in carcinogenesis, since it may deliver hypoxic and nutrient-deprived tumour tissues with enough energy to survive. Autophagy prevents cancer cells from accumulation of dysfunctional mitochondria (Guo et al. 2011). On the other hand, excessive autophagy can induce cell death and cellular senescence stopping tumour progression (Young et al. 2009). Small molecule autophagy regulators have been developed for therapeutic purposes, which can be classified as direct or indirect autophagy enhancers and autophagy inhibitors (Wu and Yan 2011).

Vital functions of mitochondria include the production of ATP. Mitochondria represent primary energy sources. As cancer cells reveal higher energy demands as normal cells, targeting mitochondrial energy production seems to be an attractive treatment target. Oxidation of glucose takes place by glycolysis (in the cytosol) and oxidative phosphorylation (in mitochondria). In the first half of the twentieth century, Otto Warburg recognized that cancer cells meet their higher energy requirement by glycolysis pointing to a defective energy metabolism in mitochondria even under anaerobic conditions (Weinhouse 1976). Up-regulated glycolysis may be an adaptation towards hypoxic environments. This is accompanied by lowered intracellular pH values facilitating tumour invasion, generation of ROS and inhibition of apoptotic signalling cascades, the latter two conferring tumour progression and resistance to chemo- and radiotherapy (Indran et al. 2011).

Several therapeutic strategies have been suggested to exploit mitochondrial functions as Achilles heel of cancer cells. The shift of cancer cells from oxidative phosphorylation to aerobic glycolysis (Warburg effect) does not imply that mitochondria play a minor role in tumour cell survival. Mitochondria supply 40–75 % of the ATP required by cancer cells (Boujrad et al. 2007). Hence, inhibition of metabolic pathways in mitochondria has emerged as treatment principle for cancer drug development. Synthetic and natural small molecule inhibitors of glycolysis, oxidative phosphorylation, mitochondrial ATP transport and glutaminolysis as well as ROS regulators have been described (Biasutto et al. 2010; Fulda et al. 2010; Hockenbery 2010; D'Souza et al. 2011; Ramsay et al. 2011; Wenner 2012).

3 Role of Reactive Oxygen Species and Iron for Artemisinin and Its Derivatives

In *Plasmodia*, the cleavage of the endoperoxide moiety of artemisinin is facilitated by haem-iron. Haemoglobin of erythrocytes serves as an amino acid source for *Plasmodium* trophozoites and schizonts. The parasites take up haemoglobin and degrade it in their food vacuoles. The release of haem-iron during haemoglobin digestion facilitates the cleavage of the endoperoxide moiety of artemisinin by a Fe(II) Fenton reaction. Thereby, ROS are generated, such as hydroxyl radicals and superoxide anions. They damage membranes of food vacuoles and lead to autodigestion. Furthermore, carbon-centred radical species are generated by the haem iron(II)-mediated decomposition of artemisinin. These highly reactive molecules are able to alkylate haem and several *Plasmodium*-specific proteins. It has also be discussed, however, haem iron(II) and oxidative stress are not to be the only determinants of artemisinin's anti-malarial activity.

Focusing on the anti-cancer activity of artemisinin and its derivatives, we compared the baseline antioxidant mRNA gene expression in the NCI cell line panel with the IC_{50} values for artesunate (Efferth et al. 2003a; Efferth and Oesch 2004; Efferth and Volm 2005). Our results speak for oxidative stress as a mechanism of artesunate against cancer cells. We found that thioredoxin reductase and catalase expression correlated significantly to the IC_{50} values for artesunate. WEHI7.2 mouse thymoma cells selected for resistance to hydrogen peroxide or transfected with thioredoxin, manganese superoxide dismutase or catalase showed resistance to artesunate as compared to the parental cell line. The microarray-based mRNA expression of dihydrodiol dehydrogenase, γ-glutamylcysteine synthase (γ-GCS; *GLCLR*), glutathione S-transferases *GSTM4, GSTT2, GSTZ1*, and microsomal glutathione S-transferase *MGST3* correlated significantly with resistance to artesunate in the NCI cell line panel. A tendency for correlation ($0.05 < p < 0.1$) was observed for *GSTA1, GSTA2, GSTP1* and *MGST1*. MSC-HL13 cells transfected with cDNAs for heavy and light subunits of γ-GCS were more resistant to artesunate than mock transfected MSV-PC4 cells (Efferth and Volm 2005). L-buthionine sulfoximine, a γ-GCS inhibitor that depletes cellular

glutathione pools completely reversed artesunate resistance in MSV-HL13 cells (Efferth and Volm 2005).

As tumour cells contain much less iron than erythrocytes, but more than other normal tissues, the question arises as to whether iron may be critical for artemisinin's action towards tumour cells. Cellular iron uptake and internalization are mediated by binding of transferrin-iron complexes to the transferrin receptor (CD71) expressed on the cell surface membrane and subsequent endocytosis. While most normal tissues are CD71-negative, CD71 is highly expressed in clinical tumours and is widely distributed among clinical tumours. We found that CD71 expression was much higher in CCRF-CEM and U373 tumour cells (48–95 %) than in peripheral mononuclear blood cells of healthy donors (<2 %) (Efferth et al. 2004). Iron(II) glycine sulfate (Ferrosanol®) and transferrin increased the cytotoxicity of free artesunate, artesunate microencapsulated in maltosyl-β-cyclodextrin, and artemisinin towards CCRF-CEM leukaemia and U373 astrocytoma cells compared with artemisinins applied without iron (Efferth et al. 2004). This effect was reversed by monoclonal antibody RVS10 against the transferrin receptor, which competes with transferrin for binding to the receptor. The IC_{50} values for eight different artemisinin derivatives in the NCI cell lines were correlated with the microarray mRNA expression of 12 genes involved in iron uptake and metabolism. The mRNA expression of mitochondrial aconitase and ceruloplasmin (ferroxidase) significantly correlated with the IC_{50} values for artemisinins. Interestingly, exposure of artemisinin and its derivatives produces no or only marginal cytotoxicity to non-tumour cells. We found that the growth of primary human fibroblasts is almost unaffected by artesunate concentrations up to 100 μM (Efferth et al. 2003b; Sertel et al. 2010a). This implies that tumours that express more CD71 than normal cells might be more affected by combination treatments of transferrin or Ferrosanol® plus artemisinin derivatives. The finding that iron(II) glycine sulfate increased the action of artemisinins is interesting, since Ferrosanol® is in clinical use for many years. Hence, artemisinins might be safely applied in combination with Ferrosanol® in a clinical setting (Efferth et al. 2004).

To further analyse the enhancement of artesunate's activity towards tumour cells by ferrous iron, we analysed the role of the transferrin receptor and the ATP-binding cassette (ABC) transporters ABCB6 and ABCB7, which are also involved in iron homeostasis (Kelter et al. 2007). Thirty-six tumour cell lines were treated with artesunate or artesunate plus Ferrosanol®. The majority of cell lines showed increased inhibition rates, for the combination of artesunate plus Ferrosanol® compared to artesunate alone. However, in 11 out of the 36 cell lines the combination treatment was not superior. Cell lines with high transferrin receptor expression significantly correlated with high degrees of modulation indicating that high transferrin receptor-expressing tumour cells would be more efficiently inhibited by this combination treatment than low transferrin receptor-expressing ones. Furthermore, we found a significant relationship between cellular response to artesunate and transferrin receptor expression in 55 cell lines of different tumour types. A significant correlation was also found for ABCB6, but not for ABCB7. Artesunate treatment of human CCRF-CEM leukaemia and MCF-7 breast cancer cells induced ABCB6 expression but repressed ABCB7 expression. Finally, artesunate inhibited proliferation and

differentiation of mouse erythroleukaemia (MEL) cells. Down-regulation of ABCB6 by antisense oligonucleotides inhibited differentiation of MEL cells indicating that artesunate and ABCB6 may cooperate (Kelter et al. 2007). In conclusion, our results indicate that ferrous iron improves the activity of artesunate in some but not all tumour cell lines. Several factors involved in iron homeostasis such as transferrin receptor and ABCB6 may contribute to this effect.

The tumour specificity of iron and artemisinin-type compounds has been shown by various groups. Human breast cancer cells were not inhibited by artemisinin plus transferrin, whereas breast cancer cells did (Singh and Lai 2001). Similarly, artemisinin linked to transferrin was more cytotoxic to leukaemia cells than to normal lymphocytes (Lai et al. 2005). Artesunate killed human papilloma virus-immortalized and transformed cervical cells with high TfR expression more efficiently than normal cervical epithelial cells (Disbrow et al. 2005).

These *in vitro* data were confirmed by data of animal experiments. Artesunate and Ferrous sulfate were co-administered to rats (Fafowora et al. 2011). Low ionic strength mitochondria were isolated from hepatic cells of the rats and assayed for protein content, changes in the absorbance of the liver mitochondria, and mitochondrial swelling. Co-administration of artesunate and ferrous sulfate resulted in increasing opening in the mitochondrial membrane transition pore.

Disbrow et al. (2005) tested three different artemisinin compounds and found that dihydroartemisinin and artesunate displayed strong cytotoxic effects on human papilloma virus-immortalized and transformed cervical cells *in vitro* with little effect on normal cervical epithelial cells. Dihydroartemisinin-induced cell death involved activation of the mitochondrial caspase pathway with resultant apoptosis. Apoptosis was p53-independent and was not the consequence of drug-induced reductions in viral oncogene expression. Due to its selective cytotoxicity, hydrophobicity, and known ability to penetrate epithelial surfaces, Disbrow et al. (2005) postulated that dihydroartemisinin might be useful for the topical treatment of mucosal papillomavirus lesions. To test this hypothesis, the authors applied dihydroartemisinin to the oral mucosa of dogs that had been challenged with the canine oral papillomavirus. Although applied only intermittently, dihydroartemisinin strongly inhibited viral-induced tumour formation. Interestingly, the dihydroartemisinin-treated, tumour-negative dogs developed antibodies against the viral L1 capsid protein, suggesting that dihydroartemisinin had inhibited tumour growth but not early rounds of papillomavirus replication. These findings indicate that dihydroartemisinin and other artemisinin derivatives may be useful for the topical treatment of epithelial papillomavirus lesions, including those that have progressed to the neoplastic state.

4 Programmed Cell Death Induction by Artemisinin and Its Derivatives

Artesunate induced apoptosis in leukemic T-cells mainly through the mitochondrial pathway via generation of ROS, a mechanism different from doxorubicin. This is confirmed by the fact that the antioxidant N-acetyl-cysteine completely blocked ROS

generation and, consequently, inhibited artesunate-induced apoptosis. Therefore, artesunate can overcome the doxorubicin-resistance and induce P-glycoprotein-overexpressing doxorubicin-resistant leukaemia cells to undergo apoptosis (Efferth et al. 2007). We also showed that artesunate can synergize with doxorubicin to enhance apoptotic cell death in leukemic T cells. This synergistic effect can be largely explained by the fact that artesunate and doxorubicin use different killing mechanisms. This raises the possibility to develop artesunate in combination with other established anticancer drugs which induce apoptosis through the pathways or mechanisms different from artesunate (Efferth et al. 2007).

In cluster analyses of microarray experiments, we found that the programmed cell death genes 2, 4, 8, and 9 (*PDCD2, PDCD4, PDCD8, PDCD9*), *BCL2-associated* athanogenes 1 and 3 (*BAG1, BAG3*), death-associated protein 6 *(DAXX), MAP*-kinase activating death domain (*MADD*), cell death-inducing *DFFA*-like effector β (*CIDEB*), and others are possible determinants of tumour cell response to artesunate (Efferth et al. 2002b, 2003b; Efferth 2006; Sertel et al. 2010b). Since apoptosis is a common mechanism of cell death for most anti-cancer drugs, this may also apply for artemisinin and its derivatives. WEHI7.2 cells transfected with the anti-apoptotic *BCL2*-gene were more resistant to artesunate than mock vector-transfected control cells (Efferth et al. 2003a), indicating that artesunate may induce apoptosis via the intrinsic mitochondrial pathway of cell death. Furthermore, we found that artesunate acts via p53-dependent and -independent pathways in isogenic p53+/+ p21WAF1/CIP1$^{+/+}$, p53$^{-/-}$ p21WAF1/CIP1$^{+/+}$, and p53$^{+/+}$ p21WAF1/CIP1$^{-/-}$ colon carcinoma cells (Efferth et al. 2003b). This was confirmed in a subsequent study with p53 wild-type TK6 and p53 mutated WTK1 lymphoblastic cells. In both cell lines, we observed a similar sensitivity towards artesunate (Efferth et al. 2004). In T-cell leukaemia cell lines, artesunate induced apoptosis by the intrinsic, but not the extrinsic pathway of apoptosis (Efferth et al. 2007).

Since chemotherapy of non-Hodgkin's lymphoma is frequently hampered by drug resistance, we investigated experimental strategies to improve cell killing rates. The monoclonal antibody rituximab specifically targets the CD20 antigen and sensitizes B-cell lymphoma cells to standard anticancer drugs. We analysed, whether a combination of rituximab and artesunate may act in a complementary manner and eventually synergize in tumour cell killing (Sieber et al. 2009). While rituximab alone was minimally cytotoxic, rituximab increased cytotoxicity to artesunate in Ramos cells. Artesunate induced apoptosis, induced Fas/CD95 expression and the formation of ROS and resulted in a breakdown of mitochondrial membrane potential. This argues for the involvement of both receptor-driven extrinsic and mitochondrial intrinsic routes of apoptosis. Rituximab increased Fas/CD95 expression and ROS formation and decreased mitochondrial membrane potential ultimately leading to increased apoptosis induced by artesunate. The transcription factors YY1 and Sp1 are upstream regulators of apoptosis by controlling the expression of apoptosis-regulating genes. YY1 and Sp1 were down-regulated and Fas/CD95 was up-regulated by rituximab and artesunate indicating that artesunate activated the Fas/CD95 pathway and that rituximab increased the susceptibility of tumour cells to artesunate-induced apoptosis. Furthermore, rituximab affected the expression of antioxidant genes. The

antibody decreased artesunate-induced up-regulation of catalase expression and increased artesunate-induced down-regulation of glutathione S-transferase-π expression. Manganese-dependent superoxide dismutase expression was not changed by artesunate. Antioxidant proteins may help to detoxify artesunate-induced ROS. Rituximab reversed the artesunate-induced expression changes of antioxidant genes and, hence, reduced the detoxification capacity of Ramos cells. The effects of rituximab on antioxidant genes represent a novel mechanism of rituximab for chemosensitization.

A connection between ROS generation, disruption of mitochondrial membrane potential and apoptosis has also been observed by other investigators. Dihydroartemisinin induced apoptosis via caspase-3-dependent mitochondrial death pathway in ASTC-a-1 lung adenocarcinoma cells (Lu et al. 2009). The authors found a collapse of mitochondrial transmembrane potential using the mitochondrial fluorescence probe rhodamine 123 and laser scanning confocal microscope and flow cytometry. Caspase-3 activities were measured with or without the broad spectrum caspase inhibitor Z-VAD-fmk pre-treatment.

Oxidative changes also occurred in Jurkat T-lymphoblastic leukaemia cells after treatment with dihydroartemisinin (Handrick et al. 2010). The disruption of the mitochondrial death pathway suggested a role of ROS and oxidative membrane changes in death signalling upstream of the mitochondria. Dihydroartemisinin induced a breakdown of the mitochondrial transmembrane potential, release of cytochrome C, activation of caspases, and DNA fragmentation indicative of apoptosis induction. Although the absence of FADD or caspase-8 did not alter apoptosis rates in Jurkat cells, over-expression of dominant-negative caspase-9 or of anti-apoptotic Bcl-xL or Bcl-2 largely decreased the cytotoxicity of dihydroartemisinin, demonstrating a role of the intrinsic death pathway. The pro-apoptotic Bcl-2 effector protein Bak and the Bcl-2 homology domain 3-only protein NOXA turned out to be important mediators of dihydroartemisinin-induced apoptosis in Jurkat cells. Dihydroartemisinin treatment triggered the expression of NOXA and the activation of Bak. Furthermore, dihydroartemisinin-induced apoptosis was completely abrogated by loss of Bak and largely reduced in cells with siRNA-mediated down-regulation of Bak or NOXA. Pro-apoptotic signalling of dihydroartemisinin also involved the formation of reactive oxygen species and membrane oxidation. Pre-treatment with the lipophilic radical scavenger vitamin E or the hydrophilic radical scavengers glutathione and N-acetyl-cysteine reduced dihydroartemisinin-induced membrane oxidation and apoptosis, respectively.

Xiao et al. (2012) explored the roles of caspase-8, -9 and -3 during artemisinin-induced apoptosis in human lung adenocarcinoma (ASTC-a-1) cells. Artemisinin treatment induces a rapid generation of ROS, and ROS-dependent apoptosis as well as the activation of caspase-8, -9 and -3 via time- and dose-dependent fashion. Inhibition of caspase-8 or -9, but not caspase-3, almost completely blocked artemisinin-induced not only activation of the caspase-8, -9 and -3 but also apoptosis. In addition, the apoptotic process triggered did not involve the Bid cleavage, tBid translocation, significant loss of mitochondrial membrane potential and cytochrome c release from mitochondria. Moreover, silencing Bax/Bak did not prevent the

artemisinin-induced cell death as well as the activation of caspase-8, -9 and -3. The authors concluded that artemisinin triggered a ROS-mediated positive feedback amplification activation loop between caspase-8 and -9 independent of mitochondria, which dominantly mediated the artemisinin-induced apoptosis via a caspase-3-independent apoptotic pathway in ASTC-a-1 cells.

Pancreatic cancer is highly resistant to the currently available chemotherapeutic agents. Less than 5 % of patients diagnosed with this disease survive more than 5 years. Du et al. (2010) reported that artesunate killed human pancreatic cancer cells *in vitro* and *in vivo*. Artesunate exhibited selective cytotoxic activity against Panc-1, BxPC-3 and CFPAC-1 pancreatic cancer cells with IC_{50} values that are 2.3- to 24-fold less than that of the normal human hepatic cells (HL-7702). The pan-caspase inhibitor zVAD-fmk did not inhibit the cytotoxic activity of artesunate. Electron microscopy of artesunate-treated cells revealed severe cytoplasmic swelling and vacuolization, swollen and internally disorganized mitochondria, dilation (but not fragmentation) of the nuclei without chromatin condensation, and cell lysis, yielding a morphotype that is typical of oncosis. Artesunate-treated cells exhibited a loss of mitochondrial membrane potential and cell death was inhibited by the ROS scavenger N-acetyl-cysteine. Importantly, artesunate produced a dose-dependent tumour regression in an *in vivo* pancreatic cancer xenografts model. The *in vivo* antitumor activity of artesunate was similar to that of the control drug, gemcitabine. The authors concluded that artesunate exhibited antitumor activity against human pancreatic cancer via a novel form of oncosis-like cell death.

The generation of ROS by artemisinin-type drugs does not only induce apoptosis and oncosis-like forms of cell death, but also autophagy. In malaria parasites, artesunate cytotoxicity originates from interactions with haem-derived iron within the food vacuole. The analogous digestive compartment of mammalian cells, the lysosome, similarly contains high levels of redox-active iron and in response to specific stimuli can initiate mitochondrial apoptosis. Hamacher-Brady et al. (2011) investigated the role of lysosomes in artesunate-induced programmed cell death and determined that in MCF-7 breast cancer cells artesunate activated lysosome-dependent mitochondrial membrane permeabilisation. Artesunate impacted endolysosomal and autophagosomal compartments, inhibiting autophagosome turnover and causing perinuclear clustering of autophagosomes, early and late endosomes, and lysosomes. Lysosomal iron chelation blocked all measured parameters of artesunate-induced cell death, whereas lysosomal iron loading enhanced death, thus identifying lysosomal iron as the lethal source of ROS upstream of mitochondrial membrane permeabilisation. Moreover, lysosomal inhibitors chloroquine and bafilomycin A1 reduced artesunate-activated cell death, evidencing a requirement for lysosomal function during programmed cell death signalling. Artesunate killing did not involve activation of the BH3-only protein, Bid, yet artesunate enhanced TNF-mediated Bid cleavage. We additionally demonstrated the lysosomal cell death pathway in T47D and MDA-MB-231 breast cancer cells. Importantly, non-tumorigenic MCF-10A cells resisted artesunate. We concluded that artesunate triggered cell death via engagement of distinct,

interconnected cell death pathways, with hierarchical signalling from lysosomes to mitochondria, suggesting a potential clinical use of artesunate for targeting lysosomes in cancer treatment.

5 Effect of Artemisinin and Derivatives on Mitochondrial Metabolism

Since mitochondria as cellular powerhouses are crucial for ATP production, their inhibition by artemisinins may offer selective and efficient ways to kill cancer cells. Overcoming multidrug resistance is a main topic of cancer chemotherapy research. As shown by Reungpatthanaphong and Mankhetkorn (2002), artemisinin and its derivatives poorly inhibited the function of P-glycoprotein and did not inhibit the function of MRP1 protein. Artemisinin, artesunate and dihydroartemisinin efficiently decreased the mitochondrial membrane potential, leading to a decrease in intracellular ATP in all cell lines tested. The compounds increased cytotoxicity of pirarubicin and doxorubicin in P-glycoprotein-overexpressing K562/adr, and in MRP1-overexpressing GLC4/adr, but not in their corresponding drug-sensitive cell lines. In this range of concentrations these compounds did not decrease the function of P-glycoprotein, suggesting a mechanism by which the drugs did not reverse multidrug resistance phenomenon at the P-glycoprotein level, but at the mitochondrial level. The disruption of the mitochondrial membrane potential and the ATP depletion caused by artemisinins indicate that metabolic changes in the mitochondria contribute to multidrug resistance, which is at least in part reversible by artemisinin compounds.

Mercer et al. (2011) have used cell models and quantitative drug metabolite analysis to define the role of the mitochondrion and cellular haem in the chemical and molecular mechanisms of cell death induced by artemisinin compounds. HeLa $\rho(0)$ cells, which are devoid of a functioning electron transport chain, were used to demonstrate that actively respiring mitochondria play an essential role in endoperoxide-induced cytotoxicity via the generation of ROS and the induction of mitochondrial dysfunction and apoptosis.

Lu et al. (2012) found that dihydroartemisinin inhibited the proliferation of human hepatocarcinoma BEL-7402 cells in a concentration-dependent manner. An analysis of the mitochondrial proteome was performed employing two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Seven mitochondrial proteins including fumarate hydratase, 60 kDa heat shock protein, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, two subunits of ATP synthase and NADPH:adrenodoxin oxidoreductase were identified to be differentially expressed between the control and dihydroartemisinin-treated groups. These results indicate that the imbalance of energy metabolism induced by dihydroartemisinin may contribute, at least in part, to its anti-cancer potential in BEL-7402 cells.

Neurotoxicity of artemisinin derivatives was discussed as a possible side effect in malaria and cancer therapy. Schmuck et al. (2002) discussed possible mechanisms of brain stem-specific neurodegeneration, in which artemisinin-sensitive neuronal brain stem cell cultures were compared with non-sensitive cultures (cortical neurons, astrocytes). Effects on the cytoskeleton of brain stem cell cultures, but not that of cortical cell cultures, were visible after 7 days. However, after a recovery period of 7 days, this effect also became visible in cortical cells and more severe in brain stem cell cultures. Neurodegeneration was induced by effects on intracellular targets such as the cytoskeleton, modulation of the energy status by mitochondrial or metabolic defects, oxidative stress or excitotoxic events. Artemisinin reduced intracellular ATP levels and the potential of the inner mitochondrial membrane below the cytotoxic concentration range in all three cell cultures, with these effects being most dominant in the brain stem cultures. Surprisingly, there were substantial effects on cortical neurons after 7 days and on astrocytes after 1 day. Artemisinin additionally induced oxidative stress, as observed as an increase of ROS and of lipid peroxidation in both neuronal cell types. Interestingly, an induction of expression of AOE was only seen in astrocytes. Here, manganese superoxide dismutase expression was increased more than 3-fold and catalase expression was increased more than 1.5-fold. In brain stem neurons, manganese superoxide dismutase expression was dose dependently decreased. Copper-zinc superoxide dismutase and glutathione peroxidase, two other antioxidant enzymes that were investigated, did not show any changes in their mRNA expression in all three cell types after exposure to artemisinin. These results demonstrate that mitochondrial ATP changes induced by artemisinin compounds may not only contribute to killing of cancer cells, but also to adverse side effects.

While the activity of artemisinins on mitochondrial pathways is still incompletely understood in mammalian cells, more hints can be found in the malaria literature. The ultrastructural changes induced by artemisinin were already studied in the 1980s by Jiang and colleagues (1985) in monkeys (*Macaca assamensis*) infected with *Plasmodium inui*. Significant changes, notably mitochondrial swelling within the parasites but not within host cells, were first observed 2.5 h after exposure to artemisinin. This suggests that the target of artemisinin may be the parasite's mitochondria. Ultrastructural changes induced in *Plasmodium falciparum* by artemisinin were studied *in vitro* by Maeno et al. (1993). Electron microscopic autoradiography was performed on infected erythrocytes that were exposed in vitro to 3H-dihydroartemisinin and 14C-artemisinin. These drugs consistently were located in food vacuoles and mitochondria. Two hours after administration, changes were observed in parasite mitochondria, rough endoplasmic reticulum, and nuclear envelope.

Krungkrai et al. (1999) assayed both asexual and sexual stages of the parasites for mitochondrial oxygen consumption by using a polarographic assay. The rate of oxygen consumption by both stages was low and not much different. Furthermore, the mitochondrial oxygen consumption by both stages was inhibited to various degrees by mammalian mitochondrial inhibitors that targeted each component of complexes I-IV of the respiratory system. The oxygen consumption by both stages

was also affected by artemisinin suggesting that *P. falciparum* in both developmental stages have functional mitochondria that operate a classical electron transport system, containing complexes I–IV.

Wang et al. (2010) also showed that artemisinin directly acts on mitochondria and it inhibits malaria in a similar way as yeast. Specifically, artemisinin and its homologues exhibited correlated activities against malaria and yeast, with the peroxide bridge playing a key role for their inhibitory action in both organisms. In addition, artemisinins were distributed to malarial mitochondria and directly impair their functions when isolated mitochondria were tested. A strikingly rapid and dramatic ROS production was induced with artemisinin in isolated yeast and malarial mitochondria, and ROS scavengers ameliorated the effects of artemisinin. Deoxyartemisinin, which lacks an endoperoxide bridge, had no effect on membrane potential or ROS production in malarial mitochondria. OZ209, a distantly related antimalarial endoperoxide, also caused ROS production and depolarization in isolated malarial mitochondria. Finally, interference of mitochondrial electron transport chain altered the sensitivity of the parasite towards artemisinin. Addition of iron chelator desferrioxamine drastically reduced electron transport chain activity as well as mitigated artemisinin-induced ROS production.

Using *Saccharamyces cerevisiae*, Li et al. (2005) demonstrated that artemisinin's inhibitory effect was mediated by disrupting the normal function of mitochondria through depolarizing their membrane potential. Moreover, in a genetic study, the electron transport chain was identified as an important player in artemisinin's action. Deletion of *NDE1* or *NDI1*, which encode mitochondrial NADH dehydrogenases, conferred resistance to artemisinin, whereas overexpression of *NDE1* or *NDI1* dramatically increased sensitivity to artemisinin. Mutations or environmental conditions that affect electron transport also altered host's sensitivity to artemisinin. Sensitivity is partially restored when the *Plasmodium falciparum NDI1* orthologue was expressed in yeast ndi1 strain. Finally, artemisinin's inhibitory effect was mediated by ROS and artemisinin's effect was primarily mediated through disruption of membrane potential by its interaction with the electron transport chain, resulting in dysfunctional mitochondria. Li et al. (2005) proposed a dual role of mitochondria played during the action of artemisinin: the electron transport chain stimulates artemisinin's effect, most likely by activating it, and the mitochondria are subsequently damaged by the locally generated free radicals.

6 Conclusions and Perspectives

During the past years, it became clear that artemisinin compounds act on malaria parasites and cancer cells by affecting mitochondrial functions. The generation of ROS, disruption of mitochondrial membrane potential and induction of apoptosis were frequently observed modes of action for artemisinin compounds. While a sufficient amount of results is available for the induction of programmed cell death by artemisinin and its derivatives, research on metabolic disruption of mitochondrial

functions is still in its infancy. There are clear hints that these compounds affect mitochondrial metabolism, but the exact mechanisms still need to be explored.

Recently, a number of highly active, novel artemisinin derivatives, *e.g.* artemisinin dimer molecules, have been described (Chow and Chan 2009; Horwedel et al. 2010). Their effects on mitochondria represent an exciting topic for research in the years to come.

References

Adjuik M, Babiker A, Garner P, Olliaro P, Taylor W, White N, International Artemisinin Study Group (2004) Artesunate combinations for treatment of malaria: meta-analysis. Lancet 363:9–17

Anfosso L, Efferth T, Albini A, Pfeffer U (2006) Microarray expression profiles of angiogenesis-related genes predict tumor cell response to artemisinins. Pharmacogenomics J 6:269–278

Bachmeier B, Fichtner I, Killian PH, Kronski E, Pfeffer U, Efferth T (2011) Development of resistance towards artesunate in MDA-MB-231 human breast cancer cells. PLoS One 6:e20550

Berdelle N, Nikolova T, Quiros S, Efferth T, Kaina B (2011) Artesunate induces oxidative DNA damage, sustained DNA double-strand breaks, and the ATM/ATR damage response in cancer cells. Mol Cancer Ther 10:2224–2233

Berger TG, Dieckmann D, Efferth T, Schultz ES, Funk JO, Baur A, Schuler G (2005) Artesunate in the treatment of metastatic uveal melanoma–first experiences. Oncol Rep 14:1599–1603

Biasutto L, Dong LF, Zoratti M, Neuzil J (2010) Mitochondrially targeted anti-cancer agents. Mitochondrion 10:670–681

Boujrad H, Gubkina O, Robert N, Krantic S, Susin SA (2007) AIF-mediated programmed necrosis: a highly regulated way to die. Cell Cycle 6:2612–2619

Chow LM, Chan TH (2009) Novel classes of dimer antitumour drug candidates. Curr Pharm Des 15:659–674

D'Souza GG, Wagle MA, Saxena V, Shah A (2011) Approaches for targeting mitochondria in cancer therapy. Biochim Biophys Acta 1807:689–696

Dell'Eva R, Pfeffer U, Vené R, Anfosso L, Forlani A, Albini A, Efferth T (2004) Inhibition of angiogenesis in vivo and growth of Kaposi's sarcoma xenograft tumors by the anti-malarial artesunate. Biochem Pharmacol 68:2359–2366

Disbrow GL, Baege AC, Kierpiec KA, Yuan H, Centeno JA, Thibodeaux CA, Hartmann D, Schlegel R (2005) Dihydroartemisinin is cytotoxic to papillomavirus-expressing epithelial cells in vitro and in vivo. Cancer Res 65:10854–10861

Du JH, Zhang HD, Ma ZJ, Ji KM (2010) Artesunate induces oncosis-like cell death in vitro and has antitumor activity against pancreatic cancer xenografts in vivo. Cancer Chemother Pharmacol 65:895–902

Efferth T (2005) Mechanistic perspectives for 1,2,4-trioxanes in anti-cancer therapy. Drug Resist Updat 8:85–97

Efferth T (2006) Molecular pharmacology and pharmacogenomics of artemisinin and its derivatives in cancer cells. Curr Drug Targets 7:407–421

Efferth T (2007) Willmar Schwabe Award 2006: antiplasmodial and antitumor activity of artemisinin–from bench to bedside. Planta Med 73:299–309

Efferth T, Kaina B (2010) Toxicity of the antimalarial artemisinin and its derivatives. Crit Rev Toxicol 40:405–421

Efferth T, Oesch F (2004) Oxidative stress response of tumor cells: microarray-based comparison between artemisinins and anthracyclines. Biochem Pharmacol 68:3–10

Efferth T, Volm M (2005) Glutathione-related enzymes contribute to resistance of tumor cells and low toxicity in normal organs to artesunate. In Vivo 19:225–232

Efferth T, Rücker G, Falkenberg M, Manns D, Olbrich A, Fabry U, Osieka R (1996) Apoptosis in KG-1a leukemic cells treated with investigational drugs. Arzneimittelforschung 46:196–200
Efferth T, Dunstan H, Sauerbrey A, Miyachi H, Chitambar CR (2001) The anti-malarial artesunate is also active against cancer. Int J Oncol 18:767–773
Efferth T, Marschall M, Wang X, Huong SM, Hauber I, Olbrich A, Kronschnabl M, Stamminger T, Huang ES (2002a) Antiviral activity of artesunate towards wild-type, recombinant, and ganciclovir-resistant human cytomegaloviruses. J Mol Med 80:233–242
Efferth T, Olbrich A, Bauer R (2002b) mRNA expression profiles for the response of human tumor cell lines to the antimalarial drugs artesunate, arteether, and artemether. Biochem Pharmacol 64:617–623
Efferth T, Briehl MM, Tome ME (2003a) Role of antioxidant genes for the activity of artesunate against tumor cells. Int J Oncol 23:1231–1235
Efferth T, Sauerbrey A, Olbrich A, Gebhart E, Rauch P, Weber HO, Hengstler JG, Halatsch ME, Volm M, Tew KD, Ross DD, Funk JO (2003b) Molecular modes of action of artesunate in tumor cell lines. Mol Pharmacol 64:382–394
Efferth T, Benakis A, Romero MR, Tomicic M, Rauh R, Steinbach D, Hafer R, Stamminger T, Oesch F, Kaina B, Marschall M (2004) Enhancement of cytotoxicity of artemisinins toward cancer cells by ferrous iron. Free Radic Biol Med 37:998–1009
Efferth T, Giaisi M, Merling A, Krammer PH, Li-Weber M (2007) Artesunate induces ROS-mediated apoptosis in doxorubicin-resistant T leukemia cells. PLoS One 2:e693
Efferth T, Kahl S, Paulus K, Adams M, Rauh R, Boechzelt H, Hao X, Kaina B, Bauer R (2008a) Phytochemistry and pharmacogenomics of natural products derived from traditional Chinese medicine and Chinese materia medica with activity against tumor cells. Mol Cancer Ther 7:152–161
Efferth T, Romero MR, Wolf DG, Stamminger T, Marin JJ, Marschall M (2008b) The antiviral activities of artemisinin and artesunate. Clin Infect Dis 47:804–811
Fafowora MV, Atanu F, Sanya O, Olorunsogo OO, Erukainure OL (2011) Effect of oral coadministration of artesunate with ferrous sulfate on rat liver mitochondrial membrane permeability transition. Drug Chem Toxicol 34:318–323
Fulda S, Galluzzi L, Kroemer G (2010) Targeting mitochondria for cancer therapy. Nat Rev Drug Discov 9:447–464
Galluzzi L, Morselli E, Kepp O, Vitale I, Rigoni A, Vacchelli E, Michaud M, Zischka H, Castedo M, Kroemer G (2010) Mitochondrial gateways to cancer. Mol Aspects Med 31:1–20
Goldsmith KC, Hogarty MD (2009) Small-molecule BH3 mimetics to antagonize Bcl-2-homolog survival functions in cancer. Curr Opin Investig Drugs 10:559–571
Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, Kamphorst JJ, Chen G, Lemons JM, Karantza V, Coller HA, Dipaola RS, Gelinas C, Rabinowitz JD, White E (2011) Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. Genes Dev 25:460–470
Hamacher-Brady A, Stein HA, Turschner S, Toegel I, Mora R, Jennewein N, Efferth T, Eils R, Brady NR (2011) Artesunate activates mitochondrial apoptosis in breast cancer cells via iron-catalyzed lysosomal reactive oxygen species production. J Biol Chem 286:6587–6601
Handrick R, Ontikatze T, Bauer KD, Freier F, Rübel A, Dürig J, Belka C, Jendrossek V (2010) Dihydroartemisinin induces apoptosis by a Bak-dependent intrinsic pathway. Mol Cancer Ther 9:2497–2510
Hockenbery DM (2010) Targeting mitochondria for cancer therapy. Environ Mol Mutagen 51:476–489
Horwedel C, Tsogoeva SB, Wei S, Efferth T (2010) Cytotoxicity of artesunic acid homo- and heterodimer molecules toward sensitive and multidrug-resistant CCRF-CEM leukemia cells. J Med Chem 53:4842–4848
Hunter AM, LaCasse EC, Korneluk RG (2007) The inhibitors of apoptosis (IAPs) as cancer targets. Apoptosis 12:1543–1568
Igney FH, Krammer PH (2002) Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer 2:277–288

Indran IR, Tufo G, Pervaiz S, Brenner C (2011) Recent advances in apoptosis, mitochondria and drug resistance in cancer cells. Biochim Biophys Acta 1807:735–745

Jansen FH, Adoubi I, KC JC, DE Cnodder T, Jansen N, Tschulakow A, Efferth T (2011) First study of oral Artenimol-R in advanced cervical cancer: clinical benefit, tolerability and tumor markers. Anticancer Res 31:4417–4422

Jiang JB, Jacobs G, Liang DS, Aikawa M (1985) Qinghaosu-induced changes in the morphology of Plasmodium inui. Am J Trop Med Hyg 34:424–428

Kelter G, Steinbach D, Konkimalla VB, Tahara T, Taketani S, Fiebig HH, Efferth T (2007) Role of transferrin receptor and the ABC transporters ABCB6 and ABCB7 for resistance and differentiation of tumor cells towards artesunate. PLoS One 2:e798

Konkimalla VB, Blunder M, Korn B, Soomro SA, Jansen H, Chang W, Posner GH, Bauer R, Efferth T (2008) Effect of artemisinins and other endoperoxides on nitric oxide-related signaling pathway in RAW 264.7 mouse macrophage cells. Nitric Oxide 19:184–191

Konkimalla VB, McCubrey JA, Efferth T (2009) The role of downstream signaling pathways of the epidermal growth factor receptor for Artesunate's activity in cancer cells. Curr Cancer Drug Targets 9:72–80

Krungkrai J, Burat D, Kudan S, Krungkrai S, Prapunwattana P (1999) Mitochondrial oxygen consumption in asexual and sexual blood stages of the human malarial parasite, Plasmodium falciparum. Southeast Asian J Trop Med Public Health 30:636–642

Lai H, Sasaki T, Singh NP, Messay A (2005) Effects of artemisinin-tagged holotransferrin on cancer cells. Life Sci 76:1267–1279

Lee J, Giordano S, Zhang J (2012) Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. Biochem J 441:523–540

Li W, Mo W, Shen D, Sun L, Wang J, Lu S, Gitschier JM, Zhou B (2005) Yeast model uncovers dual roles of mitochondria in action of artemisinin. PLoS Genet 1:e36

Li PC, Lam E, Roos WP, Zdzienicka MZ, Kaina B, Efferth T (2008) Artesunate derived from traditional Chinese medicine induces DNA damage and repair. Cancer Res 68:4347–4351

Lu YY, Chen TS, Qu JL, Pan WL, Sun L, Wei XB (2009) Dihydroartemisinin (DHA) induces caspase-3-dependent apoptosis in human lung adenocarcinoma ASTC-a-1 cells. J Biomed Sci 16:16

Lu JJ, Yang Z, Lu DZ, Wo XD, Shi JJ, Lin TQ, Wang MM, Li Y, Tang LH (2012) Dihydroartemisinin-induced inhibition of proliferation in BEL-7402 cells: an analysis of the mitochondrial proteome. Mol Med Report 6:429–433

Maeno Y, Toyoshima T, Fujioka H, Ito Y, Meshnick SR, Benakis A, Milhous WK, Aikawa M (1993) Morphologic effects of artemisinin in Plasmodium falciparum. Am J Trop Med Hyg 49:485–491

Mercer AE, Copple IM, Maggs JL, O'Neill PM, Park BK (2011) The role of heme and the mitochondrion in the chemical and molecular mechanisms of mammalian cell death induced by the artemisinin antimalarials. J Biol Chem 286:987–996

Murphy MP (2009) How mitochondria produce reactive oxygen species. Biochem J 417:1–13

Ramsay EE, Hogg PJ, Dilda PJ (2011) Mitochondrial metabolism inhibitors for cancer therapy. Pharm Res 28:2731–2744

Reungpatthanaphong P, Mankhetkorn S (2002) Modulation of multidrug resistance by artemisinin, artesunate and dihydroartemisinin in K562/adr and GLC4/adr resistant cell lines. Biol Pharm Bull 25:1555–1561

Ribeiro IR, Olliaro P (1998) Safety of artemisinin and its derivatives. A review of published and unpublished clinical trials. Med Trop (Mars) 58:50–53

Schmuck G, Roehrdanz E, Haynes RK, Kahl R (2002) Neurotoxic mode of action of artemisinin. Antimicrob Agents Chemother 46:821–827

Sertel S, Eichhorn T, Sieber S, Sauer A, Weiss J, Plinkert PK, Efferth T (2010a) Factors determining sensitivity or resistance of tumor cell lines towards artesunate. Chem Biol Interact 185:42–52

Sertel S, Eichhorn T, Simon CH, Plinkert PK, Johnson SW, Efferth T (2010b) Microarray-based mRNA expression profiling of genes associated with cytotoxicity of artesunate towards human colon, ovarian and lung cancer cell lines. Molecules 15:2886–2910

Sieber S, Gdynia G, Roth W, Bonavida B, Efferth T (2009) Combination treatment of malignant B cells using the anti-CD20 antibody rituximab and the anti-malarial artesunate. Int J Oncol 35:149–158

Singh NP, Lai H (2001) Selective toxicity of dihydroartemisinin and holotransferrin toward human breast cancer cells. Life Sci 70:49–56

Varfolomeev E, Vucic D (2011) Inhibitor of apoptosis proteins: fascinating biology leads to attractive tumor therapeutic targets. Future Oncol 7:633–648

Vogler M, Dinsdale D, Dyer MJ, Cohen GM (2009) Bcl-2 inhibitors: small molecules with a big impact on cancer therapy. Cell Death Differ 16:360–367

Wang J, Huang L, Li J, Fan Q, Long Y, Li Y, Zhou B (2010) Artemisinin directly targets malarial mitochondria through its specific mitochondrial activation. PLoS One 5:e9582

Weinhouse S (1976) The Warburg hypothesis fifty years later. Z Krebsforsch Klin Onkol Cancer Res Clin Oncol 87:115–126

Wenner CE (2012) Targeting mitochondria as a therapeutic target in cancer. J Cell Physiol 227:450–456

Wu L, Yan B (2011) Discovery of small molecules that target autophagy for cancer treatment. Curr Med Chem 18:1866–1873

Xiao F, Gao W, Wang X, Chen T (2012) Amplification activation loop between caspase-8 and -9 dominates artemisinin-induced apoptosis of ASTC-a-1 cells. Apoptosis 17:600–611

Young AR, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF, Tavaré S, Arakawa S, Shimizu S, Watt FM, Narita M (2009) Autophagy mediates the mitotic senescence transition. Genes Dev 23:798–803

Zhang E, Zhang C, Su Y, Cheng T, Shi C (2011) Newly developed strategies for multifunctional mitochondria-targeted agents in cancer therapy. Drug Discov Today 16:140–146

Chapter 7
Vitamin E Analogues as Prototypic Mitochondria-Targeting Anti-cancer Agents

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Abstract Mitochondria have emerged recently as a novel, intriguing target for anti-caner drugs, owing largely to their importance for proper growth of cancer cells. Destabilization of mitochondria often results in the induction of apoptosis in cancer cells that, consequently, may translate into suppression of tumour growth. A class of mitochondria-targeting compounds, mitocans, comprises several groups of agents with different targets within the mitochondrion. Of these, vitamin E analogues have been recently promoted as agents that disrupt mitochondria by several modes of action. These compounds hold substantial promise as potential anti-cancer drugs of clinical relevance.

Keywords Vitamin E analogues • Cancer • Mitochondria • Redox-silent • Functional domain • Anti-cancer effect • Apoptosis • Signaling pathways • α-tocopherol succinate • α-tocopheryloxyacetic acid • α-tocopheryl maleamide • Mitochondrially targeted vitamin E succinate • Complex II

1 Introduction

In spite of the recent unprecedented advancement in molecular medicine, there has been only limited progress in the search for efficient, selective anti-cancer drugs (Siegel et al. 2013). Although the hallmarks of cancer have been defined and re-defined (Hanahan and Weinberg 2000, 2011), recent findings indicate that cancer is more complex than thought, and, accordingly, the successful treatment may be harder to find than anticipated. This grim notion stems from the findings of high level of genetic and mutational differences amongst patients with the same or very similar type of tumour (Jones et al. 2008; Parsons et al. 2008), as well as within one tumour and the derived metastases in the same patient (Gerlinger et al. 2012). This heterogeneity considerably complicates the treatment (Hayden 2008), indicating that current therapies may be efficient only for some patients and, even worse, may kill only a part of the tumour cells.

Therefore, it is imperative to search for an invariant target that could be utilised across the landscape of different cancers and also could be used to selectively kill cancer cells with low or no effect on normal cells and tissues. Such a plausible target is presented by mitochondria, essential organelles that are the major source of energy as well as purveyors of cell death. Recently, there has been a focus on mitochondria as potential targets for cancer therapy, with numerous original papers and several excellent reviews (see e.g., Wallace 2012; Galluzzi et al. 2010; Fulda et al. 2010; Ralph et al. 2010a, b; Gogvadze et al. 2008). We have been coining the term 'mitocans' (standing for 'mitochondria' and 'cancer'), encompassing a class of compounds that exert anti-cancer activity by way of targeting and destabilising mitochondria, causing apoptosis, often selectively in cancer cells (Ralph et al. 2006; Neuzil et al. 2006, 2013). Of these agents, we and others have focused on intriguing types of mitocans, vitamin E (VE) analogues, that act via mitochondria, causing apoptosis selectively in cancer cells, and featuring anti-tumour activity (*e.g.* Neuzil

et al. 2007; Zhao et al. 2009; Angulo-Molina et al. 2014). In this review, we focus on VE analogues, discussing their structural features, molecular mode of activity as well as their promise as efficient anti-cancer agents.

2 Structure of Vitamin E and Its Analogues

VE is a generic term that refers to a family of eight different naturally occurring phenolic compounds (Sen et al. 2000; Elnagar et al. 2010). These agents belong to two categories, tocopherols and tocotrienols. Both groups share the aromatic chromanol group with two rings (one phenolic and one heterocyclic) and an isoprenoid chain (a 16-carbon tail) attached at the C2 position. Tocopherols differ from tocotrienols only in the aliphatic tail (Kamal-Eldin and Appelqvist 1996). Tocopherols refer to VE compounds with a saturated phytyl side chain, tocotrienols refer to compounds containing poly-unsaturated side chain. Each group includes four isoforms, named α-, β-, γ-, and δ-tocopherols or tocotrienols, which differ by the number and position of methyl groups on the chromanol ring (see the structures in Fig. 7.1) (Aggarwal et al. 2010; Elson and Yu 1994). The role of these molecules as lipophilic antioxidants *in vitro* and *in vivo* is widely accepted (Neuzil et al. 2007). It has been reported that both tocopherol and tocotrienol isoforms display a wide-range of antioxidant-related activities (McIntyre et al. 2000), with the level of phytyl chain saturation and/or chromanol ring methylation critical for the differential bioactivity of the individual isoforms (Elson and Yu 1994; McIntyre et al. 2000). In addition, non-antioxidant properties of VE family members have also been investigated (Akazawa et al. 2002).

While α-tocopherol (α-TOH) was the first VE analogue to be recognized, and was named in 1924 and synthesized in 1938 (Sen et al. 2007), the term tocotrienol was first suggested by Bunyan and colleagues (Bunyan et al. 1961), and tocotrienols were first isolated from the latex of the rubber plant in 1964 (Whittle et al. 1996). Furthermore, among the VE isomers, α-tocopherol is the most common form found in human diets; it also has the highest bioavailability. So far, α-tocopherol is the best characterized VE isomer and has been examined in a large number of studies (Ling et al. 2012). There is evidence suggesting that human tissues can convert tocotrienols to tocopherols (Qureshi et al. 2001, 2002). The differences in the aliphatic side chain may account for the differences in the efficacy and potency both *in vitro* and *in vivo*. To date, over 30,000 papers have been published on tocopherols, and about 600 on tocotrienols (Aggarwal et al. 2010). The most studied functions of these two groups of compounds are related to their antioxidant (Lee et al. 2009; Cai et al. 2012), anti-proliferative and anti-survival (Shun et al. 2004; Mudit et al. 2010), anti-inflammatory (Bachawal et al. 2010; Ivanova et al. 2011), anti-angiogenic (Inokuchi et al. 2003; Weng-Yew et al. 2009), pro-apoptotic and anti-cancer (Wu and Ng 2010; Parker et al. 1993), cardio-protective (Koba et al. 1992) and neuro-protective activity (Aggarwal et al. 2010; Sen et al. 2000).

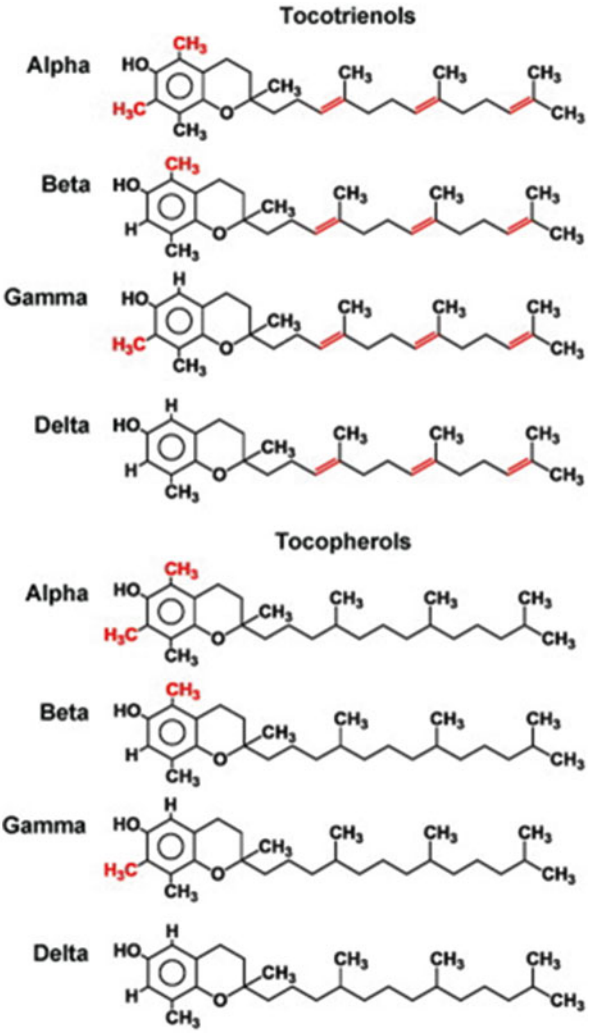

Fig. 7.1 Chemical structures of naturally occurring forms of vitamin E, tocotrienols and tocopherols

While the naturally occurring VE compounds have been a focus of numerous studies for many years since they were first recognized in 1924, semisynthetic VE analogues, a group of compounds which are derived from VE and share the same core structure with VE, have recently emerged as very important compounds with even higher biological activity, especially the anti-cancer effect. This group of compounds have been represented by agents like α-tocopheryl succinate (α-TOS), α-tocopheryl ether-linked acetic acid (α-TEA), or mitochondrially targeted vitamin E succinate (MitoVES). Their anti-cancer activity and the molecular mechanism of their biological action have been studied profoundly in recent years and will be highlighted in this paper.

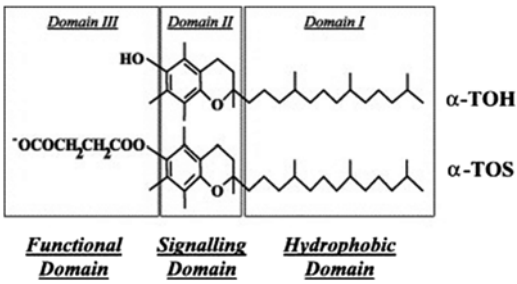

Fig. 7.2 Structures and major domains of α-TOH and α-TOS

2.1 Functional Domains of Vitamin E Analogues

VE analogues, including α-tocopheryl succinate, α-tocopherol ether-linked acetic acid, α-tocopheryl polyethylene glycol 1000 succinate, tocopheryl nicotinate, α-tocopheryl ferulate, tretinoin tocoferil or α-tocopheryl phosphate have been synthesized and their cellular effects investigated. They are examples of VE analogues modified at the tocopheryl-C6 position that do not have antioxidant activity in their intact form, unless they are hydrolysed, suggesting that individual 'regions' of the molecule of VE and its analogues can be divided into functionally different groups. Thus, the VE molecule comprises three different domains (Fig. 7.2): the hydrophobic domain (I), the signalling domain (II) and the functional domain (III) (Neuzil et al. 2007).

(i) The functional domain is the group attached at C6 of the chromanol ring. This position determines whether the molecule behaves as a redox-active or a redox-silent compound. The well documented antioxidant properties of the four tocopherol isomers and their bioavailability (in particular that of α-tocopherol) led to their testing in cancer clinical trials. In the two of the largest chemoprevention trials, VE (α-tocopherol) consumption was found to have no beneficial effect on cancer incidence, indicating little if any positive outcome concerning the use of free tocopherols in cancer prevention (Pham and Plakogiannis 2005). However, certain chemical modifications at C6, such as those resulting in the generation of ether, ester and amide analogues (with a free carboxylate) proved to endow the parental agents with pro-apoptotic and anti-neoplastic activity, which resulted in a concerted research on the anticancer efficacy of these agents (Neuzil et al. 2007).

(ii) The signalling domain is responsible for activities of the agents that are independent of the antioxidant nature of tocopherols and are given by the methylation pattern of the aromatic ring. For example, α-tocopherol has been reported to inhibit protein kinase C (PKC) by decreasing diacylglycerol levels, whereas other tocopherols with similar antioxidant efficacies do not inhibit the kinase activity. Thus, the PKC inhibitory activity of α-tocopherol is independent of its antioxidant capacity (Kunisaki et al. 1995; Tasinato et al. 1995).

(iii) The lipophilic side chain of VE compounds distinguishes between tocopherols with saturated isoprenyl units and tocotrienols with unsaturated isoprenyl units. The hydrophobic domain determines whether the molecule can bind to lipoproteins and membranes or will be degraded by phase I enzymes (Birringer et al. 2002; Neuzil et al. 2007). It has been reported that tocotrienols can be degraded to a larger extent than their counterparts with saturated side chains, and there are quantitative differences in the metabolism between individual tocopherols as well as between tocotrienols and tocopherols (Kogure et al. 2005).

2.2 *Redox-Silent Analogues of Vitamin E*

VE analogues with a modified hydroxyl group of α-TOH have been tested for their pro-apoptotic activity. The most prominent derivative of this group (and certainly the most studied one) is α-TOS (Fig. 7.2), bearing a succinyl group at position C6 of the chromanol ring. Because of its low pKa (<6), α-TOS is fully deprotonated under physiological conditions, leading to a detergent-like molecule, which destabilizes mitochondrial membranes (Neuzil et al. 2007). Dicarboxylic esters of tocopherols present the best studied compounds for structure-activity relationship (SAR). Strong apoptogens include α-TOS, α-tocopheryl oxalate and α-tocopheryl malonate, the latter two inducing non-selective cytotoxicity in mice inoculated with B16-F1 melanoma cells (Kogure et al. 2005). Even greater apoptogenic activity has been observed for unsaturated dicarboxylic acids like α-tocopheryl maleate (Birringer et al. 2003). Increasing the chain length of the dicarboxylic acid led to decreased activity as shown for α-tocopheryl glutarate and α-tocopheryl methyl glutarate (Birringer et al. 2003), or α-tocopheryl pimelate (Kogure et al. 2004) exhibiting no activity at all. The substitution pattern of the chromanol ring is often not merely related to the antioxidant properties of tocopherols (Azzi et al. 2002). An important feature of compounds with α-tocopheryl structure is their selective recognition by the hepatic α-tocopherol transfer protein (TTP). The relative affinities of the homologues of VE for TPP decrease with the loss of methylation of the chromanol ring (Hosomi et al. 1997).

2.3 *Other Vitamin E Analogues with Anti-proliferative Activity*

A number of compounds with modifications in the functional domain exhibit anti-proliferative activity and exert additional properties. For example, α-tocopheryl polyethylene glycol succinate (α-TPGS) has been used as a vehicle for drug delivery systems. As one of the components of the nano-emulsion vehicles (NEs), it showed significant pro-apoptotic effect for both murine breast and colon carcinoma cell lines, indicating built-in anti-cancer properties for such NE platforms, potentially enhancing overall anti-neoplastic effects of incorporated chemotherapeutic agents (Jordan et al. 2012). α-TPGS also showed enhanced chemosensitization and

anti-tumour efficacy against non-small cell lung cancer cell lines (Gill et al. 2012). In addition, this compound has been shown to possess anti-cancer activity against human lung carcinoma cells implanted in nude mice. The apoptosis-inducing efficacy of the compound was not due to its increased uptake by cells but rather due to increased ability to generate ROS (Youk et al. 2005).

Another example is α-tocopheryl phosphate (α-TOP), a water-soluble molecule, which has been found in animal and plant tissues (Gianello et al. 2005). It has been shown to exert more potent cellular effects than α-tocopherol itself in terms of inhibition of cell proliferation and regulation of gene expression. It may directly modulate intermediate steps of signal transduction or gene regulation (Negis et al. 2009). At a higher concentration, α-TOP induces apoptosis, as documented, for example, by its high anti-proliferative and apoptogenic effect on MG-63 cancer cells (Rezk et al. 2007). The reason for α-TOP being more efficient than α-tocopherol may be due to its better penetration across the plasma membrane (Gianello et al. 2005), and it may also be possible that the phosphorylated forms of tocopherol can be transported to the sites of action more efficiently than the non-phosphorylated ones. It is believed that α-TOP can act as a signalling molecule, modulating cell functions such that it may be a source of α-TOH that can undergo enzymatic dephosphorylation and phosphorylation reactions, modulating cell signalling pathways (Negis et al. 2005). Mixtures of α-TOP and di-α-TOP were shown to inhibit proliferation of rat aortic smooth muscle cells as well as THP-1 monocytic leukemia cells (Munteanu et al. 2004). It was proposed that α-TOS and α-TOM act in cancer cells by mimicking and substituting for α-TOP, causing permanent activation of cellular signalling (Neuzil et al. 2007).

Furthermore, two experimental α-tocopheryl esters, the all-trans retinoic acid α-tocopoheryl ester and 9-cis retinoic acid α-tocopoheryl ester, have been documented to reduce proliferation of acute promyelocytic leukemia (APL) cells. The 9-cisretinoic acid α-tocopheryl ester also inhibited proliferation of APL-derived NB4 and HT93 cells and induced their differentiation, as documented by biomarkers, such as granulocytic maturation, nitroblue tetrazolium reduction and enhanced CD11b expression (Makishima et al. 1998). Transactivation experiments with a retinoid receptor-responsive reporter construct revealed that both compounds acted as agonists of retinoic acid receptors. γ-Carboxyethyl hydroxychroman, a degradation product of γ-tocopherol often found secreted in the urine, reduces proliferation of PC-3 prostate cancer cells by inhibiting cyclin D1 expression (Galli et al. 2004).

2.4 Tocotrienol and Its Analogues

Tocotrienols are forms of VE with potent anti-proliferative activity against different types of cancer cells with little or no effect on normal cell growth or functions. Previous studies suggested that tocotrienol treatment is associated with significantly lower tumour incidence, lower tumour numbers and longer tumour latency in mammary carcinoma models (Whittle et al. 1996). However, physico-chemical and pharmacokinetic properties greatly limit their use as therapeutic agents. Chemical

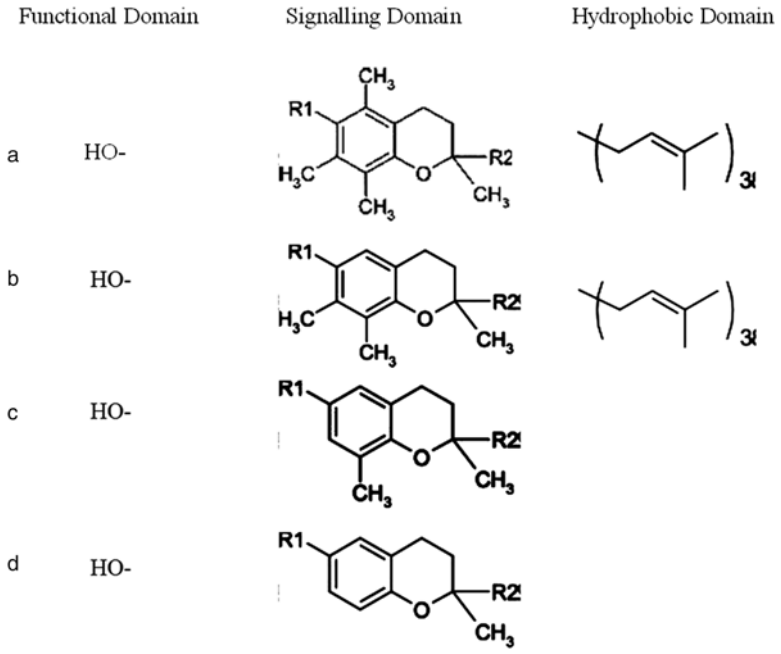

Fig. 7.3 Modification of the hydrophobic domain of tocotrienols

instability, poor water solubility, and rapid metabolism of tocotrienols are examples of obstacles that hinder the therapeutic use of these natural products and their semi-synthetic analogues (Behery et al. 2010). Tocotrienols exhibit their pro-apoptotic activity without modifications of the functional domain. The hierarchy in the signalling domain is reversed, making δ-tocotrienol (Fig. 7.3c) the most potent agent, followed by γ-tocotrienol (Fig. 7.3b) and α-tocotrienol (Fig. 7.3a) (He et al. 1997). The pro-apoptotic property of tocotrienols may be related to the inactivation of the Ras family of proteins. Recent studies indicate that both γ- and δ-tocotrienols have potent anti-proliferative and pro-apoptotic activity in several different types of pancreatic cancer cells, Panc-28, MIA PaCa-2, Panc-1 and BxPC-3. The mechanisms of the anti-cancer effects of the two tocotrienols mainly involve the Akt and the Ras/Raf/MEK/ERK signalling pathways (Shin-Kang et al. 2011). A direct inhibitory action of tocotrienols has been proposed, since the membrane-anchoring cysteine residue of Ras proteins is modified by a common structural element, a farnesyl chain. Accordingly, Ras farnesylation and RhoA prenylation were inhibited by tocotrienols in A549 cells with an activating Ras mutation (Yano et al. 2005).

It was reported that γ-tocotrienol displays potent anti-cancer activity associated with suppression of HER/ErbB receptor signalling (Ayoub et al. 2011), and a recent study showed that γ-tocotrienol enhances chemo-sensitivity of human oral cancer cells to docetaxel through the down-regulation of nuclear factor-κB (NF-κB)-regulated anti-apoptotic gene products (Kani et al. 2013). Interestingly, the number and position of methyl substituents in tocotrienols affect their hypocholesterolemic, antioxidant, and anti-tumour properties. The desmethyl tocotrienol (Fig. 7.3d),

lacking all aromatic methyl groups, shows even higher activity with the IC_{50} value of 0.9 μM. This compound has been isolated from rice bran, giving it the status of a native product (Qureshi et al. 2000).

3 Molecular Mechanism of Anti-cancer Function of Vitamin E Analogues

Advanced sequencing and bioinformatics lead to the discovery that tumours are heterogeneous. This is demonstrated by recent reports revealing the extraordinary variability of mutations in the same type of tumour from different patients (Jones et al. 2008; Parsons et al. 2008; Hayden 2008). This grim notion has been further accentuated by the finding that there are differences in the mutational signatures of different regions even in a single tumour and its metastases (Gerlinger et al. 2012). These findings underscore the low possibility of the discovery of an efficient cancer cure that would target only a single gene or a single signalling pathway. Currently, the majority of established anti-cancer drugs are either non-selective or gradually lose their efficacy because of the constant mutational changes in malignant cells. This is also likely to be the major reason why cancer incidence is either stagnating or on the rise (Siegel et al. 2012). Therefore, pursuing cancer control strategies is one of the major tasks for scientists, and discovery and development of novel selective and efficient anticancer agents for treating neoplastic diseases is of paramount importance.

VE analogues have been studied extensively for their anti-cancer potential and have been shown to possess strong pro-apoptotic and anti-cancer efficacy in different types of cancer. They are selective for malignant cells, cause destabilization of cancer cell mitochondria, and suppress cancer in pre-clinical models. The feature of selectivity for malignant cells, different molecular mechanisms of anti-cancer activity as well as their unique targets via which they exert anti-cancer activity endow VE analogues agents with a great promise to be developed into novel anti-cancer drugs to benefit cancer patients. Anti-cancer molecular mechanisms of VE analogues are summarized below.

3.1 Mitochondrial Apoptotic Pathways Induced by Vitamin E Analogues

Mitochondria are organelles important for both life and death of a cell. They are membrane-enclosed structures distributed in the cytosol of most eukaryotic cells, with the mitochondrial outer membrane (MOM) defining the entire organelle and the mitochondrial inner membrane (MIM) containing the fluid-filled matrix. Mitochondria are the major source of cellular energy with a crucial role in cellular bioenergetics and are vital for signalling of mammalian cells. However they are also central actors in cell death, critically contributing to the process of apoptosis induction and progression (Ralph et al. 2006; Neuzil et al. 2006). Furthermore,

mitochondria are also a major source of intracellular reactive oxygen species (ROS); as such, they are themselves potentially vulnerable to oxidative stress. When ROS production exceeds the capacity for detoxification and repair, oxidative damage to proteins, DNA, and phospholipids can occur, disrupting mitochondrial OXPHOS and eventually leading to the impairment of cell function and death. Accordingly, mitochondrial ROS production and oxidative damage are attractive targets for pharmacological intervention.

3.1.1 Initiation of Mitochondrial Membrane Permeabilization

Apoptosis, an organized sequence of events controlled by a network of genes, is an essential process during development and plays a key role in a variety of pathologies. There are many triggers of apoptosis, including increased levels of oxidants within the cell, damage to DNA by these oxidants or other agents, accumulation of proteins that fail to fold properly, or signalling by molecules binding to death receptors. Apoptosis induction is also a major process by which VE analogues kill cancer cells.

Various apoptotic stimuli may trigger the formation of the MOM pore (MOMP). This is a complex process that involves numerous molecular players and also serves as a target for anti-cancer drugs. During this event, both MOM and MIM are permeabilized, resulting in the "rupture" of the MOM and the release of soluble proteins from the inter-membrane space into the cytosol. This process is accompanied by the loss of the mitochondrial inner trans-membrane potential ($\Delta\Psi_{m,i}$), depletion of the cellular ATP pool and increase in the ROS levels, which, together, contribute to the cell demise. The fact that MOMP represents or is close to the commitment point in the process of cell death cascade has prompted efforts to develop agents capable of efficiently eliciting these events (Neuzil et al. 2007; Alirol and Martinou 2006).

ROS generation is important in apoptosis induction involving mitochondria. The generation of ROS is an early event occurring in response to VE analogues. Mitochondrial respiratory chain within the MIM is a major intracellular source of ROS, which cause damage to lipids, proteins and DNA, leading to alteration or loss of cellular function and, consequently, trigger or amplify the destabilization of the MOM. α-TOS is able to induce ROS accumulation in many different cancer cell lines, most probably originally in the form of superoxide anion radicals (Weber et al. 2003). Substantial accumulation of ROS in Jurkat T lymphoma cells was observed within 1 h, most likely as a result of disrupting the electron flow within the mitochondrial complex II (CII) in the respiratory chain when the cells were challenged with α-TOS (Weber et al. 2003; Dong et al. 2008).

3.1.2 Apoptotic Signalling Downstream of Mitochondria

Although the initial apoptosis triggers have not been completely resolved, the events in apoptosis induced by VE analogues downstream of mitochondria are relatively well understood. During the apoptotic process induced by VE

analogues, down-stream events of mitochondrial destabilization comprise mobilization of apoptosis mediators, including cytochrome c, the apoptosis-inducing factor (AIF), and Smac/Diablo. In turn, they set in motion a series of biochemical events that mediate the execution phase of the cell death programme resulting in the degradation of both key proteins by caspases and of genomic DNA by endonucleases.

Cytochrome c is a key player in mitochondria-dependent apoptosis, leading to caspase activation. The soluble protein is anchored to the MIM via its affinity to the mitochondria-specific phospholipid cardiolipin (CL), and the binding is disrupted upon oxidation of CL by ROS derived from the OXPHOS complexes. Increasing evidence suggests that ROS play a key role in promoting cytochrome c release from the mitochondria upon exposure of cancer cells to α-TOS, and the protein in the cytoplasm triggers activation of the caspase cascade that ultimately leads to apoptosis (Kogure et al. 2002). ROS induce dissociation of cytochrome c from CL by way of causing CL hydroperoxidation, which lowers the affinity of the phospholipid for cytochrome c, and the protein may then be released via the mitochondrial permeability transition (MPT)-dependent or MPT-independent mechanisms. ROS also promote Ca^{2+}-dependent MPT, with swelling of the mitochondrial matrix and rupture of the MOM (Kakkar and Singh 2007), and MTP-independent mechanisms involving the voltage-dependent anion channel in the MOM or an oligomeric form of Bax (Petrosillo et al. 2003).

The mitochondrial pro- and anti-apoptotic proteins, including Bax, Bak, Bcl-2, Mcl-1 and Bcl-x_L, are important modulators related to apoptotic signalling pathway, regulating the formation of a mega-channel across the MOM (Cory et al. 2003). Generation of the MPT pore and translocation of Bax from the cytosol to the mitochondria have also been suggested as an important event upon exposure of cancer cells to α-TOS. This process is likely modulated by a balance between the Bcl-2 family of pro- and anti-apoptotic proteins (Yamamoto et al. 2000; Yu et al. 2003). Over-expression of Bax results in sensitization of cells to α-TOS-induced apoptosis, whereas over-expression of Bcl-2 or Bcl-x_L protects them from α-TOS. Likewise, down-regulation of Bcl-2 by antisense oligodeoxynucleotide treatment sensitized cells to the VE analogue (Weber et al. 2003).

Probably the most compelling evidence for mitochondria as major transmitters of apoptotic signalling induced by VE analogues stems from experiments in which ρ^0 cells were found to be resistant to α-TOS (Weber et al. 2003; Wang et al. 2005). It was found that cancer cells lacking mitochondrial DNA failed to translocate cytochrome c when challenged with α-TOS, unlike the apoptosis-sensitive parental cells, and also showed low levels of phosphatidyl serine externalization and caspase-3 activation. Similar resistance of ρ^0 cells has been found for other inducers of apoptosis, including tumour necrosis factor-α (TNF-α) (Higuchi et al. 1997).

Collectively, mitochondria are the critical intracellular organelles that relay the initial apoptotic signals downstream to the apoptosis commitment stage. It needs to be noticed, though, that other organelles may also be involved in apoptosis induced by VE analogues, such as lysosomes (Neuzil et al. 2002).

3.2 *Mitochondria-Independent Apoptosis Induction by Vitamin E Analogues*

Although mitochondria are central to apoptosis induction by VE analogues via the intrinsic signalling pathways, a number of extrinsic non-mitochondrial or cytoplasmic signalling pathways have also been implicated to play a role in apoptosis induction by VE analogues in many types of tumour cells. The extrinsic signalling pathways comprise a number of mediators, and it has been recently established that death receptor, mitogen-activated protein kinase (MAPK), protein kinase C (PKC) and NF-κB signalling pathways are all related to α-TOS-triggered apoptosis (Yu et al. 2001).

3.2.1 Activation of Death Receptors by Vitamin E Analogues

Activation of the extrinsic cell death pathway is initiated by ligation of cell surface death receptors (DRs) including Fas, the TNF receptor, and the TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 (DR4) and TRAIL receptor-2 (DR5). DRs are constitutively expressed on the surface of mammalian cells, and both the Fas and TRAIL systems are effective against carcinogenesis in pre-clinical models. Impaired apoptotic signalling pathways endow some types of malignant cells with resistance to DR-mediated apoptosis, and such tumours are rather difficult to treat (O'Connell et al. 2000; Cretney et al. 2002). It has been reported that α-TOS-mediated apoptosis involves DR signalling. For example, Fas-resistant breast cancer cells were sensitized by α-TOS via mobilization of the cytosolic Fas protein to the cell surface (Turley et al. 1997). In a separate study, expression of Fas, Fas-associated death domain and caspase-8 was enhanced after α-TOS treatment in gastric cancer cells, whereas Fas antisense oligonucleotides inhibited the expression of the Fas-associated death domain protein and decreased caspase-8 activity (Wu et al. 2002).

TRAIL has attracted attention as a selective immunological apoptogen with anticancer activity. Tumour cells escape from TRAIL-induced death signalling when the balance between DRs and the 'anti-apoptogenic' decoy receptors is altered, and the latter are expressed at a higher level. It was found that the combination of TRAIL with chemotherapeutics or radiation resulted in a synergistic apoptotic response proceeding via caspase-activating signals. α-TOS and TRAIL showed a synergistic pro-apoptotic activity both *in vitro* and *in vivo* for experimental colon cancer (Weber et al. 2002). α-TOS also sensitized the resistant MM and osteosarcoma cells to TRAIL. The IC_{50} value of TRAIL treatment was greatly decreased when treating MM cells with sub-lethal doses of α-TOS, whereas an antagonistic effect of α-TOS on TRAIL sensitivity was found in the case of non-malignant mesothelial cells (Tomasetti et al. 2004). Combination of α-TOS and TRAIL resulted in enhanced apoptosis in a caspase- and p53-dependent manner (Weber et al. 2003; Tomasetti et al. 2006), and α-TOS elevated the expression of DR4 and DR5 without modulating the expression of the decoy receptors in MM cells (Tomasetti et al. 2004, 2006).

α-TOS also enhanced the sensitivity of Jurkat T lymphoma cells to TRAIL-induced apoptosis by suppression of NFκB activation (Dalen and Neuzil 2003). Thus, VE analogues may play a role in adjuvant therapy of DR-resistant cancers. These analogues can also be used alone, because they are expected to sensitize cancer cells to endogenous immunological inducers of apoptosis by cells of the immune system, thereby potentiating the natural tumour surveillance.

3.2.2 MAP Kinase Pathway and Apoptosis Induction by Vitamin E Analogues

The mitogen-activated protein kinase (MAPK) pathway is one of the most frequently studied pathways in cancer. It plays an important role in the regulation of many processes that are critical to the malignant phenotype, and a variety of genetic alterations that activate the pathway have been detected in different types of cancer. As a result, the search for compounds that inhibit the pathway has been an active area of investigation for many years (Davies and Kopetz 2013). The importance of the MAPK pathway is in the control of cellular responses to the environment and in the regulation of gene expression, cell growth, and apoptosis, making it a priority for research related to many disorders (Fang and Richardson 2005). The c-Jun N-terminal kinases (JNKs), along with Erk and p38, constitute the principle members of the MAPK family (Johnson and Lapadat 2002; Wagner and Nebreda 2009). JNKs function primarily through the activator protein-1 (AP-1) family of transcription factors to regulate a plethora of cellular processes, including cell proliferation, differentiation, survival and migration (Zhang and Selim 2012). There is also a cross-talks and integration with other signalling pathways in a cell context-specific and cell type-specific manner (Wagner and Nebreda 2009). JNKs were originally identified as the major kinase responsible for the phosphorylation of c-jun, leading to increased transcriptional activity of AP-1, again largely in response to multiple cellular stimuli, including stress events, growth factors, and cytokines (Nishina et al. 2004).

Kline's group was the first to report a role of JNK and c-jun in α-TOS-induced apoptosis. The VE analogue up-regulated c-jun expression in different types of cancer cells (Qian et al. 1996; Yu et al. 1997). α-TOS-triggered apoptosis involves a prolonged increase in c-jun expression, and AP-1 trans-activation and transfection of dominant-negative c-jun reduced α-TOS-mediated apoptosis. It was subsequently demonstrated that α-TOS enhanced ERK1/2 and JNK activity but not the p38 kinase activity (Yu et al. 2001). Increased phosphorylation and trans-activation of c-jun and ATF-2 were also observed in cells exposed to α-TOS.

Three upstream components of the JNK cascade, apoptosis signal-regulating kinase 1, growth arrest DNA damage-inducible 45β, and stress-activated protein kinase/ERK kinase-1 were all induced, and the level of phospho-JNK was also noticeably increased in prostate cancer cells in response to α-TOS (Zu et al. 2005). Dominant-negative JNK significantly reduced c-jun expression and apoptosis triggered by the agent. On the other hand, α-TOS stimulated early activation of ERK1/2

and, in turn, reduced the ERK activity concomitant with the activation of PKC in HL60 cells. However, blockage of ERK activity showed no significant effects on α-TOS-triggered apoptosis (Bang et al. 2001). Conversely, it was reported that α-TOS and α-tocopheryloxybutyric acid inhibited ERK phosphorylation and activated p38 in breast cancer cells (Akazawa et al. 2002). The discrepancy in the role of ERK activity may result from differences in the treatment time in that ERK can be rapidly and transiently induced by α-TOS, but longer exposures may lead to the suppression of ERK activation. Thus, there is strong evidence that the JNK cascade is an important modulator of apoptosis induced by α-TOS. However, it is not clear at this stage how this signalling pathway is linked to the destabilization of mitochondria by the VE analogue.

3.3 Role of Protein Kinase C in Apoptosis Induced by Vitamin E Analogues

Protein kinase C (PKC) has been a tantalizing target for drug discovery ever since it was first identified as the receptor for the tumour promoter phorbol ester in 1982 (Mochly-Rosen et al. 2012). It forms a multi-gene family of phospholipid-dependent serine/threonine protein kinases involved in modulation of divergent biological functions (Spitaler and Cantrell 2004). Much of the interest in PKC began with the discovery that members of this family of isozymes are activated in various diseases (Mochly-Rosen et al. 2012), including diabetes (Geraldes and King 2010), cancer (Totoń et al. 2011), ischaemic heart disease (Inagaki et al. 2006) and heart failure (Ferreira et al. 2011). PKC is normally present in an inactive form. Binding of cofactors to the regulatory domain induces conformational changes that result in the activation of the enzyme, which is usually associated with its membrane translocation (Basu 2003). Treatment of Jurkat cells with α-TOS caused a decrease in PKC activity by activation of PP2A, leading to hypophosphorylation of PKCα and decreased phosphorylation of Bcl-2 on Ser70 (Ruvolo et al. 1998; Neuzil et al. 2001c). Phorbol-12-myristate-13-acetate, a PKC activator, efficiently protected the cells from apoptosis induced by α-TOS, indicating an inhibitory role of PKC in the regulation of apoptosis (Neuzil et al. 2001c).

PKC isozymes can also be activated by proteolytic separation of the regulatory and the catalytic domain. Several members of the PKC family have now been identified as substrates for caspases. During apoptosis, activation of caspases results in the cleavage of PKC isozymes followed by PKC activation (Endo et al. 2000; Smith et al. 2003). It was shown that α-TOS induced apoptosis via activation of PKCβII and promoted PKCα membrane translocation, concomitant with a decline in ERK activity (Bang et al. 2001). The differences in the effects of α-TOS on PKC in relation to apoptosis might be due to the presence of specific PKC isozymes in cells of different origin, resulting in different or even opposing effects on the outcome of apoptosis.

3.4 Role of Nuclear Factor-κB in Apoptosis Induced by Vitamin E Analogues

NFκB signalling plays a critical role in cancer development and progression (Karin 2006). NFκB provides a mechanistic link between inflammation and cancer, and is a major factor controlling the ability of both pre-neoplastic and malignant cells to resist apoptosis-based tumour-surveillance mechanisms. NFκB might also regulate tumour angiogenesis and invasiveness, and the signalling pathways that mediate its activation provide attractive targets for new chemopreventive and chemotherapeutic approaches (Karin 2006). Activation of the multi-complex transcription factor NFκB is crucial for a wide variety of cellular responses. In non-stimulated cells, NFκB is sequestered in the cytoplasm by the inhibitory κB (IκB). Upon activation by a number of stimuli, the IκB protein is rapidly degraded, allowing translocation of NFκB into the nucleus and binding to cognate-response elements. In addition to its fundamental role in regulation of immune and inflammatory responses, NFκB also exerts anti-apoptotic activities.

In line with the above, it was found that NFκB activation stimulated by TNF-α was inhibited by α-TOS in Jurkat and endothelial cells (Suzuki and Packer 1993; Neuzil et al. 2001a), possibly sensitizing them to apoptosis induction. Because activation of NFκB is negatively associated with apoptosis induced by TRAIL in multiple cancer cells, agents that inhibit NFκB activation may convert TRAIL-resistant to TRAIL-sensitive cells. TRAIL may transiently activate NFκB, thereby delaying the onset of apoptosis. We found that α-TOS has the capacity to overcome this resistance by suppressing TRAIL-stimulated NFκB activation. This is based on modulating the degradation of IκB, which sensitizes cells to the effect of TRAIL (Dalen and Neuzil 2003).

4 Vitamin E Analogues, Anti-cancer Agents Targeting Mitochondria

Cancer is an ever-increasing neoplastic disease that has been a major threaten of the mankind for centuries. In 2012, over a million of Americans, representing an industrialized nation, were diagnosed with cancer, and most of them suffered more from metastatic tumours and drug side-effects rather than from the initial tumours. Despite great advances in molecular medicine and increasing understanding of molecular signalling pathways related to cancer as well as great efforts to develop better treatments, currently there is still no selective and efficient cure for most types of cancer.

As mentioned before, mitochondria have come into the focus of current research of cancer biologists, since they have emerged as an intriguing target for anti-cancer drugs, inherent to vast majority if not all cancer types. The exceptional potential of mitochondria as an emerging and perspective target for anti-cancer agents has been reinforced by the discouraging finding that there are different mutations in individual areas even in the same tumour in one patient (Gerlinger et al. 2012). This is consistent

with the idea of personalised therapy, an elusive goal at this stage, in line with the notion that tumours are unlikely to be treated by agents that target only a single gene or a single pathway. This endows mitochondria, an invariant target present in all tumours, with an exceptional momentum. Drugs that target mitochondria and exert anti-cancer activity have become a focus of recent research due to their great clinical potential. Mitochondria, whilst being the 'powerhouse' of the cell, are also reservoirs of a number of apoptosis-promoting proteins that are essential for apoptosis induction and its progression downstream of these organelles, in order for the cancer cell to shift into the commitment phase and to undergo the final demise (Galluzzi et al. 2010). It is also important to take into consideration the aberrant mitochondrial metabolism of malignant cells (Koppenol et al. 2011; Ward and Thompson 2012). Thus, the recent decade has witnessed an unprecedented focus on and discovery of novel agents that target mitochondria to induce cancer cell death, which opened a new paradigm of efficient cancer therapy and is likely to benefit cancer patients in the (near) future.

Mitochondria are proving to be worthy targets for activating specific killing of cancer cells in tumours and a diverse range of mitochondrially targeted drugs are currently in clinical trial to determine their effectiveness as anti-cancer therapies. The mechanism of action of many mitochondrially targeted anti-cancer drugs relies on their ability to disrupt the energy producing systems of cancer cell mitochondria, leading to increased generation of ROS and activation of the mitochondrially dependent cell death signalling pathways within cancer cells. We believe that targeting mitochondria for tumour treatment may lead to a potential future breakthrough in the management of neoplastic pathologies. To better classify the various group of agents that act by mitochondrial destabilisation, we proposed the term mitocans (Ralph et al. 2006; Neuzil et al. 2006).

Mitocans, an acronym for 'mitochondria and cancer', are small compounds that in many cases selectively kill cancer cells via mitochondrial destabilisation, whereby inducing apoptosis, often via generation of ROS. They are classified into eight subgroups based on the site of action of the individual agents, from the surface of the MOM to the mitochondrial matrix (Fig. 7.4). The selection of the sites also stems from their importance as targets for the development of drugs that hold substantial promise to be utilised in the clinical practice. VE analogues that act by targeting mitochondria to cause ROS production and, as well, to boost the immune surveillance, exemplify a group of mitocans. The role of ROS production and the events leading to the activation of the inflammasome and pro-inflammatory mediators induced by dying cancer cell mitochondria are later on discussed along with the evidence for their contribution to promoting immune responses against cancer (Hahn et al. 2013).

4.1 α-Tocopheryl Succinate

α-Tocopheryl succinate (α-TOS), bearing a succinyl ester at position C6 of the chromanol ring (Fig. 7.4), belongs to class 2 (BH3 mimetics group) and class 5 (electron transport chain targeting drugs) sub-groups of mitocans. It has been shown

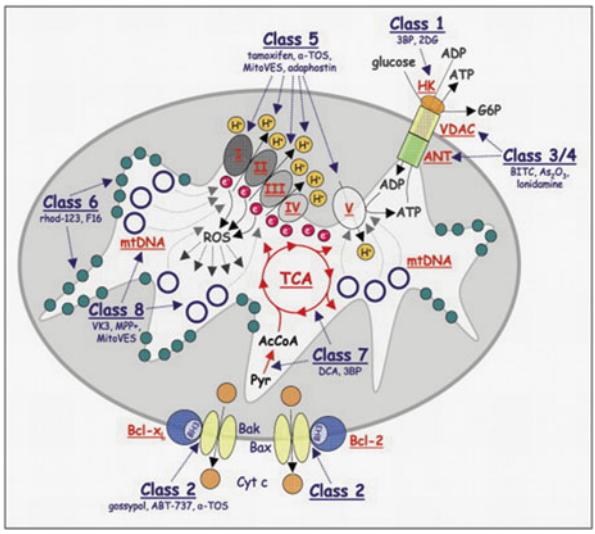

Fig. 7.4 Schematic illustration of molecular targets of individual class of mitocans. Vitamin E analogues belong to both class 2 (BH3 mimetics and related agents that impair the function of the anti-apoptotic Bcl-2 family proteins), class 5 (compounds targeting the mitochondrial electron transport chain) and class 8 (compounds targeting mtDNA)

to be a potent apoptosis inducer and growth inhibitor in a variety of cancer cells. This agent has the selectivity to kill malignant cells at concentrations non-toxic to normal cells and tissues (Neuzil et al. 2001b) and regardless whether the cells feature mutations in key tumour suppressor genes, such as p53 (Weber et al. 2002). Importantly, α-TOS also has been shown to exert anti-cancer activity in a wide range of solid tumours in pre-clinical models, including the difficult-to-treat melanomas, mesotheliomas, HER2-positive breast carcinomas, as well as prostate and colorectal tumours. So far, α-TOS has been shown to suppress tumour growth in up to 15 types of cancer (Wang et al. 2005; Neuzil et al. 2001c; Malafa et al. 2002; Stapelberg et al. 2005).

The molecular mechanism underlying the selectivity of α-TOS and similar agents for malignant cells results from at least two mechanisms. One relates to the ester structure of α-TOS and is due to the generally higher levels of esterases in normal cells such as hepatocytes, colonocytes, fibroblasts or cardiomyocytes that cleave α-TOS to produce vitamin E (Don and Hogg 2004; Fariss et al. 2001). Another reason for the cancer cell-specific toxicity of α-TOS may be related to the inherent property of many inducers of apoptosis to trigger programmed cell death by initially inducing cells to accumulate ROS that, in turn, cause a cascade of subsequent reactions leading to the transition of the cell into the apoptosis commitment phase (Simon et al. 2000). α-TOS has been reported to induce ROS accumulation in many different cancer cell lines, most probably resulting in the generation of superoxide anion radicals (Kogure et al. 2001, 2002; Malafa et al. 2002; Swettenham et al. 2005). In addition, it has been reported that cancer cells feature lower antioxidant defences than normal cells. For example, malignant cells express lower levels of manganese superoxide dismutase (MnSOD) compared to non-malignant cells. Moreover, we have found that proliferating endothelial cells,

unlike their confluent (growth-arrested) counterparts, are susceptible to α-TOS-induced apoptosis (Neuzil et al. 2001a), endowing α-TOS the ability to selectively kill proliferating endothelial cells and inhibit angiogenesis in mouse tumour models (Dong et al. 2007).

An important discovery demonstrated that α-TOS has a unique target to perform its biological (apoptogenic) activity. Research from Neuzil's group found that α-TOS induces cancer cell apoptosis by targeting the mitochondrial complex II (CII). More specifically, α-TOS inhibits the succinate quinone reductase (SQR) activity of CII by interacting with the proximal and distal ubiquinone (UbQ) binding site (Q_P and Q_D, respectively) (Dong et al. 2008). Additional work documented that CII is also a target for α-TOS in a pre-clinical tumour model, since tumours with mutant CII (a stop codon mutation in the CII's subunit SDHC) were not responsive to the agent unlike the wild-type or reconstituted CII tumours (Dong et al. 2009). This is the first time that a molecular target of α-TOS has been defined. CII as the target indicates that the agent may be an efficient anti-cancer drug since genes coding for the four subunits of CII only rarely mutate (for example there is only one mutation in CII per one million breast cancer patients).

Recent studies from Gogvadze's group also emphasized the importance of mitochondria as a target for α-TOS. The agent was shown by these researchers to trigger cancer cell apoptosis via targeting mitochondria, more specifically by stimulating rapid entry of Ca^{2+} into the cytosol, compromising the Ca^{2+} buffering capacity of mitochondria and sensitising them to mitochondrial permeability transition and subsequent apoptotic cell death. This mechanism was reported for neuroblastoma cells that were found to be killed by α-TOS irrespective of their *MycN* oncogene expression level and amplification (Kruspig et al. 2012).

Collectively, there is ample evidence for α-TOS (and similar agents) to kill cancer cells by destabilising mitochondria. Therefore, it seems a logical next step to modify the drugs in such a way that they accumulate in mitochondria, i.e. in the proximity of their molecular target.

4.2 Mitochondrially Targeted Vitamin E Succinate

Although α-TOS acts on mitochondria, it does not discriminate between the different membranous intracellular compartments. Therefore, we modified the structure of α-TOS in order to generate a variant of the agent that would be directly targeted to mitochondria, so that its apoptogenic activity would be increased. Based on the previous work of synthesis and testing of a series of mitochondrially targeted antioxidants by tagging them with the positively charged triphenylphosphonium group (TPP^+) (Murphy and Smith 2007), producing very efficient redox-active compounds (Kelso et al. 2001; James et al. 2007), our group prepared the mitochondrially targeted vitamin E succinate (MitoVES) by adding the TPP^+ group to the hydrophobic chain of vitamin E succinate. The result of this modification was the preferential

localisation of the agent in mitochondria, greatly enhancing its pro-apoptotic and anti-cancer activity (Dong et al. 2011a, b; Rohlena et al. 2011).

MitoVES, besides accumulating in mitochondria, has another advantage: its mitochondrial accumulation is based on the $\Delta\Psi_{m,i}$, which is considerably higher (in negative values) in cancer cells than in normal cells (Modica-Napolitano and Aprille 2001; Fantin et al. 2002). Due to the chemical structure of MitoVES, the compound is expected to be positioned such that the positive charge of the TPP^+ group is adjacent to the interface of the MIM and matrix and its hydrophobic alkyl chain spans the MIM with the tocopheryl succinyl group juxtapositioned to its molecular target, the UbQ-site of CII. Our recent research documents that the prototypic compound of such a targeted VE analogue, MitoVES, is some 1–2 orders of magnitude more efficient in apoptosis induction than the untargeted parental compound α-TOS, while maintaining selectivity for malignant cells (Dong et al. 2011a, b). One reason is that at relatively low levels, high percentage of the MitoVES pool (90-95 %) accumulates in mitochondria, resulting in very fast generation of ROS (within minutes) and changes in mitochondrial morphology (10–15 min). This then causes the modulation of expression of Bcl-2 family proteins. More specifically, we found that the ROS generated in response to MitoVES activates the Mst1 kinase that, in turn, phosphorylates the transcription factor FoxO1. Phosphorylated FoxO1 then translocates to the nucleus where it activates transcription of the BH3-only protein Noxa that, in turn, diverts Mcl-2 from Bak that can then form a channel in the MOM (Dong et al. 2011a, b; Valis et al. 2011).

MitoVES proved to be superior in suppression of experimental tumours compared to the untargeted analogue, as we showed for breast cancer, colon cancer and mesothelioma (Dong et al. 2011a, b; Kovarova et al. 2014). Based on the prototypic MitoVES targeting the mitochondrial CII, we propose that mitochondrially targeted delivery of anti-cancer agents offers a new paradigm for increasing the efficacy of compounds with anti-cancer activity (Figs. 7.5, 7.6). Accordingly, preparation of novel anti-cancer agents by tagging other compounds that target mitochondrial complexes is imminent and we are conducting relevant experiments.

An interesting way how to possibly suppress tumour promotion avoiding the many mutations in cancer cells is targeting angiogenesis. In this regard, we found that MitoVES efficiently kills proliferating endothelial cells (ECs) but not contact-arrested ECs or ECs deficient in mitochondrial DNA. It also suppressed angiogenesis *in vitro* by inducing accumulation of ROS and induction of apoptosis in proliferating/angiogenic ECs. Resistance of arrested ECs was ascribed, at least in part, to lower $\Delta\Psi_{m,i}$ of quiescent ECs compared with their proliferating counterparts, resulting in the lower level of mitochondrial uptake of MitoVES. (Rohlena et al. 2011).

Additional studies on energy-related mitochondrial function using isolated mitochondria demonstrated that MitoVES stimulates basal respiration and ATP hydrolysis, but inhibits net state 3 (ADP-stimulated) respiration and Ca^{2+} uptake by collapsing $\Psi\Delta_{m,i}$ at low doses, acting as an uncoupler (1–5 μM). At higher doses (> 5 μM), MitoVES targets the SQR activity of the mitochondrial CII. Uncoupled

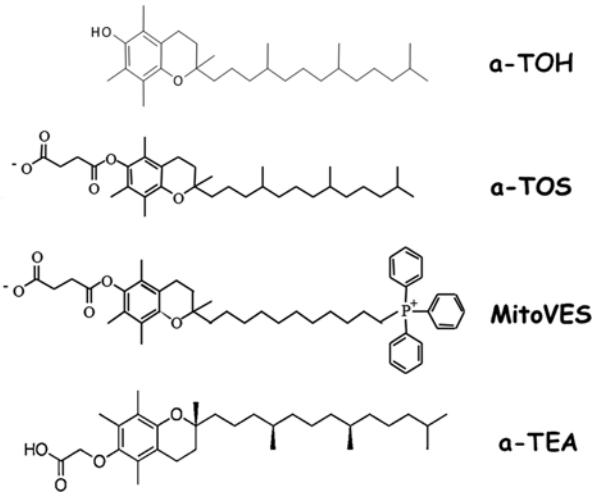

Fig. 7.5 Structures of mitochondria-untargeted and -targeted vitamin E analogues

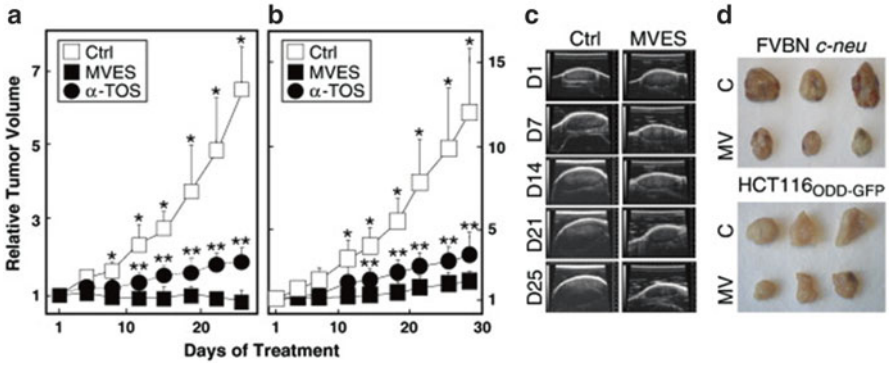

Fig. 7.6 MitoVES suppresses tumour progression. (**a**) FVB/N c-neu mice with breast carcinomas and (**b**) Balb c nu/nu mice with xenografts derived from HCT116 cells were treated by ip injection of 1–2 μmol MitoVES or 15 μmol α-TOS per mouse every 3–4 days, and tumors were visualized and their volume was quantified using ultrasound imaging (panel (**c**) shows representative ultrasound images of tumours acquired on given days, panel (**d**) documents representative tumours at the end of the experiment. (Adapted from Dong et al. 2011a, b)

mitochondrial respiration and basal respiration of SMPs were inhibited by VE analogues with the following efficacy: MitoVES > α-TEA > α-TOS (Rodríguez-Enríquez et al. 2012). The same authors also showed that MitoVES inhibited oxidative phosphorylation and induced ATP depletion in rodent and human cancer cells more potently than in normal rat hepatocytes. This is consistent with our recent data that *in vivo*, cancer cell mitochondria are a preferred target of MitoVES compared to normal cells (such as found for kidney, heart or liver; Truksa et al. unpublished data). Collectively, these findings are consistent with and corroborate the notion that targeting of anti-cancer agents to mitochondria enhances their anti-cancer efficacy and, as shown for MitoVES, maintains their selectivity for malignant cells.

4.3 α-Tocopheryloxyacetic Acid

α-TEA is an ether analogue of VE with potent anti-cancer actions via activation of pro-apoptotic pathways and suppression of pro-survival pathways both *in vitro* and *in vivo*. It has been shown to suppress tumour growth in various murine and human xenograft tumour models, including melanoma, breast, lung, prostate, and ovarian cancers (Jia et al. 2008; Yu et al. 2006; Dong et al. 2012). α-TEA has also been shown to exhibit anti-metastatic activities in xenografts, syngeneic and spontaneous mouse models of breast cancer. Importantly, similar to α-TOS, α-TEA has the ability to possess anti-cancer properties that are selective for cancer cells, to reduce tumour burden and metastases *in vivo* with no or very low toxicity to normal tissues (Yu et al. 2006; Lawson et al. 2004; Latimer et al. 2009; Hahn et al. 2009; Shun et al. 2010). This gives α-TEA a great potential for future drug development for clinical use.

α-TEA is a stable semi-synthetic analogue of naturally occurring VE. It is derived from α-tocopherol by a chemical modification, replacing the hydroxyl group C6 of the phenolic ring with an acetic acid residue linked via an ether bond (Fig. 7.5). This modification makes α-TEA redox-silent, while making it active against tumours (Lawson et al. 2003; Hahn et al. 2006). The presence of the non-cleavable ether bond ensures the stability of α-TEA, allowing it to be delivered via the oral route in a biologically active form (Dong et al. 2012). An earlier report documented that α-TEA, when given orally (incorporated into mouse chow), significantly inhibited the progression of breast cancers and strongly reduced the incidence of spontaneous lung metastases before and after primary tumour establishment without general toxicity (Lawson et al. 2003). A number of reports from different groups have showed that apoptosis is a primary mode of α-TEA-induced tumour cell death (Kline et al. 2001; Neuzil et al. 2004). However, since the antitumor activity of α-TEA could not be completely blocked using pan-caspase or caspase-specific inhibitors (Jia et al. 2008), additional pathway(s) may be involved in α-TEA-mediated tumour cell killing, such as mitochondria-independent signalling pathways.

It has been reported that α-TEA induces apoptosis in MCF-7 and HCC-1954 breast cancer cells via TRAIL/DR5-induced activation of caspase-8 that is relayed to mitochondria-dependent pro-apoptotic pathway by increasing the DR5 and TRAIL protein levels, and via suppression of the anti-apoptotic protein c-FLIP by decreasing its levels. This mitochondria-dependent apoptosis signalling pathway involves the initial activation of caspase-8 followed by cleavage of Bid to tBid, activation of the Bax protein (its translocation to the MOM) and increased mitochondrial permeability transition. The data suggest that that α-TEA has the potential as a treatment for human breast cancer either as a stand-alone drug or in combination with the recombinant TRAIL protein or antagonistic antibodies to the TRAIL receptor (Yu et al. 2010). Mechanistic studies showed that the major events necessary and sufficient for inducing apoptosis of cancer cells with α-TEA include the activation of pro-apoptotic mechanisms such as signalling via the Fas receptor/Fas ligand, and endoplasmic reticulum (ER) stress involving JNK/CHOP/DR5 and p73/Noxa,

leading to caspase-8 activation followed by mitochondria-dependent apoptotic cascade of reactions (Hahn et al. 2009, 2011; Shun et al. 2010). One report documented that α-TEA disrupts the cholesterol-rich micro-domains, acting cooperatively with a selective estrogen-receptor modulator to reduce pro-survival mediators, and induces DR5-mediated mitochondria-dependent apoptosis via the ER stress-mediated pro-apoptotic pJNK/CHOP/DR5 amplification loop (Lawson et al. 2003; Tiwary et al. 2010).

A previous report suggested that α-TEA suppresses the phosphatidylinositol-3-kinase (PI3K)/Akt/ERK pathways via JNK-mediated down-regulation of insulin-receptor substrate (IRS-1) (Tiwary et al. 2011b). It induced apoptosis in human MCF-7 and HCC-1954 breast cancer cells by suppressing constitutively active basal levels of pAKT, pERK, p-mTOR, and their downstream targets. In addition, α-TEA increased levels of pIRS-1 (Ser-307), a phosphorylation site correlated with insulin receptor substrate-1 (IRS-1) inactivation, as well as of total IRS-1. Down-regulation of the IRS-1/PI3K pathways via JNK are critical for α-TEA and α-TEA + MEK or mTOR inhibitor-induced apoptosis in human MCF-7 and HCC-1954 breast cancer cells (Tiwary et al. 2011b). α-TEA has also been shown to induce cancer cell death, at least in part, by down-regulation of members of the EGFR family (Shun et al. 2010).

Collectively, there is ample data documenting the potential of α-TEA as an anti-cancer agent. Except for some specific activities in cancer cells, the ether can be considered as a stable 'homologue' of the prototypic anti-cancer VE analogue, α-TOS. From the application point of view, the great advantage of α-TEA is that it can be applied orally, while α-TOS has to reach the site of the tumour while bypassing the alimentary system, where it is completely hydrolysed to the non-apoptogenic vitamin E.

5 Vitamin E Analogues as Stimulants of the Immune System

Recent research demonstrated that, in addition to the direct cytotoxic effects, α-TEA stimulates anti-tumour immune responses, resulting in higher level of infiltration of activated T cells in the tumour microenvironment and increased ratios of $CD4^+$ and $CD8^+$ T cells to regulatory T cells in the tumour, respectively (Hahn et al. 2011; Tiwary et al. 2011a). Moreover, autophagy has been found to be involved in the immune system stimulation by α-TEA, since autophagy plays an essential role in the major histocompatibility complex (MHC) class II-restricted antigen presentation (Münz 2009), and recently its role in MHC class I–restricted stimulation of $CD8^+$ T cells (cross-presentation) has been documented (Li et al. 2008; Kepp et al. 2009). A recent report indicates that α-TEA triggers tumour cell autophagy and improves cross-presentation of tumour antigens to the immune system. α-TEA stimulated both apoptosis and autophagy in murine mammary and lung cancer cells. These findings suggest that both autophagy and apoptosis signalling programmes

are activated during α-TEA-induced tumour cell killing (Li et al. 2012), endowing the agent with a very interesting pattern of bioactivities.

6 Conclusions

Taken together, VE analogues are potent anti-cancer agents that selectively kill cancer cells with the advantage of limited side effects on normal cells, as also shown for high level of toxicity of the agents to cancer tissues and low (if any) deleterious effects on normal tissues. Mitochondria act as targets for VE analogues, relaying the selective apoptotic signals shifting cancer cells to the commitment phase of programmed cell death. The VE analogues α-TOS, α-TEA and MitoVES epitomize mitocans, a large group of compounds acting as anti-cancer agents by destabilizing mitochondria. These compounds present a source of very promising, potentially highly effective and selective lead structures hopefully leading to exciting new developments in cancer therapy. The predominant mechanism of action whereby mitochondria-targeted anticancer drugs kill cancer cells is linked to the ability of these drugs to disrupt the energy-producing systems of cancer cells (concentrated in mitochondria), leading to increased accumulation of ROS and the activation of the mitochondria-dependent death signalling pathways. There is little doubt that, given recent advances in anti-cancer research, mitocans will become an integral part of modern weaponry in the fight to eliminate cancer, although there is still a lot of work to achieve this goal. Notwithstanding this, the authors are optimistic and can 'see the light at the end of the (apparently) long tunnel'.

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References

Aggarwal BB, Sundaram C, Prasad S, Kannappan R (2010) Tocotrienols, the vitamin E of the 21st century: its potential against cancer and other chronic diseases. Biochem Pharmacol 80:1613–1631

Akazawa A, Nishikawa K, Suzuki K, Asano R, Kumadaki I, Satoh H, Hagiwara K, Shin SJ, Yano T (2002) Induction of apoptosis in a human breast cancer cell overexpressing ErbB-2 receptor by α-tocopheryloxybutyric acid. Jpn J Pharmacol 89:417–421

Alirol E, Martinou JC (2006) Mitochondria and cancer: is there a morphological connection? Oncogene 25:4706–4716

Angulo-Molina A, Reyes-Leyva J, López-Malo A, Hernández J (2014) The Role of a-tocopheryl succinate (a-TOS) as a potential anticancer agent. Nutr Cancer 66:167–176

Ayoub NM, Bachawal SV, Sylvester PW (2011) γ-Tocotrienol inhibits HGF-dependent mitogenesis and Met activation in highly malignant mammary tumour cells. Cell Prolif 44:516–526

Azzi A, Ricciarelli R, Zingg JM (2002) Non-antioxidant molecular functions of α-tocopherol (vitamin E). FEBS Lett 22:8–10

Bachawal SV, Wali VB, Sylvester PW (2010) Combined γ-tocotrienol and erlotinib/gefitinib treatment suppresses Stat and Akt signalling in murine mammary tumor cells. Anticancer Res 30:429–437

Bang OS, Park JH, Kang SS (2001) Activation of PKC but not of ERK is required for vitamin E-succinate-induced apoptosis of HL-60 cells. Biochem Biophys Res Commun 288:789–797

Basu A (2003) Involvement of protein kinase C-delta in DNA damage-induced apoptosis. J Cell Mol Med 7:341–350

Behery FA, Elnagar AY, Akl MR, Wali VB, Abuasal B, Kaddoumi A, Sylvester PW, El Sayed KA (2010) Redox-silent tocotrienol esters as breast cancer proliferation and migration inhibitors. Bioorg Med Chem 18:8066–8075

Birringer M, Pfluger P, Kluth D, Landes N, Brigelius-Flohé R (2002) Identities and differences in the metabolism of tocotrienols and tocopherols in HepG2 cells. J Nutr 132:3113–3118

Birringer M, EyTina JH, Salvatore BA, Neuzil J (2003) Vitamin E analogues as inducers of apoptosis: structure-function relation. Br J Cancer 88:1948–1955

Bunyan J, McHale D, Green J, Marcinkiewicz S (1961) Biological potencies of ε- and ζ-1-tocopherol and 5-methyltocol. Br J Nutr 15:253–257

Cai X, Khdour OM, Jaruvangsanti J, Hecht SM (2012) Simplified bicyclic pyridinol analogues protect mitochondrial function. Bioorg Med Chem 20:3584–3595

Cory S, Huang DC, Adams JM (2003) The Bcl-2 family: roles in cell survival and oncogenesis. Oncogene 22:8590–8607

Cretney E, Takeda K, Yagita H, Glaccum M, Peschon JJ, Smyth MJ (2002) Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. J Immunol 168:1356–1361

Dalen H, Neuzil J (2003) α-Tocopheryl succinate sensitises T lymphoma cells to TRAIL killing by suppressing NF-κB activation. Br J Cancer 88:153–158

Davies MA, Kopetz S (2013) Overcoming resistance to MAPK pathway inhibitors. J Natl Cancer Inst 105:9–10

Don AS, Hogg PJ (2004) Mitochondria as cancer drug targets. Trends Mol Med 10:372–378

Dong LF, Swettenham E, Eliasson J, Wang XF, Gold M, Medunic Y, Stantic M, Low P, Prochazka L, Witting PK, Turanek J, Akporiaye ET, Ralph SJ, Neuzil J (2007) Vitamin E analogues inhibit angiogenesis by selective induction of apoptosis in proliferating endothelial cells: the role of oxidative stress. Cancer Res 67:11906–11913

Dong LF, Low P, Dyason JC, Wang XF, Prochazka L, Witting PK, Freeman R, Swettenham E, Valis K, Liu J, Zobalova R, Turanek J, Spitz DR, Domann FE, Scheffler IE, Ralph SJ, Neuzil J (2008) α-Tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II. Oncogene 27:4324–4335

Dong LF, Freeman R, Liu J, Zobalova R, Marin-Hernandez A, Stantic M, Rohlena J, Rodriguez-Enriquez S, Valis K, Butcher B, Goodwin J, Brunk UT, Witting PK, Moreno-Sanchez R, Scheffler IE, Ralph SJ, Neuzil J (2009) Suppression of tumour growth *in vivo* by the mitocan α-tocopheryl succinate requires respiratory complex II. Clin Cancer Res 15:1593–1600

Dong LF, Jameson VJ, Tilly D, Cerny J, Mahdavian E, Marín-Hernández A, Hernández-Esquivel L, Rodríguez-Enríquez S, Stursa J, Witting PK, Stantic B, Rohlena J, Truksa J, Kluckova K, Dyason JC, Ledvina M, Salvatore BA, Moreno-Sánchez R, Coster MJ, Ralph SJ, Smith RA, Neuzil J (2011a) Mitochondrial targeting of vitamin E succinate enhances its pro-apoptotic and anti-cancer activity via mitochondrial complex II. J Biol Chem 286:3717–3728

Dong LF, Jameson VJ, Tilly D, Prochazka L, Rohlena J, Valis K, Truksa J, Zobalova R, Mahdavian E, Kluckova K, Stantic M, Stursa J, Freeman R, Witting PK, Norberg E, Goodwin J, Salvatore BA, Novotna J, Turanek J, Ledvina M, Hozak P, Zhivotovsky B, Coster MJ, Ralph SJ, Smith RA, Neuzil J (2011b) Mitochondrial targeting of α-tocopheryl succinate enhances its pro-apoptotic efficacy: a new paradigm for effective cancer therapy. Free Radic Biol Med 50:1546–1555

Dong LF, Grant G, Massa H, Zobalova R, Akporiaye E, Neuzil J (2012) a-Tocopheryloxyacetic acid is superior to a-tocopheryl succinate in suppressing HER2-high breast carcinomas due to its higher stability. Int J Cancer 131:1052–1058

Elnagar AY, Wali VB, Sylvester PW, El Sayed KA (2010) Design and preliminary structure-activity relationship of redox-silent semisynthetic tocotrienol analogues as inhibitors for breast cancer proliferation and invasion. Bioorg Med Chem 18:755–768

Elson CE, Yu SG (1994) The chemoprevention of cancer by mevalonate-derived constituents of fruits and Vegetables. J Nutr 124:607–614

Endo K, Oki E, Biedermann V, Kojima H, Yoshida K, Johannes FJ, Kufe D, Datta R (2000) Proteolytic cleavage and activation of protein kinase Cμ by caspase-3 in the apoptotic response of cells to 1-β-D-arabinofuranosylcytosine and other genotoxic agents. J Biol Chem 275:18476–18481

Fang JY, Richardson BC (2005) The MAPK signalling pathways and colorectal cancer. Lancet Oncol 6:322–327

Fantin VR, Berardi MJ, Scorrano L, Korsmeyer SJ, Leder P (2002) A novel mitochondriotoxic small molecule that selectively inhibits tumor cell growth. Cancer Cell 2:29–42

Fariss MW, Nicholls-Grzemski FA, Tirmenstein MA, Zhang JG (2001) Enhanced antioxidant and cytoprotective abilities of vitamin E succinate is associated with a rapid uptake advantage in rat hepatocytes and mitochondria. Free Radic Biol Med 31:530–541

Ferreira JC, Brum PC, Mochly-Rosen D (2011) βIIPKC and εPKC isozymes as potential pharmacological targets in cardiac hypertrophy and heart failure. J Mol Cell Cardiol 51:479–484

Fulda S, Galluzzi L, Kroemer G (2010) Targeting mitochondria for cancer therapy. Nat Rev Drug Discov 9:447–464

Galli F, Stabile AM, Betti M, Conte C, Pistilli A, Rende M, Floridi A, Azzi A (2004) The effect of α- and γ-tocopherol and their carboxyethyl hydroxychroman metabolites on prostate cancer cell proliferation. Arch Biochem Biophys 423:97–102

Galluzzi L, Morselli E, Kepp O, Vitale I, Rigoni A, Vacchelli E, Michaud M, Zischka H, Castedo M, Kroemer G (2010) Mitochondrial gateways to cancer. Mol Aspects Med 31:1–20

Geraldes P, King GL (2010) Activation of protein kinase C isoforms and its impact on diabetic complications. Circ Res 106:1319–1331

Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, Martinez P, Matthews N, Stewart A, Tarpey P, Varela I, Phillimore B, Begum S, McDonald NQ, Butler A, Jones D, Raine K, Latimer C, Santos CR, Nohadani M, Eklund AC, Spencer-Dene B, Clark G, Pickering L, Stamp G, Gore M, Szallasi Z, Downward J, Futreal PA, Swanton C (2012) Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 366:883–892

Gianello R, Libinaki R, Azzi A, Gavin PD, Negis Y, Zingg JM, Holt P, Keah HH, Griffey A, Smallridge A, West SM, Ogru E (2005) α-Tocopheryl phosphate: a novel, natural form of vitamin E. Free Radic Biol Med 39:970–976

Gill KK, Kaddoumi A, Nazzal S (2012) Mixed micelles of PEG(2000)-DSPE and vitamin-E TPGS for concurrent delivery of paclitaxel and parthenolide: enhanced chemosensitization and antitumor efficacy against non-small cell lung cancer (NSCLC) cell lines. Eur J Pharm Sci 46:64–71

Gogvadze V, Orrenius S, Zhivotovsky B (2008) Mitochondria in cancer cells: what is so special about them? Trends Cell Biol 18:165–173

Hahn T, Szabo L, Gold M, Ramanathapuram L, Hurley LH, Akporiaye ET (2006) Dietary administration of the pro-apoptotic vitamin E analogue α-tocopheryloxyacetic acid inhibits metastatic murine breast cancer. Cancer Res 66:9374–9378

Hahn T, Fried K, Hurley LH, Akporiaye ET (2009) Orally active α-tocopheryloxyacetic acid suppresses tumor growth and multiplicity of spontaneous murine breast cancer. Mol Cancer Ther 8:1570–1578

Hahn T, Jagadish B, Mash EA, Garrison K, Akporiaye ET (2011) α-Tocopheryloxyacetic acid: a novel chemotherapeutic that stimulates the antitumor immune response. Breast Cancer Res 13:R4

Hahn T, Polanczyk MJ, Borodovsky A, Ramanathapuram LV, Akporiaye ET, Ralph SJ (2013) Use of anti-cancer drugs, mitocans, to enhance the immune responses against tumors. Curr Pharm Biotechnol 14:357–376

Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100:57–70

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674

Hayden E (2008) Cancer complexity slows quest for cure. Nature 455:148

He L, Mo H, Hadisusilo S, Qureshi AA, Elson CE (1997) Isoprenoids suppress the growth of murine B16 melanomas in vitro and in vivo. J Nutr 127:668–674

Higuchi M, Aggarwal BB, Yeh ET (1997) Activation of CPP32-like protease in tumor necrosis factor-induced apoptosis is dependent on mitochondrial function. J Clin Invest 99:1751–1758

Hosomi A, Arita M, Sato Y, Kiyose C, Ueda T, Igarashi O, Arai H, Inoue K (1997) Affinity for α-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. FEBS Lett 409:105–108

Inagaki K, Churchill E, Mochly-Rosen D (2006) Epsilon protein kinase C as a potential therapeutic target for the ischemic heart. Cardiovasc Res 70:222–230

Inokuchi H, Hirokane H, Tsuzuki T, Nakagawa K, Igarashi M, Miyazawa T (2003) Anti-angiogenic activity of tocotrienol. Biosci Biotechnol Biochem 67:1623–1627

Ivanova AN, Belsky YP, Spivak AY, Danilets MG, Belska NV, Chalitova RR, Ligatcheva AA, Uchasova EG (2011) In vitro effect of combined hybrid molecules from vitamin E analogues and betulinic acid on macrophage activity. Bull Exp Biol Med 151:694–697

James AM, Sharpley MS, Manas AR, Frerman FE, Hirst J, Smith RA, Murphy MP (2007) Interaction of the mitochondria-targeted antioxidant MitoQ with phospholipid bilayers and ubiquinone oxidoreductases. J Biol Chem 282:14708–14718

Jia L, Yu W, Wang P, Sanders BG, Kline K (2008) In vivo and in vitro studies of anticancer actions of α-TEA for human prostate cancer cells. Prostate 68:849–860

Johnson GL, Lapadat R (2002) Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 298:1911–1912

Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 321:1801–1806

Jordan M, Nayel A, Brownlow B, Elbayoumi T (2012) Development and evaluation of tocopherol-rich argan oil-based nanoemulsions as vehicles possessing anticancer activity. J Biomed Nanotechnol 8:944–956

Kakkar P, Singh BK (2007) Mitochondria: A hub of redox activities and cellular distress control. Mol Cell Biochem 305:235–253

Kamal-Eldin A, Appelqvist LA (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31:671–701

Kani K, Momota Y, Harada M, Yamamura Y, Aota K, Yamanoi T, Takano H, Motegi K, Azuma M (2013) γ-Tocotrienol enhances the chemosensitivity of human oral cancer cells to docetaxel through the downregulation of the expression of NF-κB-regulated anti-apoptotic gene products. Int J Oncol 42:75–82

Karin M (2006) Nuclear factor-κB in cancer development and progression. Nature 441:431–436

Kelso GF, Porteous CM, Coulter CV, Hughes G, Porteous WK, Ledgerwood EC, Smith RA, Murphy MP (2001) Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. J Biol Chem 276:4588–4596

Kepp O, Tesniere A, Schlemmer F, Michaud M, Senovilla L, Zitvogel L, Kroemer G (2009) Immunogenic cell death modalities and their impact on cancer treatment. Apoptosis 14:364–375

Kline K, Yu W, Sanders BG (2001) Vitamin E: mechanisms of action as tumor cell growth inhibitors. J Nutr 131:161S–163S

Koba K, Abe K, Ikeda I, Suhano M (1992) Effects of α-tocopherol and tocotrienols on blood pressure and linoleic acid metabolism in spontaneously hypertensive rat (SHR). Biosci Biotechnol Biochem 56:1420–1423

Kogure K, Morita M, Nakashima S, Hama S, Tokumura A, Fukuzawa K (2001) Superoxide is responsible for apoptosis in rat vascular smooth muscle cells induced by α-tocopheryl hemisuccinate. Biochim Biophys Acta 1528:25–30

Kogure K, Hama S, Manabe S, Tokumura A, Fukuzawa K (2002) High cytotoxicity of α-tocopheryl hemisuccinate to cancer cells is due to failure of their antioxidative defense systems. Cancer Lett 186:151–156

Kogure K, Yamauchi I, Tokumura A, Kondou K, Tanaka N, Takaishi Y, Fukuzawa K (2004) Novel antioxidants isolated from plants of the genera Ferula, Inula, Prangos and Rheum collected in Uzbekistan. Phytomedicine 11:645–651

Kogure K, Manabe S, Suzuki I, Tokumura A, Fukuzawa K (2005) Cytotoxicity of α-tocopheryl succinate, malonate and oxalate in normal and cancer cells *in vitro* and their anti-cancer effects on mouse melanoma *in vivo*. J Nutr Sci Vitaminol 51:392–397

Koppenol WH, Bounds PL, Dang CV (2011) Otto Warburg's contributions to current concepts of cancer metabolism. Nat Rev Cancer 11:325–337

Kovarova J, Bajzikova M, Vondrusova M, Stursa J, Goodwin J, Nguyen M, Zobalova R, Alizadeh E, Truksa J, Tomasetti M, Dong LF, Neuzil J (2014) Efficient suppression of malignant mesothelioma by mitochondrially targeted of α-tocopheryl succinate. Redox Rep 19:16–25

Kruspig B, Nilchian A, Bejarano I, Orrenius S, Zhivotovsky B, Gogvadze V (2012) Targeting mitochondria by α-tocopheryl succinate kills neuroblastoma cells irrespective of MycN oncogene expression. Cell Mol Life Sci 69:2091–2099

Kunisaki M, Bursell SE, Clermont AC, Ishii H, Ballas LM, Jirousek MR, Umeda F, Nawata H, King GL (1995) Vitamin E prevents diabetes-induced abnormal retinal blood flow via the diacylglycerol-protein kinase C pathway. Am J Physiol 269:E239–E246

Latimer P, Menchaca M, Snyder RM, Yu W, Gilbert BE, Sanders BG, Kline K (2009) Aerosol delivery of liposomal formulated paclitaxel and vitamin E analog reduces murine mammary tumor burden and metastases. Exp Biol Med 234:1244–1252

Lawson KA, Anderson K, Menchaca M, Atkinson J, Sun L, Knight V, Gilbert BE, Conti C, Sanders BG, Kline K (2003) Novel vitamin E analogue decreases syngeneic mouse mammary tumor burden and reduces lung metastasis. Mol Cancer Ther 2:437–444

Lawson KA, Anderson K, Simmons-Menchaca M, Atkinson J, Sun L, Sanders BG, Kline K (2004) Comparison of vitamin E derivatives α-TEA and VES in reduction of mouse mammary tumor burden and metastasis. Exp Biol Med 229:954–963

Lee SP, Mar GY, Ng LT (2009) Effects of tocotrienol-rich fraction on exercise endurance capacity and oxidative stress in forced swimming rats. Eur J Appl Physiol 107:587–595

Li Y, Wang LX, Yang G, Hao F, Urba WJ, Hu HM (2008) Efficient cross-presentation depends on autophagy in tumor cells. Cancer Res 68:6889–6895

Li Y, Hahn T, Garrison K, Cui ZH, Thorburn A, Thorburn J, Hu HM, Akporiaye ET (2012) The vitamin E analogue α-TEA stimulates tumor autophagy and enhances antigen cross-presentation. Cancer Res 72:3535–3545

Ling MT, Luk SU, Al-Ejeh F, Khanna KK (2012) Tocotrienol as a potential anticancer agent. Carcinogenesis 33:233–239

Makishima M, Umesono K, Shudo K, Naoe T, Kishi K, Honma Y (1998) Induction of differentiation in acute promyelocytic leukemia cells by 9-cis retinoic acid α-tocopherol ester (9-cis tretinoin tocoferil). Blood 91:4715–4726

Malafa MP, Fokum FD, Mowlavi A, Abusief M, King M (2002) Vitamin E inhibits melanoma growth in mice. Surgery 131:85–91

McIntyre BS, Briski KP, Gapor A, Sylvester PW (2000) Antiproliferative and apoptotic effects of tocopherols and tocotrienols on preneoplastic and neoplastic mouse mammary epithelial cells. Proc Soc Exp Biol Med 224:292–301

Mochly-Rosen D, Das K, Grimes KV (2012) Protein kinase C, an elusive therapeutic target? Nat Rev Drug Discov 11:937–957

Modica-Napolitano JS, Aprille JR (2001) Delocalized lipophilic cations selectively target the mitochondria of carcinoma cells. Adv Drug Deliv Rev 49:63–70

Mudit M, Behery FA, Wali VB, Sylvester PW, El Sayed KA (2010) Synthesis of fluorescent analogues of the anticancer natural products 4-hydroxyphenylmethylene hydantoin and δ-tocotrienol. Nat Prod Commun 5:1623–1626

Munteanu A, Zingg JM, Ogru E, Libinaki R, Gianello R, West S, Negis Y, Azzi A (2004) Modulation of cell proliferation and gene expression by α-tocopheryl phosphates: relevance to atherosclerosis and inflammation. Biochem Biophys Res Commun 318:311–316

Münz C (2009) Enhancing immunity through autophagy. Annu Rev Immunol 423–449

Murphy MP, Smith RA (2007) Targeting antioxidants to mitochondria by conjugation to lipophilic cations. Annu Rev Pharmacol Toxicol 629–656

Negis Y, Zingg JM, Ogru E, Gianello R, Libinaki R, Azzi A (2005) On the existence of cellular tocopheryl phosphate, its synthesis, degradation and cellular roles: a hypothesis. IUBMB Life 57:23–25

Negis Y, Zingg JM, Libinaki R, Meydani M, Azzi A (2009) Vitamin E and cancer. Nutr Cancer 61:875–878

Neuzil J, Schröder A, von Hundelshausen P, Zernecke A, Weber T, Gellert N, Weber C (2001a) Inhibition of inflammatory endothelial responses by a pathway involving caspase activation and p65 cleavage. Biochemistry 40:4686–4692

Neuzil J, Weber T, Gellert N, Weber C (2001b) Selective cancer cell killing by α-tocopheryl succinate. Br J Cancer 84:87–89

Neuzil J, Weber T, Schröder A, Lu M, Ostermann G, Gellert N, Mayne GC, Olejnicka B, Nègre-Salvayre A, Stícha M, Coffey RJ, Weber C (2001c) Induction of cancer cell apoptosis by α-tocopheryl succinate: molecular pathways and structural requirements. FASEB J 15:403–415

Neuzil J, Zhao M, Ostermann G, Sticha M, Gellert N, Weber C, Eaton JW, Brunk UT (2002) α-Tocopheryl succinate, an agent with in vivo anti-tumour activity, induces apoptosis by causing lysosomal instability. Biochem J 362:709–715

Neuzil J, Tomasetti M, Mellick AS, Alleva R, Salvatore BA, Birringer M, Fariss MW (2004) Vitamin E analogues: a new class of inducers of apoptosis with selective anti-cancer effects. Curr Cancer Drug Targets 4:355–372

Neuzil J, Wang XF, Dong LF, Low P, Ralph SJ (2006) Molecular mechanism of 'mitocan'-induced apoptosis in cancer cells epitomizes the multiple roles of reactive oxygen species and Bcl-2 family proteins. FEBS Lett 580:5125–5129

Neuzil J, Tomasetti M, Zhao Y, Dong LF, Birringer M, Wang XF, Low P, Wu K, Salvatore BA, Ralph SJ (2007) Vitamin E analogs, a novel group of 'mitocans', as anticancer agents: the importance of being redox-silent. Mol Pharmacol 71:1185–1199

Neuzil J, Dong LF, Rohlena J, Truksa J, Ralph SJ (2013) Classification of mitocans, anti-cancer drugs acting on mitochondria. Mitochondrion 13:199–208

Nishina H, Wada T, Katada T (2004) Physiological roles of SAPK/JNK signaling pathway. J Biochem 136:123–126

O'Connell J, Bennett MW, Nally K, Houston A, O'Sullivan GC, Shanahan F (2000) Altered mechanisms of apoptosis in colon cancer: Fas resistance and counterattack in the tumour-immune conflict. Ann N Y Acad Sci 910:178–192

Parker RA, Pearce BC, Clark RW, Gordon DA, Wright JJ (1993) Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J Biol Chem 268:11230–11238

Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) An integrated genomic analysis of human glioblastoma multiforme. Science 321:1807–1812

Petrosillo G, Ruggiero FM, Paradies G (2003) Role of reactive oxygen species and cardiolipin in the release of cytochrome c from mitochondria. FASEB J 17:2202–2208

Pham DQ, Plakogiannis R (2005) Vitamin E supplementation in cardiovascular disease and cancer prevention: Part 1. Ann Pharmacother 39:1870–1878

Qian M, Sanders BG, Kline K (1996) RRR-α-tocopheryl succinate induces apoptosis in avian retrovirus-transformed lymphoid cells. Nutr Cancer 25:9–26

Qureshi AA, Mo H, Packer L, Peterson DM (2000) Isolation and identification of novel tocotrienols from rice bran with hypocholesterolemic, antioxidant, and antitumor properties. J Agric Food Chem 48:3130–3140

Qureshi AA, Sami SA, Salser WA, Khan FA (2001) Synergistic effect of tocotrienol-rich fraction (TRF(25)) of rice bran and lovastatin on lipid parameters in hypercholesterolemic humans. J Nutr Biochem 12:318–329

Qureshi AA, Sami SA, Salser WA, Khan FA (2002) Dose-dependent suppression of serum cholesterol by tocotrienol-rich fraction (TRF25) of rice bran in hypercholesterolemic humans. Atherosclerosis 161:199–207

Ralph SJ, Low P, Dong L, Lawen A, Neuzil J (2006) Mitocans: mitochondrial targeted anti-cancer drugs as improved therapies and related patent documents. Recent Patents Anticancer Drug Discov 1:327–346

Ralph SJ, Rodríguez-Enríquez S, Neuzil J, Moreno-Sánchez R (2010a) Bioenergetic pathways in tumor mitochondria as targets for cancer therapy and the importance of the ROS-induced apoptotic trigger. Mol Aspects Med 31:29–59

Ralph SJ, Rodríguez-Enríquez S, Neuzil J, Saavedra E, Moreno-Sánchez R (2010b) The causes of cancer revisited: "mitochondrial malignancy" and ROS-induced oncogenic transformation - why mitochondria are targets for cancer therapy. Mol Aspects Med 31:145–170

Rezk BM, van der Vijgh WJ, Bast A, Haenen GR (2007) α-Tocopheryl phosphate is a novel apoptotic agent. Front Biosci 12:2013–2019

Rodríguez-Enríquez S, Hernández-Esquivel L, Marín-Hernández A, Dong LF, Akporiaye ET, Neuzil J, Ralph SJ, Moreno-Sánchez R (2012) Molecular mechanism for the selective impairment of cancer mitochondrial function by a mitochondrially targeted vitamin E analogue. Biochim Biophys Acta 1817:1597–1607

Rohlena J, Dong LF, Kluckova K, Zobalova R, Goodwin J, Tilly D, Stursa J, Pecinova A, Philimonenko A, Hozak P, Banerjee J, Ledvina M, Sen CK, Houstek J, Coster MJ, Neuzil J (2011) Mitochondrially targeted α-tocopheryl succinate is antiangiogenic: potential benefit against tumor angiogenesis but caution against wound healing. Antioxid Redox Signal 15:2923–2935

Ruvolo PP, Deng X, Carr BK, May WS (1998) A functional role for mitochondrial protein kinase C-α in Bcl2 phosphorylation and suppression of apoptosis. J Biol Chem 273:25436–25442

Sen CK, Khanna S, Roy S, Packer L (2000) Molecular basis of vitamin E action. Tocotrienol potently inhibits glutamate-induced pp 60(c-Src) kinase activation and death of HT4 neuronal cells. J Biol Chem 275:13049–13055

Sen CK, Khanna S, Rink C, Roy S (2007) Tocotrienols: the emerging face of natural vitamin E. Vitam Horm 76:203–261

Shin-Kang S, Ramsauer VP, Lightner J, Chakraborty K, Stone W, Campbell S, Reddy SA, Krishnan K (2011) Tocotrienols inhibit AKT and ERK activation and suppress pancreatic cancer cell proliferation by suppressing the ErbB2 pathway. Free Radic Biol Med 51:1164–1174

Shun MC, Yu W, Gapor A, Parsons R, Atkinson J, Sanders BG, Kline K (2004) Pro-apoptotic mechanisms of action of a novel vitamin E analog (α-TEA) and a naturally occurring form of vitamin E (δ-tocotrienol) in MDA-MB-435 human breast cancer cells. Nutr Cancer 48:95–105

Shun MC, Yu W, Park SK, Sanders BG, Kline K (2010) Downregulation of epidermal growth factor receptor expression contributes to α-TEA's proapoptotic effects in human ovarian cancer cell lines. J Oncol 824571

Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, Cooper D, Gansler T, Lerro C, Fedewa S, Lin C, Leach C, Cannady RS, Cho H, Scoppa S, Hachey M, Kirch R, Jemal A, Ward E (2012) Cancer treatment and survivorship statistics. CA Cancer J Clin 62:220–241

Siegel R, Naishadham D, Jemal A (2013) Cancer statistics, 2013. CA Cancer J Clin 63:11–30

Simon HU, Haj-Yehia A, Levi-Schaffer F (2000) Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis 5:415–418

Smith L, Wang Z, Smith JB (2003) Caspase processing activates atypical protein kinase C zeta by relieving autoinhibition and destabilizes the protein. Biochem J 375:663–761

Spitaler M, Cantrell DA (2004) Protein kinase C and beyond. Nat Immunol 5:785–790

Stapelberg M, Gellert N, Swettenham E, Tomasetti M, Witting PK, Procopio A, Neuzil J (2005) α-Tocopheryl succinate inhibits malignant mesothelioma by disrupting the fibroblast growth factor autocrine loop: mechanism and the role of oxidative stress. J Biol Chem 280:25369–25376

Suzuki YJ, Packer L (1993) Inhibition of NF-κB DNA binding activity by α-tocopheryl succinate. Biochem Mol Biol Int 31:693–700

Swettenham E, Witting PK, Salvatore BA, Neuzil J (2005) α-Tocopheryl succinate selectively induces apoptosis in neuroblastoma cells: potential therapy of malignancies of the nervous system? J Neurochem 94:1448–1456

Tasinato A, Boscoboinik D, Bartoli GM, Maroni P, Azzi A (1995) d-α-Tocopherol inhibition of vascular smooth muscle cell proliferation occurs at physiological concentrations, correlates with protein kinase C inhibition, and is independent of its antioxidant properties. Proc Natl Acad Sci U S A 92:12190–12194

Tiwary R, Yu W, Li J, Park SK, Sanders BG, Kline K (2010) Role of endoplasmic reticulum stress in α-TEA mediated TRAIL/DR5 death receptor dependent apoptosis. PLoS One 5:e11865

Tiwary R, Yu W, deGraffenried LA, Sanders BG, Kline K (2011a) Targeting cholesterol-rich microdomains to circumvent tamoxifen-resistant breast cancer. Breast Cancer Res 13:R120

Tiwary R, Yu W, Sanders BG, Kline K (2011b) α-TEA cooperates with MEK or mTOR inhibitors to induce apoptosis via targeting IRS/PI3K pathways. Br J Cancer 104:101–109

Tomasetti M, Rippo MR, Alleva R, Moretti S, Andera L, Neuzil J, Procopio A (2004) α-Tocopheryl succinate and TRAIL selectively synergise in induction of apoptosis in human malignant mesothelioma cells. Br J Cancer 90:1644–1653

Tomasetti M, Andera L, Alleva R, Borghi B, Neuzil J, Procopio A (2006) α-Tocopheryl succinate induces DR4 and DR5 expression by a p53-dependent route: implication for sensitisation of resistant cancer cells to TRAIL apoptosis. FEBS Lett 580:1925–1931

Totoń E, Ignatowicz E, Skrzeczkowska K, Rybczyńska M (2011) Protein kinase Cε as a cancer marker and target for anticancer therapy. Pharmacol Rep 63:19–29

Turley JM, Ruscetti FW, Kim SJ, Fu T, Gou FV, Birchenall-Roberts MC (1997) Vitamin E succinate inhibits proliferation of BT-20 human breast cancer cells: increased binding of cyclin A negatively regulates E2F transactivation activity. Cancer Res 57:2668–2675

Valis K, Prochazka L, Boura E, Chladova M, Obsil T, Rohlena J, Truksa J, Dong LF, Ralph SJ, Neuzil J (2011) Hippo/Mst1 stimulates transcription of *NOXA* in a FoxO1-dependent manner. Cancer Res 71:946–954

Wagner EF, Nebreda AR (2009) Signal integration by JNK and p38 MAPK pathways in cancer development. Nat Rev Cancer 9:537–549

Wallace DC (2012) Mitochondria and cancer. Nat Rev Cancer 12:685–698

Wang XF, Witting PK, Salvatore BA, Neuzil J (2005) Vitamin E analogs trigger apoptosis in HER2/erbB2-overexpressing breast cancer cells by signaling via the mitochondrial pathway. Biochem Biophys Res Commun 326:282–289

Ward PS, Thompson CB (2012) Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. Cancer Cell 21:297–308

Weber T, Lu M, Andera L, Lahm H, Gellert N, Fariss MW, Korinek V, Sattler W, Ucker DS, Terman A, Schröder A, Erl W, Brunk UT, Coffey RJ, Weber C, Neuzil J (2002) Vitamin E succinate is a potent novel anti-neoplastic agent with high selectivity and cooperativity with

tumor necrosis factor-related apoptosis-inducing ligand (Apo2 ligand) in vivo. Clin Cancer Res 8:863–869

Weber T, Dalen H, Andera L, Nègre-Salvayre A, Augé N, Sticha M, Lloret A, Terman A, Witting PK, Higuchi M, Plasilova M, Zivny J, Gellert N, Weber C, Neuzil J (2003) Mitochondria play a central role in apoptosis induced by α-tocopheryl succinate, an agent with antineoplastic activity: comparison with receptor-mediated pro-apoptotic signalling. Biochemistry 42:4277–4291

Weng-Yew W, Selvaduray KR, Ming CH, Nesaretnam K (2009) Suppression of tumour growth by palm tocotrienol via the attenuation of angiogenesis. Nutr Cancer 61:367–373

Whittle KJ, Dunphy PJ, Pennock JF (1996) The isolation and properties of δ-tocotrienol from Hevea latex. Biochem J 100:138–145

Wu SJ, Ng LT (2010) Tocotrienols inhibited growth and induced apoptosis in human HeLa cells through the cell cycle signalling pathway. Integr Cancer Ther 9:66–72

Wu K, Li Y, Zhao Y, Shan YJ, Xia W, Yu WP, Zhao L (2002) Roles of Fas signaling pathway in vitamin E succinate-induced apoptosis in human gastric cancer SGC-7901 cells. World J Gastroenterol 8:982–986

Yamamoto S, Tamai H, Ishisaka R, Kanno T, Arita K, Kobuchi H, Utsumi K (2000) Mechanism of α-tocopheryl succinate-induced apoptosis of promyelocytic leukemia cells. Free Radic Res 33:407–418

Yano Y, Satoh H, Fukumoto K, Kumadaki I, Ichikawa T, Yamada K, Hagiwara K, Yano T (2005) Induction of cytotoxicity in human lung adenocarcinoma cells by 6-O-carboxypropyl-α-tocotrienol, a redox-silent derivative of α-tocotrienol. Int J Cancer 115:839–846

Youk HJ, Lee E, Choi MK, Lee YJ, Chung JH, Kim SH, Lee CH, Lim SJ (2005) Enhanced anticancer efficacy of α-tocopheryl succinate by conjugation with polyethylene glycol. J Control Release 107:43–52

Yu W, Sanders BG, Kline K (1997) RRR-α-tocopheryl succinate inhibits EL4 thymic lymphoma cell growth by inducing apoptosis and DNA synthesis arrest. Nutr Cancer 27:92–101

Yu W, Liao QY, Hantash FM, Sanders BG, Kline K (2001) Activation of extracellular signal-regulated kinase and c-Jun-NH2-terminal kinase but not p38 mitogen-activated protein kinases is required for RRR- α-tocopheryl succinate-induced apoptosis of human breast cancer cells. Cancer Res 61:6569–6576

Yu W, Sanders BG, Kline K (2003) RRR-α-tocopheryl succinate-induced apoptosis of human breast cancer cells involves Bax translocation to mitochondria. Cancer Res 63:2483–2491

Yu W, Shun MC, Anderson K, Chen H, Sanders BG, Kline K (2006) α-TEA inhibits survival and enhances death pathways in cisplatin sensitive and resistant human ovarian cancer cells. Apoptosis 11:1813–1823

Yu W, Tiwary R, Li J, Park SK, Jia L, Xiong A, Simmons-Menchaca M, Sanders BG, Kline K (2010) α-TEA induces apoptosis of human breast cancer cells via activation of TRAIL/DR5 death receptor pathway. Mol Carcinog 49:964–973

Zhang JY, Selim MA (2012) The role of the c-Jun N-terminal kinase signaling pathway in skin cancer. Am J Cancer Res 2:691–698

Zhao Y, Neuzil J, Wu K (2009) Vitamin E analogues as mitochondria-targeting compounds: from the bench to the bedside? Mol Nutr Food Res 53:129–139

Zu K, Hawthorn L, Ip C (2005) Up-regulation of c-Jun-NH2-kinase pathway contributes to the induction of mitochondria-mediated apoptosis by α-tocopheryl succinate in human prostate cancer cells. Mol Cancer Ther 4:43–50

Chapter 8
Targeting Mitochondria of Cancer Cells: Mechanisms and Compounds

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Contents

Abstract Mitochondria are being proposed and tested as plausible targets for cancer therapy. There are several reasons for this recent approach. Perhaps the most important one is the fact that mitochondria comprise potent inducers of apoptosis, therefore disruption of mitochondria with ensuing apoptotic cell death is a promising strategy for cancer therapy. Importantly, too, mitochondria of cancer cells differ from these organelles in normal cells, in particular in their altered bioenergetics, the former utilizing for their energetic needs aerobic glycolysis. Further, mitochondria of cancer cells are characterized by greater negative potential across the inner

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membrane, also contributing to the potential design and testing of cancer cell-selective compounds. Some of the agents that target mitochondria of cancer cells, based on their differences compared to mitochondria of normal cells, are currently undergoing pre-clinical and clinical testing, which gives hope to the potential establishment of efficient and selective anti-cancer agents.

Keywords Mitochondria • Cancer cell • Metabolism • Apoptosis • Oxidative stress • Mitochondrial dysfunction • Therapeutic targets

1 Introduction

Mitochondria are cellular organelles that play pivotal roles in cellular metabolism, redox signaling, calcium homeostasis, and regulation of cell proliferation and cell death. Mitochondria have unique ultra-structural features that differ from other cellular organelles as they are enclosed by double membranes with certain unique lipid compositions. The mitochondrial membranes and matrix contain important molecules and metabolic enzymes, including those involved in electron transport, oxidative phosphorylation (OXPHOS), and the tricarboxylic acid (TCA) cycle. The mitochondrial DNA (mtDNA) contains genes that encode mitochondrial rRNA and tRNA as well as 13 proteins which, together with the nuclear DNA-encoded protein components, are necessary for the assembly of the oxidative phosphorylation machinery. Mitochondrial functions are coordinated with their dynamic behaviors and tightly regulated by balanced fusion and fission events. The replication of mtDNA and biogenesis of mitochondria are not necessarily synchronous with the cell cycle events, and thus mitochondria may replicate without cell division. Due to their diverse roles in the production of ATP and metabolic intermediates, generation of reactive oxygen species (ROS), and regulation of apoptosis, mitochondria are involved in a variety of pathological processes including neurodegenerative diseases, cancer, ischemia/reperfusion injury, aging, obesity, diabetes, and other disease conditions. The complex regulatory mechanisms involved in mitochondrial energetics, epigenetics, and genetics have been reviewed recently (Wallace and Fan 2010; Gogvadze 2011; Heller et al. 2012).

Mitochondria contribute to many critical cellular metabolic pathways, including oxidative phosphorylation, the TCA cycle (Krebs cycle), lipid metabolism, biosynthesis of iron sulfur center and heme, and metabolism of amino acids (Smith et al. 2012). Oxidative phosphorylation generates the bulk of cellular ATP to meet the energy requirements for normal cell proliferation and maintenance. The mitochondrial electron transport chain (ETC) complexes (I–V) are the key molecular machineries that execute the biochemical processes of respiration and ATP synthesis. ETC accepts electrons from NADH and succinate via complex I and complex II, respectively, and delivers them through complexes III and IV to the terminal electron acceptor oxygen, forming water and pumping proton into the intermembrane space to generate an electrochemical gradient across the mitochondrial inner membrane. The energy potential stored in this transmembrane proton gradient

can be used to drive ATP synthesis by complex V (ATP synthase). The TCA cycle is the major source of NADH that feed electrons into the ETC via complex I. Acetyl-CoA is a key metabolite from pyruvate catalyzed by pyruvate dehydrogenase, and subsequently reacts with oxaloacetate to form citrate and fuel to TCA cycle. The enzymes of the TCA cycle are mainly located in the mitochondrial matrix. Succinate dehydrogenase associated with the inner mitochondrial membrane functions as part of ETC Complex II.

Mitochondria are also a major intracellular source of reactive oxygen species (ROS), which are mainly produced at Complex I and III of the respiratory chain, although complex II may also be a site of ROS generation under certain conditions. Associated with the electron transport process during oxidative phosphorylation, some of the electrons may leak or "escape" from the ETC complexes, and are captured by molecular oxygen to form superoxide radicals. Superoxide can then be converted to other forms of ROS such as hydrogen peroxide or react with nitric oxide to form peroxynitrite, a reactive nitrogen species (RNS). Interestingly, mitochondrial respiratory chain is a major source of cellular free radicals and at the same time, a vulnerable target of free radicals (Ott et al. 2007). ROS/RNS are implicated to play a significant role in regulating a wide variety of physiological functions. At moderate levels, ROS/RNS may function as signaling molecules and affect many biological processes such as cell proliferation, mobility, epithelial–mesenchymal transition (EMT), angiogenesis and metabolic coupling of stromal and epithelial cells. This can be accomplished by transcriptional regulation through certain redox-sensitive transcription factors including NF-κB, p53, Nrf-2, TGF-β and HIF, or by directly modifying protein/enzyme molecules such as TRX, AKT, PTEN, MAPK, PI3K, and Bcl-2 (Trachootham et al. 2008a; Cui 2012; Ray et al. 2012). At a sustained high level beyond the cellular antioxidant buffering capacity, ROS/RNS can cause various damages to biolipids, DNA, and proteins, leading to injury of cellular components and cell death (Trachootham et al. 2009; Bae et al. 2011).

Besides their prominent role in cellular energy metabolism, mitochondria also function as a key modulator of the cell death pathways including apoptosis, necrosis, and autophagy. Both apoptotic and non-apoptotic cell death pathways need the involvement of mitochondria and alterations in mitochondrial membranes (Wenner 2012). The intrinsic pathway of apoptosis is initiated at mitochondria, leading to a release of multiple mitochondrial proteins such as cytochrome c, apoptosis-inducing factor (AIF), endonuclease G, and Smac/DIABLO. The release of such mitochondrial apoptotic factors in turn activates the caspase cascades, leading to protein degradation, DNA cleavage, and cell death. In the extrinsic apoptotic pathway (also known as death receptor pathway), apoptosis is triggered by ligand-induced activation of death receptors on the cell surface. Such death receptors include CD95/Fas (the receptor of CD95L/FasL) and the TNF-related apoptosis inducing ligand (TRAIL) receptors. The extrinsic apoptotic pathway can activate a subset of proteases, which then cause a destruction of cellular proteins leading to cell death. The extrinsic cell death process may cross-talk with the intrinsic pathway by caspase-mediated cleavage of Bid, which is translocated to mitochondria to induce the release of apoptosis factors (Kroemer et al. 2007). Autophagy is a special cell death pathway which also involves mitochondria. The inclusion of mitochondria within the lysosomal structure is a characteristic

change. Furthermore, accumulating evidence suggests that whether cells undergo apoptosis or necrosis is in part determined by their energy status. The mitochondrial membrane potential (MMP) can be an important parameter for necrotic cell death. Considering the crucial roles of mitochondria in various cell death pathways, it is not surprising that compounds that target mitochondria may profoundly affect cell viability by inducing apoptosis, necrosis, or autophagy.

2 Mitochondrial Dysfunction in Cancer Cells

Mitochondrial dysfunction may be considered as a hallmark of transformed cancer cells, and can be categorized into primary and secondary dysfunctions (Smith et al. 2012). Primary dysfunction can be caused by mutations of mtDNA or nuclear genes that code for mitochondrial proteins, directly leading to mitochondria defects. Secondary mitochondrial dysfunction may be caused by signaling events that originate outside the mitochondria and indirectly affect mitochondrial functions. Mitochondrial dysfunction in cancer cells may lead to metabolic reprogramming to maintain that promotes tumor cell survival and proliferation. Mitochondrial dysfunction may also contribute to elevated ROS, which can stimulate cell proliferation and also function as a constant source of mutagen to induce DNA damage and genetic instability, leading to cancer development and progression (Storz 2005).

2.1 Mitochondrial Dysfunction and Alterations in Energy Metabolism

Cancer cells are active in glycolysis even in the presence of oxygen. This phenomenon of active aerobic glycolysis, known as the Warburg effect, was first observed in the 1920s and has since been shown to occur in various types of cancer cells. Warburg attributed this phenomenon to defective mitochondria or respiratory injury and considered this metabolic change as a critical event in cancer development (Warburg 1956). The observation that cancer cells actively utilize glucose has led to the development of ^{18}F-deoxyglucose positron emission tomography (FDG-PET), a sensitive imaging technology for cancer diagnosis and for monitoring therapeutic responses. FDG-PET scan is largely based on high glucose uptake by cancer cells in vivo (Gambhir 2002). The prevalence of high glycolysis in cancer cells has led to the hypothesis that dysfunctional mitochondria are the root of cancer (Koppenol et al. 2011). However, why cancer cells are addicted to aerobic glycolysis and the underlying molecular mechanisms still remain elusive. In many cancer cells, mitochondria are still able to carry out oxidative phosphorylation. Glycolysis may be still highly active even in cancer cells with competent mitochondrial function and active oxidative phosphorylation. As such, blocking a single energy metabolic pathway might not be highly effective in killing cancer cells (Moreno-Sanchez et al. 2010). Given the

catabolic and anabolic demands of proliferating tumor cells, it is believed that mitochondrial impairment would be disadvantageous for cancer cell survival, (Romero-Garcia et al. 2011). Nevertheless, the high glycolytic activity in cancer cells may provide certain metabolic advantages over their normal counterparts. Firstly, active glycolysis enables cancer cells to survive under the harsh microenvironment such as hypoxia (Gogvadze 2011). Secondly, the metabolic intermediates from glycolysis provide cells with an important source of precursors needed for biosynthesis of nucleotides, lipids, and NADPH, also of which are important for cell proliferation and redox balance (DeBerardinis et al. 2008). Thirdly, the acidification of microenvironment due to lactate production during glycolysis may facilitate tumor invasion and metastasis (Gatenby and Gillies 2004; Weinberg and Chandel 2009). Interestingly, it has been found that certain mitochondrial proteins encoded by nuclear genes can be tumor suppressors and their abnormalities may be involved in tumor development. For instance, mutations in succinate dehydrogenase (SDH) and fumarate hydratase (FH) are linked to phaeochromocytoma, paraganglioma, leiomyoma, and renal cell carcinoma (Ward and Thompson 2012).

Activation of the PI3K/AKT pathway is one of the most common alterations in human cancer, and this pathway plays a major role in promoting glucose uptake and glycolysis (Gottlieb and Tomlinson 2005; Weinberg and Chandel 2009). The PI3K/AKT pathway promotes glucose flux into the biosynthetic pathways in the mitochondria through affecting the activity of pyruvate dehydrogenase (PDH) complex that converts pyruvate into acetyl-CoA for metabolism via the TCA cycle. PI3K/AKT oncogenic activity seems to play a key role in reprogramming cellular energy metabolism. It is known that downstream of PI3K/AKT pathway, activation of the mTORC pathway has profound effect on nutrient uptake and mitochondrial metabolism (Elstrom et al. 2004; Buzzai et al. 2005). Another key protein involved in glycolysis is HIF-1α, which plays a pivotal role in reprogramming of cancer metabolism by activating the transcription of glucose transporters (GLUT 1, GLUT 4) and many of the glycolytic enzymes. Activation of HIF-1α seems able to induce the Warburg effect and promote aggressiveness of malignant cells (Bhaskar et al. 2009). When mitochondrial respiration in tumor cells is inhibited, accumulation of TCA cycle substrates might serve as a signal for stimulation of glycolysis. Succinate was shown to inhibit HIF-1α prolyl hydroxylases in the cytosol, leading to stabilization and activation of HIF-1α, which then stimulates the expression of lactate dehydrogenase to accelerate the conversion of pyruvate into lactate (Robey et al. 2005), thus diminishing the utilization of pyruvate by the mitochondria. In addition, HIF-1α can modulate cytochrome oxidase (COX) expression and alter the subunit composition of COX to affect its activity under hypoxic conditions. For instance, expression of the COX4–2 subunit is increased, whereas the COX4–1 subunit is degraded by activation of the mitochondrial protease LON, a downstream enzyme of HIF-1α (Semenza et al. 1996). Similarly, the oncogene Myc also plays a major role in promoting glycolysis by transcriptionally enhancing the expression of glycolytic enzymes (Dang 2012).

The tumor suppressor p53 is a key regulator of cell cycle checkpoints and DAN repair, and is often deregulated or mutated in cancer cells. Accumulating evidence

suggest that p53 not only functions as a guardian for the nuclear genome but also plays a significant role in maintaining mitochondrial genomic integrity (Gogvadze et al. 2008). Loss of p53 function may lead to mtDNA mutations, and thus has a major effect on cellular metabolism including glycolysis (Bakhanashvili et al. 2008). The wild-type p53 represses GLUT1 and GLUT4 gene transcription, while mutations within the DNA binding domain of p53 release the repressive effect on GLUT transcription, leading to high glucose uptake and increased glucose metabolism (Ralph et al. 2010). The p53 downstream gene TIGAR (TP53-induced glycolysis and apoptosis regulator) is able to significantly alter glucose metabolism at the step involving phosphofructose. Wild-type p53 can down-regulate, while mutant p53 can enhance phosphoglycerate mutase (PGM) activity and glycolytic flux (Schwartzenberg-Bar-Yoseph et al. 2004). Loss of p53 function leads to a decrease of mitochondrial respiration and increased glycolysis. In addition, p53 has recently been shown to directly regulate OXPHOS in cancer cells through transcriptional upregulation of its downstream gene SCO2 (Shen et al. 2012). Knockout of p53 in HCT116 colon cancer cells resulted in lower expression of SCO2, leading to abated OXPHOS activity which was compensated by an increase in glycolysis (Madan et al. 2011). Furthermore, p53 has been reported to directly increase GLS2 (glutaminase 2) activity and accelerate the TCA metabolism by enhancing the flow of glutamine into the TCA cycle (Madan et al. 2011). In addition, p53 seems able to inhibit the pentose phosphate pathway through binding to glucose-6-phosphate dehydrogenase (G6PD), and to inhibit fatty acid metabolism through upregulation of AMPK activity (Suzuki et al. 2010). Several key oncogenes including c-Myc and Ras have been shown to regulate cellular metabolism. Their potential roles in affecting mitochondrial functions and energy metabolism warrant further study.

Taken together, multiple regulatory pathways participate in the metabolic regulation in cancer cells and are closely associated with mitochondrial dysfunction. Metabolic reprogramming may render cancer cells highly dependent on certain metabolic enzymes and thus may provide a biological basis for developing novel and effective therapeutic approaches to cancer therapy. Several agents inhibiting glycolytic enzymes have been in various stages of preclinical and clinical studies (Pelicano et al. 2006; Zhang et al. 2010; Maddocks and Vousden 2011).

2.2 Mitochondrial Dysfunction and Oxidative Stress

Mitochondria consume the majority of oxygen used by the cells, and are considered the major site of ROS generation. Nearly 2 % of oxygen consumed by cells is used through the ETC to generate superoxide, which can then be converted to other ROS. If not detoxified, high levels of ROS may cause significant damage to proteins, lipids, nuclear acids and other biomolecules, while a mild level of oxidative stress stimulate cell proliferation. As such, both ROS-mediated genotoxicity (DNA damage) and ROS-mediated signaling may contribute to tumor initiation and progression (Pathania et al. 2009; Chen et al. 2010). It has been recognized that increased ROS stress in cancer cells is correlated with aggressiveness of tumors

and poor prognosis (Fogg et al. 2011). Compelling evidence suggests that cancer cells tend to have elevated levels of ROS compared to the normal cells of same tissue origins (Patel and Chiplunkar 2007; Kumar et al. 2008). Mitochondrial dysfunction has been proposed as one of the main reasons for elevated ROS in cancer cells (Szatrowski and Nathan 1991). The hypoxic environment in the rapidly growing tumor mass may facilitate ROS production since a lower oxygen tension may cause dysfunction of ETC and more electron leakage from the respiratory complexes. Prolonged hypoxia and glucose deprivation are reported to increase mitochondrial ROS in various cell types (Brandon et al. 2006; Pani et al. 2009). A sustained ROS increase could result in mtDNA mutations leading to further defect in mitochondrial respiration, more electron leakage and higher ROS generation (Martens et al. 2005; Isaev et al. 2008), and mitochondrial oxidative stress has been linked to a vicious cycle of ROS generation, DNA mutations, and oncogene deregulation, leading to cell transformation and malignancy (Gogvadze 2011).

A series of studies suggest that oncogenic K-Ras may induce the expression of NRF2, PGC-1α and TFAM associated with mitochondrial dysfunction manifested by low mitochondrial respiration, decreased ATP generation, AMPK activation, and high glycolysis (Ralph et al. 2010). More recently, K-Ras is shown to cause mitochondrial dysfunction leading to elevated ROS generation (Moiseeva et al. 2009; Yun et al. 2009). In addition, microarray analysis revealed that K-ras transformed cells had low copy number of mitochondrial OXPHOS components. It has been suggested that a high level of K-Ras induces cell senescence while sustained expression of a low level of K-Ras induces carcinogenesis (Janssen-Heininger et al. 2008; Moiseeva et al. 2009). Severe ROS stress may contribute to K-Ras-induced senescence.

Another oncogene Myc also impacts mitochondrial function and promote glycolysis in cancer cells. Myc induces expression of mitochondrial biogenesis gene TFAM and glutaminolysis gene GLS, leading to increased oxygen consumption and elevated ROS (Chiaradonna et al. 2006; Sarkisian et al. 2007). Interestingly, Myc also promotes the expression of several mitochondrial antioxidant molecules such as SOD2, PRX 6, and PRX III, and thus provides a mechanism for the transformed cells to sustain oxidative stress. Inhibition of Myc-induced oxidative stress by vitamin C, which prevent glutathione depletion, can delay Myc-mediated cell transformation (Morrish et al. 2008; Wise et al. 2008), suggesting that ROS may play a significant role in cell transformation. It was recently hypothesized that Myc may cause mitochondria functional transition from basal maintaining level (low oxygen consumption, low ROS leakage from ETC, low ATP generation) to an activated state (high oxygen consumption, high ROS production, and high ATP generation) (Dang et al. 2005; Kc et al. 2005).

Loss of p53 function may play an important role in mtDNA depletion, mitochondrial dysfunction, and ROS alteration. P53 seems to affect mitochondrial ROS homeostasis through its influence on mtDNA replication and repair. In one study, thymic lymphomas derived from p53-/- mice showed an abnormal mitochondrial biogenesis, increased mtDNA copy number, high ROS and glycolytic activity (Ralph et al. 2010). These observations suggest that mutation or loss of p53 is associated with alterations in mitochondrial function, energy metabolism, and ROS generation,

and thereby may promote malignant phenotype. As such, mitochondrial oxidative stress due to loss of p53 may contribute to the development of the hallmarks of cancer (Samper et al. 2009), including immortalization and transformation (Hanahan and Weinberg 2000), uncontrolled proliferation (Behrend et al. 2003), disruption of cell survival/death signaling (Pervaiz and Clement 2004; Achanta et al. 2005; Clerkin et al. 2008), epithelial–mesenchymal transition and metastasis (Trachootham et al. 2008a, b), angiogenesis and chemo-resistance (Achanta et al. 2005; Radisky 2005).

The elevated ROS associated with mitochondrial dysfunction in cancer cells may be exploited for therapeutic purpose. Pharmacological agents may be used to selectively induce ROS-mediated death in cancer cells by increasing the already high intrinsic ROS level above the threshold that triggers cell death, while normal cells with low basal ROS may be able to sustain the same treatment due to lower basal ROS output (Sullivan et al. 2008). Indeed, certain drugs used in clinical treatment of cancer such as cisplatin, vinblastine, and doxorubicin exert their cytotoxic action in part by inducing ROS generation. Arsenic trioxide, which is capable of impacting the mitochondrial respiratory chain and promoting superoxide generation, has been shown to effectively kill leukemia cells with relatively low toxicity to normal cells (Wang and Choudhary 2011). However, for certain cancer cells, especially those in advanced disease stages with sustain oxidative stress, it is possible that these malignant cells might have up-regulated their antioxidant systems and become less sensitive to ROS-promoting agents. In fact, antioxidant molecules such as SOD2, glutathione, Nrf-2, and GPX are often overexpressed in malignant cells (Pelicano et al. 2003). Therefore, abrogation or inhibition of such antioxidant systems may represent a more effective strategy than using ROS-generating agents to kill these malignant cells. It has been suggested that the combination of ROS-generating agents and drugs that abolish the antioxidant systems would be an effective therapeutic strategy (Janssen et al. 1998; Plymate et al. 2003; Irwin et al. 2013).

2.3 Mitochondrial Dysfunction and Apoptosis

Besides their roles in cellular energy metabolism and redox homeostasis, mitochondria also play a key role in regulation of cell death. Apoptosis is a complex cell death process regulated by mitochondria and is characterized by cell shrinkage, nuclear condensation, chromatin fragmentation, and generation of apoptotic bodies. Execution of apoptosis is orchestrated in part by mitochondria, which release apoptosis factors such as cytochrome c, AIF, Smac/DIABLO, and endonuclease G into the cytoplasm, followed by activation of the caspase cascade (Smith et al. 2011). Malignant cells harness various mechanisms to suppress the intrinsic and extrinsic apoptotic signaling to escape cell death, which is a hallmark of cancer. It is believed that evasion of the normal apoptotic pathways is necessary for tumorigenesis (Gogvadze et al. 2006; Low et al. 2011).

One of the central events that control cellular commitment to apoptotic cell death is mitochondrial outer membrane permeabilization (MOMP), which allows

apoptotic factors to be released to cytosol. This process is in part controlled by the Bcl-2 family (Ferrin et al. 2011; Fogg et al. 2011). The anti-apoptotic members of the Bcl-2 family members such as Bcl-2, Bcl-XL, and Mcl-1 are pivotal regulators of apoptotic cell death, and are often overexpressed in cancers of different origins (Davids and Letai 2012). Bcl-2 family can be divided into 3 subgroups based on their functional and structural features: (a) proteins that promote cell survival and contain multi BH domains such as Bcl-2 and Bcl-XL; (b) proteins that contain only BH3 domain with pro-apoptotic function such as Bim and Puma; (c) proteins that activate the effecters of apoptotic pathways such as Bax and Bak. Mutations or deregulation of the Bcl-2 family members are commonly observed in human tumor samples. Unfortunately, these changes may render tumor cells refractory to chemotherapies. The permeability of the outer mitochondrial membrane can be regulated by the cross-talk between the pro- and anti-apoptotic proteins. Anti-apoptotic proteins (Bcl-2, Bcl-XL, Mcl-1) interact with the pro-apoptotic proteins (Bax, Bak), thereby preventing the oligomerization of the pro-apoptotic proteins to induce MOMP. Targeting the Bcl-2 family of proteins and the combination of such targeting agents with the conventional anticancer drugs may be effective therapeutic strategies.

A key cell pathway, which may engage either necrotic or apoptotic cell death, is the induction of mitochondrial permeability transition (MPT) involving multiple protein components and complex regulatory processes. MPT can be a sudden permeabilization of the inner mitochondrial membrane in response to various stimuli such as oxidative stress, calcium overload, hypoxia, or certain cytotoxic chemicals (Straten and Andersen 2010). These stimuli may cause a dissipation of the mitochondrial transmembrane potential, loss of ATP generation, and formation of mitochondrial permeability transition pore (MPTP), leading to the release of mitochondrial apoptotic factors (McCommis and Baines 2012). The mitochondrial voltage-dependent anion channel (VDAC) seems to play a crucial role in regulating MPTP. VDAC not only forms the main interface between the mitochondrial and cellular metabolic processes thus controlling a cross-talk between mitochondria and the rest of the cell, but is also involved in regulating cell death through direct interaction between hexokinase 2 (HK2), Bcl-2 family proteins such as Bcl-2 or Bcl-XL (Shoshan-Barmatz et al. 2010; Arbel et al. 2012), peripheral benzodiazepine receptor (PBR), and the translocator protein TSPO. Hexokinase is a key glycolytic enzyme associated with VDAC on the mitochondrial outer membrane, and thereby gains access to the mitochondrial ATP during glucose metabolism. In vitro and in vivo studies suggested that the mitochondria-bound HK2 is elevated in cancer cells and protects against apoptosis by inhibiting cytochrome c release. The mitochondrial-bound HK2 also protects against Bax-induced apoptosis (Arbel et al. 2012). Interestingly, a recent study revealed that a single mutation in VDAC1 prevented HK-mediated protection against apoptosis (Pedersen et al. 2002; Arzoine et al. 2009). Another study showed that both anti- and pro-apoptotic Bcl-2 family proteins interact with VDAC and regulate MPTP. Bcl-2/Bcl-XL over-expression blocked As_2O_3-induced VDAC dimerization (Shoshan-Barmatz et al. 2009), and cisplatin-induced apoptosis through activation of BAX was inhibited in VDAC-depleted cells (Zheng et al. 2004). The close association between TSPO and VDAC

is also thought to play a role in cellular apoptosis. A TSPO antagonist PK11195 can induce mitochondrial depolarization and cytochrome c release in primary chronic lymphocytic leukemia (CLL) cells, while such PK11195-induced apoptosis could be suppressed by an inhibitor of VDAC, suggesting a possible interaction between TSPO and VDAC (Tajeddine et al. 2008). Since Bcl-2 family proteins, VDAC and their associated proteins are often altered in cancer cells with mitochondrial dysfunction, these changes provide opportunities for therapeutic intervention.

3 Compounds Targeting Mitochondrial Dysfunction in Cancer Cells

3.1 Targeting Metabolic Alternations Associated with Mitochondrial Dysfunction

Cancer cells with mitochondrial dysfunction typically undergo a series of metabolic changes to support the energetic and biosynthetic requirements for rapid cell proliferation. Therefore, specific targeting these altered metabolic pathways provides an attractive strategy to preferentially kill cancer cells. Altered expression and activities of enzymes in glycolysis and the TCA cycle have been observed in various cancers (Santidrian et al. 2007). One strategy is to inhibit glycolysis, which may cause depletion of ATP, or force the cancer cells to use the "normal" OXPHOS metabolic pathway and thus disrupt their survival and growth. Some of the compounds described below have indeed been shown to effectively kill cancer cells with high glycolytic phenotype, and may also enhance their sensitivity towards other chemotherapeutic agents. A major caveat with glycolytic inhibition is that cancer cells may exhibit flexibility in metabolism and use alternative metabolic pathways when glycolysis is inhibited. Therefore, combinational strategies that target multiple metabolic pathways such as glutamine and fatty acid metabolism may be required to eliminate therapeutic resistance. Figure 8.1 illustrates several key compounds that target metabolic pathways in cancer cells.

3-Bromoyruvate: Many tumor cells exhibit increased glucose uptake and active glycolysis with overexpression of hexokinase (HK), especially HK2. Hexokinase is the first enzyme in the glycolytic pathway that converts glucose to glucose-6-phosphate via transfer of a phosphate group from ATP to the 6-carbon of glucose. Under physiological condition, a portion of HK2 is associated with mitochondria through VDAC to form a complex that seems to prevent apoptosis, thus contributing to drug resistance (Kroemer and Pouyssegur 2008; DeBerardinis and Thompson 2012). A synthetic brominated derivative of pyruvic acid, 3-bromopyruvate (3-BrPA), has been found to selectively kill hepatocellular carcinoma cells in vitro, leaving normal hepatocytes undamaged (Mathupala et al. 2010). Moreover, systemic delivery of 3-BrPA suppressed "metastatic" lung tumors with no apparent toxicity to the animals (Mathupala et al. 2010). Analysis of potential cellular targets for 3-BrPA revealed that this

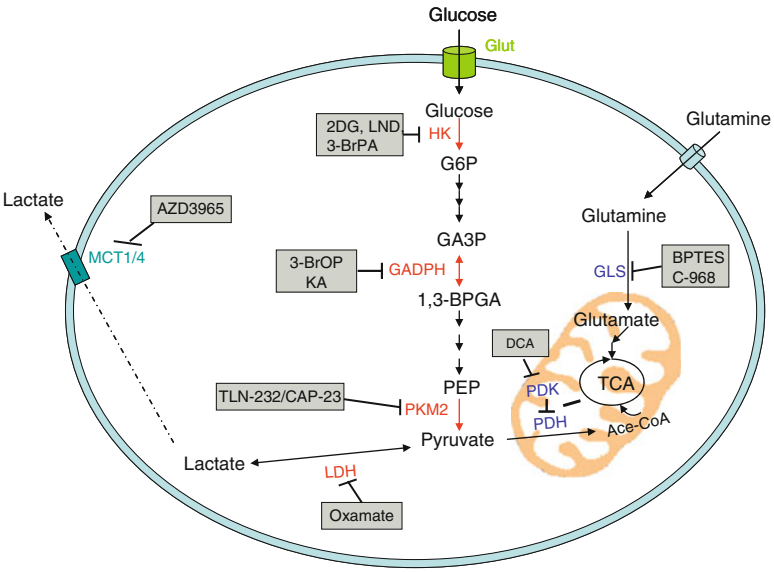

Fig. 8.1 Compounds targeting cancer cells with mitochondrial dysfunction and altered energy metabolism. Compounds that target glucose and glutamine metabolism in cancer cells are shown in gray boxes. Glycolysis is a series of metabolic processes, driven by specific enzymes, and generated 2 molecules of ATP per glucose with a production of 2 molecules of pyruvate, which is further metabolize in the mitochondria through TCA cycle under normal conditions. In cancer cells, pyruvate is mainly converted into lactate. Glutaminolysis converts glutamine into glutamate which also feed the TCA cycle for further metabolism. *Glut* glucose transporter, *MCT1/4* monocarboxylate transporter 1 and 4, *G6P* Glucose-6-phosphate, *GA3P* Glyceraldehyde-3-phosphate, *1,3-BPGA* 1, 3-bisphosphoglycerate. Chemical inhibitors are indicated in *bold*: *2DG* 2-Deoxy-Glucose, *LND* Lonidamine, *3-BrPA* 3-Bromopyruvate, *3-BrOP* 3-bromopyruvate propyl ester, *KA* koningic acid, *DCA* Dichloroacetate, *BPTES* bis-2-(5-phenylacetimido-1,2,4,thiadiazol-2-yl) ethyl sulfide

compound suppressed the glycolytic capacity of the tumor, inhibited the activity of mitochondria-bound hexokinase, and also suppressed respiration of isolated mitochondria (Geschwind et al. 2002). 3-BrPA is a highly reactive electrophilic alkylator and may react with multiple proteins including the glycolytic enzymes HK and GAPDH (Gogvadze 2011), the mitochondrial succinate dehydrogenase (Ganapathy-Kanniappan et al. 2010a), and certain endoplasmic reticulum proteins (Dschietzig et al. 2009). Recent work found 3-BrPA causes a covalent modification of mitochondria-bound HK2, leading to disruption of interaction between HK2 and apoptosis-inducing factor (AIF). AIF released from the mitochondria leads to induction of cell death, revealing another mechanism of action of 3-BrPA (Ganapathy-Kanniappan et al. 2010a, b). A chemopotentiating function/activity of 3-BrPA has also been shown in combination with platinum drugs (Chen et al. 2009b) and with 5-fluorouracil (Ihrlund et al. 2008) through the synergetic effect of glycolysis inhibition and DNA damage. Recent studies showed that the 3-BrPA derivative 3-BrOP preferentially inhibits GAPDH, and is highly effective in killing stem-like cancer cells, especially when it is combined with a conventional anticancer drug BCNU (Zhao et al. 2011; Yuan et al. 2013).

Dichloroacetate: The use of dichloroacetate (DCA) to treat certain metabolic abnormalities, administered as an ionic complex with sodium or other cations, has been in clinical practice for decades. As shown in Fig. 8.1, DCA is an inhibitor of pyruvate dehydrogenase kinase (PDK) and is able to stimulate the activity of pyruvate dehydrogenase (PDH) via suppressing the inhibitory phosphorylation by PDK, thus enhancing the conversion of pyruvate to acetyl-CoA to enter the TCA cycle and facilitate mitochondrial oxidative phosphorylation. Interestingly, DCA has been shown to inhibit cancer cell proliferation, increase apoptosis and decrease mitochondrial membrane potential (Sanchez-Arago and Cuezva 2011). PDK II seems to be an isozyme constitutively expressed in most tissues and is sensitivity to DCA. A knockdown of PDK II by siRNA showed similar effect as DCA (Bonnet et al. 2007). DCA treatment leads to inhibition of cancer growth in vitro and in vivo (Bonnet et al. 2007). In preclinical studies, DCA has shown promising antitumor activity and is currently being tested in clinical trials against metastatic solid tumors including gliomas and glioblastoma (Cao et al. 2008).

Oxamate: Lactate dehydrogenase (LDH) is an enzyme that catalyzes the reversible conversion of pyruvate to lactate and NADH to NAD^+. The c-Myc oncogene was reported to up-regulate the expression of LDHA in tumor cells and promote glycolytic activity (Michelakis et al. 2008; Stacpoole et al. 2008). LDH expression and activity is found to increase in malignant cells compared to normal tissues (Lewis et al. 2000). HIF-1α, a central regulator in cells under hypoxic conditions, also up-regulates LDHA expression, which is positively associated with active cell proliferation and poor prognosis in breast cancer (Gimeno et al. 1976; Balinsky et al. 1983). Thus LDH may be a key enzyme that affects glycolysis and a potential target for cancer therapy. Inhibition of LDH might force cancer cells to utilize pyruvate in mitochondrial OXPHOS by shuttling pyruvate into the TCA cycle. Oxamate has been shown to competitively inhibit LDH in vitro using recombinant LDH-A (Thornburg et al. 2008). This compound was shown to suppress the glycolytic flux in HeLa cells (cervical adenocarcinoma cells), and is selective toxicity to fibroblasts transformed with H-Ras (Thornburg et al. 2008). Interestingly, a combination of oxamate and taxol seems to have synergistic effect on the breast cancer cell line MDA-MB-435 (Ramanathan et al. 2005). It should be noted that oxamate may also inhibit aspartate aminotransferase, an enzyme involved in transfer of α-amino group between aspartate and glutamate and plays a critical in amino acid metabolism and electron transfer. As such, the anticancer activity of oxamate should not be entirely attributed to inhibition of LDH.

TLN-232: Formerly known as CAP-232, TLN-232 is a synthetic cyclic peptide of 7 amino acids and has entered clinic trials in metastatic melanoma and renal cell carcinoma. This compound exhibits broad antitumor activity in vitro and in vivo (Zhou et al. 2010). Mechanistically, TLN-232 targets pyruvate kinase isoenzyme type 2 (PKM2), which is a dimeric isoform of pyruvate kinase and the preferentially expressed in tumor cells (Tejeda et al. 2007). PKM2 is a glycolytic enzyme that converts phosphoenolpyruvate (PEP) to pyruvate, with concomitant generation of ATP. The primary role of PKM2 in proliferation cells is to facilitate anabolic metabolism as a glycolytic switch (Steinberg et al. 1999). TLN-232 can cause nuclear translocation

of PKM2, resulting in alteration of cellular glycolysis. The redistribution of PKM2 in the cells seems to correlate with the cytotoxicity of TLN-232. Incubation of the highly glycolytic glioma cells (SF-188) with TLN-232 resulted in a dose-dependent inhibition of PKM2 enzymatic activity associated with a cleavage and activation of caspases and PARP, leading to apoptosis.

3.2 Targeting Redox Alternations Associated with Mitochondrial Dysfunction

Reactive oxygen species (ROS) are products of cellular metabolism, and may have beneficial or harmful effects on the cells, depending on their levels and subcellular localizations. In cancer cells with mitochondrial dysfunction, high levels of ROS are often observed due in part to an increase in electron leakage from the respiratory chain. It is believed that ROS may promote tumorigenesis due to induction of mutation, and induce drug resistance due to overexpression of antioxidant molecules (e.g. SOD2, Prx III, GPX4, glutathione) as a consequence of adaptation to oxidative stress (Ward and Thompson 2012). Paradoxically, ROS manipulation is considered a valuable approach to cancer prevention and treatment. Both antioxidants and pro-oxidants have been tested experimentally and clinically with various results. Since cancer cells often have higher levels of ROS than normal cells, they are intrinsically under oxidative stress and thus may be highly sensitive to further ROS stress by redox-modulating agents. A key to successful design of effective cancer therapeutics exploiting oxidative stress is to effectively break the cellular redox balance in cancer cells to induce apoptosis before antioxidant adaptation, while exerting minimum toxic effect on normal cells due to their low basal ROS output (Smith et al. 2011). Figure 8.2 illustrates several compounds that target redox balance with promising anticancer activity.

β-Phenethyl isothiocyanate (PEITC): PEITC is a natural product found in cruciferous vegetables such as watercress, cabbage, and broccoli. It shows promising anticancer activity through a ROS-modulating mechanism. Accumulating research shows that PEITC can selectively kill cancer cells, including those resistant to standard chemotherapeutic agents such as fludarabine, cisplatin, and Gleevec, with low toxicity to normal cells. Furthermore, PEITC is also effective in tumor xenograft models and in primary cancer cells from leukemia patients (Trachootham et al. 2009). The highly electrophilic chemical property of PEITC seems to be the base of its biochemical reactions with various cellular targets. Among the many mechanisms proposed, the ability of PEITC to disable cellular antioxidant GSH system seems to be the most prominent mechanism contributing to the anticancer activity of this compound. PEITC can conjugate with GSH and cause its rapid export from the cells leading to a severe depletion of intracellular GSH pool, and can also inhibit the redox-modulating enzyme glutathione peroxidase (GPX) (Trachootham et al. 2006, 2008a, b, 2009; Zhang et al. 2008). The depletion of GSH and inhibition of GPX effectively abrogate the cellular antioxidant capacity

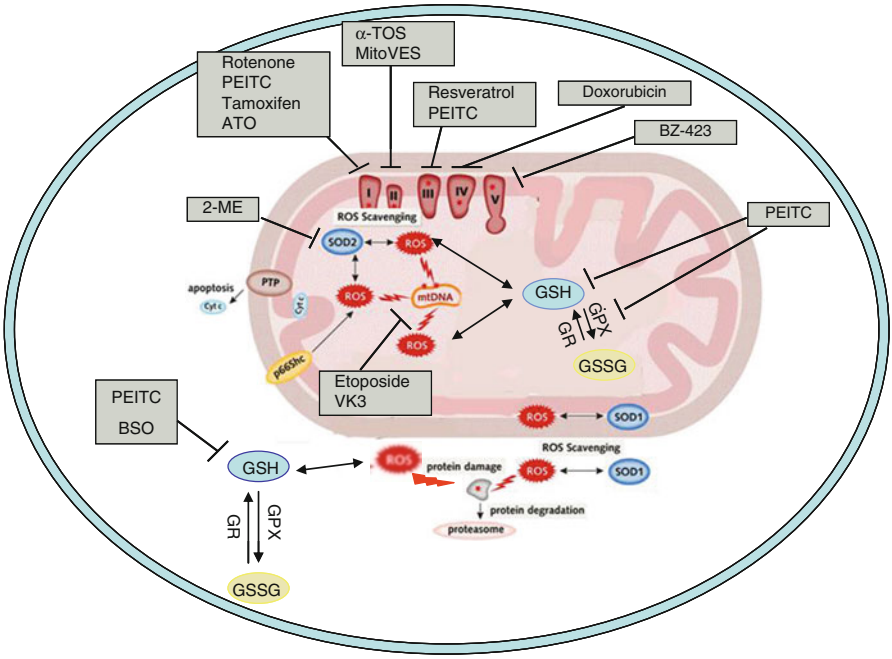

Fig. 8.2 Compounds targeting redox imbalance in cancer cells with mitochondrial dysfunction. Chemical inhibitors are indicated in *bold. PEITC* phenethyl isothiocyanate, *ATO* arsenic trioxide, *α-TOS* α-Tocopheryl succinate, *2-ME* 2-Methoxy-Estradiol, *VK3* vitamine K3, *BSO* buthionine sulphoximine, *BZ-423* 1,4-benzodiazepine

and thus cause a severe ROS accumulation in cancer cells, leading to oxidative damage and cell death. Since normal cells have a lower basal ROS output and thus are less dependent on the GSH antioxidant system, PEITC has minimum cytotoxicity effect on normal cells at the concentrations that are toxic to cancer cells (Estrela et al. 2006; Trachootham et al. 2009). Recent studies suggest that PEITC may also target the mitochondrial respiration chain components, cause complex disruption, and promote electron leakage and ROS generation (Trachootham et al. 2009). It should be noted that PEITC is mechanistically different from the glutathione-depleting agent buthionine sulfoximine (BSO), which inhibit glutathione synthesis and causes slow depletion of cellular GSH, while PEITC deprives both cytosolic and mitochondrial GSH pool rapidly, leaving cancer cells under tremendous oxidative stress in an acute manner early as 1 h (Brown et al. 2010; Chen et al. 2011). The rapid action of PEITC to cause oxidative damage in cancer cells leave little time for adaptation. As such, PEITC may be more effective than BSO in eliminating cancer cells.

Arsenic Trioxide: Arsenic trioxide (ATO) is a highly effective compound for treatment of acute promyelocytic leukemia (APL) as a single agent, and has also been included in several front-line studies to overcome chemotherapy resistance

and decrease chemotherapy-related side effects. Interestingly, ATO seems to have concentration-dependent mechanisms of action. At the concentration range of 0.1–0.5 mM, ATO induces partial differentiation, catabolism and proteasomal degradation of PML and PML-RARalpha, while at higher concentrations (0.5–2.0 mmol/l), ATO stimulates ROS generation and activates caspases, which in turn induce apoptosis (Chen et al. 2011). Interfering with the mitochondrial electron transport chain is likely an important mechanism by which ATO promotes ROS generation (Lengfelder et al. 2012). In leukemia cells, ATO is capable of inhibiting mitochondrial respiration, resulting in a substantial decrease of oxygen consumption as early as 3 h and a concurrent increase in ROS generation. The mitochondrial respiration-deficient cells (rho-0 cells) seem relatively resistant to ATO treatment compared to their respiration functional counterparts, further suggesting that the mitochondrial respiratory chain activity may be an important target of ATO (Pelicano et al. 2003).

2-Methoxyestradiol (2-ME): 2-methoxyestradiol is a natural estrogen metabolite with promising anticancer activity (Pelicano et al. 2003). Treatment of Ewing sarcoma cells with 2-ME leads to elevated cellular ROS, disruption of mitochondrial membrane potential, cytochrome c release, caspase activation, and apoptosis (Fotopoulou et al. 2010). The proposed mechanisms of action of 2-ME include disruption of microtubules and inhibition of mitochondrial superoxide dismutase (SOD2). A recent study suggested that after 3 months of chronic incubation with 2-ME, the level of mitochondrial SOD2 protein was significant upregulated in the resistant pancreatic cells compared to the parental cells, and silencing of SOD2 re-sensitized the resistant cells to 2-ME (Djavaheri-Mergny et al. 2003). Since 2-ME has limited bioavailability and rapid metabolic degradation, development of special formulation and analogues to increase bioavailability and combination with other drugs seem to be attractive strategies to enhance its therapeutic activity in vivo. Such therapeutic strategies are under clinical evaluation in various tumor types including breast cancer, pancreatic cancer, and metastatic renal cancer (Zhou and Du 2012).

3.3 Targeting Apoptosis Resistance Associated with Mitochondrial Dysfunction

Many anticancer agents kill tumor cells by inducing apoptosis. However, dysfunction of apoptotic pathways is often observed in cancer cells and contributes to chemoresistance. Thus, reengaging the mitochondrial apoptosis pathway by activating apoptotic effectors in cancer cells presents an attractive therapeutic approach. Mitochondrial outer membrane permeablization (MOMP) links the intra-mitochondrial death factors to the cytosolic apoptotic machinery and presents a critical event in induction of apoptosis. Besides Bcl-2 family members, certain porin components such as VDAC, ANT, CyPD are also involved in the regulation of MOMP. Several agents including BH3-mimetics targeting MOMP are currently

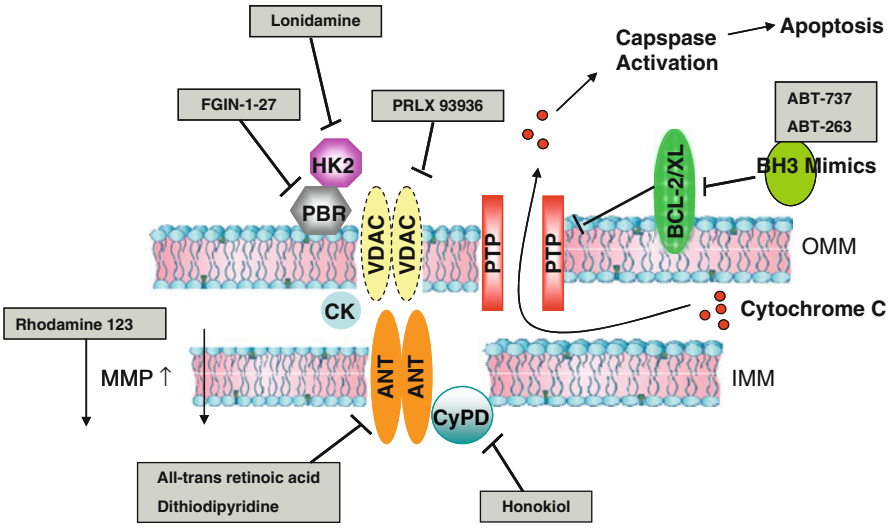

Fig. 8.3 Compounds targeting molecules involve in mitochondrial apoptosis. Compounds targeting apoptosis pathways in cancer cells are shown in *gray boxes. OMM* outer mitochondrial membrane, *IMM* inner mitochondrial membrane, *VDAC* voltage-dependent anion channel

under clinical trials as potential anticancer drugs. Another group of compounds that are selectively accumulate in the mitochondria of cancer cells due to elevated MMP also shows promising antitumor activity. Figure 8.3 illustrates compounds that induce apoptosis by targeting various mitochondrial apoptotic components in cancer cells.

3.3.1 Compounds Affecting Mitochondrial Membrane Permeability

ABT-737: This compound was developed through a rational nuclear magnetic resonance-based screening and structure-based design. As a BH3 mimic, ABT-737 binds to Bcl-2, Bcl-Xl and Bcl-W (but not Mcl-1), and shows potent anti-tumor effects as a single agent in a variety of cancer types including solid tumors and leukemia with high expression of Bcl-2 family members (Harrison et al. 2011; Bruce et al. 2012). And ABT-737 in combination with other chemotherapeutic agents such as Vorinostat showed strong synergic effects and significantly lowered the side effects (Konopleva et al. 2006; High et al. 2010).

PRLX 93936: A structural analogue of erastin, PRLX 93936 is a small molecule exhibiting promising anticancer activity in multiple tumor models. This compound shows positive activity against tumors with limited treatment options such as malignant melanoma. Mass spectrometry-based proteomic experiments showed that PRLX 93936 could bind to VDAC and induce rapid mitochondrial membrane polarization, affect ion flux, disturb cell cycle, and ultimately induce caspase-dependent

apoptosis. It is currently in clinical phase I/II trials for treatment of solid tumors (Sahasrabudhe et al. 2008).

3.3.2 Compounds Targeting Mitochondria Based on Altered Transmembrane Potential

Rhodamine-123: Due to its lipophilic and cationic properties, rhodamine-123 can readily cross the double mitochondrial membranes and accumulate in the mitochondrial matrix where the microenvironment is negatively charged (Hikita et al. 2010). Due to the fact that mitochondrial membrane potential is often elevated in cancer cells compared to normal cells, rhodamine-123 is preferentially taken up by malignant cells and accumulated in their mitochondria. This compound showed modest anticancer activity in animal tumor models, and this selective toxicity could be further potentiated by combination with 2-deoxyglucose, a glycolytic inhibitor (Lampidis et al. 1985). A phase I clinical trial of rhodamine-123 was carried in hormone refractory prostate cancer patients to determine the maximum tolerated dose (MTD) and its safety/toxicity profile (Herr et al. 1988). More recently, the mitochondrial targeting character of rhodamine-123 has also been utilized to facilitate therapeutic payload to the mitochondria by using rhodamine-123 conjugated polymers (Jones et al. 2005).

Table 8.1 shows a list of compounds under clinical development that directly or indirectly target mitochondria.

4 Summary

Mitochondria play important roles in regulating fundamental cellular functions. Dysfunction of mitochondria is involved in various physiological and pathological processes that are pivotal in tumorigenesis and malignance progression. As such, mitochondrial dysfunction may be regarded as a hallmark of cancer. Integrated with oncogenes and tumor suppressor deregulation, mitochondrial dysfunction may cause extensive metabolic reprogramming, elevated ROS stress and alterations in apoptosis pathways. The key pathophysiological differences between mitochondria in cancer cells and normal cells provide a biological basis for developing novel strategies to selectively target cancer cells. Compounds that target mitochondria (Mitocans) show promising anticancer activity and selectivity (Biswas et al. 2011). These therapeutic strategies and the relevant compounds provide a new possibility to improve cancer treatment outcomes, and investigation of the underlying mechanisms may gain new insights into mitochondrial biology and their role in cancer development. It is extremely important to identify Mitocans that can discriminate the difference between cancer and normal mitochondria. Combination of Mitocans with conventional drugs may further increase therapeutic activity and reduce toxic side effects. In addition, recent study suggests that an

Table 8.1 Clinical trial compounds target at mitochondria

Compound	Cellular target	Clinical status	References
Reagents targeting IAP proteins			
AEG35156	XIAP antisense	Phase I/II	Diehn et al. (2009)
GDC-0917; LCL-161	Monovalent IAP antagonist	Phase I	LaCasse et al. (2006)
BV6, Comp3, CompA	IAP antagonist	Preclinical	Vucic (2008), Fulda and Vucic (2012)
MV1, LBW242, CS3			Vince et al. (2007)
Reagents targeting mitochondrial metabolism			
3-BrOP	GAPDH, OXPHOS, HKII	Preclinical	Varfolomeev et al. (2007)
Lonidamine	HKII, Ant	Phase IV	Dell'Antone (2009)
Oxamate	LDH	Preclinical	Akers et al. (2011)
2-deoxyglucose	HKII	Phase I/II	Gorlach et al. (1995)
DCA	PDK	Phase I	Maher et al. (2004)
Reagents targeting mitochondria			
Honokiol	Mitochondrial permeability transition pore	Preclinical	McFate et al. (2008)
Betulinic acid	Mitochondrial permeability transition pore	Phase I/II	Chen et al. (2009a)
Rhodamine 123	Mitochondrial transmembrane potential	Phase I	Hsu et al. (2012)
MKT-077	mtDNA, mitochondrial transmembrane potential	Phase I	Jones et al. (2005)
2-ME	Cu–ZnSOD, MnSOD	Phase I/II	Britten et al. (2000)
Curcumin	Bax, Bcl2, Bcl-XL	Phase I/II	Harrison et al. (2011)
ABT-263	Bcl2 inhibitors	Phase I/II	Carroll et al. (2011)
ABT-737	Bcl2 inhibitors	Preclinical	Rudin et al. (2012)
Gossypol	Bcl-2, Bcl-XL	Phase I/II	Vaux (2008)
HA14-1	BcL-2	Preclinical	Van Poznak et al. (2001)
Obatoclax	Bcl-2, Bcl-XL	Phase I/II	Simonin et al. (2009)
As_2O_3	OXPHOS, Ant, VDAC	Phase IV	Parikh et al. (2010), Paik et al. (2011)
Bullatacin	OXPHOS	Preclinical	Powell et al. (2010) Li et al. (2012)
Antimycin A	OXPHOS	Preclinical	Holschneider et al. (1994)
5-Aminolevulinic acid	OXPHOS	Phase I	Wolvetang et al. (1994)
Resveratrol	OXPHOS	Phase I/II	Kashtan et al. (1999)
Rhodamine-123	OXPHOS	Phase I	Li et al. (2006)
α-TOS	OXPHOS, Bcl2, Bcl-XL	Preclinical	Lampidis et al. (1983)
MitoVES	OXPHOS,	Preclinical	Neuzil et al. (2007)
Doxorubicin	OXPHOS	Phase IV	Dong et al. (2011)
Tamoxifen	OXPHOS, antagonist of the estrogen receptor	Phase IV	Oliveira and Wallace (2006)
Photofrin	OXPHOS	Phase III	James et al. (2012)
Fenretinid	OXPHOS	Phase III	Wu et al. (2009)

(continued)

Table 8.1 (continued)

Compound	Cellular target	Clinical status	References
3,30-Diindolylmethane	OXPHOS	Preclinical	Villablanca et al. (2011)
Atpenins	OXPHOS	Preclinical	Yin et al. (2012)
Bz-423	OXPHOS	Phase II/III	Miyadera et al. (2003)
PEITCs	GSH, OXPHOS	Preclinical	Blatt et al. (2009)
BITC	GSH, OXPHOS	Preclinical	Chen et al. (2011)
all-*trans* retinoic acid	Ant	phase II	Antony et al. (2012)
Dithiodipyridine	Ant	Preclinical	Iland et al. (2012)
GSAO	Ant	Phase I	Lifson et al. (2004)
EGCG	Hsp70, Hsp90, p68	Preclinical	Elliott et al. (2012)
Ro5-4864	PBR	Preclinical	Shanafelt et al. (2009, 2013)
Menadione	Redox-cycling agent	Phase I/II	Lee et al. (2009)
Metformin	AMPK, OXPHOS	Phase I/II	Lim et al. (2005)
Vinblastine	DNA	Phase I/II	Esteva et al. (2012)
Etoposide	DNA	phase IV	Delord et al. (2012)
Motexafin gadolinium	Akt	Phase III	Wang et al. (2012)
TP187	OXPHOS	Preclinical	Edelman et al. (2011)
Phloretin	GLUT-2	Preclinical	Millard et al. (2010)
Troglitazone	PPARγ, OXPHOS, AMPK	Phase I/II	Wu et al. (2009)

appropriate level of ROS may be important for maintaining cancer cell stemness (Biasutto et al. 2010). Certain compounds such as 3-BrOP, BCNU, and arthenolide show unique ability to selectively eliminate cancer stem cells and provide a new possibility to eradicate tumors in vivo (Yuan et al. 2013). Overall, a comprehensive understanding of mitochondrial functions and their alterations in cancer cells will be essential to the development of mitochondrial targeting agents for effective cancer treatment.

References

Achanta G, Sasaki R, Feng L, Carew JS, Lu W, Pelicano H, Keating MJ, Huang P (2005) Novel role of p53 in maintaining mitochondrial genetic stability through interaction with DNA Pol gamma. EMBO J 24:3482–3492

Akers LJ, Fang W, Levy AG, Franklin AR, Huang P, Zweidler-McKay PA (2011) Targeting glycolysis in leukemia: a novel inhibitor 3-BrOP in combination with rapamycin. Leuk Res 35:814–820

Antony ML, Kim SH, Singh SV (2012) Critical role of p53 upregulated modulator of apoptosis in benzyl isothiocyanate-induced apoptotic cell death. PLoS One 7:e32267

Arbel N, Ben-Hail D, Shoshan-Barmatz V (2012) Mediation of the antiapoptotic activity of Bcl-xL protein upon interaction with VDAC1 protein. J Biol Chem 287(27):23152–23161

Arzoine L, Zilberberg N, Ben-Romano R, Shoshan-Barmatz V (2009) Voltage-dependent anion channel 1-based peptides interact with hexokinase to prevent its anti-apoptotic activity. J Biol Chem 284:3946–3955

Bae YS, Oh H, Rhee SG, Yoo YD (2011) Regulation of reactive oxygen species generation in cell signaling. Mol Cells 32:491–509

Bakhanashvili M, Grinberg S, Bonda E, Simon AJ, Moshitch-Moshkovitz S, Rahav G (2008) p53 in mitochondria enhances the accuracy of DNA synthesis. Cell Death Differ 15:1865–1874

Balinsky D, Platz CE, Lewis JW (1983) Isozyme patterns of normal, benign, and malignant human breast tissues. Cancer Res 43:5895–5901

Behrend L, Henderson G, Zwacka RM (2003) Reactive oxygen species in oncogenic transformation. Biochem Soc Trans 31:1441–1444

Bhaskar PT, Nogueira V, Patra KC, Jeon SM, Park Y, Robey RB, Hay N (2009) mTORC1 hyperactivity inhibits serum deprivation-induced apoptosis via increased hexokinase II and GLUT1 expression, sustained Mcl-1 expression, and glycogen synthase kinase 3beta inhibition. Mol Cell Biol 29:5136–5147

Biasutto L, Dong LF, Zoratti M, Neuzil J (2010) Mitochondrially targeted anti-cancer agents. Mitochondrion 10:670–681

Biswas S, Dodwadkar NS, Sawant RR, Koshkaryev A, Torchilin VP (2011) Surface modification of liposomes with rhodamine-123-conjugated polymer results in enhanced mitochondrial targeting. J Drug Target 19:552–561

Blatt NB, Boitano AE, Lyssiotis CA, Opipari AW Jr, Glick GD (2009) Bz-423 superoxide signals B cell apoptosis via Mcl-1, Bak, and Bax. Biochem Pharmacol 78:966–973

Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Harry G, Hashimoto K, Porter CJ, Andrade MA, Thebaud B, Michelakis ED (2007) A mitochondria-K+channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 11:37–51

Brandon M, Baldi P, Wallace DC (2006) Mitochondrial mutations in cancer. Oncogene 25:4647–4662

Britten CD, Rowinsky EK, Baker SD, Weiss GR, Smith L, Stephenson J, Rothenberg M, Smetzer L, Cramer J, Collins W, Von Hoff DD, Eckhardt SG (2000) A phase I and pharmacokinetic study of the mitochondrial-specific rhodacyanine dye analog MKT 077. Clin Cancer Res 6:42–49

Brown KK, Cox AG, Hampton MB (2010) Mitochondrial respiratory chain involvement in peroxiredoxin 3 oxidation by phenethyl isothiocyanate and auranofin. FEBS Lett 584:1257–1262

Bruce JY, Eickhoff J, Pili R, Logan T, Carducci M, Arnott J, Treston A, Wilding G, Liu G (2012) A phase II study of 2-methoxyestradiol nanocrystal colloidal dispersion alone and in combination with sunitinib malate in patients with metastatic renal cell carcinoma progressing on sunitinib malate. Invest New Drugs 30:794–802

Buzzai M, Bauer DE, Jones RG, Deberardinis RJ, Hatzivassiliou G, Elstrom RL, Thompson CB (2005) The glucose dependence of Akt-transformed cells can be reversed by pharmacologic activation of fatty acid beta-oxidation. Oncogene 24:4165–4173

Cao W, Yacoub S, Shiverick KT, Namiki K, Sakai Y, Porvasnik S, Urbanek C, Rosser CJ (2008) Dichloroacetate (DCA) sensitizes both wild-type and over expressing Bcl-2 prostate cancer cells in vitro to radiation. Prostate 68:1223–1231

Carroll RE, Benya RV, Turgeon DK, Vareed S, Neuman M, Rodriguez L, Kakarala M, Carpenter PM, McLaren C, Meyskens FL Jr, Brenner DE (2011) Phase IIa clinical trial of curcumin for the prevention of colorectal neoplasia. Cancer Prev Res (Phila) 4:354–364

Chen G, Izzo J, Demizu Y, Wang F, Guha S, Wu X, Hung MC, Ajani JA, Huang P (2009a) Different redox states in malignant and nonmalignant esophageal epithelial cells and differential cytotoxic responses to bile acid and honokiol. Antioxid Redox Signal 11:1083–1095

Chen Z, Zhang H, Lu W, Huang P (2009b) Role of mitochondria-associated hexokinase II in cancer cell death induced by 3-bromopyruvate. Biochim Biophys Acta 1787:553–560

Chen G, Wang F, Trachootham D, Huang P (2010) Preferential killing of cancer cells with mitochondrial dysfunction by natural compounds. Mitochondrion 10:614–625

Chen G, Chen Z, Hu Y, Huang P (2011) Inhibition of mitochondrial respiration and rapid depletion of mitochondrial glutathione by beta-phenethyl isothiocyanate: mechanisms for anti-leukemia activity. Antioxid Redox Signal 15:2911–2921

Chiaradonna F, Sacco E, Manzoni R, Giorgio M, Vanoni M, Alberghina L (2006) Ras-dependent carbon metabolism and transformation in mouse fibroblasts. Oncogene 25:5391–5404
Clerkin JS, Naughton R, Quiney C, Cotter TG (2008) Mechanisms of ROS modulated cell survival during carcinogenesis. Cancer Lett 266:30–36
Cui X (2012) Reactive oxygen species: the Achilles' heel of cancer cells? Antioxid Redox Signal 16:1212–1214
Dang CV (2012) MYC on the path to cancer. Cell 149:22–35
Dang CV, Li F, Lee LA (2005) Could MYC induction of mitochondrial biogenesis be linked to ROS production and genomic instability? Cell Cycle 4:1465–1466
Davids MS, Letai A (2012) Targeting the B-cell lymphoma/leukemia 2 family in cancer. J Clin Oncol 30:3127–3135
DeBerardinis RJ, Thompson CB (2012) Cellular metabolism and disease: what do metabolic outliers teach us? Cell 148:1132–1144
DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB (2008) The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab 7:11–20
Dell'Antone P (2009) Targets of 3-bromopyruvate, a new, energy depleting, anticancer agent. Med Chem 5:491–496
Delord JP, Bennouna J, Mourey L, Bougaret J, Brandely-Talbot M, Ferre P (2012) Vinflunine oral pharmacokinetics and absolute bioavailability of soft and hard gelatin capsules: results of two phase I trials. Clin Pharmacokinet 51:357–364
Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, Qian D, Lam JS, Ailles LE, Wong M, Joshua B, Kaplan MJ, Wapnir I, Dirbas FM, Somlo G, Garberoglio C, Paz B, Shen J, Lau SK, Quake SR, Brown JM, Weissman IL, Clarke MF (2009) Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 458:780–783
Djavaheri-Mergny M, Wietzerbin J, Besancon F (2003) 2-Methoxyestradiol induces apoptosis in Ewing sarcoma cells through mitochondrial hydrogen peroxide production. Oncogene 22:2558–2567
Dong LF, Jameson VJ, Tilly D, Cerny J, Mahdavian E, Marin-Hernandez A, Hernandez-Esquivel L, Rodriguez-Enriquez S, Stursa J, Witting PK, Stantic B, Rohlena J, Truksa J, Kluckova K, Dyason JC, Ledvina M, Salvatore BA, Moreno-Sanchez R, Coster MJ, Ralph SJ, Smith RA, Neuzil J (2011) Mitochondrial targeting of vitamin E succinate enhances its pro-apoptotic and anti-cancer activity via mitochondrial complex II. J Biol Chem 286:3717–3728
Dschietzig T, Bartsch C, Baumann G, Stangl K (2009) RXFP1-inactive relaxin activates human glucocorticoid receptor: further investigations into the relaxin-GR pathway. Regul Pept 154:77–84
Edelman MJ, Otterson G, Leach J, Malpass T, Salgia R, Jones D, Mody TD, Govindan R (2011) Multicenter phase II trial of Motexafin gadolinium and pemetrexed for second-line treatment in patients with non-small cell lung cancer. J Thorac Oncol 6:786–789
Elliott MA, Ford SJ, Prasad E, Dick LJ, Farmer H, Hogg PJ, Halbert GW (2012) Pharmaceutical development of the novel arsenical based cancer therapeutic GSAO for Phase I clinical trial. Int J Pharm 426:67–75
Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR, Zhuang H, Cinalli RM, Alavi A, Rudin CM, Thompson CB (2004) Akt stimulates aerobic glycolysis in cancer cells. Cancer Res 64:3892–3899
Esteva FJ, Moulder SL, Gonzalez-Angulo AM, Ensor J, Murray JL, Green MC, Koenig KB, Lee MH, Hortobagyi GN, Yeung SC (2013) Phase I trial of exemestane in combination with metformin and rosiglitazone in nondiabetic obese postmenopausal women with hormone receptor-positive metastatic breast cancer. Cancer Chemother Pharmacol 71(1):63–72
Estrela JM, Ortega A, Obrador E (2006) Glutathione in cancer biology and therapy. Crit Rev Clin Lab Sci 43:143–181
Ferrin G, Linares CI, Muntane J (2011) Mitochondrial drug targets in cell death and cancer. Curr Pharm Des 17:2002–2016
Fogg VC, Lanning NJ, Mackeigan JP (2011) Mitochondria in cancer: at the crossroads of life and death. Chin J Cancer 30:526–539

Fotopoulou C, Baumunk D, Schmidt SC, Schumacher G (2010) Additive growth inhibition after combined treatment of 2-methoxyestradiol and conventional chemotherapeutic agents in human pancreatic cancer cells. Anticancer Res 30:4619–4624

Fulda S, Vucic D (2012) Targeting IAP proteins for therapeutic intervention in cancer. Nat Rev Drug Discov 11:109–124

Gambhir SS (2002) Molecular imaging of cancer with positron emission tomography. Nat Rev Cancer 2:683–693

Ganapathy-Kanniappan S, Geschwind JF, Kunjithapatham R, Buijs M, Syed LH, Rao PP, Ota S, Kwak BK, Loffroy R, Vali M (2010a) 3-Bromopyruvate induces endoplasmic reticulum stress, overcomes autophagy and causes apoptosis in human HCC cell lines. Anticancer Res 30:923–935

Ganapathy-Kanniappan S, Vali M, Kunjithapatham R, Buijs M, Syed LH, Rao PP, Ota S, Kwak BK, Loffroy R, Geschwind JF (2010b) 3-bromopyruvate: a new targeted antiglycolytic agent and a promise for cancer therapy. Curr Pharm Biotechnol 11:510–517

Gatenby RA, Gillies RJ (2004) Why do cancers have high aerobic glycolysis? Nat Rev Cancer 4:891–899

Geschwind JF, Ko YH, Torbenson MS, Magee C, Pedersen PL (2002) Novel therapy for liver cancer: direct intraarterial injection of a potent inhibitor of ATP production. Cancer Res 62:3909–3913

Gimeno AL, Goldraij A, Gimeno MF, Santillan de Torres R (1976) Effects of oxamate, an inhibitor of lactate dahydrogenase, upon the spontaneous or oxytocin-induced motility and over pyruvate levels of uterine horns isolated from ovariectomized or estrus rats. Reproducción 3(1–2):5–14

Gogvadze V (2011) Targeting mitochondria in fighting cancer. Curr Pharm Des 17:4034–4046

Gogvadze V, Orrenius S, Zhivotovsky B (2006) Multiple pathways of cytochrome c release from mitochondria in apoptosis. Biochim Biophys Acta 1757:639–647

Gogvadze V, Orrenius S, Zhivotovsky B (2008) Mitochondria in cancer cells: what is so special about them? Trends Cell Biol 18:165–173

Gorlach A, Bolling B, Holtermann G, Schwachofer J, Carlsson J, Acker H (1995) Changes in growth, po(2) and ph after exposure to oxamate – studies of 2 human tumor-cell lines growing as multicellular spheroids. Int J Oncol 7:831–839

Gottlieb E, Tomlinson IP (2005) Mitochondrial tumour suppressors: a genetic and biochemical update. Nat Rev Cancer 5:857–866

Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100:57–70

Harrison MR, Hahn NM, Pili R, Oh WK, Hammers H, Sweeney C, Kim K, Perlman S, Arnott J, Sidor C, Wilding G, Liu G (2011) A phase II study of 2-methoxyestradiol (2ME2) NanoCrystal(R) dispersion (NCD) in patients with taxane-refractory, metastatic castrate-resistant prostate cancer (CRPC). Invest New Drugs 29:1465–1474

Heller A, Brockhoff G, Goepferich A (2012) Targeting drugs to mitochondria. Eur J Pharm Biopharm 82:1–18

Herr HW, Huffman JL, Huryk R, Heston WD, Melamed MR, Whitmore WF Jr (1988) Anticarcinoma activity of rhodamine 123 against a murine renal adenocarcinoma. Cancer Res 48:2061–2063

High LM, Szymanska B, Wilczynska-Kalak U, Barber N, O'Brien R, Khaw SL, Vikstrom IB, Roberts AW, Lock RB (2010) The Bcl-2 homology domain 3 mimetic ABT-737 targets the apoptotic machinery in acute lymphoblastic leukemia resulting in synergistic in vitro and in vivo interactions with established drugs. Mol Pharmacol 77:483–494

Hikita H, Takehara T, Shimizu S, Kodama T, Shigekawa M, Iwase K, Hosui A, Miyagi T, Tatsumi T, Ishida H, Li W, Kanto T, Hiramatsu N, Hayashi N (2010) The Bcl-xL inhibitor, ABT-737, efficiently induces apoptosis and suppresses growth of hepatoma cells in combination with sorafenib. Hepatology 52:1310–1321

Holschneider CH, Johnson MT, Knox RM, Rezai A, Ryan WJ, Montz FJ (1994) Bullatacin–in vivo and in vitro experience in an ovarian cancer model. Cancer Chemother Pharmacol 34:166–170

Hsu TI, Wang MC, Chen SY, Huang ST, Yeh YM, Su WC, Chang WC, Hung JJ (2012) Betulinic Acid Decreases Specificity Protein 1 (Sp1) Level via Increasing the Sumoylation of Sp1 to Inhibit Lung Cancer Growth. Mol Pharmacol 82:1115–1128

Ihrlund LS, Hernlund E, Khan O, Shoshan MC (2008) 3-Bromopyruvate as inhibitor of tumour cell energy metabolism and chemopotentiator of platinum drugs. Mol Oncol 2:94–101

Iland HJ, Bradstock K, Supple SG, Catalano A, Collins M, Hertzberg M, Browett P, Grigg A, Firkin F, Hugman A, Reynolds J, Di Iulio J, Tiley C, Taylor K, Filshie R, Seldon M, Taper J, Szer J, Moore J, Bashford J, Seymour JF (2012) All-trans-retinoic acid, idarubicin, and IV arsenic trioxide as initial therapy in acute promyelocytic leukemia (APML4). Blood 120:1570–1580, quiz 1752

Irwin ME, Rivera-Del Valle N, Chandra J (2013) Redox control of leukemia: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal 18(11):1349–1383

Isaev NK, Stelmashook EV, Dirnagl U, Plotnikov EY, Kuvshinova EA, Zorov DB (2008) Mitochondrial free radical production induced by glucose deprivation in cerebellar granule neurons. Biochemistry (Mosc) 73:149–155

James R, Ahmed F, Cunnick G (2012) The efficacy of tamoxifen in the treatment of primary gynecomastia: an observational study of tamoxifen versus observation alone. Breast J 18:620–621

Janssen AM, Bosman CB, Sier CF, Griffioen G, Kubben FJ, Lamers CB, van Krieken JH, van de Velde CJ, Verspaget HW (1998) Superoxide dismutases in relation to the overall survival of colorectal cancer patients. Br J Cancer 78:1051–1057

Janssen-Heininger YM, Mossman BT, Heintz NH, Forman HJ, Kalyanaraman B, Finkel T, Stamler JS, Rhee SG, van der Vliet A (2008) Redox-based regulation of signal transduction: principles, pitfalls, and promises. Free Radic Biol Med 45:1–17

Jones LW, Narayan KS, Shapiro CE, Sweatman TW (2005) Rhodamine-123: therapy for hormone refractory prostate cancer, a phase I clinical trial. J Chemother 17:435–440

Kashtan H, Konikoff F, Haddad R, Skornick Y (1999) Photodynamic therapy of cancer of the esophagus using systemic aminolevulinic acid and a non laser light source: a phase I/II study. Gastrointest Endosc 49:760–764

Kc S, Carcamo JM, Golde DW (2005) Vitamin C enters mitochondria via facilitative glucose transporter 1 (Glut1) and confers mitochondrial protection against oxidative injury. FASEB J 19:1657–1667

Konopleva M, Contractor R, Tsao T, Samudio I, Ruvolo PP, Kitada S, Deng X, Zhai D, Shi YX, Sneed T, Verhaegen M, Soengas M, Ruvolo VR, McQueen T, Schober WD, Watt JC, Jiffar T, Ling X, Marini FC, Harris D, Dietrich M, Estrov Z, McCubrey J, May WS, Reed JC, Andreeff M (2006) Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell 10:375–388

Koppenol WH, Bounds PL, Dang CV (2011) Otto Warburg's contributions to current concepts of cancer metabolism. Nat Rev Cancer 11:325–337

Kroemer G, Pouyssegur J (2008) Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell 13:472–482

Kroemer G, Galluzzi L, Brenner C (2007) Mitochondrial membrane permeabilization in cell death. Physiol Rev 87:99–163

Kumar B, Koul S, Khandrika L, Meacham RB, Koul HK (2008) Oxidative stress is inherent in prostate cancer cells and is required for aggressive phenotype. Cancer Res 68:1777–1785

LaCasse EC, Cherton-Horvat GG, Hewitt KE, Jerome LJ, Morris SJ, Kandimalla ER, Yu D, Wang H, Wang W, Zhang R, Agrawal S, Gillard JW, Durkin JP (2006) Preclinical characterization of AEG35156/GEM 640, a second-generation antisense oligonucleotide targeting X-linked inhibitor of apoptosis. Clin Cancer Res 12:5231–5241

Lampidis TJ, Bernal SD, Summerhayes IC, Chen LB (1983) Selective toxicity of rhodamine 123 in carcinoma cells in vitro. Cancer Res 43:716–720

Lampidis TJ, Munck JN, Krishan A, Tapiero H (1985) Reversal of resistance to rhodamine 123 in adriamycin-resistant Friend leukemia cells. Cancer Res 45:2626–2631

Lee SW, Lee JT, Lee MG, Lee HW, Ahn SJ, Lee YJ, Lee YL, Yoo J, Ahn BC, Ha JH (2009) In vitro antiproliferative characteristics of flavonoids and diazepam on SNU-C4 colorectal adenocarcinoma cells. J Nat Med 63:124–129

Lengfelder E, Hofmann WK, Nowak D (2012) Impact of arsenic trioxide in the treatment of acute promyelocytic leukemia. Leukemia 26:433–442

Lewis BC, Prescott JE, Campbell SE, Shim H, Orlowski RZ, Dang CV (2000) Tumor induction by the c-Myc target genes rcl and lactate dehydrogenase A. Cancer Res 60:6178–6183
Li Y, Liu J, Liu X, Xing K, Wang Y, Li F, Yao L (2006) Resveratrol-induced cell inhibition of growth and apoptosis in MCF7 human breast cancer cells are associated with modulation of phosphorylated Akt and caspase-9. Appl Biochem Biotechnol 135:181–192
Li N, Song Y, Zhou J, Fang B (2012) Arsenic trioxide improves hematopoiesis in refractory severe aplastic anemia. J Hematol Oncol 5:61
Lifson JD, Rossio JL, Piatak M Jr, Bess J Jr, Chertova E, Schneider DK, Coalter VJ, Poore B, Kiser RF, Imming RJ, Scarzello AJ, Henderson LE, Alvord WG, Hirsch VM, Benveniste RE, Arthur LO (2004) Evaluation of the safety, immunogenicity, and protective efficacy of whole inactivated simian immunodeficiency virus (SIV) vaccines with conformationally and functionally intact envelope glycoproteins. AIDS Res Hum Retroviruses 20:772–787
Lim D, Morgan RJ Jr, Akman S, Margolin K, Carr BI, Leong L, Odujinrin O, Doroshow JH (2005) Phase I trial of menadiol diphosphate (vitamin K3) in advanced malignancy. Invest New Drugs 23:235–239
Low IC, Kang J, Pervaiz S (2011) Bcl-2: a prime regulator of mitochondrial redox metabolism in cancer cells. Antioxid Redox Signal 15:2975–2987
Madan E, Gogna R, Bhatt M, Pati U, Kuppusamy P, Mahdi AA (2011) Regulation of glucose metabolism by p53: emerging new roles for the tumor suppressor. Oncotarget 2:948–957
Maddocks OD, Vousden KH (2011) Metabolic regulation by p53. J Mol Med (Berl) 89:237–245
Maher JC, Krishan A, Lampidis TJ (2004) Greater cell cycle inhibition and cytotoxicity induced by 2-deoxy-D-glucose in tumor cells treated under hypoxic vs aerobic conditions. Cancer Chemother Pharmacol 53:116–122
Martens GA, Cai Y, Hinke S, Stange G, Van de Casteele M, Pipeleers D (2005) Glucose suppresses superoxide generation in metabolically responsive pancreatic beta cells. J Biol Chem 280:20389–20396
Mathupala SP, Ko YH, Pedersen PL (2010) The pivotal roles of mitochondria in cancer: Warburg and beyond and encouraging prospects for effective therapies. Biochim Biophys Acta 1797:1225–1230
McCommis KS, Baines CP (2012) The role of VDAC in cell death: friend or foe? Biochim Biophys Acta 1818:1444–1450
McFate T, Mohyeldin A, Lu H, Thakar J, Henriques J, Halim ND, Wu H, Schell MJ, Tsang TM, Teahan O, Zhou S, Califano JA, Jeoung NH, Harris RA, Verma A (2008) Pyruvate dehydrogenase complex activity controls metabolic and malignant phenotype in cancer cells. J Biol Chem 283:22700–22708
Michelakis ED, Webster L, Mackey JR (2008) Dichloroacetate (DCA) as a potential metabolic-targeting therapy for cancer. Br J Cancer 99:989–994
Millard M, Pathania D, Shabaik Y, Taheri L, Deng J, Neamati N (2010) Preclinical evaluation of novel triphenylphosphonium salts with broad-spectrum activity. PLoS One 5
Miyadera H, Shiomi K, Ui H, Yamaguchi Y, Masuma R, Tomoda H, Miyoshi H, Osanai A, Kita K, Omura S (2003) Atpenins, potent and specific inhibitors of mitochondrial complex II (succinate-ubiquinone oxidoreductase). Proc Natl Acad Sci U S A 100:473–477
Moiseeva O, Bourdeau V, Roux A, Deschenes-Simard X, Ferbeyre G (2009) Mitochondrial dysfunction contributes to oncogene-induced senescence. Mol Cell Biol 29:4495–4507
Moreno-Sanchez R, Saavedra E, Rodriguez-Enriquez S, Gallardo-Perez JC, Quezada H, Westerhoff HV (2010) Metabolic control analysis indicates a change of strategy in the treatment of cancer. Mitochondrion 10:626–639
Morrish F, Neretti N, Sedivy JM, Hockenbery DM (2008) The oncogene c-Myc coordinates regulation of metabolic networks to enable rapid cell cycle entry. Cell Cycle 7:1054–1066
Neuzil J, Dyason JC, Freeman R, Dong LF, Prochazka L, Wang XF, Scheffler I, Ralph SJ (2007) Mitocans as anti-cancer agents targeting mitochondria: lessons from studies with vitamin E analogues, inhibitors of complex II. J Bioenerg Biomembr 39:65–72
Oliveira PJ, Wallace KB (2006) Depletion of adenine nucleotide translocator protein in heart mitochondria from doxorubicin-treated rats–relevance for mitochondrial dysfunction. Toxicology 220:160–168

Ott M, Gogvadze V, Orrenius S, Zhivotovsky B (2007) Mitochondria, oxidative stress and cell death. Apoptosis 12:913–922

Paik PK, Rudin CM, Pietanza MC, Brown A, Rizvi NA, Takebe N, Travis W, James L, Ginsberg MS, Juergens R, Markus S, Tyson L, Subzwari S, Kris MG, Krug LM (2011) A phase II study of obatoclax mesylate, a Bcl-2 antagonist, plus topotecan in relapsed small cell lung cancer. Lung Cancer 74:481–485

Pani G, Giannoni E, Galeotti T, Chiarugi P (2009) Redox-based escape mechanism from death: the cancer lesson. Antioxid Redox Signal 11:2791–2806

Parikh SA, Kantarjian H, Schimmer A, Walsh W, Asatiani E, El-Shami K, Winton E, Verstovsek S (2010) Phase II study of obatoclax mesylate (GX15-070), a small-molecule BCL-2 family antagonist, for patients with myelofibrosis. Clin Lymphoma Myeloma Leuk 10:285–289

Patel S, Chiplunkar S (2007) Role of cyclooxygenase-2 in tumor progression and immune regulation in lung cancer. Indian J Biochem Biophys 44:419–428

Pathania D, Millard M, Neamati N (2009) Opportunities in discovery and delivery of anticancer drugs targeting mitochondria and cancer cell metabolism. Adv Drug Deliv Rev 61:1250–1275

Pedersen PL, Mathupala S, Rempel A, Geschwind JF, Ko YH (2002) Mitochondrial bound type II hexokinase: a key player in the growth and survival of many cancers and an ideal prospect for therapeutic intervention. Biochim Biophys Acta 1555:14–20

Pelicano H, Feng L, Zhou Y, Carew JS, Hileman EO, Plunkett W, Keating MJ, Huang P (2003) Inhibition of mitochondrial respiration: a novel strategy to enhance drug-induced apoptosis in human leukemia cells by a reactive oxygen species-mediated mechanism. J Biol Chem 278:37832–37839

Pelicano H, Martin DS, Xu RH, Huang P (2006) Glycolysis inhibition for anticancer treatment. Oncogene 25:4633–4646

Pervaiz S, Clement MV (2004) Tumor intracellular redox status and drug resistance–serendipity or a causal relationship? Curr Pharm Des 10:1969–1977

Plymate SR, Haugk KH, Sprenger CC, Nelson PS, Tennant MK, Zhang Y, Oberley LW, Zhong W, Drivdahl R, Oberley TD (2003) Increased manganese superoxide dismutase (SOD-2) is part of the mechanism for prostate tumor suppression by Mac25/insulin-like growth factor binding-protein-related protein-1. Oncogene 22:1024–1034

Powell BL, Moser B, Stock W, Gallagher RE, Willman CL, Stone RM, Rowe JM, Coutre S, Feusner JH, Gregory J, Couban S, Appelbaum FR, Tallman MS, Larson RA (2010) Arsenic trioxide improves event-free and overall survival for adults with acute promyelocytic leukemia: North American Leukemia Intergroup Study C9710. Blood 116:3751–3757

Radisky DC (2005) Epithelial-mesenchymal transition. J Cell Sci 118:4325–4326

Ralph SJ, Rodriguez-Enriquez S, Neuzil J, Saavedra E, Moreno-Sanchez R (2010) The causes of cancer revisited: "mitochondrial malignancy" and ROS-induced oncogenic transformation – why mitochondria are targets for cancer therapy. Mol Aspects Med 31:145–170

Ramanathan A, Wang C, Schreiber SL (2005) Perturbational profiling of a cell-line model of tumorigenesis by using metabolic measurements. Proc Natl Acad Sci U S A 102:5992–5997

Ray PD, Huang BW, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24:981–990

Robey IF, Lien AD, Welsh SJ, Baggett BK, Gillies RJ (2005) Hypoxia-inducible factor-1alpha and the glycolytic phenotype in tumors. Neoplasia 7:324–330

Romero-Garcia S, Lopez-Gonzalez JS, Baez-Viveros JL, Aguilar-Cazares D, Prado-Garcia H (2011) Tumor cell metabolism: an integral view. Cancer Biol Ther 12:939–948

Rudin CM, Hann CL, Garon EB, Ribeiro de Oliveira M, Bonomi PD, Camidge DR, Chu Q, Giaccone G, Khaira D, Ramalingam SS, Ranson MR, Dive C, McKeegan EM, Chyla BJ, Dowell BL, Chakravartty A, Nolan CE, Rudersdorf N, Busman TA, Mabry MH, Krivoshik AP, Humerickhouse RA, Shapiro GI, Gandhi L (2012) Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. Clin Cancer Res 18:3163–3169

Sahasrabudhe SR, Lai S, Pierce M, Clemens C, Venkat R, Rebentisch M, Senina A, Becklin R, Richards SK, Erkkila T, Robbins PB (2008) Selective in vitro and in vivo anti-tumor activity of

PRLX 93936 in biological models of melanoma and ovarian cancer. J Clin Oncol ASCO Annu Meeting Proc (Post Meeting Ed) 26:14586
Samper E, Morgado L, Estrada JC, Bernad A, Hubbard A, Cadenas S, Melov S (2009) Increase in mitochondrial biogenesis, oxidative stress, and glycolysis in murine lymphomas. Free Radic Biol Med 46:387–396
Sanchez-Arago M, Cuezva JM (2011) The bioenergetic signature of isogenic colon cancer cells predicts the cell death response to treatment with 3-bromopyruvate, iodoacetate or 5-fluorouracil. J Transl Med 9:19
Santidrian AF, Cosialls AM, Coll-Mulet L, Iglesias-Serret D, de Frias M, Gonzalez-Girones DM, Campas C, Domingo A, Pons G, Gil J (2007) The potential anticancer agent PK11195 induces apoptosis irrespective of p53 and ATM status in chronic lymphocytic leukemia cells. Haematologica 92:1631–1638
Sarkisian CJ, Keister BA, Stairs DB, Boxer RB, Moody SE, Chodosh LA (2007) Dose-dependent oncogene-induced senescence in vivo and its evasion during mammary tumorigenesis. Nat Cell Biol 9:493–505
Schwartzenberg-Bar-Yoseph F, Armoni M, Karnieli E (2004) The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. Cancer Res 64:2627–2633
Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P, Giallongo A (1996) Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. J Biol Chem 271:32529–32537
Shanafelt TD, Call TG, Zent CS, LaPlant B, Bowen DA, Roos M, Secreto CR, Ghosh AK, Kabat BF, Lee MJ, Yang CS, Jelinek DF, Erlichman C, Kay NE (2009) Phase I trial of daily oral Polyphenon E in patients with asymptomatic Rai stage 0 to II chronic lymphocytic leukemia. J Clin Oncol 27:3808–3814
Shanafelt TD, Call TG, Zent CS, Leis JF, Laplant B, Bowen DA, Roos M, Laumann K, Ghosh AK, Lesnick C, Lee MJ, Yang CS, Jelinek DF, Erlichman C, Kay NE (2013) Phase 2 trial of daily, oral polyphenon E in patients with asymptomatic, Rai stage 0 to II chronic lymphocytic leukemia. Cancer 119(2):363–370
Shen L, Sun X, Fu Z, Yang G, Li J, Yao L (2012) The fundamental role of the p53 pathway in tumor metabolism and its implication in tumor therapy. Clin Cancer Res 18:1561–1567
Shoshan-Barmatz V, Zakar M, Rosenthal K, Abu-Hamad S (2009) Key regions of VDAC1 functioning in apoptosis induction and regulation by hexokinase. Biochim Biophys Acta 1787:421–430
Shoshan-Barmatz V, De Pinto V, Zweckstetter M, Raviv Z, Keinan N, Arbel N (2010) VDAC, a multi-functional mitochondrial protein regulating cell life and death. Mol Aspects Med 31:227–285
Simonin K, Brotin E, Dufort S, Dutoit S, Goux D, N'Diaye M, Denoyelle C, Gauduchon P, Poulain L (2009) Mcl-1 is an important determinant of the apoptotic response to the BH3-mimetic molecule HA14-1 in cisplatin-resistant ovarian carcinoma cells. Mol Cancer Ther 8:3162–3170
Smith DG, Magwere T, Burchill SA (2011) Oxidative stress and therapeutic opportunities: focus on the Ewing's sarcoma family of tumors. Expert Rev Anticancer Ther 11:229–249
Smith RA, Hartley RC, Cocheme HM, Murphy MP (2012) Mitochondrial pharmacology. Trends Pharmacol Sci 33:341–352
Stacpoole PW, Kurtz TL, Han Z, Langaee T (2008) Role of dichloroacetate in the treatment of genetic mitochondrial diseases. Adv Drug Deliv Rev 60:1478–1487
Steinberg P, Klingelhoffer A, Schafer A, Wust G, Weisse G, Oesch F, Eigenbrodt E (1999) Expression of pyruvate kinase M2 in preneoplastic hepatic foci of N-nitrosomorpholine-treated rats. Virchows Arch 434:213–220
Storz P (2005) Reactive oxygen species in tumor progression. Front Biosci 10:1881–1896
Straten P, Andersen MH (2010) The anti-apoptotic members of the Bcl-2 family are attractive tumor-associated antigens. Oncotarget 1:239–245
Sullivan R, Pare GC, Frederiksen LJ, Semenza GL, Graham CH (2008) Hypoxia-induced resistance to anticancer drugs is associated with decreased senescence and requires hypoxia-inducible factor-1 activity. Mol Cancer Ther 7:1961–1973

Suzuki S, Tanaka T, Poyurovsky MV, Nagano H, Mayama T, Ohkubo S, Lokshin M, Hosokawa H, Nakayama T, Suzuki Y, Sugano S, Sato E, Nagao T, Yokote K, Tatsuno I, Prives C (2010) Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species. Proc Natl Acad Sci U S A 107:7461–7466

Szatrowski TP, Nathan CF (1991) Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res 51:794–798

Tajeddine N, Galluzzi L, Kepp O, Hangen E, Morselli E, Senovilla L, Araujo N, Pinna G, Larochette N, Zamzami N, Modjtahedi N, Harel-Bellan A, Kroemer G (2008) Hierarchical involvement of Bak, VDAC1 and Bax in cisplatin-induced cell death. Oncogene 27:4221–4232

Tejeda M, Gaal D, Hullan L, Schwab R, Szokoloczi O, Keri G (2007) Antitumor activity of the somatostatin structural derivative (TT-232), against mouse and human melanoma tumor models. Anticancer Res 27:4015–4019

Thornburg JM, Nelson KK, Clem BF, Lane AN, Arumugam S, Simmons A, Eaton JW, Telang S, Chesney J (2008) Targeting aspartate aminotransferase in breast cancer. Breast Cancer Res 10:R84

Trachootham D, Zhou Y, Zhang H, Demizu Y, Chen Z, Pelicano H, Chiao PJ, Achanta G, Arlinghaus RB, Liu J, Huang P (2006) Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. Cancer Cell 10:241–252

Trachootham D, Lu W, Ogasawara MA, Nilsa RD, Huang P (2008a) Redox regulation of cell survival. Antioxid Redox Signal 10:1343–1374

Trachootham D, Zhang H, Zhang W, Feng L, Du M, Zhou Y, Chen Z, Pelicano H, Plunkett W, Wierda WG, Keating MJ, Huang P (2008b) Effective elimination of fludarabine-resistant CLL cells by PEITC through a redox-mediated mechanism. Blood 112:1912–1922

Trachootham D, Alexandre J, Huang P (2009) Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov 8:579–591

Van Poznak C, Seidman AD, Reidenberg MM, Moasser MM, Sklarin N, Van Zee K, Borgen P, Gollub M, Bacotti D, Yao TJ, Bloch R, Ligueros M, Sonenberg M, Norton L, Hudis C (2001) Oral gossypol in the treatment of patients with refractory metastatic breast cancer: a phase I/II clinical trial. Breast Cancer Res Treat 66:239–248

Varfolomeev E, Blankenship JW, Wayson SM, Fedorova AV, Kayagaki N, Garg P, Zobel K, Dynek JN, Elliott LO, Wallweber HJ, Flygare JA, Fairbrother WJ, Deshayes K, Dixit VM, Vucic D (2007) IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. Cell 131:669–681

Vaux DL (2008) ABT-737, proving to be a great tool even before it is proven in the clinic. Cell Death Differ 15:807–808

Villablanca JG, London WB, Naranjo A, McGrady P, Ames MM, Reid JM, McGovern RM, Buhrow SA, Jackson H, Stranzinger E, Kitchen BJ, Sondel PM, Parisi MT, Shulkin B, Yanik GA, Cohn SL, Reynolds CP (2011) Phase II study of oral capsular 4-hydroxyphenylretinamide (4-HPR/fenretinide) in pediatric patients with refractory or recurrent neuroblastoma: a report from the Children's Oncology Group. Clin Cancer Res 17:6858–6866

Vince JE, Wong WW, Khan N, Feltham R, Chau D, Ahmed AU, Benetatos CA, Chunduru SK, Condon SM, McKinlay M, Brink R, Leverkus M, Tergaonkar V, Schneider P, Callus BA, Koentgen F, Vaux DL, Silke J (2007) IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. Cell 131:682–693

Vucic D (2008) Targeting IAP (inhibitor of apoptosis) proteins for therapeutic intervention in tumors. Curr Cancer Drug Targets 8:110–117

Wallace DC, Fan W (2010) Energetics, epigenetics, mitochondrial genetics. Mitochondrion 10:12–31

Wang HC, Choudhary S (2011) Reactive oxygen species-mediated therapeutic control of bladder cancer. Nat Rev Urol 8:608–616

Wang L, Wu S, Ou G, Bi N, Li W, Ren H, Cao J, Liang J, Li J, Zhou Z, Lv J, Zhang X (2012) Randomized phase II study of concurrent cisplatin/etoposide or paclitaxel/carboplatin and thoracic radiotherapy in patients with stage III non-small cell lung cancer. Lung Cancer 77:89–96

Warburg O (1956) On the origin of cancer cells. Science 123:309–314
Ward PS, Thompson CB (2012) Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. Cancer Cell 21:297–308
Weinberg F, Chandel NS (2009) Mitochondrial metabolism and cancer. Ann N Y Acad Sci 1177:66–73
Wenner CE (2012) Targeting mitochondria as a therapeutic target in cancer. J Cell Physiol 227:450–456
Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB, Thompson CB (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci U S A 105:18782–18787
Wolvetang EJ, Johnson KL, Krauer K, Ralph SJ, Linnane AW (1994) Mitochondrial respiratory chain inhibitors induce apoptosis. FEBS Lett 339:40–44
Wu CH, Ho YS, Tsai CY, Wang YJ, Tseng H, Wei PL, Lee CH, Liu RS, Lin SY (2009) In vitro and in vivo study of phloretin-induced apoptosis in human liver cancer cells involving inhibition of type II glucose transporter. Int J Cancer 124:2210–2219
Yin XF, Chen J, Mao W, Wang YH, Chen MH (2012) A selective aryl hydrocarbon receptor modulator 3,3′-Diindolylmethane inhibits gastric cancer cell growth. J Exp Clin Cancer Res 31:46
Yuan S, Wang F, Chen G, Zhang H, Feng L, Wang L, Colman H, Keating MJ, Li X, Xu RH, Wang J, Huang P (2013) Effective elimination of cancer stem cells by a novel drug combination strategy. Stem Cells 31:23–34
Yun J, Rago C, Cheong I, Pagliarini R, Angenendt P, Rajagopalan H, Schmidt K, Willson JK, Markowitz S, Zhou S, Diaz LA Jr, Velculescu VE, Lengauer C, Kinzler KW, Vogelstein B, Papadopoulos N (2009) Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells. Science 325:1555–1559
Zhang H, Trachootham D, Lu W, Carew J, Giles FJ, Keating MJ, Arlinghaus RB, Huang P (2008) Effective killing of Gleevec-resistant CML cells with T315I mutation by a natural compound PEITC through redox-mediated mechanism. Leukemia 22:1191–1199
Zhang XD, Qin ZH, Wang J (2010) The role of p53 in cell metabolism. Acta Pharmacol Sin 31:1208–1212
Zhao Y, Liu Q, Wang X, Zhou L, Wang Q, Zhang Y (2011) Surface display of Aeromonas hydrophila GAPDH in attenuated Vibrio anguillarum to develop a Noval multivalent vector vaccine. Mar Biotechnol (NY) 13:963–970
Zheng Y, Shi Y, Tian C, Jiang C, Jin H, Chen J, Almasan A, Tang H, Chen Q (2004) Essential role of the voltage-dependent anion channel (VDAC) in mitochondrial permeability transition pore opening and cytochrome c release induced by arsenic trioxide. Oncogene 23:1239–1247
Zhou J, Du Y (2012) Acquisition of resistance of pancreatic cancer cells to 2-methoxyestradiol is associated with the upregulation of manganese superoxide dismutase. Mol Cancer Res 10:768–777
Zhou M, Zhao Y, Ding Y, Liu H, Liu Z, Fodstad O, Riker AI, Kamarajugadda S, Lu J, Owen LB, Ledoux SP, Tan M (2010) Warburg effect in chemosensitivity: targeting lactate dehydrogenase-A re-sensitizes taxol-resistant cancer cells to taxol. Mol Cancer 9:33

Chapter 9
Targets and Strategies for the Mitochondrial Assault on Cancer

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Contents

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Abstract Mitochondria-directed anti-cancer agents ("mitocans") represent a novel approach to cancer therapy. To optimize their effectiveness researchers need to identify appropriate targets and mechanisms of action, and to adopt a molecular design and/or delivery strategy ensuring that the drugs act at the mitochondrial level. Here we review: (i) the major options available for mitochondrial targeting: so far most studies have taken advantage of the high mitochondrial transmembrane potential; (ii) one set of possible targets: membrane channels exploited as transducers of a death signal; (iii) the induction of redox stress at mitochondria as a promising approach to cancerous cell destruction.

Keywords Mitocans • Mitochondrial ion channels • Mitochondria-targeting • Apoptosis • Necrosis • VDAC • Kv1.3 • Permeability transition pore • Ion homeostasis • Oxidative stress • ROS • Triphenylphosphonium • Polyphenols • Plant antioxidants

1 Introduction

Mitochondria are emerging as a target of chemopreventive or chemotherapeutic intervention largely because of the sheer complexity of cancer. Estimates of independent mutations in tumour cells range from a minimum of 4–7 (Balmain et al. 1993) up to several thousands (Stoler et al. 1999). They lead to the dysregulation of hundred of genes and of multiple interacting signalling pathways. The mutational profile of what is clinically the same cancer may differ from an individual to the next, depending on the genetic background and on gene-environment interactions, so that only about 20 % of patients, on average, responds optimally to any given chemotherapeutic treatment. Despite this complexity, much effort continues to be devoted to targeted therapy approaches, whereby a single component of the intricate web is chosen as the bull's eye. Despite the effort put into target validation this approach has so far generally failed to deliver efficacious and affordable therapies. Even targeting whole processes, rather than single molecules, may not be enough. In an important example, repressing angiogenesis may be counterproductive because it may lead to enhanced selection by hypoxia of cells with genetic characteristics unfavourable for the patient (Voss et al. 2010).

One can think of two ways to improve this situation. One is "personalized medicine": determining the exact genetic and epigenetic characteristics of any individual tumour, and developing a taylor-made suite of treatments. The other is to focus on a target which is present and relatively invariant in all cells, but has assumed a peculiar role in cancer cell physiology, or else can be manipulated so as to convey a cytotoxic effect specifically to cancer cells (Ralph and Neuzil 2009). The Readers of this book already know that this target is the mitochondrion. In this chapter we briefly discuss some approaches to specific pharmacological intervention on mitochondria, and then focus on two promising strategies we address in our research: the exploitation of mitochondrial channels to obtain outer and/or inner membrane permeabilization and the induction of mitochondrial oxidative stress. In both cases the goal is the selective demise of cancerous cells.

2 Mitochondrial Targeting

Anti-cancer agents that induce cell death via mitochondria are termed "mitocans", and can be classified into subfamilies depending on their specific target (Neuzil et al. 2013). Directing the active moieties to mitochondria would be expected, by definition, to improve their effectiveness, as exemplified by mitoVES, a mitochondria-targeted vitamin E analog (Dong et al. 2011a, b; Rodríguez-Enríquez et al. 2012). Several strategies have been devised to achieve "mitochondriotropicity" (Biasutto et al. 2010; Smith et al. 2011, 2012). The most widely used is probably that of linking the "cargo" to a membrane-permeant lipophilic cation, often triphenylphosphonium (TPP), which will cause the construct to accumulate in compartments held at more negative electrical potentials, such as the mitochondrial matrix (Murphy and Smith 2007; Hoye et al. 2008; Smith et al. 2012). This strategy, originally developed by V.P. Skulachev, has been used by his group to convey plastoquinones to mitochondria both *in vitro* and *in vivo*. Used at low concentrations, these compounds have had signal success as antioxidants in a number of pathology models (Skulachev et al. 2009, 2011). Berberine and palmatine, penetrating cations of plant origin, have recently been used instead of TPP (Lyamzaev et al. 2011; Chernyak et al. 2013). Positive results have been obtained also by the group of M.P. Murphy and R.A. Smith, who have adopted an analogous approach (Smith and Murphy 2010; Smith et al. 2011, 2012).

Charged peptides can also be used either to affect mitochondria directly or to "ship" molecules to mitochondria, and may be more versatile (Szeto and Schiller 2011; Yousif et al. 2009a, b; Kelley et al. 2011). TPP groups can be bound to pro-apoptotic cell-penetrating peptides, increasing their effectiveness (Abu-Gosh et al. 2009; Kolevzon et al. 2011; Kelley et al. 2011). Peptides of viral origin (Brabant et al. 2009; Silic-Benussi et al. 2009; Lecoeur et al. 2012) may be particularly promising as anti-cancer agents, in part because they may induce the mitochondrial permeability transition (MPT) and thereby cause cell death (Deniaud et al. 2006). Some of these approaches have already been tested in vivo and offer considerable promise (reviewed in Smith et al. 2012).

Weak acids are also expected to accumulate into mitochondria, although at lower in/out ratios than permeant cations, because of the basic-inside ΔpH maintained across the inner mitochondrial membrane (IMM). While these strategies are based on extensive physico-chemical properties of the mitochondria, other approaches may take advantage of specific transport pathways to promote irreversible uptake of suitable constructs. In a pioneering study carried out more than 20 years ago a mitochondrial leader peptide was used to direct import of a DNA sequence into isolated mitochondria (Vestweber and Schatz 1989). This approach has attracted renewed interest in more recent years (Flierl et al. 2003; Mileshina et al. 2011).

To better insure specificity of action, mitochondriotropic pro-drugs may be designed in such a way that a targeting portion is eliminated by a reaction taking place exclusively in mitochondria, thus releasing the active compound only where desired. One such construct was the carboxyester of lipoic acid and a TPP-carrying alcohol. The ester moiety withstood cytosolic esterases but was lysed by

mitochondrial aldehyde dehydrogenase (Ripcke et al. 2009). Other studies exploited the fatty acid β-oxidation pathway (Anders 1995, 2011; Roser et al. 2010). Kagan's group used the peroxidase activity of the cytochrome c – cardiolipin complex which forms in apoptotic cells by devising a mitochondriotropic substrate of the complex which would release NO. The compound induces "suicide" of the peroxidase, since NO inhibits it by binding to heme iron (Stoyanovsky et al. 2008; Belikova et al. 2009; Prime et al. 2009; Atkinson et al. 2011). In a related approach, a mitochondria-targeted precursor has been photoactivated by UV irradiation to release an uncoupler with precise spatial control (Chalmers et al. 2012).

Instead of targeting the molecules themselves, one may deliver them selectively to mitochondria using mitochondria-targeted liposomes or nanoparticles (D'Souza and Weissig 2009; Biasutto et al. 2010). Dequalinium, a dication in which the two charges are separated by a lipophilic chain, assembles into vesicular "DQAsomes" which have been developed and elaborated mainly by Weissig's group (Weissig 2011, 2012). These structures can penetrate into cells and reach mitochondria. They are being considered as a vehicle for the delivery of DNA (Weissig and Torchilin 2001; D'Souza et al. 2003; Vaidya et al. 2009a; Lyrawati et al. 2011) and, in cancer, cell death inducers to mitochondria. Some success has been reported in this latter case for the mitochondrial delivery of paclitaxel (PTX) (which acts as an inducer of the mitochondrial permeability transition) (D'Souza et al. 2008). Improved systems incorporate in the DQAsomes PEG chains to facilitate membrane permeation, and ligands to be recognized by receptors abundantly expressed by cancer cells, such as those for folate (Vaidya et al. 2009b) or transferrin (Vaidya and Vyas 2012).

In a mouse xenograft model these vesicles proved capable of delivering paclitaxel to the tumour more efficiently than plain DQAsomes or paclitaxel as such, and to exert significant antitumour activity. These vehicles are somewhat analogous to the "Multifunctional Envelope-type Nano Devices" (MEND) (Yamada et al. 2012; Nakamura et al. 2012) and analogous multi-functionalized vesicles (Sawant and Torchilin 2011), which utilize a multiplicity of surface groups to optimize delivery to the intended target(s). Harashima's group has developed a sophisticated variation on this theme, the "Dual-Function mitoPORTER" vesicles (Yasuzaki et al. 2010; Yamada et al. 2011; Yamada and Harashima 2012). In this system the cargo to be delivered to mitochondria (nucleic acids, proteins, toxins) is enclosed in an inner membrane made of a mito-fusogenic lipid (DOPE: 1,2-dioleoyl-sn-glycero-3-phosphatidyl ethanolamine) bearing fusogenic and mito-targeting octa-arginine (R8) peptides which act in Tat-like fashion. This assembly is furthermore enclosed within another membrane system, also decorated with R8, which promotes fusion with the endosomal membrane and thus delivery of the inner particle to the cell cytoplasm and eventually to the mitochondria. When administered to cultured cells, these constructs are internalized by macropinocytosis and have been shown to deliver their cargo to the mitochondrial matrix.

Torchilin and colleagues have produced PTX-loaded liposomes bearing PEG-PE chains with a Rhodamine 123 or a triphenylphosphonium group attached which showed an efficient colocalization with mitochondria and demonstrated enhanced cytotoxicity and anti-tumour efficacy (compared to control unmodified

liposomes) in *in vitro* and *in vivo* experiments (Biswas et al. 2011, 2012a). Mitochondria-directed dendrimers have also been recently produced by conjugation with TPP groups, and have been shown to effectively target mitochondria in cultured cells (Biswas et al. 2012b). With essentially the same strategy, Marrache and Dhar (2012) have designed solid block-polymer nanoparticles containing PEG and TPP groups on a poly(D,L-lactic-co-glycolic acid) base. These particles could be loaded with various mitochondria-active compounds and proved capable of delivering them in various *in vitro* cellular systems.

Alterations of mitochondrial metabolism in cancer are offering new perspectives for therapeutic approaches (Pathania et al. 2009; Solaini et al. 2011; Barbosa et al. 2012). The challenge is down to develop targeted drugs which may exploit these alterations.

3 Mitochondrial Channels as Targets

The complex membrane system of mitochondria is their key functional feature. The "impermeable" inner membrane allows the formation of an electrochemical proton gradient which in turn drives the aerobic synthesis of ATP. The "semipermeable" outer membrane (OMM) serves to enclose a periplasmic space where proteins with fundamental roles in cell death are stored until a sufficiently strong pro-apoptotic signal arrives.

A still imperfectly defined, multi-factorial process leads to the loss of the permeability barrier posed by the OMM in cells committed to apoptosis (or at least to so-called "intrinsic" apoptosis). A generally accepted feature of this process is the involvement of proteins of the Bcl-2 family, the most notorious pro-apoptotic ones being Bax and Bak. Bax/Bak functional mimetics are therefore a tool whose development is being actively pursued as a potential means to eliminate cancer cells (Leber et al. 2010; Shamas-Din et al. 2011; Billard 2013; Hartman and Czyz 2012). Pro-apoptotic Bax oligomers display ion channel activity in phospholipid bilayers and this oligomerization has been proposed to underlie OMM permeabilization (Tait and Green 2010).

However, recent work by our group challenges the hypothesis that Bax alone is sufficient to induce cytochrome c release, given that a single point mutant of Bax did not mediate cell death in Bax/Bak-less mouse embryonic fibroblasts despite forming ion channels with properties similar to WT Bax (Szabò et al. 2011; Brustovetsky et al. 2010). A pore (mitochondrial apoptosis-induced channel, MAC) with an estimated diameter sufficient to allow the passage of cytochrome c was detected by patch clamp (Martinez-Caballero et al. 2009; Dejean et al. 2010). MAC activity was inhibited in cells over-expressing Bcl-2 and was similar to the activity of pure Bax in planar bilayer (BLM) experiments. The timing of cyt c release in apoptotic cells correlated with the onset of MAC activity and with the translocation of Bax to mitochondrial membranes, suggesting that MAC includes Bax. This does not exclude the participation of other proteins/factors.

The major protein of the OMM is porin, often referred to as "voltage-dependent anion channel" (VDAC) because of its properties. A conspicuous literature supports its involvement in apoptosis and hence its potential usefulness as a target for a mitochondrial therapy of cancer (Sect. 3.1).

A marvelous array of specialized proteins conducts and controls the intense traffic of ions, metabolites, nucleotides and proteins across the IMM, insuring that its "tightness" is not lost. When the IMM becomes freely permeable to solutes, the consequences for the cell can be catastrophic. The prime example of such a catastrophe is the Permeability Transition (discussed in Sect. 3.3), which is considered to bear heavy responsibilities in the tissue damage caused by, e.g., ischemia/reperfusion and oxidative stress. While much effort is directed towards finding ways to prevent this phenomenon when noxious, the selective induction of IMM permeabilization in cancer cells is a strategy definitely worth pursuing in oncotherapy. Other inner membrane mitochondrial (IMM) channels may participate in cell death. We mention these channels only very briefly, (in Sect. 3.4), given that their role is indirect, hypothetical, or both. We devote more space to the involvement of IMM voltage-dependent Kv channels, whose role and mechanism in apoptosis we have studied, and which offer a definite perspective of pharmacological intervention (Sect. 3.2).

3.1 VDAC

3.1.1 Structure and Properties

VDAC1, one of the three isoforms of the most abundant protein of the OMM, was the first mitochondrial channel to be discovered (Schein et al. 1976; Colombini 1979), and has been the object of a large number of studies. We will refer to VDAC1 as "VDAC" unless we want to emphasize the isoform involved. Many excellent reviews of the field are available, including comprehensive, detailed recent ones (Shoshan-Barmatz et al. 2010a; Shoshan-Barmatz and Golan 2012; Shoshan-Barmatz and Ben-Hail 2012; Colombini and Mannella 2012) and a special issue of *Biochim Biophys Acta* dedicated to "VDAC structure, function and regulation of mitochondrial metabolism" (Vol. 1818, June 2012). We will concentrate therefore our overview on the aspects of greatest relevance for the theme of this book, i.e. the role of VDACs in apoptosis and their candidacy as anti-cancer pharmacological targets.

Some 30 years after the field was opened by the studies by Schein and Colombini, and much intervening work seeking to clarify its architecture, the structure of mammalian VDAC has been reported in 2008 (Bayrhuber et al. 2008; Hiller et al. 2008; Ujwal et al. 2008, 2009; Raschle et al. 2009; Choudhary et al. 2010; Yu et al. 2012). The channel was confirmed to be a β-barrel, composed of 19 β-strands and an N-terminal α-helix adhering to the inner wall of the pore but possessing considerable freedom of motion (Villinger et al. 2010). The N-terminal segment is a crucial region, involved in many molecular interactions. The barrel measures 3.1–3.5 nm

Table 9.1 Major intrinsic biochemical/biophysical characteristics of VDAC1

Property	References[a]
Structure: β-barrel, composed of 19 β-strands and an N-terminal α-helix	Bayrhuber et al. (2008), Hiller et al. (2008), Ujwal et al. (2008), Raschle et al. (2009), Yu et al. (2012)
Conductance: ohmic with substates. Highest conductance is ~ 4–5 nS in symm. 1 M KCl, ~ 0.9 nS in 0.15 M KCl	De Pinto et al. (1987), Blachly-Dyson et al. (1993), Mirzabekov et al. (1993), Benz (1990)
Steep voltage dependence: the channel closes partially and occasionally completely when at voltages higher than ~20 mV of either polarity	Colombini (1989), Popp et al. (1996), Báthori et al. (1998)
Slight anion selectivity for the maximal conductance state(s): P_{Cl^-}/P_{K^+} ~ 1–4.5	Colombini (1989), De Pinto et al. (1987)
Anion selectivity is determined largely by exposed charges on the inside walls of the pore	Adelsberger-Mangan and Colombini (1987), Blachly-Dyson et al. (1990), Peng et al. (1992), Ujwal et al. (2008), Choudhary et al. (2010)
Cation selectivity of the majority of substates;	Colombini (1989), Benz et al. (1990), Benz and Brdiczka (1992)
Occasionally also of high-conductance states	Pavlov et al. (2005a)
The channel conducts a range of ions and metabolites, including	Hodge and Colombini 1997; Rostovtseva et al. 2002.
Ca^{2+}	Gincel et al. (2001), Rapizzi et al. (2002), Israelson et al. (2007), De Stefani et al. (2012)
ATP	Rostovtseva and Colombini (1996, 1997), Rostovtseva and Bezrukov (1998)
Superoxide	Han et al. (2003), Lustgarten et al. (2012)
Variable kinetic behaviour, with often "slow" gating kinetics but also fast transitions between substates	Mirzabekov et al. (1993), Báthori et al. (1998)

[a]Only exemplary references are cited

(it is slightly elliptical) in the plane of the membrane, and ~4 nm in the perpendicular dimension. The path for solute passage measures approximately 1.5 by 1 nm.

The channel properties of the purified and reconstituted protein have been thoroughly defined in studies using mostly the planar bilayer technique. In experiments of this type VDAC reproducibly forms a large, voltage-dependent pore, whose characteristics – rather well conserved among species – are summarized in (Table 9.1).

3.1.2 Regulation and Modulation

A clear-cut identification of VDAC activity has not been reported in patch-clamp experiments purportedly recording from the OMM. VDAC might actually be a strictly regulated pore, rather than a permanently open one. In fact VDAC, once

seen as the inert hole of a sieve, has come to be considered, at the other extreme, as a master gatekeeper regulating the flux of metabolites and ions between the mitochondria and the cytoplasm. The truth is probably somewhere in between. Despite its abundance, VDAC may indeed pose a limit to the traffic of nutrients and metabolites across the OMM, since its downregulation by shRNA has a dramatic effect on cell growth (Abu-Hamad et al. 2006; Koren et al. 2010). VDAC can be modulated in various ways (Das et al. 2012; Kerner et al. 2012). It can be Ser-Thr phosphorylated at various positions (at least *in vitro*) by GSK3β, PKA, PKCε, Nek1 (never-in-mitosis A (Nima)-related kinase) and other kinases and also by tyrosine kinases (Salvi et al. 2005). Phosphorylation is thought to regulate interaction with cytoskeletal components and is considered to be in most cases pro-apoptotic.

A list of factors potentially modulating VDAC (directly or indirectly) includes pyridine dinucleotides, glutamate, ATP and ADP, lipids including cholesterol, phosphatidylethanolamine and cardiolipin, possibly Ca^{2+}. Proteins like VDAC itself, creatine kinase, hexokinase I and II, the ANT, c-Raf kinase, eNOS, tubulin, G-actin, gelsolin, dynein light chain, mtHSP70, the TSPO (formerly mtBzR), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), aldolase, mutant (G37R) SOD1, BclxL, Bax, possibly t-Bid, COX-I are part of its interactome. These interactions constitute the basis of the involvement of VDAC in cell death and in cancer.

Overexpression of Hexokinase-2 (HK2) and its association with VDAC are key features of glycolytic cancers (Wolf et al. 2011). HK2 binding to the conduit channeling ATP out of mitochondria provides a metabolic benefit to cancer cells (Warburg effect) and it antagonizes cell death via inhibition of Bax-induced cyt c release (Pastorino et al. 2002; Gall et al. 2011) and/or inhibition of the Mitochondrial Permeability Transition (MPT) (Chiara et al. 2008). Phosphorylation of VDAC (on Thr51) by GSK3β prevents binding of Hexokinase to VDAC (Pastorino and Hoek 2008). One of the ways the pro-life kinase Akt is understood to produce its effects is by migrating to mitochondria, phosphorylating GSK3β (on Ser9), thereby inhibiting it and thus promoting HK-VDAC interaction (Bijur and Jope 2003; Pastorino et al. 2005). This pro-life association of VDAC and HK appears furthermore to be dependent on Cyclophilin D (Machida et al. 2006). HK2 can be dissociated from mitochondria by peptides modeled on either HK2 or VDAC1 sequences, and by small molecules such as clotrimazole, bifonazole (both fungicides, in the tens-of-μM range) and methyl jasmonate. The mechanism by which HK detachment favours cell death is not yet clear and may comprise more than one factor. Proposals include disruption of aerobic glycolysis and of the energy balance of the cell, regulation of ROS production, altered interaction of Bcl2 family proteins with mitochondria, facilitation of VDAC oligomer formation (reviewed in: Shoshan-Barmatz et al. 2010a; Shoshan-Barmatz and Golan 2012), and MPTP opening (since death is influenced by expression of Cyp D and by Cyclosporin A) (Chiara et al. 2008). Regardless, the interaction of HK2 and VDAC may well represent an Achilles' heel of cancer.

3.1.3 VDAC and Apoptosis

The role of VDAC in cell death is multi-faceted and complex (McCommis and Baines 2012; Shoshan-Barmatz and Golan 2012; Shoshan-Barmatz and Ben-Hail 2012; Shoshan-Barmatz and Mizrachi 2012). Proteins of the Bcl2 family have been found to interact with VDAC1 and/or VDAC2. Tsujimoto's group first reported that pro-apoptotic Bax and Bak could stimulate the efflux of cyt c from VDAC-containing liposomes via formation of a large pore comprising VDAC and Bax/Bak (Tsujimoto and Shimizu 2000). The reality of such a cooperative interaction between Bax and VDAC is however controversial. For example, VDAC1 and VDAC3 were found to be dispensable for the formation of the mitochondrial apoptosis-induced channel (MAC), proposed to correspond to the conduit for cytochrome c release (Martinez-Caballero et al. 2009), which requires instead the presence of Bax and/or Bak.

Tsujimoto's group was also the first to report that Bcl2 and BclxL inhibited VDAC via their BH4 domain, and ascribed to this inhibition the protection from apoptotic death afforded by BclxL (Shimizu and Tsujimoto 2000). Binding has been confirmed by structural studies (Malia and Wagner 2007; Hiller et al. 2008; Arbel and Shoshan-Barmatz 2010; Abu-Hamad et al. 2009), and is considered to have an anti-apoptotic significance. In fact, a peptide derived from the BH4 domain of BclxL was found to have anti-apoptotic action, reducing I/R injury in isolated rat hearts (Sugioka et al. 2003; Ono et al. 2005) and apoptosis in isolated human islets (Klein et al. 2004), while, conversely, expression of peptides copying VDAC sequences antagonized the protection offered by Bcl2 (Arbel and Shoshan-Barmatz 2010) or BclxL (Arbel et al. 2012) against staurosporine-induced apoptosis. The potential biomedical relevance of these studies is evident.

The formation of dimers and higher oligomers of VDAC1 has been confirmed by several studies (Shoshan-Barmatz et al. 2010b; Mader et al. 2010; Geula et al. 2012). These oligomers have been proposed to form the conduit for the efflux of cytochrome c from the mitochondrial periplasmic space of pre-apoptotic cells (Zalk et al. 2005; Abu-Hamad et al. 2008, 2009; Shoshan-Barmatz et al. 2006, 2008, 2010a).

Overexpression of VDAC1 has been found to be conducive to cell death (Rapizzi et al. 2002; Zaid et al. 2005; Mader et al. 2010; Sharaf el dein et al. 2012). This may be rationalized in terms of an increased formation of pro-apoptotic oligomers, or of an increase in the fraction of channels not bound to HK, equivalent to a partial detachment of HK from its VDAC binding sites. In contrast with the view that BclxL inhibits VDAC thereby antagonizing apoptosis (see above), block of VDAC by the phosphorothioate oligonucleotide G3139 (Tan 2012) or by avicins (plant saponins with anticancer activity) (Haridas et al. 2007) has been observed to be pro-apoptotic.

VDAC2 has been heavily implicated in the regulation of Bak activity. Korsmeyer's group reported (Cheng et al. 2003) that this isoform (but not VDAC1) inhibits Bak activation and apoptosis, as later confirmed by other groups. In lymphocytes, VDAC2 deletion is fatal but can be rescued by the simultaneous deletion of Bak (Ren et al. 2009). In cells lacking VDAC2, Bak relocates from the OMM to the ER (Raghavan et al. 2012). Along with the evidence in favor of the participation of VDAC's in the processes of cell death, results have been presented which argue

against the whole notion. In their paper reporting the irrelevance of VDAC isoforms for the MPT (see below), Baines et al. (2007) also reported that Bax-induced cyt c release from mitochondria isolated from WT or VDAC1^{-}, VDAC3^{-} and VDAC1/VDAC3-null cells was the same. MEFs lacking either one or both of these isoforms showed no difference in their apoptotic response to Bax overexpression, staurosporine or TNFα (lack of VDAC2 is fatal; Cheng et al. 2003).

3.1.4 VDAC2 and VDAC3

VDAC2 and VDAC3 (Messina et al. 2012; Raghavan et al. 2012) are much less abundant than VDAC1, and less studied. Despite its comparatively low abundance, VDAC2 is the major isoform in bovine testis and spermatozoa, where it is located in part in the cellular membrane of the head and it undergoes tyrosine phosphorylation during capacitation (in human spermatozoa). The porin has been shown to be associated with the outer dense fiber of the sperm flagellum, a non-membranous compartment (Hinsch et al. 2004). The axoneme and mitochondria of VDAC3^{-} mice are altered (Sampson et al. 2001), and VDAC2 and VDAC3 are very important for male fertility (Kwon et al. 2013). This suggests a function other than pore formation. VDAC3, purified and reconstituted after expression in yeast (it has never been obtained as a reasonably pure preparation from native mammalian tissue), gave rise to channel activity only with difficulty (Xu et al. 1999), and whether this scant activity was really due to VDAC3 is doubtful (Messina et al. 2012). All three isoforms reportedly permeabilized liposomes with similar solute size exclusion thresholds (Xu et al. 1999). VDAC2 forms pores resembling those of VDAC1 (Menzel et al. 2009; Xu et al. 1999). VDAC2 has been reported to associate with Ryanodine Receptor 2 at SR/mitochondria junctions in the heart, and to be of major importance for the direct transfer of Ca^{2+} from the SR to mitochondria (Min et al. 2012). VDAC1 has in turn been reported to interact with the IP3 receptor and to channel Ca^{2+} from the ER to mitochondria selectively, transferring an apoptotic signal (Hajnóczky et al. 2002; De Stefani et al. 2012). The fact that VDAC2 deletion is embryonically lethal (while deletion of VDAC1 or VDAC3 is not, and does not seem to induce alterations of apoptosis) and its interaction with Bak (see above) single out VDAC2 for special consideration as a target for mitocans (Raghavan et al. 2012).

3.1.5 VDAC and Cancer

The role of the VDAC-HK assembly in aerobic glycolysis and the relevance of VDACs (at least VDAC1 and VDAC2) for apoptosis automatically provide these proteins with oncological relevance (Shoshan-Barmatz and Golan 2012; Shoshan-Barmatz and Mizrachi 2012). Expression of VDACs has been found to be higher in cancerous than in normal cells, and may change with chemotherapy. A high VDAC level is an unfavourable prognostic factor (Grills et al. 2011). VDAC downregulation by RNA interference is an obstacle to cancer growth (Koren et al. 2010). This

may seem at odds with the finding that VDAC overexpression induces apoptosis (see above), a discrepancy illustrating the point that the functional meaning of a biological parameter depends on context. The upregulation of VDAC in cancer goes hand-in-hand with that of HK2 and may be considered as a tassel of the upregulation of glycolysis.

The development of antitumoural drugs targeting VDAC and its interactions is still in its infancy, but shows promise (Shoshan-Barmatz and Golan 2012). A major target is the HK-VDAC complex (Galluzzi et al. 2008; Simamura et al. 2008; Mathupala and Pedersen 2010); peptides interfering with this association and small molecules doing the same (clotrimazole, bifonazole, methyl jasmonate) have already been mentioned. The HK-VDAC complex, or more specifically HK, has also been considered a prime target for the alkylating agent 3-Bromopyruvate, reported to be very effective on *in vitro* and *in vivo* models (Pedersen 2012; Shoshan 2012; Ko et al. 2012; Cardaci et al. 2012). The targets of this compound are however several, including GAPDH (Tang et al. 2012), components of the respiratory chain (Rodrigues-Ferreira et al. 2012), the ER and lysosomes. Its remarkable specificity for cancerous cells may be attributed in part to the presence of members of the glycolytic pathway (HK, GADPH) among the targets, but mostly to facilitated entry into glycolitic cells via the lactate transporter (Ko et al. 2004; Queirós et al. 2012).

Erastin has been found to selectively induce the death of cells with mutations in the oncogenes HRas, KRas and BRaf. The cytotoxic effect was found to depend on VDAC2/3: RNAi-mediated knockdown of these proteins rescued the cells, and Erastin was found to bind to VDAC2 and to increase the permeability of liposomes containing it (Yagoda et al. 2007; Bauer et al. 2011). A recent publication seems to reconduce Erastin-induced death to an oxidative form of cell death (nicknamed "ferroptosis"; possibly related to "autoschizis"; Jamison et al. 2002), which depends on iron and is potentiated by erastin inhibition of the cystine/glutamate antiporter which supplies substrate for glutathione synthesis (Dixon et al. 2012). How VDAC2/3 would fit into such a mechanism is not clear, but oxidative stress and VDACs have been linked in other studies.

Furonaphtoquinones (FNQs) induced apoptosis of HeLa cells and increased production of H_2O_2, a production that was reduced by anti-VDAC antibodies and by VDAC-targeting siRNA. The expression level of VDAC, FNQ-induced H_2O_2 and cell death were found to be correlated (Simamura et al. 2006). VDAC has been identified as part of a complex having oxidoreductase activity involved in Paraquat toxicity (Shimada et al. 2009). Notwithstanding the above, a systematic search for compounds acting at the level of VDAC to antagonize cancer remains to be performed.

3.2 The Potassium Channel Kv1.3

Voltage-gated potassium channels (Kv) comprise a large family of channels which are expressed in both excitable and non-excitable cells (Armstrong 2003; Gutman et al. 2003; Yu et al. 2005). They control the resting plasma membrane potential

and the frequency of action potentials in excitable cells. In non-excitable tissues, such as pancreatic islets, the immune system, and epithelial cells, they are involved in feedback regulation of the potential, and thus in processes ranging from secretion to cell proliferation. Downstream events are the result of a signalling cascade which most often involves modulation of voltage-dependent Ca^{2+} channels and variations of cellular Ca^{2+} levels. Each Kv gene encodes a protein subunit, four of which may form either homotetramers or heterotetramers within the same family (Kv1 – Kv12). Functional diversity of Kv activities is enhanced by heterotetramerization and the association of accessory proteins, such as the β-subunits which can modulate gating properties and assist multimerization (Torres et al. 2007; Pongs and Schwarz 2010). Alternative splicing and posttranslational modifications also contribute to variety of activity, and various mechanisms have been proposed to regulate protein expression itself.

A characteristic of Kv channels is the presence of a vestibule which helps to gather and concentrate cations for eventual transport through the pore, due to the presence of a set of acidic aminoacids which impart an overall negative charge to the vestibule walls. These conserved residues can interact with a strategic basic (positive) aminoacid present in the peptide toxins of certain venoms – such as ChTx, MgTx, IbTx, ShK – which can therefore "plug" and block the channel (Yu et al. 2004; Rauer et al. 1999). Other toxins approach instead the channel via the membrane lipid bilayer and interact with the voltage sensor (Lee and MacKinnon 2004) or enter the channel and block it at the level of the selectivity filter (Zimin et al. 2010). This may well be the mechanism of potent small molecule (membrane-permeant) inhibitors of Kv1.3, notably Psora-4 (EC_{50} 3 nM) (Vennekamp et al. 2004), PAP-1 (EC_{50} 2 nM) (Schmitz et al. 2005) and clofazimine (EC_{50} 300 nM) (Ren et al. 2008).

Kv members are known to be located in the plasmamembrane, but Kv1.3 is present in the IMM as well (see below). This channel was first discovered in lymphocytes, but it is expressed also in the CNS, macrophages, kidney, testis, adipose tissue, osteoclasts, liver and skeletal muscle as well (Gutman et al. 2003). Kv1.3 has been implicated in the regulation of cell proliferation (Cahalan and Chandy 2009), apoptosis (Szabò et al. 2010; Gulbins et al. 2010; Szabò et al 2004), cell volume (Lang et al. 1999, 2004), and neurotransmitter release in excitable cells (Shoudai et al. 2007). Furthermore, Kv1.3 seems to participate in the pathways regulating energy homeostasis and body weight by a still poorly clarified mechanism (Xu et al. 2003). One intriguing possibility is that the mitochondrial Kv1.3 rather than the PM-located channel is involved in energy homeostasis.

Clarification of its patho-physiological roles and the discovery of powerful inhibitors made this channel a promising pharmacological target (Beraud and Chandy 2011) for various diseases, including autoimmune diseases (Wulff et al. 2009; Chi et al. 2012), diabetes (Choi and Hahn 2010) and cancer (Arcangeli et al. 2009, 2012; Felipe et al. 2012).

3.2.1 Mitochondrial Localization of Kv1.3

Localization of Kv1.3 to the IMM in lymphocytes and subsequently in other cell types has been demonstrated by various techniques: patch clamp (see below), immunogold transmission electron microscopy in lymphocytes (Szabò et al. 2005), macrophages (Vicente et al. 2006) and neurons of the postsynaptic medial nucleus of the trapezoid body (Gazula et al. 2010), immunofluorescence in gerbil hippocampus (Bednarczyk et al. 2010) and Western blot in the same preparation as well as in PC-3 prostate cancer cells, in MCF-7 breast adenocarcinoma (Gulbins et al. 2010), in SAOS-2 osteosarcoma, in B16F10 melanoma cell lines (Leanza et al. 2012a) and in J774 macrophages (Leanza et al. 2012b). In all these cases the channel protein was found in genetically non-manipulated cell lines or tissues, either healthy or cancerous. Kv1.5 also has a dual localization in the PM and in mitochondria at least in macrophages (Leanza et al. 2012b) and another Kv channel, Kv10.1, has recently been described as a functional channel in the nuclear membrane beside being active in the PM (Chen et al. 2011).

3.2.2 Pathophysiological Roles of mtKv1.3

mtKv1.3 is expected to participate in regulation of mitochondrial membrane potential, volume and ROS production, similarly to other K^+ channels found in the IMM. The role of mtKv1.3 under physiological conditions has not been elucidated in detail, but a crucial role for this channel in apoptosis became evident first in lymphocytes and later in other systems as well. Expression of a mitochondria-targeted Kv1.3 construct was sufficient to sensitize apoptosis-resistant CTLL-2 T lymphocytes, which lack Kv channels. MtKv1.3 has been identified as a target of Bax and physical interaction between the two proteins in apoptotic cells has been demonstrated (Szabò et al. 2008, 2011). Incubating Kv1.3-positive isolated mitochondria with Bax triggered apoptotic events including membrane potential changes (hyperpolarization followed by depolarization due to the opening of MPTP), ROS production and cyt c release, whereas Kv1.3-deficient mitochondria were resistant. Highly conserved Bax lysine 128 protrudes into the intermembrane space (Annis et al. 2005) and mimics a crucial lysine in Kv1.3-blocking peptide toxins. Mutation of Bax at K128 (BaxK128E) abrogated its effects on Kv1.3 and mitochondria, as well as in Bax/Bak-less double knock-out (DKO) mouse embryonic fibroblasts, indicating a toxin-like action of Bax on Kv1.3 to trigger at least some of the mitochondrial changes typical of apoptosis. These findings are not in contradiction with the view that Bax contributes to cyt c release, but indicate that a K^+ channel-dependent event is important for the release of cyt c and that expression of mtKv1.3 sensitizes cells to apoptosis. Blocking the IMM channel(s) would not *per se* be expected to result in permeabilisation of the OMM to proteins. This might however be obtained with the intermediacy of ROS, which may prompt Bax migration to mitochondria and/or the

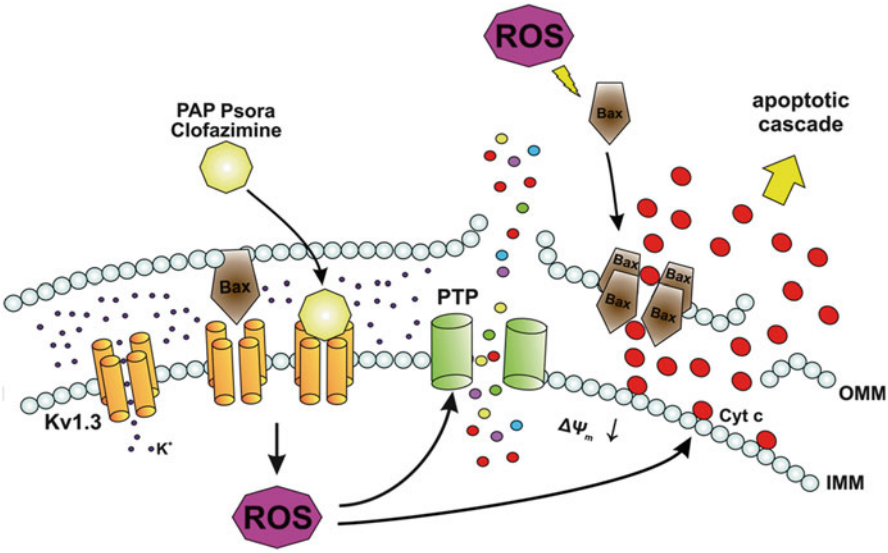

Fig. 9.1 Mitochondrial Kv1.3 in death induction. A cartoon illustrating the role of mtKv1.3 in cell death. Normally mtKv1.3 acts as a conduit for the passive flow of K^+ ions (*small* and *purple*) across the IMM, i.e., as a component of the machinery controlling mitochondrial volume, ion homeostasis and transmembrane potential. In cells challenged by a pro-apoptotic stimulus, Bax begins to migrate to the mitochondria, undergoing a conformational change and inserting into the OMM. There, it can interact via a protruding lysine residue with Kv1.3 and other Kv-family channels in the IMM, blocking K^+ flux. This causes a transient hyperpolarisation and hence enhanced production of ROS by the respiratory chain. These ROS can further stimulate Bax recruitment and/or induce the mitochondrial permeability transition. The consequence is loss of cytochrome c (*large*, *red*) via Bax oligomers or OMM lacerations caused by swelling of the matrix compartment following MPTP induction. The latter event also results in the collapse of the potential and in the dispersion into the cytoplasm of small molecules (*multicoloured*) such as NADH and Krebs cycle intermediates, hampering respiration. Pharmacological inhibition of mtKv1.3 with membrane-permeant small molecules is sufficient to induce the same phenomena and cell death in cancerous cells, which are already subjected to a higher-than-normal redox stress

onset of the MPT, which provides a mechanism alternative to Bax oligomerization for cytochrome c efflux. In accordance with our findings, platelets, in which Kv1.3 is the exclusive Kv channel expressed, were resistant to apoptosis in Kv1.3 knockout mice (McCloskey et al. 2010).

Recently we reported for the first time that Psora-4, PAP-1 and clofazimine, three distinct membrane-permeant inhibitors of Kv1.3, induce death by directly targeting the mitochondrial channel in multiple human and mouse cancer cell lines, while membrane-impermeant, selective and high-affinity Kv1.3 inhibitors ShK or MgTx did not induce apoptosis, further proving the crucial role of mtKv1.3 for this process (Leanza et al. 2012a). Importantly, the membrane-permeant drugs killed cells also in the absence of Bax and Bak, a result in agreement with the current mechanistic model for mtKv1.3 action (Fig. 9.1). Genetic deficiency or siRNA-mediated downregulation of Kv1.3 abrogated the effects of the drugs, proving specificity of their action via

Kv1.3. Intraperitoneal injection of clofazimine reduced tumour size by 90 % in an orthotopic melanoma B16F10 mouse model *in vivo*, while no adverse effects were observed in several healthy tissues. Similar results were obtained for primary human cancer cells from patients with chronic lymphocytic leukemia (Leanza et al. 2013).

The selective action of these drugs on tumour cells may be related to the observed higher expression of Kv1.3 in cancer lines/tissues with respect to healthy ones, but other factors are also expected to contribute and are under investigation. In any case, the fact that clofazimine is already used in the clinic for the treatment of e.g. leprosis (Ren et al. 2008) and shows an excellent safety profile emphasizes the possibility to exploit mtKv1.3 targeting for therapy.

In summary, inhibition of mtKv1.3 results in the generation of ROS and facilitation of cyt c release. It may therefore be expected that inhibition of other Kv-family channels present in the IMM of other cell types would promote apoptosis in a similar manner. It is to note that indeed the action of Bax is not restricted to Kv1.3: Kv1.1, mtKv1.5 and mtKCa1.1 can also interact with Bax (Cheng et al. 2011; Leanza et al. 2012b). In macrophages, which express both Kv1.3 and Kv1.5 in their mitochondria, down-expression of both channels is required to prevent staurosporine-induced apoptosis (Leanza et al. 2012b). Thus, beside those of the plasmamembrane, mitochondrial voltage-gated K^+ channels are emerging as therapeutic targets for oncological diseases.

3.3 MPTP

The Mitochondrial Permeability Transition Pore (MPTP) is to date known only indirectly. Its properties are deduced from those of the phenomenon it causes – the Permeability Transition – and from electrophysiological observations of a mitoplast channel believed to coincide with the MPTP. The MPT has been traditionally defined as the unselective permeabilisation of the inner mitochondrial membrane to solutes of MW up to 1.5 KDa, caused by operation of a wide pore. This permeabilisation results in a "bioenergetic catastrophe": the transmembrane electrochemical proton gradient is dissipated, ATP synthesis ceases, respiratory substrates are lost from the mitochondrial matrix. If MPTP opening is widespread and sustained, cell death, generally classified nowadays as a type of necrosis, ensues. A variety of stresses and dysfunctions can impinge on mitochondria causing them to undergo this rather brutal alteration, which is now considered as a target for intervention in pathologies ranging from infarct to neurodegeneration.

In the absence of definitive evidence on its molecular composition, the MPTP must be defined operationally on the basis of its pharmaceutical and biophysical properties. This is no easy task, because the MPT is a many-faceted, multi-factor phenomenon with no absolute dependence on any one parameter. A minimum set of properties may nonetheless be subjectively chosen, such that any phenomenon attributed to the MPTP ought to display them. This list might include: (1) a requirement for the presence of Ca^{2+} in the matrix; (2) dependence on matrix pH: acidification inhibits; (3) voltage dependence: the MPTP will open upon depolarization if the mitochondria have been suitably primed by Ca^{2+} loading or other treatments;

Table 9.2 A tabulation of reviews on the MPTP[a]

Focused on	Reviews
General properties and possible functions	Zoratti and Szabò (1995), Bernardi and Petronilli (1996), Bernardi et al. (1998, 2006), Nowikovsky et al. (2009), Azzolin et al. (2010), Ricchelli et al. (2011), Uribe-Carvajal et al. (2011), Bernardi and von Stockum (2012), Vianello et al. (2012)
Electrophysiology	Zoratti and Szabó (1994), Zoratti et al. (2009, 2010)
Molecular composition	Halestrap et al. (2002), Halestrap and Brenner (2003), Grimm and Brdiczka (2007), Leung and Halestrap (2008), Halestrap (2009), Baines (2009b), Forte and Bernardi (2005), Zoratti et al. (2005), Brenner and Moulin (2012)
Regulation	Bernardi (1996), Fontaine and Bernardi (1999), Bernardi and Rasola (2007), Di Lisa and Bernardi (2009), Zorov et al. (2009), Rasola et al. (2010a), Miura et al. 2010; Miura and Tanno 2012, Belliere et al. (2012), Papanicolaou et al. 2012
Involvement in cell death	Lemasters et al. (1998, 2009), Tsujimoto et al. (2006), Tsujimoto and Shimizu (2007), Grimm and Brdiczka (2007), Rasola and Bernardi (2007, 2011), Giorgio et al. (2010), Kinnally et al. (2011), Dorn (2010a, b, 2011), Baines (2011), Miura and Tanno (2012)
Involvement in autophagy	Kim et al. (2007), Aviv et al. (2011), Gottlieb et al. (2011)
Involvement in dystrophy	Bernardi and Bonaldo (2008)
Involvement in I/R injury	Halestrap et al. (1997, 2004), Di Lisa et al. (2003), Di Lisa and Bernardi (2006), Halestrap and Pasdois (2009), Baines (2009a), Halestrap (2010), Ruiz-Meana et al. (2010), Miura et al. (2010), Gouriou et al. (2011), Garcia-Dorado et al. (2012), Webster (2012), Oerlemans et al. (2013), Kalogeris et al. (2012), Sloan et al. (2012), Griffiths (2012), Kim et al. (2012)
Involvement in cardioprotection (conditioning)	Halestrap et al. (2007), Murphy and Steenbergen (2008, 2011), Penna et al. (2008, 2013), Pagliaro and Penna (2011), Di Lisa et al. (2011a, b), Boengler et al. (2011), Sanada et al (2011), Cour et al. (2011)
Targeting the MPTP for ischemic damage and cardiac disease	Liem et al. (2007), Javadov and Karmazyn (2007), Andreadou et al. (2008), Javadov et al. (2009, 2011), Morin et al. (2009), Di Lisa et al. (2011a), Hausenloy et al. (2012), Oerlemans et al. (2013)
Involvement in neurodegeneration	Friberg and Wieloch (2002), Du and Yan (2010), Martin (2010a, b, c, 2011, 2012)
Targeting the MPTP for neurodegeneration	Tatton and Chalmers-Redman (1998), Martin (2010c), Azarashvili et al. (2010)
Targeting the MPTP for cancer	Armstrong (2006), Ralph et al. (2006), Hail and Lotan (2009), Fulda et al. (2010), Gogvadze (2011), Javadov et al. (2011)

[a]Most reviews cover various aspects and provide at least some general information on the MPT

(4) redox sensitivity: oxidative stress favours the MPT. Many reviews covering the various aspects of MPTP physiology and pharmacology, the various hypotheses about its composition, its role in cell death, autophagy and in assorted pathologies have been published, and most are tabulated in Table 9.2. We provide here a brief overview and focus on the relevance of this phenomenon for cancer. The MPT has

been clearly recognized as one of the mitochondrial processes one can hope to exploit for cancer therapy (see Table 9.2 for reviews).

3.3.1 Properties

Information on the properties of the open MPTP has been gleaned from biochemical experiments following mitochondrial swelling (water follows the suspension medium osmolite into the matrix, once the IMM has become permeable to the osmolite itself) and the permeation of tracers as well as from electrophysiological work. Most have been known since Hunter and Haworth's fundamental work (Haworth and Hunter 1979, 1980, 2000; Hunter and Haworth 1979a, b). Solute exclusion studies suggested a diameter of 2–3 nm and indicate that the pore is unselective, or only poorly selective. A high-conductance channel (dubbed "Mitochondrial MegaChannel", MMC) has been identified as the MPTP on the basis of its pharmacological profile (Zoratti and Szabò 1994, 1995; Martinucci et al. 2000; De Marchi et al. 2009a; Zoratti et al. 2010) and proposed to consist of a dimer of cooperating, mildly anion-selective, substate-rich pores which can occasionally turn into irregular, cation-selective channels.

The idea that MPTPs of different sizes may exist in different mitochondria and that the size of the pore may depend on the MPT-inducing conditions has been around for a long time (Pfeiffer et al. 1995; Broekemeier et al. 1998). A major line of evidence consists in the observation that the time course of mitochondrial swelling does not always coincide with that of other phenomena thought to be associated with the MPT (Zoratti and Szabò 1995; Novgorodov and Gudz 1996). This vision of the MPTP as a "variable pore" is consistent with its putatively "accidental" nature, i.e. with the concept that it is formed by membrane components whose main task is something else. What these components may be is still, after 40 years of work, an unsolved riddle.

3.3.2 Composition

The idea that the adenine nucleotide translocator (ANT) may be the centerpiece of a molecular complex – comprising also VDAC, HK or creatine kinase, the OMM protein TSPO (or peripheral benzodiazepine receptor), and cyclophilin D, the mitochondrial matrix isoform of a family of peptidyl-prolyl cis-trans isomerases – which would form the MPT is old (Hunter and Haworth 1979a; Le Quoc and Le Quoc 1988; rev.: Zoratti and Szabò 1995) and entrenched, but it has not stood the test of genetic experiments. It was much weakened when it was shown that the MPT took place also in mouse liver mitochondria in which the expression of both ANT-1 and ANT-2 isoforms had been eliminated (Kokoszka et al. 2004). To accommodate the undeniable effects of ANT ligands (atractyloside (ATR) favours, and bongkrekate (BGK) inhibits, MPT development) the carrier is now considered by many to be one of the regulatory components of the pore (Zhivotovsky et al. 2009; Biasutto et al. 2010; Baines 2009b). Another, definitive blow came from the observation that the MPTP

was still present, with much the usual characteristics, in cells in which the three VDAC isoforms had been eliminated by pairwise gene knockout (and siRNA silencing of the third) (Krauskopf et al. 2006; Baines et al. 2007; De Marchi et al. 2008). Cyclophilin D (Cyp D) was confirmed to be a component of the MPTP by studies showing that its deletion decreased MPT-related damage in ischemia (Baines et al. 2005; Nakagawa et al. 2005; Schinzel et al. 2005). Subsequent studies confirmed this and the regulatory role of the isomerase: the MPT can still be elicited in the absence of CypD, although it requires more drastic conditions (Basso et al. 2005; De Marchi et al. 2006). Cyp D has recently been found to act by masking an inhibitory Pi-binding site (Basso et al. 2008; Li et al. 2012). Thus, Pi would appear to be the real MPTP inhibitor in the presence of the Cyp D ligand cyclosporine A (CSA); the role of the latter would be to unmask the regulatory site by causing CypD detachment. Finally, the TSPO has also been found to be disposable for the MPT (Sileikyte et al. 2011), although it definitely retains the role of a regulatory component.

The collapse of the ANT-VDAC model has opened the way to other more or less speculative models. The phosphate carrier (PiC) and at least two other members of the "tripartite" carrier family – the aspartate-glutamate exchanger and the ornithine-citrulline exchanger – have been proposed as candidates (Baines 2009b). The similarity between the pore activity observed upon reconstitution of components of the mitochondrial protein import system and the MPTP channel points to a possible identification of the former with the latter (Zoratti et al. 2010). Other candidates include inorganic polyphosphate (Pavlov et al. 2005b) and unspecific aggregates of membrane proteins caused by oxidative stress (Kowaltowski et al. 2001). Recently, Cyclophilin D has been found to interact with the lateral stalk of the F_0F_1 ATP synthase (Giorgio et al. 2009, 2010). Azarashvili's group on the other hand has reported that ligands of the TSPO (see above) promote both opening of the MPTP and dephosphorylation of subunit c of the ATP synthase in rat brain mitochondria (Krestinina et al. 2009). These findings point to the possibility that the ATP synthase itself might form the MPTP (Giorgio et al. (2013); Bernardi (2013)).

3.3.3 Regulation

The MPT in isolated mitochondria is favoured by Ca^{2+} loading in conjunction with inducers such as Pi, sources of ROS, thiol cross-linkers and acylating agents, $\Delta\psi$-reducing and surface potential-modifying agents, fatty acids, ligands stabilizing the C conformation of the ANT (ATR, CATR, acyl-CoA's, pyridoxal-5-phosphate) and compounds that can deplete the mitochondria of protective molecules (e.g. ADP depletion by PPi). Pore opening can be inhibited, or open pores closed, by agents that counteract the action of inducers. For example, radical scavengers will protect against oxidation of thiol groups, dithiothreitol will reverse it. In addition, as already mentioned above, the MPT is classically inhibited by CSA (although many papers describe or refer to CSA-insensitive forms of the MPTP), matrix acidification, adenine nucleotides, divalent cations other than Ca^{2+}, compounds that stabilize the

M conformation of the ANT (BGK, matrix ADP). Which parts of the electron transport chain are used may also have important consequences on MPT induction (see below). It is worth repeating that since the MPT is influenced by many factors, the effects described in the literature are rarely all-or-nothing; their magnitude depends on conditions.

Unless inhibitors are administered or Ca^{2+} is sequestered, prolonged closure after prolonged opening of the full-size MPTP does not readily occur *in vitro*. Endogenous protective factors such as NAD(P)H and ADP are rapidly lost through the open pores, along with Krebs cycle intermediates. Swelling may result in the loss of cytochrome c upon rupture of the OMM, and respiration may be permanently impaired. This scenario may not apply to brief openings.

While Ca^{2+} is a key effector at least *in vitro*, MPT induction by oxidizing conditions has long been known and indeed it is considered by some to be an essential feature of the MPT (Kowaltowski et al. 2001; Juhaszova et al. 2008). Ca^{2+}-treated mitochondria produce more ROS (Maciel et al. 2001) and this enhanced ROS production may well contribute to MPTP opening. Thus, antioxidants antagonize the MPT, and pro-oxidants promote it (De Marchi et al. 2009a).

The origins of the perduring interest in the MPT can be traced to the discovery that Cyclosporin A is a powerful inhibitor (Broekemeier et al. 1989; Crompton et al. 1988; Fournier et al. 1987). This cyclic endecapeptide – an inhibitor of calcineurin and widely used immunosuppressant – is effective on the MPT induced by a variety of agents, although the inhibitory effect may be partial, transient, or both, and its characteristics may depend on experimental details (Zoratti et al. 2005). The favored mechanistic model for the effect on the MPT is formulated in terms of MPT-permissive binding of CypD to the MPTP precursor. CSA is thought to inhibit by competing for CypD, with which it forms a complex no longer able to bind to the MPTP precursor. This model has been a key element for the candidacy of two proteins of the IMM – the ANT and, very recently, the F_0F_1 ATP synthase – as MPTP precursors (see above). While CypD is universally recognized to play a role in the MPT, this role is now recognized to be "merely" that of enhancing the sensitivity of the process to Ca^{2+}, as proposed early on (Halestrap et al. 1997). Definitive evidence came from the demonstration that Cyp D-null mitochondria could still undergo the MPT at higher Ca^{2+} loads (Basso et al. 2005). The pore responsible for permeabilization was shown to have essentially the same properties as the one observed in WT mitochondria, implying that CypD has a permissive role, but does not intervene to determine the properties of the pore once it has been activated (De Marchi et al. 2006).

Voltage dependence can be summarized by stating that the pore tends to open upon depolarization. Electrophysiological observations at the single-channel level on isolated, washed-out membranes indicate that this is an intrinsic property of the pore, independent of binding or blocking events or of secondary phenomena such as ROS production by uncoupler-treated mitochondria.

For several years in the 1980s the MPTP was thought to be due to membrane "defects" caused by the accumulation of lysophospholipids and free long-chain fatty acids in the IMM upon activation of Ca^{2+}-sensitive mitochondrial phospholipase

A2 (Zoratti and Szabò 1995). While this model has been largely abandoned, it remains true that the MPT is facilitated by fatty acids, prominently by arachidonic acid (Wieckowski et al. 2000; Penzo et al. 2002; Scorrano et al. 2001), and fatty acid acyl-CoAs (Furuno et al. 2001; Oyanagi et al. 2011).

A cross-talk between apoptosis effectors, in particular Bax, and the MPTP has been proposed in several studies (Roy et al. 2009; Brustovetsky et al. 2010; Li et al. 2008, 2010; Cheng et al. 2011; Whelan et al. 2012; Tischner et al. 2012; Kumarswamy and Chandna 2009), but our group did not find evidence for a direct interaction in studies with isolated mitochondria (De Marchi et al. 2004; Campello et al. 2005). Anti-apoptotic BclxL has been reported to antagonize opening of the MPTP as observed indirectly as a collapse of the mitochondrial potential and directly as channel activity in patch-clamp experiments (Jordán et al. 2004; Tornero et al. 2011). The MPTP is reportedly activated, under conditions of oxidative stress and I/R injury, by p53, which accumulates in mitochondria and induces necrosis by interacting with Cyp D (Vaseva et al. 2012).

In a physiological context, the MPT is subject to regulation by cellular phosphorylation pathways. The processes taking place during post-ischemic reperfusion and pre- and post-conditioning, and the modulation (repression) of the MPTP in cancerous cells have been investigated in some detail (Table 9.2). Hypoxic preconditioning (HPC) apparently increases a basal rate of opening of the MPTP in cardiac myocytes (Hausenloy et al. 2004, 2010). The model is coherent with current paradigms: a major signal emanating from hypoxic mitochondria is increased ROS generation, due to the respiratory chain being in a highly reduced state and enhanced mono-electronic "leakage" to residual oxygen. ROS promote MPTP opening, and MPTP opening may itself contribute to oxidative stress: ROS increased under HPC conditions in WT myocytes, but not in $CypD^{-/-}$ cells, pointing to a positive-feedback on ROS production (indeed, death by sustained simulated hypoxia/reperfusion was reduced by HPC in the case of WT cells, but not for $CypD^{-/-}$ cells).

This somewhat paradoxical increase of ROS in hypoxia determines a negative feedback response by the cell, which antagonizes the MPT and thus affords protection from reperfusion damage or, in preconditioning, in subsequent ischemic episodes. ROS contribute – by direct action or by inhibiting redox-sensitive phosphatases – to the activation of the so-called RISK (Reperfusion Injury Salvage Kinase) kinases, a group including Akt, Erk1/2, PKC-ε, PKG and P70s6K. These kinases in turn phosphorylate and inactivate GSK3β (Glycogen Synthase Kinase-3β; also involved in the canonical Wnt signalling pathway). GSK3β is constitutively active, and its inactivation results in desensitization of the mitochondria to MPT induction (Miura et al. 2010; Yang et al. 2010; Rasola et al. 2010a; Hausenloy et al. 2009, 2011; Pagliaro and Penna 2011). Importantly, Cyp D is phosphorylated by GSK3β (Rasola et al. 2010b). Other kinases, including PKC-ε (Garlid et al. 2009), Akt (Rasola et al. 2010a) and PKA (Pediaditakis et al. 2010) may act directly on mitochondria protecting from the MPT. CAMK-II on the other hand facilitates MPT onset in ischemized cardiac myocytes, reportedly by increasing Ca^{2+} influx mediated by the mitochondrial Ca^{2+} uniporter (MCU) into the mitochondria (Odagiri et al. 2009; Joiner et al. 2012).

Much the same signalling pathways may be involved in desensitizing cancer cells to MPT induction (Rasola et al. 2010a). In cancer models containing constitutively active ERK, this kinase acts through the GSK3β-Cyp D axis to repress cell death by MPTP inducers such as arachidonic acid or BH3 mimetic EM20-25 (Rasola et al. 2010b). MPTP desensitization in cancer cells seems to be also mediated by TRAP-1 (Tumour necrosis factor Receptor-Associated Protein-1), a chaperone homologous to Hsp90, which is localized in mitochondria in tumoural but not in healthy cells (Altieri et al. 2012, 2013a, b; Matassa et al. 2012). This protein and mitochondrial Hsp90 and Hsp60 interact with Cyp D and antagonize its MPT-facilitating action. TRAP-1 and the Hsp's are therefore new targets for mitocan development (Altieri 2013a, b; Kang 2012). TRAP-1 is a substrate of mitochondrial PTEN-Induced Kinase-1 (PINK1), and its phosphorylation by PINK1 protects against apoptosis induction by oxidative stress.

3.3.4 Physio-pathological Relevance

Ca^{2+}-induced IMM permeabilization has been observed in yeast, fungi, plant, fly, amphibian, fish and, obviously, mammalian mitochondria (Azzolin et al. 2010; Uribe-Carvajal et al. 2011; von Stockum et al. 2011; Bernardi and von Stockum 2012; Stella et al. 2011; Vianello et al. 2012). While the characteristics of the phenomenon vary depending on the system, its conservation suggests a physiological role for a controlled IMM permeabilisation. What this role may be remains an open question. A long-standing proposal is that the MPTP may act as a Ca^{2+} release channel whose brief openings would allow the mitochondria to get rid of excess Ca^{2+} accumulated because of thermodynamics (Bernardi and Petronilli 1996; Bernardi and von Stockum 2012). In support, Molkentin's group (Elrod et al. 2010) has reported that $CypD^{-/-}$ mice suffer from cardiac defects associated with alteration of MPTP-mediated Ca^{2+} efflux and consequent overload of the mitochondrial matrix.

The MPT is a signal for autophagic elimination (mitophagy) (Kim et al. 2007; Aviv et al. 2011; Gottlieb and Gustafsson 2011). Surprisingly, collagen VI deficiency-linked Ullrich congenital muscle dystrophy turned out to be associated with increased cell death caused by abnormal occurrence of the MPT (Irwin et al. 2003; Bernardi and Bonaldo 2008) and in fact can be contrasted by administration of CSA (Angelin et al. 2008; Merlini et al. 2008, 2011). In this pathology autophagy is defective, preventing the elimination of dysfunctional mitochondria, and its reactivation ameliorated the dystrophic phenotype (Grumati et al. 2010).

Autophagy is involved in development, in caloric limitation-induced lifespan extension, in opposing aging, obesity, neurodegeneration, and in a number of other major conditions and diseases (Ravikumar et al. 2010; Murrow and Debnath 2013). Autophagy seems to intervene to protect cells during I/R, and also to be a key process in the phenomenon of ischemic preconditioning. Its upregulation is associated with cancer (White et al. 2010; Morselli et al. 2011; Kongara and Karantza 2012; Jain et al. 2013). Although its function in these major pathologies is still debated, it may mainly represent a pro-survival/proliferation strategy.

Defects in autophagy are associated with inflammation, an oncogenic factor. On the other hand, direct or indirect induction of the MPT is a pharmacological strategy against cancer (see below).

The MPT is most notorious for its key role in the mechanism of ischemia/reperfusion damage (Table 9.2). During reperfusion, the conditions in the cell cytoplasm are nearly ideal for the onset of the MPT. Indeed, ablation of Cyp D or treatment with MPTP inhibitors produces clear protective effects (Table 9.2) (Hausenloy et al. 2003; Javadov et al. 2003; Baines et al. 2005; Nakagawa et al. 2005; Schinzel et al. 2005). In general, the MPT may be suspected to play a role in all degenerative, necrotic diseases. It is important for neurodegeneration in pathologies such as Alzheimer's, Parkinson's and ALS, acute consequences of stroke, cardiac arrest, brain trauma, insulin-induced hypoglycaemia and epileptic crises, and ethanol-induced death of neurons in mouse pups. In mice, lack of Cyp D protected neurons in experimental autoimmune encephalomyelitis, a model of multiple sclerosis, and muscle cells from atrophy following denervation, but caused behavioural disturbances.

In cancer, signalling pathways are activated which desensitize the mitochondria to MPT induction (Rasola et al. 2010b; Traba et al. 2012) (see above, also for the connection with autophagy and a protective mitochondrial chaperone network in cancer), while chemotherapeutic agents causing oxidative stress may activate signals causing cancer cell death via the MPT (Chiara et al. 2012). Transplant patients, treated with CSA to prevent transplant rejection, have a high incidence of cancer not only because of the drug's immunosuppressive action, but also because CSA inhibits the MPTP (Norman et al. 2010).

3.3.5 Pharmacology

The list of MPT inhibitors, of great interest because of the role of the MPT in cardiac damage and other pathological situations, is too long to be recapitulated here. Recently, a powerful derivative of CSA has been obtained by one of the mitochondria-targeting strategies mentioned in Sect. 2, i.e. conjugation of a triphenylphosphonium group (Malouitre et al. 2009; Dube et al. 2012). Non-immunosupressive but still MPT-inhibiting analogues of CSA are available: NIM811 (Waldmeier et al. 2002) and Debio-025 (Alisporivir) (Gomez et al. 2007; Tiepolo et al. 2009). They, and Sanglifehrin A, another cyclophilin-binding compound which however is immunosuppressive, have been tested as cardiac protectors (Oerlemans et al. 2013). Antamanide, produced by *Amanita phalloides*, likewise inhibits the MPT by binding to Cyclophilin D (Azzolin et al. 2011). In this context, it is interesting that specific inhibition of MPTP-associated "necroptosis" can result in cell death by "classical" apoptotic OMM permeabilisation (Han et al. 2009), highlighting the concept that necrosis and apoptosis may function as reciprocal back-up mechanisms.

For oncological applications MPT inducers are more relevant, although other mechanisms may account for chemotherapeutic action at least in part and in some cases, and the likelihood of noxious side-effects, for example on the nervous

system, must be kept in mind. A large number of compounds, many of which natural products, have been shown to induce the MPT in cultured cells, often as a consequence of oxidative stress and/or disruption of Ca^{2+} homeostasis. The effect is often observed at relatively high concentrations. One class of such compounds is that of mitochondria-penetrating peptides, such as mastoparan-like sequences, peptides of the innate immunity systems or the molecules developed by Kelley's group (Jones et al. 2008; Risso et al. 2002; Horton et al. 2012). The MPT can be activated (or inhibited) indirectly, by acting on the signalling pathways which modulate its occurrence. For example, induction of oxidative stress by gold complex AUL12, which inhibits respiratory Complex I, can lead to activation of GSK3α/β, favouring MPT opening (Chiara et al. 2012). Prominent among these candidate drugs are peptides and small molecules disrupting the interaction of HK2 with the mitochondrial membrane system (see above): jasmonates (Flescher 2005, 2007; Raviv et al. 2013); arsenite (Larochette et al. 1999); TNF or TRAIL and Actinomycin D (Pastorino et al. 1996; Kim et al. 2002); lonidamine, betulinic acid, the synthetic retinoid CD437 (Costantini et al. 2000; Lena et al. 2009; Javadov et al. 2011); further natural compounds like berberine (Pereira et al. 2007, 2008), chelerythrine (Wan et al. 2008; Funakoshi et al. 2011), stevioside (Paul et al. 2012), cinnamaldehyde (Ka et al. 2003), andrographolide (Chen et al. 2012), neosergeolide (Cavalcanti et al. 2012), 7-xylosyl-10-deacetylpaclitaxel (Jiang et al. 2011), honokiol (Li et al. 2007), α-bisabolol (Cavalieri et al. 2009), shikonin (Han et al. 2007; Xuan and Hu 2009).

3.4 Other Mitochondrial Channels as Possible Oncological Targets

In addition to the above-mentioned Kv1.3, VDAC and MPTP, several other proved or putative mitochondrial ion channels have been proposed to impact on cell survival and as such might play a role during tumourigenesis and may eventually become oncological/pharmacological targets. These include the large- and intermediate-conductance calcium-dependent (BK_{Ca} and IK_{Ca}, respectively), the ATP-dependent and the two-pore TASK3 potassium channels, uncoupling protein UCP2, calcium uniporter MCU and the magnesium-selective channel Mrs2. For reasons of space, we only mention briefly here a few points of interest.

IK_{Ca} (KCa3.1) is expressed in various tissues including epithelial- and endothelial tissues, the immune system, sensory neurons and microglia but not in excitable tissues. As for the other Ca^{2+}-activated K^+ channels, plasma membrane IKCa provides a feedback mechanism whereby cytoplasmic Ca^{2+} increase results in a hyperpolarizing response counteracting depolarization associated with Ca^{2+} influx and sustaining Ca^{2+} entry (Cahalan and Chandy 2009). This circuit is important for progression through the cell cycle. Given the widespread presence of the channel in cancer cells, it is therefore under consideration as a target for anti-tumoural as well as immunosuppressive intervention (e.g. Panyi 2005). IK_{Ca} activity has been recorded from the inner mitochondrial membranes of human colon carcinoma cells (De Marchi et al. 2009b), of

HeLa (human cervix adenocarcinoma) cells and of mouse embryonic fibroblasts (Sassi et al. 2010).

MtIK$_{Ca}$ was selectively inhibited by membrane-permeant inhibitors clotrimazole and TRAM34. Given the proposed role for other mitoK$^+$ channels in cell death, the membrane permeant TRAM34 was used in an attempt to induce cell death and the effect of recombinant Bax on channel activity was studied. In contrast to BK$_{Ca}$ (KCa1.1) and Kv1.3, IK$_{Ca}$ was not inhibited by Bax (Sassi et al. 2010). Interestingly, while TRAM-34 used alone at 2–80 μM concentration did not induce apoptosis (Sassi et al. 2010; Quast et al. 2012), in combination with the death receptor ligand TRAIL it synergistically increased the sensitivity to TRAIL of melanoma cells (Quast et al. 2012). TRAM34 induced a hyperpolarization of the mitochondrial membrane (as expected if a positive charge-carrying influx is inhibited), confirming that functional IK$_{Ca}$ is expressed in the IMM. Interestingly, application of TRAM34 alone induced translocation of Bax to mitochondria, representing an early step of apoptosis. Given that both TRAM34 and TRAIL have a relatively good safety profile, co-administration of the two drugs might be exploited for melanoma treatment. TRAM34 and clotrimazole have also been shown to decrease cell viability of epidermoid cancer cells when applied together with cisplatin, apparently via an action on the PM-located IK$_{Ca}$, involved in apoptotic volume decrease (Lee et al. 2008).

The long-sought molecular identification of the mitochondrial Ca^{2+} "uniporter", responsible for the low-affinity uptake of calcium into the mitochondrial matrix driven by the negative membrane potential, has recently been achieved (De Stefani et al. 2011; Baughman et al. 2011). MCU participates in the control of Ca^{2+} signaling, and may thus be a very useful tool to influence the myriad cellular calcium-dependent processes, ranging from proliferation to exocytosis to cell death (Rizzuto et al. 2012). Subthreshold apoptotic signals were shown to synergize with cytosolic Ca^{2+} waves (Pinton et al. 2001), evoked by a physiological stimulation, in opening of the permeability transition pore which ultimately leads to release of proapoptotic factors and cell death. Cells overexpressing MCU underwent more pronounced apoptosis upon challenging with H_2O_2 and C_2-ceramide (De Stefani et al. 2011). A recent finding identified an MCU-targeting microRNA, miR-25, whose overexpression in colon cancer cells resulted in MCU downregulation, impaired calcium uptake and increased resistance to apoptosis (Marchi et al. 2013). Thus, MCU seems to be an important factor in cell death, and its specific pharmacological activators, if identified, might become useful anti-cancer tools.

4 Oxidative Stress as a Strategy Against Cancer

4.1 Oxidative Stress and Cancer

In most cells, mitochondria are the main site of Reactive Oxygen Species (ROS) production. Radicals are produced via one-electron transfer to oxygen from sites of the respiratory chain (Starkov 2008; Murphy 2009), and, under appropriate

circumstances, by diversion from cytochrome c to oxygen with the intermediacy of p66Shc (Pinton and Rizzuto 2008; Pani et al. 2009; Di Lisa et al. 2009) or as a product of Monoamino Oxidase activity (Di Lisa et al. 2009). These ROS carry a considerable part of the blame for aging (Nakanishi and Wu 2009; Blagosklonny 2008), neurodegenerative disorders (Lee et al. 2009; Sayre et al. 2008), and ischemia/reperfusion damage (Di Lisa et al. 2009; Szeto 2008).

Increased production of reactive oxygen species (ROS) is important for the maintenance and evolution of the cancerous phenotype (Fiaschi and Chiarugi 2012). The effect of ROS may vary according to the stage of carcinogenesis; in the tumour initiation phase, ROS may cause DNA damage and mutagenesis, while in established cancer ROS acting as signal mediators may stimulate proliferation, conferring growth advantage. ROS, and more specifically H_2O_2, are a recognized mitogenic factor (Groeger et al. 2009; Verschoor et al. 2010), acting via transactivation of growth factor receptors and/or of downstream effectors such as the Akt pathway. These effects are largely ascribed to the inhibition, via sensitive cysteine residues, of phosphatases (PTEN and phosphatases of the MAPK cascade). Redox-sensitive transcription factors, notably AP-1, NF-kB and p53, are also activated by ROS (Trachootham et al. 2008; Verschoor et al. 2010; Morgan and Liu 2011). Mitochondrial ROS production increases the metastatic potential of cells (Ishikawa et al. 2008; Pani et al. 2009; Hyoudou et al. 2008, 2009; Pelicano et al. 2009) and anti-oxidant polyphenols decrease cell shedding from cancer spheroids in culture (a model of the metastatic process) (Günther et al. 2007). For an in-depth discussion the Reader is referred to several excellent recent reviews (Ralph et al. 2010; Trachootham et al. 2009; Verschoor et al. 2010).

An increased production of ROS has been reported for several different cancer types (Verschoor et al. 2010; López-Lázaro 2010). Judging from the effects of increased catalase activity, H_2O_2 appears to be the relevant specie (Szatrowski and Nathan 1991; Hyoudou et al. 2008, 2009; Policastro et al. 2004). Alterations in antioxidant enzyme activities have also been reported in many tumour cell lines and tumour samples (Oberley and Oberley 1997; Chung-man et al. 2001; Policastro et al. 2004; Gokul et al. 2010), resulting in increased oxidative stress.

The interaction of ROS with HIF is very relevant. ROS can induce pseudohypoxia, i.e. HIF activation (Chandel et al. 2000; Jezek et al. 2010; Tormos and Chandel 2010). Hypoxia is known to lead to a paradoxical increase of mitochondrial ROS production (see above and: Guzy and Schumacker 2006; Korde et al. 2011). HIF levels are normally regulated by prolyl oxidases (prolyl hydroxylase domain enzymes, PHDs) which hydroxylate HIF1α prolines, and by FIH (Factor Inhibiting HIF), which hydroxylates HIF1α asparagines. These modifications result in HIF1α polyubiquitination and degradation. An increase in cytosolic H_2O_2, downstream of mitochondrial superoxide production due to hypoxia or to mitochondrial dysfunction, is thought to lead to oxidation of the Fe^{2+} used by PHDs and FIH as an essential cofactor, and thus to their inactivation and to the consequent stabilization of HIF, with the attendant downstream effects, including tumour vascularization (Solaini et al. 2010; Jezek et al. 2010; Kobliakov 2010; Tertil et al. 2010). On the other hand it is clear that ROS can induce cell

death and thus act as anti-cancer agents (Ueda et al. 2002; Circu and Aw 2010; Panieri et al. 2013).

Cell death is simplistically classified as either apoptosis or necrosis; we maintain this simplified distinction, even though today it is clear that it can occur via a continuum of processes (Proskuryakov and Gabai 2010; Zhivotovsky and Orrenius 2010). Apoptosis consists in a coordinated, well-regulated series of enzymatic events, and ROS action in its induction likewise consists in discrete, well-definable events at the molecular level, rather than in the indiscriminate damage that may take place at higher levels of oxidative stress and which may result instead in death via necrotic pathways or "autoschizis" (see below).

Elevated ROS production can be a cause as well as a consequence (Wu et al. 2010) of genetic damage. Damage may be due to alteration of the nucleotide pool, with subsequent incorporation of oxidized nucleotides (Rai 2010), as well as to direct attack onto the chromatin-bound nuclear DNA or mitochondrial DNA, much more vulnerable because it is not packed into histones. When not repaired, these lesions lead to cellular senescence, genome instability, mitochondrial dysfunction and pathologies such as neurodegeneration, cancer and aging. When first the redox defences, and then the DNA repair machinery cannot meet the challenge, cells may resort to "suicide" to avoid organismal damage.

Activation of kinase-based pro-apoptotic signaling by ROS is of fundamental importance (Biasutto et al. 2011; Ueda et al. 2002; Takeda et al. 2011). A major such pathway is the stress response of the JNK and p38 MAPK cascades. An apical kinase (MAPKKK) in this system is Apoptosis Signal-Regulating Kinase 1 (ASK1). Among the many ASK1-associated proteins is thioredoxin; ROS cause thioredoxin oxidation on cysteine residues, and its dissociation from ASK1 (Saitoh et al. 1998). A signalosome then assembles, and ASK1 is activated by phosphorylation at a conserved threonine residue. Transcription factors such as c-Jun, p53 and p73, other kinases and members of the Bcl-2 family act downstream of JNK and p38 to induce apoptosis (Takeda et al. 2011).

Another set of pro-apoptotic kinases responding to oxidative stress is that of the appropriately named Death-Associated Protein Kinases (DAPK; 5 members) (Bialik and Kimchi 2006; Takeda et al. 2011), whose downregulation is associated with many cancers (Michie et al. 2010). Under oxidizing conditions DAPK binds to and activates PKD, which in turn induces the JNK pathway, and seems to be required for oxidative stress-induced necrotic death (Eisenberg-Lerner and Kimchi 2007).

Bax, one of the key players in "mitochondrial" apoptosis, can be activated by a number of signals, including, importantly, oxidative stress (Ghibelli and Diederich 2010). Homodimerization via formation of an SS bridge between cysteins and phosphorylation at Thr167 by redox-sensitive JNK/p38 (see above), required for mitochondrial translocation, account for this. Redox modification of the catalytic cysteine in caspases by H_2O_2 has also emerged as a relevant feature in the control of apoptosis (Katoh et al. 2004). Pro-caspase 9 belonging to the mitochondrial pool can form dimers via disulfide bridges and perform auto-processing to generate the active enzyme.

Necrosis, long considered as a sort of uncontrolled, haphazard form of cell death, characteristically determined by elevated redox stress, is now understood to comprise a set of interacting signalling cascades and biochemical phenomena forming a continuum with other forms of cell death (Kroemer et al. 2009). Depletion of ATP is often cited as a key factor shifting cell death towards necrosis rather than apoptosis. Necrosis can be classified into subtypes, still in the process of consolidation in usage. "Accidental necrosis" is induced by severe damage with profound ionic imbalance and cellular swelling. It is now distinguished from necroptosis (involving RIPK1 and also the MPT), "programmed necrosis" involving the MPT and AIF and EndoG release from the mitochondria, and secondary necrosis, the latter following on the heels of apoptosis, all leading to morphologically similar end results (Vanden Berghe et al. 2010). "Autoschizis" is used by some researchers to indicate pro-oxidant-induced death (Jamison et al. 2002). The molecular mechanisms of autoschizis have not been characterized in detail, but they are considered to involve widespread damage to cellular components by the oxidant species generated.

Both anti-oxidant and pro-oxidant approaches have thus been proposed to antagonize cancer (Trachootham et al. 2009; Ralph et al. 2010, 2011; Wang and Yi 2008). Anti-oxidants are expected to be useful for cancer prevention, while pro-oxidants are desirable in an oncological context to (selectively) induce cancer cell death. These considerations explain the booming interest in the development of pharmacological tools allowing the modulation of the mitochondrial redox state, which in most studies take the form of mitochondria-targeted redox-active compounds (Smith and Murphy 2010; Biasutto et al. 2010; Dong et al. 2011a, b).

Preferential accumulation into mitochondria has been sought by the strategies mentioned in Sect. 2. The most popular approach has been the conjugation to the membrane-permeable triphenylphosphonium cation. Selectivity towards tumour cells can be achieved thanks to the higher mitochondrial potential ($\Delta\Psi$) of the latter compared to their non-tumoural counterpart (Chen 1988; Bonnet et al. 2007). Cancer cells produce their ATP mostly by glycolysis (the Warburg effect), exhibiting reduced oxidative phosphorylation activity; the mitochondrial electrochemical proton gradient is thus dissipated less efficiently, and the transmembrane potential increased (Modica-Napolitano and Aprille 2001). Moreover, mitochondrial membrane potential seems to be correlated to tumour aggressiveness. Cells with higher $\Delta\Psi$ have higher levels of Vascular Endothelial Growth Factor and matrix metalloproteinase 7, and increased invasive behaviour compared to cancer cells with lower $\Delta\Psi$ (Heerdt et al. 2005).

At least in the case of MitoVES (Dong et al. 2011a, b) and of polyphenol-TPP conjugates synthesised by our group (Sassi et al. 2012) these compounds can indeed act as pro-oxidant cell killers. Since cancerous cells in many cases have a higher-than-normal basal rate of ROS production, despite their enhanced redox defences an additional oxidative stress may push them over the brink of death more easily than normal cells (López-Lázaro 2010; Wondrak 2009; Leanza et al. 2013).

4.2 Vitamin E Derivatives

The vitamin E analogue α-tocopheryl succinate (α-TOS) has been reported to selectively kill cancer cells, causing ROS generation through inhibition of mitochondrial complex II (Neuzil et al. 2001; Ralph et al. 2011). The mechanistic model envisions α-TOS displacing ubiquinone from its binding site(s) in complex II. Consequently, electron transfer is blocked; instead of binding to their natural acceptor, electrons react with oxygen and generate superoxide anion, leading to apoptosis induction. Cancer cell selectivity can be achieved thanks to higher esterase levels in normal cells (which lead to regeneration of vitamin E), and lower pH in the tumour microenvironment, which favours protonation of the carboxy group and thus a greater internalization of α-TOS.

To increase the efficacy of this anticancer agent, a mitochondrially targeted vitamin E derivative, mitoVES, was obtained by conjugation of α-TOS to a triphenylphosphonium cation via an aliphatic chain. MitoVES turned out to be more effective than its untargeted analog α-TOS in selectively killing cancer cells. An 11-carbon chain was found to be optimal, presumably because it localizes the derivative into the ideal position across the interface of inner mitochondrial membrane and matrix, optimizing its efficacy (Dong et al. 2011a, b).

4.3 Polyphenol-Triphenylphosphonium Conjugates

Polyphenols are plant-derived redox-active compounds with potentially useful biological properties; their effects are mediated by interaction with cellular and mitochondrial components, and by modulation of redox processes (Biasutto et al. 2011). Polyphenols are generally regarded as antioxidants, but their ability to act as pro-oxidants as well is inherent in their structure and chemico-physical properties. Which mode of action will predominate depends on the circumstances, e.g. the pH and the presence of Cu and Fe ions (De Marchi et al. 2009a; Halliwell 2008).

Mitochondria-targeted derivatives of quercetin and resveratrol (both frequently studied representatives of the polyphenol superfamily) have been produced (Mattarei et al. 2008; Biasutto et al. 2008). These compounds accumulated in cultured cells and isolated or *in situ* mitochondria as expected (Fig. 9.2). Quercetin and resveratrol have both been reported to kill cancerous cells *in vitro* (Murakami et al. 2008; Kundu and Surh 2008; Athar et al. 2009), but high concentrations (tens of μM) were needed, which are not expected to be reached *in vivo*, due to the low bioavailability of polyphenols (Landete 2012). When tested on cultured cells at concentrations in the low μM range, mitochondriotropic derivatives of both resveratrol and quercetin turned out to act as cytostatic/cytotoxic agents. Interestingly, this toxicity was selective for fast-growing cells (Mattarei et al. 2008; Biasutto et al. 2008; Sassi et al. 2012 and unpublished results). The corresponding parent polyphenols had no significant effect at the same concentrations.

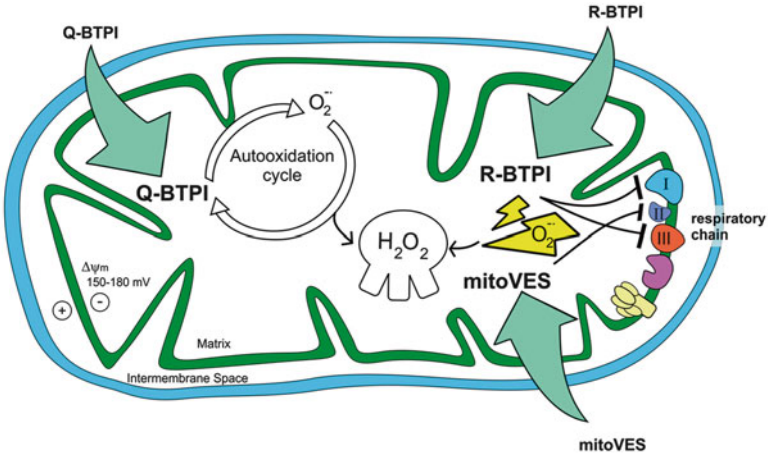

Fig. 9.2 Mitochondriotropic compounds acting as pro-oxidants to precipitate cell death. MitoVES and TPP-linked quercetin and resveratrol derivatives (Q-BTPI and R-BTPI, respectively) are driven to mitochondria, where they induce generation of superoxide and redox stress by different mechanisms. MitoVES and R-BTPI interact with complexes of the respiratory chain: mitoVES with complex II and R-BTPI more markedly with complexes I and III. These interactions result in the diversion of unpaired electrons to oxygen, with the production of superoxide. Q-BTPI undergoes autooxidation, reducing oxygen to superoxide in a chain process. The ensuing oxidative stress, to which cancerous cells are particularly susceptible, causes apoptosis or necrosis. The transmembrane potential-driven accumulation of the compounds into mitochondria is a key element, greatly increasing the efficacy of the derivatives with respect to the parent (non-mitochondriotropic) compounds

Cytotoxicity is due to ROS generation upon accumulation of the compounds into mitochondria. The mechanism underlying pro-oxidant behaviour, however, is different for quercetin and resveratrol derivatives. Quercetin derivatives produce ROS through an autooxidation process (Sassi et al. 2012). Since cell death was largely prevented by externally added membrane-permeating catalase, the toxicant is H_2O_2, or reactive species derived from it. Also, permeant superoxide dismutase rescued the cells. This behaviour, together with the observation that the presence of free phenolic hydroxyls and accumulation in mitochondria were both necessary to induce oxidative stress, led us to hypothesise a chain "autooxidation" mechanism of H_2O_2 production, in which dismutation of superoxide constitutes a termination step. Superoxide anion is generated by a cyclic reaction via transfer of an electron from the semiquinone of the quercetin derivative to oxygen, and in turn regenerates the semiquinone by oxidizing a quercetin moiety, with the concomitant production of H_2O_2 (Sassi et al. 2012). Furthermore, 7-O–(4-triphenylphosphoniumbutyl)quercetin iodide (Q-7BTPI) inhibits Glutathione Peroxidase, an effect expected to accentuate oxidative stress by interfering with the elimination of H_2O_2. Incubation of cells with low μM concentrations of mitochondriotropic resveratrol derivatives also causes cell death and ROS production, but the underlying mechanism is different. H_2O_2 is the toxicant, since externally

added membrane-permeant catalase again largely prevented cell death. However superoxide dismutase in this case potentiated toxicity, and effectiveness was increased if resveratrol hydroxyls were acetylated or methylated (Sassi et al. 2014). This suggests that autooxidation of the polyphenolic nucleus is not involved. The compounds caused also Cyclosporin A- and ROS-independent depolarization of *in situ* mitochondria. ROS generation is due to interaction of the derivatives with respiratory chain complexes (especially complex I and III) and the ATP synthase (Sassi et al. 2014). These mitochondriotropic polyphenol derivatives are currently being tested in *in vivo* tumour models.

5 Conclusions

The development of "mitocans" is proceeding at a fast pace: the concept is by now established, and promising leads are being followed with the hope they may turn into clinically useful medical devices. Imaginative new vehicles and constructs offer the perspective of both highly cancer-selective and highly mitochondrion-specific targeting. The two major strategies to provoke the death of cancerous cells rely on paroxysmal oxidative stress and/or "tearing up" mitochondrial membranes. ROaSting hectic cancer cells while sparing the more sedate populations seems now a goal within range. Mitochondrial channels are emerging as mediators of cell death. Besides VDAC, known to have an important role in cancer's metabolic peculiarities, and the permeability transition pore – the lurking all-purpose executioner waiting to be summoned – inner membrane potassium channels are now clamouring for a piece of the action. New recruits, in particular the MCU, are warming up to join the fray. Other aspects of mitochondrial physiology, such as mitochondrial turnover and mitochondrial fission-fusion, are demanding more attention in an oncological context. The next few years ought to be exciting.

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References

Abu-Gosh SE, Kolvazon N, Tirosh B, Ringel I, Yavin E (2009) Multiple triphenylphosphonium cations shuttle a hydrophilic peptide into mitochondria. Mol Pharm 6:1138–1144

Abu-Hamad S, Sivan S, Shoshan-Barmatz V (2006) The expression level of the voltage-dependent anion channel controls life and death of the cell. Proc Natl Acad Sci U S A 103:5787–5792

Abu-Hamad S, Zaid H, Israelson A, Nahon E, Shoshan-Barmatz V (2008) Hexokinase-I protection against apoptotic cell death is mediated via interaction with the voltage-dependent anion channel-1: mapping the site of binding. J Biol Chem 283:13482–13490

Abu-Hamad S, Arbel N, Calo D, Arzoine L, Israelson A, Keinan N, Ben-Romano R, Friedman O, Shoshan-Barmatz V (2009) The VDAC1 N-terminus is essential both for apoptosis and the protective effect of anti-apoptotic proteins. J Cell Sci 122:1906–1916

Adelsberger-Mangan DM, Colombini M (1987) Elimination and restoration of voltage dependence in the mitochondrial channel, VDAC, by graded modification with succinic anhydride. J Membr Biol 98:157–168

Altieri DC (2013a) Hsp90 regulation of mitochondrial protein folding: from organelle integrity to cellular homeostasis. Cell Mol Life Sci 70(14):2463–2472

Altieri DC (2013b) Mitochondrial HSP90s and tumor cell metabolism. Autophagy 9:244–245

Altieri DC, Stein GS, Lian JB, Languino LR (2012) TRAP-1, the mitochondrial Hsp90. Biochim Biophys Acta 1823:767–773

Anders MW (1995) Mitochondrial bioactivation of cysteine S-conjugates and 4-thiaalkanoates: implications for mitochondrial dysfunction and mitochondrial diseases. Biochim Biophys Acta 1271:51–57

Anders MW (2011) Putting bioactivation reactions to work: targeting antioxidants to mitochondria. Chem Biol Interact 192:8–13

Andreadou I, Iliodromitis EK, Koufaki M, Kremastinos DT (2008) Pharmacological pre- and post- conditioning agents: reperfusion-injury of the heart revisited. Mini Rev Med Chem 8:952–959

Angelin A, Bonaldo P, Bernardi P (2008) Altered threshold of the mitochondrial permeability transition pore in Ullrich congenital muscular dystrophy. Biochim Biophys Acta 1777: 893–896

Annis MG, Soucie EL, Dlugosz PJ, Cruz-Aguado JA, Penn LZ, Leber B, Andrews DW (2005) Bax forms multispanning monomers that oligomerize to permeabilize membranes during apoptosis. EMBO J 24:2096–2103

Arbel N, Shoshan-Barmatz V (2010) Voltage-dependent anion channel 1-based peptides interact with Bcl-2 to prevent antiapoptotic activity. J Biol Chem 285:6053–6062

Arbel N, Ben-Hail D, Shoshan-Barmatz V (2012) Mediation of the antiapoptotic activity of Bcl-xL protein upon interaction with VDAC1 protein. J Biol Chem 287:23152–23161

Arcangeli A, Crociani O, Lastraioli E, Masi A, Pillozzi S, Becchetti A (2009) Targeting ion channels in cancer: a novel frontier in antineoplastic therapy. Curr Med Chem 16:66–93

Arcangeli A, Pillozzi S, Becchetti A (2012) Targeting ion channels in leukemias: a new challenge for treatment. Curr Med Chem 19:683–696

Armstrong CM (2003) Voltage-gated K channels. Sci STKE 2003(188), re10.

Armstrong JS (2006) Mitochondria: a target for cancer therapy. Br J Pharmacol 147:239–248

Athar M, Back JH, Kopelovich L, Bickers DR, Kim AL (2009) Multiple molecular targets of resveratrol: anti-carcinogenic mechanisms. Arch Biochem Biophys 486:95–102

Atkinson J, Kapralov AA, Yanamala N, Tyurina YY, Amoscato AA, Pearce L, Peterson J, Huang Z, Jiang J, Samhan-Arias AK, Maeda A, Feng W, Wasserloos K, Belikova NA, Tyurin VA, Wang H, Fletcher J, Wang Y, Vlasova II, Klein-Seetharaman J, Stoyanovsky DA, Bayîr H, Pitt BR, Epperly MW, Greenberger JS, Kagan VE (2011) A mitochondria-targeted inhibitor of cytochrome c peroxidase mitigates radiation-induced death. Nat Commun 2:497

Aviv Y, Shaw J, Gang H, Kirshenbaum LA (2011) Regulation of autophagy in the heart: "you only live twice". Antioxid Redox Signal 14:2245–2250

Azarashvili T, Stricker R, Reiser G (2010) The mitochondria permeability transition pore complex in the brain with interacting proteins - promising targets for protection in neurodegenerative diseases. Biol Chem 391:619–629

Azzolin L, von Stockum S, Basso E, Petronilli V, Forte MA, Bernardi P (2010) The mitochondrial permeability transition from yeast to mammals. FEBS Lett 584:2504–2509

Azzolin L, Antolini N, Calderan A, Ruzza P, Sciacovelli M, Marin O, Mammi S, Bernardi P, Rasola A (2011) Antamanide, a derivative of Amanita phalloides, is a novel inhibitor of the mitochondrial permeability transition pore. PLoS One 6:e16280

Baines CP (2009a) The mitochondrial permeability transition pore and ischemia-reperfusion injury. Basic Res Cardiol 104:181–188

Baines CP (2009b) The molecular composition of the mitochondrial permeability transition pore. J Mol Cell Cardiol 46:850–857
Baines CP (2011) The mitochondrial permeability transition pore and the cardiac necrotic program. Pediatr Cardiol 32:258–262
Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, Brunskill EW, Sayen MR, Gottlieb RA, Dorn GW, Robbins J, Molkentin JD (2005) Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. Nature 434:658–662
Baines CP, Kaiser RA, Sheiko T, Craigen WJ, Molkentin JD (2007) Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. Nat Cell Biol 9:550–555
Balmain A, Barrett JC, Moses H, Renan MJ (1993) How many mutations are required for tumorigenesis? Implications for human cancer data. Mol Carcinog 7:139–146
Barbosa IA, Machado NG, Skildum AJ, Scott PM, Oliveira PJ (2012) Mitochondrial remodeling in cancer metabolism and survival: potential for new therapies. Biochim Biophys Acta 1826: 238–254
Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P (2005) Properties of the permeability transition pore in mitochondria devoid of cyclophilin D. J Biol Chem 280:18558–18561
Basso E, Petronilli V, Forte MA, Bernardi P (2008) Phosphate is essential for inhibition of the mitochondrial permeability transition pore by cyclosporin A and by cyclophilin D ablation. J Biol Chem 283:26307–26311
Báthori G, Szabó I, Schmehl I, Tombola F, Messina A, De Pinto V, Zoratti M (1998) Novel aspects of the electrophysiology of mitochondrial porin. Biochem Biophys Res Commun 243:258–263
Bauer AJ, Gieschler S, Lemberg KM, McDermott AE, Stockwell BR (2011) Functional model of metabolite gating by human voltage-dependent anion channel 2. Biochemistry 50:3408–3410
Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, Koteliansky V, Mootha VK (2011) Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature 476:341–345
Bayrhuber M, Meins T, Habeck M, Becker S, Giller K, Villinger S, Vonrhein C, Griesinger C, Zweckstetter M, Zeth K (2008) Structure of the human voltage-dependent anion channel. Proc Natl Acad Sci U S A 105:15370–15375
Bednarczyk P, Kowalczyk JE, Beresewicz M, Dołowy K, Szewczyk A, Zabłocka B (2010) Identification of a voltage-gated potassium channel in gerbil hippocampal mitochondria. Biochem Biophys Res Commun 397:614–620
Belikova NA, Jiang J, Stoyanovsky DA, Glumac A, Bayir H, Greenberger JS, Kagan VE (2009) Mitochondria-targeted (2-hydroxyamino-vinyl)-triphenyl-phosphonium releases NO(.) and protects mouse embryonic cells against irradiation-induced apoptosis. FEBS Lett 583:1945–1950
Belliere J, Devun F, Cottet-Rousselle C, Batandier C, Leverve X, Fontaine E (2012) Prerequisites for ubiquinone analogs to prevent mitochondrial permeability transition-induced cell death. J Bioenerg Biomembr 44:207–212
Benz R (1990) Biophysical properties of porin pores from mitochondrial outer membrane of eukaryotic cells. Experientia 46:131–137
Benz R, Brdiczka D (1992) The cation-selective substate of the mitochondrial outer membrane pore: single-channel conductance and influence on intermembrane and peripheral kinases. J Bioenerg Biomembr 24:33–39
Benz R, Kottke M, Brdiczka D (1990) The cationically selective state of the mitochondrial outer membrane pore: a study with intact mitochondria and reconstituted mitochondrial porin. Biochim Biophys Acta 1022:311–318
Beraud E, Chandy KG (2011) Therapeutic potential of peptide toxins that target ion channels. Inflamm Allergy Drug Targets 10:322–342
Bernardi P (1996) The permeability transition pore. Control points of a cyclosporin A-sensitive mitochondrial channel involved in cell death. Biochim Biophys Acta 1275:5–9
Bernardi P (2013) The mitochondrial permeability transition pore: a mystery solved? Front Physiol 4:95. doi:10.3389/fphys.2013.00095

Bernardi P, Bonaldo P (2008) Dysfunction of mitochondria and sarcoplasmic reticulum in the pathogenesis of collagen VI muscular dystrophies. Ann N Y Acad Sci 1147:303–311
Bernardi P, Petronilli V (1996) The permeability transition pore as a mitochondrial calcium release channel: a critical appraisal. J Bioenerg Biomembr 28:131–138
Bernardi P, Rasola A (2007) Calcium and cell death: the mitochondrial connection. Subcell Biochem 45:481–506
Bernardi P, von Stockum S (2012) The permeability transition pore as a Ca^{2+} release channel: new answers to an old question. Cell Calcium 52:22–27
Bernardi P, Colonna R, Costantini P, Eriksson O, Fontaine E, Ichas F, Massari S, Nicolli A, Petronilli V, Scorrano L (1998) The mitochondrial permeability transition. Biofactors 8:273–281
Bernardi P, Krauskopf A, Basso E, Petronilli V, Blachly-Dyson E, Di Lisa F, Forte MA (2006) The mitochondrial permeability transition from in vitro artifact to disease target. FEBS J 273:2077–2099
Bialik S, Kimchi A (2006) The death-associated protein kinases: structure, function, and beyond. Annu Rev Biochem 75:189–210
Biasutto L, Mattarei A, Marotta E, Bradaschia A, Sassi N, Garbisa S, Zoratti M, Paradisi C (2008) Development of mitochondria-targeted derivatives of resveratrol. Bioorg Med Chem Lett 18:5594–5597
Biasutto L, Dong LF, Zoratti M, Neuzil J (2010) Mitochondrially targeted anti-cancer agents. Mitochondrion 10:670–681. Erratum in: Mitochondrion (2011),11:996
Biasutto L, Szabò I, Zoratti M (2011) Mitochondrial effects of plant-made compounds. Antioxid Redox Signal 15:3039–3059
Bijur GN, Jope RS (2003) Rapid accumulation of Akt in mitochondria following phosphatidylinositol 3-kinase activation. J Neurochem 87:1427–1435
Billard C (2013) BH3 mimetics: status of the field and new developments. Mol Cancer Ther 12:1691–1700
Biswas S, Dodwadkar NS, Sawant RR, Koshkaryev A, Torchilin VP (2011) Surface modification of liposomes with rhodamine-123-conjugated polymer results in enhanced mitochondrial targeting. J Drug Target 19:552–561
Biswas S, Dodwadkar NS, Deshpande PP, Torchilin VP (2012a) Liposomes loaded with paclitaxel and modified with novel triphenylphosphonium-PEG-PE conjugate possess low toxicity, target mitochondria and demonstrate enhanced antitumor effects in vitro and in vivo. J Control Release 159:393–402
Biswas S, Dodwadkar NS, Piroyan A, Torchilin VP (2012b) Surface conjugation of triphenylphosphonium to target poly(amidoamine) dendrimers to mitochondria. Biomaterials 33:4773–4782
Blachly-Dyson E, Peng S, Colombini M, Forte M (1990) Selectivity changes in site-directed mutants of the VDAC ion channel: structural implications. Science 247:1233–1236
Blachly-Dyson E, Zambronicz EB, Yu WH, Adams V, McCabe ER, Adelman J, Colombini M, Forte M (1993) Cloning and functional expression in yeast of two human isoforms of the outer mitochondrial membrane channel, the voltage-dependent anion channel. J Biol Chem 268:1835–1841
Blagosklonny MV (2008) Aging: ROS or TOR. Cell Cycle 7:3344–3354
Boengler K, Heusch G, Schulz R (2011) Mitochondria in postconditioning. Antioxid Redox Signal 14:863–880
Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Bonnet S, Harry G, Hashimoto K, Porter CJ, Andrade MA, Thebaud B, Michelakis ED (2007) A mitochondria-K^+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 11:37–51
Brabant M, Baux L, Casimir R, Briand JP, Chaloin O, Porceddu M, Buron N, Chauvier D, Lassalle M, Lecoeur H, Langonné A, Dupont S, Déas O, Brenner C, Rebouillat D, Muller S, Borgne-Sanchez A, Jacotot E (2009) A flavivirus protein M-derived peptide directly permeabilizes mitochondrial membranes, triggers cell death and reduces human tumor growth in nude mice. Apoptosis 14:1190–1203
Brenner C, Moulin M (2012) Physiological roles of the permeability transition pore. Circ Res 111:1237–1247

Broekemeier KM, Dempsey ME, Pfeiffer DR (1989) Cyclosporin A is a potent inhibitor of the inner membrane permeability transition in liver mitochondria. J Biol Chem 264:7826–7830
Broekemeier KM, Klocek CK, Pfeiffer DR (1998) Proton selective substate of the mitochondrial permeability transition pore: regulation by the redox state of the electron transport chain. Biochemistry 37:13059–13065
Brustovetsky T, Li T, Yang Y, Zhang JT, Antonsson B, Brustovetsky N (2010) BAX insertion, oligomerization, and outer membrane permeabilization in brain mitochondria: role of permeability transition and SH-redox regulation. Biochim Biophys Acta 1797:1795–1806
Cahalan MD, Chandy KG (2009) The functional network of ion channels in T lymphocytes. Immunol Rev 231:59–87
Campello S, De Marchi U, Szabò I, Tombola F, Martinou JC, Zoratti M (2005) The properties of the mitochondrial megachannel in mitoplasts from human colon carcinoma cells are not influenced by Bax. FEBS Lett 579:3695–3700
Cardaci S, Desideri E, Ciriolo MR (2012) Targeting aerobic glycolysis: 3-bromopyruvate as a promising anticancer drug. J Bioenerg Biomembr 44:17–29
Cavalcanti BC, da Costa PM, Carvalho AA, Rodrigues FA, Amorim RC, Silva EC, Pohlit AM, Costa-Lotufo LV, Moraes MO, Pessoa C (2012) Involvement of intrinsic mitochondrial pathway in neosergeolide-induced apoptosis of human HL-60 leukemia cells: the role of mitochondrial permeability transition pore and DNA damage. Pharm Biol 50:980–993
Cavalieri E, Bergamini C, Mariotto S, Leoni S, Perbellini L, Darra E, Suzuki H, Fato R, Lenaz G (2009) Involvement of mitochondrial permeability transition pore opening in alpha-bisabolol induced apoptosis. FEBS J 276:3990–4000
Chalmers S, Caldwell ST, Quin C, Prime TA, James AM, Cairns AG, Murphy MP, McCarron JG, Hartley RC (2012) Selective uncoupling of individual mitochondria within a cell using a mitochondria-targeted photoactivated protonophore. J Am Chem Soc 134:758–761
Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, Schumacker PT (2000) Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O2 sensing. J Biol Chem 275:25130–25138
Chen LB (1988) Mitochondrial membrane potential in living cells. Annu Rev Cell Biol 4:155–181
Chen Y, Sánchez A, Rubio ME, Kohl T, Pardo LA, Stühmer W (2011) Functional K(v)10.1 channels localize to the inner nuclear membrane. PLoS One 6:e19257
Chen W, Feng L, Nie H, Zheng X (2012) Andrographolide induces autophagic cell death in human liver cancer cells through cyclophilin D-mediated mitochondrial permeability transition pore. Carcinogenesis 33:2190–2198
Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ (2003) VDAC2 inhibits BAK activation and mitochondrial apoptosis. Science 301:513–517
Cheng Y, Gulbins E, Siemen D (2011) Activation of the permeability transition pore by Bax via inhibition of the mitochondrial BK channel. Cell Physiol Biochem 27:191–200
Chernyak BV, Antonenko YN, Domnina LV, Ivanova OY, Lyamzaev KG, Pustovidko AV, Rokitskaya TI, Severina II, Simonyan RA, Trendeleva TA, Zvyagilskaya RA (2013) Novel penetrating cations for targeting mitochondria. Curr Pharm Des 19(15):2795–2806
Chi V, Pennington MW, Norton RS, Tarcha EJ, Londono LM, Sims-Fahey B, Upadhyay SK, Lakey JT, Iadonato S, Wulff H, Beeton C, Chandy KG (2012) Development of a sea anemone toxin as an immunomodulator for therapy of autoimmune diseases. Toxicon 59:529–546
Chiara F, Castellaro D, Marin O, Petronilli V, Brusilow WS, Juhaszova M, Sollott SJ, Forte M, Bernardi P, Rasola A (2008) Hexokinase II detachment from mitochondria triggers apoptosis through the permeability transition pore independent of voltage-dependent anion channels. PLoS One 3:e1852
Chiara F, Gambalunga A, Sciacovelli M, Nicolli A, Ronconi L, Fregona D, Bernardi P, Rasola A, Trevisan A (2012) Chemotherapeutic induction of mitochondrial oxidative stress activates

GSK-3α/β and Bax, leading to permeability transition pore opening and tumor cell death. Cell Death Dis 3:e444
Choi BH, Hahn SJ (2010) Kv1.3: a potential pharmacological target for diabetes. Acta Pharmacol Sin 31:1031–1035
Choudhary OP, Ujwal R, Kowallis W, Coalson R, Abramson J, Grabe M (2010) The electrostatics of VDAC: implications for selectivity and gating. J Mol Biol 396:580–592
Chung-man Ho J, Zheng S, Comhair SA, Farver C, Erzurum SC (2001) Differential expression of manganese superoxide dismutase and catalase in lung cancer. Cancer Res 61:8578–8585
Circu ML, Aw TY (2010) Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med 48:749–762
Colombini M (1979) A candidate for the permeability pathway of the outer mitochondrial membrane. Nature 279:643–645
Colombini M (1989) Voltage gating in the mitochondrial channel, VDAC. J Membr Biol 111: 103–111
Colombini M, Mannella CA (2012) VDAC, The early days. Biochim Biophys Acta 1818: 1438–1443
Costantini P, Jacotot E, Decaudin D, Kroemer G (2000) Mitochondrion as a novel target of anticancer chemotherapy. J Natl Cancer Inst 92:1042–1053
Cour M, Gomez L, Mewton N, Ovize M, Argaud L (2011) Postconditioning: from the bench to bedside. J Cardiovasc Pharmacol Ther 16:117–130
Crompton M, Ellinger H, Costi A (1988) Inhibition by cyclosporin A of a Ca^{2+}-dependent pore in heart mitochondria activated by inorganic phosphate and oxidative stress. Biochem J 255:357–360
D'Souza GG, Weissig V (2009) Subcellular targeting: a new frontier for drug-loaded pharmaceutical nanocarriers and the concept of the magic bullet. Expert Opin Drug Deliv 6:1135–1148
D'Souza GG, Rammohan R, Cheng SM, Torchilin VP, Weissig V (2003) DQAsome-mediated delivery of plasmid DNA toward mitochondria in living cells. J Control Release 92:189–197
D'Souza GG, Cheng SM, Boddapati SV, Horobin RW, Weissig V (2008) Nanocarrier-assisted subcellular targeting to the site of mitochondria improves the pro-apoptotic activity of paclitaxel. J Drug Target 16:578–585
Das S, Steenbergen C, Murphy E (2012) Does the voltage dependent anion channel modulate cardiac ischemia-reperfusion injury? Biochim Biophys Acta 1818:1451–1456
De Marchi U, Campello S, Szabò I, Tombola F, Martinou JC, Zoratti M (2004) Bax does not directly participate in the $Ca^{(2+)}$-induced permeability transition of isolated mitochondria. J Biol Chem 279:37415–37422
De Marchi U, Basso E, Szabò I, Zoratti M (2006) Electrophysiological characterization of the Cyclophilin D-deleted mitochondrial permeability transition pore. Mol Membr Biol 23:521–530
De Marchi U, Szabò I, Cereghetti GM, Hoxha P, Craigen WJ, Zoratti M (2008) A maxi-chloride channel in the inner membrane of mammalian mitochondria. Biochim Biophys Acta 1777:1438–1448
De Marchi U, Biasutto L, Garbisa S, Toninello A, Zoratti M (2009a) Quercetin can act either as an inhibitor or an inducer of the mitochondrial permeability transition pore: a demonstration of the ambivalent redox character of polyphenols. Biochim Biophys Acta 1787:1425–1432
De Marchi U, Sassi N, Fioretti B, Catacuzzeno L, Cereghetti GM, Szabò I, Zoratti M (2009b) Intermediate conductance Ca^{2+}-activated potassium channel (KCa3.1) in the inner mitochondrial membrane of human colon cancer cells. Cell Calcium 45:509–516
De Pinto V, Ludwig O, Krause J, Benz R, Palmieri F (1987) Porin pores of mitochondrial outer membranes from high and low eukaryotic cells: biochemical and biophysical characterization. Biochim Biophys Acta 894:109–119
De Stefani D, Raffaello A, Teardo E, Szabò I, Rizzuto R (2011) A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature 476:336–340
De Stefani D, Bononi A, Romagnoli A, Messina A, De Pinto V, Pinton P, Rizzuto R (2012) VDAC1 selectively transfers apoptotic Ca^{2+} signals to mitochondria. Cell Death Differ 19:267–273

Dejean LM, Ryu SY, Martinez-Caballero S, Teijido O, Peixoto PM, Kinnally KW (2010) MAC and Bcl-2 family proteins conspire in a deadly plot. Biochim Biophys Acta 1797:1231–1238

Deniaud A, Hoebeke J, Briand JP, Muller S, Jacotot E, Brenner C (2006) Peptido-targeting of the mitochondrial transition pore complex for therapeutic apoptosis induction. Curr Pharm Des 12:4501–4511

Di Lisa F, Bernardi P (2006) Mitochondria and ischemia-reperfusion injury of the heart: fixing a hole. Cardiovasc Res 70:191–199

Di Lisa F, Bernardi P (2009) A CaPful of mechanisms regulating the mitochondrial permeability transition. J Mol Cell Cardiol 46:775–780

Di Lisa F, Canton M, Menabò R, Dodoni G, Bernardi P (2003) Mitochondria and reperfusion injury. The role of permeability transition. Basic Res Cardiol 98:235–241

Di Lisa F, Kaludercic N, Carpi A, Menabò R, Giorgio M (2009) Mitochondrial pathways for ROS formation and myocardial injury: the relevance of p66(Shc) and monoamine oxidase. Basic Res Cardiol 104:131–139

Di Lisa F, Carpi A, Giorgio V, Bernardi P (2011a) The mitochondrial permeability transition pore and cyclophilin D in cardioprotection. Biochim Biophys Acta 1813:1316–1322

Di Lisa F, Canton M, Carpi A, Kaludercic N, Menabò R, Menazza S, Semenzato M (2011b) Mitochondrial injury and protection in ischemic pre- and postconditioning. Antioxid Redox Signal 14:881–891

Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, Morrison B 3rd, Stockwell BR (2012) Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 149:1060–1072

Dong LF, Jameson VJ, Tilly D, Cerny J, Mahdavian E, Marín-Hernández A, Hernández-Esquivel L, Rodríguez-Enríquez S, Stursa J, Witting PK, Stantic B, Rohlena J, Truksa J, Kluckova K, Dyason JC, Ledvina M, Salvatore BA, Moreno-Sánchez R, Coster MJ, Ralph SJ, Smith RA, Neuzil J (2011a) Mitochondrial targeting of vitamin E succinate enhances its pro-apoptotic and anti-cancer activity via mitochondrial complex II. J Biol Chem 286:3717–3728

Dong LF, Jameson VJ, Tilly D, Prochazka L, Rohlena J, Valis K, Truksa J, Zobalova R, Mahdavian E, Kluckova K, Stantic M, Stursa J, Freeman R, Witting PK, Norberg E, Goodwin J, Salvatore BA, Novotna J, Turanek J, Ledvina M, Hozak P, Zhivotovsky B, Coster MJ, Ralph SJ, Smith RA, Neuzil J (2011b) Mitochondrial targeting of α-tocopheryl succinate enhances its pro-apoptotic efficacy: a new paradigm for effective cancer therapy. Free Radic Biol Med 50:1546–1555

Dorn GW 2nd (2010a) Mechanisms of non-apoptotic programmed cell death in diabetes and heart failure. Cell Cycle 9:3442–3448

Dorn GW 2nd (2010b) Mitochondrial pruning by Nix and BNip3: an essential function for cardiac-expressed death factors. J Cardiovasc Transl Res 3:374–383

Dorn GW 2nd (2011) Nix nought nothing: fairy tale or real deal. J Mol Cell Cardiol 51:497–500

Du H, Yan SS (2010) Mitochondrial permeability transition pore in Alzheimer's disease: cyclophilin D and amyloid beta. Biochim Biophys Acta 1802:198–204

Dube H, Selwood D, Malouitre S, Capano M, Simone MI, Crompton M (2012) A mitochondrial-targeted cyclosporin A with high binding affinity for cyclophilin D yields improved cytoprotection of cardiomyocytes. Biochem J 441:901–907

Eisenberg-Lerner A, Kimchi A (2007) DAP kinase regulates JNK signaling by binding and activating protein kinase D under oxidative stress. Cell Death Differ 14:1908–1915

Elrod JW, Wong R, Mishra S, Vagnozzi RJ, Sakthievel B, Goonasekera SA, Karch J, Gabel S, Farber J, Force T, Brown JH, Murphy E, Molkentin JD (2010) Cyclophilin D controls mitochondrial pore-dependent Ca(2+) exchange, metabolic flexibility, and propensity for heart failure in mice. J Clin Invest 120:3680–3687

Felipe A, Bielanska J, Comes N, Vallejo A, Roig S, Ramón Y, Cajal S, Condom E, Hernández-Losa J, Ferreres JC (2012) Targeting the voltage-dependent K(+) channels Kv1.3 and Kv1.5 as tumor biomarkers for cancer detection and prevention. Curr Med Chem 19:661–674

Fiaschi T, Chiarugi P (2012) Oxidative stress, tumor microenvironment, and metabolic reprogramming: a diabolic liaison. Int J Cell Biol 2012:762825

Flescher E (2005) Jasmonates–a new family of anti-cancer agents. Anticancer Drugs 16:911–916
Flescher E (2007) Jasmonates in cancer therapy. Cancer Lett 245:1–10
Flierl A, Jackson C, Cottrell B, Murdock D, Seibel P, Wallace DC (2003) Targeted delivery of DNA to the mitochondrial compartment via import sequence-conjugated peptide nucleic acid. Mol Ther 7:550–557
Fontaine E, Bernardi P (1999) Progress on the mitochondrial permeability transition pore: regulation by complex I and ubiquinone analogs. J Bioenerg Biomembr 31:335–345
Forte M, Bernardi P (2005) Genetic dissection of the permeability transition pore. J Bioenerg Biomembr 37:121–128
Fournier N, Ducet G, Crevat A (1987) Action of cyclosporine on mitochondrial calcium fluxes. J Bioenerg Biomembr 19:297–303
Friberg H, Wieloch T (2002) Mitochondrial permeability transition in acute neurodegeneration. Biochimie 84:241–250
Fulda S, Galluzzi L, Kroemer G (2010) Targeting mitochondria for cancer therapy. Nat Rev Drug Discov 9:447–464
Funakoshi T, Aki T, Nakayama H, Watanuki Y, Imori S, Uemura K (2011) Reactive oxygen species-independent rapid initiation of mitochondrial apoptotic pathway by chelerythrine. Toxicol In Vitro 25:1581–1587
Furuno T, Kanno T, Arita K, Asami M, Utsumi T, Doi Y, Inoue M, Utsumi K (2001) Roles of long chain fatty acids and carnitine in mitochondrial membrane permeability transition. Biochem Pharmacol 62:1037–1046
Gall JM, Wong V, Pimental DR, Havasi A, Wang Z, Pastorino JG, Bonegio RG, Schwartz JH, Borkan SC (2011) Hexokinase regulates Bax-mediated mitochondrial membrane injury following ischemic stress. Kidney Int 79:1207–1216
Galluzzi L, Kepp O, Tajeddine N, Kroemer G (2008) Disruption of the hexokinase-VDAC complex for tumor therapy. Oncogene 27:4633–4635
Garcia-Dorado D, Ruiz-Meana M, Inserte J, Rodriguez-Sinovas A, Piper HM (2012) Calcium-mediated cell death during myocardial reperfusion. Cardiovasc Res 94:168–180
Garlid KD, Costa AD, Quinlan CL, Pierre SV, Dos Santos P (2009) Cardioprotective signaling to mitochondria. J Mol Cell Cardiol 46:858–866
Gazula VR, Strumbos JG, Mei X, Chen H, Rahner C, Kaczmarek LK (2010) Localization of Kv1.3 channels in presynaptic terminals of brainstem auditory neurons. J Comp Neurol 518:3205–3220
Geula S, Naveed H, Liang J, Shoshan-Barmatz V (2012) Structure-based analysis of VDAC1 protein: defining oligomer contact sites. J Biol Chem 287:2179–2190
Ghibelli L, Diederich M (2010) Multistep and multitask Bax activation. Mitochondrion 10:604–613
Gincel D, Zaid H, Shoshan-Barmatz V (2001) Calcium binding and translocation by the voltage-dependent anion channel: a possible regulatory mechanism in mitochondrial function. Biochem J 358:147–155
Giorgio V, Bisetto E, Soriano ME, Dabbeni-Sala F, Basso E, Petronilli V, Forte MA, Bernardi P, Lippe G (2009) Cyclophilin D modulates mitochondrial F0F1-ATP synthase by interacting with the lateral stalk of the complex. J Biol Chem 284:33982–33988
Giorgio V, Soriano ME, Basso E, Bisetto E, Lippe G, Forte MA, Bernardi P (2010) Cyclophilin D in mitochondrial pathophysiology. Biochim Biophys Acta 1797:1113–1118
Giorgio V, von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M, Glick GD, Petronilli V, Zoratti M, Szabó I, Lippe G, Bernardi P (2013) Dimers of mitochondrial ATP synthase form the permeability transition pore. Proc Natl Acad Sci U S A 110:5887–5892
Gogvadze V (2011) Targeting mitochondria in fighting cancer. Curr Pharm Des 17:4034–4046
Gokul S, Patil V, Jailkhani R, Hallikeri K, Kattappagari K (2010) Oxidant-antioxidant status in blood and tumor tissue of oral squamous cell carcinoma patients. Oral Dis 16:29–33
Gomez L, Thibault H, Gharib A, Dumont JM, Vuagniaux G, Scalfaro P, Derumeaux G, Ovize M (2007) Inhibition of mitochondrial permeability transition improves functional recovery and reduces mortality following acute myocardial infarction in mice. Am J Physiol Heart Circ Physiol 293:H1654–H1661

Gottlieb RA, Gustafsson AB (2011) Mitochondrial turnover in the heart. Biochim Biophys Acta 1813:1295–1301
Gottlieb RA, Mentzer RM Jr, Linton PJ (2011) Impaired mitophagy at the heart of injury. Autophagy 7:1573–1574
Gouriou Y, Demaurex N, Bijlenga P, De Marchi U (2011) Mitochondrial calcium handling during ischemia-induced cell death in neurons. Biochimie 93:2060–2067
Griffiths EJ (2012) Mitochondria and heart disease. Adv Exp Med Biol 942:249–267
Grills C, Jithesh PV, Blayney J, Zhang SD, Fennell DA (2011) Gene expression meta-analysis identifies VDAC1 as a predictor of poor outcome in early stage non-small cell lung cancer. PLoS One 6:e14635
Grimm S, Brdiczka D (2007) The permeability transition pore in cell death. Apoptosis 12:841–855
Groeger G, Quiney C, Cotter TG (2009) Hydrogen peroxide as a cell-survival signaling molecule. Antioxid Redox Signal 11:2655–2671
Grumati P, Coletto L, Sabatelli P, Cescon M, Angelin A, Bertaggia E, Blaauw B, Urciuolo A, Tiepolo T, Merlini L, Maraldi NM, Bernardi P, Sandri M, Bonaldo P (2010) Autophagy is defective in collagen VI muscular dystrophies, and its reactivation rescues myofiber degeneration. Nat Med 16:1313–1320
Gulbins E, Sassi N, Grassmè H, Zoratti M, Szabò I (2010) Role of Kv1.3 mitochondrial potassium channel in apoptotic signalling in lymphocytes. Biochim Biophys Acta 1797:1251–1259
Günther S, Ruhe C, Derikito MG, Böse G, Sauer H, Wartenberg M (2007) Polyphenols prevent cell shedding from mouse mammary cancer spheroids and inhibit cancer cell invasion in confrontation cultures derived from embryonic stem cells. Cancer Lett 250:25–35
Gutman GA, Chandy KG, Adelman JP, Aiyar J, Bayliss DA, Clapham DE, Covarriubias M, Desir GV, Furuichi K, Ganetzky B, Garcia ML, Grissmer S, Jan LY, Karschin A, Kim D, Kuperschmidt S, Kurachi Y, Lazdunski M, Lesage F, Lester HA, McKinnon D, Nichols CG, O'Kelly I, Robbins J, Robertson GA, Rudy B, Sanguinetti M, Seino S, Stuehmer W, Tamkun MM, Vandenberg CA, Wei A, Wulff H, Wymore RS, International Union of Pharmacology (2003) International Union of Pharmacology. XLI. Compendium of voltage-gated ion channels: potassium channels. Pharmacol Rev 55:583–586
Guzy RD, Schumacker PT (2006) Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. Exp Physiol 91:807–819
Hail N Jr, Lotan R (2009) Cancer chemoprevention and mitochondria: targeting apoptosis in transformed cells via the disruption of mitochondrial bioenergetics/redox state. Mol Nutr Food Res 53:49–67
Hajnóczky G, Csordás G, Yi M (2002) Old players in a new role: mitochondria-associated membranes, VDAC, and ryanodine receptors as contributors to calcium signal propagation from endoplasmic reticulum to the mitochondria. Cell Calcium 32:363–377
Halestrap AP (2009) What is the mitochondrial permeability transition pore? J Mol Cell Cardiol 46:821–831
Halestrap AP (2010) A pore way to die: the role of mitochondria in reperfusion injury and cardioprotection. Biochem Soc Trans 38:841–860
Halestrap AP, Brenner C (2003) The adenine nucleotide translocase: a central component of the mitochondrial permeability transition pore and key player in cell death. Curr Med Chem 10:1507–1525
Halestrap AP, Pasdois P (2009) The role of the mitochondrial permeability transition pore in heart disease. Biochim Biophys Acta 1787:1402–1415
Halestrap AP, Connern CP, Griffiths EJ, Kerr PM (1997) Cyclosporin A binding to mitochondrial cyclophilin inhibits the permeability transition pore and protects hearts from ischaemia/reperfusion injury. Mol Cell Biochem 174:167–172
Halestrap AP, McStay GP, Clarke SJ (2002) The permeability transition pore complex: another view. Biochimie 84:153–166
Halestrap AP, Clarke SJ, Javadov SA (2004) Mitochondrial permeability transition pore opening during myocardial reperfusion–a target for cardioprotection. Cardiovasc Res 61:372–385

Halestrap AP, Clarke SJ, Khaliulin I (2007) The role of mitochondria in protection of the heart by preconditioning. Biochim Biophys Acta 1767:1007–1031
Halliwell B (2008) Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? Arch Biochem Biophys 476:107–112
Han D, Antunes F, Canali R, Rettori D, Cadenas E (2003) Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. J Biol Chem 278:5557–5563
Han W, Li L, Qiu S, Lu Q, Pan Q, Gu Y, Luo J, Hu X (2007) Shikonin circumvents cancer drug resistance by induction of a necroptotic death. Mol Cancer Ther 6:1641–1649
Han W, Xie J, Li L, Liu Z, Hu X (2009) Necrostatin-1 reverts shikonin-induced necroptosis to apoptosis. Apoptosis 14:674–686
Haridas V, Li X, Mizumachi T, Higuchi M, Lemeshko VV, Colombini M, Gutterman JU (2007) Avicins, a novel plant-derived metabolite lowers energy metabolism in tumor cells by targeting the outer mitochondrial membrane. Mitochondrion 7:234–240
Hartman ML, Czyz M (2012) Pro-apoptotic activity of BH3-only proteins and BH3 mimetics: from theory to potential cancer therapy. Anticancer Agents Med Chem 12:966–981
Hausenloy DJ, Duchen MR, Yellon DM (2003) Inhibiting mitochondrial permeability transition pore opening at reperfusion protects against ischaemia-reperfusion injury. Cardiovasc Res 60:617–625
Hausenloy D, Wynne A, Duchen M, Yellon D (2004) Transient mitochondrial permeability transition pore opening mediates preconditioning-induced protection. Circulation 109:1714–1717
Hausenloy DJ, Ong SB, Yellon DM (2009) The mitochondrial permeability transition pore as a target for preconditioning and postconditioning. Basic Res Cardiol 104:189–202
Hausenloy DJ, Lim SY, Ong SG, Davidson SM, Yellon DM (2010) Mitochondrial cyclophilin-D as a critical mediator of ischaemic preconditioning. Cardiovasc Res 88:67–74
Hausenloy DJ, Lecour S, Yellon DM (2011) Reperfusion injury salvage kinase and survivor activating factor enhancement prosurvival signaling pathways in ischemic postconditioning: two sides of the same coin. Antioxid Redox Signal 14:893–907
Hausenloy DJ, Boston-Griffiths EA, Yellon DM (2012) Cyclosporin A and cardioprotection: from investigative tool to therapeutic agent. Br J Pharmacol 165:1235–1245
Haworth RA, Hunter DR (1979) The Ca^{2+}-induced membrane transition in mitochondria. II. Nature of the Ca2+ trigger site. Arch Biochem Biophys 195:460–467
Haworth RA, Hunter DR (1980) Allosteric inhibition of the Ca^{2+}-activated hydrophilic channel of the mitochondrial inner membrane by nucleotides. J Membr Biol 54:231–236
Haworth RA, Hunter DR (2000) Control of the mitochondrial permeability transition pore by high-affinity ADP binding at the ADP/ATP translocase in permeabilized mitochondria. J Bioenerg Biomembr 32:91–96
Heerdt BG, Houston MA, Augenlicht LH (2005) The intrinsic mitochondrial membrane potential of colonic carcinoma cells is linked to the probability of tumor progression. Cancer Res 65:9861–9867
Hiller S, Garces RG, Malia TJ, Orekhov VY, Colombini M, Wagner G (2008) Solution structure of the integral human membrane protein VDAC-1 in detergent micelles. Science 321:1206–1210
Hinsch KD, De Pinto V, Aires VA, Schneider X, Messina A, Hinsch E (2004) Voltage-dependent anion-selective channels VDAC2 and VDAC3 are abundant proteins in bovine outer dense fibers, a cytoskeletal component of the sperm flagellum. J Biol Chem 279:15281–15288
Hodge T, Colombini M (1997) Regulation of metabolite flux through voltage-gating of VDAC channels. J Membr Biol 157:271–279
Horton KL, Pereira MP, Stewart KM, Fonseca SB, Kelley SO (2012) Tuning the activity of mitochondria-penetrating peptides for delivery or disruption. Chembiochem 13:476–485
Hoye AT, Davoren JE, Wipf P, Fink MP, Kagan VE (2008) Targeting mitochondria. Acc Chem Res 41:87–97
Hunter DR, Haworth RA (1979a) The Ca2+-induced membrane transition in mitochondria. I. The protective mechanisms. Arch Biochem Biophys 195:453–459

Hunter DR, Haworth RA (1979b) The Ca2+-induced membrane transition in mitochondria. III. Transitional Ca2+ release. Arch Biochem Biophys 195:468–477

Hyoudou K, Nishikawa M, Kobayashi Y, Ikemura M, Yamashita F, Hashida M (2008) SOD derivatives prevent metastatic tumor growth aggravated by tumor removal. Clin Exp Metastasis 25:531–536

Hyoudou K, Nishikawa M, Ikemura M, Kobayashi Y, Mendelsohn A, Miyazaki N, Tabata Y, Yamashita F, Hashida M (2009) Prevention of pulmonary metastasis from subcutaneous tumors by binary system based sustained delivery of catalase. J Control Release 137:110–115

Irwin WA, Bergamin N, Sabatelli P, Reggiani C, Megighian A, Merlini L, Braghetta P, Columbaro M, Volpin D, Bressan GM, Bernardi P, Bonaldo P (2003) Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. Nat Genet 35:367–371

Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H, Nakada K, Honma Y, Hayashi J (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 320:661–664

Israelson A, Abu-Hamad S, Zaid H, Nahon E, Shoshan-Barmatz V (2007) Localization of the voltage-dependent anion channel-1 Ca2+-binding sites. Cell Calcium 41:235–244

Jain MV, Paczulla AM, Klonisch T, Dimgba FN, Rao SB, Roberg K, Schweizer F, Lengerke C, Davoodpour P, Palicharla VR, Maddika S, Los M (2013) Interconnections between apoptotic, autophagic and necrotic pathways: implications for cancer therapy development. J Cell Mol Med 17(1):12–29

Jamison JM, Gilloteaux J, Taper HS, Calderon PB, Summers JL (2002) Autoschizis: a novel cell death. Biochem Pharmacol 63:1773–1783

Javadov S, Karmazyn M (2007) Mitochondrial permeability transition pore opening as an endpoint to initiate cell death and as a putative target for cardioprotection. Cell Physiol Biochem 20:1–22

Javadov SA, Clarke S, Das M, Griffiths EJ, Lim KH, Halestrap AP (2003) Ischaemic preconditioning inhibits opening of mitochondrial permeability transition pores in the reperfused rat heart. J Physiol 549:513–524

Javadov S, Karmazyn M, Escobales N (2009) Mitochondrial permeability transition pore opening as a promising therapeutic target in cardiac diseases. J Pharmacol Exp Ther 330:670–678

Javadov S, Hunter JC, Barreto-Torres G, Parodi-Rullan R (2011) Targeting the mitochondrial permeability transition: cardiac ischemia-reperfusion versus carcinogenesis. Cell Physiol Biochem 27:179–190

Jezek P, Plecitá-Hlavatá L, Smolková K, Rossignol R (2010) Distinctions and similarities of cell bioenergetics and the role of mitochondria in hypoxia, cancer, and embryonic development. Int J Biochem Cell Biol 42:604–622

Jiang S, Zu Y, Wang Z, Zhang Y, Fu Y (2011) Involvement of mitochondrial permeability transition pore opening in 7-xylosyl-10-deacetylpaclitaxel-induced apoptosis. Planta Med 77: 1005–1012

Joiner ML, Koval OM, Li J, He BJ, Allamargot C, Gao Z, Luczak ED, Hall DD, Fink BD, Chen B, Yang J, Moore SA, Scholz TD, Strack S, Mohler PJ, Sivitz WI, Song LS, Anderson ME (2012) CaMKII determines mitochondrial stress responses in heart. Nature 491:269–273

Jones S, Martel C, Belzacq-Casagrande AS, Brenner C, Howl J (2008) Mitoparan and target-selective chimeric analogues: membrane translocation and intracellular redistribution induces mitochondrial apoptosis. Biochim Biophys Acta 1783:849–863

Jordán J, Galindo MF, Tornero D, González-García C, Ceña V (2004) Bcl-x L blocks mitochondrial multiple conductance channel activation and inhibits 6-OHDA-induced death in SH-SY5Y cells. J Neurochem 89:124–133

Juhaszova M, Wang S, Zorov DB, Nuss HB, Gleichmann M, Mattson MP, Sollott SJ (2008) The identity and regulation of the mitochondrial permeability transition pore: where the known meets the unknown. Ann N Y Acad Sci 1123:197–212

Ka H, Park HJ, Jung HJ, Choi JW, Cho KS, Ha J, Lee KT (2003) Cinnamaldehyde induces apoptosis by ROS-mediated mitochondrial permeability transition in human promyelocytic leukemia HL-60 cells. Cancer Lett 196:143–152

Kalogeris T, Baines CP, Krenz M, Korthuis RJ (2012) Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol 298:229–317

Kang BH (2012) TRAP1 regulation of mitochondrial life or death decision in cancer cells and mitochondria-targeted TRAP1 inhibitors. BMB Rep 45:1–6

Katoh I, Tomimori Y, Ikawa Y, Kurata S (2004) Dimerization and processing of procaspase-9 by redox stress in mitochondria. J Biol Chem 279:15515–15523

Kelley SO, Stewart KM, Mourtada R (2011) Development of novel peptides for mitochondrial drug delivery: amino acids featuring delocalized lipophilic cations. Pharm Res 28: 2808–2819

Kerner J, Lee K, Tandler B, Hoppel CL (2012) VDAC proteomics: post-translation modifications. Biochim Biophys Acta 1818:1520–1525

Kim YS, Schwabe RF, Qian T, Lemasters JJ, Brenner DA (2002) TRAIL-mediated apoptosis requires NF-kappaB inhibition and the mitochondrial permeability transition in human hepatoma cells. Hepatology 36:1498–1508

Kim I, Rodriguez-Enriquez S, Lemasters JJ (2007) Selective degradation of mitochondria by mitophagy. Arch Biochem Biophys 462:245–253

Kim JS, Wang JH, Lemasters JJ (2012) Mitochondrial permeability transition in rat hepatocytes after anoxia/reoxygenation: role of Ca2+-dependent mitochondrial formation of reactive oxygen species. Am J Physiol Gastrointest Liver Physiol 302:G723–G731

Kinnally KW, Peixoto PM, Ryu SY, Dejean LM (2011) Is mPTP the gatekeeper for necrosis, apoptosis, or both? Biochim Biophys Acta 1813:616–622

Klein D, Ribeiro MM, Mendoza V, Jayaraman S, Kenyon NS, Pileggi A, Molano RD, Inverardi L, Ricordi C, Pastori RL (2004) Delivery of Bcl-XL or its BH4 domain by protein transduction inhibits apoptosis in human islets. Biochem Biophys Res Commun 323:473–478

Ko YH, Smith BL, Wang Y, Pomper MG, Rini DA, Torbenson MS, Hullihen J, Pedersen PL (2004) Advanced cancers: eradication in all cases using 3-bromopyruvate therapy to deplete ATP. Biochem Biophys Res Commun 324:269–275

Ko YH, Verhoeven HA, Lee MJ, Corbin DJ, Vogl TJ, Pedersen PL (2012) A translational study "case report" on the small molecule "energy blocker" 3-bromopyruvate (3BP) as a potent anticancer agent: from bench side to bedside. J Bioenerg Biomembr 44:163–170

Kobliakov VA (2010) Mechanisms of tumor promotion by reactive oxygen species. Biochemistry (Mosc) 75:675–685

Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP, MacGregor GR, Wallace DC (2004) The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. Nature 427:461–465

Kolevzon N, Kuflik U, Shmuel M, Benhamron S, Ringel I, Yavin E (2011) Multiple triphenylphosphonium cations as a platform for the delivery of a pro-apoptotic peptide. Pharm Res 28:2780–2789

Kongara S, Karantza V (2012) The interplay between autophagy and ROS in tumorigenesis. Front Oncol 2:171

Korde AS, Yadav VR, Zheng YM, Wang YX (2011) Primary role of mitochondrial Rieske iron-sulfur protein in hypoxic ROS production in pulmonary artery myocytes. Free Radic Biol Med 50:945–952

Koren I, Raviv Z, Shoshan-Barmatz V (2010) Downregulation of voltage-dependent anion channel-1 expression by RNA interference prevents cancer cell growth in vivo. Cancer Biol Ther 9:1046–1052

Kowaltowski AJ, Castilho RF, Vercesi AE (2001) Mitochondrial permeability transition and oxidative stress. FEBS Lett 495:12–15

Krauskopf A, Eriksson O, Craigen WJ, Forte MA, Bernardi P (2006) Properties of the permeability transition in VDAC1$^{-/-}$ mitochondria. Biochim Biophys Acta 1757:590–595

Krestinina OV, Grachev DE, Odinokova IV, Reiser G, Evtodienko YV, Azarashvili TS (2009) Effect of peripheral benzodiazepine receptor (PBR/TSPO) ligands on opening of Ca^{2+}-induced pore and phosphorylation of 3.5-kDa polypeptide in rat brain mitochondria. Biochemistry (Moscow) 74:421–429

Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke EH, Blagosklonny MV, El-Deiry WS, Golstein P, Green DR, Hengartner M, Knight RA, Kumar S, Lipton SA, Malorni W, Nunez G, Peter ME, Tschopp J, Yuan J, Piacentini M, Zhivotovsky B, Melino G (2009) Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ 16:3–11

Kumarswamy R, Chandna S (2009) Putative partners in Bax mediated cytochrome-c release: ANT, CypD, VDAC or none of them? Mitochondrion 9:1–8

Kundu JK, Surh YJ (2008) Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. Cancer Lett 269:243–261

Kwon WS, Park YJ, Mohamed ES, Pang MG (2013) Voltage-dependent anion channels are a key factor of male fertility. Fertil Steril 99(2):354–361

Landete JM (2012) Updated knowledge about polyphenols: functions, bioavailability, metabolism, and health. Crit Rev Food Sci Nutr 52:936–948

Lang F, Uhlemann AC, Lepple-Wienhues A, Szabo I, Siemen D, Nilius B, Gulbins E (1999) Cell volume regulatory mechanisms in apoptotic cell death. Herz 24:232–235

Lang F, Gulbins E, Szabo I, Lepple-Wienhues A, Huber SM, Duranton C, Lang KS, Lang PA, Wieder T (2004) Cell volume and the regulation of apoptotic cell death. J Mol Recognit 17:473–480

Larochette N, Decaudin D, Jacotot E, Brenner C, Marzo I, Susin SA, Zamzami N, Xie Z, Reed J, Kroemer G (1999) Arsenite induces apoptosis via a direct effect on the mitochondrial permeability transition pore. Exp Cell Res 249:413–421

Le Quoc K, Le Quoc D (1988) Involvement of the ADP/ATP carrier in calcium-induced perturbations of the mitochondrial inner membrane permeability: importance of the orientation of the nucleotide binding site. Arch Biochem Biophys 265:249–257

Leanza L, Henry B, Sassi N, Zoratti M, Chandy KG, Gulbins E, Szabò I (2012a) Inhibitors of mitochondrial Kv1.3 channels induce Bax/Bak-independent death of cancer cells. EMBO Mol Med 4:577–593

Leanza L, Zoratti M, Gulbins E, Szabò I (2012b) Induction of apoptosis in macrophages via Kv1.3 and Kv1.5 potassium channels. Curr Med Chem 19:5394–5404

Leanza L, Trentin L, Becker KA, Frezzato F, Zoratti M, Semenzato G, Gulbins E, Szabo I (2013) Clofazimine, Psora-4 and PAP-1, inhibitors of the potassium channel Kv1.3, as a new and selective therapeutic strategy in chronic lymphocytic leukemia. Leukemia 27:1782–1785

Leber B, Geng F, Kale J, Andrews DW (2010) Drugs targeting Bcl-2 family members as an emerging strategy in cancer. Expert Rev Mol Med 12:e28

Lecoeur H, Borgne-Sanchez A, Chaloin O, El-Khoury R, Brabant M, Langonné A, Porceddu M, Brière JJ, Buron N, Rebouillat D, Péchoux C, Deniaud A, Brenner C, Briand JP, Muller S, Rustin P, Jacotot E (2012) HIV-1 Tat protein directly induces mitochondrial membrane permeabilization and inactivates cytochrome c oxidase. Cell Death Dis 3:e282

Lee SY, MacKinnon R (2004) A membrane-access mechanism of ion channel inhibition by voltage sensor toxins from spider venom. Nature 430:232–235

Lee EL, Hasegawa Y, Shimizu T, Okada Y (2008) IK1 channel activity contributes to cisplatin sensitivity of human epidermoid cancer cells. Am J Physiol Cell Physiol 294:C1398–C1406

Lee J, Boo JH, Ryu H (2009) The failure of mitochondria leads to neurodegeneration: do mitochondria need a jump start? Adv Drug Deliv Rev 30:1316–1323

Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, Crowe RA, Cascio WE, Bradham CA, Brenner DA, Herman B (1998) The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. Biochim Biophys Acta 1366:177–196

Lemasters JJ, Theruvath TP, Zhong Z, Nieminen AL (2009) Mitochondrial calcium and the permeability transition in cell death. Biochim Biophys Acta 1787:1395–1401

Lena A, Rechichi M, Salvetti A, Bartoli B, Vecchio D, Scarcelli V, Amoroso R, Benvenuti L, Gagliardi R, Gremigni V, Rossi L (2009) Drugs targeting the mitochondrial pore act as cytotoxic and cytostatic agents in temozolomide-resistant glioma cells. J Transl Med 7:13

Leung AW, Halestrap AP (2008) Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore. Biochim Biophys Acta 1777:946–952
Li L, Han W, Gu Y, Qiu S, Lu Q, Jin J, Luo J, Hu X (2007) Honokiol induces a necrotic cell death through the mitochondrial permeability transition pore. Cancer Res 67:4894–4903
Li T, Brustovetsky T, Antonsson B, Brustovetsky N (2008) Oligomeric BAX induces mitochondrial permeability transition and complete cytochrome c release without oxidative stress. Biochim Biophys Acta 1777:1409–1421
Li T, Brustovetsky T, Antonsson B, Brustovetsky N (2010) Dissimilar mechanisms of cytochrome c release induced by octyl glucoside-activated BAX and by BAX activated with truncated BID. Biochim Biophys Acta 1797:52–62
Li B, Chauvin C, De Paulis D, De Oliveira F, Gharib A, Vial G, Lablanche S, Leverve X, Bernardi P, Ovize M, Fontaine E (2012) Inhibition of complex I regulates the mitochondrial permeability transition through a phosphate-sensitive inhibitory site masked by cyclophilin D. Biochim Biophys Acta 1817:1628–1634
Liem DA, Honda HM, Zhang J, Woo D, Ping P (2007) Past and present course of cardioprotection against ischemia-reperfusion injury. J Appl Physiol 103:2129–2136
López-Lázaro M (2010) A new view of carcinogenesis and an alternative approach to cancer therapy. Mol Med 16:144–153
Lustgarten MS, Bhattacharya A, Muller FL, Jang YC, Shimizu T, Shirasawa T, Richardson A, Van Remmen H (2012) Complex I generated, mitochondrial matrix-directed superoxide is released from the mitochondria through voltage dependent anion channels. Biochem Biophys Res Commun 422:515–521
Lyamzaev KG, Pustovidko AV, Simonyan RA, Rokitskaya TI, Domnina LV, Ivanova OY, Severina II, Sumbatyan NV, Korshunova GA, Tashlitsky VN, Roginsky VA, Antonenko YN, Skulachev MV, Chernyak BV, Skulachev VP (2011) Novel mitochondria-targeted antioxidants: plastoquinone conjugated with cationic plant alkaloids berberine and palmatine. Pharm Res 28:2883–2895
Lyrawati D, Trounson A, Cram D (2011) Expression of GFP in the mitochondrial compartment using DQAsome-mediated delivery of an artificial mini-mitochondrial genome. Pharm Res 28:2848–2862
Machida K, Ohta Y, Osada H (2006) Suppression of apoptosis by cyclophilin D via stabilization of hexokinase II mitochondrial binding in cancer cells. J Biol Chem 281:14314–14320
Maciel EN, Vercesi AE, Castilho RF (2001) Oxidative stress in Ca^{2+}-induced membrane permeability transition in brain mitochondria. J Neurochem 79:1237–1245
Mader A, Abu-Hamad S, Arbel N, Gutiérrez-Aguilar M, Shoshan-Barmatz V (2010) Dominant-negative VDAC1 mutants reveal oligomeric VDAC1 to be the active unit in mitochondria-mediated apoptosis. Biochem J 429:147–155
Malia TJ, Wagner G (2007) NMR structural investigation of the mitochondrial outer membrane protein VDAC and its interaction with antiapoptotic Bcl-xL. Biochemistry 46:514–525
Malouitre S, Dube H, Selwood D, Crompton M (2009) Mitochondrial targeting of cyclosporin A enables selective inhibition of cyclophilin-D and enhanced cytoprotection after glucose and oxygen deprivation. Biochem J 425:137–148
Marchi S, Lupini L, Patergnani S, Rimessi A, Missiroli S, Bonora M, Bononi A, Corrà F, Giorgi C, De Marchi E, Poletti F, Gafà R, Lanza G, Negrini M, Rizzuto R, Pinton P (2013) Downregulation of the mitochondrial calcium uniporter by cancer-related miR-25. Curr Biol 23:58–63
Marrache S, Dhar S (2012) Engineering of blended nanoparticle platform for delivery of mitochondria-acting therapeutics. Proc Natl Acad Sci U S A 109:16288–16293
Martin LJ (2010a) Mitochondrial pathobiology in Parkinson's disease and amyotrophic lateral sclerosis. J Alzheimers Dis 20(Suppl 2):S335–S356
Martin LJ (2010b) Mitochondrial and cell death mechanisms in neurodegenerative diseases. Pharmaceuticals (Basel) 3:839–915
Martin LJ (2010c) The mitochondrial permeability transition pore: a molecular target for amyotrophic lateral sclerosis therapy. Biochim Biophys Acta 1802:186–197
Martin LJ (2011) Mitochondrial pathobiology in ALS. J Bioenerg Biomembr 43:569–579

Martin LJ (2012) Biology of mitochondria in neurodegenerative diseases. Prog Mol Biol Transl Sci 107:355–415

Martinez-Caballero S, Dejean LM, Kinnally MS, Oh KJ, Mannella CA, Kinnally KW (2009) Assembly of the mitochondrial apoptosis-induced channel, MAC. J Biol Chem 284: 12235–12245

Martinucci S, Szabò I, Tombola F, Zoratti M (2000) Ca^{2+}-reversible inhibition of the mitochondrial megachannel by ubiquinone analogues. FEBS Lett 480:89–94

Matassa DS, Amoroso MR, Maddalena F, Landriscina M, Esposito F (2012) New insights into TRAP1 pathway. Am J Cancer Res 2:235–248

Mathupala SP, Pedersen PL (2010) Voltage dependent anion channel-1 (VDAC-1) as an anti-cancer target. Cancer Biol Ther 9:1053–1056

Mattarei A, Biasutto L, Marotta E, De Marchi U, Sassi N, Garbisa S, Zoratti M, Paradisi C (2008) A mitochondriotropic derivative of quercetin: a strategy to increase the effectiveness of polyphenols. Chembiochem 9:2633–2642

McCloskey C, Jones S, Amisten S, Snowden RT, Kaczmarek LK, Erlinge D, Goodall AH, Forsythe ID, Mahaut-Smith MP (2010) Kv1.3 is the exclusive voltage-gated K+ channel of platelets and megakaryocytes: roles in membrane potential, Ca^{2+} signalling and platelet count. J Physiol 588:1399–1406

McCommis KS, Baines CP (2012) The role of VDAC in cell death: friend or foe? Biochim Biophys Acta 1818:1444–1450

Menzel VA, Cassará MC, Benz R, de Pinto V, Messina A, Cunsolo V, Saletti R, Hinsch KD, Hinsch E (2009) Molecular and functional characterization of VDAC2 purified from mammal spermatozoa. Biosci Rep 29:351–362

Merlini L, Angelin A, Tiepolo T, Braghetta P, Sabatelli P, Zamparelli A, Ferlini A, Maraldi NM, Bonaldo P, Bernardi P (2008) Cyclosporin A corrects mitochondrial dysfunction and muscle apoptosis in patients with collagen VI myopathies. Proc Natl Acad Sci U S A 105:5225–5229

Merlini L, Sabatelli P, Armaroli A, Gnudi S, Angelin A, Grumati P, Michelini ME, Franchella A, Gualandi F, Bertini E, Maraldi NM, Ferlini A, Bonaldo P, Bernardi P (2011) Cyclosporine A in Ullrich congenital muscular dystrophy: long-term results. Oxid Med Cell Longev 2011:139194

Messina A, Reina S, Guarino F, De Pinto V (2012) VDAC isoforms in mammals. Biochim Biophys Acta 1818:1466–1476

Michie AM, McCaig AM, Nakagawa R, Vukovic M (2010) Death-associated protein kinase (DAPK) and signal transduction: regulation in cancer. FEBS J 277:74–80

Mileshina D, Ibrahim N, Boesch P, Lightowlers RN, Dietrich A, Weber-Lotfi F (2011) Mitochondrial transfection for studying organellar DNA repair, genome maintenance and aging. Mech Ageing Dev 132:412–423

Min CK, Yeom DR, Lee KE, Kwon HK, Kang M, Kim YS, Park ZY, Jeon H, Kim do H (2012) Coupling of ryanodine receptor 2 and voltage-dependent anion channel 2 is essential for Ca^{2+} transfer from the sarcoplasmic reticulum to the mitochondria in the heart. Biochem J 447:371–379

Mirzabekov T, Ballarin C, Nicolini M, Zatta P, Sorgato MC (1993) Reconstitution of the native mitochondrial outer membrane in planar bilayers. Comparison with the outer membrane in a patch pipette and effect of aluminum compounds. J Membr Biol 133:129–143

Miura T, Tanno M (2012) The mPTP and its regulatory proteins: final common targets of signalling pathways for protection against necrosis. Cardiovasc Res 94:181–189

Miura T, Tanno M, Sato T (2010) Mitochondrial kinase signalling pathways in myocardial protection from ischaemia/reperfusion-induced necrosis. Cardiovasc Res 88:7–15

Modica-Napolitano JS, Aprille JR (2001) Delocalized lipophilic cations selectively target the mitochondria of carcinoma cells. Adv Drug Deliv Rev 49:63–70

Morgan MJ, Liu ZG (2011) Crosstalk of reactive oxygen species and NF-κB signaling. Cell Res 21:103–115

Morin D, Assaly R, Paradis S, Berdeaux A (2009) Inhibition of mitochondrial membrane permeability as a putative pharmacological target for cardioprotection. Curr Med Chem 16:4382–4398

Morselli E, Galluzzi L, Kepp O, Mariño G, Michaud M, Vitale I, Maiuri MC, Kroemer G (2011) Oncosuppressive functions of autophagy. Antioxid Redox Signal 14:2251–2269

Murakami A, Ashida H, Terao J (2008) Multitargeted cancer prevention by quercetin. Cancer Lett 269:315–325

Murphy MP (2009) How mitochondria produce reactive oxygen species. Biochem J 417:1–13

Murphy MP, Smith RA (2007) Targeting antioxidants to mitochondria by conjugation to lipophilic cations. Annu Rev Pharmacol Toxicol 47:629–656

Murphy E, Steenbergen C (2008) Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiol Rev 88:581–609

Murphy E, Steenbergen C (2011) What makes the mitochondria a killer? Can we condition them to be less destructive? Biochim Biophys Acta 1813:1302–1308

Murrow L, Debnath J (2013) Autophagy as a stress-response and quality-control mechanism: implications for cell injury and human disease. Annu Rev Pathol 8:105–137

Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H, Inohara H, Kubo T, Tsujimoto Y (2005) Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. Nature 434:652–658

Nakamura T, Akita H, Yamada Y, Hatakeyama H, Harashima H (2012) A multifunctional envelope-type nanodevice for use in nanomedicine: concept and applications. Acc Chem Res 45:1113–1121

Nakanishi H, Wu Z (2009) Microglia-aging: roles of microglial lysosome- and mitochondria-derived reactive oxygen species in brain aging. Behav Brain Res 201:1–7

Neuzil J, Weber T, Gellert N, Weber C (2001) Selective cancer cell killing by alpha-tocopheryl succinate. Br J Cancer 84:87–89

Neuzil J, Dong LF, Rohlena J, Truksa J, Ralph SJ (2013) Classification of mitocans, anti-cancer drugs acting on mitochondria. Mitochondrion 13(3):199–208

Norman KG, Canter JA, Shi M, Milne GL, Morrow JD, Sligh JE (2010) Cyclosporine A suppresses keratinocyte cell death through MPTP inhibition in a model for skin cancer in organ transplant recipients. Mitochondrion 10:94–101

Novgorodov SA, Gudz TI (1996) Permeability transition pore of the inner mitochondrial membrane can operate in two open states with different selectivities. J Bioenerg Biomembr 28:139–146

Nowikovsky K, Schweyen RJ, Bernardi P (2009) Pathophysiology of mitochondrial volume homeostasis: potassium transport and permeability transition. Biochim Biophys Acta 1787:345–350

Oberley TD, Oberley LW (1997) Antioxidant enzyme levels in cancer. Histol Histopathol 12:525–535

Odagiri K, Katoh H, Kawashima H, Tanaka T, Ohtani H, Saotome M, Urushida T, Satoh H, Hayashi H (2009) Local control of mitochondrial membrane potential, permeability transition pore and reactive oxygen species by calcium and calmodulin in rat ventricular myocytes. J Mol Cell Cardiol 46:989–997

Oerlemans MI, Koudstaal S, Chamuleau SA, de Kleijn DP, Doevendans PA, Sluijter JP (2013) Targeting cell death in the reperfused heart: pharmacological approaches for cardioprotection. Int J Cardiol 165:410–422

Ono M, Sawa Y, Ryugo M, Alechine AN, Shimizu S, Sugioka R, Tsujimoto Y, Matsuda H (2005) BH4 peptide derivative from Bcl-xL attenuates ischemia/reperfusion injury thorough anti-apoptotic mechanism in rat hearts. Eur J Cardiothorac Surg 27:117–1121

Oyanagi E, Yano H, Uchida M, Utsumi K, Sasaki J (2011) Protective action of L-carnitine on cardiac mitochondrial function and structure against fatty acid stress. Biochem Biophys Res Commun 412:61–67

Pagliaro P, Penna C (2011) Cardiac postconditioning. Antioxid Redox Signal 14:777–779

Pani G, Koch OR, Galeotti T (2009) The p53-p66shc-Manganese Superoxide Dismutase (MnSOD) network: a mitochondrial intrigue to generate reactive oxygen species. Int J Biochem Cell Biol 41:1002–1005

Panieri E, Gogvadze V, Norberg E, Venkatesh R, Orrenius S, Zhivotovsky B (2013) Reactive oxygen species generated in different compartments induce cell death, survival, or senescence. Free Radic Biol Med 57:176–187

Panyi G (2005) Biophysical and pharmacological aspects of K^+ channels in T lymphocytes. Eur Biophys J 34:515–529

Papanicolaou KN, Phillippo MM, Walsh K (2012) Mitofusins and the mitochondrial permeability transition: the potential downside of mitochondrial fusion. Am J Physiol Heart Circ Physiol 303:H243–H255

Pastorino JG, Hoek JB (2008) Regulation of hexokinase binding to VDAC. J Bioenerg Biomembr 40:171–182

Pastorino JG, Simbula G, Yamamoto K, Glascott PA Jr, Rothman RJ, Farber JL (1996) The cytotoxicity of tumor necrosis factor depends on induction of the mitochondrial permeability transition. J Biol Chem 271:29792–29798

Pastorino JG, Shulga N, Hoek JB (2002) Mitochondrial binding of hexokinase II inhibits Bax-induced cytochrome c release and apoptosis. J Biol Chem 277:7610–7618

Pastorino JG, Hoek JB, Shulga N (2005) Activation of glycogen synthase kinase 3beta disrupts the binding of hexokinase II to mitochondria by phosphorylating voltage-dependent anion channel and potentiates chemotherapy-induced cytotoxicity. Cancer Res 65:10545–10554

Pathania D, Millard M, Neamati N (2009) Opportunities in discovery and delivery of anticancer drugs targeting mitochondria and cancer cell metabolism. Adv Drug Deliv Rev 61:1250–1275

Paul S, Sengupta S, Bandyopadhyay TK, Bhattacharyya A (2012) Stevioside induced ROS-mediated apoptosis through mitochondrial pathway in human breast cancer cell line MCF-7. Nutr Cancer 64:1087–1094

Pavlov E, Grigoriev SM, Dejean LM, Zweihorn CL, Mannella CA, Kinnally KW (2005a) The mitochondrial channel VDAC has a cation-selective open state. Biochim Biophys Acta 1710:96–102

Pavlov E, Zakharian E, Bladen C, Diao CT, Grimbly C, Reusch RN, French RJ (2005b) A large, voltage-dependent channel, isolated from mitochondria by water-free chloroform extraction. Biophys J 88:2614–2625

Pedersen PL (2012) 3-Bromopyruvate (3BP) a fast acting, promising, powerful, specific, and effective "small molecule" anti-cancer agent taken from labside to bedside: introduction to a special issue. J Bioenerg Biomembr 44:1–6

Pediaditakis P, Kim JS, He L, Zhang X, Graves LM, Lemasters JJ (2010) Inhibition of the mitochondrial permeability transition by protein kinase A in rat liver mitochondria and hepatocytes. Biochem J 431:411–421

Pelicano H, Lu W, Zhou Y, Zhang W, Chen Z, Hu Y, Huang P (2009) Mitochondrial dysfunction and reactive oxygen species imbalance promote breast cancer cell motility through a CXCL14-mediated mechanism. Cancer Res 69:2375–2383

Peng S, Blachly-Dyson E, Forte M, Colombini M (1992) Large scale rearrangement of protein domains is associated with voltage gating of the VDAC channel. Biophys J 62:123–131

Penna C, Mancardi D, Raimondo S, Geuna S, Pagliaro P (2008) The paradigm of postconditioning to protect the heart. J Cell Mol Med 12:435–458

Penna C, Perrelli MG, Pagliaro P (2013) Mitochondrial pathways, permeability transition pore, and redox signaling in cardioprotection: therapeutic implications. Antioxid Redox Signal 18:556–599

Penzo D, Tagliapietra C, Colonna R, Petronilli V, Bernardi P (2002) Effects of fatty acids on mitochondria: implications for cell death. Biochim Biophys Acta 1555:160–165

Pereira GC, Branco AF, Matos JA, Pereira SL, Parke D, Perkins EL, Serafim TL, Sardão VA, Santos MS, Moreno AJ, Holy J, Oliveira PJ (2007) Mitochondrially targeted effects of berberine [Natural Yellow 18, 5,6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo(5,6-a) quinolizinium] on K1735-M2 mouse melanoma cells: comparison with direct effects on isolated mitochondrial fractions. J Pharmacol Exp Ther 323:636–649

Pereira CV, Machado NG, Oliveira PJ (2008) Mechanisms of berberine (natural yellow 18)-induced mitochondrial dysfunction: interaction with the adenine nucleotide translocator. Toxicol Sci 105:408–417

Pfeiffer DR, Gudz TI, Novgorodov SA, Erdahl WL (1995) The peptide mastoparan is a potent facilitator of the mitochondrial permeability transition. J Biol Chem 270:4923–4932

Pinton P, Rizzuto R (2008) p66Shc, oxidative stress and aging: importing a lifespan determinant into mitochondria. Cell Cycle 7:304–308

Pinton P, Ferrari D, Rapizzi E, Di Virgilio F, Pozzan T, Rizzuto R (2001) The Ca2+ concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action. EMBO J 20:2690–2701

Policastro L, Molinari B, Larcher F, Blanco P, Podhajcer OL, Costa CS, Rojas P, Durán H (2004) Imbalance of antioxidant enzymes in tumor cells and inhibition of proliferation and malignant features by scavenging hydrogen peroxide. Mol Carcinog 39:103–113

Pongs O, Schwarz JR (2010) Ancillary subunits associated with voltage-dependent K^+ channels. Physiol Rev 90:755–796

Popp B, Court DA, Benz R, Neupert W, Lill R (1996) The role of the N and C termini of recombinant Neurospora mitochondrial porin in channel formation and voltage-dependent gating. J Biol Chem 271:13593–13599

Prime TA, Blaikie FH, Evans C, Nadtochiy SM, James AM, Dahm CC, Vitturi DA, Patel RP, Hiley CR, Abakumova I, Requejo R, Chouchani ET, Hurd TR, Garvey JF, Taylor CT, Brookes PS, Smith RA, Murphy MP (2009) A mitochondria-targeted S-nitrosothiol modulates respiration, nitrosates thiols, and protects against ischemia-reperfusion injury. Proc Natl Acad Sci U S A 106:10764–10769

Proskuryakov SY, Gabai VL (2010) Mechanisms of tumor cell necrosis. Curr Pharm Des 16:56–68

Quast SA, Berger A, Buttstädt N, Friebel K, Schönherr R, Eberle J (2012) General Sensitization of melanoma cells for TRAIL-induced apoptosis by the potassium channel inhibitor TRAM-34 depends on release of SMAC. PLoS One 7:e39290

Queirós O, Preto A, Pacheco A, Pinheiro C, Azevedo-Silva J, Moreira R, Pedro M, Ko YH, Pedersen PL, Baltazar F, Casal M (2012) Butyrate activates the monocarboxylate transporter MCT4 expression in breast cancer cells and enhances the antitumor activity of 3-bromopyruvate. J Bioenerg Biomembr 44:141–153

Raghavan A, Sheiko T, Graham BH, Craigen WJ (2012) Voltage-dependant anion channels: novel insights into isoform function through genetic models. Biochim Biophys Acta 1818:1477–1485

Rai P (2010) Oxidation in the nucleotide pool, the DNA damage response and cellular senescence: defective bricks build a defective house. Mutat Res 703:71–81

Ralph SJ, Neuzil J (2009) Mitochondria as targets for cancer therapy. Mol Nutr Food Res 53:9–28

Ralph SJ, Low P, Dong L, Lawen A, Neuzil J (2006) Mitocans: mitochondrial targeted anti-cancer drugs as improved therapies and related patent documents. Recent Pat Anticancer Drug Discov 1:327–346

Ralph SJ, Rodríguez-Enríquez S, Neuzil J, Saavedra E, Moreno-Sánchez R (2010) The causes of cancer revisited: "mitochondrial malignancy" and ROS-induced oncogenic transformation - why mitochondria are targets for cancer therapy. Mol Aspects Med 31:145–170

Ralph SJ, Moreno-Sánchez R, Neuzil J, Rodríguez-Enríquez S (2011) Inhibitors of succinate: quinone reductase/Complex II regulate production of mitochondrial reactive oxygen species and protect normal cells from ischemic damage but induce specific cancer cell death. Pharm Res 28:2695–2730

Rapizzi E, Pinton P, Szabadkai G, Wieckowski MR, Vandecasteele G, Baird G, Tuft RA, Fogarty KE, Rizzuto R (2002) Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca^{2+} microdomains to mitochondria. J Cell Biol 159:613–624

Raschle T, Hiller S, Yu TY, Rice AJ, Walz T, Wagner G (2009) Structural and functional characterization of the integral membrane protein VDAC-1 in lipid bilayer nanodiscs. J Am Chem Soc 131:17777–17779

Rasola A, Bernardi P (2007) The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis. Apoptosis 12:815–833

Rasola A, Bernardi P (2011) Mitochondrial permeability transition in Ca^{2+}-dependent apoptosis and necrosis. Cell Calcium 50:222–233

Rasola A, Sciacovelli M, Chiara F, Pantic B, Brusilow WS, Bernardi P (2010a) Activation of mitochondrial ERK protects cancer cells from death through inhibition of the permeability transition. Proc Natl Acad Sci U S A 107:726–731

Rasola A, Sciacovelli M, Pantic B, Bernardi P (2010b) Signal transduction to the permeability transition pore. FEBS Lett 584:1989–1996

Rauer H, Pennington M, Cahalan M, Chandy KG (1999) Structural conservation of the pores of calcium-activated and voltage-gated potassium channels determined by a sea anemone toxin. J Biol Chem 274:21885–21892

Ravikumar B, Sarkar S, Davies JE, Futter M, Garcia-Arencibia M, Green-Thompson ZW, Jimenez-Sanchez M, Korolchuk VI, Lichtenberg M, Luo S, Massey DC, Menzies FM, Moreau K, Narayanan U, Renna M, Siddiqi FH, Underwood BR, Winslow AR, Rubinsztein DC (2010) Regulation of Mammalian autophagy in physiology and pathophysiology. Physiol Rev 90:1383–1435

Raviv Z, Cohen S, Reischer-Pelech D (2013) The anti-cancer activities of jasmonates. Cancer Chemother Pharmacol 71(2):275–285

Ren YR, Pan F, Parvez S, Fleig A, Chong CR, Xu J, Dang Y, Zhang J, Jiang H, Penner R, Liu JO (2008) Clofazimine inhibits human Kv1.3 potassium channel by perturbing calcium oscillation in T lymphocytes. PLoS One 3:e4009

Ren D, Kim H, Tu HC, Westergard TD, Fisher JK, Rubens JA, Korsmeyer SJ, Hsieh JJ, Cheng EH (2009) The VDAC2-BAK rheostat controls thymocyte survival. Sci Signal 2:ra48

Ricchelli F, Sileikytė J, Bernardi P (2011) Shedding light on the mitochondrial permeability transition. Biochim Biophys Acta 1807:482–490

Ripcke J, Zarse K, Ristow M, Birringer M (2009) Small-molecule targeting of the mitochondrial compartment with an endogenously cleaved reversible tag. Chembiochem 10: 1689–1696

Risso A, Braidot E, Sordano MC, Vianello A, Macrì F, Skerlavaj B, Zanetti M, Gennaro R, Bernardi P (2002) BMAP-28, an antibiotic peptide of innate immunity, induces cell death through opening of the mitochondrial permeability transition pore. Mol Cell Biol 22:1926–1935

Rizzuto R, De Stefani D, Raffaello A, Mammucari C (2012) Mitochondria as sensors and regulators of calcium signalling. Nat Rev Mol Cell Biol 13:566–578

Rodrigues-Ferreira C, da Silva AP, Galina A (2012) Effect of the antitumoral alkylating agent 3-bromopyruvate on mitochondrial respiration: role of mitochondrially bound hexokinase. J Bioenerg Biomembr 44:39–49

Rodríguez-Enríquez S, Hernández-Esquivel L, Marín-Hernández A, Dong LF, Akporiaye ET, Neuzil J, Ralph SJ, Moreno-Sánchez R (2012) Molecular mechanism for the selective impairment of cancer mitochondrial function by a mitochondrially targeted vitamin E analogue. Biochim Biophys Acta 1817:1597–1607

Roser KS, Brookes PS, Wojtovich AP, Olson LP, Shojaie J, Parton RL, Anders MW (2010) Mitochondrial biotransformation of omega-(phenoxy)alkanoic acids, 3-(phenoxy)acrylic acids, and omega-(1-methyl-1H-imidazol-2-ylthio)alkanoic acids: a prodrug strategy for targeting cytoprotective antioxidants to mitochondria. Bioorg Med Chem 18:1441–1448

Rostovtseva TK, Bezrukov SM (1998) ATP transport through a single mitochondrial channel, VDAC, studied by current fluctuation analysis. Biophys J 74:2365–2373

Rostovtseva T, Colombini M (1996) ATP flux is controlled by a voltage-gated channel from the mitochondrial outer membrane. J Biol Chem 271:28006–28008

Rostovtseva T, Colombini M (1997) VDAC channels mediate and gate the flow of ATP: implications for the regulation of mitochondrial function. Biophys J 72:1954–1962

Rostovtseva TK, Komarov A, Bezrukov SM, Colombini M (2002) VDAC channels differentiate between natural metabolites and synthetic molecules. J Membr Biol 187:147–156

Roy SS, Madesh M, Davies E, Antonsson B, Danial N, Hajnóczky G (2009) Bad targets the permeability transition pore independent of Bax or Bak to switch between Ca^{2+}-dependent cell survival and death. Mol Cell 33:377–388

Ruiz-Meana M, Fernandez-Sanz C, Garcia-Dorado D (2010) The SR-mitochondria interaction: a new player in cardiac pathophysiology. Cardiovasc Res 88:30–39

Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, Ichijo H (1998) Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J 17:2596–2606

Salvi M, Brunati AM, Toninello A (2005) Tyrosine phosphorylation in mitochondria: a new frontier in mitochondrial signaling. Free Radic Biol Med 38:1267–1277

Sampson MJ, Decker WK, Beaudet al, Ruitenbeek W, Armstrong D, Hicks MJ, Craigen WJ (2001) Immotile sperm and infertility in mice lacking mitochondrial voltage-dependent anion channel type 3. J Biol Chem 276:39206–39212

Sanada S, Komuro I, Kitakaze M (2011) Pathophysiology of myocardial reperfusion injury: preconditioning, postconditioning, and translational aspects of protective measures. Am J Physiol Heart Circ Physiol 301:H1723–H1741

Sassi N, De Marchi U, Fioretti B, Biasutto L, Gulbins E, Francolini F, Szabò I, Zoratti M (2010) An investigation of the occurrence and properties of the mitochondrial intermediate conductance Ca2+-activated K^+ channel mtKCa3.1. Biochim Biophys Acta 1797:1260–1267

Sassi N, Biasutto L, Mattarei A, Carraro M, Giorgio V, Citta A, Bernardi P, Garbisa S, Szabò I, Paradisi C, Zoratti M (2012) Cytotoxicity of a mitochondriotropic quercetin derivative: Mechanisms. Biochim Biophys Acta 1817:1095–1106

Sassi N, Mattarei A, Azzolini M, Bernardi P, Szabo I, Paradisi C, Zoratti M, Biasutto L (2014) Mitochondria-targeted resveratrol derivatives act as cytotoxic pro-oxidants. Curr Pharm Des 20:172–179

Sawant RR, Torchilin VP (2011) Design and synthesis of novel functional lipid-based bioconjugates for drug delivery and other applications. Methods Mol Biol 751:357–378

Sayre LM, Perry G, Smith MA (2008) Oxidative stress and neurotoxicity. Chem Res Toxicol 21:172–188

Schein SJ, Colombini M, Finkelstein A (1976) Reconstitution in planar lipid bilayers of a voltage-dependent anion-selective channel obtained from paramecium mitochondria. J Membr Biol 30:99–120

Schinzel AC, Takeuchi O, Huang Z, Fisher JK, Zhou Z, Rubens J, Hetz C, Danial NN, Moskowitz MA, Korsmeyer SJ (2005) Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. Proc Natl Acad Sci U S A 102:12005–12010

Schmitz A, Sankaranarayanan A, Azam P, Schmidt-Lassen K, Homerick D, Hänsel W, Wulff H (2005) Design of PAP-1, a selective small molecule Kv1.3 blocker, for the suppression of effector memory T cells in autoimmune diseases. Mol Pharmacol 68:1254–1270

Scorrano L, Penzo D, Petronilli V, Pagano F, Bernardi P (2001) Arachidonic acid causes cell death through the mitochondrial permeability transition. Implications for tumor necrosis factor-alpha aopototic signaling. J Biol Chem 276:12035–12040

Shamas-Din A, Brahmbhatt H, Leber B, Andrews DW (2011) BH3-only proteins: orchestrators of apoptosis. Biochim Biophys Acta 1813:508–520

Sharaf el dein O, Gallerne C, Brenner C, Lemaire C (2012) Increased expression of VDAC1 sensitizes carcinoma cells to apoptosis induced by DNA cross-linking agents. Biochem Pharmacol 83:1172–1182

Shimada H, Hirai K, Simamura E, Hatta T, Iwakiri H, Mizuki K, Hatta T, Sawasaki T, Matsunaga S, Endo Y, Shimizu S (2009) Paraquat toxicity induced by voltage-dependent anion channel 1 acts as an NADH-dependent oxidoreductase. J Biol Chem 284:28642–28649

Shimizu S, Tsujimoto Y (2000) Proapoptotic BH3-only Bcl-2 family members induce cytochrome c release, but not mitochondrial membrane potential loss, and do not directly modulate voltage-dependent anion channel activity. Proc Natl Acad Sci U S A 97:577–582

Shoshan MC (2012) 3-Bromopyruvate: targets and outcomes. J Bioenerg Biomembr 44:7–15
Shoshan-Barmatz V, Ben-Hail D (2012) VDAC, a multi-functional mitochondrial protein as a pharmacological target. Mitochondrion 12:24–34
Shoshan-Barmatz V, Golan M (2012) Mitochondrial VDAC1: function in cell life and death and a target for cancer therapy. Curr Med Chem 19:714–735
Shoshan-Barmatz V, Mizrachi D (2012) VDAC1: from structure to cancer therapy. Front Oncol 2:164
Shoshan-Barmatz V, Israelson A, Brdiczka D, Sheu SS (2006) The voltage-dependent anion channel (VDAC): function in intracellular signalling, cell life and cell death. Curr Pharm Des 12:2249–2270
Shoshan-Barmatz V, Keinan N, Zaid H (2008) Uncovering the role of VDAC in the regulation of cell life and death. J Bioenerg Biomembr 40:183–191
Shoshan-Barmatz V, De Pinto V, Zweckstetter M, Raviv Z, Keinan N, Arbel N (2010a) VDAC, a multi-functional mitochondrial protein regulating cell life and death. Mol Aspects Med 31:227–285
Shoshan-Barmatz V, Keinan N, Abu-Hamad S, Tyomkin D, Aram L (2010b) Apoptosis is regulated by the VDAC1 N-terminal region and by VDAC oligomerization: release of cytochrome c, AIF and Smac/Diablo. Biochim Biophys Acta 1797:1281–1291
Shoudai K, Nonaka K, Maeda M, Wang ZM, Jeong HJ, Higashi H, Murayama N, Akaike N (2007) Effects of various K+channel blockers on spontaneous glycine release at rat spinal neurons. Brain Res 1157:11–22
Sileikyte J, Petronilli V, Zulian A, Dabbeni-Sala F, Tognon G, Nikolov P, Bernardi P, Ricchelli F (2011) Regulation of the inner membrane mitochondrial permeability transition by the outer membrane translocator protein (peripheral benzodiazepine receptor). J Biol Chem 286:1046–1053
Silic-Benussi M, Cannizzaro E, Venerando A, Cavallari I, Petronilli V, La Rocca N, Marin O, Chieco-Bianchi L, Di Lisa F, D'Agostino DM, Bernardi P, Ciminale V (2009) Modulation of mitochondrial K(+) permeability and reactive oxygen species production by the p13 protein of human T-cell leukemia virus type 1. Biochim Biophys Acta 1787:947–954
Simamura E, Hirai K, Shimada H, Koyama J, Niwa Y, Shimizu S (2006) Furanonaphthoquinones cause apoptosis of cancer cells by inducing the production of reactive oxygen species by the mitochondrial voltage-dependent anion channel. Cancer Biol Ther 5:1523–1529
Simamura E, Shimada H, Hatta T, Hirai K (2008) Mitochondrial voltage-dependent anion channels (VDACs) as novel pharmacological targets for anti-cancer agents. J Bioenerg Biomembr 40:213–217
Skulachev VP (2012) Mitochondria-targeted antioxidants as promising drugs for treatment of age-related brain diseases. J Alzheimers Dis 28:283–289
Skulachev VP, Anisimov VN, Antonenko YN, Bakeeva LE, Chernyak BV, Erichev VP, Filenko OF, Kalinina NI, Kapelko VI, Kolosova NG, Kopnin BP, Korshunova GA, Lichinitser MR, Obukhova LA, Pasyukova EG, Pisarenko OI, Roginsky VA, Ruuge EK, Senin II, Severina II, Skulachev MV, Spivak IM, Tashlitsky VN, Tkachuk VA, Vyssokikh MY, Yaguzhinsky LS, Zorov DB (2009) An attempt to prevent senescence: a mitochondrial approach. Biochim Biophys Acta 1787:437–461
Skulachev MV, Antonenko YN, Anisimov VN, Chernyak BV, Cherepanov DA, Chistyakov VA, Egorov MV, Kolosova NG, Korshunova GA, Lyamzaev KG, Plotnikov EY, Roginsky VA, Savchenko AY, Severina II, Severin FF, Shkurat TP, Tashlitsky VN, Shidlovsky KM, Vyssokikh MY, Zamyatnin AA Jr, Zorov DB, Skulachev VP (2011) Mitochondrial-targeted plastoquinone derivatives. Effect on senescence and acute age-related pathologies. Curr Drug Targets 12:800–826
Sloan RC, Moukdar F, Frasier CR, Patel HD, Bostian PA, Lust RM, Brown DA (2012) Mitochondrial permeability transition in the diabetic heart: contributions of thiol redox state and mitochondrial calcium to augmented reperfusion injury. J Mol Cell Cardiol 52:1009–1018
Smith RA, Murphy MP (2010) Animal and human studies with the mitochondria-targeted antioxidant MitoQ. Ann N Y Acad Sci 1201:96–103

Smith RA, Hartley RC, Murphy MP (2011) Mitochondria-targeted small molecule therapeutics and probes. Antioxid Redox Signal 15:3021–3038
Smith RA, Hartley RC, Cochemé HM, Murphy MP (2012) Mitochondrial pharmacology. Trends Pharmacol Sci 33:341–352
Solaini G, Baracca A, Lenaz G, Sgarbi G (2010) Hypoxia and mitochondrial oxidative metabolism. Biochim Biophys Acta 1797:1171–1177
Solaini G, Sgarbi G, Baracca A (2011) Oxidative phosphorylation in cancer cells. Biochim Biophys Acta 1807:534–542
Starkov AA (2008) The role of mitochondria in reactive oxygen species metabolism and signaling. Ann N Y Acad Sci 1147:37–52
Stella C, Burgos I, Chapela S, Gamondi O (2011) Ischemia–reperfusion: a look from yeast mitochondria. Curr Med Chem 18:3476–3484
Stoler DL, Chen N, Basik M, Kahlenberg MS, Rodriguez-Bigas MA, Petrelli NJ, Anderson GR (1999) The onset and extent of genomic instability in sporadic colorectal tumor progression. Proc Natl Acad Sci U S A 96:15121–15126
Stoyanovsky DA, Vlasova II, Belikova NA, Kapralov A, Tyurin V, Greenberger JS, Kagan VE (2008) Activation of NO donors in mitochondria: peroxidase metabolism of (2-hydroxyaminovinyl)-triphenyl-phosphonium by cytochrome c releases NO and protects cells against apoptosis. FEBS Lett 582:725–728
Sugioka R, Shimizu S, Funatsu T, Tamagawa H, Sawa Y, Kawakami T, Tsujimoto Y (2003) BH4-domain peptide from Bcl-xL exerts anti-apoptotic activity in vivo. Oncogene 22:8432–8440
Szabò I, Adams C, Gulbins E (2004) Ion channels and membrane rafts in apoptosis. Pflugers Arch 448:304–312
Szabò I, Bock J, Jekle A, Soddemann M, Adams C, Lang F, Zoratti M, Gulbins E (2005) A novel potassium channel in lymphocyte mitochondria. J Biol Chem 280:12790–12798
Szabò I, Bock J, Grassmé H, Soddemann M, Wilker B, Lang F, Zoratti M, Gulbins E (2008) Mitochondrial potassium channel Kv1.3 mediates Bax-induced apoptosis in lymphocytes. Proc Natl Acad Sci U S A 105:14861–14866
Szabò I, Zoratti M, Gulbins E (2010) Contribution of voltage-gated potassium channels to the regulation of apoptosis. FEBS Lett 584:2049–2056
Szabò I, Soddemann M, Leanza L, Zoratti M, Gulbins E (2011) Single-point mutations of a lysine residue change function of Bax and Bcl-xL expressed in Bax- and Bak-less mouse embryonic fibroblasts: novel insights into the molecular mechanisms of Bax-induced apoptosis. Cell Death Differ 18:427–438
Szatrowski TP, Nathan CF (1991) Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res 51:794–798
Szeto HH (2008) Mitochondria-targeted cytoprotective peptides for ischemia-reperfusion injury. Antiox Redox Signal 10:601–619
Szeto HH, Schiller PW (2011) Novel therapies targeting inner mitochondrial membrane–from discovery to clinical development. Pharm Res 28:2669–2679
Tait SW, Green DR (2010) Mitochondria and cell death: outer membrane permeabilization and beyond. Nat Rev Mol Cell Biol 11:621–632
Takeda K, Naguro I, Nishitoh H, Matsuzawa A, Ichijo H (2011) Apoptosis signaling kinases: from stress response to health outcomes. Antioxid Redox Signal 15:719–761
Tan W (2012) VDAC blockage by phosphorothioate oligonucleotides and its implication in apoptosis. Biochim Biophys Acta 1818:1555–1561
Tang Z, Yuan S, Hu Y, Zhang H, Wu W, Zeng Z, Yang J, Yun J, Xu R, Huang P (2012) Over-expression of GAPDH in human colorectal carcinoma as a preferred target of 3-bromopyruvate propyl ester. J Bioenerg Biomembr 44:117–125
Tatton WG, Chalmers-Redman RM (1998) Mitochondria in neurodegenerative apoptosis: an opportunity for therapy? Ann Neurol 44(3 Suppl 1):S134–S141
Tertil M, Jozkowicz A, Dulak J (2010) Oxidative stress in tumor angiogenesis- therapeutic targets. Curr Pharm Des 16:3877–3894
Tiepolo T, Angelin A, Palma E, Sabatelli P, Merlini L, Nicolosi L, Finetti F, Braghetta P, Vuagniaux G, Dumont JM, Baldari CT, Bonaldo P, Bernardi P (2009) The cyclophilin inhibitor Debio 025

normalizes mitochondrial function, muscle apoptosis and ultrastructural defects in Col6a1$^{-/-}$ myopathic mice. Br J Pharmacol 157:1045–1052
Tischner D, Manzl C, Soratroi C, Villunger A, Krumschnabel G (2012) Necrosis-like death can engage multiple pro-apoptotic Bcl-2 protein family members. Apoptosis 17:1197–1209
Tormos KV, Chandel NS (2010) Inter-connection between mitochondria and HIFs. J Cell Mol Med 14:795–804
Tornero D, Posadas I, Ceña V (2011) Bcl-x(L) blocks a mitochondrial inner membrane channel and prevents Ca^{2+} overload-mediated cell death. PLoS One 6:e20423
Torres YP, Morera FJ, Carvacho I, Latorre R (2007) A marriage of convenience: beta-subunits and voltage-dependent K^+ channels. J Biol Chem 282:24485–24489
Traba J, Del Arco A, Duchen MR, Szabadkai G, Satrústegui J (2012) SCaMC-1 promotes cancer cell survival by desensitizing mitochondrial permeability transition via ATP/ADP-mediated matrix Ca^{2+} buffering. Cell Death Differ 19:650–660
Trachootham D, Lu W, Ogasawara MA, Nilsa RD, Huang P (2008) Redox regulation of cell survival. Antioxid Redox Signal 10:1343–1374
Trachootham D, Alexandre J, Huang P (2009) Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov 8:579–591
Tsujimoto Y, Shimizu S (2000) VDAC regulation by the Bcl-2 family of proteins. Cell Death Differ 7:1174–1181
Tsujimoto Y, Shimizu S (2007) Role of the mitochondrial membrane permeability transition in cell death. Apoptosis 12:835–840
Tsujimoto Y, Nakagawa T, Shimizu S (2006) Mitochondrial membrane permeability transition and cell death. Biochim Biophys Acta 1757:1297–1300
Ueda S, Masutani H, Nakamura H, Tanaka T, Ueno M, Yodoi J (2002) Redox control of cell death. Antioxid Redox Signal 4:405–414
Ujwal R, Cascio D, Colletier JP, Faham S, Zhang J, Toro L, Ping P, Abramson J (2008) The crystal structure of mouse VDAC1 at 2.3 A resolution reveals mechanistic insights into metabolite gating. Proc Natl Acad Sci U S A 105:17742–17747
Ujwal R, Cascio D, Chaptal V, Ping P, Abramson J (2009) Crystal packing analysis of murine VDAC1 crystals in a lipidic environment reveals novel insights on oligomerization and orientation. Channels (Austin) 3:167–170
Uribe-Carvajal S, Luévano-Martínez LA, Guerrero-Castillo S, Cabrera-Orefice A, Corona-de-la-Peña NA, Gutiérrez-Aguilar M (2011) Mitochondrial unselective channels throughout the eukaryotic domain. Mitochondrion 11:382–390
Vaidya B, Vyas SP (2012) Transferrin coupled vesicular system for intracellular drug delivery for the treatment of cancer: development and characterization. J Drug Target 20:372–380
Vaidya B, Mishra N, Dube D, Tiwari S, Vyas SP (2009a) Targeted nucleic acid delivery to mitochondria. Curr Gene Ther 9:475–486
Vaidya B, Paliwal R, Rai S, Khatri K, Goyal AK, Mishra N, Vyas SP (2009b) Cell-selective mitochondrial targeting: a new approach for cancer therapy. Cancer Ther 7:141–148
Vanden Berghe T, Vanlangenakker N, Parthoens E, Deckers W, Devos M, Festjens N, Guerin CJ, Brunk UT, Declercq W, Vandenabeele P (2010) Necroptosis, necrosis and secondary necrosis converge on similar cellular disintegration features. Cell Death Differ 17:922–930
Vaseva AV, Marchenko ND, Ji K, Tsirka SE, Holzmann S, Moll UM (2012) p53 opens the mitochondrial permeability transition pore to trigger necrosis. Cell 149:1536–1548
Vennekamp J, Wulff H, Beeton C, Calabresi PA, Grissmer S, Hänsel W, Chandy KG (2004) Kv1.3-blocking 5-phenylalkoxypsoralens: a new class of immunomodulators. Mol Pharmacol 65:1364–1374
Verschoor ML, Wilson LA, Singh G (2010) Mechanisms associated with mitochondrial-generated reactive oxygen species in cancer. Can J Physiol Pharmacol 88:204–219
Vestweber D, Schatz G (1989) DNA-protein conjugates can enter mitochondria via the protein import pathway. Nature 338:170–172
Vianello A, Casolo V, Petrussa E, Peresson C, Patui S, Bertolini A, Passamonti S, Braidot E, Zancani M (2012) The mitochondrial permeability transition pore (PTP) – an example of multiple molecular exaptation? Biochim Biophys Acta 1817:2072–2086

Vicente R, Escalada A, Villalonga N, Texidó L, Roura-Ferrer M, Martín-Satué M, López-Iglesias C, Soler C, Solsona C, Tamkun MM, Felipe A (2006) Association of Kv1.5 and Kv1.3 contributes to the major voltage-dependent K^+ channel in macrophages. J Biol Chem 281:37675–37685

Villinger S, Briones R, Giller K, Zachariae U, Lange A, de Groot BL, Griesinger C, Becker S, Zweckstetter M (2010) Functional dynamics in the voltage-dependent anion channel. Proc Natl Acad Sci U S A 107:22546–22551

von Stockum S, Basso E, Petronilli V, Sabatelli P, Forte MA, Bernardi P (2011) Properties of Ca^{2+} transport in mitochondria of *Drosophila melanogaster*. J Biol Chem 286:41163–41170

Voss MJ, Niggemann B, Zänker KS, Entschladen F (2010) Tumour reactions to hypoxia. Curr Mol Med 10:381–386

Waldmeier PC, Feldtrauer JJ, Qian T, Lemasters JJ (2002) Inhibition of the mitochondrial permeability transition by the nonimmunosuppressive cyclosporin derivative NIM811. Mol Pharmacol 62:22–29

Wan KF, Chan SL, Sukumaran SK, Lee MC, Yu VC (2008) Chelerythrine induces apoptosis through a Bax/Bak-independent mitochondrial mechanism. J Biol Chem 283:8423–8433

Wang J, Yi J (2008) Cancer cell killing via ROS: to increase or decrease, that is the question. Cancer Biol Ther 7:1875–1884

Webster KA (2012) Mitochondrial membrane permeabilization and cell death during myocardial infarction: roles of calcium and reactive oxygen species. Future Cardiol 8:863–884

Weissig V (2011) From serendipity to mitochondria-targeted nanocarriers. Pharm Res 28:2657–2668

Weissig V (2012) Mitochondria-specific nanocarriers for improving the proapoptotic activity of small molecules. Methods Enzymol 508:131–155

Weissig V, Torchilin VP (2001) Towards mitochondrial gene therapy: DQAsomes as a strategy. J Drug Target 9:1–13

Whelan RS, Konstantinidis K, Wei AC, Chen Y, Reyna DE, Jha S, Yang Y, Calvert JW, Lindsten T, Thompson CB, Crow MT, Gavathiotis E, Dorn GW 2nd, O'Rourke B, Kitsis RN (2012) Bax regulates primary necrosis through mitochondrial dynamics. Proc Natl Acad Sci U S A 109:6566–6571

White E, Karp C, Strohecker AM, Guo Y, Mathew R (2010) Role of autophagy in suppression of inflammation and cancer. Curr Opin Cell Biol 22:212–217

Wieckowski MR, Brdiczka D, Wojtczak L (2000) Long-chain fatty acids promote opening of the reconstituted mitochondrial permeability transition pore. FEBS Lett 484:61–64

Wolf A, Agnihotri S, Micallef J, Mukherjee J, Sabha N, Cairns R, Hawkins C, Guha A (2011) Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme. J Exp Med 208:313–326

Wondrak GT (2009) Redox-directed cancer therapeutics: molecular mechanisms and opportunities. Antioxid Redox Signal 11:3013–3069

Wu SB, Ma YS, Wu YT, Chen YC, Wei YH (2010) Mitochondrial DNA mutation-elicited oxidative stress, oxidative damage, and altered gene expression in cultured cells of patients with MERRF syndrome. Mol Neurobiol 41:256–266

Wulff H, Castle NA, Pardo LA (2009) Voltage-gated potassium channels as therapeutic targets. Nat Rev Drug Discov 8:982–1001

Xu X, Decker W, Sampson MJ, Craigen WJ, Colombini M (1999) Mouse VDAC isoforms expressed in yeast: channel properties and their roles in mitochondrial outer membrane permeability. J Membr Biol 170:89–102

Xu J, Koni PA, Wang P, Li G, Kaczmarek L, Wu Y, Li Y, Flavell RA, Desir GV (2003) The voltage gated potassium channel Kv1.3 regulates energy homeostasis and body weight. Hum Mol Genet 12:551–559

Xuan Y, Hu X (2009) Naturally-occurring shikonin analogues–a class of necroptotic inducers that circumvent cancer drug resistance. Cancer Lett 274:233–242

Yagoda N, von Rechenberg M, Zaganjor E, Bauer AJ, Yang WS, Fridman DJ, Wolpaw AJ, Smukste I, Peltier JM, Boniface JJ, Smith R, Lessnick SL, Sahasrabudhe S, Stockwell BR (2007) RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. Nature 447:864–868

Yamada Y, Harashima H (2012) Delivery of bioactive molecules to the mitochondrial genome using a membrane-fusing, liposome-based carrier, DF-MITO-Porter. Biomaterials 33:1589–1595
Yamada Y, Furukawa R, Yasuzaki Y, Harashima H (2011) Dual function MITO-Porter, a nano carrier integrating both efficient cytoplasmic delivery and mitochondrial macromolecule delivery. Mol Ther 19:1449–1456
Yamada Y, Akita H, Harashima H (2012) Multifunctional envelope-type nano device (MEND) for organelle targeting via a stepwise membrane fusion process. Methods Enzymol 509:301–326
Yang X, Cohen MV, Downey JM (2010) Mechanism of cardioprotection by early ischemic preconditioning. Cardiovasc Drugs Ther 24:225–234
Yasuzaki Y, Yamada Y, Harashima H (2010) Mitochondrial matrix delivery using MITO-Porter, a liposome-based carrier that specifies fusion with mitochondrial membranes. Biochem Biophys Res Commun 397:181–186
Yousif LF, Stewart KM, Horton KL, Kelley SO (2009a) Mitochondria-penetrating peptides: sequence effects and model cargo transport. Chembiochem 10:2081–2088
Yousif LF, Stewart KM, Kelley SO (2009b) Targeting mitochondria with organelle-specific compounds: strategies and applications. Chembiochem 10:1939–1950
Yu K, Fu W, Liu H, Luo X, Chen KX, Ding J, Shen J, Jiang H (2004) Computational simulations of interactions of scorpion toxins with the voltage-gated potassium ion channel. Biophys J 86:3542–3555
Yu FH, Yarov-Yarovoy V, Gutman GA, Catterall WA (2005) Overview of molecular relationships in the voltage-gated ion channel superfamily. Pharmacol Rev 57:387–395
Yu TY, Raschle T, Hiller S, Wagner G (2012) Solution NMR spectroscopic characterization of human VDAC-2 in detergent micelles and lipid bilayer nanodiscs. Biochim Biophys Acta 1818:1562–1569
Zaid H, Abu-Hamad S, Israelson A, Nathan I, Shoshan-Barmatz V (2005) The voltage-dependent anion channel-1 modulates apoptotic cell death. Cell Death Differ 12:751–760
Zalk R, Israelson A, Garty ES, Azoulay-Zohar H, Shoshan-Barmatz V (2005) Oligomeric states of the voltage-dependent anion channel and cytochrome c release from mitochondria. Biochem J 386:73–83
Zhivotovsky B, Orrenius S (2010) Cell death mechanisms: cross-talk and role in disease. Exp Cell Res 316:1374–1383
Zhivotovsky B, Galluzzi L, Kepp O, Kroemer G (2009) Adenine nucleotide translocase: a component of the phylogenetically conserved cell death machinery. Cell Death Differ 16:1419–1425
Zimin PI, Garic B, Bodendiek SB, Mahieux C, Wulff H, Zhorov BS (2010) Potassium channel block by a tripartite complex of two cationophilic ligands and a potassium ion. Mol Pharmacol 78:588–599
Zoratti M, Szabó I (1994) Electrophysiology of the inner mitochondrial membrane. J Bioenerg Biomembr 26:543–553
Zoratti M, Szabò I (1995) The mitochondrial permeability transition. Biochim Biophys Acta 1241:139–176
Zoratti M, Szabò I, De Marchi U (2005) Mitochondrial permeability transitions: how many doors to the house? Biochim Biophys Acta 1706:40–52
Zoratti M, De Marchi U, Gulbins E, Szabò I (2009) Novel channels of the inner mitochondrial membrane. Biochim Biophys Acta 1787:351–363
Zoratti M, De Marchi U, Biasutto L, Szabò I (2010) Electrophysiology clarifies the megariddles of the mitochondrial permeability transition pore. FEBS Lett 584:1997–2004
Zorov DB, Juhaszova M, Yaniv Y, Nuss HB, Wang S, Sollott SJ (2009) Regulation and pharmacology of the mitochondrial permeability transition pore. Cardiovasc Res 83:213–225

Chapter 10
Emerging Anti-cancer Targets in Mitochondria

Petr Ježek, Katarína Smolková, Aleš Dvořák, and Tomáš Olejár

Contents

Abstract Metabolic cancer therapy should target the most aggressive and malignant tumour cells within solid tumours. Targeting of mitochondrial isocitrate dehydrogenase-2 (IDH2) by inhibitors or silencing elements would eliminate tumour cells surviving the harsh conditions of hypoxia and intermittent aglycemia as well as in progressive stages of malignancies with mutant IDH2 or IDH1, producing the oncometabolite D-2-hydroxyglutarate. Strategies for its withdrawal should also be exploited. Also, cancer-specific mitochondria-located enzymes participating in aerobic glycolysis are suitable targets of anti-cancer strategies, such as inhibition of hexokinase-2 by the small molecule 3-bromopyruvate or by post-transcriptional repression of hexokinase-2 by microRNA-143. Similarly, dichloroacetate (DCA) has been considered as a potential cancer therapeutic agent, being an efficient inhibitor of four isoforms of pyruvate dehydrogenase kinases

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(PDK), which by phosphorylating the E1 subunit inhibit pyruvate dehydrogenase and channel pyruvate to lactate. This, in turn, establishes the glycolytic phenotype of cancer cells. Other targets include for example the inhibitor protein of the mitochondrial ATP synthase and the facilitator of its degradation, the immediate-response factor 1 (IEX1), for which strategies dependent on the specific conditions should be exploited. However, there has been a limited number of clinical studies documenting the significance of mitochondrial targeting in cancer therapies.

Keywords Mitochondria • Krebs cycle • Glycolysis • Isocitrate dehydrogenase-2 • Hexokinase-2 • Pyruvate dehydrogenase kinase • Warburg phenotype • Oxidative phosphorylation • Reductive carboxylation glutaminolysis • 3-Br-pyruvate • Dichloroacetate • Immediate early responsive gene X1 • Hypoxia-induced factor 1

1 Introduction

A drawback of current cancer therapies lies in an unwanted 'selection' of the most aggressive tumour cells, which may cause tumour recurrence. The ideal metabolic cancer therapy would, in turn, target the most aggressive and malignant tumour cells within solid tumours, while the most optimal therapeutic modality would target cancer stem cells. In order to develop such a therapy in the future, clearly, the most vulnerable targets specific for the most malignant tumour cells ought to be established. Recent investigation of dysregulatory mechanisms that endow tumour cell with an 'invincible' status frequently involve changes affecting mitochondrial enzymes and regulatory proteins (Barbosa et al. 2012; Smolková et al. 2011a; Zhao et al. 2011; Ramsay et al. 2011; Shih et al. 2012; Smolková and Ježek 2012; Teicher et al. 2012; Wenner 2012). Thus, targeting mitochondria in cancer cells appears to be a promising and strategic approach (Zhao et al. 2011; Ramsay et al. 2011). Conceivably, cancer-specific glycolytic enzymes should be simultaneously targeted in order to completely eliminate tumour cells.

In this chapter we focus on three key mitochondrial targets that have already been established to contribute to the survival of highly malignant tumours. These targets include the mitochondrial isocitrate dehydrogenase-2 (IDH2), hexokinase-2 (HK2), and cancer-specific isoforms of pyruvate dehydrogenase kinase (PDK). The emerging knowledge on other mitochondrial proteins that may represent anti-cancer targets or prediction markers, such as the immediate early response gene X-1 (IEX1), is also briefly discussed.

2 Isocitrate Dehydrogenase-2

During malignant transformation, cells undergo stages of gene expression reprogramming and mutagenesis that alter their metabolic phenotype(s) (Yuneva 2008; Bellance et al. 2009; Mullen et al. 2011; Smolková et al. 2011a). Initial stimuli (not

all known) deregulate the 'information' signalling and activate oncogenes and/or cancer stem cells, resulting in a partial glycolytic "Warburg" phenotype, in which pyruvate is diverted from oxidative phosphorylation (OXPHOS) to lactate, at least to some extent. Higher proliferation and impaired angiogenesis subsequently cause hypoxia in certain regions within a growing tumour, and the hypoxia-mediated metabolic re-programming (such as that promoted by the hypoxia-induced factor, HIF; Denko 2008; Ježek et al. 2010; Shaw 2006) further promotes the glycolytic phenotype. The sustained high rate of cell proliferation, however, results frequently in aglycemia, initiating the revival of OXPHOS in conjunction with the promotion of general glutaminolysis (Yuneva et al. 2007; DeBerardinis et al. 2008; Mullen et al. 2011; Smolková et al. 2011a; Smolková and Ježek 2012; Teicher et al. 2012), a mitochondrial pathway of glutamine or serine utilisation.

Glutaminolysis *per se*, defined as a pathway converting glutamine to 2-oxoglutarate (2OG), can be followed by truncated Krebs cycle or by setting the Krebs cycle in the reverse direction, both with concomitant citrate efflux from the mitochondrial matrix. Therefore, we further use a term (general) glutaminolysis for the description of all metabolic reactions that are involved. In this sense, general glutaminolysis can proceed either as the classical OXPHOS-linked glutaminolysis (Smolková et al. 2011a; Smolková and Ježek 2012), encompassing the forward Krebs cycle truncated after citrate synthase; or as the *reductive carboxylation* ("anoxic") *glutaminolysis* (RCG), utilising the only two enzymes of the Krebs cycle that can act in the reverse mode: IDH2 and the reversed aconitase reaction (Fig. 10.1). OXPHOS-linked glutaminolysis can fully compensate for the cellular ATP demand. On the contrary RCG is not an ATP-generating pathway and cannot support proliferation without additional ATP supply, such as that produced by glycolysis. RCG can act either transiently until the glycolytic ATP pool is consumed, providing anoxic/hypoxic tumours to survive during a critical period until glucose supply and utilisation is restored; or it must act in parallel with OXPHOS-lined glutaminolysis. Both modes of glutaminolysis provide pyruvate, lactate, and the NADPH pool, normally supplied by glucose metabolism via the pentose phosphate pathway. Citrate extrusion from mitochondria (allowed among other factors by the reversed IDH2 reaction) and NADPH are required for lipid biosynthesis, and the produced alanine is used for cytosolic protein synthesis.

Recently, it has become clear that the initial RCG may give rise due to IDH2 and cytosolic IDH1 heterozygous mutations to the production of the oncometabolite D-2-hydroxy-glutarate (2HG), which further accelerates tumourigenesis in grade 2 and 3 gliomas (Chou et al. 2012; Fu et al. 2012; Ichimura 2012; Jenkins et al. 2012; Theeler et al. 2012), secondary glioblastomas (less frequently in primary glioblastomas) (Ducray et al. 2009; Yan et al. 2009; Guo et al. 2011; Horbinski et al. 2010; Amary et al. 2011; Capper et al. 2011; Krell et al. 2011; Mellai et al. 2011; Reitman et al. 2011; Ward et al. 2012; Yang et al. 2012), acute myeloid leukemia (AML, Dang et al. 2010), T-cell angioimmunoblastic lymphoma (Cairns et al. 2012), chondrosarcoma (Amary et al. 2011), and intrahepatic cholangiocarcinomas (Wang et al. 2013). It may also play a role in colorectal (Sjoblom et al. 2006) and prostate cancer (Ghiam et al. 2012). The reverse reaction of mitochondrial IDH2 (with mutated Arg-172 and Arg-140) and the cytosolic IDH1 (with mutated Arg-132) produces

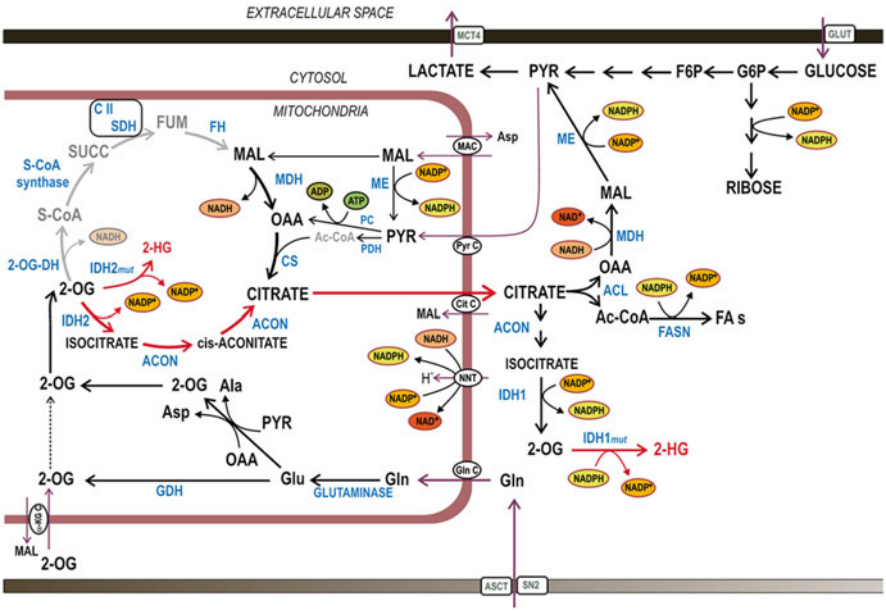

Fig. 10.1 Reductive carboxylation glutaminolysis in cancer cells. Glutaminolysis *per se* principally provides 2-oxoglutarate (2OG) for the Krebs cycle, starting by glutamine import from the extracellular space by the glutamine transporters ASCT2 and SN2. Glutamine is then deaminated to glutamate by a phosphate-dependent glutaminase or transaminated to yield glutamate (Glu) via the nucleotide biosynthesis pathway (not shown). Glutamate is further metabolised by the transaminases activity of glutamate dehydrogenase (GDH) to yield 2OG which then supplies the Krebs cycle. Cancer cells with deregulated c-Myc do not efficiently metabolise 2OG by 2OG dehydrogenase (2OG-DH), but employ reductive carboxylation glutaminolysis (RCG, *red arrows*). Under conditions of RCG the reaction of the isocitrate dehydrogenase isoform-2 (IDH2) is re-directed to NADPH-dependent 'reverse' reaction converting 2OG to isocitrate, or to 2-hydroxyglutarate (2HG), when IDH2 mutants occur (IDH2*mut*) in later stages of tumorigenesis. 2HG is an oncometabolite deregulating cell epigenetics. Consequently, 2OG and isocitrate are converted to citrate by aconitase (ACON). NADPH is readily generated by the mitochondrial malic enzyme and/or mitochondrial nicotinamide nucleotide transhydrogenase (NNT). This pathway is completely independent of the regular forward Krebs cycle, thus on oxygen levels and OXPHOS. However, it itself cannot support proliferation without ATP produced by glycolysis or by other means, such as OXPHOS glutaminolysis, which metabolises 2OG to citrate. In both glutaminolytic modes, citrate is exported from the mitochondrial matrix to the cytosol as a precursor for *de novo* synthesis of fatty acids (FAs) and lipids. Similarly, cytosolic isocitrate dehydrogenase isoform-1 (IDH1), when mutated (IDH1mut) forms the oncometabolite 2HG, when at least some non-mutant IDH1 is present, which forms 2OG that can be converted to 2HG. *Further abbreviations*: *Cit C* citrate carrier, *FH* fumarate hydratase (fumarase), *FUM* fumarate, *Gln C* glutamine carrier, *MAC* malate aspartate "shuttle", representing the oxoglutarate carrier (2-OG C) and glutamate aspartate carrier, *Pyr C* pyruvate carrier, *S-CoA* succinyl-CoA, *SUCC* succinate, *SDH* succinate dehydrogenase, *MAL* malate, *MDH* malate dehydrogenase, *OAA* oxaloacetate, *PYR* pyruvate, *CS* citrate synthase, *PC* pyruvate carboxylase, *ACL* ATP-citrate lyase, *FASN* fatty acid synthase

2HG from 2-oxoglutarate (Dang et al. 2009; Gross et al. 2010; Capper et al. 2011; Jin et al. 2011; Metallo et al. 2011; Xu et al. 2011; Ward et al. 2010, 2012, 2013; Choi et al. 2012; Fathi et al. 2012). 2HG further promotes neoplasia by competitive inhibition of histone demethylation (Duncan et al. 2012) and 5-methylcytosine hydroxylation, leading to genome-wide alternations in the methylation of histones and DNA (Jin et al. 2011; Chou et al. 2012; Duncan et al. 2012; Lu et al. 2012; Sasaki et al. 2012; Shih et al. 2012). The resulting inhibition of histone demethylation is sufficient to block differentiation of non-transformed cells (Lu et al. 2012). Also, 2HG accumulating in IDH1/2 mutant brain tumours promotes oncogenic transformation by stimulating prolyl-4-hydroxylase activity, whereby lowering the HIFα levels (Koivunen et al. 2012). Surprisingly, it has also been reported that the glioblastoma SF188 cells produce 2HG under hypoxia, albeit lacking the IDH1/2 mutations (Wise et al. 2011). It should be noted that RCG is inactivated under condition of particular IDH1 or IDH2 mutants (Leonardi et al. 2012), since 2HG is not metabolised by aconitase.

RCG has also been detected in human osteosarcoma 143B cells, in which the mitochondrial DNA (mtDNA) encoded a loss-of-function mutation in the respiratory chain complex III (CYTB 143B cells) (Mullen et al. 2011). Because only low-level RCG was detected in wild-type 143B cells, the authors suggested that the impairment of OXPHOS, such as given by mutant mitochondrial DNA, induces RCG. Silencing of either IDH1 or IDH2 reduced the growth of both wild-type and CYTB 143B cells (Mullen et al. 2011). Moreover, unlike in wild-type 143B cells, *de novo* fatty acid synthesis from glutamine as a precursor is prevalent in CYTB 143B cells. RCG in fumarate hydratase-devoid UOK262 cells, which are defective in respiration, has also been identified, typically in parallel with OXPHOS glutaminolysis (Mullen et al. 2011). Interestingly, inhibition of respiration in mouse embryonic fibroblasts *via* administration of antimycin, rotenone or metformin induces high 2OG utilisation towards RCG (Mullen et al. 2011).

Melanomas require RCG for lipogenesis (Lian et al. 2012), and functional IDH2 prevents the loss of 5-methylcytosine hydroxylation in melanomas (Juratli et al. 2012). IDH2 was found down-regulated in early phase development of colon carcinoma and upregulated in its advanced phase (Lv et al. 2012). RCG thus may play a significant role in colon cancer as well. All these data, including ours (Smolková et al. 2011b), suggest a hypothesis assuming that reductive RCG is a common cellular response to impaired mitochondrial metabolism, which proceeds in different tumours. RCG then hinders the oxidising capacity of the Krebs cycle, *i.e.*, its ability to utilise 2OG oxidatively for ATP synthesis via OXPHOS. Targeting RCG by genetic (silencing) and/or pharmacological means is a prerequisite to the future design of a new class of emerging anti-cancer drugs. One must make a distinction between such targeting in the earlier phase of oncogenic development and the occurrence of IDH1/2 mutations. In the later stage when 2HG becomes detectable, strategies how to compensate for the adverse effects of 2HG ought to be developed (Choi et al. 2012).

RCG was predicted in 1994 by Sazanov and Jackson (1994) (see also Des Rosiers et al. 1994) as the reductive carboxylation reaction by IDH2 that converts

2-oxoglutarate (2OG) to isocitrate while oxidising NADPH to $NADP^+$. This "reverse" IDH2 reaction mode should proceed more efficiently *in vivo* when followed by the reverse aconitase reaction and the subsequent citrate export from the mitochondrial matrix (Fig. 10.1). In general, the direction of the wild-type IDH2 reaction is given by the $NADP^+$/NADPH ratio in the mitochondrial matrix. Although the sirtuin SIRT3-based activation regulates the mitochondrial redox status by deacetylation of Lys-413 of human IDH2 (Yu et al. 2012), it is not known whether this deacetylation also activates the reverse (NADPH-dependent) IDH2 reaction. Reductive carboxylation was demonstrated for IDH2 by Comte at al. (2002) and was documented in transformed brown adipocytes (Yoo et al. 2008). Its existence was later demonstrated in the pediatric glioma SF188 cells (Ward et al. 2010; Wise et al. 2011) and UOK262 cells (from a renal tumour in a patient with hereditary leiomyomatosis; these cells are defective in respiration and devoid of fumarate hydratase activity) (Mullen et al. 2011).

Reductive carboxylation accompanied by citrate efflux has been found also in quiescent fibroblasts, being further enhanced in the contact-inhibited cells (Lemons et al. 2010). IDH2 silencing in SF188 cells results in diminished conversion of glutamine to citrate (Ward et al. 2010; Wise et al. 2011). Hypoxia was found to increase RCG in SF188 cells in a HIF-dependent manner (Wise et al. 2011). SF188 cells were able to proliferate at 0.5 % O_2 even if such hypoxic conditions substantially diminished the glucose-based production of citrate, *i.e.*, OXPHOS, promoting the Krebs cycle contribution to the ATP production (Wise et al. 2011). Further, reductive carboxylation was detected in the osteosarcoma 143B cells with a loss-of-function mutation in respiratory chain complex III (Mullen et al. 2011). RCG is therefore considered as a common cellular response to impaired mitochondrial metabolism.

The reductive carboxylation reaction and the overall RCG may indeed proceed together with general OXPHOS glutaminolysis, involving the Krebs cycle truncated to export citrate into the matrix. The best evidence was obtained by tracking the metabolites of ^{13}C-labelled glutamine, such as the appearance of ^{13}C-citrate (Yoo et al. 2008; Ward et al. 2010; Mullen et al. 2011; Wise et al. 2011; Lian et al. 2012). These demonstrations of RCG in cancer cells are consistent with the recent finding that mutant IDH2 in gliomas, AML and other cancer types (see above) also produces 2HG from 2OG by "alternate" reductive carboxylation. Because these mutants are heterozygous, both RCG and the production of 2HG might occur simultaneously. Interestingly, cells with mutant IDH2 are capable to form 2HG, but cytosolic IDH1 mutants always require a wild-type IDH1 homodimeric partner for 2HG production (Ward et al. 2013). We therefore predict that in heterozygous IDH2 mutant cells, the RCG reaction pathway involves the 'non-mutant', NADPH-dependent ('reverse') IDH2 reaction followed by isocitrate conversion to citrate and by citrate export. The mutant IDH2 (but maybe also wild-type IDH2; Wise et al. 2011) acting also in a 'reverse' mode, however, produces 2HG, which cannot be metabolised by aconitase (Leonardi et al. 2012). Its fate is currently unknown. There is therefore no metabolic activity for the depletion of 2HG, its excessive accumulation yielding accelerated tumorigenesis (Xu et al. 2011; Chou et al. 2012; Duncan et al. 2012; Koivunen et al. 2012; Lu et al. 2012; Sasaki et al. 2012; Shih et al. 2012).

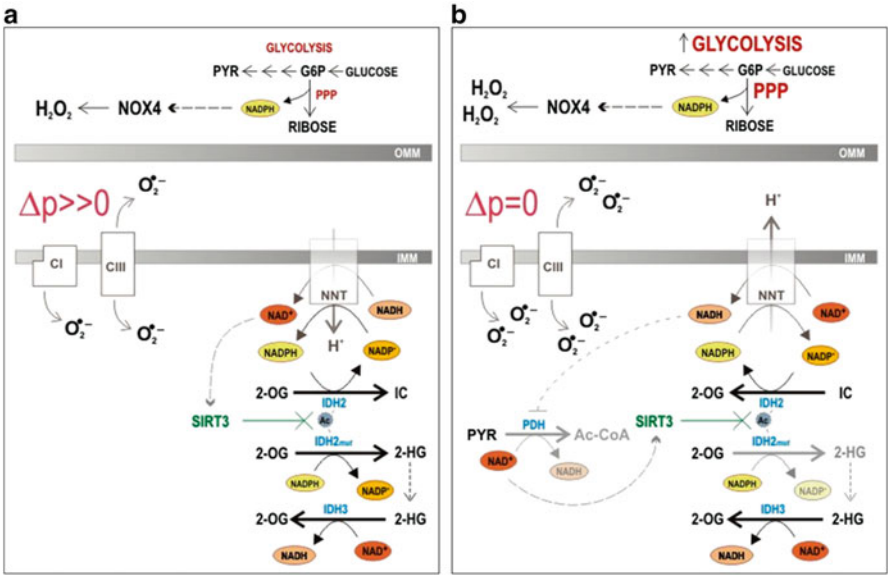

Fig. 10.2 The role of mitochondrial nicotinamide nucleotide transhydrogenase (*NNT*) in promoting NADPH-dependent (reverse) IDH2 reaction and the 2-oxoglutarate/2-D-hydroxyglutarate dissipative cycle. (**a**) Typical NNT reaction direction producing NADPH at sufficient proton-motive force (Δp; composed of inner the mitochondrial membrane, IMM, potential plus ΔpH, expressed in mV). Besides the matrix malic enzyme, NNT provides further supply of NADPH for the reverse IDH2 reaction, thus promoting RCG. Even at diminished complex I respiration and hence NAD^+ formation, NNT also supplies NAD^+ for the activation of the sirtuin SIRT3-mediated deacetylation of IDH2. IDH2 in the forward $NADP^+$-dependent mode is activated by SIRT3, and we hypothesise that its reversed NADPH-dependent reaction is activated by SIRT3 as well. The 2-oxoglutarate/2-D-hydroxyglutarate (2OG/2HG) dissipative cycle would be hypothetically possible when the Krebs cycle enzyme, isocitrate dehydrogenase isoform-3 (IDH3) metabolises 2HG at the expense of NAD^+. Since such cycle consumes the exact amounts of NADPH and NAD^+ produced by NNT, it may excessively consume (dissipate) Δp to zero values. (**b**) The reverse NNT reaction direction that consumes NADPH is established at very low Δp values. However, it should necessarily lead neither to the forward $NADP^+$-dependent IDH2 reaction, such as displayed in the scheme not to the abolishment of RCG, since the matrix malic enzyme can still to supply sufficient NADPH. Nevertheless the 2OG/2HG dissipative cycle might be diminished by the NAD^+ shortage, unless it is balanced, e.g. by complex I respiration. The upper part of the schemes illustrates the role of the elevated metabolic flux via the pentose phosphate shuttle, of which the first enzyme forms NADPH used by constitutively expressed NADPH oxidase isoform-4 (NOX4) to produce H_2O_2. This mechanism, together with dormant mitochondria, low OXPHOS and slow respiration, and hence high formation of mitochondrial superoxide, typically contributes to oxidative stress in tumour cells

Enzymatic degradation of 2HG could e considered as an efficient strategy for the therapy of cancers bearing IDH1/2 mutations.

Figure 10.1 depicts that the formation of NADPH by the matrix malic enzyme should be sufficient to drive the reverse IDH2 reaction. Neither NADH/NAD^+ nor NADPH/$NADP^+$ can be imported from the cytosol. Another enzyme capable of NADPH formation from $NADP^+$ at the expense of NADH and proton-motive force

is the mitochondrial nicotinamide nucleotide transhydrogenase (NNT) (Albracht et al. 2011), which can also provide NAD^+ for possible SIRT3-mediated activation of IDH2, even if the complex I-dependent NAD^+ formation is retarded (Fig. 10.2a). NNT may cause that the possible 2HG cycling, proceeding as the conversion of 2OG to 2HG by the well established reverse IDH2 reaction at the expense of NADPH together with the still hypothetical (forward) conversion of 2HG back to 2OG via IDH3 at the expense of NAD^+, would dissipate the proton-motive force (Sazanov and Jackson 1994; Smolková and Ježek 2012). Consequently, the oncometabolite 2HG would also act as an 'uncoupler' suppressing the remaining ATP synthesis.

Under conditions of almost no proton-motive force, NNT might reverse its reaction, which would still allow for 2HG cycling (Fig. 10.2b). The consumption of NADPH in the matrix consequently shifts the homeostasis of ROS towards oxidative stress, which is, in turn, anti-oxidatively balanced by the NADPH production by the mitochondrial malic enzyme (Bellance et al. 2009; Smolková et al. 2011a) and NNT (Sazanov and Jackson 1994; Pedersen et al. 2008). Since the mitochondrial IDH2 reaction is driven mainly by the NADPH/$NADP^+$ ratio, the NNT activity and NADPH formation rate significantly influence the IDH2 reaction rate and its direction. Silencing of NNT in rat pheochromocytoma PC12 cells decreased the NADPH/$NADP^+$ ratio, which was accompanied by the increase of oxidative stress as indicated by the increased level of GSSG (Yin et al. 2012). The initial NADH accumulation after NNT knock-down also sensitised PDH to phosphorylation and hence inactivation, which led to the limited acetyl-CoA formation and to retarded Krebs cycle. On the other hand, prolonged NNT inactivation caused a drop in the NADH/NAD^+ ratio, facilitating the SIRT3 activity.

3 Strategies of Defense Against Isocitrate Dehydrogenase-2-Related Oncogenesis

To date, there have been no clinical trials for IDH2-related cancer therapy. In spite of its emerging widespread occurrence, strategies to target glutaminolysis have not been fully developed. Aminooxyacetate is an inhibitor of glutamate pyruvate transaminase, which converts glutamine to 2OG (Fig. 10.1). It was reported to inhibit the proliferation of the SF188 glioblastoma cells as well as the growth of breast cancer MDA-MB-231 xenografts in mice (Wise et al. 2008; Thornburg et al. 2008). Suppression of glutaminolysis by aminooxyacetate led to the TNF-related apoptosis-inducing ligand (TRAIL)-promoted cell death in melanomas (Qin et al. 2010a). Nevertheless, there has been no clinical development of transaminase inhibitors (Ramsay et al. 2011).

An ideal drug should affect only cancer-specific proteins. From this point of view, IDH2 appears a valuable target, since its inhibition in healthy tissue would lead merely to oxidative stress (Smolková and Ježek 2012) that could be defended against by suitable antioxidants. Targeting IDH2 would eliminate tumour cells

surviving at harsh conditions of hypoxia and intermittent aglycemia as well as in progressive stages of malignancies with mutant IDH2 or IDH1 producing the oncometabolite 2HG. Thus, a potential of the IDH2 inhibitor oxalomalate (hydroxyoxalosuccinic acid) should be evaluated, in spite of its inhibitory ability with regard to aconitase and inducible nitric oxide synthase (Irace et al. 2007). Further, IDH2 silencing elements on proper carries could well substitute small molecule therapeutic approaches based targeting IDH2. Also strategies for withdrawal of adverse effects of 2HG should be exploited. Both natural and synthetic manners of 2HG degradation would facilitate therapy of cancers bearing IDH1/2 mutations.

4 Hexokinase Isoform-2 (HK2)

Proliferative states as diverse as the embryonal development and malignancies rely on aerobic glycolysis and preferentially express the HK2 isoform, found to be regulated in part epigenetically (Mathupala et al. 2010; Pedersen 2007; Wolf et al. 2011a, b). Activation of the PI3K-Akt pathway promotes aerobic glycolysis also by allowing HK2 binding to the voltage-dependent anion channel (VDAC), which forms pores in the outer mitochondrial membrane (Mathupala et al. 2010; Pedersen 2007). Interestingly, SIRT3-mediated deacetylation of cyclophilin D induces dissociation of HK2 from mitochondria (Shulga et al. 2010). In turn, migration of the ATF2 transcription factor to mitochondria triggered by PKCε perturbs the HK1/VDAC complex (Lau et al. 2012). Thus, ATP exported by the ADP/ATP carrier (AAC) from the mitochondrial matrix upon active OXPHOS is immediately available by glycolysis, as hexokinase controls its ultimately first step, irreversibly converting glucose to glucose-6-phosphate (Fig. 10.3).

Expression of HK2 was found to be elevated in response to interleukin 6 (Ando et al. 2010). Also a p53-inducible protein, the Tp53-induced glycolysis and apoptosis regulator (TIGAR) exerting fructose-2,6-bisphosphatase activity, which promotes glucose-6-phosphate flux into the pentose phosphate pathway, is relocated to mitochondria during hypoxia, forming a complex with HK2, whereby increasing its activity (Cheung et al. 2012). MicroRNA-143 (MiR143) of the fragile chromosome 5 site, which is frequently deleted in cancer, was found to down-regulate HK2 as its 3′URT contains a MiR143 cognate sites (Fang et al. 2012; Gregersen et al. 2012; Jiang et al. 2012; Peschiaroli et al. 2013). Consequently, the loss of MiR143-facilitates repression of HK2 to promote the glycolytic (Warburg) phenotype. HK2 repression by MiR143 was observed in colon cancer cells (Gregersen et al. 2012), human lung cancer (Fang et al. 2012), head and neck squamous cell carcinoma-derived cell lines (Peschiaroli et al. 2013), and breast cancer cells (Jiang et al. 2012). HK2 expression has been proposed as an independent predictor of hepatocellular carcinoma survival (Kwee et al. 2012) and a marker of poor prognosis was found in patients expressing HK2 in hepatocellular carcinoma (Gong et al. 2012) and human brain metastases as well as breast cancer (Palmieri et al. 2009).

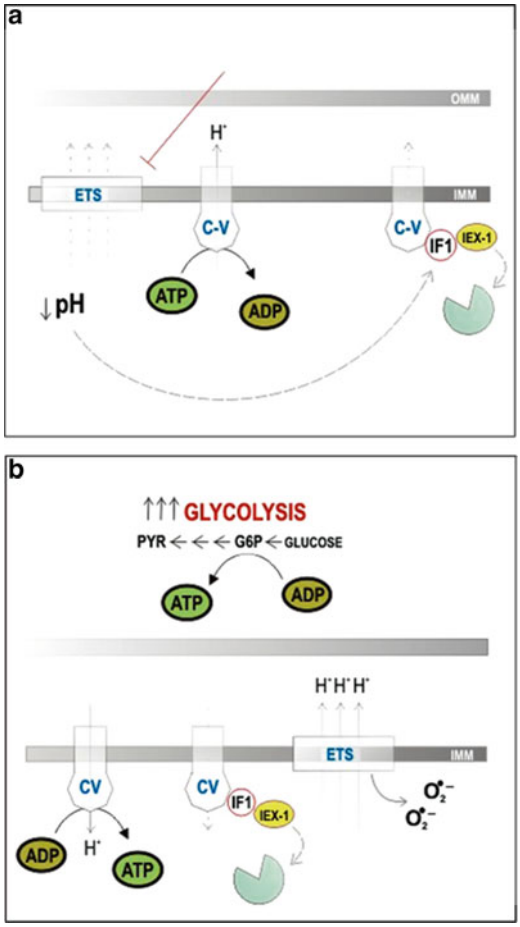

Fig. 10.3 Possible role of IEX1 and the inhibitory factor IF1 in regulation of ATP synthesis and oxidative stress. (**a**) Pathological situation of dormant OXPHOS involves the canonical function of IF1 at the reversed H^+ flow via ATP synthase due to ATP hydrolysis. The concomitantly lower matrix pH then activates IF1, which stops ATP hydrolysis, thus preventing ATP depletion. Under these conditions, IEX1-mediated IF1 degradation further promotes ATP hydrolysis and H^+ pumping by ATP synthase. (**b**) OXPHOS conditions in cancer cell are indicated when yet unknown stimuli activate IF1, thus inhibiting ATP synthesis leading to state-4-like conditions, which evoke higher superoxide mitochondrial formation and increased glycolysis. In this case, IEX1-mediated IF1 degradation acts in an anti-oxidative manner and helps promote OXPHOS

5 HK2 as the Therapeutic Target in Aerobic Glycolysis

Aerobic glycolysis in general is a target of clinical and experimental anti-cancer strategies (Upadhyay et al. 2013). Inhibition of mitochondria-localised HK2, being at the beginning of the glycolytic pathway, belongs to promising approaches in current oncology. 3-Bromopyruvate (3BP) has been under intensive experimental

investigation *in vitro* and *in vivo* (Cardaci et al. 2012). In addition to HK2 inhibition, 3BP negatively influences glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Ganapathy-Kanniappan et al. 2013) and antagonises the effect of lactate and pyruvate (El Sayed et al. 2012a, b). 3BP is known to induce apoptotic cell death in hepatocellular carcinoma (Kim et al. 2007a, b) and in hepatoma (Kim et al. 2008) cell lines, MCF-7 breast cancer cells (Liu et al. 2009), melanoma cell lines (Qin et al. 2010a, b) and multiple myeloma cells (Nakano et al. 2012). Synergic antiproliferative effect of 3BP was recorded with 2-deoxyglucose in pancreatic cancer cells (Xiao et al. 2013), and with platinum drugs in hepatocellular cancer (Ihrlund et al. 2008). Using systemic animal model, 3BP suppressed the aggressive EBV3-induced lymphomas in mice (Schaefer et al. 2012), and effectively prolonged the survival of mice with mesothelioma; this effect was potentiated by cisplatin (Zhang et al. 2009). Complete suppression of hepatocellular cancer by 3BP without significant toxicity has been reported (Ko et al. 2004). In combination with heat shock protein HSP90 inhibitors, 3BP inhibited transgenic spontaneous pancreatic cancer formation and metastasis in mice (Cao et al. 2008a, b).

Before human clinical trials, potential liver and gastrointestinal toxicity must be considered (Chang et al. 2007; Vali et al. 2007). Notwithstanding, the first case of the use of 3BPin a human subject human suffering from hepatocellular cancer was reported to significantly prolong the patient's survival ((Ko et al. 2012). 3BP was delivered using the transcatheter arterial chemoembolisation method following previous sorafenib/gemcitabine/cisplatin treatment. These promising results corroborate the necessity to conduct a sufficient number of clinical trials to find the proper and safe therapeutic approach utilising this simple agent.

Therapeutic targeting of HK2 should be also beneficial for glioblastoma multifome, the most aggressive type of brain tumour (Wolf et al. 2011a, b). 3BP therapy was successful also for multiple myeloma cells (Nakano et al. 2012). A combined treatment with iodoacetate, an inhibitor of G3PDH, and 3BP was reported for pancreatic cancer cells (Bhardwaj et al. 2010). Though 3BP has never been employed in clinical studies (Mathupala et al. 2010; Shoshan 2012), a combined therapy using promotion of oxidative stress via a D-amino acid oxidase and HK2 targeting by 3BP, together with citrate significantly inhibited angiogenesis (El Sayed et al. 2012a, b); this finding is of considerable translational value.

Direct delivery of ATP by AAC to VDAC-bound HK2 can be targeted by 4-(N-cysteinyl-acetyl) amino)phenylarsonous acid arising *in vivo* from the prodrugs 4-(N-(S-glutathionylacetyl) amino) phenylarsonous acid (GSAO) and 4-(N-(S-penicillaminylacetyl)amino) phenylarsonous acid (PENAO) (Don et al. 2003; Park et al 2012). GSAO has been tested in a phase I clinical trial due to its anti-angiogenic capacity (Elliott et al. 2012). Everolimus induced an increase in MiR143 and decreased in HK2 in pancreatic cancer cells (Liu et al. 2013). Trastuzumab, which down-regulates AKT/PI3K and MAPK signalling and is curative in HER2-high breast cancer patients, was reported to reduce HK2 in MDA-MB-453 xenografts via HER2 signalling, which is upstream of AKT/PI3K and MAPK (Smith et al. 2013). Clotrimazole induces a dose-dependent decrease in

glucose uptake in breast cancer cells (Furtado et al. 2012). A combined therapy with short hairpin RNA (shRNA) for HK2 and ^{131}I for the human sodium iodide symporter was reported for treatment of anaplastic thyroid carcinoma cells (Kim et al. 2011). These examples point to HK2 as a valuable target, in particular when the treatment is of a combinatorial nature, also potentially involving approaches making use of the tumour suppressor nature of MiR143.

6 Pyruvate Dehydrogenase Kinase Isoforms

The mitochondrial matrix pyruvate dehydrogenase (PDH) complex converts pyruvate to acetyl-coenzyme A (acetyl-CoA), which enters the Krebs cycle (Patel and Korotchkina 2003, 2006). This reaction is irreversible and unique within the cell and is therefore carefully controlled to maintain the glucose and lipid homeostasis. PDH activity is regulated by its products, NADH and acetyl-CoA, which inhibit pyruvate decarboxylation and further reactions of PDH by acetylating its E2 subunit. Additional key regulation includes phosphorylation/dephosphorylation of the E1 subunit [P21] by Ser/Thr kinases, the pyruvate dehydrogenase kinases (PDK), which by phosphorylating the E1 subunit inhibit the PDH activity (Patel and Korotchkina 2003, 2006).

PDKs are allosterically activated by E2 acetylation, thus reinforcing PDH inactivation by product inhibition. Reactivation of E1 by dephosphorylation is accomplished by pyruvate dehydrogenase phosphatases. Mammalian tissues express four isoenzymes of PDK and two isoenzymes of pyruvate dehydrogenase phosphatase with different tissue distribution, specific activities and sensitivities to effectors (Patel and Korotchkina 2006; Roche and Hiromasa 2007). Three sites are phosphorylated within E1: Ser-264 (site 1), Ser-271 (site 2), and Ser-203 (site 3); only PDK1 modifies site 3 (Korotchkina and Patel 2001). PDKs are recruited to the inner lipoyl (L2) domain of the E2 subunit only when a lipoyl group is covalently attached to Lys-173 of L2, while binding affinities decrease in the order of PDK3 > PDK1 ~ PDK2 > PDK4, hence the highest activity is exhibited by PDK3 (Tuganova et al. 2002). Another reason is that PDK3 is not inhibited by pyruvate (Baker et al. 2000). An efficient inhibitor of PDKs is represented by dichloroacetate (DCA) (Hur et al. 2013), which binds to the invariant DW-motif (Li et al. 2009).

Suppression of OXPHOS is an inherent part of adaptation to hypoxia. Thus, expression of PDK1 (Cairns et al. 2007; Kim et al. 2006) as well as PDK3 (Lu et al. 2008) increases in response to HIF1α stabilisation. PDK1 was found to be induced also in the Von Hippel-Lindau factor-deficient cells (Papandreou et al. 2006) and in response to deregulated c-Myc (Kim et al. 2007a, b). PDK1-induced inactivation of PDH during hypoxia effectively decreases respiration (Papandreou et al. 2006). The resulting inhibition provides a growth advantage because it makes pyruvate available to LDH, thus accelerating NADH re-oxidation and ATP production by glycolysis. PDK1 inhibition results in increased hypoxic cell

death in head and neck carcinoma (McFate et al. 2008), illustrating that down-regulation of respiration is required for cell survival during hypoxia. Tyrosine phosphorylation by outer mitochondrial membrane tyrosine kinases FGFR1 and FLT3 (which themselves may represent anti-cancer targets) enhances PDK1 kinase activity by promoting ATP binding and binding to the PDH complex (Hitosugi et al. 2011). Expression of PDK2 was found to be elevated in liver, kidney, and mammary gland during starvation (Wu et al. 2000), whereas high fat diet and diabetic development reportedly enhanced PDK4 expression (Holness et al. 2000). PDK4 gene expression was also found to be induced by the thyroid hormone (T3), glucocorticoids, and long chain fatty acids, while T3 acts via the PGC1α co-activator (Attia et al. 2010).

PDK1 immunochemical staining significantly correlated with gastric tumour invasion, the presence of a positive metastatic lymph node and larger tumour size, and it has been suggested to that PDK1 could be used as specific prognostic marker for more advanced gastric cancer (Hur et al. 2013). In colorectal tumours, HIF-responsible PDK3 was increased unlike PDK1 compared to the adjacent normal tissue (Lu et al. 2011).

Considering our hypothesis of several 'waves' of oncogenesis (Smolková et al. 2011a), when oncogenic gene re-programming is followed by hypoxic (namely HIF) re-programming, this is followed by re-establishment of OXPHOS and/or glutaminolysis. This speculation is consistent with the sequence of such waves ib renal cell carcinoma: upregulation of PDK-1 was recognised as an early event in the development of renal cell carcinoma, but not for the progression toward an aggressive phenotype, for which glutaminolysis is required (Baumunk et al. 2013).

7 Strategies to Defense Oncogenesis Related to Pyruvate Dehydrogenase Kinase Isoforms

DCA is an efficient inhibitor of PDK (Hur et al. 2013; Michelakis et al. 2008). Moreover, while allowing more 2OG to be produced, the prolyl hydroxylase domain (PHD) enzymes are activated, thus inhibiting HIF1α stabilisation and concomitant HIF-related signalling (Sutendra et al. 2013) that otherwise further strengthens the glycolytic (Warburg) phenotype. The resulting increase of the Krebs cycle turnover and OXPHOS pre-determine that more superoxide and consequently H_2O_2 is produced in mitochondria, which inn turn activates p53 and GSK3β (Sutendra et al. 2013). Such a normalisation of pseudo-hypoxic signalling leads also to the suppression of angiogenesis. Interestingly, p53 was reported to negatively regulate PDK2 (Contractor and Harris 2012).

DCA is a generic agent, used in the past, mostly to treat congenital lactic acidosis-related mitochondrial defects (Berendzen et al. 2006; Stacpoole et al. 1997, 2003). However, dose-limited toxicity described in some cases must be also considered (Kaufmann et al. 2006). Potential use of DCA in neoplasias was investigated in the

past. In the B6C3F1 mice, the agent caused hepatoproliferative lesions including adenomas and hepatocellular carcinomas (Bull et al. 1990; Nelson et al. 1990). However, in 2007 DCA was found to induce apoptosis, decrease proliferation in several cell lines and inhibit tumour growth in the athymic nude mice (Bonnet et al. 2007). DCA induced mitochondria-initiated apoptosis also in cells of endometrial cancer (Wong et al. 2008), ovarian (Saed et al. 2011), breast (Sun et al. 2011), and colorectal cancer (Kumar et al. 2012); as well as in neuroblastomas (Vella et al. 2012), gliomas(Morfouace et al. 2012), and in hematological malignancies (Madhok et al. 2010). DCA reduced HeLa and pancreatic cancer cell proliferation and viability (Anderson et al. 2009). Anti-tumour synergy of DCA was reported with cisplatin (Olszewski et al. 2010; Xie et al. 2011), 5-fluorouracil (Tong et al. 2011), omeprazole (Ishiguro et al. 2012a), or omeprazole with tamoxifen (Ishiguro et al. 2012b). Successful co-treatment of metastatic melanomas with DCA and a the pro-oxidative drug elesclomol was reported (Kluza et al. 2012). Further, DCA inhibits growth and sensitises to radiation in prostatic cancer cells (Cao et al. 2008a, b).

DCA has been considered a potential cancer therapeutic agent for several malignant tumours originating from the endometrium, breast and brain (Michelakis et al. 2008, 2010; Sun et al. 2010; Wong et al. 2008), as well as for glioma cancer stem cells (Morfouace et al. 2012). Such treatment could be applied to gastric cancer, which represents the second highest cause of cancer-related deaths worldwide (Jemal et al. 2007). Interestingly, DCA was found to overcome sorafenib resistance of hepatocellular carcinoma (Shen et al. 2013). Unfortunately, as a generic agent, DCA is not a focus of pharmaceutical companies and to date, only 16 clinical trials have been planned or conducted worldwide (www.clinicaltrials.gov). Of these, only six are in the field of oncology, of which some were terminated due to toxicity. To date, mostly individual case reports have been published. Complete remission of non-Hodgkin lymphoma lasting 4 years has been reported after the disease progressed during rituximab plus cytoxan, hydroxy-daunomycin, oncovin and prednisone treatment (Strum et al. 2013). Single case of cholangiocarcinoma treated with DCA, stable for 3 months, has reported (Ishiguro et al. 2012b).

The largest cohort of ‘five’ adult glioblastoma patients treated by DCA after surgical intervention, radiotherapy and several chemotherapies was reported by Michelakis et al. (2010). The patients were followed up for 15 months, and reversible peripheral neuropathy was observed as the only apparent sign of toxicity. From the efficacy point of view, four patients remained stable or the tumour regression was observed after combining DCA with temozolomide. One patient died after 3 months due to intracranial hypertension. Furthermore, intermittent intracranial re-surgeries allowed pre- and post-DCA evaluation of certain ‘metabolic’ or apoptotic markers in different time schedules. Thus, in two patients, before DCA treatment, HK2 was co-localised to mitochondria in primary cultures contributing to cell hyperpolarisation and resistance to apoptosis (Pastorino and Hoek 2008). However, cell lines isolated from these patients following chronic DCA therapy normalised the diffuse HK2 positivity. Three patients also exhibited increase of apoptosis (TUNEL assay), PDH activity, and p53 positivity after DCA

treatment in parallel to the decrease of PCNA. Also HIF1-positivity decreased post-DCA treatment. In an *ex vivo* experiment, H_2O_2 and 2OG production was increased in the glioblastoma cells after addition of DCA in a dose-dependent manner (Michelakis et al. 2010).

The above data point to a very promising direction of future cancer treatment via metabolic intervention of the equilibrium between aerobic glycolysis and OXPHOS. However, the reported example of experimentally effective generic agent DCA shows a necessity of further dose-escalating studies with larger cohorts to find safe and effective protocols to avoid unnecessary toxicity that can be a result of self-administered treatment (Brandsma et al. 2010).

8 Other Important Mitochondrial Anti-cancer Targets

Other targets related to mitochondria are emerging, even if not yet completely understood from the point of view of their molecular physiology/pathology. Important targets are related to mitochondria-initiated apoptosis (Barbosa et al. 2012; Wenner 2012), that are out of scope of this review (Dong et al. 2008; Kluckova et al. 2013; Neuzil et al. 2001), including the benzodiazepine receptor, termed also the translocator protein TSPO, a mitochondrial drug- and cholesterol-binding protein (Batarseh and Papadopoulos 2010; Mukherjee and Das 2012).

The immediate early responsive gene X1 (IEX1) represents an important factor of potential future use in cancer gene therapy (or at least as a complex prognostic factor with outcomes dependent on the tumour type and stage). IEX1 (also known as IER3) is not a transcriptional factor, in spite of belonging to the family of *c-fos*, *c-jun* and *c-myc* genes (Arlt and Schäfer 2011; Kapoor 2012). Like the other members, IEX1 can be transcriptionally activated within minutes and multiple-way activation is possible also without the need of protein synthesis. IEX1 is a stress gene up-regulated e.g. by the NFκB (Huang et al. 2002) and ERK/MAPK pathways (Letourneux et al. 2006). It cay be pro-apoptotic when acting in the nucleus (Shen et al. 2006) or anti-apoptotic when acting in mitochondria (Shen et al. 2009). Its trans-membrane domain allows IEX1 localisation also to the endoplasmic reticulum. In general IEX acts distinctly in different cell types and tumour stages, affecting the cell cycle, differentiation and survival, or, on the other hand, promoting apoptosis (Kapoor 2012). IEX1 is constitutively expressed in epithelia such as of the skin, trachea, as well as gastrointestinal and genitourinary epithelia, all having a direct contact with the external environment (Feldmann et al. 2001).

It has been suggested that mitochondrial IEX1 protects from apoptosis, reportedly also by diminishing the mitochondrial production of ROS (Shen et al. 2009). When IEX1 migrates to the mitochondrial matrix, it associates with the C-terminus of the inhibitory protein of the F_1 domain of H^+ATP synthase (IF1), thus sensitising IF1 for cleavage by an unknown protease (Shen et al. 2009). Hypothetically, IEX1 therefore prevents the slow-down of ATP synthesis by IF1, including the blockage of the possible reverse H^+ flux via F_OF_1ATP synthase when ATP hydrolysis drives

H^+ pumping out of the matrix. This pro-OXPHOS function of IEX1 could be employed to reduce the glycolytic phenotype of cancer cells and facilitate their apoptosis (by increasing ROS, besides the direct effect in the nucleus). These possibilities have been documented in HeLa cells, where IEX1 over-expression enhanced their apoptotic sensitivity to chemotherapeutic agents (Arlt et al. 2001). Similarly, radiation-induced glioma apoptosis was enhanced by IEX1 over-expression (Yamashita et al. 2009). In turn, the FDA-approved 26S-proteasome inhibitor bortezomib sensitises malignant lymphocytes to TNFα-mediated apoptosis *via* blocking NFκB activation and thereby IEX1 expression (Huang et al. 2002).

Theoretically, not only IEX1 over-expression but also IF1 silencing could decrease the Warburg phenotype (Formentini et al. 2012; Fujikawa et al. 2012; Sánchez-Aragó et al. 2012a), especially when targeting directly cancer cells. This is important, since IF1 inhibits ATP synthase, hence OXPHOS, and the increase in the proton-motive force becomes closer to the maximum state-4 values. Consequently, IF1 silencing while maintaining sufficient respiration increases ATP levels. However, since IF1 also inhibits the hydrolytic activity of ATP synthase at acid pH that might be established at the reversed H^+ flow (H^+ pumping) at the expense of ATP, a stable IF1 knock-down in the presence of an uncoupler drastically decreased the matrix and hence cellular ATP (Sánchez-Aragó et al. 2012b). The same could be predicted for IEX1 over-expression. While more work has to be carried out in particular to understand the molecular mechanism and cancer cell selectivity, IEX1 appears as an attractive, thus far unexplored, target for cancer therapy.

9 Conclusion

The equilibrium between aerobic glycolysis and oxidative phosphorylation, as well as between glutaminolysis and mitochondrial oncometabolite formation in neoplastic cells seem to be promising targets of treatment of the pathology. Improving OXPHOS may eliminate also side-effects like cancer-related cachexia. However, only a few clinical experiments proving the significance of this approach have been conducted in humans to date. Unfortunately, certain chemicals affecting metabolism acquiring and exhibiting experimental efficacy are well-known, including small and/or generic molecules. Therefore, there is not much interest of pharmaceutical companies, therefore extensive clinical testing cannot be expected in this field. The investigators should focus on finding new molecules and new possible mechanisms of targeting, such as using silencing elements on proper carriers, and acquire progress from the viewpoint of possible patent protection and subsequent investment from pharmaceutical business. Notwithstanding these logistic difficulties, mitochondria offer a number of thus far underexplored targets, whose clinical utilization can make a change in cancer management.

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References

Albracht SPJ, Meijer AJ, Rydström J (2011) Mammalian NADH: ubiquinone oxidoreductase (Complex I) and nicotinamide nucleotide transhydrogenase (Nnt) together regulate the mitochondrial production of H_2O_2 – implications for their role in disease, especially cancer. J Bioenerg Biomembr 43:541–564

Amary MF, Bacsi K, Maggiani F, Damato S, Halai D, Berisha F, Pollock R, O'Donnell P, Grigoriadis A, Diss T, Eskandarpour M, Presneau N, Hogendoorn PC, Futreal A, Tirabosco R, Flanagan AM (2011) IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. J Pathol 224:334–343

Anderson KM, Jajeh J, Guinan P, Rubenstein M (2009) In vitro effects of dichloroacetate and CO_2 on hypoxic HeLa cells. Anticancer Res 29:4579–4588

Ando M, Uehara I, Kogure K, Asano Y, Nakajima W, Abe Y, Kawauchi K, Tanaka N (2010) Interleukin 6 enhances glycolysis through expression of the glycolytic enzymes hexokinase 2 and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3. J Nippon Med Sch 77:97–105

Arlt A, Schäfer H (2011) Role of the immediate early response 3 (IER3) gene in cellular stress response, inflammation and tumorigenesis. Eur J Cell Biol 90:545–552

Arlt A, Grobe O, Sieke A, Kruse ML, Fölsch UR, Schmidt WE, Schäfer H (2001) Expression of the NF-kappa B target gene IEX-1 (p22/PRG1) does not prevent cell death but instead triggers apoptosis in Hela cells. Oncogene 20:69–76

Attia RR, Connnaughton S, Boone LR, Wang F, Elam MB, Ness GC, Cook GA, Park EA (2010) Regulation of pyruvate dehydrogenase kinase 4 (PDK4) by thyroid hormone: role of the peroxisome proliferator-activated receptor gamma coactivator (PGC-1 α). J Biol Chem 285:2375–2385

Baker JC, Yan X, Peng T, Kasten S, Roche TE (2000) Marked differences between two isoforms of human pyruvate dehydrogenase kinase. J Biol Chem 275:15773–15781

Barbosa IA, Machado NG, Skildum AJ, Scott PM, Oliveira PJ (2012) Mitochondrial remodeling in cancer metabolism and survival: potential for new therapies. Biochim Biophys Acta 1826:238–254

Batarseh A, Papadopoulos V (2010) Regulation of translocator protein 18kDa (TSPO) expression in health and disease states. Mol Cell Endocrinol 327:1–12

Baumunk D, Reichelt U, Hildebrandt J, Krause H, Ebbing J, Cash H, Miller K, Schostak M, Weikert S (2013) Expression parameters of the metabolic pathway genes pyruvate dehydrogenase kinase-1 (PDK-1) and DJ-1/PARK7 in renal cell carcinoma (RCC). World J Urol 31:1191–1196

Bellance N, Lestienne P, Rossignol R (2009) Mitochondria: from bioenergetics to the metabolic regulation of carcinogenesis. Front Biosci 14:4015–4034

Berendzen K, Theriaque DW, Shuster J, Stacpoole PW (2006) Therapeutic potential of dichloroacetate for pyruvate dehydrogenase complex deficiency. Mitochondrion 6:126–135

Bhardwaj V, Rizvi N, Lai MB, Lai JC, Bhushan A (2010) Glycolytic enzyme inhibitors affect pancreatic cancer survival by modulating its signaling and energetics. Anticancer Res 30:743–749

Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Bonnet S, Harry G, Hashimoto K, Porter CJ, Andrade MA, Thebaud B, Michelakis ED (2007) A mitochondria-K+channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 11:37–51

Brandsma D, Dorlo TP, Haanen JH, Beijnen JH, Boogerd W (2010) Severe encephalopathy and polyneuropathy induced by dichloroacetate. J Neurol 257:2099–2100

Bull RJ, Sanchez IM, Nelson MA, Larson JL, Lansing AJ (1990) Liver tumor induction in B6C3F1 mice by dichloroacetate and trichloroacetate. Toxicology 63:341–359

Cairns RA, Papandreou I, Sutphin PD, Denko NC (2007) Metabolic targeting of hypoxia and HIF1 in solid tumors can enhance cytotoxic chemotherapy. Proc Natl Acad Sci U S A 104:9445–9450

Cairns RA, Iqbal J, Lemonnier F, Kucuk C, de Leval L, Jais JP, Parrens M, Martin A, Xerri L, Brousset P, Chan LC, Chan WC, Gaulard P, Mak TW (2012) IDH2 mutations are frequent in angioimmunoblastic T-cell lymphoma. Blood 119:1901–1903

Cao W, Yacoub S, Shiverick KT, Namiki K, Sakai Y, Porvasnik S, Urbanek C, Rosser CJ (2008a) Dichloroacetate (DCA) sensitizes both wild-type and over expressing Bcl-2 prostate cancer cells in vitro to radiation. Prostate 68:1223–1231

Cao X, Jia G, Zhang T, Yang M, Wang B, Wassenaar PA, Cheng H, Knopp MV, Sun D (2008b) Non-invasive MRI tumor imaging and synergistic anticancer effect of HSP90 inhibitor and glycolysis inhibitor in RIP1-Tag2 transgenic pancreatic tumor model. Cancer Chemother Pharmacol 62:985–994

Capper D, Simon M, Langhans CD, Okun JG, Tonn JC, Weller M, Deimling AV, Hartmann C (2011) 2-Hydroxyglutarate concentration in serum from patients with gliomas does not correlate with IDH1/2 mutation status or tumor size. Int J Cancer 131:766–768

Cardaci S, Desideri E, Ciriolo MR (2012) Targeting aerobic glycolysis: 3-bromopyruvate as a promising anticancer drug. J Bioenerg Biomembr 44:17–29

Chang JM, Chung JW, Jae HJ, Eh H, Son KR, Lee KC, Park JH (2007) Local toxicity of hepatic arterial infusion of hexokinase II inhibitor, 3-bromopyruvate: in vivo investigation in normal rabbit model. Acad Radiol 14:85–92

Cheung EC, Ludwig RL, Vousden KH (2012) Mitochondrial localization of TIGAR under hypoxia stimulates HK2 and lowers ROS and cell death. Proc Natl Acad Sci U S A 109:20491–20496

Choi C, Ganji SK, DeBerardinis RJ, Hatanpaa KJ, Rakheja D, Kovacs Z, Yang XL, Mashimo T, Raisanen JM, Marin-Valencia I, Pascual JM, Madden CJ, Mickey BE, Malloy CR, Bachoo RM, Maher EA (2012) 2-hydroxyglutarate detection by magnetic resonance spectroscopy in IDH-mutated patients with gliomas. Nat Med 18:624–629

Chou AP, Chowdhury R, Li S, Chen W, Kim AJ, Piccioni DE, Selfridge JM, Mody RR, Chang S, Lalezari S, Lin J, Sanchez DE, Wilson RW, Garrett MC, Harry B, Mottahedeh J, Nghiemphu PL, Kornblum HI, Mischel PS, Prins RM, Yong WH, Cloughesy T, Nelson SF, Liau LM, Lai A (2012) Identification of retinol binding protein 1 promoter hypermethylation in isocitrate dehydrogenase 1 and 2 mutant gliomas. J Natl Cancer Inst 104:1458–1469

Comte B, Vincent G, Bouchard B, Benderdour M, Des Rosiers C (2002) Reverse flux through cardiac NADP(+)-isocitrate dehydrogenase under normoxia and ischemia. Am J Physiol 283:H1505–H1514

Contractor T, Harris CR (2012) p53 negatively regulates transcription of the pyruvate dehydrogenase kinase Pdk2. Cancer Res 72:560–567

Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liau LM, Rabinowitz JD, Cantley LC, Thompson CB, Vander Heiden MG, Su SM (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 462:739–744

Dang L, Jin S, Su SM (2010) IDH mutations in glioma and acute myeloid leukemia. Trends Mol Med 16:387–397

DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB (2008) The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab 7:11–20

Denko NC (2008) Hypoxia, HIF1 and glucose metabolism in the solid tumour. Nat Rev Cancer 8:705–713

Des Rosiers C, Fernandez CA, David F, Brunengraber H (1994) Reversibility of the mitochondrial isocitrate dehydrogenase reaction in the perfused rat liver. Evidence from isotopomer analysis of citric acid cycle intermediate. J Biol Chem 269:27179–27182

Don AS, Kisker O, Dilda P, Donoghue N, Zhao X, Decollogne S, Creighton B, Flynn E, Folkman J, Hogg PJ (2003) A peptide trivalent arsenical inhibits tumor angiogenesis by perturbing mitochondrial function in angiogenic endothelial cells. Cancer Cell 3:497–509

Dong LF, Low P, Dyason JC, Wang XF, Prochazka L, Witting PK, Freeman R, Swettenham E, Valis K, Liu J, Zobalova R, Turanek R, Spitz DR, Domann FE, Scheffler IE, Ralph SJ, Neuzil J (2008) α-Tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II. Oncogene 27:4324–4335

Ducray F, Marie Y, Sanson M (2009) IDH1 and IDH2 mutations in gliomas. New Engl J Med 360:2248–2249

Duncan CG, Barwick BG, Jin G, Rago C, Kapoor-Vazirani P, Powell DR, Chi JT, Bigner DD, Vertino PM, Yan H (2012) A heterozygous IDH1R132H/WT mutation induces genome-wide alterations in DNA methylation. Genome Res 22:2339–2355

El Sayed SM, El-Magd RM, Shishido Y, Chung SP, Diem TH, Sakai T, Watanabe H, Kagami S, Fukui K (2012a) 3-Bromopyruvate antagonizes effects of lactate and pyruvate, synergizes with citrate and exerts novel anti-glioma effects. J Bioenerg Biomembr 44:61–79

El Sayed SM, El-Magd RM, Shishido Y, Yorita K, Chung SP, Tran DH, Sakai T, Watanabe H, Kagami S, Fukui K (2012b) D-amino acid oxidase-induced oxidative stress, 3-bromopyruvate and citrate inhibit angiogenesis, exhibiting potent anticancer effects. J Bioenerg Biomembr 44:513–523

Elliott MA, Ford SJ, Prasad E, Dick LJ, Farmer H, Hogg PJ, Halbert GW (2012) Pharmaceutical development of the novel arsenical based cancer therapeutic GSAO for Phase I clinical trial. Int J Pharm 426:67–75

Fang R, Xiao T, Fang Z, Sun Y, Li F, Gao Y, Feng Y, Li L, Wang Y, Liu X, Chen H, Liu XY, Ji H (2012) MicroRNA-143 (miR-143) regulates cancer glycolysis via targeting hexokinase 2 gene. J Biol Chem 287:23227–23235

Fathi AT, Sadrzadeh H, Borger DR, Ballen KK, Amrein PC, Attar EC, Foster J, Burke M, Lopez HU, Matulis CR, Edmonds KM, Iafrate AJ, Straley KS, Yen KE, Agresta S, Schenkein DP, Hill C, Emadi A, Neuberg DS, Stone RM, Chen YB (2012) Prospective serial evaluation of 2-hydroxyglutarate, during treatment of newly diagnosed acute myeloid leukemia, to assess disease activity and therapeutic response. Blood 120:4649–4652

Feldmann KA, Pittelkow MR, Roche PC, Kumar R, Grande JP (2001) Expression of an immediate early gene, IEX-1, in human tissues. Histochem Cell Biol 115:489–497

Formentini L, Sánchez-Aragó M, Sánchez-Cenizo L, Cuezva JM (2012) The mitochondrial ATPase inhibitory factor 1 triggers a ROS-mediated retrograde pro-survival and proliferative response. Mol Cell 45:731–742

Fu Y, Zheng S, Zheng Y, Huang R, An N, Liang A, Hu C (2012) Glioma derived isocitrate dehydrogenase-2 mutations induced up-regulation of HIF-1α and β-catenin signaling: possible impact on glioma cell metastasis and chemo-resistance. Int J Biochem Cell Biol 44:770–775

Fujikawa M, Imamura H, Nakamura J, Yoshida M (2012) Assessing actual contribution of IF1, inhibitor of mitochondrial FoF1, to ATP homeostasis, cell growth, mitochondrial morphology, and cell viability. J Biol Chem 287:18781–18787

Furtado CM, Marcondes MC, Sola-Penna M, de Souza ML, Zancan P (2012) Clotrimazole preferentially inhibits human breast cancer cell proliferation, viability and glycolysis. PLoS One 7:e30462

Ganapathy-Kanniappan S, Kunjithapatham R, Geschwind JF (2013) Anticancer efficacy of the metabolic blocker 3-bromopyruvate: specific molecular targeting. Anticancer Res 33:13–20

Ghiam AF, Cairns RA, Thoms J, Dal Pra A, Ahmed O, Meng A, Mak TW, Bristow RG (2012) IDH mutation status in prostate cancer. Oncogene 31:3826

Gong L, Cui Z, Chen P, Han H, Peng J, Leng X (2012) Reduced survival of patients with hepatocellular carcinoma expressing hexokinase II. Med Oncol 29:909–914

Gregersen LH, Jacobsen A, Frankel LB, Wen J, Krogh A, Lund AH (2012) MicroRNA-143 down-regulates hexokinase 2 in colon cancer cells. BMC Cancer 12:232

Gross S, Cairns RA, Minden MD, Driggers EM, Bittinger MA, Jang HG, Sasaki M, Jin S, Schenkein DP, Su SM, Dang L, Fantin VR, Mak TW (2010) Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. J Exp Med 207:339–344

Guo CC, Pirozzi CJ, Lopez GY, Yan H (2011) Isocitrate dehydrogenase mutations in gliomas: mechanisms, biomarkers and therapeutic target. Curr Opin Neurol 24:648–652

Hitosugi T, Fan J, Chung TW, Lythgoe K, Wang X, Xie J, Ge Q, Gu TL, Polakiewicz RD, Roesel JL, Chen GZ, Boggon TJ, Lonial S, Fu H, Khuri FR, Kang S, Chen J (2011) Tyrosine phosphorylation of mitochondrial pyruvate dehydrogenase kinase 1 is important for cancer metabolism. Mol Cell 44:864–877

Holness MJ, Kraus A, Harris RA, Sugden MC (2000) Targeted upregulation of pyruvate dehydrogenase kinase (PDK)-4 in slow-twitch skeletal muscle underlies the stable modification of the regulatory characteristics of PDK induced by high-fat feeding. Diabetes 49:775–781

Horbinski C, Kelly L, Nikiforov YE, Durso MB, Nikiforova MN (2010) Detection of IDH1 and IDH2 mutations by fluorescence melting curve analysis as a diagnostic tool for brain biopsies. J Mol Diagn 12:487–492

Huang YH, Wu JY, Zhang Y, Wu MX (2002) Synergistic and opposing regulation of the stress-responsive gene IEX-1 by p53, c-Myc, and multiple NF-κB/rel complexes. Oncogene 21:6819–6828

Hur H, Xuan Y, Kim YB, Lee G, Shim W, Yun J, Ham IH, Han SU (2013) Expression of pyruvate dehydrogenase kinase-1 in gastric cancer as a potential therapeutic target. Int J Oncol 42:44–54

Ichimura K (2012) Molecular pathogenesis of IDH mutations in gliomas. Brain Tumor Pathol 29:131–139

Ihrlund LS, Hernlund E, Khan O, Shoshan MC (2008) 3-Bromopyruvate as inhibitor of tumour cell energy metabolism and chemopotentiator of platinum drugs. Mol Oncol 2:94–101

Irace C, Esposito G, Maffettone C, Rossi A, Festa M, Iuvone T, Santamaria R, Sautebin L, Carnuccio R, Colonna A (2007) Oxalomalate affects the inducible nitric oxide synthase expression and activity. Life Sci 80:1282–1291

Ishiguro T, Ishiguro M, Ishiguro R, Iwai S (2012a) Cotreatment with dichloroacetate and omeprazole exhibits a synergistic antiproliferative effect on malignant tumors. Oncol Lett 3:726–728

Ishiguro T, Ishiguro R, Ishiguro M, Iwai S (2012b) Co-treatment of dichloroacetate, omeprazole and tamoxifen exhibited synergistically antiproliferative effect on malignant tumors: in vivo experiments and a case report. Hepatogastroenterol 59:994–996

Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ (2007) Cancer statistics. CA Cancer J Clin 57:43–66

Jenkins RB, Xiao Y, Sicotte H, Decker PA, Kollmeyer TM, Hansen HM, Kosel ML, Zheng S, Walsh KM, Rice T, Bracci P, McCoy LS, Smirnov I, Patoka JS, Hsuang G, Wiemels JL, Tihan T, Pico AR, Prados MD, Chang SM, Berger MS, Caron AA, Fink SR, Halder C, Rynearson AL, Fridley BL, Buckner JC, O'Neill BP, Giannini C, Lachance DH, Wiencke JK, Eckel-Passow JE, Wrensch MR (2012) A low-frequency variant at 8q24.21 is strongly associated with risk of oligodendroglial tumors and astrocytomas with IDH1 or IDH2 mutation. Nat Genet 44:1122–1125

Ježek P, Plecitá-Hlavatá L, Smolková K, Rossignol R (2010) Distinctions and similarities of cell bioenergetics and role of mitochondria in hypoxia, cancer, and embryonic development. Int J Biochem Cell Biol 42:604–622

Jiang S, Zhang LF, Zhang HW, Hu S, Lu MH, Liang S, Li B, Li Y, Li D, Wang ED, Liu MF (2012) A novel miR-155/miR-143 cascade controls glycolysis by regulating hexokinase 2 in breast cancer cells. EMBO J 31:1985–1998

Jin G, Reitman ZJ, Spasojevic I, Batinic-Haberle I, Yang J, Schmidt-Kittler O, Bigner DD, Yan H (2011) 2-hydroxyglutarate production, but not dominant negative function, is conferred by glioma-derived NADP-dependent isocitrate dehydrogenase mutations. PLoS One 6:e16812

Juratli TA, Kirsch M, Robel K, Soucek S, Geiger K, von Kummer R, Schackert G, Krex D (2012) IDH mutations as an early and consistent marker in low-grade astrocytomas WHO grade II and their consecutive secondary high-grade gliomas. J Neurooncol 108:403–410

Kapoor S (2012) Emerging role of IEX-1 in tumor pathogenesis and prognosis. Ultrastruct Pathol 36:285

Kaufmann P, Engelstad K, Wei Y, Jhung S, Sano MC, Shungu DC, Millar WS, Hong X, Gooch CL, Mao X, Pascual JM, Hirano M, Stacpoole PW, DiMauro S, De Vivo DC (2006) Dichloroacetate causes toxic neuropathy in MELAS: a randomized, controlled clinical trial. Neurology 66:324–330

Kim JW, Tchernyshyov I, Semenza GL, Dang CV (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3:177–185

Kim JW, Gao P, Liu YC, Semenza GL, Dang CV (2007a) Hypoxia-inducible factor I and dysregulated c-myc cooperatively induce vascular endothelial growth factor and metabolic switches hexokinase 2 and pyruvate dehydrogenase kinase 1. Mol Cell Biol 27:7381–7393

Kim W, Yoon JH, Jeong JM, Cheon GJ, Lee TS, Yang JI, Park SC, Lee HS (2007b) Apoptosis-inducing antitumor efficacy of hexokinase II inhibitor in hepatocellular carcinoma. Mol Cancer Ther 6:2554–2562

Kim JS, Ahn KJ, Kim JA, Kim HM, Lee JD, Lee JM, Kim SJ, Park JH (2008) Role of reactive oxygen species-mediated mitochondrial dysregulation in 3-bromopyruvate induced cell death in hepatoma cells: ROS-mediated cell death by 3-BrPA. J Bioenerg Biomembr 40:607–618

Kim JE, Ahn BC, Hwang MH, Jeon YH, Jeong SY, Lee SW, Lee J (2011) Combined RNA interference of hexokinase II and (131)I-sodium iodide symporter gene therapy for anaplastic thyroid carcinoma. J Nucl Med 52:1756–1763

Kluckova K, Bezawork-Geleta A, Rohlena J, Dong L, Neuzil J (2013) Mitochondrial complex II, a novel target for anticancer agents. Biochim Biophys Acta 1827:552–564

Kluza J, Corazao-Rozas P, Touil Y, Jendoubi M, Maire C, Guerreschi P, Jonneaux A, Ballot C, Balayssac S, Valable S, Corroyer-Dulmont A, Bernaudin M, Malet-Martino M, de Lassalle EM, Maboudou P, Formstecher P, Polakowska R, Mortier L, Marchetti P (2012) Inactivation of the HIF-1α/PDK3 signaling axis drives melanoma toward mitochondrial oxidative metabolism and potentiates the therapeutic activity of pro-oxidants. Cancer Res 72:5035–5047

Ko YH, Smith BL, Wang Y, Pomper MG, Rini DA, Torbenson MS, Hullihen J, Pedersen PL (2004) Advanced cancers: eradication in all cases using 3-bromopyruvate therapy to deplete ATP. Biochem Biophys Res Commun 324:269–275

Ko YH, Verhoeven HA, Lee MJ, Corbin DJ, Vogl TJ, Pedersen PL (2012) A translational study "case report" on the small molecule "energy blocker" 3-bromopyruvate (3BP) as a potent anticancer agent: from bench side to bedside. J Bioenerg Biomembr 44:163–170

Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkissoon S, Losman JA, Joensuu P, Bergmann U, Gross S, Travins J, Weiss S, Looper R, Ligon KL, Verhaak RG, Yan H, Kaelin WG Jr (2012) Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. Nature 483:484–488

Korotchkina LG, Patel MS (2001) Site specificity of four pyruvate dehydrogenase kinase isoenzymes toward the three phosphorylation sites of human pyruvate dehydrogenase. J Biol Chem 276:37223–37229

Krell D, Assoku M, Galloway M, Mulholland P, Tomlinson I, Bardella C (2011) Screen for IDH1, IDH2, IDH3, D2HGDH and L2HGDH mutations in glioblastoma. PLoS One 6:e19868

Kumar A, Kant S, Singh SM (2012) Novel molecular mechanisms of antitumor action of dichloroacetate against T cell lymphoma: implication of altered glucose metabolism, pH homeostasis and cell survival regulation. Chem Biol Interact 199:29–37

Kwee SA, Hernandez B, Chan O, Wong L (2012) Choline kinase α and hexokinase-2 protein expression in hepatocellular carcinoma: association with survival. PLoS One 7:e46591

Lau E, Kluger H, Varsano T, Lee K, Scheffler I, Rimm DL, Ideker T, Ronai ZA (2012) PKCε promotes oncogenic functions of ATF2 in the nucleus while blocking its apoptotic function at mitochondria. Cell 148:543–555

Lemons JM, Lemons S, Feng XJ, Bennett BD, Legesse-Miller A, Johnson EL, Raitman I, Pollina EA, Rabitz HA, Rabinowitz JD, Coller HA (2010) Quiescent fibroblasts exhibit high metabolic activity. PLoS Biol 8:e1000514

Leonardi R, Subramanian C, Jackowski S, Rock CO (2012) Cancer-associated isocitrate dehydrogenase mutations inactivate NADPH-dependent reductive carboxylation. J Biol Chem 287:14615–14620

Letourneux C, Rocher G, Porteu F (2006) B56-containing PP2A dephosphorylate ERK and their activity is controlled by the early gene IEX-1 and ERK. EMBO J 25:727–738

Li J, Kato M, Chuang DT (2009) Pivotal role of the C-terminal DW-motif in mediating inhibition of pyruvate dehydrogenase kinase 2 by dichloroacetate. J Biol Chem 284:34458–34467

Lian CG, Xu Y, Ceol C, Wu F, Larson A, Dresser K, Xu W, Tan L, Hu Y, Zhan Q, Lee CW, Hu D, Lian BQ, Kleffel S, Yang Y, Neiswender J, Khorasani AJ, Fang R, Lezcano C, Duncan LM, Scolyer RA, Thompson JF, Kakavand H, Houvras Y, Zon LI, Mihm MC Jr, Kaiser UB, Schatton T, Woda BA, Murphy GF, Shi YG (2012) Loss of 5-hydroxymethylcytosine is an epigenetic hallmark of melanoma. Cell 150:1135–1146

Liu XH, Zheng XF, Wang YL (2009) Inhibitive effect of 3-bromopyruvic acid on human breast cancer MCF-7 cells involves cell cycle arrest and apoptotic induction. Chin Med J 122:1681–1685

Liu L, Gong L, Zhang Y, Li N (2013) Glycolysis in Panc-1 human pancreatic cancer cells is inhibited by everolimus. Exp Ther Med 5:338–342

Lu CW, Lin SC, Chen KF, Lai YY, Tsai SJ (2008) Induction of pyruvate dehydrogenase kinase-3 by hypoxia-inducible factor-1 promotes metabolic switch and drug resistance. J Biol Chem 283:28106–28114

Lu CW, Lin SC, Chien CW, Lin SC, Lee CT, Lin BW, Lee JC, Tsai SJ (2011) Overexpression of pyruvate dehydrogenase kinase 3 increases drug resistance and early recurrence in colon cancer. Am J Pathol 179:1405–1414

Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, Edwards CR, Khanin R, Figueroa ME, Melnick A, Wellen KE, O'Rourke DM, Berger SL, Chan TA, Levine RL, Mellinghoff IK, Thompson CB (2012) IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature 483:474–478

Lv Q, Xing S, Li Z, Li J, Gong P, Xu X, Chang L, Jin X, Gao F, Li W, Zhang G, Yang J, Zhang X (2012) Altered expression levels of IDH2 are involved in the development of colon cancer. Exp Ther Med 45:801–806

Madhok BM, Yeluri S, Perry SL, Hughes TA, Jayne DG (2010) Dichloroacetate induces apoptosis and cell-cycle arrest in colorectal cancer cells. Br J Cancer 102:1746–1752

Mathupala SP, Ko YH, Pedersen PL (2010) The pivotal roles of mitochondria in cancer: Warburg and beyond and encouraging prospects for effective therapies. Biochim Biophys Acta 1797:1225–1230

McFate T, Mohyeldin A, Lu H, Thakar J, Henriques J, Halim ND, Wu H, Schell MJ, Tsang TM, Teahan O, Zhou S, Califano JA, Jeoung NH, Harris RA, Verma A (2008) Pyruvate dehydrogenase complex activity controls metabolic and malignant phenotype in cancer cells. J Biol Chem 283:22700–22708

Mellai M, Piazzi A, Caldera V, Monzeglio O, Cassoni P, Valente G, Schiffer D (2011) IDH1 and IDH2 mutations, immunohistochemistry and associations in a series of brain tumors. J Neuro-Oncol 105:345–357

Metallo CM, Gameiro PA, Bell EL, Mattaini KR, Yang J, Hiller K, Jewell CM, Johnson ZR, Irvine DJ, Guarente L, Kelleher JK, Vander Heiden MG, Iliopoulos O, Stephanopoulos G (2011) Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. Nature 481:380–384

Michelakis ED, Webster L, Mackey JR (2008) Dichloroacetate as a potential metabolic-targeting therapy for cancer. Br J Cancer 99:989–994

Michelakis ED, Sutendra G, Dromparis P, Webster L, Haromy A, Niven E, Maguire C, Gammer TL, Mackey JR, Fulton D, Abdulkarim B, McMurtry MS, Petruk KC (2010) Metabolic modulation of glioblastoma with dichloroacetate. Sci Transl Med 2:31–34

Morfouace M, Lalier L, Bahut M, Bonnamain V, Naveilhan P, Guette C, Oliver L, Gueguen N, Reynier P, Vallette FM (2012) Comparison of spheroids formed by rat glioma stem cells and neural stem cells reveals differences in glucose metabolism and promising therapeutic applications. J Biol Chem 287:33664–33674

Mukherjee S, Das SK (2012) Translocator protein (TSPO) in breast cancer. Curr Mol Med 12:443–457

Mullen AR, Wheaton WW, Jin ES, Chen PH, Sullivan LB, Cheng T, Yang Y, Linehan WM, Chandel NS, Deberardinis RJ (2011) Reductive carboxylation supports growth in tumour cells with defective mitochondria. Nature 48:385–388

Nakano A, Miki H, Nakamura S, Harada T, Oda A, Amou H, Fujii S, Kagawa K, Takeuchi K, Ozaki S, Matsumoto T, Abe M (2012) Up-regulation of hexokinase II in myeloma cells: targeting myeloma cells with 3-bromopyruvate. J Bioenerg Biomembr 44:31–38

Nelson MA, Sanchez IM, Bull RJ, Sylvester SR (1990) Increased expression of c-myc and c-Ha-ras in dichloroacetate and trichloroacetate-induced liver tumors in B6C3F1 mice. Toxicology 64:47–57

Neuzil J, Weber T, Schröder A, Lu M, Ostermann G, Gellert N, Olejnicka B, Nègre-Salvayre A, Stícha M, Coffey RJ, Weber C (2001) Induction of cancer cell apoptosis by α-tocopheryl succinate: molecular pathways and structural requirements. FASEB J 15:403–415

Olszewski U, Poulsen TT, Ulsperger E, Poulsen HS, Geissler K, Hamilton G (2010) In vitro cytotoxicity of combinations of dichloroacetate with anticancer platinum compounds. Clin Pharmacol 2:177–183

Palmieri D, Fitzgerald D, Shreeve SM, Hua E, Bronder JL, Weil RJ, Davis S, Stark AM, Merino MJ, Kurek R, Mehdorn HM, Davis G, Steinberg SM, Meltzer PS, Aldape K, Steeg PS (2009) Analyses of resected human brain metastases of breast cancer reveal the association between up-regulation of hexokinase 2 and poor prognosis. Mol Cancer Res 7:1438–1445

Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 3:187–197

Park D, Chiu J, Perrone GG, Dilda PJ, Hogg PJ (2012) The tumour metabolism inhibitors GSAO and PENAO react with cysteines 57 and 257 of mitochondrial adenine nucleotide translocase. Cancer Cell Int 12:11

Pastorino JG, Hoek JB (2008) Regulation of hexokinase binding to VDAC. J Bioenerg Biomembr 40:171–182

Patel MS, Korotchkina LG (2003) The biochemistry of the pyruvate dehydrogenase complex. Biochem Mol Biol Edu 31:5–15

Patel MS, Korotchkina LG (2006) Regulation of the pyruvate dehydrogenase complex. Biochem Soc Trans 34:217–222

Pedersen PL (2007) Warburg, me and Hexokinase 2: multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the "Warburg Effect", i.e., elevated glycolysis in the presence of oxygen. J Bioenerg Biomembr 39:211–222

Pedersen A, Karlsson GB, Rydström J (2008) Proton-translocating transhydrogenase: an update of unsolved and controversial tissues. J Bioenerg Biomembr 40:463–473

Peschiaroli A, Giacobbe A, Formosa A, Markert EK, Bongiorno-Borbone L, Levine AJ, Candi E, D'Alessandro A, Zolla L, Finazzi Agrò A, Melino G (2013) miR-143 regulates hexokinase 2 expression in cancer cells. Oncogene 32:797–802

Qin JZ, Xin H, Nickoloff BJ (2010a) Targeting glutamine metabolism sensitizes melanoma cells to TRAIL-induced death. Biochem Biophys Res Commun 398:146–152

Qin JZ, Xin H, Nickoloff BJ (2010b) 3-Bromopyruvate induces necrotic cell death in sensitive melanoma cell lines. Biochem Biophys Res Commun 396:495–500

Ramsay EE, Hogg PJ, Dilda PJ (2011) Mitochondrial metabolism inhibitors for cancer therapy. Pharm Res 28:2731–2744

Reitman ZJ, Jin G, Karoly ED, Spasojevic I, Yang J, Kinzler KW, He Y, Bigner DD, Vogelstein B, Yan H (2011) Profiling the effects of isocitrate dehydrogenase 1 and 2 mutations on the cellular metabolome. Proc Natl Acad Sci U S A 108:3270–3275

Roche TE, Hiromasa Y (2007) Pyruvate dehydrogenase kinase regulatory mechanisms and inhibition in treating diabetes, heart ischemia, and cancer. Cell Mol Life Sci 64:830–849

Saed GM, Fletcher NM, Jiang ZL, Abu-Soud HM, Diamond MP (2011) Dichloroacetate induces apoptosis of epithelial ovarian cancer cells through a mechanism involving modulation of oxidative stress. Reprod Sci 18:1253–1261

Sánchez-Aragó M, Formentini L, Cuezva JM (2012a) Mitochondria-mediated energy adaption in cancer: the H(+)-ATP synthase-geared switch of metabolism in human tumors. Antioxid Redox Signal 19(3):285–298

Sánchez-Aragó M, Formentini L, García-Bermúdez J, Cuezva JM (2012b) IF1 reprograms energy metabolism and signals the oncogenic phenotype in cancer. Cell Cycle 11:2963–2964

Sasaki M, Knobbe CB, Munger JC, Lind EF, Brenner D, Brüstle A, Harris IS, Holmes R, Wakeham A, Haight J, You-Ten A, Li WY, Schalm S, Su SM, Virtanen C, Reifenberger G, Ohashi PS, Barber DL, Figueroa ME, Melnick A, Zúñiga-Pflücker JC, Mak TW (2012) IDH1(R132H) mutation increases murine haematopoietic progenitors and alters epigenetics. Nature 488:656–659

Sazanov LA, Jackson JB (1994) Proton-translocating transhydrogenase and NAD- and NADP-linked isocitrate dehydrogenases operate in a substrate cycle which contributes to fine regulation of the tricarboxylic acid cycle activity in mitochondria. FEBS Lett 344:109–116

Schaefer NG, Geschwind JF, Engles J, Buchanan JW, Wahl RL (2012) Systemic administration of 3-bromopyruvate in treating disseminated aggressive lymphoma. Transl Res 159:51–57

Shaw RJ (2006) Glucose metabolism and cancer. Curr Opin Cell Biol 18:598–608

Shen L, Guo J, Santos-Berrios C, Wu MX (2006) Distinct domains for anti- and pro-apoptotic activities of IEX-1. J Biol Chem 281:15304–15311

Shen L, Zhi L, Hu W, Wu MX (2009) IEX-1 targets mitochondrial F1F0-ATPase inhibitor for degradation. Cell Death Differ 16:603–612

Shen YC, Ou DL, Hsu C, Lin KL, Chang CY, Lin CY, Liu SH, Cheng AL (2013) Activating oxidative phosphorylation by a pyruvate dehydrogenase kinase inhibitor overcomes sorafenib resistance of hepatocellular carcinoma. Br J Cancer 108:72–81

Shih AH, Abdel-Wahab O, Patel JP, Levine RL (2012) The role of mutations in epigenetic regulators in myeloid malignancies. Nat Rev Cancer 12:599–612

Shoshan MC (2012) 3-Bromopyruvate: targets and outcomes. J Bioenerg Biomembr 44:7–15

Shulga N, Wilson-Smith R, Pastorino JG (2010) Sirtuin-3 deacetylation of cyclophilin D induces dissociation of hexokinase II from the mitochondria. J Cell Sci 123:894–902

Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, Szabo S, Buckhaults P, Farrell C, Meeh P, Markowitz SD, Willis J, Dawson D, Willson JK, Gazdar AF, Hartigan J, Wu L, Liu C, Parmigiani G, Park BH, Bachman KE, Papadopoulos N, Vogelstein B, Kinzler KW, Velculescu VE (2006) The consensus coding sequences of human breast and colorectal cancers. Science 314:268–274

Smith TA, Appleyard MV, Sharp S, Fleming IN, Murray K, Thompson AM (2013) Response to trastuzumab by HER2 expressing breast tumour xenografts is accompanied by decreased hexokinase II, GLUT1 and ^{18}F-FDG incorporation and changes in ^{31}P-NMR-detectable phosphomonoesters. Cancer Chemother Pharmacol 71(2):473–480

Smolková K, Ježek P (2012) The role of mitochondrial NADPH-dependent isocitrate dehydrogenase in cancer cells. Int J Cell Biol 2012:273947

Smolková K, Plecitá–Hlavatá L, Bellance N, Benard G, Rossignol R, Ježek P (2011a) Waves of gene regulation suppress and then restore oxidative phosphorylation in cancer cells. Int J Biochem Cell Biol 43:950–958

Smolková K, Dvořák A, Zelenka J, Vítek L, Ježek P (2011b) Reductive carboxylation pathway and redox potential in cancer cells with IDH2 silencing. Free Radic Biol Med 51:S121

Stacpoole PW, Barnes CL, Hurbanis MD, Cannon SL, Kerr DS (1997) Treatment of congenital lactic acidosis with dichloroacetate. Arch Dis Child 77:535–541

Stacpoole PW, Nagaraja NV, Hutson AD (2003) Efficacy of dichloroacetate as a lactate-lowering drug. J Clin Pharmacol 43:683–691

Strum SB, Adalsteinsson O, Black RR, Segal D, Peress NL, Waldenfels J (2013) Case report: sodium dichloroacetate (DCA) inhibition of the "Warburg effect" in a human cancer patient: complete response in non-Hodgkin's lymphoma after disease progression with rituximab-CHOP. J Bioenerg Biomembr 45:307–315

Sun RC, Fadia M, Dahlstrom JE, Parish CR, Board PG, Blackburn AC (2010) Reversal of the glycolytic phenotype by dichloroacetate inhibits metastatic breast cancer cell growth in vitro and in vivo. Breast Cancer Res Treat 120:253–260

Sun RC, Board PG, Blackburn AC (2011) Targeting metabolism with arsenic trioxide and dichloroacetate in breast cancer cells. Mol Cancer 10:142

Sutendra G, Dromparis P, Kinnaird A, Stenson TH, Haromy A, Parker JM, McMurtry MS, Michelakis ED (2013) Mitochondrial activation by inhibition of PDKII suppresses HIF1a signaling and angiogenesis in cancer. Oncogene 32:1638–1650

Teicher BA, Linehan WM, Helman LJ (2012) Targeting cancer metabolism. Clin Cancer Res 18:5537–5545

Theeler BJ, Yung WK, Fuller GN, De Groot JF (2012) Moving toward molecular classification of diffuse gliomas in adults. Neurology 79:1917–1926

Thornburg J, Nelson K, Clem B, Lane A, Arumugam S, Simmons A, Eaton JW, Telang S, Chesney J (2008) Targeting aspartate aminotransferase in breast cancer. Breast Cancer Res 10:R84

Tong J, Xie G, He J, Li J, Pan F, Liang H (2011) Synergistic antitumor effect of dichloroacetate in combination with 5-fluorouracil in colorectal cancer. J Biomed Biotechnol 2011:740564

Tuganova A, Boulatnikov I, Popov KM (2002) Interaction between the individual isoenzymes of pyruvate dehydrogenase kinase and the inner lipoyl-bearing domain of transacetylase component of pyruvate dehydrogenase complex. Biochem J 366:129–136

Upadhyay M, Samal J, Kandpal M, Singh OV, Vivekandan P (2013) The Warburg effect: insights from the past decade. Pharmacol Ther 137:318–330

Vali M, Liapi E, Kowalski J, Hong K, Khwaja A, Torbenson MS, Georgiades C, Geschwind JF (2007) Intra-arterial therapy with a new potent inhibitor of tumor metabolism (3-bromopyruvate): identification of therapeutic dose and method of injection in an animal model of liver cancer. J Vasc Interv Radiol 18:95–101

Vella S, Conti M, Tasso R, Cancedda R, Pagano A (2012) Dichloroacetate inhibits neuroblastoma growth by specifically acting against malignant undifferentiated cells. Int J Cancer 130:1484–1493

Wang P, Dong Q, Zhang C, Kuan PF, Liu Y, Jeck WR, Andersen JB, Jiang W, Savich GL, Tan TX, Auman JT, Hoskins JM, Misher AD, Moser CD, Yourstone SM, Kim JW, Cibulskis K, Getz G, Hunt HV, Thorgeirsson SS, Roberts LR, Ye D, Guan KL, Xiong Y, Qin LX, Chiang DY (2013) Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. Oncogene 32:3091–3100

Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Coller HA, Cross JR, Fantin VR, Hedvat CV, Perl AE, Rabinowitz JD, Carroll M, Su SM, Sharp KA, Levine RL, Thompson CB (2010) The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting α-ketoglutarate to 2-hydroxyglutarate. Cancer Cell 17:225–234

Ward PS, Cross JR, Lu C, Weigert O, Abel-Wahab O, Levine RL, Weinstock DM, Sharp KA, Thompson CB (2012) Identification of additional IDH mutations associated with oncometabolite R(-)-2-hydroxyglutarate production. Oncogene 31:2491–2498

Ward PS, Lu C, Cross JR, Abdel-Wahab O, Levine RL, Schwartz GK, Thompson CB (2013) The potential for isocitrate dehydrogenase mutations to produce 2-hydroxyglutarate depends on allele specificity and subcellular compartmentalization. J Biol Chem 288:3804–3815

Wenner CE (2012) Targeting mitochondria as a therapeutic target in cancer. J Cell Physiol 227:450–456

Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Zudkoff M, McMahon SB, Thompson CB (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci U S A 105:18782–18787

Wise DR, Ward PS, Shay JE, Cross JR, Gruber JJ, Sachdeva UM, Platt JM, Dematteo RG, Simon MC, Thompson CB (2011) Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α-ketoglutarate to citrate to support cell growth and viability. Proc Natl Acad Sci U S A 108:19611–19616

Wolf A, Agnihotri S, Micallef J, Mukherjee J, Sabha N, Cairns R, Hawkins C, Guha A (2011a) Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme. J Exp Med 208:313–326

Wolf A, Agnihotri S, Munoz D, Guha A (2011b) Developmental profile and regulation of the glycolytic enzyme hexokinase 2 in normal brain and glioblastoma multiforme. Neurobiol Dis 44:84–91

Wong JY, Huggins GS, Debidda M, Munshi NC, De Vivo I (2008) Dichloroacetate induces apoptosis in endometrial cancer cells. Gynecol Oncol 109:394–402

Wu P, Blair PV, Sato J, Jaskiewicz J, Popov KM, Harris RA (2000) Starvation increases the amount of pyruvate dehydrogenase kinase in several mammalian tissues. Arch Biochem Biophys 381:1–7

Xiao H, Li S, Zhang D, Liu T, Yu M, Wang F (2013) Separate and concurrent use of 2-deoxy-D-glucose and 3-bromopyruvate in pancreatic cancer cells. Oncol Rep 29:329–334

Xie J, Wang BS, Yu DH, Lu Q, Ma J, Qi H, Fang C, Chen HZ (2011) Dichloroacetate shifts the metabolism from glycolysis to glucose oxidation and exhibits synergistic growth inhibition with cisplatin in HeLa cells. Int J Oncol 38:409–417

Xu XW, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Wang P, Xiao MT, Liu LX, Jiang WQ, Liu J, Zhang JY, Wang B, Frye S, Zhang Y, Xu YH, Lei QY, Guan KL, Zhao SM, Xiong Y (2011) Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. Cancer Cell 19:17–30

Yamashita K, Nakashima S, You F, Hayashi S, Iwama T (2009) Overexpression of immediate early gene X-1 (IEX-1) enhances gamma-radiation-induced apoptosis of human glioma cell line, U87-MG. Neuropathology 29:20–24

Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD (2009) IDH1 and IDH2 mutations in gliomas. New Engl J Med 360:765–773

Yang H, Ye D, Guan KL, Xiong Y (2012) IDH1 and IDH2 mutations in tumorigenesis: mechanistic insights and clinical perspectives. Clin Cancer Res 18:5562–5571

Yin F, Sancheti H, Cadenas E (2012) Silencing of nicotinamide nucleotide transhydrogenase impairs cellular redox homeostasis and energy metabolism in PC12 cells. Biochim Biophys Acta 1817:401–409

Yoo H, Antoniewicz MR, Stephanopoulos G, Kelleher JK (2008) Quantifying reductive carboxylation flux of glutamine to lipid in a brown adipocyte cell line. J Biol Chem 283:20621–20627

Yu W, Dittenhafer-Reed KE, Denu JM (2012) SIRT3 protein deacetylates isocitrate dehydrogenase 2 (IDH2) and regulates mitochondrial redox status. J Biol Chem 287:14078–14086

Yuneva M (2008) Finding an "Achilles' heel" of cancer: the role of glucose and glutamine metabolism in the survival of transformed cells. Cell Cycle 7:2083–2089

Yuneva M, Zamboni N, Oefner P, Sachidanandam R, Lazebnik Y (2007) Deficiency in glutamine but not glucose induces MYC dependent apoptosis in human cells. J Cell Biol 178:93–105

Zhang X, Varin E, Briand M, Allouche S, Heutte N, Schwartz L, Poulain L, Icard P (2009) Novel therapy for malignant pleural mesothelioma based on anti-energetic effect: an experimental study using 3-bromopyruvate on nude mice. Anticancer Res 29:1443–1448

Zhao Y, Liu H, Riker AI, Fodstad O, Ledoux SP, Wilson GL, Ming T (2011) Emerging metabolic targets in cancer therapy. Front Biosci 16:1844–1860

Chapter 11
Relevance of Mitochondrial Functions and Plasticity in Tumor Biology

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Abstract In this chapter we discuss the relevance of mitochondrial functions and plasticity in tumor biology. In 1920, Otto Warburg first hypothesized that mitochondrial impairment is a leading cause of cancer although he recognized the existence of oxidative tumors. Likewise, Weinhouse (1951) and others found that deficient mitochondrial respiration is not an obligatory feature of cancer and Peter Vaupel suggested in the 90s that tumor oxygenation rather than OXPHOS capacity was the limiting factor of mitochondrial energy production in cancer. Recent studies now clearly indicate that mitochondria are highly functional in mice tumors and the field of oncobioenergetics identified MYC, Oct1 and RAS as pro-OXPHOS oncogenes. In addition, cancer cells adaptation to aglycemia, metabolic symbiosis between hypoxic and non-hypoxic tumor regions as well the reverse Warburg hypothesis support the crucial role of mitochondrial plasticity in the survival of a subclass of tumors. Therefore, mitochondria are now considered as potential targets for anti-cancer therapy and tentative strategies including a bioenergetic profile characterization of the tumor and the subsequent adapted bioenergetic modulation could be considered for cancer killing.

Keywords Metabolic remodeling • Oncobioenergetics • Warburg effect • Mitochondria • Metabolic therapy

1 Introduction

Rapid progress in mitochondrial research recently demonstrated a critical role for mitochondria in the initiation and the progression of cancer. Several mitochondrial functions play a role in cancer biology, including apoptosis, energetics, calcium signaling, and ROS generation. Recent observations also reported changes in mitochondrial form in cancer cells. Hence, the link between mitochondrial dynamics, energy production and tumorigenesis remains to be clarified. Moreover, a key role of mitochondria in tumor survival could be explained by their potential to derive energy from various sources of carbon, as fatty acids, amino-acids or ketone bodies. Such metabolic flexibility conferred by the mitochondrion raise the question of mitochondrial adaptation to tumor energy needs and energy substrate type and disponibility. Little is known on the signals and downstream pathways which govern such mitochondrial adaptations and transformations. Few adaptive loops including redox sensors, kinases and transcription factors were deciphered, but their implication in physiology and pathology remains elusive. Mitoplasticity could play a central role in cancer progression and survival so that fundamental research on adaptation and transformation biology of the mitochondrion could allow the design of innovative therapies, notably in cancer.

2 The Biochemistry of Energy Production

In most human tissues, mitochondria provide the energy necessary for cell growth, and biological activities. It has been estimated that about 90 % of mammalian oxygen consumption is mitochondrial, which primarily serves to

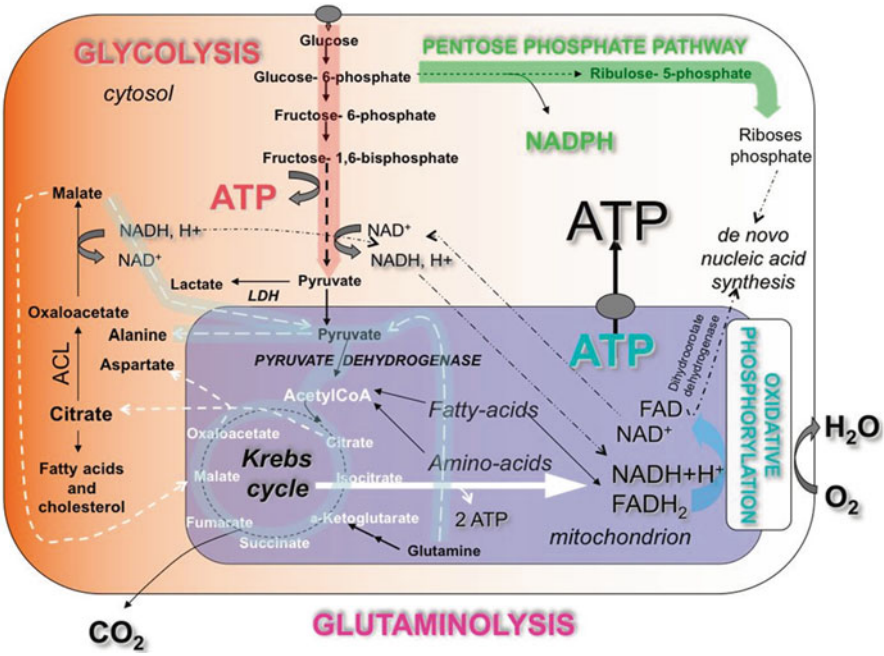

Fig. 11.1 Main pathways of cellular and mitochondrial energy metabolism. The two main metabolic pathways, *i.e.* glycolysis and oxidative phosphorylation are linked by the enzyme complex pyruvate dehydrogenase. Briefly, glucose is transported inside the cell and oxidized to pyruvate. Under aerobic conditions, the complete oxidation of pyruvate occurs through the TCA cycle to produce NADH, H^+ and/or $FADH_2$. These reduced equivalents are oxidized further by the mitochondrial respiratory chain

synthesize ATP, although in variable levels according to the tissue considered and the organism's activity status (Benard et al. 2006). Mitochondria intervene in the ultimate phase of cellular catabolism, following the enzymatic reactions of intermediate metabolism that degrade carbohydrates, fats and proteins into smaller molecules such as pyruvate, fatty acids and amino acids, respectively (Fig. 11.1). Mitochondria further transform these energetic elements into NADH and/or $FADH_2$, through ß-oxidation and the Krebs cycle. Those reduced equivalents are then degraded by the mitochondrial respiratory chain in a global energy converting process called oxidative phosphorylation (OXPHOS) where the electrons liberated by the oxidation of NADH and $FADH_2$ are passed along a series of carriers regrouped under the name of "respiratory chain" or "electron transport chain" (ETC), and ultimately transferred to molecular oxygen (Fig. 11.2). ETC is located in mitochondrial inner membrane, with enrichment in the cristae. ETC consists of four enzyme complexes (complexes I – IV), and two mobile electron carriers (coenzyme Q and cytochrome c). These complexes are composed of numerous subunits encoded by both nuclear genes and mitochondrial DNA at the exception of complex II (nuclear only). It was demonstrated that

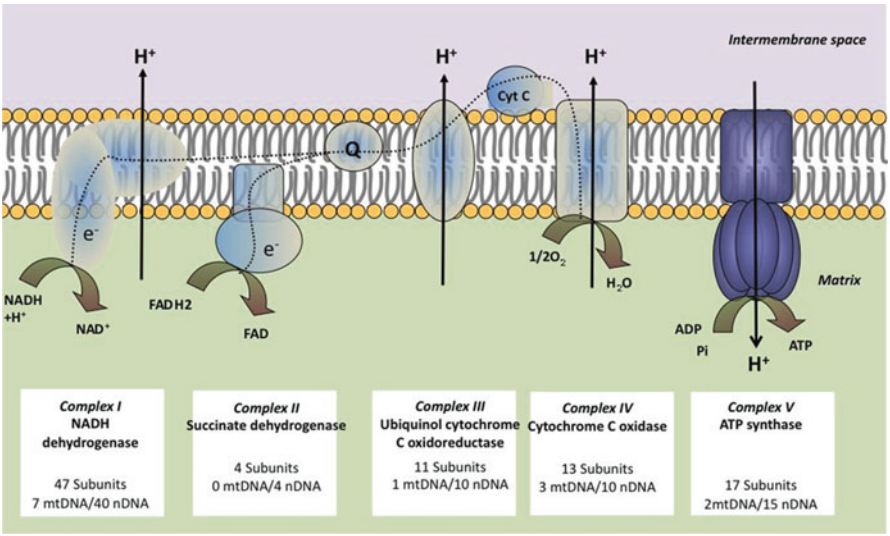

Fig. 11.2 Mitochondrial respiratory chain. For mammals, the respiratory chain consists of four enzyme complexes (complexes I – IV) and two intermediary substrates (coenzyme Q and cytochrome c). The $NADH + H^+$ and $FADH_2$ produced by the intermediate metabolism are oxidized further by the mitochondrial respiratory chain to establish an electrochemical gradient of protons, which is finally used by the F1F0-ATP synthase (complex V) to produce ATP, the only form of energy used by the cell. In this simple representation of the respiratory chain, the supramolecular organization (supercomplexes, dimers) is not shown

these complexes can assemble into supramolecular assemblies called "supercomplexes" or respirasome.

In presence of energy substrate (NADH or $FADH_2$), the transfer of electrons from complex I (or II) to complex IV mediates the extrusion of protons from the matrix to the inter-membrane space, thus generating an electrochemical gradient of protons ($\Delta\mu_{H^+}$) which is finally used by the F_1–F_o ATP synthase (*i.e.* complex V) to produce adenosine triphosphate (ATP) the energetic currency of the cell. This gradient has two components: an electric potential ($\Delta\Psi$) and a chemical potential ($\Delta\mu_{H^+}$) that can also be expressed as a pH gradient (ΔpH). According to the chemiosmotic theory (Mitchell 1961), $\Delta\mu_{H^+} = \Delta\Psi - Z\Delta pH$, with $Z = -2.303$ RT/F. Under physiological conditions, mitochondrial energy production can alternate between two energy steady-states: basically, at state 4, respiration is slow and ATP is not produced ($\Delta\Psi$ is high), while during state 3, respiration is faster and ATP is largely produced ($\Delta\Psi$ is lower). In particular conditions, such as mitochondrial inner membrane permeabilization or the use of a chemical uncoupler, $\Delta\Psi$ can be totally dispersed. As a consequence, respiration is accelerated and ATP production annihilated. The inhibition of respiratory chain complexes also generally decreases $\Delta\Psi$. Under physiological conditions, it is considered that mitochondria produce ATP in an

intermediate state lying between state 3 and state 4. ATP is the only form of energy used by the cell, and when produced in the mitochondrion it is exported to the cytosol by the adenine nucleotide translocator (ANT) in exchange for cytosolic ADP. Generally, the transport of energy metabolites, nucleotides and cofactors into and out of the mitochondrial matrix is performed by transporters located in the inner membrane (Palmieri et al. 1996). Mitochondria also contain "shuttle" systems that permit the transport of NADH.

3 The Regulation of Cellular and Mitochondrial Energy Production

In mammalian cells, energy homeostasis requires a constant coordination between cell activity, nutrient availability and the regulation of energy transformation processes. This is obtained via a complex system of signaling linking energy sensing and nutrient sensing to cellular effectors that include kinases and transcription factors. The AMP-activated protein kinases are activated upon alterations in the cellular AMP/ATP ratio, which is dictated by the balance between energy supply (ATP production) and energy demand (ATP consumption). When activated by AMP, the AMPK initiate a cascade of phosphorylation to switch on the catabolic pathways that produce ATP (glycolysis, oxidative phosphorylation via the stimulation of mitochondrial biogenesis), and to switch-off the anabolic pathways that consume ATP (protein synthesis, fatty acid synthesis, cholesterol synthesis) (for review see (Hardie 2007)). More recently, it was discovered that AMPK further regulate energy metabolism through the activation of the sirtuin SIRT1 (Canto and Auwerx 2009; Canto et al. 2009) and the modulation of the activity of downstream SIRT1 targets that include the peroxisome proliferator-activated receptor-y coactivator l-α (PGC1α), and the forkhead box O1 (FOXO1) and O3 (FOXO3a) transcription factors. In the present chapter we will discuss how mitochondrial dynamics interacts with the regulatory pathway of energy metabolism governed by AMPK and sirtuins, and reciprocally. The regulation of mitochondrial energy production is concerted and multi-site (Benard et al. 2010). The different levels of OXPHOS regulation include (1) the direct modulation of respiratory chain kinetic parameters, (2) modulation of OXPHOS intrinsic efficiency by changes in the basal proton conductance or the induced proton conductance, (3) possible changes in the morphological state of the mitochondrial compartment (as discussed here), (4) modulation of mitochondrial biogenesis and degradation, and (5) *in situ* regulation of mitochondrial heterogeneity by the cellular and the mitochondrial microenvironment. Most of these regulatory mechanisms (Fig. 11.3) of mitochondrial energy production were discovered at the level of the respiratory chain and its surrounding lipid environment. Below, we discuss whether and how changes in mitochondrial fusion and fission impact these conserved mechanisms of bioenergetic regulation.

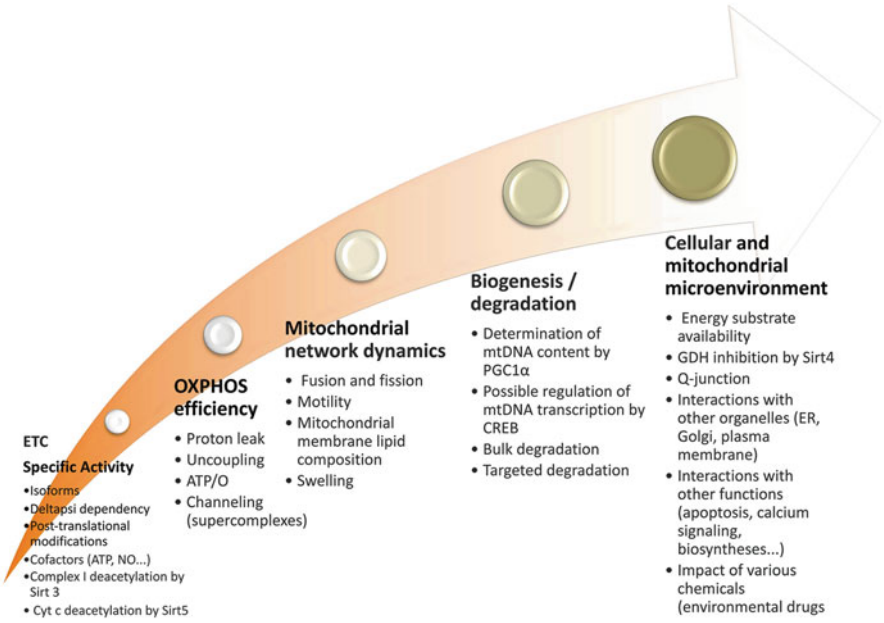

Fig. 11.3 Multi-site regulation of mitochondrial oxidative phosphorylation. The modulation of OXPHOS capacity and activity occurs at different levels to adapt mitochondrial energy production to the cellular needs and environmental bioenergetic constraints

4 Mitochondrial Structure and Dynamics

In the past decade, the development of fluorescence microscopy has allowed the gathering of a three dimensional view of the mitochondrion in living human cells (Bereiter-Hahn and Voth 1994; Griparic and Van Der Bliek 2001; Yaffe 1999). In these studies, the mitochondria looked as an organelle that appeared more like a wide network of long tubules rather than a collection of small individual vesicles. In Fig. 11.4, we see the arborescence of the mitochondrial network in living human cells. Mitochondrial dynamics is performed by fusion proteins and fission proteins, which could reveal new possibilities for the control of mitochondrial energy production and cell viability (Benard and Karbowski 2009). In mammals, the proteins involved in mitochondrial fusion include the mitofusins MFN1 and MFN2, and OPA1 (different isoforms of OPA1 are generated by alternative splicing). SLP2 (Stoml2), MarchV, Bax and Bak interact with MFN2 to regulate fusion. In addition, different ATP-dependent or ATP independent proteases regulate fusion via OPA1 processing (Ehses et al. 2009). Low mitochondrial ATP levels, or the dissipation of the mitochondrial electric membrane potential across the inner membrane induce OPA1 cleavage by PARL and the matrix AAA (*m*-AAA) protease (Duvezin-Caubet et al. 2006). OMA1 mediates OPA1 processing if *m*-AAA proteases are absent or

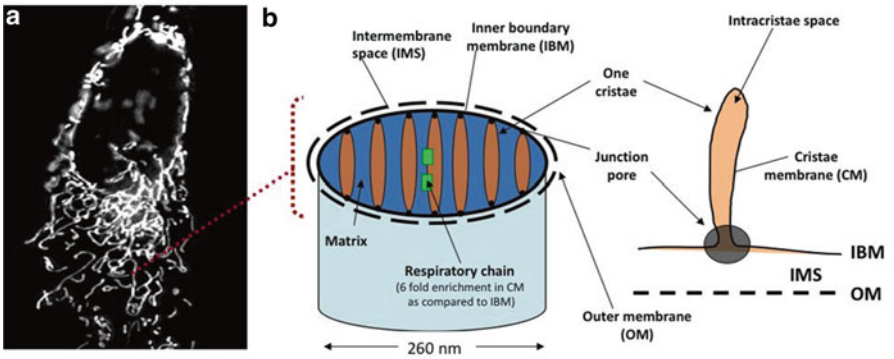

Fig. 11.4 Mitochondrial structure. (**a**) Overview of the mitochondrial network in living human HeLa cells. The mitochondrial network was imaged by fluorescence microscopy (bi-photonic), using a matrix-targeted GFP. (**b**) Internal organization of the mitochondrial network; section of a tubule. This scheme illustrates the "cristae junction model" of mitochondrial interior. In this view, the pores could serve to regulate the release of cytochrome c during apoptosis

mitochondrial activities are impaired (Ehses et al. 2009). PISD and mito-PLD are proteins involved in the metabolism of phospholipid and could regulate mitochondrial fusion via changes in membrane composition (Furt and Moreau 2009). The role of MIB is unclear, and might also participate to mitochondrial fusion via MFN2 binding. The proteins involved in mitochondrial fission include FIS1 and DRP1. MFF also plays a role in mitochondrial fission. The regulators of fission include MARCHV, Mff, Bcl-w and different kinases required for the phosphorylation of DRP1. DRP1 can also be nitrosylated. So far, the interaction between mitochondrial dynamics and mitochondrial energetics remain unclear.

To investigate the link between mitochondrial energy production and organellar shape changes, appropriate methods are needed to quantify changes both in mitochondrial form and function. Firstly, one can observe the shape of the mitochondrial network by epifluorescence or confocal microscopy in living cells using various fluorescent probes targeted to the mitochondrial matrix (GFPs, mitotrackers, TMRM, etc.). For instance, inhibited fusion leads to a fragmented mitochondrial network while inhibited fission generates long tubules with signs of hyperfusion. The morphometric analysis of the mitochondrial compartment can be performed on microscopy images by using an automated computerized method to assess the length and branching degree of the mitochondrial particles (Koopman et al. 2005). This method gives a quantitative evaluation based on the measurement of a form factor (combined measure of length and degree of branching), an aspect ratio (measure of length), and the overall content of mitochondria in the cell. In situations of altered fusion and fission (as occurs in neurological diseases caused by mutations in OPA1 and Mfn2, or DRP1, respectively) the viscosity of the mitochondrial matrix can also change (Benard and Rossignol 2008a; Koopman et al. 2008). Likewise, a test of mitochondrial fusion is available on cells containing different fluorescent proteins

targeted to the mitochondrial matrix (matrix red fluorescent protein; mtRFP) or matrix green fluorescent protein (mtGFP). These two types of cells are fused with polyethylene glycol (PEG) to allow cell membrane fusion and the resulting polykaryons are analyzed by confocal microscopy on the basis of their level of mtRFP and mtGFP colocalization. The mixing of green and red mitochondrial matrix-targeted proteins due to mitochondrial fusion leads to cells containing "yellow mitochondria" (Legros et al. 2002). Yet, the polethylene glycol used for cell membrane fusion might also interfer with mitochondrial membrane fusion and perturbate the assay. Another possibility to measure mitochondrial fusion is to use mitochondrially targeted photoconvertible GFP (mito-Dendra), which changes color from green to red once activated by a blue laser. The mixing of the activated GFP with mitochondrial tubules generates yellow regions which can be counted as function of time to evaluate fusion, fission and transport (Koutsopoulos et al. 2010). Similar assays were also developed to investigate automatically the activity of mitochondrial fusion and fission (Jourdain and Martinou 2010). To further assess the activity of mitochondrial fission, it is also possible to use the so-called "CCCP-assay" which inhibits fusion and allows fission to proceed. The time required, or the CCCP amount needed to visualize fission gives a measure of its activity. This test is indirect and considers that OPA1 processing is unchanged in the different conditions. This test was used in several reports (Ehses et al. 2009; Ishihara et al. 2003, 2006).

5 Mitochondrial Impairment in Cancer: One Century of Warburg Hypothesis

In the 1920s, Otto Warburg evidenced that tumor cells consume large amounts of glucose and convert it mostly to lactic acid despite the presence of oxygen. This led him to hypothesize that cancer arises from the impairment of mitochondrial oxidative metabolism. *"The aerobic glycolysis of the tumour cell is derived in any case from a disturbance of the respiration."* This famous Warburg hypothesis seduced the medical community in that a single metabolic specificity of cancer cells, the high dependency on glycolysis for energy production, could eventually be targeted to cure the disease. Over 90 years of cancer biology research has been conducted with the basis of this predominant Warburg theory. The exclusive respiratory impairment hypothesis is now challenged by an increasing number of bioenergetic studies which already started in the 1950s, when Sidney Weinhouse published experimental evidences in opposition to the Warburg hypothesis. The major argument raised by Weinhouse was that tumors and non-neoplastic tissues show no difference in their ability to convert glucose and fatty acids to carbon dioxide, a process that requires respiration and functional mitochondria.

Likewise, Gregg showed that cell respiration increased concomitantly with the increase in glucose consumption in normal and transformed cells, and that HeLa cells presented with a high respiratory rate. It is noteworthy that Warburg himself recognized that respiration was not totally impaired and could even be efficient in a subset of tumor, the frequency of which still remain undetermined. "*As a rule, the*

respiration of the tumour cell is small, but in recent years tumour cells with a large respiration have also been found…". Yet, this finding of Warburg remained underestimated so that an accepted consensus on a general mitochondrial impairment in cancer cells had emerged in the 90s and remained highly cited since then. Nevertheless, in the last decade, several groups reported accumulating evidences of functional mitochondria in tumors and demonstrations of a strong dependency of cancer cells survival on mitochondrial oxidative metabolism. This short *argumentum* aims to clarify the implication and the importance of mitochondria in cancer biology. We discuss the status and the variability of mitochondrial content and functionality in cancer. A special attention was given to the rationale for targeting mitochondria in anti-cancer strategies.

6 Mitochondrial Content and Efficiency Is Enhanced in a Subset of Human Tumors

The changes in mitochondrial content, composition and bioenergetics parameters including respiration and proton-motive force observed in various tumor-derived cell lines were extensively reviewed in previous articles (Bellance et al. 2009a; Jose et al. 2011; Smolkova et al. 2011). However, a large number of those studies were performed on tumor derived cell culture models which might exhibit a strong dependency on glycolysis not only due to their cancer nature. Indeed, there is a strong impact of the artificial culture conditions on energy metabolism and on mitochondrial physiology, as revealed by cell biology investigations in senescence research. One example of the impact of cell culture on cell biology is the so-called 'culture shock' (Gnaiger and Kemp 1990; Gstraunthaler et al. 1999; Sherr and Depinho 2000) which modulates the activity of the CDK inhibitors p16INK4a and p21Cip1, the p53 inducer p19ARF, and p53 itself. For this reason, we focused our argumentation on the few reports of mitochondrial changes in excised human and mice tumors. Firstly, to evaluate mitochondrial representation in tumors different groups have measured the mitochondrial DNA (mtDNA) content in cancer regions and performed a comparison with normal cells (Table 11.1). In this manner, a decrease in mtDNA copy number was reported in many types of cancers, including ovarian

Table 11.1 Variation of the mtDNA content in tumors

Cancer type	Up-regulated	Down-regulated	References
Type I endometrial cancer	×		Cormio et al. (2009)
Primary leukemia cells	×		Carew et al. (2004)
Arsenical skin cancer	×		Lee et al. (2011)
Thyroidoncocytoma	×		Baris et al. (2004), Savagner et al. (2003)
Kidney carcinoma		×	Hervouet et al. (2005)
Renal cell carcinoma		×	Simonnet et al. (2003)
Lung carcinoma	×		Bellance et al. (2009b)

(Bonnet et al. 2007), gastric (Bouzier et al. 1998) and hepatocellular (Burgess et al. 2008), suggesting that the decreased mtDNA copy number may contribute to, or associate with tumorigenesis. Furthermore, Meierhofer et al. (Canto et al. 2009) reported a significant reduction of mitochondrial enzyme activities and of mtDNA copy number in 34 out of 37 renal cell carcinoma tissues, as compared with adjacent noncancerous tissues. Similar observations were made on the reduced activity of respiratory chain complexes and the diminished mtDNA content in renal tumors (Capuano et al. 1996; Heddi et al. 1996; Simonnet et al. 2002). This alteration of OXPHOS capacity was related to the aggressiveness of the tumors, suggesting a progressive shift of energy metabolism toward glycolysis during tumorigenesis in kidney tumors. In breast cancer, another study confirmed these findings and observed a decrease in mtDNA content. In rat gliomas, the mitochondrial content is also decreased (Ehses et al. 2009).

However, other authors have observed opposite results in favor of an increase of mitochondrial capacity, notably in breast cancer (Shen et al. 2010). This controversial situation was found for other types of cancer as an increased mtDNA content was reported both in chronic lymphocytic leukemias (Carew et al. 2004) and in lung cancer (Hosgood et al. 2010). These opposite findings do not permit to conclude unequivocally on the status of mitochondrial content in cancer and point towards variability of mitochondrial content and capacity in tumors, rather than a simple general repression. The field of mitochondrial-cancer research still lacks of large-scale studies on different types of tumors which could allow the estimation of the variability of mitochondrial content and functionality as well as solicitation in cancer tissues. Alternatively, prospective cohort studies have tried to assess the possible role of mtDNA copy number in blood cells as a risk factor for cancer. The results were extremely variable and suggested again a large disparity between cancer types and even within one type of tumor between different individuals. Some authors found a positive association between high mtDNA content and the risk of both lung cancer (Hosgood et al. 2010) and non-Hodgkin lymphomas (Lan et al. 2008). Lynch et al. also found an association between high mtDNA copy number and pancreatic cancer incidence (Lynch et al. 2011). This association was significant for the cases diagnosed during the first 7 years of follow-up, but not for cases occurring during this follow-up of 7 years or greater. In striking contrast, no association between leukocyte mtDNA copy number and the risk of developing gastric cancer has been found on a large population-based prospective cohort (Liao et al. 2011). Interestingly, the authors found an association between low levels of mtDNA copy number and the risk for gastric cancer among earlier diagnosed cases (and not when the time between sample collection and cancer diagnosis increased). These authors concluded on a possible early disease effect on mtDNA copy number levels. Yet, such association studies lack of demonstration so that a causality link between mitochondrial content and cancer risk could be clearly shown. Of note, in the above cited studies, mitochondrial DNA content does not necessarily reflect the organelle content as defects in mtDNA translation, replication or in nuclear-encoded mitochondrial protein content alteration as well as mitophagy, may decrease the number of mitochondria or the OXPHOS protein content.

To understand the underlying mechanism of mitochondrial content variability in tumors, some authors have analyzed the status of mitochondrial biogenesis, notably by measuring the expression level of the master regulator PGC1α. It has been reported that in breast cancer PGC1α was expressed at lower levels as compared to normal background tissue (Jiang et al. 2003). This could suggest an important role for this factor in refining the cancer cell's metabolic profile and also that a therapeutic gain might be obtained from the reactivation of mitochondrial biogenesis in cancer cells. This hypothesis was tested with success in different studies (Bellance et al. 2009b; Wang and Moraes 2011) although the biogenic drugs used in these studies may produce off-targets effects responsible for cancer killing.

In addition to biogenesis reduction the low efficiency of the OXPHOS system observed in the Warburg type of cancer cells could be explained by the alteration of respiratory chain complexes specific activity, possibly caused by cancer specific protein regulations such as post-translational modifications or ROS-induced alterations. Yet little is known on this particular aspect of OXPHOS dysregulation and only one study reported a functional alteration of the respiratory chain complex I in cancer (Canto et al. 2009). Furthermore, abnormalities in the overall morphology of the mitochondrial network and its internal organization have been reported in numerous cancers, including human astrocytomas (Chen et al. 2012), carcinomas (Fischer et al. 2000), Warthin's tumor (Fryer et al. 2002), xenografted gliomas (Ehses et al. 2009), malignant glioma cells (Furt and Moreau 2009) and HeLa cells (Garber 2010). Most of these studies reported heterogeneous ultrastructural abnormalities, such as organellar swelling with a disorganization and distortion of the cristae and partial or even total cristolysis. Those might underline an activity defect of the OXPHOS system as such ultrastructural alterations of the mitochondrion are commonly found in mitochondrial diseases. Abnormalities in mitochondrial fusion-fission were also proposed from the analysis of astrocytomas sections by electron microscopy (Chen et al. 2012). Such structural defects in the mitochondria are frequently seen in cancer cells, although their precise origin and participation in tumor progression are not well understood. An analysis of mitochondrial morphogenesis in cancer cells could help to clarify this question.

Thus, it is clear that mitochondrial dysfunctions exist in some tumors but this feature is not a hallmark of cancer since numerous studies have demonstrated that tumor mitochondria can be functional with regard to respiration and ATP synthesis, exhibiting normal respiratory control ratios and normal capabilities for the oxidation of respiratory substrates (Eakin et al. 1972). As soon as in 1976, in a critical analysis of Warburg's theory, Sidney Weinhouse concluded: "*no substantial evidence has been found that would indicate a respiratory defect, either in the machinery of electron transport, or in the coupling of respiration with ATP formation, or in the unique presence or absence of mitochondrial enzymes or cofactors involved in electron transport*". In accordance with this, a comprehensive study of mitochondrial function in slowly growing hepatomas showed almost no differences in respiratory parameters as compared to normal liver mitochondria (Pedersen et al. 1970). In some tumors, oxygen consumption was found to be similar, or even higher than

in the normal tissue. In 1979 Reitzer demonstrated that cancer cells can derive 96 % of their ATP from oxidative phosphorylation using glutamine as energy substrate (Reitzer et al. 1979). In a study performed on human lung tumors (*Non-small-cell lung carcinoma* (*NSCLC*)), it was found that 70 % of the NSCLC presented with a decreased expression of the pyruvate dehydrogenase complex but 30 % of the tumors revealed a higher expression as compared to non-cancer tissue (Koukourakis et al. 2005). They could also explain the difficulty to detect some tumors using PET scan, as OXPHOS tumors would derive energy from glutamine and other oxidative substrates oxidation rather than from glucose. More recently, this distinction was found while comparing the metabolic profile of SiHa human cervix squamous carcinoma cells with WiDr human colorectal adenocarcinoma cells. The first one is of the OXPHOS type and can oxidize lactate while the second one is more glycolytic and generate lactate (Koukourakis et al. 2005). Other studies have indeed shown that tumor-derived cell lines can use glycolysis or OXPHOS to variable extent, so that some cancer cells have been classified as 'glycolytic' and other as 'OXPHOS' depending on their utilization of these two systems of energy production. One recent study (Weinberg et al. 2010) indicates that a reduction of mitochondrial content in pre-cancer tissues reduces the tumorigenic potential of K-RAS mutations in mice. This strongly suggests that mitochondrial metabolism is required for tumorigenesis, in contrast with Warburg hypothesis.

7 Mitochondrial Structural Adaptation Is Observed in Cancer Cells Confronted to Aglycemia

Already in 1979, Reitzer and colleagues published an article entitled "*Evidence that glutamine, not sugar, is the major energy source for cultured Hela cells*", which demonstrated that oxidative phosphorylation was used preferentially to produce ATP in cervical carcinoma cells (Reitzer et al. 1979). Griguer and colleagues also identified several glioma cell lines that were highly dependent on the mitochondrial OXPHOS pathway to produce ATP (Griguer et al. 2005). Glioma stem cells (Vlashi et al. 2011) also rely mainly on oxidative phosphorylation to derive energy. These findings are in apparent contrast with the field of "oncobioenergetics" (Hamanaka and Chandel 2012; Shaw 2006) which suggests that most oncogenes trigger the enhancement of glycolysis and the reduction of oxidative phosphorylation. This apparent contradiction can be resolved when considering that in addition to the oncogenes, other regulators of carcinogenesis participate to the re-shaping of energy metabolism (Hamanaka and Chandel 2012; Jose et al. 2011). In particular, the microenvironment of tumors was designated as a key regulator of the bioenergetic profile of cancer cells and we give some examples below.

Firstly, glucose deprivation leads to a profound modification of the energy pathways toward oxidative metabolism, with upregulation of respiratory chain proteins and higher branching of the mitochondrial network in osteosarcoma (143B) and hepatocellular carcinoma (HEPG2) (Plecita-Hlavata et al. 2008; Rossignol et al. 2004).

Likewise, a subclass of glioma cells which utilize glycolysis preferentially (i.e. glycolytic gliomas) can switch from aerobic glycolysis to OXPHOS under limiting glucose conditions (Bouzier et al. 1998, 2005), as observed in cervical cancer cells, breast carcinoma cells, hepatoma cells and pancreatic cancer cells (Plecita-Hlavata et al. 2008; Rossignol et al. 2004; Smolkova et al. 2010). In breast cancer cells, the expression level of the Complex IV subunit one was increased by a factor of 6 after only 4 days of growth in aglycemia, and this phenomenon was associated with a threefold increase in respiration, while no significant change was measured in the corresponding non-cancer cells (Smolkova et al. 2010). In this study we observed a significant increase (+30 %) in the mitochondrial to nucleus areas in HTB-126 cancer cells grown under aglycemic conditions for 4 days. These results suggested a stimulation of mitochondrial biogenesis induced by glucose deprivation, specifically in the cancer cells. In addition to OXPHOS machinery enhancement, glioblastoma cells exposed to glucose withdrawal showed an increase in glutamine metabolism, which was explained by a large increase in glutamate dehydrogenase activity (Yang et al. 2009). This adaptation was linked to the Akt pathway since inhibition of Akt signaling increased GDH activity, whereas overexpression of Akt suppressed it. In that study, GDH activation resulted from the loss of glycolysis because it could be mimicked with the glycolytic inhibitor 2-deoxyglucose and reversed with a pyruvate analogue.

In addition to glutaminolysis, the β-oxidation of fatty acids is also activated upon glucose deprivation (Buzzai et al. 2005) and the oncogenic kinase Akt blocks these oxidative by-pass in presence of glucose (Fig. 11.5b). Akt is central to bioenergetics remodeling as it promotes GLUT1 recruitment to plasma membrane, activates hexokinase association with the mitochondrion and phosphorylates ATP citrate lyase with downstream activation of fatty acid synthesis. Akt also inhibits fatty acid β-oxidation via the inhibition of carnitine palmitoyltransferase (CPT1A). Of particular importance for mitoplasticity, defects in mitochondrial respiration can lead to activation of Akt (Pelicano et al. 2006). Pelicano et al. (2006) showed that respiration deficiency induced by mitochondrial mutagenesis, pharmacologic inhibition, or hypoxia causes inactivation of PTEN a negative regulator of Akt.

An intriguing question raised by all these studies concerns the mechanisms involved in the enhancement of OXPHOS observed in HeLa, 143B, HEPG2, HTB126 and other cancer cells shifted from glucose to glutamine-galactose medium (Fig. 11.5a). So far, the molecular pathways involved in this adaptation remain unresolved. Three possible stimuli should be distinguished: (1) the direct effect of glucose removal, (2) the effect of prolonged glucose deprivation, and (3) the secondary effect of mitochondrial re-activation through glutaminolysis. These stimuli activate different signaling pathways which might work synergistically to generate the observed OXPHOS enhancement. Concerning the first possibility, it is known that glucose inhibits OXPHOS in cancer cells, and that removal of this Crabtree effect could liberate OXPHOS and possibly restore a feed-back activation of OXPHOS biogenesis. We demonstrated the reversibility of the Crabtree effect in breast cancer cells (Smolkova et al. 2010). The underlying mechanisms still remain unresolved, and current theories suggest that some intermediates of glucose oxidation could

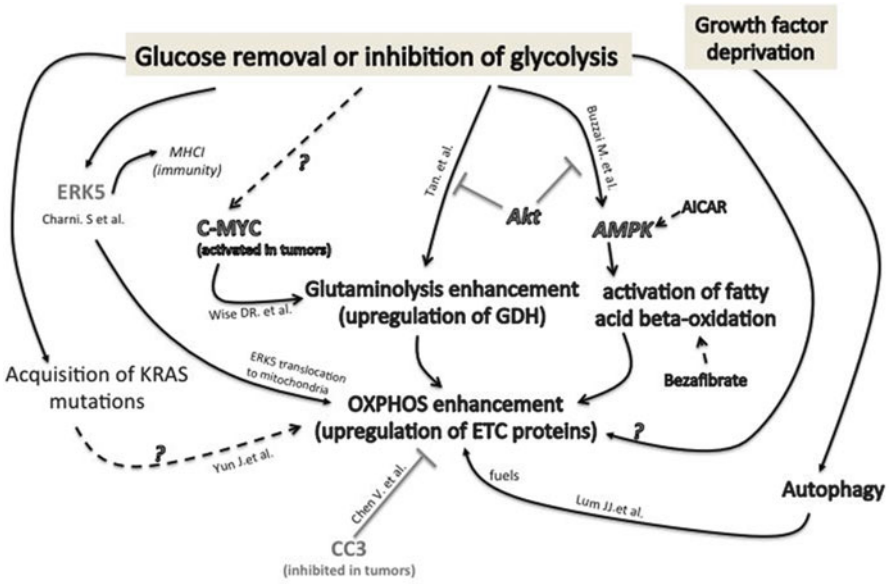

Fig. 11.5 Cancer cells adaptation to aglycemia. The numerous signalling pathways by which cancer cells can adapt to glucose deprivation are shown in this figure (they were identified in different cell types and might not be considered as a unique response to glucose deprivation). In hemotopoietic cells the MAP kinase ERK5 was involved in the adaptation to growth in galactose medium and this was associated with a modulation of MHCI expression (markers of self-immunity). The oncogene MYC could also participate to cell survival in absence of glucose as oncogenic levels of MYC induce a transcriptional program that promotes glutaminolysis and could trigger cellular addiction to glutamine as a bioenergetic substrate. The activation of the oncogenic kinase Akt blocks the ability of cancer cells to consume non-glycolytic energy substrates and thereby sensitizes these cells to death in conditions of glucose withdrawal. AICAR, an activator of AMPK can reverse this sensitization, notably through the stimulation of fatty-acid consumption for energy production. Another mechanism of OXPHOS activation was evidenced in cancer cells submitted to growth factor removal. In this situation autophagy is activated and amino-acids are oxidized by OXPHOS to produce ATP and maintain energy homeostasis. It was also shown that cells grown in absence of glucose acquire mutations in KRAS which could trigger the enhancement of OXPHOS. Lastly, CC3 was shown to negatively regulate the metabolic adaptation of tumor cells to glucose limitation. The silencing of CC3 improves cancer cell survival in absence of glucose. The mechanism by which the loss of CC3 stimulates OXPHOS includes the upregulation of OXPHOS proteins but the molecular details still remain unknown

inhibit mitochondrial oxidative phosphorylation. Biochemical studies performed in yeast suggest that fructose-1,6-bisphosphate (F-1,6-BP) could play a determining role in this process (Diaz-Ruiz et al. 2008). Hepatoma cells show a 50-fold increase in this metabolite concentration following 5 mM glucose addition to the cells (Rodriguez-Enriquez et al. 2001). Interestingly, addition of galactose did not change the level of F-1,6-BP in that study, and we also observed the suppression of the Crabtree effect when glucose was replaced by galactose. It is important to notice that galactose is not used as a fuel by cancer cells but serves for nucleic acid synthesis, while glutamine is consumed by the mitochondria to produce ATP.

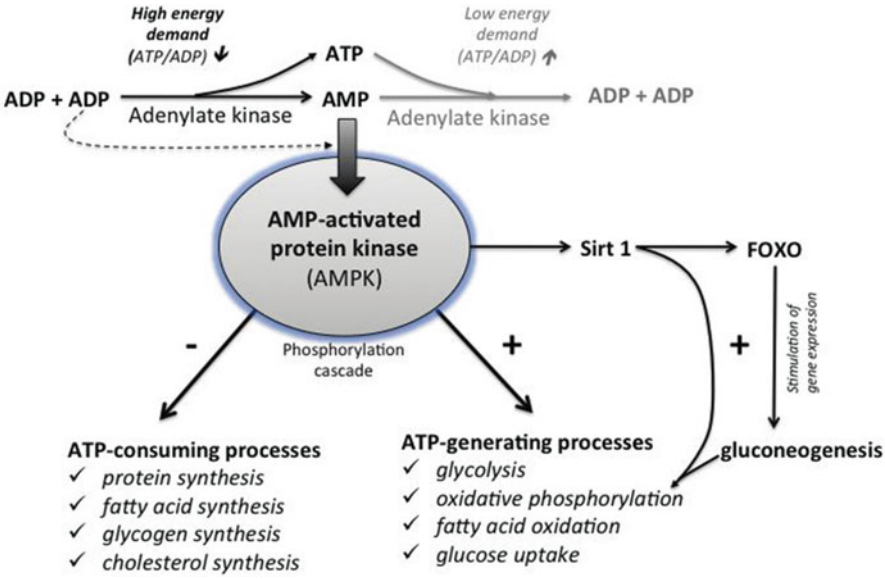

Fig. 11.6 Regulation of energy metabolism by AMPK. The AMP-activated protein kinase is a master regulator of energy metabolism. In conditions of intense ATP expenditure, the ratio of [AMP][ADP]/[ATP] increases and the AMPK is activated by AMP and ADP. This activation stimulates several processes leading to ATP production and inhibits the reactions which consume ATP. Therefore, AMPK plays a central role in Mitoplasticity as AMPK controls mitochondrial biogenesis and mitophagy

This phenomenon was demonstrated in HeLa cells or in CHO cells using C_{13}-labeled glucose and glutamine (Donnely and Scheffler 1976; Reitzer et al. 1979). Thus, to compare the bioenergetic status of cancer cells and non-cancer cells it is essential to compare the cells while in the "glucose" and the "glucose-deprived" conditions. Moreover, the rapid loss of HTB-126 cancer cell viability induced by various inhibitors of the OXPHOS system was observed only in glucose-deprived medium (galactose and glutamine are present in this medium), further emphasizing the fact that ATP is derived from OXPHOS in this medium.

The second mechanism by which OXPHOS biogenesis could occur in cancer cells grown in galactose medium is more complex and involves the potency of glucose deprivation to activate a large number of signaling pathways involving specific kinases capable of stimulating OXPHOS biogenesis and intermediate metabolism. Namely, the AMPK, p38 MAPK, and PI3K/PKB have been linked to the regulation of glucose uptake elicited by diverse hormonal stimuli in several cell types (Czech and Corvera 1999; Fryer et al. 2002; Kanda and Watanabe 2005; Sweeney et al. 1999). AMPK is a metabolic-sensing protein that is activated by an increase in the AMP/ATP ratio. AMPK is activated by allosteric binding of AMP and by phosphorylation by one of the upstream AMPK kinases. Once activated, AMPK regulates ATP levels by suppressing biosynthetic pathways, such as fatty acid and cholesterol biosynthesis, as well as by activating ATP-generating catabolic pathways such as fatty acid oxidation and glycolysis (Fig. 11.6).

Other intracellular signaling systems that may be involved in metabolic response to glucose deprivation are the stress-activated p38 MAPK (Cheung and Hart 2008) and the survival pathway PI3K/PKB (Vanhaesebroeck and Alessi 2000). As discussed above, the role of Akt in the activation of GDH and of β-oxidation upon inhibition of glycolysis could occur parallel to OXPHOS upregulation (Yang et al. 2009).

The third mechanism considers the possibility that the higher energy needs of cancer cells for supporting their deregulated growth and extensive biosyntheses, stimulates the OXPHOS system when the fuel for energy production switches from glycolytic to oxidative-only. Under non-stringent conditions, glucose is the preferential energy substrate of cancer cells, and its consumption is typically increased by the expression of more rapid isoforms of the glycolytic pathway (Mathupala et al. 1997). This result could indicate that the strong energy demand of cancer cells dictates the upregulation of whatever energy pathway is used, as determined by the type and availability of the energy substrate. This peculiarity has been observed for other cancer cells HeLa and 143B (Rossignol et al. 2004), and HepG2 (Plecita-Hlavata et al. 2008) and could designate the underlying pathway of metabolic remodeling as a potential target for anti-cancer therapy.

The role of PDH also remains to be evaluated in cells grown in absence of glucose. DCA is a pyruvate dehydrogenase kinase inhibitor, and typically results in increased pyruvate dehydrogenase activity. The group of Bonnet et al. (2007) explained the anti-cancer effect of DCA by a complex mechanism which involved the re-sensitization of cancer cells to apoptotic stimuli via the stimulation of mitochondrial respiration and the subsequent (cancer specific) upregulation of a potassium channel (Kv1.5). However, during the adaptation of cancer cells to growth in absence of glucose, PDH re-activation might be required to provide acetyl-coA to the Krebs cycle enzyme alpha-ketoglutarate dehydrogenase (KDH) since the carbon source and energy source is glutamine and this amino-acid enter the Krebs cycle at the level of KDH. This underestimated role of PDH could explain why we observed a strong upregulation of PDH in HeLa cells forced to grow in absence of glucose (Rossignol et al. 2004). Most of the experiments aiming at the evaluation of DCA cancer toxicity were performed in high-glucose media and in these conditions DCA induced apoptosis. However, given the potential key role of PDH in absence of glucose, DCA could favor the utilization of glutamine by the Krebs cycle and might benefit the growth of cancer cells in conditions of glucose withdrawal. This hypothesis remains to be evaluated.

Besides glucose, another key player in the definition of a cancer cell's metabolic profile is oxygen tension (p_{O2}). Hypoxia is a common feature of the microenvironment of cancer cells, and tumor oxygenation can be severely compromised as compared to normal tissue (Vaupel et al. 2007). Thus, it seems more appropriate to investigate the impact of hypoxia and aglycemia on cancer cells, which typically encounter this type of stress *in situ*. Vaupel and coworkers observed low values for intratumoral oxygen tension ranging between 3 and 10 mmHg in breast malignant tissue while non-cancer tissue exhibited higher values approximating 50 mmHg (Vaupel et al. 2003). The "Gatenby and Gillies" microenvironmental model of carcinogenesis (Gatenby and Gillies 2004, 2008) considers that pre-cancer cells are typically found in tissue regions where oxygen and glucose delivery is low. This might

have pre-adapted energy metabolism to a life of uncontrolled growth and deregulated cell death. Gatenby and Gillies further proposed that during tumor growth, angiogenesis leads to the growth of inadequate microvasculature, which results in intermittent oxygen and glucose deprivation in cancer cells along with acidification of the extracellular space (Gatenby and Gillies 2008). Also, Okunieff et al. showed that although glucose supply may be adequate to small murine fibrosarcoma tumors (~76 mm^3), the metabolic baseline of large tumors (~300 mm^3) includes glucose deficit in addition to hypoxia. Although the impact of aglycemia, hypoxia and acidification on cancer cell metabolic remodeling was analyzed in parcellar studies, their combined interaction has been insufficiently investigated. Different levels of hypoxia are observed in tumors, leading Vaupel and colleagues to propose the "Janus face" model whereby metabolic adaptations are thought to occur when oxygen levels decrease below 1 %, while more drastic hypoxia (below 0.1 %) could trigger the generation of new genetic variants and resistance to apoptosis (Vaupel 2008; Vaupel and Mayer 2005). Another advantage of tumor cell deviant metabolism is acidification of extracellular medium, which inhibits the anti-tumoral immune response (Fischer et al. 2000). In a previous study, we conducted a longitudinal bioenergetic analysis of oxygen and glucose deprivation in human breast carcinoma cells (HTB-126) and breast non-cancer cells (HTB-125).

We mimicked *in vitro* the tumor microenvironmental conditions of tumor oxygen limitation (less than 1 % O_2; $p_{O2} < 7$ mmHg or 1 kPa) and glucose deprivation (no glucose; replaced by galactose and glutamine (Reitzer et al. 1979; Rossignol et al. 2004). We observed that the specific downregulation of OXPHOS triggered by hypoxia in cancer cells did no longer occur when glutamine was the only substrate for growth, i.e., when ATP can only be produced by mitochondria. Hence, under conditions of increased mitochondrial energy demand, 1 % O_2 hypoxia cannot downregulate the OXPHOS system as occurs in glucose medium. This observation suggests that cancer cells' metabolic apparatus ultimately comprises interactions and coordination between nutrient sensing and oxygen sensing. In non-cancer cells, glucose-deprivation did not change the stimulatory effect of hypoxia, and OXPHOS remained intact after 6 days of growth in 1 % O_2. Of note, stimulation of OXPHOS by glucose deprivation and hypoxia was additive in cancer cells.

Recent studies in the field of immunology have revealed the existence of biochemical pathways involved in the switch of glycolytic to oxidative metabolism associated to the modulation of the immune response. The group of Martin Villalba has shown the role of the ERK5 kinase in the coordinated modulation of energy metabolism and the MHC-I markers of self-immunity. In culture conditions that force respiration in leukemia cells these authors observed the upregulation of MHC-I transcription and protein levels at the cell surface, whereas opposite changes were observed in the same cells forced to perform fermentation as well as in leukemia cells lacking a functional mitochondrial respiratory chain. Forced respiration triggered the increased expression of the MAPK ERK5, which activated MHC-I gene promoters, and ERK5 accumulation in mitochondria. Such respiration-induced MHC-I upregulation was reversed upon short hairpin RNA-mediated ERK5 downregulation and by inactive mutants of ERK5. Moreover, short hairpin RNA for

ERK5 leukemia cells did not tolerate forced respiration. This work revealed that the expression of ERK5 and MHC-I is linked to cell energy metabolism.

In most of the above presented studies, mitochondrial adaptation occurred only in cancer cells forced to grow in absence of glucose while the non-cancer cells survived without adaptation. This specificity could be exploited as a therapeutic target although the determinants remain unclear. We could speculate that the higher energy demand of the cancer cells confronted to their lower OXPHOS capacity (versus their normal counterpart cells; at least for the "Warburg type" of cancer cells) could trigger the need to upregulate OXPHOS to allow survival in absence of glucose. In our work (Jose et al. 2012; Rossignol et al. 2004; Smolkova et al. 2010), the non-cancer cells possessed a higher contribution of OXPHOS to ATP synthesis so that upregulation might not be needed in situations of glucose withdrawal.

Then, the laboratory of Glick and Ferrara showed that bone marrow cells proliferating after transplantation increased aerobic glycolysis but not OXPHOS, whereas T cells proliferating in response to allo-antigens during graft-versus-host disease increased both aerobic glycolysis and OXPHOS. Alloreactive T cells also exhibited a hyperpolarized mitochondrial membrane potential, increased superoxide production, and decreased amounts of antioxidants, whereas proliferating BM cells did not. These findings challenged the current paradigm that activated T cells meet their increased demands for ATP through aerobic glycolysis, and identified the possibility that bioenergetic and redox characteristics could be selectively exploited as a therapeutic strategy for immune disorders. Lastly, the group of Ericka Pearce also demonstrated the importance of OXPHOS for the physiology of lymphocytes CD8 T cells, which have a crucial role in immunity. These authors showed that (TNF) receptor-associated factor 6 (TRAF6) regulates CD8 T(M)-cell development after infection by modulating fatty acid metabolism through OXPHOS. This bioenergetic phenomenon is crucial to maintain the memory function of the immune system.

8 The Rewiring of Pre-existing Metabolic Routes Accommodates Energy Needs, Anabolic Needs and ROS Management in Cancer Cells: Role of Hif1α, ChREBP, PDK and PKM2

Cancer cells are forced to continuously rewire alternative metabolic routes for their energetic and anabolic needs due to the fluctuation in substrates and oxygen levels in the growing tumor. Under conditions of glucose deprivation, mitochondrial activity is increased to sustain ATP production and lipid and protein synthesis. As discussed above, cancer cells can switch from aerobic glycolysis to OXPHOS under limiting glucose conditions. However, when glucose is available in large amounts, mitochondrial respiration is reduced by the crabtree effect and the F1Fo-ATP

synthase activity can even be reversed at the expense of glycolytic ATP. Although this particular functioning of the OXPHOS system (no longer OXPHOS) can deplete ATP and precipitate necrosis, it is limited by the mitochondrial protein inhibitor factor 1 (IF1), an endogenous F1Fo-ATPase inhibitor (Domenis et al. 2011). The high energetic needs of tumor cells are often correlated with increasing expression and inhibitory efficacy of IF1. Moreover, IF1 appears to have a role in defining the conformation of the F1F0-ATP synthase and mitochondrial cristae's structure and function (Cabezon et al. 2003; Campanella et al. 2008). The density of the cristae in IF1 overexpressing HeLa cells delayed the release of cytochrome *c* from intracristae space and increased survival after staurosporine treatment (Tan 2011). In this regard, recent experimental evidence has shed some light on a critical impact of mitochondrial morphology on the control of important mitochondrial functions including apoptosis and oxidative phosphorylation (Benard and Rossignol 2008b; Cereghetti and Scorrano 2006). In particular, de-regulated mitochondrial fusion and fission can now be regarded as important factors in cancer onset and progression, as discussed above. Several studies showed elevated levels of ($\Delta\mu_{H^+}$) in cancer cells despite a lower respiration rate and ATP synthesis that correlates with the tumorigenic potential of the cells (Heerdt et al. 2005).

The maintenance and even elevation of the electrochemical gradient of protons ($\Delta\mu_{H^+}$) can be done via the F_1F_0-ATP synthase working in reverse. The mechanism by which this reversible enzyme switches from the ATP synthetic to the ATP hydrolytic activity is still not well understood, but in *E. coli*, yeast, and mammalian cells it has been suggested to be a response to the proton motive force and the ADP/ATP balance. ATP hydrolytic activity, therefore, predominates at high cellular ATP levels and low membrane potential, forcing the reverse pumping of protons to generate a membrane potential (Suzuki et al. 2003).

As discussed above, intense glycolysis can force OXPHOS to work in reverse mode and this may not be associated with adaptive changes of the OXPHOS system, as none was reported. In contrast, hypoxia promotes glycolysis via the stabilization of the hypoxia-inducible factor 1 alpha (HIF1α) and alters OXPHOS via PDK1 activation and the subsequent inhibition of PDH (Kim et al. 2006; Papandreou et al. 2006; Semenza 2007; Wigfield et al. 2008). HIF1α is central to mitoplasticity as Fukuda et al. revealed that this transcription factor regulates the expression of the COX4-1 and COX4-2 isoforms. Under conditions of reduced oxygen availability, hypoxia-inducible factor 1 reciprocally regulates COX4 subunit expression by activating transcription of the genes encoding COX4-2 and LON, a mitochondrial protease that is required for COX4-1 degradation. This phenomenon is thought to optimize the efficiency of respiration at low oxygen concentration.

HIF1α also regulates the amount of mitochondria as stimulation of HIF1α ubiquitination by the Von-Hippel-Lindau factor and the subsequent proteasomal degradation of HIF1α restores the amount of mitochondria in cancer cells (Hervouet et al. 2008). In addition, HIF1α inhibits PDH via the activation of PDK1 which further inhibits OXPHOS capacity (Papandreou et al. 2006; Wigfield et al. 2008). Accordingly, increased levels of HIF1α were associated with the accumulation of

Krebs cycle intermediates (Pollard et al. 2005). It is well known that the stabilization of HIF1α stimulates glycolysis in various cell types (Lu et al. 2002; Marin-Hernandez et al. 2009) so that HIF1α can be considered as a master regulator of the balance between glycolysis and OXPHOS.

The rewiring of energy metabolism in cancer cells was recently shown to modulate the balance between anabolism and catabolism. For instance, it is proposed that pyruvate synthesis occurs primarily by the isoform 2 of pyruvate kinase (PKM2) in cancer cells (Mazurek 2010). The tetrameric form of PKM2 channels pyruvate to lactate and the formation of PKM2 tetramers is stimulated by the glycolytic intermediate F-1,6-BP. The dimeric form of PKM2 retards pyruvate formation and allows the accumulation of upstream glycolytic intermediates, promoting their distribution into the biosynthetic pathways. This regulation of enzyme activity may constitute a molecular switch that diverts glucose metabolites from energy production to anabolic processes in the pentose phosphate pathway and the generation of NADPH. Such a switch between glycolysis and pentose-phosphate pathway (PPP) was analyzed by Bensaad et al. in the context of cancer and the role of TIGAR in ROS homeostasy (Bensaad et al. 2006). Likewise, the work by Vaughn and Deshmukh (Vaughn and Deshmukh 2008) revealed the importance of glucose utilization through the PPP for neurons and cancer cells to control cytochrome *c* redox state and thereby modulate apoptosis. In addition, Herrero-Mendez et al. (2009) described the role for E3 ubiquitin ligase APC/C-Cdh1 in promoting the degradation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, isoform 3 (PFKFB3), which in turns allows glucose to be consumed through the PPP to avoid oxidative stress (whilst this work was performed in neurons, it could describe an universal mechanism that regulates this glycolytic/PPP shift). Besides, Smolkova and colleagues reviewed the fate of glutamine in cancer metabolism and evoke two principal pathways of glutamine utilization in cancer cells, one utilizing OXPHOS and another one operating more in hypoxic conditions.

The first pathway, dependent on NADPH and independent of respiration, employs only two enzymes of the Krebs cycle which act in a reverse mode and convert αKG to citrate by reductive carboxylation. However, such "reductive glutaminolysis" cannot support proliferation without ATP produced by glycolysis. Either it can act transiently until the glycolytic ATP pool is consumed, providing anoxic/hypoxic tumors to survive during a critical period until glucose utilization is restored, or it must act in parallel with the oxidative mode. In the 'reductive carboxylation glutaminolysis', 2-oxoglutarate and NADPH are consumed by isocitrate dehydrogenase isoform 2 (IDH2) followed by the reverse aconitase reaction and citrate efflux (Mullen et al. 2012). This pathway feeds fatty acid synthesis but does not produce ATP by substrate phosphorylation (via Krebs) or by NADH oxidation (via OXPHOS). Therefore, glycolysis is required to produce ATP in the reductive mode of glutaminolysis (Mullen et al. 2012).

The oxidative mode utilizes the forward-running Krebs cycle truncated after citrate synthase at citrate extrusion. In both modes, citrate is extruded from mitochondria and converted to oxaloacetate and acetyl-CoA by ACL. Acetyl-CoA is

then used to produce fatty acids by fatty acid synthase (FASN) and cholesterol for general lipid synthesis. OXPHOS glutaminolysis can compensate for deficiencies in cellular ATP. Both glutaminolytic modes provide pyruvate, lactate and the NADPH pool, normally supplied by glucose metabolism via the pentose phosphate pathway. Increasing anti-cancer therapeutic approaches target metabolism to selectively kill tumor cells. Glucose catabolism is the pathway the most targeted in this end but the pentose phosphate pathway, TCA cycle, and glutaminolysis are increasingly the focus of research as the cancer cells are not equally sensitive to agents interfering with glucose metabolism. Lastly, a recent player to consider in the field of energy homeostasy is the carbohydrate-response element-binding protein (CREBP), the deletion of which alters substrate utilization and produces an energy-deficient liver (Burgess et al. 2008). The KO of this transcription factor in mice induced an increase in PDH activity. This was explained by a diminished pyruvate dehydrogenase kinase activity. In the liver of these mice, the greater pyruvate dehydrogenase complex activity caused a stimulation of lactate and pyruvate oxidation, and it significantly impaired fatty acid oxidation. It remains to be shown whether CREBP could participate to the maintenance of energy homeostasy in cancer cells and whether it could play a role during the shift between glucose and galactose media where the needs in PDH activity are strongly increased.

9 Cellular and Mitochondrial Adaptations to the Type of Available Energy Substrate: Ras and CC3 Modulate Metabolic Flexibility in Cancer Cells

The impact of various oncogenic mutations on energy metabolism discussed above could seem paradoxical as they can disable or, at the opposite, improve the metabolic flexibility, i.e. the capacity to derive energy from multiple sources. Yet, nutrient addiction is the Achilles' heel of numerous tumor cells and is often proposed as a rationale for metabolic therapy. Alterations in phosphoinositide 3-kinase (PI3K)/serine-threonine kinase AKT/mammalian target of rapamycin (mTOR) signaling are perhaps the most frequent events observed in solid tumors. Constitutive activation of the oncogenic kinase AKT addicts glioblastoma cells to glucose by interfering with the induction of fatty acid oxidation when glucose is withdrawn (Buzzai et al. 2005). Another well-known example of oncogene that causes nutrient addiction is c-Myc that is frequently deregulated in tumors. Induction of c-Myc expression in MEFs results in the induction of glutamine transporters, glutaminase and lactate dehydrogenase A (LDH A) (Wise et al. 2008).

Some transformating mutations can bypass the addiction of cancer cells to glucose (i.e. metabolic rigidity). It has been shown that KRAS or BRAF mutations increase glucose uptake and glycolysis and at the same time, permit cells to survive in low-glucose conditions. However, the mechanisms which support survival in low glucose conditions remain controversial as conflicting results were published

recently. For instance, the activation of RAS was shown to confer survival in low glucose via the enhancement of glucose uptake by GLUT1 and the enhancement of glycolysis along with the alteration of respiratory chain activity (Baracca et al. 2010; Demaria et al. 2010; Yun et al. 2009). Conversely, the activation of STAT3 was shown to stimulate respiratory chain activity in another study (Gough et al. 2009), while more recently, Ras activation was associated with mitophagy and the reduction of OXPHOS capacity (Kim et al. 2011). This important issue is further discussed below.

Firstly, Ras oncogenic mutations were associated with mitochondrial protein downregulation mediated by STAT3. The study of Demaria and colleagues (2010) evidenced a reduced mitochondrial activity in MEFs with constitutively active STAT3 (*Stat3*$^{C/C}$MEFs). The significant down-regulation of nuclear-encoded genes involved in mitochondrial function observed in these cells together with their decreased PDH activity led to the reduction of mitochondrial respiration. Mitochondrial ATP production and basal respiratory chain activity were also reduced in these cells, as well as the protein levels of representative components of the Electron Transport Chain (ETC), particularly those belonging to complexes IV and V. Lastly, mitochondrial morphology and total area were normal in these cells, suggesting a specific degradation of COX and CV components. Accordingly, the recent study by Kim J. and colleagues (2011) also reported that K-Ras mediates functional loss of mitochondria during cell transformation to overcome an energy deficit resulting from glucose deficiency. In this study, the decrease of respiration was explained by the increased formation of acidic vesicles enclosing mitochondria, during which autophagy-related proteins such as Beclin 1, Atg5, LC3-II and vacuolar ATPases were induced. The blockade of autophagy recovered respiratory protein expression and respiratory activity. However, in this work, K-Ras (V12) did not induce the expression of GLUT1, the low Km glucose transporter. Lastly, the interaction between Ras, STAT3 and the mitochondrion was analyzed in immortalized STAT3-deficient mouse embryo fibroblasts (Gough et al. 2009). In this work STAT3 was detected in mitochondria of primary liver tissue, non-transformed MCF10A mammary epithelial cells, and T24 bladder carcinoma cells. In contrast with the two above cited studies, mitochondrial STAT3 augmented here the electron transport chain activity.

Mitochondrial STAT3 also contributed to Ras-dependent cellular transformation by augmenting electron transport chain activity, particularly that of complexes II and V, accompanied somewhat paradoxically by shifted energy production to favor fermentation. In a fourth study the link between STAT3 and mitochondria was further investigated (Wegrzyn et al. 2009) in non-cancer tissues such as liver and heart. In striking contrast with the study of Demaria et al. where constitutively active STAT3 triggered a reduction of mitochondrial activity in MEFs, the absence of STAT3 in heart and liver was associated with a significant decrease of complex I and II activities. Moreover, in the study of Wegrzyn et al. (2009) state 3 respiration was reduced by 70 % in STAT3$^{-/-}$ cells with glutamate as a substrate for complex I and by 50 % in cells with succinate as a substrate for complex II. The authors concluded that the most likely mechanism by which STAT3 exerts its actions is not

as a transcription factor that regulates nuclear gene expression, but rather through its localization in the mitochondria. The effects of STAT3 potentially represent a general mechanism by which this protein can influence cell metabolism and tumorigenesis.

Already in 1999, Onhami and coworkers showed that the blockade of K-Ras with antisense in pancreatic cancer cells modified (increased) the expression pattern of 11 mitochondrial genes (Ohnami et al. 1999). Those included mitochondrial 16 s rRNA gene, cytochrome oxidase subunit 1, NADH dehydrogenase subunit 4 and ATP synthase. Lastly, the group of Weinberg investigated the impact of the K-Ras mutation on mitochondria and proposed that this organelle serves mainly for the production of ROS which are essential for anchorage independent growth (Weinberg et al. 2010). In this study, human colon cancer cells were able to consume glutamine through respiration but the contribution of mitochondria to cellular ATP production was not assessed. Interestingly, this study revealed that mice harboring a knock-out of TFAM with an activated form of K-Ras had smaller tumors and displayed fewer Ki67 positive cells. Therefore, strategies aiming at the reduction of mitochondrial biogenesis should be tested for their capacity to reduce tumor growth.

In contrast to Ras, CC3/TIP30 is a tumor suppressor with reduced or absent expression in a variety of aggressive tumors. Yet, CC3 could also be involved in the control of metabolic flexibility as Chen et al. (2012) demonstrated that the silencing of CC3 in HeLa cells strongly improves survival and allows superior metabolic adaptation in response to glucose limitation. These authors showed that silenced CC3 increases mitochondrial respiration and expression of mitochondrial proteins of the ETC along while maintaining high levels of c-Myc expression and downstream GLUT1 and PKM2. Moreover, loss of CC3 represses AMPK activation by low glucose. Lastly, metabolic flexibility might also include the capacity of deriving energy from the amino acids obtained by autophagy. In transformed fibroblasts, the Simian Virus 40 Small T (Small T) carrying cells are more resistant to glucose deprivation-induced cell death than cells lacking ST. ST antigen protects cancer cells from glucose deprivation by maintaining energy homeostasis with activation of AMPK, inhibition of mTOR and induction of autophagy as an alternate energy source (Kumar and Rangarajan 2009).

The above described changes in mitochondrial energy metabolism raise the question of mitochondrial integrity in cancer cells: is mitochondrial functional or dysfunctional in tumors?

10 Towards Tumor Adapted Anti-cancer Bioenergetics Strategies

The peculiarities of energy production in tumors could designate targets of choice for innovative anti-cancer therapy (Fig. 11.7). Three groups of tentative bioenergetic strategies could be distinguished: (1) approaches aiming at specific tumor energy desequilibrium, either by (1.1) the blockade of the primary pathways of energy

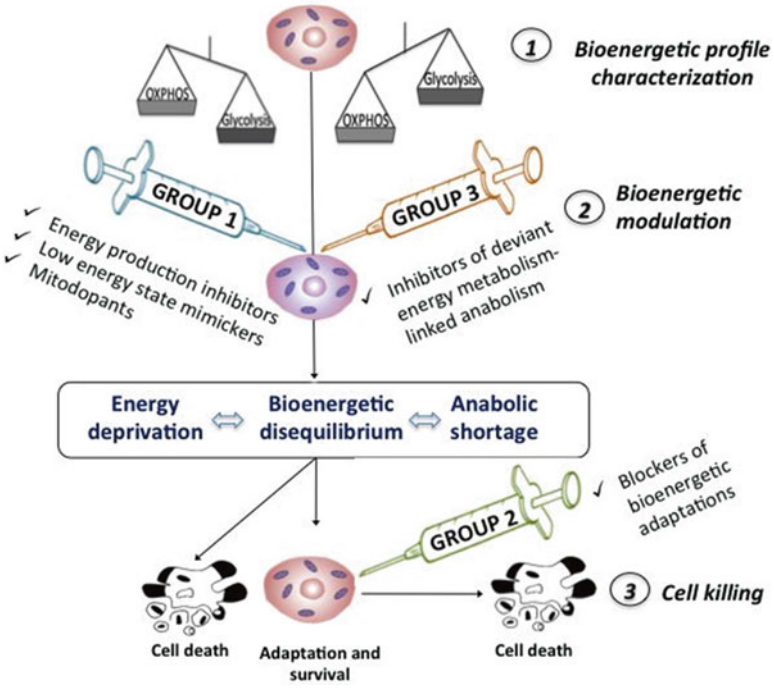

Fig. 11.7 Tentative strategies for anti-cancer bioenergetic therapies. The first step consists in the evaluation of the bioenergetic profile to identify both the primary pathways of tumor energy production and the predominant energy substrates used for ATP synthesis. To be developed-non-invasive methods should allow to evaluate this profile *in situ* via imaging or secretomics techniques as well as with dedicated micro-electrodes. Then, three groups of molecules could be used to inhibit cancer energetics. In Group 1 therapies, glycolytic cells should receive a treatment aiming at the inhibition of glycolysis while OXPHOS cancer cells should be attacked with OXPHOS inhibitors. The main difficulty will be to discover drugs specific to tumors or to deliver doses that do not impact the surrounding non-cancer tissue. In group 3 therapies, the molecules should further block some anabolic pathways linked to the deviations of energy metabolism (as the ATP-citrate lyase which utilizes the citrate evaded from the Krebs cycle to produce lipids). Therefore, group 1 and group 3 therapies will destabilize the tumor bioenergetic profile, promote energy deprivation and alter deviant anabolic processes. However, cancer cells can use alternative sources of energy when the primary pathway are blocked, a process known as metabolic flexibility which requires sometimes metabolic adaptations. Therefore, Group 2 therapies (in combination with group 1 and/or 3) will aim at the blockade of such adaptations to fully alter cancer cells energy production and to promote ultimate cell death

production or by (1.2) mimicking a low energy state; (2) coupled strategies focused on the inhibition of the adaptive response of tumors to the bioenergetic crisis provoked by the first group of approaches; (3) approaches focused on the inhibition of anabolic reactions branched on the pathways involved in energy metabolism.

Concerning the first group of potential therapies, different possibilities must be considered according to the variable bioenergetic profile of tumors:

(i) glycolytic inhibitors (such as 2-deoxyglucose, 3-bromopyruvate or lonidamine), (ii) OXPHOS inhibitors (glutaminase, other ones to be developed), (iii) OXPHOS enhancers (resveratrol, bezafibrate, rosiglitazone, dichloroacetate, α-lipoic acid), (iv) combination of glycolytic and OXPHOS inhibitors, (v) mimickers of low energy state (as AICAR or metformin). Regarding the second group, more research is needed to decipher the molecular pathways involved in cancer cell adaptation to specific bioenergetic impairment in order to identify new drugs. For example, proteins involved in energy sensing like AMPK, AKT and m-TOR could be counteracted to escape a reestablishment of energetic equilibrium. Lastly, the group of drugs focused on anabolic pathways linked to energy pathways could include to be developed-inhibitors of the ATP citrate lyase or of fatty acid synthase. It must be stressed here that some of the above cited drugs might lack target specificity so that multiple mechanisms could explain their mode of action. For instance, the glycolytic inhibitor 3-bromopyruvate was also shown to inhibit mitochondrial respiratory chain (Ko et al. 2001). Same for resveratrol which not only activates mitochondrial biogenesis but also play a role as antioxidant and regulator of several intracellular pathways (Pervaiz and Holme 2009). Ideally, these bioenergetic modulators aiming at the reduction of tumor energy production could be combined with more classic chemotherapeutic agents to promote synergistic killing effects. Interestingly, drugs able to inhibit both OXPHOS and glycolysis, albeit with cancer specificity, might produce the best anti-cancer killing effect, as recently shown for the compound Bezielle (Chen et al. 2012).

As a first step, it is crucial to establish the bioenergetic profile of the tumor. This could be done on a series of biopsies by determining the bioenergetic signature as proposed by Cuezva et al. (2004, 2002). Yet, multiple biopsies should be performed to take into consideration the intra-tumor bioenergetic variability. Additional approaches could take into consideration the non-invasive imaging techniques which allow to assess glucose and oxygen consumption in tissues as shown for the brain (Vaishnavi et al. 2010). Oxidative tumors could be detected and might correspond to the tumor non-detected by PET-scan. Hence, one could propose to develop oxidative markers (glutamine based for instance) visualized by PET-scan to assess the ratio of glucose/glutamine utilization by the tumor. Then, brainstorming must occur to choose the best approach, as current knowledge in the field does not permit to conclude on this question. Should we attack a glycolytic tumor with solely glycolytic inhibitors or should we add OXPHOS inhibitors to avoid tumor bioenergetic escape? What will happen with the remaining cancer stem cells, what are their bioenergetics Achille's heel and how to kill them? Of particular importance, the bioenergetic profile of the tumors might not only give indications on what drug should be used but it could also help to avoid chemoresistance as difference in cellular mitochondrial content were previously associated with differences in drug-resistance. This is the case for the HeLa derivative cell line lacking mtDNA (ρ^0) which was more resistant to adriamycin and PDT-induced cytotoxicity.

Regarding the killing of 'OXPHOS tumors' we should discover new OXPHOS inhibitors specific to those cancer cells with no or limited adverse effects on non-cancer tissue which utilize OXPHOS to major extent. Mitochondrial pharmacology

has recently developed chemical compounds and nano-objects able to accumulate in mitochondria and capable of delivering active compounds there (D'souza et al. 2003). The DQasomes are one example of such available technology and could be used in future strategies. More research on the specificities of mitochondrial structure in tumors might also allow to discover new targets. Current OXPHOS strategies of cancer focusing on the development of glutaminase inhibitors or of Krebs cycle inhibitors were discussed in a recent review (Garber 2010).

To foster industrial research on OXPHOS inhibitors specific of cancer cells one should consider the recent studies which provide a rationale for mitochondria-targeting strategies of tumors. For instance, the introduction of mtDNA mutation in human cancer cells *in vitro* activates both the metastatic potential and the tumorigenicity of these cells in soft-agar and in xenografted tumors (Ishikawa et al. 2008; Petros et al. 2005). However, this pro-tumorigenic effect occurs only for moderate inhibition of the respiratory chain, and stronger inhibitions trigger the desired therapeutic effect; they inhibit tumor growth. This was shown in two independent studies using different cancer cells with variable load of mtDNA mutations: while the heteroplasmic clones exhibited pro-tumorigenic features, the homoplasmic cancer cells showed reduced growth and altered tumorigenic potential (Gasparre et al. 2011; Park et al. 2009). These studies highlight the Importance of the degree of mitochondrial inhibition for promoting pro-tumorigenic and anti-tumorigenic effects. Below a threshold of 80 % of mutant load in mitochondrial DNA, these mutations stimulate tumor growth. Above 80 %, the accumulation of mtDNA defects and the consecutive inhibition of mitochondrial respiration reduce tumor growth. These observations are in complete adequation with our findings of the existence a biochemical threshold effect that governs the phenotypic expression of mtDNA mutations and of biochemical defects in respiratory chain function (Benard et al. 2006, 2008; Faustin et al. 2004; Rossignol et al. 1999, 2000, 2003). For instance, we demonstrated that human cells can handle a 70 % inhibition of complex I without reduction in the overall flux of respiration and of ATP production. However, above this threshold of inhibition, the reduced CI activity entails a drop in respiration and cellular alterations are observed (Benard et al. 2007). Differences in threshold value (as observed between rat tissues) could provide differences in sensitivity to drugs targeting OXPHOS, since cells with a lower threshold will appear more sensitive and could loose viability at lower doses of these drugs. All these studies indicate that blocking OXPHOS functions can be anti-tumorigenic only when the inhibition level outranges the biochemical threshold value.

While most mitochondria-targeted anti-cancer strategies often attempt to inhibit mitochondrial capacity (as proposed for OXPHOS tumors), other groups choose to increase mitochondrial content to alter the proliferative potential of cancer cells in glycolytic tumors. For example, stable overexpression of mitochondrial frataxin in colon cancer cell lines increased aconitase activity, mitochondrial membrane potential, cellular respiration, and ATP content. Such OXPHOS improvement reduced

growth rates and colony formation capacity in soft agar assays, and tumor formation when injected into nude mice (Schulz et al. 2006). Inhibition of L-alanine production impaired D-glucose uptake of LLC1 Lewis lung carcinoma cells and increased mitochondrial metabolism leading to an impairment of anchorage-dependent and anchorage-independent growth rates (Beuster et al. 2011). The utilization of mitodopants to reduce cancer cell growth showed promising results *in vitro* (Bellance et al. 2009b; Wang and Moraes 2011) but lack of *in vivo* data.

11 Conclusions and Future Directions

Even though the mitochondrion appears like a tempting target for the design of bioenergetics anti-cancer therapies, the huge disparity in the reported mitochondrial alterations among tumors could interfere with the efficacy of the potential mitochondrion-targeted treatments. There is a need for large-scale studies on the bioenergetics of human tumors to derive knowledge useful for therapy. It is important to consider cancer specificity as embraced by the so-called personalized medicine since tumors are able to derive energy by variable means. As an example, OXPHOS inhibition was proven to be efficient decreasing tumorigenesis in mice models while opposite approaches performed *in vitro* and *in vivo* revealed that OXPHOS enhancement (chemically-induced increase in mitochondrial content or stimulation of PDH activity) can also overcome the deregulated proliferation of cancer cells. We need to evaluate the extent to which those opposing strategies could work on the same cancer types and between different individuals. Also, fundamental research on the peculiarities of mitochondrial turnover and dynamics in cancer cell is lacking and could help to discover specificities of these processes which might unravel new protein targets. Again, one needs first to better characterize the mitochondrial alterations and to adopt a case-by-case therapeutic strategy. The optimization and the adaptation of diagnostic tools used for mitochondrial evaluation could be considered for clinical cancer evaluation and the deciphering of the bioenergetics profile. Mitochondrial functionality better than mitochondrial content could predict the cell fate in face of a bioenergetic drug (Table 11.2). The specificities of the proposed bioenergetics therapies might consider the phenomenon of biochemical threshold which determines the overall impact of a drug active at the level of respiratory chain. Research on tumor bioenergetics escape will also reveal the adaptive mechanisms by which cancer cells control their metabolic flexibility when the primary pathway of energy production is blocked and this will provide new targets of choice. Additional compensatory mechanisms involved in the potential resistance to bioenergetics therapy exist at the level the pathways involved in the response to low energy crisis and blockade of its main actors, as Akt or AMPK might also be considered in combination with bioenergetic therapy.

Table 11.2 Bioenergetic drugs for cancer therapy

Pathway	Compound	Target	Effect	Cancer
	2-desoxyglucose	Hexokinase 2/Glut 1-3	Inhibits glycolysis	Solid tumors (lung, breast, prostate, gastric)
	Lonidamine	Hexokinase 2	Inhibits glycolysis	Lung, prostate
Glycolysis	3-bromopyruvate	Hexokinase 2	Inhibits glycolysis	Fibrolamellar hepatocellular carcinoma
	TLN-232	Pyruvate kinase	Inhibits glycolysis	Melanoma, renal cell carcinoma
	Dichloroacetate	Pyruvate dehydrogenase kinase 1	Inhibits glycolysis	Solid tumors (lung, glioma, GBM)
	CPI-613	Pyruvate dehydrogenase	Inhibits pyruvate dehydrogenase	Pancreatic and other
	Resveratrol	Respiratory complex	OXPHOS enhancers	Colon, breast, lung
Mitochondria	Bezafibrate	Agonist PPARα	OXPHOS enhancers	Hepatocarcinome, breast
	Rosiglitazone	Mitochondrial biogenesis	OXPHOS enhancers	Prostate, liposarcoma
	Alpha-lipoic acid	Antioxidant	OXPHOS enhancers	Breast, prostate, adult solid tumors
mTORC1	Temsirolimus, everolimus	mTORC1	Inhibits mTORC1	Solid tumors (metastatic and non-metastatic)
	Ridaforolimus, others rapalogues	mTORC1	Inhibits mTORC1	Solid tumors (pancreatic, endometrial, glioblastoma), lymphoma
Hypoxia	PX-478	HIF1α	Inhibits HIF signalling	Solid tumours and lymphoma
	Tirapazamine, other bioreductive	Hypoxia	Resensitizes cells to other treatments	Solid tumours (cervical, SCLC, NSCLC)
PI3K	GDC-0941, PX866	PI3K	Inhibits PI3K	Solid tumours (metastatic, breast, non-Hodgkin's lymphoma)
AKT	Perifosine, GSK690693	AKT	Inhibits AKT	Solid tumours (renal cancer, NSCLC) lymphoma
AMPK	Metformin	AMPK, complex I	Activates AMPK	Solid tumors, lymphoma
	AICAR	Increased p21	Activates AMPK	Solid tumors

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References

Baracca A, Chiaradonna F, Sgarbi G, Solaini G, Alberghina L, Lenaz G (2010) Mitochondrial Complex I decrease is responsible for bioenergetic dysfunction in K-ras transformed cells. Biochim Biophys Acta 1797:314–323

Baris O, Savagner F, Nasser V, Loriod B, Granjeaud S, Guyetant S, Franc B, Rodien P, Rohmer V, Bertucci F, Birnbaum D, Malthiery Y, Reynier P, Houlgatte R (2004) Transcriptional profiling reveals coordinated up-regulation of oxidative metabolism genes in thyroid oncocytic tumors. J Clin Endocrinol Metab 89:994–1005

Beckner ME, Gobbel GT, Abounader R, Burovic F, Agostino NR, Laterra J, Pollack IF (2005) Glycolytic glioma cells with active glycogen synthase are sensitive to PTEN and inhibitors of PI3K and gluconeogenesis. Lab Invest 85:1457–1470

Bellance N, Benard G, Furt F, Begueret H, Smolkova K, Passerieux E, Delage JP, Baste JM, Moreau P, Rossignol R (2009a) Bioenergetics of lung tumors: alteration of mitochondrial biogenesis and respiratory capacity. Int J Biochem Cell Biol 41:2566–2577

Bellance N, Lestienne P, Rossignol R (2009b) Mitochondria: from bioenergetics to the metabolic regulation of carcinogenesis. Front Biosci 14:4015–4034

Benard G, Karbowski M (2009) Mitochondrial fusion and division: regulation and role in cell viability. Semin Cell Dev Biol 20:365–374

Benard G, Rossignol R (2008a) Mitochondrial fluidity matters. Focus on "Inherited complex I deficiency is associated with faster protein diffusion in the matrix of moving mitochondria". Am J Physiol Cell Physiol 294:C1123

Benard G, Rossignol R (2008b) Ultrastructure of the mitochondrion and its bearing on function and bioenergetics. Antioxid Redox Signal 10:1313–1342

Benard G, Faustin B, Passerieux E, Galinier A, Rocher C, Bellance N, Delage JP, Casteilla L, Letellier T, Rossignol R (2006) Physiological diversity of mitochondrial oxidative phosphorylation. Am J Physiol Cell Physiol 291:C1172–C1182

Benard G, Bellance N, James D, Parrone P, Fernandez H, Letellier T, Rossignol R (2007) Mitochondrial bioenergetics and structural network organization. J Cell Sci 120:838–848

Benard G, Faustin B, Galinier A, Rocher C, Bellance N, Smolkova K, Casteilla L, Rossignol R, Letellier T (2008) Functional dynamic compartmentalization of respiratory chain intermediate substrates: implications for the control of energy production and mitochondrial diseases. Int J Biochem Cell Biol 40:1543–1554

Benard G, Bellance N, Jose C, Melser S, Nouette-Gaulain K, Rossignol R (2010) Multi-site control of cellular and mitochondrial energy production. Biochim Biophys Acta 1797:698–709

Bensaad K, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R, Gottlieb E, Vousden KH (2006) TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell 126:107–120

Bereiter-Hahn J, Voth M (1994) Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. Microsc Res Tech 27:198–219

Beuster G, Zarse K, Kaleta C, Thierbach R, Kiehntopf M, Steinberg P, Schuster S, Ristow M (2011) Inhibition of alanine aminotransferase in silico and in vivo promotes mitochondrial metabolism to impair malignant growth. J Biol Chem 286:22323–22330

Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Bonnet S, Harry G, Hashimoto K, Porter CJ, Andrade MA, Thebaud B, Michelakis ED (2007) A mitochondria-K+channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 11:37–51

Bouzier AK, Voisin P, Goodwin R, Canioni P, Merle M (1998) Glucose and lactate metabolism in C6 glioma cells: evidence for the preferential utilization of lactate for cell oxidative metabolism. Dev Neurosci 20:331–338

Burgess SC, Iizuka K, Jeoung NH, Harris RA, Kashiwaya Y, Veech RL, Kitazume T, Uyeda K (2008) Carbohydrate-response element-binding protein deletion alters substrate utilization producing an energy-deficient liver. J Biol Chem 283:1670–1678

Buzzai M, Bauer DE, Jones RG et al (2005) The glucose dependence of Akt-transformed cells can be reversed by pharmacologic activation of fatty acid β-oxidation. Oncogene 24:4165–4173

Cabezon E, Montgomery MG, Leslie AG, Walker JE (2003) The structure of bovine F1-ATPase in complex with its regulatory protein IF1. Nat Struct Biol 10:744–750

Campanella M, Casswell E, Chong S, Farah Z, Wieckowski MR, Abramov AY, Tinker A, Duchen MR (2008) Regulation of mitochondrial structure and function by the F1Fo-ATPase inhibitor protein, IF1. Cell Metab 8:13–25

Canto C, Auwerx J (2009) PGC-1α, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr Opin Lipidol 20:98–105

Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J (2009) AMPK regulates energy expenditure by modulating NAD(+) metabolism and SIRT1 activity. Nature 458:1056–1060

Capuano F, Varone D, D'eri N, Russo E, Tommasi S, Montemurro S, Prete F, Papa S (1996) Oxidative phosphorylation and F(O)F(1) ATP synthase activity of human hepatocellular carcinoma. Biochem Mol Biol Int 38:1013–1022

Carew JS, Nawrocki ST, Xu RH, Dunner K, Mcconkey DJ, Wierda WG, Keating MJ, Huang P (2004) Increased mitochondrial biogenesis in primary leukemia cells: the role of endogenous nitric oxide and impact on sensitivity to fludarabine. Leukemia 18:1934–1940

Cereghetti GM, Scorrano L (2006) The many shapes of mitochondrial death. Oncogene 25:4717–4724

Chen V, Staub RE, Fong S, Tagliaferri M, Cohen I, Shtivelman E (2012) Bezielle selectively targets mitochondria of cancer cells to inhibit glycolysis and OXPHOS. PLoS One 7:e30300

Cheung WD, Hart GW (2008) AMP-activated protein kinase and p38 MAPK activate O-GlcNAcylation of neuronal proteins during glucose deprivation. J Biol Chem 283:13009–13020

Cormio A, Guerra F, Cormio G, Pesce V, Fracasso F, Loizzi V, Cantatore P, Selvaggi L, Gadaleta MN (2009) The PGC-1α-dependent pathway of mitochondrial biogenesis is upregulated in type I endometrial cancer. Biochem Biophys Res Commun 390:1182–1185

Cuezva JM, Krajewska M, De Heredia ML, Krajewski S, Santamaria G, Kim H, Zapata JM, Marusawa H, Chamorro M, Reed JC (2002) The bioenergetic signature of cancer: a marker of tumor progression. Cancer Res 62:6674–6681

Cuezva JM, Chen G, Alonso AM, Isidoro A, Misek DE, Hanash SM, Beer DG (2004) The bioenergetic signature of lung adenocarcinomas is a molecular marker of cancer diagnosis and prognosis. Carcinogenesis 25:1157–1163

Czech MP, Corvera S (1999) Signaling mechanisms that regulate glucose transport. J Biol Chem 274:1865–1868

Demaria M, Giorgi C, Lebiedzinska M, Esposito G, D'angeli L, Bartoli A, Gough DJ, Turkson J, Levy DE, Watson CJ, Wieckowski MR, Provero P, Pinton P, Poli V (2010) A STAT3-mediated metabolic switch is involved in tumour transformation and STAT3 addiction. Aging 2:823–842

Diaz-Ruiz R, Averet N, Araiza D, Pinson B, Uribe-Carvajal S, Devin A, Rigoulet M (2008) Mitochondrial oxidative phosphorylation is regulated by fructose 1,6-bisphosphate. A possible role in Crabtree effect induction? J Biol Chem 283:26948–26955

Domenis R, Comelli M, Bisetto E, Mavelli I (2011) Mitochondrial bioenergetic profile and responses to metabolic inhibition in human hepatocarcinoma cell lines with distinct differentiation characteristics. J Bioenerg Biomembr 43:493–505

Donnely M, Scheffler I (1976) Energy metabolism in respiration deficient and wild type Chinese hamster fibroblasts in culture. J Cell Physiol 89:39–52

D'souza GG, Rammohan R, Cheng SM, Torchilin VP, Weissig V (2003) DQAsome-mediated delivery of plasmid DNA toward mitochondria in living cells. J Control Release 92:189–197

Duvezin-Caubet S, Jagasia R, Wagener J, Hofmann S, Trifunovic A, Hansson A, Chomyn A, Bauer MF, Attardi G, Larsson NG, Neupert W, Reichert AS (2006) Proteolytic processing of OPA1 links mitochondrial dysfunction to alterations in mitochondrial morphology. J Biol Chem 281:37972–37979

Eakin RT, Morgan LO, Gregg CT, Matwiyoff NA (1972) Carbon-13 nuclear magnetic resonance spectroscopy of living cells and their metabolism of a specifically labeled 13C substrate. FEBS Lett 28:259–264

Ehses S, Raschke I, Mancuso G, Bernacchia A, Geimer S, Tondera D, Martinou JC, Westermann B, Rugarli EI, Langer T (2009) Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1. J Cell Biol 187:1023–1036

Faustin B, Rossignol R, Rocher C, Benard G, Malgat M, Letellier T (2004) Mobilization of adenine nucleotide translocators as molecular bases of the biochemical threshold effect observed in mitochondrial diseases. J Biol Chem 279:20411–20421

Fischer B, Muller B, Fisch P, Kreutz W (2000) An acidic microenvironment inhibits antitumoral non-major histocompatibility complex-restricted cytotoxicity: implications for cancer immunotherapy. J Immunother 23:196–207

Fryer LG, Foufelle F, Barnes K, Baldwin SA, Woods A, Carling D (2002) Characterization of the role of the AMP-activated protein kinase in the stimulation of glucose transport in skeletal muscle cells. Biochem J 363:167–174

Furt F, Moreau P (2009) Importance of lipid metabolism for intracellular and mitochondrial membrane fusion/fission processes. Int J Biochem Cell Biol 41:1828–1836

Garber K (2010) Oncology's energetic pipeline. Nat Biotechnol 28:888–891

Gasparre G, Kurelac I, Capristo M, Iommarini L, Ghelli A, Ceccarelli C, Nicoletti G, Nanni P, De Giovanni C, Scotlandi K, Betts CM, Carelli V, Lollini PL, Romeo G, Rugolo M, Porcelli AM (2011) A mutation threshold distinguishes the antitumorigenic effects of the mitochondrial gene MTND1, an oncojanusfunction. Cancer Res 71:6220–6229

Gatenby RA, Gillies RJ (2004) Why do cancers have high aerobic glycolysis? Nat Rev Cancer 4:891–899

Gatenby RA, Gillies RJ (2008) A microenvironmental model of carcinogenesis. Nat Rev Cancer 8:56–61

Gnaiger E, Kemp RB (1990) Anaerobic metabolism in aerobic mammalian cells: information from the ratio of calorimetric heat flux and respirometric oxygen flux. Biochim Biophys Acta 1016:328–332

Gough DJ, Corlett A, Schlessinger K, Wegrzyn J, Larner AC, Levy DE (2009) Mitochondrial STAT3 supports Ras-dependent oncogenic transformation. Science 324:1713–1716

Griguer CE, Oliva CR, Gillespie GY (2005) Glucose metabolism heterogeneity in human and mouse malignant glioma cell lines. J Neurooncol 74:123–133

Griparic L, Van Der Bliek AM (2001) The many shapes of mitochondrial membranes. Traffic 2:235–244

Gstraunthaler G, Seppi T, Pfaller W (1999) Impact of culture conditions, culture media volumes, and glucose content on metabolic properties of renal epithelial cell cultures. Are renal cells in tissue culture hypoxic? Cell Physiol Biochem 9:150–172

Hamanaka RB, Chandel NS (2012) Targeting glucose metabolism for cancer therapy. J Exp Med 209:211–215

Hardie DG (2007) AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat Rev Mol Cell Biol 8:774–785

Heddi A, Faure-Vigny H, Wallace DC, Stepien G (1996) Coordinate expression of nuclear and mitochondrial genes involved in energy production in carcinoma and oncocytoma. Biochim Biophys Acta 1316:203–209

Heerdt BG, Houston MA, Augenlicht LH (2005) The intrinsic mitochondrial membrane potential of colonic carcinoma cells is linked to the probability of tumor progression. Cancer Res 65:9861–9867

Herrero-Mendez A, Almeida A, Fernandez E, Maestre C, Moncada S, Bolanos JP (2009) The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. Nat Cell Biol 11:747–752

Hervouet E, Demont J, Pecina P, Vojtiskova A, Houstek J, Simonnet H, Godinot C (2005) A new role for the von Hippel-Lindau tumor suppressor protein: stimulation of mitochondrial oxidative phosphorylation complex biogenesis. Carcinogenesis 26:531–539

Hervouet E, Cizkova A, Demont J, Vojtiskova A, Pecina P, Franssen-Van Hal NL, Keijer J, Simonnet H, Ivanek R, Kmoch S, Godinot C, Houstek J (2008) HIF and reactive oxygen species regulate oxidative phosphorylation in cancer. Carcinogenesis 29:1528–1537

Hosgood HD 3rd, Liu CS, Rothman N, Weinstein SJ, Bonner MR, Shen M, Lim U, Virtamo J, Cheng WL, Albanes D, Lan Q (2010) Mitochondrial DNA copy number and lung cancer risk in a prospective cohort study. Carcinogenesis 31:847–849

Ishihara N, Jofuku A, Eura Y, Mihara K (2003) Regulation of mitochondrial morphology by membrane potential, and DRP1-dependent division and FZO1-dependent fusion reaction in mammalian cells. Biochem Biophys Res Commun 301:891–898

Ishihara N, Fujita Y, Oka T, Mihara K (2006) Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. EMBO J 25:2966–2977

Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H, Nakada K, Honma Y, Hayashi J (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 320:661–664

Jiang WG, Douglas-Jones A, Mansel RE (2003) Expression of peroxisome-proliferator activated receptor-γ (PPARγ) and the PPARγ co-activator, PGC-1, in human breast cancer correlates with clinical outcomes. Int J Cancer 106(5):752–757

Jose C, Bellance N, Rossignol R (2011) Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? Biochim Biophys Acta 1807:552–561

Jose C, Bellance N, Chatelain EH, Benard G, Nouette-Gaulain K, Rossignol R (2012) Antiproliferative activity of levobupivacaine and aminoimidazole carboxamide ribonucleotide on human cancer cells of variable bioenergetic profile. Mitochondrion 12:100–109

Jourdain A, Martinou JC (2010) Mitochondrial dynamics: quantifying mitochondrial fusion in vitro. BMC Biol 8:99

Kanda Y, Watanabe Y (2005) Thrombin-induced glucose transport via Src-p38 MAPK pathway in vascular smooth muscle cells. Br J Pharmacol 146:60–67

Kim JW, Tchernyshyov I, Semenza GL, Dang CV (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3:177–185

Kim JH, Kim HY, Lee YK, Yoon YS, Xu WG, Yoon JK, Choi SE, Ko YG, Kim MJ, Lee SJ, Wang HJ, Yoon G (2011) Involvement of mitophagy in oncogenic K-Ras-induced transformation: overcoming a cellular energy deficit from glucose deficiency. Autophagy 7:1187–1198

Ko YH, Pedersen PL, Geschwind JF (2001) Glucose catabolism in the rabbit VX2 tumor model for liver cancer: characterization and targeting hexokinase. Cancer Lett 173:83–91

Koopman WJ, Visch HJ, Verkaart S, Van Den Heuvel LW, Smeitink JA, Willems PH (2005) Mitochondrial network complexity and pathological decrease in complex I activity are tightly correlated in isolated human complex I deficiency. Am J Physiol Cell Physiol 289:C881–C890

Koopman WJ, Distelmaier F, Hink MA, Verkaart S, Wijers M, Fransen J, Smeitink JA, Willems PH (2008) Inherited complex I deficiency is associated with faster protein diffusion in the matrix of moving mitochondria. Am J Physiol Cell Physiol 294:C1124–C1132

Koukourakis MI, Giatromanolaki A, Sivridis E, Gatter KC, Harris AL (2005) Pyruvate dehydrogenase and pyruvate dehydrogenase kinase expression in non small cell lung cancer and tumor-associated stroma. Neoplasia 7:1–6

Koutsopoulos OS, Laine D, Osellame L, Chudakov DM, Parton RG, Frazier AE, Ryan MT (2010) Human Miltons associate with mitochondria and induce microtubule-dependent remodeling of mitochondrial networks. Biochim Biophys Acta 1803:564–574

Kumar SH, Rangarajan A (2009) Simian virus 40 small T antigen activates AMPK and triggers autophagy to protect cancer cells from nutrient deprivation. J Virol 83:8565–8574

Lan Q, Lim U, Liu CS, Weinstein SJ, Chanock S, Bonner MR, Virtamo J, Albanes D, Rothman N (2008) A prospective study of mitochondrial DNA copy number and risk of non-Hodgkin lymphoma. Blood 112:4247–4249

Lee CH, Wu SB, Hong CH, Liao WT, Wu CY, Chen GS, Wei YH, Yu HS (2011) Aberrant cell proliferation by enhanced mitochondrial biogenesis via mtTFA in arsenical skin cancers. Am J Pathol 178:2066–2076

Legros F, Lombes A, Frachon P, Rojo M (2002) Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. Mol Biol Cell 13:4343–4354

Liao LM, Baccarelli A, Shu XO, Gao YT, Ji BT, Yang G, Li HL, Hoxha M, Dioni L, Rothman N, Zheng W, Chow WH (2011) Mitochondrial DNA copy number and risk of gastric cancer: a report from the Shanghai Women's Health Study. Cancer Epidemiol Biomarkers Prev 20:1944–1949

Lu H, Forbes RA, Verma A (2002) Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. J Biol Chem 277:23111–23115

Lynch SM, Weinstein SJ, Virtamo J, Lan Q, Liu CS, Cheng WL, Rothman N, Albanes D, Stolzenberg-Solomon RZ (2011) Mitochondrial DNA copy number and pancreatic cancer in the α-tocopherol beta-carotene cancer prevention study. Cancer Prev Res 4:1912–1919

Marin-Hernandez A, Gallardo-Perez JC, Ralph SJ, Rodriguez-Enriquez S, Moreno-Sanchez R (2009) HIF-1α modulates energy metabolism in cancer cells by inducing over-expression of specific glycolytic isoforms. Mini Rev Med Chem 9:1084–1101

Mathupala SP, Rempel A, Pedersen PL (1997) Aberrant glycolytic metabolism of cancer cells: a remarkable coordination of genetic, transcriptional, post-translational, and mutational events that lead to a critical role for type II hexokinase. J Bioenerg Biomembr 29:339–343

Mazurek S (2010) Pyruvate kinase type M2: a key regulator of the metabolic budget system in tumor cells. Int J Biochem Cell Biol 43:969–980

Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 191:144–148

Mullen AR, Wheaton WW, Jin ES, Chen PH, Sullivan LB, Cheng T, Yang Y, Linehan WM, Chandel NS, Deberardinis RJ (2012) Reductive carboxylation supports growth in tumour cells with defective mitochondria. Nature 481:385–388

Ohnami S, Matsumoto N, Nakano M, Aoki K, Nagasaki K, Sugimura T, Terada M, Yoshida T (1999) Identification of genes showing differential expression in antisense K-ras-transduced pancreatic cancer cells with suppressed tumorigenicity. Cancer Res 59:5565–5571

Palmieri F, Bisaccia F, Capobianco L, Dolce V, Fiermonte G, Iacobazzi V, Indiveri C, Palmieri L (1996) Mitochondrial metabolite transporters. Biochim Biophys Acta 1275:127–132

Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 3:187–197

Park JS, Sharma LK, Li H, Xiang R, Holstein D, Wu J, Lechleiter J, Naylor SL, Deng JJ, Lu J, Bai Y (2009) A heteroplasmic, not homoplasmic, mitochondrial DNA mutation promotes tumorigenesis via alteration in reactive oxygen species generation and apoptosis. Hum Mol Genet 18:1578–1589

Pedersen PL, Greenawalt JW, Chan TL, Morris HP (1970) A comparison of some ultrastructural and biochemical properties of mitochondria from Morris hepatomas 9618A, 7800, and 3924A. Cancer Res 30:2620–2626

Pelicano H, Xu RH, Du M, Feng L, Sasaki R, Carew JS, Hu Y, Ramdas L, Hu L, Keating MJ, Zhang W, Plunkett W, Huang P (2006) Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism. J Cell Biol 175:913–923

Pervaiz S, Holme AL (2009) Resveratrol: its biologic targets and functional activity. Antioxid Redox Signal 11:2851–2897

Petros JA, Baumann AK, Ruiz-Pesini E, Amin MB, Sun CQ, Hall J, Lim S, Issa MM, Flanders WD, Hosseini SH, Marshall FF, Wallace DC (2005) mtDNA mutations increase tumorigenicity in prostate cancer. Proc Natl Acad Sci U S A 102:719–724

Plecita-Hlavata L, Lessard M, Santorova J, Bewersdorf J, Jezek P (2008) Mitochondrial oxidative phosphorylation and energetic status are reflected by morphology of mitochondrial network in INS-1E and HEP-G2 cells viewed by 4Pi microscopy. Biochim Biophys Acta 1777:834–846

Pollard PJ, Briere JJ, Alam NA, Barwell J, Barclay E, Wortham NC, Hunt T, Mitchell M, Olpin S, Moat SJ, Hargreaves IP, Heales SJ, Chung YL, Griffiths JR, Dalgleish A, Mcgrath JA, Gleeson

MJ, Hodgson SV, Poulsom R, Rustin P, Tomlinson IP (2005) Accumulation of Krebs cycle intermediates and over-expression of HIF1α in tumours which result from germline FH and SDH mutations. Hum Mol Genet 14:2231–2239

Reitzer L, Wice B, Kennel D (1979) Evidence that glutamine, not sugar, is the major energy source for cultured Hela cells. J Biol Chem 254:2669–2676

Rodriguez-Enriquez S, Juarez O, Rodriguez-Zavala JS, Moreno-Sanchez R (2001) Multisite control of the Crabtree effect in ascites hepatoma cells. Eur J Biochem 268:2512–2519

Rossignol R, Malgat M, Mazat JP, Letellier T (1999) Threshold effect and tissue specificity. Implication for mitochondrial cytopathies. J Biol Chem 274:33426–33432

Rossignol R, Letellier T, Malgat M, Rocher C, Mazat JP (2000) Tissular variation in the control of oxidative phosphorylations, implication for mitochondrial diseases. Biochem J 347:45–53

Rossignol R, Faustin B, Rocher C, Malgat M, Mazat JP, Letellier T (2003) Mitochondrial threshold effects. Biochem J 370:751–762

Rossignol R, Gilkerson R, Aggeler R, Yamagata K, Remington SJ, Capaldi RA (2004) Energy substrate modulates mitochondrial structure and oxidative capacity in cancer cells. Cancer Res 64:985–993

Savagner F, Mirebeau D, Jacques C, Guyetant S, Morgan C, Franc B, Reynier P, Malthiery Y (2003) PGC-1-related coactivator and targets are upregulated in thyroid oncocytoma. Biochem Biophys Res Commun 310:779–784

Schulz TJ, Thierbach R, Voigt A, Drewes G, Mietzner B, Steinberg P, Pfeiffer AF, Ristow M (2006) Induction of oxidative metabolism by mitochondrial frataxin inhibits cancer growth: Otto Warburg revisited. J Biol Chem 281:977–981

Semenza GL (2007) Oxygen-dependent regulation of mitochondrial respiration by hypoxia-inducible factor 1. Biochem J 405:1–9

Shaw RJ (2006) Glucose metabolism and cancer. Curr Opin Cell Biol 18:598–608

Shen J, Platek M, Mahasneh A, Ambrosone CB, Zhao H (2010) Mitochondrial copy number and risk of breast cancer: a pilot study. Mitochondrion 10:62–68

Sherr CJ, Depinho RA (2000) Cellular senescence: mitotic clock or culture shock? Cell 102:407–410

Simonnet H, Alazard N, Pfeiffer K, Gallou C, Beroud C, Demont J, Bouvier R, Schagger H, Godinot C (2002) Low mitochondrial respiratory chain content correlates with tumor aggressiveness in renal cell carcinoma. Carcinogenesis 23:759–768

Simonnet H, Demont J, Pfeiffer K, Guenaneche L, Bouvier R, Brandt U, Schagger H, Godinot C (2003) Mitochondrial complex I is deficient in renal oncocytomas. Carcinogenesis 24(9): 1461–1466. Epub 4 Jul 2003

Smolkova K, Bellance N, Scandurra F, Genot E, Gnaiger E, Plecita-Hlavata L, Jezek P, Rossignol R (2010) Mitochondrial bioenergetic adaptations of breast cancer cells to aglycemia and hypoxia. J Bioenerg Biomembr 42:55–67

Smolkova K, Plecita-Hlavata L, Bellance N, Benard G, Rossignol R, Jezek P (2011) Waves of gene regulation suppress and then restore oxidative phosphorylation in cancer cells. Int J Biochem Cell Biol 43:950–968

Suzuki T, Murakami T, Iino R, Suzuki J, Ono S, Shirakihara Y, Yoshida M (2003) F0F1-ATPase/synthase is geared to the synthesis mode by conformational rearrangement of epsilon subunit in response to proton motive force and ADP/ATP balance. J Biol Chem 278:46840–46846

Sweeney G, Somwar R, Ramlal T, Volchuk A, Ueyama A, Klip A (1999) An inhibitor of p38 mitogen-activated protein kinase prevents insulin-stimulated glucose transport but not glucose transporter translocation in 3T3-L1 adipocytes and L6 myotubes. J Biol Chem 274:10071–10078

Tan C (2011) The cell biology of the mitochondrial protein IF1, University College London

Vaishnavi SN, Vlassenko AG, Rundle MM, Snyder AZ, Mintun MA, Raichle ME (2010) Regional aerobic glycolysis in the human brain. Proc Natl Acad Sci U S A 107:17757–17762

Vanhaesebroeck B, Alessi DR (2000) The PI3K-PDK1 connection: more than just a road to PKB. Biochem J 346:561–576

Vaughn AE, Deshmukh M (2008) Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome c. Nat Cell Biol 10:1477–1483

Vaupel P (2008) Hypoxia and aggressive tumor phenotype: implications for therapy and prognosis. Oncologist 13:S21–S26

Vaupel P, Mayer A (2005) Effects of anemia and hypoxia on tumor biology. In: Bokemeyer C, Ludwig H (eds) Anemia in cancer. European school of oncology scientific updates. Elsevier, Philadelphia, pp 47–54

Vaupel P, Mayer A, Briest S, Hockel M (2003) Oxygenation gain factor: a novel parameter characterizing the association between hemoglobin level and the oxygenation status of breast cancers. Cancer Res 63:7634–7637

Vaupel P, Hockel M, Mayer A (2007) Detection and characterization of tumor hypoxia using pO2 histography. Antioxid Redox Signal 9:1221–1235

Vlashi E, Lagadec C, Vergnes L, Matsutani T, Masui K, Poulou M, Popescu R, Della Donna L, Evers P, Dekmezian C, Reue K, Christofk H, Mischel PS, Pajonk F (2011) Metabolic state of glioma stem cells and nontumorigenic cells. Proc Natl Acad Sci U S A 108:16062–16067

Wang X, Moraes CT (2011) Increases in mitochondrial biogenesis impair carcinogenesis at multiple levels. Mol Oncol 5:399–409

Wegrzyn J, Potla R, Chwae YJ, Sepuri NB, Zhang Q, Koeck T, Derecka M, Szczepanek K, Szelag M, Gornicka A, Moh A, Moghaddas S, Chen Q, Bobbili S, Cichy J, Dulak J, Baker DP, Wolfman A, Stuehr D, Hassan MO, Fu XY, Avadhani N, Drake JI, Fawcett P, Lesnefsky EJ, Larner AC (2009) Function of mitochondrial Stat3 in cellular respiration. Science 323:793–797

Weinberg F, Hamanaka R, Wheaton WW, Weinberg S, Joseph J, Lopez M, Kalyanaraman B, Mutlu GM, Budinger GR, Chandel NS (2010) Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. Proc Natl Acad Sci U S A 107:8788–8793

Weinhouse S (1951) Studies on the fate of isotopically labeled metabolites in the oxidative metabolism of tumors. Cancer Res 11:585–591

Wigfield SM, Winter SC, Giatromanolaki A, Taylor J, Koukourakis ML, Harris AL (2008) PDK-1 regulates lactate production in hypoxia and is associated with poor prognosis in head and neck squamous cancer. Br J Cancer 98:1975–1984

Wise DR, Deberardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, Mcmahon SB, Thompson CB (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci U S A 105:18782–18787

Yaffe MP (1999) Dynamic mitochondria. Nat Cell Biol 1:E149–E150

Yang C, Sudderth J, Dang T, Bachoo RM, Mcdonald JG, Deberardinis RJ (2009) Glioblastoma cells require glutamate dehydrogenase to survive impairments of glucose metabolism or Akt signaling. Cancer Res 69:7986–7993

Yun J, Rago C, Cheong I, Pagliarini R, Angenendt P, Rajagopalan H, Schmidt K, Willson JK, Markowitz S, Zhou S, Diaz LA Jr, Velculescu VE, Lengauer C, Kinzler KW, Vogelstein B, Papadopoulos N (2009) Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells. Science 325:1555–1559

Chapter 12
Crosstalk Between p53 and Mitochondrial Metabolism

Koji Itahana and Shazib Pervaiz

Contents

Abstract After being obtained from bacteria, eukaryotic mitochondria acquired a myriad of metabolic functions during evolution to coordinate energy efficiency and demand with host cells during cell proliferation and growth arrest for maintaining cellular homeostasis as well as functions in decisions of cell death and survival. To achieve this, mitochondria and host cells have developed tight communications, and recent evidence suggests that tumour suppressor p53 actively participates in

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these communications. p53 influences mitochondrial metabolism by activating or repressing the transcription of target genes as well as directly interacting with proteins in different cellular compartments, including mitochondria. This review discusses recent findings of p53-mediated regulation of cellular metabolism, such as oxidative phosphorylation, glutamine and fatty acid metabolism, autophagy, glycolysis, and reactive oxygen species, to better understand the tumour suppressive functions of p53, which may facilitate the identification of novel therapeutic targets and strategies.

Keywords p53 • Mitochondria • Metabolism • Oxidative phosphorylation • Glycolysis • Glutamine • Fatty acids • Autophagy • Reactive oxygen species • Cancer • Tumor suppressor • ARF

1 Introduction

TP53 is a well-known tumour suppressor gene that is mutated in more than 50 % of cancer types. p53 is a transcription factor whose activation in response to stress triggers numerous genes required for protection against cancer. These transcriptional targets of p53 control important tumour suppressor pathways, such as apoptosis, cell cycle arrest, senescence, and DNA repair to maintain cellular genomic integrity and to avoid unwanted proliferation of cells with unrepaired damaged DNA. Because of this, p53 has been described as 'the guardian of the genome'. Historically, the study of p53 has focused on functions that determine cell fate, such as cell cycle arrest, senescence, and apoptosis in response to DNA damage and oncogenic insults. However, recent evidence suggests that p53 impacts a wide variety of metabolic processes for maintaining cellular homeostasis, and to that end there is substantial evidence to implicate p53 in the maintenance of mitochondrial metabolism as well as the effect of mitochondrial metabolism on regulating p53 biology. This review focuses on recent findings regarding the regulation of p53 in mitochondrial metabolism, including oxidative phosphorylation, glutamine and fatty acid metabolism, and reactive oxygen species (ROS), in addition to other cellular metabolism functions, such as autophagy and glycolysis. Importantly, these functions are closely connected to one other to maintain cellular homeostasis.

2 p53 and Oxidative Phosphorylation

In eukaryotes, mitochondria are the site of oxidative phosphorylation for the generation of the cellular energy source, ATP. Using a proton gradient generated by the electron transport chain, ATP is synthesized by the ATP synthase FoF1 complex at the inner mitochondrial membrane. In normal human cells, glucose is the primary energy source, and mitochondria efficiently yield 32–34 ATP molecules during the aerobic oxidation of one glucose molecule. Therefore, it is important for cells to

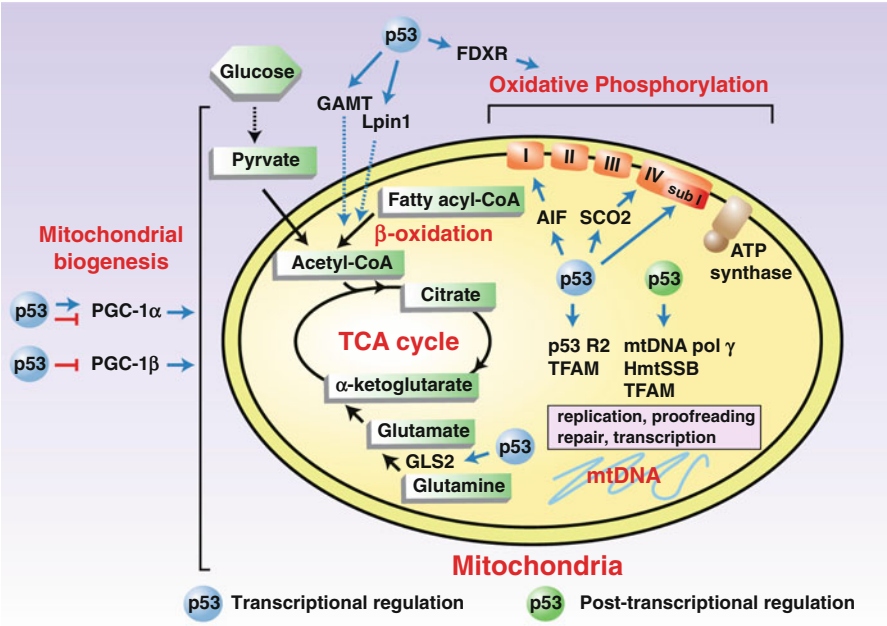

Fig. 12.1 p53 Regulates oxidative phosphorylation, glutamine metabolism and fatty acid oxidation. p53 enhances mitochondrial functions by inducing genes involved in oxidative phosphorylation, mtDNA integrity, and mitochondrial biogenesis (as shown by p53 in *blue*) as well as directly interacting with proteins involved in mtDNA integrity (as shown by p53 in *green*). p53 also affects glutamine metabolism and fatty acid oxidation by inducing target genes involved in these metabolic pathways (as shown by p53 in *blue*)

maintain healthy mitochondria for proper oxidative phosphorylation. Recent evidence suggests that p53 positively regulates oxidative phosphorylation either directly or indirectly (Fig. 12.1). Mouse embryos that lack p53 display weakened cytochrome c oxidase (Complex IV) staining and reduced ATP content (Ibrahim et al. 1998). Mitochondrial Complex IV activity is decreased in HCT116 ($p53^{-/-}$) cells compared to HCT116 ($p53^{+/+}$) cells (Zhou et al. 2003). Skeletal muscle from *p53* knockout (KO) mice shows diminished mitochondrial content and levels of proliferator-activated receptor-gamma co-activator (PGC)-1α which stimulates mitochondrial biogenesis (Saleem et al. 2009). p53 has been shown to translocate to the mitochondria during apoptosis (Marchenko et al. 2000), and several lines of evidence suggest that the presence of p53 in mitochondria also contributes to mitochondrial health and activity. p53 in mitochondria maintains mitochondrial genetic stability through binding to mtDNA polymerase gamma to enhance its function in mitochondrial DNA replication (Achanta et al. 2005), repair (Nithipongvanitch et al. 2007), and proofreading during replication (Bakhanashvili et al. 2009). Mitochondrial p53 also participates in the incorporation step (de Souza-Pinto et al. 2004) and the glycosylase

step of the base excision repair of mitochondrial DNA (Chen et al. 2006). In addition, the interaction between p53 and mitochondrial single-strand binding protein (HmtSSB) increases the p53 3′→5′ exonuclease activity on mitochondrial DNA (Wong et al. 2009). p53 also interacts with mitochondrial transcription factor A (TFAM), which is necessary for both transcription and maintenance of the mitochondrial DNA (Yoshida et al. 2003). These data show the important role of mitochondrial p53 in maintaining mitochondrial integrity; however, it is not clear how p53 enters the mitochondrial matrix via the inner mitochondrial membrane without having obvious mitochondrial targeting sequences.

As a transcription factor in the nucleus, p53 plays an important role in enhancing oxidative phosphorylation and maintaining mitochondrial integrity. For example, p53 directly activates the transcription of *cytochrome c oxidase (SCO2)* gene, which is required for the assembly of Complex IV (Matoba et al. 2006). Indeed, decreased oxidative phosphorylation in *p53* null cells was rescued by physiological levels of SCO2 expression (Matoba et al. 2006; Ma et al. 2007). Several other p53 target genes promoting oxidative phosphorylation have been also reported. For example, mutations of *RRM2B* encoding p53-controlled ribonucleotide reductase (p53R2), identified as a direct p53 target gene (Tanaka et al. 2000), cause mitochondrial DNA depletion (Bourdon et al. 2007). Furthermore, the loss of p53 results in the reduction of p53R2, mtDNA copy number, and mitochondrial mass (Kulawiec et al. 2009; Lebedeva et al. 2009). p53 also directly activates the transcription of *TFAM* gene to increase mitochondrial DNA content (Park et al. 2009a). There are other p53 target genes that encode mitochondrial proteins, but it is unclear whether these targets are involved in p53-mediated regulation of oxidative phosphorylation. For example, *apoptosis inducing factor (AIF)* has been reported as a p53 target gene (Stambolsky et al. 2006). Although AIF is a well-known apoptosis regulator that translocates to the nucleus during apoptosis and participates in apoptotic chromatinolysis, AIF is also essential for mitochondrial complex I function, and *AIF* deficiency compromises oxidative phosphorylation (Vahsen et al. 2004). p53 also targets the transcription of *ferrodoxin reductase (FDXR)* gene (Hwang et al. 2001), a mitochondrial NADPH P450 reductase gene required for iron-sulfur protein and haem biogenesis, both of which are essential for oxidative phosphorylation. There is also evidence, albeit indirect, that p53 induces the transcription of a gene encoding subunit I of Complex IV (Okamura et al. 1999).

Despite substantial evidence indicating the positive effect of p53 on oxidative phosphorylation and mitochondrial integrity, this concept has been challenged by studies in telomerase knockout mice (Sahin et al. 2011). Telomere dysfunction in these mice induces p53 activation, leading to many features suggestive of aging, mitochondrial dysfunction, and reduced oxidative phosphorylation. These changes were found to be the result of p53-mediated repression of the master positive regulator genes of mitochondrial biogenesis, *PGC-1n and PGC-1β* (Sahin et al. 2011). These data suggest that p53-regulated genes affecting oxidative phosphorylation and mitochondrial integrity may be highly cell type specific. It is also noteworthy that reduced oxidative phosphorylation in p53 null cells is easily compensated for by

glycolysis to maintain the overall cellular levels of ATP (Matoba et al. 2006). The physiological impact of the loss of p53 activity on oxidative phosphorylation and mitochondrial integrity in different tissue types remains to be elucidated.

3 p53 and Glutamine and Fatty Acid Metabolism

Although glucose is the major energy source for most cell types, including cancer cells, cells can also use fatty acids and amino acids such as glutamine as fuel for oxidative phosphorylation. Glutamine circulates in the blood as the most abundant amino acid and functions as a nitrogen donor and bioenergetic substrate. Highly proliferative cells, including cancer cells, require additional supplies of biosynthetic precursors like glutamine for glucose-independent metabolic processes (Le et al. 2012). For example, the oncoprotein Myc, which is frequently overexpressed in cancer cells, activates genes involved in mitochondrial biogenesis and glutamine metabolism (Gao et al. 2009). Interestingly, p53 is also involved in enhancing glutamine metabolism (Fig. 12.1). For example, p53 has also been shown to directly induce the *glutaminase 2 (GLS2)* gene, which encodes a mitochondrial glutaminase catalysing the hydrolysis of glutamine to glutamate to enhance glutamine metabolism (Hu et al. 2010; Suzuki et al. 2010). The induction of GLS2 by p53 increases the production of α-ketoglutarate, a substrate for the TCA cycle, and enhances oxidative phosphorylation (Hu et al. 2010).

Fatty acids are also important metabolites for oxidative phosphorylation. In addition to their contribution to lipid synthesis and protein modification, fatty acids serve as an energy source by being metabolized in the mitochondria by β-oxidation to generate acetyl-CoA, the entry molecule for the TCA cycle. p53 also participates in enhancing fatty acids metabolism (Fig. 12.1). For example, the AMP-activated protein kinase (AMPK) activator AICAR, which mimics starvation signals, enhances fatty acid β-oxidation in $p53^{+/+}$ mouse embryonic fibroblasts (MEFs), but not in $p53^{-/-}$ MEFs (Buzzai et al. 2007). Recently, *guanidinoacetate methyltransferase (GAMT)*, a gene encoding enzyme involved in creatine synthesis, has been reported as a p53 target gene (Ide et al. 2009). In response to glucose starvation, p53-dependent induction of GMAT increases fatty acid oxidation by increasing the levels of creatine, which can stimulate AMPK phosphorylation and activation (Ide et al. 2009). These data are consistent with studies showing that AMPK activates fatty acid oxidation (Hardie and Pan 2002), and AMPK-induces p53 activation promotes cellular survival in response to glucose deprivation (Jones et al. 2005). Another p53 target gene involved in fatty acid oxidation is *Lpin1*, which is essential for adipocyte development and fat metabolism (Assaily et al. 2011). Indeed, p53 induces fatty acid oxidation during glucose deprivation through the induction of Lpin1 (Assaily et al. 2011). These data suggest that, in response to glucose shortages, p53 is able to induce several genes that contribute to the usage of alternative energy sources, such as glutamine and fatty acids, to maintain cellular energy homeostasis.

4 p53 and Autophagy

Although p53 regulates genes involved in the use of alternative fuels for maintaining cellular homeostasis, what happens when all available fuel sources become limited? As a last resort in these settings, cells will begin breaking down their own components to use as energy. This step has been termed autophagy and is a controlled lysosomal catabolic process that degrades proteins and organelles. Autophagy also functions as a tumour suppressive system to digest damaged organelles, such as mitochondria, to maintain cellular homeostasis (Mizushima and Komatsu 2011). Although it is plausible to speculate that p53 promotes autophagy to remove damaged organelles in normal circumstances as a tumour suppressor as well as to provide extra energy during starvation, evidence suggests that autophagy regulation by p53 is complex (Fig. 12.2).

Indeed, p53 is capable of inducing and inhibiting autophagy depending on the cellular context and extrinsic stress. It was first reported that p53 enhances autophagy by inhibiting mTOR pathways through the activation of AMPK (Feng et al. 2005), and AMPK was subsequently confirmed as a p53 target gene product (Feng et al. 2007).

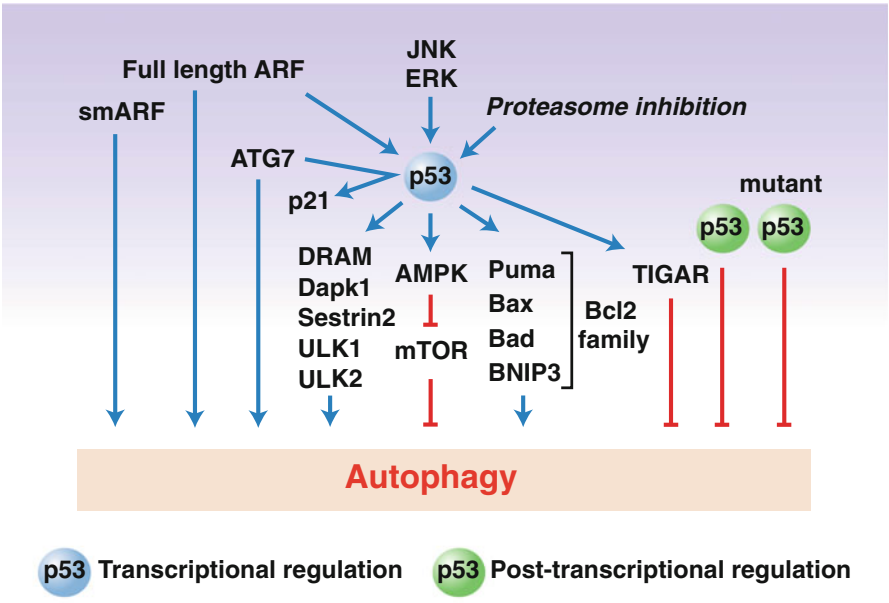

Fig. 12.2 Regulation of autophagy by p53. The regulation of autophagy by p53 is complex. p53 enhances autophagy by inducing the transcription of a number of autophagy related genes (as shown by p53 in *blue*) while cytoplasmic p53 (as shown by p53 in *green*) inhibits autophagy. ARF, an upstream regulator of p53, also enhances autophagy, and ATG7, a core autophagy regulator, induces p21 via activating p53 through physical interaction, leading to cell survival during nutrient deprivation

p53 also induces autophagy by activating the transcription of *DRAM* gene, which encodes a lysosomal protein (Crighton et al. 2006). Other p53 target genes that mediate the induction of autophagy include *sestrin-2* (Budanov and Karin 2008; Maiuri et al. 2009), autophagy-initiation kinases *ULK1* and *ULK2* (Gao et al. 2011), *Dapk1* (Martoriati et al. 2005; Harrison et al. 2008), several well-known pro-apoptotic Bcl-2 family genes, such as *Puma* (Yee et al. 2009), *Bax* (Yee et al. 2009), and *Bad* (Maiuri et al. 2007), in addition to *BNIP3* (Fei et al. 2004), whose product induces selective mitochondrial autophagy by competing with Beclin 1 for binding to Bcl2, thereby releasing Beclin 1 to trigger autophagy (Zhang et al. 2008). Furthermore, mitogen-activated protein kinase (MAPK) family proteins, such as extracellular signal-related kinase (ERK) and c-Jun N-terminal kinase (JNK), induce autophagy through p53 activation (Cheng et al. 2008; Park et al. 2009b). In addition, proteasome inhibition also induces autophagy in a p53-dependent manner (Du et al. 2009). In a *p53*-deficient Myc-induced lymphoma mouse model, the restoration of p53 using a switchable p53ER system results in the induction of autophagy, supporting the idea that p53 induces autophagy *in vivo* (Amaravadi et al. 2007). The p53 family member, p73, also induces autophagy (Crighton et al. 2007), and several p73 targets involved in autophagy (ATG5, ATG7, and UVRAG) have been reported (Rosenbluth and Pietenpol 2009).

Recently, the tumour suppressor alternative reading frame protein (ARF), which is an upstream positive regulator of p53, has also been reported to induce autophagy. Full-length ARF is localized in both the nucleolus and mitochondria (Itahana et al. 2008; Itahana and Zhang 2008, 2010; Pimkina et al. 2009; Irvine et al. 2010), while the short form of ARF (smARF) is localized primarily in mitochondria (Reef et al. 2006). Whereas smARF induces autophagy in a p53-independent manner (Reef et al. 2006), full-length ARF can induce autophagy in both a p53-dependent and -independent manner (Abida and Gu 2008; Pimkina et al. 2009). The impact of ARF on mitochondrial metabolism is still largely unknown.

The concept that p53 enhances autophagy was recently challenged by studies on cytoplasmic p53 (Tasdemir et al. 2008a). Either depletion or inhibition of p53 induced autophagy in human, mouse, and nematode cells, and cytoplasmic, but not nuclear, p53 was able to repress the enhanced autophagy in *p53* null cells (Tasdemir et al. 2008a). p53-mediated inhibition of autophagy occurs in the G0/G1 phase (Tasdemir et al. 2008b), and hot-spot DNA binding mutants of p53 in the cytoplasm also inhibit autophagy (Morselli et al. 2008). In *C. elegans*, deficiency of the p53 orthologue gene *CEP-1* results in lifespan extension, which depended on the ability of CEP1 to inhibit autophagy (Tavernarakis et al. 2008). In some cases, nuclear p53 also represses autophagy. For example, *TP53-induced glycolysis and apoptosis regulator (TIGAR)*, a p53 target gene, whose product decreases ROS production upon nutrient deprivation, thereby inhibiting autophagy (Bensaad et al. 2009). These data suggest that, depending on the cell context, p53 could engage or inhibit autophagy, however the description of these contextual differences remains to be clarified. Despite that fact, recent data indicate that both of these p53-mediated effects contribute to cell survival after nutrient deprivation (Scherz-Shouval et al. 2010), suggesting that p53 maintains better autophagic homeostasis by adjusting the rate of autophagy under limited nutrient

supply (Scherz-Shouval et al. 2010). Reciprocally, it has been recently shown that autophagy gene product ATG7 regulates p53 to induce cell cycle arrest through induction of p21 leading to cell survival during nutrient deprivation (Lee et al. 2012), further supporting the important role of p53 in autophagic homeostasis.

5 p53 and Glycolysis

As eluded to earlier, glucose is the major cellular energy source. After entering the cell, glucose undergoes glycolysis, the sequence of reactions that converts glucose into pyruvate in the cytoplasm with the concomitant production of 2 ATP molecules per molecule of glucose. When oxygen is available, the pyruvate is further and completely oxidized in the mitochondria, generating 32–34 ATP per glucose through the citric acid cycle and oxidative phosphorylation. Therefore, there is an intricate interplay between oxidative phosphorylation and glycolysis with evidence of reciprocal reinforcement. When oxygen is limited, pyruvate is instead converted into lactate in the cytoplasm via fermentation by the enzyme lactate dehydrogenase. Although oxidative phosphorylation produces greater ATP numbers than glycolysis, glycolysis provides metabolic intermediates in biosynthetic reactions and can be initiated more quickly leading to faster ATP production. Interestingly, the majority of cancer cells tend to acquire ATP using the more rapid glycolysis method even in the presence of ample oxygen levels to meet their high energy demands. This phenomenon is known as the 'Warburg effect'. Although cancer cells do use oxidative phosphorylation, enhanced aerobic glycolysis has been observed in most types of cancers and is recognized as a hallmark of cancer.

Although p53 enhances mitochondrial DNA integrity, mitochondrial biogenesis, and oxidative phosphorylation to contribute to mitochondrial ATP generation in many cases, recent evidence suggests that p53 inhibits glycolysis (Fig. 12.3). Indeed, this property fits well with the p53 tumour-suppressive function against the enhanced glycolytic phenotype in cancer. p53 induces TIGAR, an enzyme that dephosphorylates either fructose-1,6-bisphosphate or fructose-2,6-bisphosphate into fructose-6-phosphate (Bensaad et al. 2006). This TIGAR-mediated activity inhibits glycolysis by counteracting the step where fructose-6-bisphosphate is phosphorylated into fructose-1,6-bisphosphate by phosphofructokinase (PFK). Therefore, TIGAR blocks glycolysis at the fructose-6-phosphate stage to push glucose catabolism toward the pentose phosphate pathway (PPP) (Bensaad et al. 2006), which generates metabolites used for the synthesis of nucleotides, nucleic acids, fatty acids, and aromatic amino acids. Although TIGAR can enhance the PPP pathway, it has recently been shown that *p53*$^{-/-}$ MEFs have enhanced PPP glucose flux with higher glucose consumption compared to wild-type MEFs (Jiang et al. 2011), suggesting that there are other p53 targets that regulate PPP. Indeed, it has been shown that cytoplasmic p53 inhibits the PPP pathway by binding to and preventing the formation of the active dimer of glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the PPP (Jiang et al. 2011).

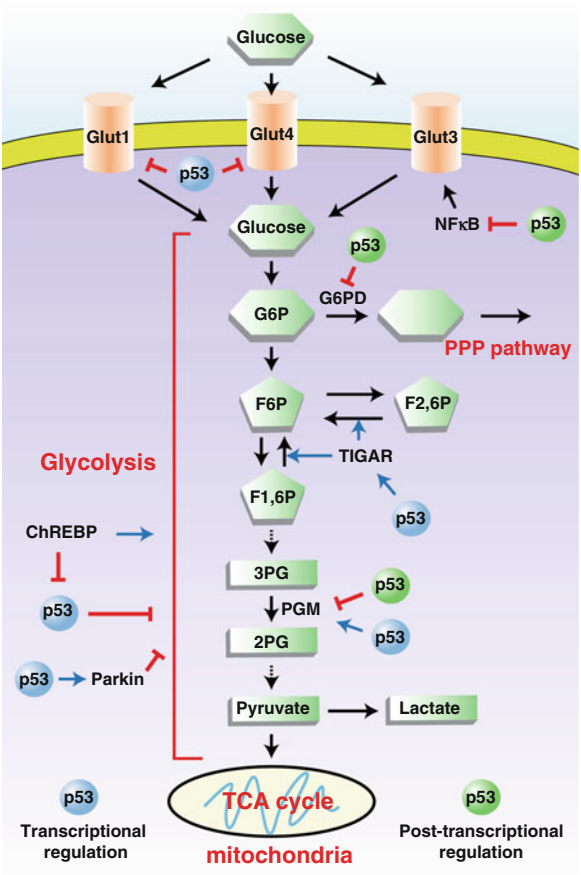

Fig. 12.3 Regulation of glycolysis by p53. p53 inhibits glycolysis by either repressing the transcription of glucose transporters directly or indirectly or by modulating the activity of the particular glycolysis step via transcription-dependent (as shown by p53 in *blue*) or transcription-independent manner (as shown by p53 in *green*)

p53 also stimulates the transcription of the Parkinson's disease-associated gene, *Parkin (PARK2)*, and its deficiency activates glycolysis and reduces mitochondrial respiration (Zhang et al. 2011). Furthermore, p53 can suppress glycolysis by directly repressing the transcription of glucose transporter gene, *Glut1* and *Glut4* (Schwartzenberg-Bar-Yoseph et al. 2004), by indirectly down-regulating *Glut3* by inhibiting NF-κB (Kawauchi et al. 2008a, b), by repressing the insulin receptor promoter (Webster et al. 1996), or by down-regulating the glycolytic enzyme, phosphoglycerate mutase (PGM), in manner that is independent of p53 transcriptional activity (Kondoh et al. 2005). Reciprocally, the glucose-responsive transcription factor, carbohydrate responsive element binding protein (ChREBP), enhances aerobic glycolysis via partially suppressing the ability of p53 to inhibit glycolysis (Tong et al. 2009). Although much evidence suggests that p53 reduces glycolysis, there are other cases where p53 activates transcription of glycolytic enzyme genes. For example, p53 directly transactivates the transcription of *PGM* gene in cardiac myocytes

(Ruiz-Lozano et al. 1999), and mutant p53 activates transcription of *hexokinase II* gene (Mathupala et al. 1997); however, it is not yet clear whether the p53-mediated induction of these proteins translates into changes in glycolysis.

6 p53 and Reactive Oxygen Species

Mitochondria are the primary source of ROS within a cell. Although excessive ROS are capable of damaging DNA, proteins, and lipids, and initiating apoptosis, ROS also play roles in important cellular process, such as functioning as second messengers for cell signalling and mediating protein modifications. Recent evidence suggests that p53 has both an antioxidant role, to protect cells from ROS, and a pro-oxidant role, to enhance apoptosis when stress reaches maximum levels (Maillet and Pervaiz 2012) (Fig. 12.4). Conversely, ROS can also directly impact p53 activity (Fig. 12.4). For

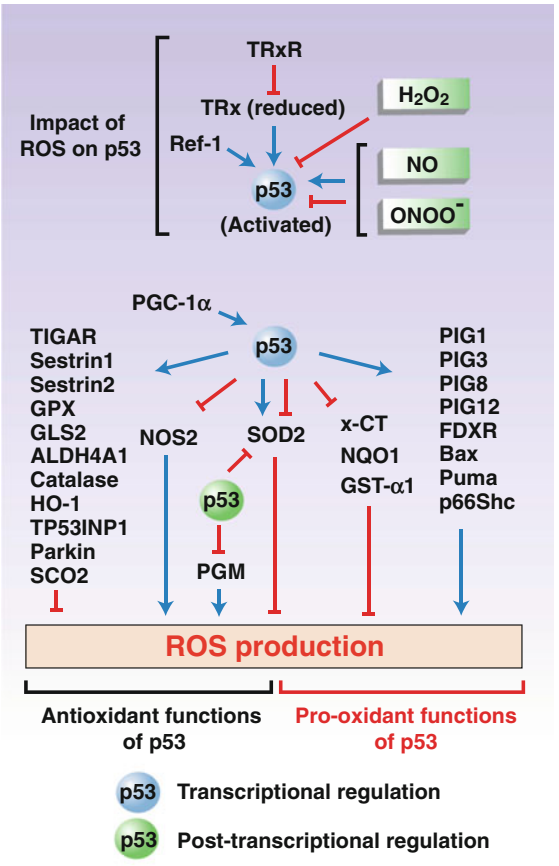

Fig. 12.4 Impact of oxidation on p53 and regulation of ROS by p53. *Upper panel*: oxidation of p53 by ROS results in p53 inactivation, which is reversed by antioxidants. *Lower panel*: basal p53 levels in the absence of stress primarily maintain antioxidant enzymes and repress pro-oxidant enzymes to protect cells from oxidative stress, while p53 can promote pro- or antioxidant functions depending on the cellular context when the cells experience significant stress. Transcriptional regulation of p53 (as shown by p53 in *blue*) and post-transcriptional regulation of p53 (as shown by p53 in *green*) are indicated

example, in general, excess ROS reduces the DNA binding activity of p53 (Hainaut and Milner 1993; Russo et al. 1995) by posttranslational modifications, such as glutathionylation of cysteine residues (Velu et al. 2007). On the other hand, p53 oxidation is reversed, and p53 is activated by antioxidant proteins, such as Redox factor-1 (Ref-1) (Jayaraman et al. 1997) or thioredoxin (TRx), which reduce oxidized cysteine groups on proteins (Ueno et al. 1999; Ravi et al. 2005). Consistent with this, thioredoxin reductase (TrxR), which converts TRx to its oxidized form, reduces p53 activity (Moos et al. 2003; Cassidy et al. 2006). Another p53-mediated modification is tyrosine nitration by nitric oxide (NO) and peroxynitrite (ONOO-) produced by NO and $O2^{\cdot -}$. These ROS have been reported to either inactivate (Cobbs et al. 2003) or activate p53 (Yakovlev et al. 2010), and this dual functionality has been explained by studies showing that lower concentrations will activate p53 whereas higher concentrations will inactivate p53 (Calmels et al. 1997). Although the cysteine nitration of p53 has not yet been reported, the cysteine nitration of MDM2, a critical negative regulator of p53 by acting as a ubiquitin E3 ligase, has been reported to inhibit MDM2-p53 binding (Schonhoff et al. 2002).

Although ROS modulate p53 functions, p53 also modulates ROS through several distinct mechanisms. For example, it has been shown that basal levels of p53 are sufficient for the up-regulation of several antioxidant genes, and the depletion of endogenous p53 results in DNA oxidation and genomic instability, which can be rescued by the addition of antioxidant n-acetyl cysteine (NAC) (Sablina et al. 2005). Furthermore, p53 null mice exhibit increased oxidative damage to DNA, leading to genomic instability (Griffiths et al. 1997; Bishop et al. 2003). Several lines of evidence suggest that p53 directly transactivates genes coding various antioxidant enzymes. Some of these enzymes include glutathione peroxidase (GPX), which scavenges hydrogen peroxide or organic hydroperoxide (Tan et al. 1999), aldehyde dehydrogenase 4 family member A1 (ALDH4A1), a mitochondrial-matrix NAD+-dependent enzyme suggested to inhibit ROS produced by proline metabolism (Yoon et al. 2004), and catalase, the enzyme responsible for converting hydrogen peroxide into water and oxygen. p53 knockdown also results in a decrease in catalase and an increase in intracellular ROS *in vivo* (O'Connor et al. 2008). Other transcriptional target genes of p53 involved in antioxidant activity include *sestrin1* and *sestrin2*, which are essential for the regeneration of over-oxidized peroxiredoxins (Budanov and Karin 2008) that are involved in the decomposition of hydrogen peroxide (Finkel 2003), *tumour protein p53-inducible nuclear protein 1 (TP53INP1)* (Cano et al. 2009), and *Parkin* (Zhang et al. 2011), whose product inhibits glycolysis. p53 is able to inhibit NO production by repressing a gene of *the inducible nitric oxide synthase (iNOS)*, *NOS2* (Forrester et al. 1996), and higher levels of NOS2 have been observed in *p53* null mice (Ambs et al. 1998). p53 also induces antioxidant defence by regulating glycolysis. As mentioned above, p53 induces the transactivation of *TIGAR* gene, whose product enhances the PPP and promote the production of NADPH (Bensaad et al. 2006). NADPH is a hydride (hydrogen anion) donor in a variety of enzymatic processes and acts as an antioxidant to reduce oxidized glutathione for ROS scavenging. Thus, TIGAR decreases ROS levels (Bensaad et al. 2009). Similarly, the repression of the glycolysis enzyme gene, *PGM*, by p53

(Kondoh et al. 2005) also contributes to an increase in the PPP. On the other hand, another report shows that p53 reduces the production of NADPH from the PPP pathway by binding to and inhibiting G6PD (Jiang et al. 2011). Thus, the impact on ROS by the inhibition of G6PD remains to be determined. The ability of p53 to transactivate *SCO2* may also contribute to antioxidant activity, as human cell lines with the *SCO2* gene homozygously disrupted, shows increased intracellular oxygen and nicotinamide adenine dinucleotide (NADH) levels, which results in increased ROS and oxidative DNA damage (Sung et al. 2010).

In response to stress, p53 induces senescence and apoptosis depending on the cellular context. Indeed, p53 is capable of enhancing ROS when oxidative stress reaches critical levels (Sablina et al. 2005). An increase of ROS levels during stress plays an important role in enhancing p53-dependent senescence and apoptosis (Macip et al. 2003), and even necrosis via induction of *cathepsin Q* gene by p53 (Tu et al. 2009). ROS induction is known to contribute to apoptosis (Johnson et al. 1996), and a set of p53-induced genes (PIG) that impact ROS production during apoptosis have been identified (Polyak et al. 1997). These gene products include pro-oxidant enzymes, such as p53 induced gene 6 (proline oxidase 1) (Donald et al. 2001; Rivera and Maxwell 2005), a mitochondrial protein that catalyses the first step in proline degradation and is required for p53-mediated apoptosis via the calcium-dependent serine-threonine phosphatase, calcineurin. Other gene products include p53-induced gene 3 (TP53I3/PIG3) (Flatt et al. 2000), quinone oxidoreductase, which has a role in the activation of DNA damage checkpoints (Porte et al. 2009; Lee et al. 2010), PIG1, a member of the galectin family that can stimulate superoxide production (Yamaoka et al. 1995), and PIG8, a quinone known to generate ROS and can be induced by etoposide (Lehar et al. 1996).

In the mouse model, the hypomorphic allele of *p53*, *p53515C* (encoding p53R172P), rescues embryonic lethality of *MDM2*$^{-/-}$ mice. However, *MDM2*$^{-/-}$ *p53*$^{515C/515C}$ mice exhibits elevated ROS levels and the induction of the *PIG1*, *PIG8*, and *PIG12* genes. These mice succumbs to haematopoietic cell death after birth, and the lethality is partially rescued by antioxidant treatment (Abbas et al. 2010), suggesting the importance of p53-mediated ROS production for this type of cell death *in vivo*. Another p53-inducible pro-oxidant enzyme is FDXR, a mitochondrial protein that transfers an electron from NADPH to cytochrome P450 through ferredoxin and sensitizes cells to ROS-mediated p53-dependent apoptosis (Liu and Chen 2002). p53 also induces pro-apoptotic BCL-2 family proteins, such as Bax and Puma (Liu et al. 2005), which increase ROS generation, as well as the pro-oxidant protein p66Shc (Trinei et al. 2002; Giorgio et al. 2005), which is a splice variant of p52shc/p46shc, a signal transducer involved in RAS signalling. Interestingly, the depletion of p66Shc has been shown to reduce ROS levels and extend the mouse life span by about 30 % (Migliaccio et al. 1999).

p53 counteracts the antioxidant Nrf2 transcription factor, which transactivates genes with promoters containing antioxidant response cis-elements (AREs), such as *cysteine/glutamate transporter (x-CT)*, *NAD(P)H quinone oxidoreductase (NQO1)*, and *glutathione S-transferase α1(GST-α1)* (Faraonio et al. 2006). p53 also directly represses ARE promoters to induce ROS production and enhances apoptosis during

stress (Faraonio et al. 2006). p53 also represses a gene of key antioxidant enzymes, mitochondrial superoxide dismutase 2 (SOD2), which catalyses the dismutation of superoxide into oxygen and hydrogen peroxide to enhance p53-induced apoptosis (Pani et al. 2000; Drane et al. 2001; Dhar et al. 2006). Consistent with the ability of p53 to suppress antioxidant enzymes, Bmi1 deficiency in neurons results in abnormally high ROS concentrations due to the accumulation of p53 on the promoters for repressing antioxidant genes such as *X-CT* and *SOD2* (Chatoo et al. 2009). p53-mediated suppression of SOD2 has also been suggested by the direct interaction between p53 and SOD2 in mitochondria (Zhao et al. 2005). On the contrary, some reports have shown that p53 induces *SOD2* (Hussain et al. 2004; Li et al. 2010); however, an additional report shows that low p53 concentrations increases SOD2 expression while high concentrations suppresses it (Dhar et al. 2010). One additional report shows that p53-mediated induction of SOD2 and GPX causes an imbalance of antioxidant enzymes and increases ROS to contribute to p53-mediated apoptosis (Hussain et al. 2004). Clearly, the physiological regulation of SOD2 by p53 requires further investigation.

Interestingly, recent results have shown that p53 activation could lead to cell survival rather than apoptosis (Janicke et al. 2008). Indeed, p53 may act as antioxidant even when cells are exposed to significant oxidative stress depending on the cellular context. For, example, p53 can protect cells from death mediated by oxidative stress, but not by DNA damaging agents, through induction of a gene of the antioxidant enzyme, haeme-oxygenase-1 (HO-1), which catalyses the degradation of haem into bilirubin and CO (Nam and Sabapathy 2011). Similarly, p53 protects cells from NO-mediated apoptosis in smooth muscle cells (Kim et al. 2008; Popowich et al. 2010); however, NO can induce apoptosis in a p53 dependent manner in lymphoblastoid and fibroblast cells (McLaughlin and Demple 2005), suggesting that NO-mediated cell death mediated by p53 is cell type specific. The transcriptional co-activator PGC-1-α can activate p53 through direct binding and rescue cells from glucose starvation-mediated cell death by reducing ROS via activating antioxidant regulators downstream from p53 (Sen et al. 2011). p53 also transactivates *GLS2* to facilitate glutamine metabolism and lowers ROS levels by increasing reduced glutathione levels to protect cells from cell death mediated by oxidative stress (Hu et al. 2010; Suzuki et al. 2010). The p53 target, *ALDH4A1,* also protects cells from oxidative stress-mediated death by reducing ROS (Yoon et al. 2004).

Some discrepancy remains regarding how p53 regulates these pro- and antioxidant factors, as the regulation could depend on either cell context or the mechanism of p53 activation. However, there is some consensus in that physiological p53 levels without stress primarily maintain antioxidant enzymes to protect cells from oxidative stress, while p53 can promote pro- or antioxidant functions depending on the cellular context when the cells experience significant stress. Interestingly, these biologically distinct effects of p53 on cellular redox homeostasis are congruent with the divergent effects of an altered redox milieu on cell fate decisions. For example, a pro-oxidant state is an invariable finding in many cancers (Cerutti 1985), and depending upon the level of oxidative stress and the specific type of ROS, the effects range from pro-survival to pro-death (Pervaiz et al. 1999; Clement and Pervaiz

2001; Pervaiz and Clement 2002, 2007; Clement et al. 2003). On the one hand, the intrinsic antioxidant defence in cancer cells may protect them from death mediated by chemotherapeutic drugs that execute cells via intracellular ROS generation. Conversely, ROS-inducing effect of p53 might serve to push cells already in a slight 'pro-oxidant' milieu toward execution. A better understanding of the intricate cross-talk between p53 and cellular redox status as well as the effect of loss of function mutations of the tumour suppressor could provide potential leads that could be exploited for the design and development of novel therapeutic strategies.

7 Conclusions and Future Directions

After aerobic bacteria colonized primordial eukaryotic cells more than a billion years ago, mitochondria acquired wide-ranging functions involved in many cellular processes during evolution. Some of these include apoptosis, cell growth, cell division, lipid peroxidation, autophagy, glycolysis, ROS regulation, bioenergetic and biosynthetic metabolism, and ATP production by oxidative phosphorylation. During the evolution of the p53 family members from sea anemones to Homo sapiens (Rasmussen-Torvik et al. 2010), p53 has acquired many mechanisms for regulating mitochondrial metabolism, which, if deregulated, could negatively impact aging, cancer patients, and neurodegenerative disease patients. After the discovery of p53 30 years ago, many p53 target genes were identified and were found to participate in a myriad of cellular processes, including the regulation of oxidative phosphorylation, glycolysis, autophagy, glutamine and fatty acid metabolism, and ROS production. Generally, basal levels of p53 enhance oxidative phosphorylation, glutamine and fatty acid metabolism, antioxidant defences, and autophagy, while inhibiting glycolysis. In contrast, p53 accumulation after significant stress can result in either pro-oxidant function leading to cell death or antioxidant functions to counteract death, depending on the cellular context. Because the metabolic pathways described above are closely connected and p53 regulates many genes at the same time, the actions of p53 for a particular metabolic process are likely different for each cell type and context. This may explain why results published by different groups appear to be conceptually controversial, as discussed in this review. Many studies suggest that p53 regulates metabolism through both transcriptional activation and direct protein interactions in different cellular compartments, including mitochondria. However, it is still unclear which signals dictate the localization of p53 to different cellular compartments. The role of the metabolic function of p53 in tumour suppression has been recently demonstrated *in vivo* (Li et al. 2012). Mice bearing lysine to arginine mutations at three p53 acetylation sites, which lead to abrogated p53 functions in cell cycle arrest, apoptosis, and senescence do not show the same early onset of tumours as p53 null mice. Interestingly, these mice still retain the metabolic functions of p53, such as inhibiting glycolysis, reducing ROS, and the ability to induce *TIGAR*, *Aldh4h*, *Gamt*, *GLS2*, *Gpx1*, *sestrin1*, and *sestrin2* (Li et al. 2012), suggesting that p53-mediated

changes in cell-cycle, apoptosis, and senescence are dispensable for preventing the early onset of tumours, while the metabolic-related functions are associated with tumour suppression.

Recently, a gain-of-function p53 mutant was shown to transactivate particular genes (Freed-Pastor et al. 2012) and interact with several binding partners in different cellular compartments (Muller et al. 2009). In this respect, it is worth comparing the role of mutant p53 in the metabolic functions reported for wild-type p53, as mutant p53 is often overexpressed in cancer cells and may impact particular metabolic processes to a greater degree stoichiometrically than wild-type p53 (Goh et al. 2011). Although p53-mediated antioxidant functions protect cells from DNA damage and genomic instability caused by oxidative damage, these functions may interfere with the cell's ability to undergo apoptosis and senescence even after severe DNA damage and oncogenic insults. Therefore, the antioxidant function of p53 may inhibit the efficacy of cancer drugs. Indeed, p53 has several metabolic functions that may be considered beneficial to cancer cells, such as antioxidant protection against apoptosis or enhancing autophagy for cell survival. For example, it has been recently shown that serine starvation in cancer cells induces oxidative stress which is attenuated by p53-mediated metabolic remodelling that includes the efficient synthesis of major antioxidant glutathione (Maddocks et al. 2013). Because half of all cancer types contain wild-type p53, understanding the metabolic role of wild-type p53 within particular cancer types will provide important information for clinical oncology.

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References

Abbas HA, Maccio DR, Coskun S, Jackson JG, Hazen AL, Sills TM, You MJ, Hirschi KK, Lozano G (2010) Mdm2 is required for survival of hematopoietic stem cells/progenitors via dampening of ROS-induced p53 activity. Cell Stem Cell 7:606–617

Abida WM, Gu W (2008) p53-Dependent and p53-independent activation of autophagy by ARF. Cancer Res 68:352–357

Achanta G, Sasaki R, Feng L, Carew JS, Lu W, Pelicano H, Keating MJ, Huang P (2005) Novel role of p53 in maintaining mitochondrial genetic stability through interaction with DNA Pol gamma. EMBO J 24:3482–3492

Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, Thomas-Tikhonenko A, Thompson CB (2007) Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. J Clin Invest 117:326–336

Ambs S, Ogunfusika MO, Merriam WG, Bennett WP, Billiar TR, Harris CC (1998) Up-regulation of inducible nitric oxide synthase expression in cancer-prone p53 knockout mice. Proc Natl Acad Sci U S A 95:8823–8828

Assaily W, Rubinger DA, Wheaton K, Lin Y, Ma W, Xuan W, Brown-Endres L, Tsuchihara K, Mak TW, Benchimol S (2011) ROS-mediated p53 induction of Lpin1 regulates fatty acid oxidation in response to nutritional stress. Mol Cell 44:491–501

Bakhanashvili M, Grinberg S, Bonda E, Rahav G (2009) Excision of nucleoside analogs in mitochondria by p53 protein. AIDS 23:779–788

Bensaad K, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R, Gottlieb E, Vousden KH (2006) TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell 126:107–120

Bensaad K, Cheung EC, Vousden KH (2009) Modulation of intracellular ROS levels by TIGAR controls autophagy. EMBO J 28:3015–3026

Bishop AJ, Hollander MC, Kosaras B, Sidman RL, Fornace AJ Jr, Schiestl RH (2003) Atm-, p53-, and Gadd45a-deficient mice show an increased frequency of homologous recombination at different stages during development. Cancer Res 63:5335–5343

Bourdon A, Minai L, Serre V, Jais JP, Sarzi E, Aubert S, Chretien D, de Lonlay P, Paquis-Flucklinger V, Arakawa H, Nakamura Y, Munnich A, Rotig A (2007) Mutation of RRM2B, encoding p53-controlled ribonucleotide reductase (p53R2), causes severe mitochondrial DNA depletion. Nat Genet 39:776–780

Budanov AV, Karin M (2008) p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. Cell 134:451–460

Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, Viollet B, Thompson CB (2007) Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumour cell growth. Cancer Res 67:6745–6752

Calmels S, Hainaut P, Ohshima H (1997) Nitric oxide induces conformational and functional modifications of wild-type p53 tumour suppressor protein. Cancer Res 57:3365–3369

Cano CE, Gommeaux J, Pietri S, Culcasi M, Garcia S, Seux M, Barelier S, Vasseur S, Spoto RP, Pebusque MJ, Dusetti NJ, Iovanna JL, Carrier A (2009) Tumour protein 53-induced nuclear protein 1 is a major mediator of p53 antioxidant function. Cancer Res 69:219–226

Cassidy PB, Edes K, Nelson CC, Parsawar K, Fitzpatrick FA, Moos PJ (2006) Thioredoxin reductase is required for the inactivation of tumour suppressor p53 and for apoptosis induced by endogenous electrophiles. Carcinogenesis 27:2538–2549

Cerutti PA (1985) Prooxidant states and tumour promotion. Science 227:375–381

Chatoo W, Abdouh M, David J, Champagne MP, Ferreira J, Rodier F, Bernier G (2009) The polycomb group gene Bmi1 regulates antioxidant defenses in neurons by repressing p53 pro-oxidant activity. J Neurosci 29:529–542

Chen D, Yu Z, Zhu Z, Lopez CD (2006) The p53 pathway promotes efficient mitochondrial DNA base excision repair in colorectal cancer cells. Cancer Res 66:3485–3494

Cheng Y, Qiu F, Tashiro S, Onodera S, Ikejima T (2008) ERK and JNK mediate TNFalpha-induced p53 activation in apoptotic and autophagic L929 cell death. Biochem Biophys Res Commun 376:483–488

Clement MV, Pervaiz S (2001) Intracellular superoxide and hydrogen peroxide concentrations: a critical balance that determines survival or death. Redox Rep 6:211–214

Clement MV, Hirpara JL, Pervaiz S (2003) Decrease in intracellular superoxide sensitizes Bcl-2-overexpressing tumour cells to receptor and drug-induced apoptosis independent of the mitochondria. Cell Death Differ 10:1273–1285

Cobbs CS, Whisenhunt TR, Wesemann DR, Harkins LE, Van Meir EG, Samanta M (2003) Inactivation of wild-type p53 protein function by reactive oxygen and nitrogen species in malignant glioma cells. Cancer Res 63:8670–8673

Crighton D, Wilkinson S, O'Prey J, Syed N, Smith P, Harrison PR, Gasco M, Garrone O, Crook T, Ryan KM (2006) DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. Cell 126:121–134

Crighton D, O'Prey J, Bell HS, Ryan KM (2007) p73 regulates DRAM-independent autophagy that does not contribute to programmed cell death. Cell Death Differ 14:1071–1079

de Souza-Pinto NC, Harris CC, Bohr VA (2004) p53 functions in the incorporation step in DNA base excision repair in mouse liver mitochondria. Oncogene 23:6559–6568

Dhar SK, Xu Y, Chen Y, St Clair DK (2006) Specificity protein 1-dependent p53-mediated suppression of human manganese superoxide dismutase gene expression. J Biol Chem 281:21698–21709

Dhar SK, Xu Y, St Clair DK (2010) Nuclear factor kappaB- and specificity protein 1-dependent p53-mediated bi-directional regulation of the human manganese superoxide dismutase gene. J Biol Chem 285:9835–9846

Donald SP, Sun XY, Hu CA, Yu J, Mei JM, Valle D, Phang JM (2001) Proline oxidase, encoded by p53-induced gene-6, catalyzes the generation of proline-dependent reactive oxygen species. Cancer Res 61:1810–1815

Drane P, Bravard A, Bouvard V, May E (2001) Reciprocal down-regulation of p53 and SOD2 gene expression-implication in p53 mediated apoptosis. Oncogene 20:430–439

Du Y, Yang D, Li L, Luo G, Li T, Fan X, Wang Q, Zhang X, Wang Y, Le W (2009) An insight into the mechanistic role of p53-mediated autophagy induction in response to proteasomal inhibition-induced neurotoxicity. Autophagy 5:663–675

Faraonio R, Vergara P, Di Marzo D, Pierantoni MG, Napolitano M, Russo T, Cimino F (2006) p53 suppresses the Nrf2-dependent transcription of antioxidant response genes. J Biol Chem 281:39776–39784

Fei P, Wang W, Kim SH, Wang S, Burns TF, Sax JK, Buzzai M, Dicker DT, McKenna WG, Bernhard EJ, El-Deiry WS (2004) Bnip3L is induced by p53 under hypoxia, and its knockdown promotes tumour growth. Cancer Cell 6:597–609

Feng Z, Zhang H, Levine AJ, Jin S (2005) The coordinate regulation of the p53 and mTOR pathways in cells. Proc Natl Acad Sci U S A 102:8204–8209

Feng Z, Hu W, de Stanchina E, Teresky AK, Jin S, Lowe S, Levine AJ (2007) The regulation of AMPK beta1, TSC2, and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1-AKT-mTOR pathways. Cancer Res 67:3043–3053

Finkel T (2003) Oxidant signals and oxidative stress. Curr Opin Cell Biol 15:247–254

Flatt PM, Polyak K, Tang LJ, Scatena CD, Westfall MD, Rubinstein LA, Yu J, Kinzler KW, Vogelstein B, Hill DE, Pietenpol JA (2000) p53-dependent expression of PIG3 during proliferation, genotoxic stress, and reversible growth arrest. Cancer Lett 156:63–72

Forrester K, Ambs S, Lupold SE, Kapust RB, Spillare EA, Weinberg WC, Felley-Bosco E, Wang XW, Geller DA, Tzeng E, Billiar TR, Harris CC (1996) Nitric oxide-induced p53 accumulation and regulation of inducible nitric oxide synthase expression by wild-type p53. Proc Natl Acad Sci U S A 93:2442–2447

Freed-Pastor WA, Mizuno H, Zhao X, Langerod A, Moon SH, Rodriguez-Barrueco R, Barsotti A, Chicas A, Li W, Polotskaia A, Bissell MJ, Osborne TF, Tian B, Lowe SW, Silva JM, Borresen-Dale AL, Levine AJ, Bargonetti J, Prives C (2012) Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. Cell 148:244–258

Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT, Dang CV (2009) c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature 458:762–765

Gao W, Shen Z, Shang L, Wang X (2011) Upregulation of human autophagy-initiation kinase ULK1 by tumour suppressor p53 contributes to DNA-damage-induced cell death. Cell Death Differ 18:1598–1607

Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C, Pelliccia G, Luzi L, Minucci S, Marcaccio M, Pinton P, Rizzuto R, Bernardi P, Paolucci F, Pelicci PG (2005) Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. Cell 122:221–233

Goh AM, Coffill CR, Lane DP (2011) The role of mutant p53 in human cancer. J Pathol 223:116–126

Griffiths SD, Clarke AR, Healy LE, Ross G, Ford AM, Hooper ML, Wyllie AH, Greaves M (1997) Absence of p53 permits propagation of mutant cells following genotoxic damage. Oncogene 14:523–531

Hainaut P, Milner J (1993) Redox modulation of p53 conformation and sequence-specific DNA binding in vitro. Cancer Res 53:4469–4473

Hardie DG, Pan DA (2002) Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. Biochem Soc Trans 30:1064–1070

Harrison B, Kraus M, Burch L, Stevens C, Craig A, Gordon-Weeks P, Hupp TR (2008) DAPK-1 binding to a linear peptide motif in MAP1B stimulates autophagy and membrane blebbing. J Biol Chem 283:9999–10014

Hu W, Zhang C, Wu R, Sun Y, Levine A, Feng Z (2010) Glutaminase 2, a novel p53 target gene regulating energy metabolism and antioxidant function. Proc Natl Acad Sci U S A 107:7455–7460

Hussain SP, Amstad P, He P, Robles A, Lupold S, Kaneko I, Ichimiya M, Sengupta S, Mechanic L, Okamura S, Hofseth LJ, Moake M, Nagashima M, Forrester KS, Harris CC (2004) p53-induced up-regulation of MnSOD and GPx but not catalase increases oxidative stress and apoptosis. Cancer Res 64:2350–2356

Hwang PM, Bunz F, Yu J, Rago C, Chan TA, Murphy MP, Kelso GF, Smith RA, Kinzler KW, Vogelstein B (2001) Ferredoxin reductase affects p53-dependent, 5-fluorouracil-induced apoptosis in colorectal cancer cells. Nat Med 7:1111–1117

Ibrahim MM, Razmara M, Nguyen D, Donahue RJ, Wubah JA, Knudsen TB (1998) Altered expression of mitochondrial 16S ribosomal RNA in p53-deficient mouse embryos revealed by differential display. Biochim Biophys Acta 1403:254–264

Ide T, Brown-Endres L, Chu K, Ongusaha PP, Ohtsuka T, El-Deiry WS, Aaronson SA, Lee SW (2009) GAMT, a p53-inducible modulator of apoptosis, is critical for the adaptive response to nutrient stress. Mol Cell 36:379–392

Irvine M, Philipsz S, Frausto M, Mijatov B, Gallagher SJ, Fung C, Becker TM, Kefford RF, Rizos H (2010) Amino terminal hydrophobic import signals target the p14(ARF) tumour suppressor to the mitochondria. Cell Cycle 9:829–839

Itahana K, Zhang Y (2008) Mitochondrial p32 is a critical mediator of ARF-induced apoptosis. Cancer Cell 13:542–553

Itahana K, Zhang Y (2010) Mitochondrial targeting signals: another barcode in p14ARF? Cell Cycle 9:864–865

Itahana K, Clegg HV, Zhang Y (2008) ARF in the mitochondria: the last frontier? Cell Cycle 7:3641–3646

Janicke RU, Sohn D, Schulze-Osthoff K (2008) The dark side of a tumour suppressor: anti-apoptotic p53. Cell Death Differ 15:959–976

Jayaraman L, Murthy KG, Zhu C, Curran T, Xanthoudakis S, Prives C (1997) Identification of redox/repair protein Ref-1 as a potent activator of p53. Genes Dev 11:558–570

Jiang P, Du W, Wang X, Mancuso A, Gao X, Wu M, Yang X (2011) p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase. Nat Cell Biol 13:310–316

Johnson TM, Yu ZX, Ferrans VJ, Lowenstein RA, Finkel T (1996) Reactive oxygen species are downstream mediators of p53-dependent apoptosis. Proc Natl Acad Sci U S A 93:11848–11852

Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, Birnbaum MJ, Thompson CB (2005) AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol Cell 18:283–293

Kawauchi K, Araki K, Tobiume K, Tanaka N (2008a) Activated p53 induces NF-kappaB DNA binding but suppresses its transcriptional activation. Biochem Biophys Res Commun 372:137–141

Kawauchi K, Araki K, Tobiume K, Tanaka N (2008b) p53 regulates glucose metabolism through an IKK-NF-kappaB pathway and inhibits cell transformation. Nat Cell Biol 10:611–618

Kim YM, Choi BM, Kim YS, Kwon YG, Kibbe MR, Billiar TR, Tzeng E (2008) Protective effect of p53 in vascular smooth muscle cells against nitric oxide-induced apoptosis is mediated by up-regulation of heme oxygenase-2. BMB Rep 41:164–169

Kondoh H, Lleonart ME, Gil J, Wang J, Degan P, Peters G, Martinez D, Carnero A, Beach D (2005) Glycolytic enzymes can modulate cellular life span. Cancer Res 65:177–185

Kulawiec M, Ayyasamy V, Singh KK (2009) p53 regulates mtDNA copy number and mitocheckpoint pathway. J Carcinog 8:8

Le A, Lane AN, Hamaker M, Bose S, Gouw A, Barbi J, Tsukamoto T, Rojas CJ, Slusher BS, Zhang H, Zimmerman LJ, Liebler DC, Slebos RJ, Lorkiewicz PK, Higashi RM, Fan TW, Dang CV (2012) Glucose-independent glutamine metabolism via TCA cycling for proliferation and survival in B cells. Cell Metab 15:110–121

Lebedeva MA, Eaton JS, Shadel GS (2009) Loss of p53 causes mitochondrial DNA depletion and altered mitochondrial reactive oxygen species homeostasis. Biochim Biophys Acta 1787:328–334

Lee JH, Kang Y, Khare V, Jin ZY, Kang MY, Yoon Y, Hyun JW, Chung MH, Cho SI, Jun JY, Chang IY, You HJ (2010) The p53-inducible gene 3 (PIG3) contributes to early cellular response to DNA damage. Oncogene 29:1431–1450

Lee IH, Kawai Y, Fergusson MM, Rovira II, Bishop AJ, Motoyama N, Cao L, Finkel T (2012) Atg7 modulates p53 activity to regulate cell cycle and survival during metabolic stress. Science 336:225–228

Lehar SM, Nacht M, Jacks T, Vater CA, Chittenden T, Guild BC (1996) Identification and cloning of EI24, a gene induced by p53 in etoposide-treated cells. Oncogene 12:1181–1187

Li Z, Shi K, Guan L, Cao T, Jiang Q, Yang Y, Xu C (2010) ROS leads to MnSOD upregulation through ERK2 translocation and p53 activation in selenite-induced apoptosis of NB4 cells. FEBS Lett 584:2291–2297

Li T, Kon N, Jiang L, Tan M, Ludwig T, Zhao Y, Baer R, Gu W (2012) Tumour suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence. Cell 149:1269–1283

Liu G, Chen X (2002) The ferredoxin reductase gene is regulated by the p53 family and sensitizes cells to oxidative stress-induced apoptosis. Oncogene 21:7195–7204

Liu Z, Lu H, Shi H, Du Y, Yu J, Gu S, Chen X, Liu KJ, Hu CA (2005) PUMA overexpression induces reactive oxygen species generation and proteasome-mediated stathmin degradation in colorectal cancer cells. Cancer Res 65:1647–1654

Ma W, Sung HJ, Park JY, Matoba S, Hwang PM (2007) A pivotal role for p53: balancing aerobic respiration and glycolysis. J Bioenerg Biomembr 39:243–246

Macip S, Igarashi M, Berggren P, Yu J, Lee SW, Aaronson SA (2003) Influence of induced reactive oxygen species in p53-mediated cell fate decisions. Mol Cell Biol 23:8576–8585

Maddocks OD, Berkers CR, Mason SM, Zheng L, Blyth K, Gottlieb E, Vousden KH (2013) Serine starvation induces stress and p53-dependent metabolic remodelling in cancer cells. Nature 493:542–546

Maillet A, Pervaiz S (2012) Redox regulation of p53, redox effectors regulated by p53: a subtle balance. Antioxid Redox Signal 16:1285–1294

Maiuri MC, Criollo A, Tasdemir E, Vicencio JM, Tajeddine N, Hickman JA, Geneste O, Kroemer G (2007) BH3-only proteins and BH3 mimetics induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-X(L). Autophagy 3:374–376

Maiuri MC, Malik SA, Morselli E, Kepp O, Criollo A, Mouchel PL, Carnuccio R, Kroemer G (2009) Stimulation of autophagy by the p53 target gene Sestrin2. Cell Cycle 8:1571–1576

Marchenko ND, Zaika A, Moll UM (2000) Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. J Biol Chem 275:16202–16212

Martoriati A, Doumont G, Alcalay M, Bellefroid E, Pelicci PG, Marine JC (2005) dapk1, encoding an activator of a p19ARF-p53-mediated apoptotic checkpoint, is a transcription target of p53. Oncogene 24:1461–1466

Mathupala SP, Heese C, Pedersen PL (1997) Glucose catabolism in cancer cells. The type II hexokinase promoter contains functionally active response elements for the tumour suppressor p53. J Biol Chem 272:22776–22780

Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurley PJ, Bunz F, Hwang PM (2006) p53 regulates mitochondrial respiration. Science 312:1650–1653

McLaughlin LM, Demple B (2005) Nitric oxide-induced apoptosis in lymphoblastoid and fibroblast cells dependent on the phosphorylation and activation of p53. Cancer Res 65:6097–6104

Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, Pelicci PG (1999) The p66shc adaptor protein controls oxidative stress response and life span in mammals. Nature 402:309–313

Mizushima N, Komatsu M (2011) Autophagy: renovation of cells and tissues. Cell 147:728–741

Moos PJ, Edes K, Cassidy P, Massuda E, Fitzpatrick FA (2003) Electrophilic prostaglandins and lipid aldehydes repress redox-sensitive transcription factors p53 and hypoxia-inducible factor by impairing the selenoprotein thioredoxin reductase. J Biol Chem 278:745–750

Morselli E, Tasdemir E, Maiuri MC, Galluzzi L, Kepp O, Criollo A, Vicencio JM, Soussi T, Kroemer G (2008) Mutant p53 protein localized in the cytoplasm inhibits autophagy. Cell Cycle 7:3056–3061

Muller PA, Caswell PT, Doyle B, Iwanicki MP, Tan EH, Karim S, Lukashchuk N, Gillespie DA, Ludwig RL, Gosselin P, Cromer A, Brugge JS, Sansom OJ, Norman JC, Vousden KH (2009) Mutant p53 drives invasion by promoting integrin recycling. Cell 139:1327–1341

Nam SY, Sabapathy K (2011) p53 promotes cellular survival in a context-dependent manner by directly inducing the expression of haeme-oxygenase-1. Oncogene 30:4476–4486

Nithipongvanitch R, Ittarat W, Velez JM, Zhao R, St Clair DK, Oberley TD (2007) Evidence for p53 as guardian of the cardiomyocyte mitochondrial genome following acute adriamycin treatment. J Histochem Cytochem 55:629–639

O'Connor JC, Wallace DM, O'Brien CJ, Cotter TG (2008) A novel antioxidant function for the tumour-suppressor gene p53 in the retinal ganglion cell. Invest Ophthalmol Vis Sci 49:4237–4244

Okamura S, Ng CC, Koyama K, Takei Y, Arakawa H, Monden M, Nakamura Y (1999) Identification of seven genes regulated by wild-type p53 in a colon cancer cell line carrying a well-controlled wild-type p53 expression system. Oncol Res 11:281–285

Pani G, Bedogni B, Anzevino R, Colavitti R, Palazzotti B, Borrello S, Galeotti T (2000) Deregulated manganese superoxide dismutase expression and resistance to oxidative injury in p53-deficient cells. Cancer Res 60:4654–4660

Park JY, Wang PY, Matsumoto T, Sung HJ, Ma W, Choi JW, Anderson SA, Leary SC, Balaban RS, Kang JG, Hwang PM (2009a) p53 improves aerobic exercise capacity and augments skeletal muscle mitochondrial DNA content. Circ Res 105:705–712, 711 p following 712

Park KJ, Lee SH, Lee CH, Jang JY, Chung J, Kwon MH, Kim YS (2009b) Upregulation of Beclin-1 expression and phosphorylation of Bcl-2 and p53 are involved in the JNK-mediated autophagic cell death. Biochem Biophys Res Commun 382:726–729

Pervaiz S, Clement MV (2002) A permissive apoptotic environment: function of a decrease in intracellular superoxide anion and cytosolic acidification. Biochem Biophys Res Commun 290:1145–1150

Pervaiz S, Clement MV (2007) Superoxide anion: oncogenic reactive oxygen species? Int J Biochem Cell Biol 39:1297–1304

Pervaiz S, Ramalingam JK, Hirpara JL, Clement MV (1999) Superoxide anion inhibits drug-induced tumour cell death. FEBS Lett 459:343–348

Pimkina J, Humbey O, Zilfou JT, Jarnik M, Murphy ME (2009) ARF induces autophagy by virtue of interaction with Bcl-xl. J Biol Chem 284:2803–2810

Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B (1997) A model for p53-induced apoptosis. Nature 389:300–305

Popowich DA, Vavra AK, Walsh CP, Bhikhapurwala HA, Rossi NB, Jiang Q, Aalami OO, Kibbe MR (2010) Regulation of reactive oxygen species by p53: implications for nitric oxide-mediated apoptosis. Am J Physiol Heart Circ Physiol 298:H2192–H2200

Porte S, Valencia E, Yakovtseva EA, Borras E, Shafqat N, Debreczeny JE, Pike AC, Oppermann U, Farres J, Fita I, Pares X (2009) Three-dimensional structure and enzymatic function of proapoptotic human p53-inducible quinone oxidoreductase PIG3. J Biol Chem 284:17194–17205

Rasmussen-Torvik LJ, Alonso A, Li M, Kao W, Kottgen A, Yan Y, Couper D, Boerwinkle E, Bielinski SJ, Pankow JS (2010) Impact of repeated measures and sample selection on genome-wide association studies of fasting glucose. Genet Epidemiol 34:665–673

Ravi D, Muniyappa H, Das KC (2005) Endogenous thioredoxin is required for redox cycling of anthracyclines and p53-dependent apoptosis in cancer cells. J Biol Chem 280:40084–40096

Reef S, Zalckvar E, Shifman O, Bialik S, Sabanay H, Oren M, Kimchi A (2006) A short mitochondrial form of p19ARF induces autophagy and caspase-independent cell death. Mol Cell 22:463–475

Rivera A, Maxwell SA (2005) The p53-induced gene-6 (proline oxidase) mediates apoptosis through a calcineurin-dependent pathway. J Biol Chem 280:29346–29354

Rosenbluth JM, Pietenpol JA (2009) mTOR regulates autophagy-associated genes downstream of p73. Autophagy 5:114–116

Ruiz-Lozano P, Hixon ML, Wagner MW, Flores AI, Ikawa S, Baldwin AS Jr, Chien KR, Gualberto A (1999) p53 is a transcriptional activator of the muscle-specific phosphoglycerate mutase gene and contributes in vivo to the control of its cardiac expression. Cell Growth Differ 10:295–306

Russo T, Zambrano N, Esposito F, Ammendola R, Cimino F, Fiscella M, Jackman J, O'Connor PM, Anderson CW, Appella E (1995) A p53-independent pathway for activation of WAF1/CIP1 expression following oxidative stress. J Biol Chem 270:29386–29391

Sablina AA, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE, Chumakov PM (2005) The antioxidant function of the p53 tumour suppressor. Nat Med 11:1306–1313

Sahin E, Colla S, Liesa M, Moslehi J, Muller FL, Guo M, Cooper M, Kotton D, Fabian AJ, Walkey C, Maser RS, Tonon G, Foerster F, Xiong R, Wang YA, Shukla SA, Jaskelioff M, Martin ES, Heffernan TP, Protopopov A, Ivanova E, Mahoney JE, Kost-Alimova M, Perry SR, Bronson R, Liao R, Mulligan R, Shirihai OS, Chin L, DePinho RA (2011) Telomere dysfunction induces metabolic and mitochondrial compromise. Nature 470:359–365

Saleem A, Adhihetty PJ, Hood DA (2009) Role of p53 in mitochondrial biogenesis and apoptosis in skeletal muscle. Physiol Genomics 37:58–66

Scherz-Shouval R, Weidberg H, Gonen C, Wilder S, Elazar Z, Oren M (2010) p53-dependent regulation of autophagy protein LC3 supports cancer cell survival under prolonged starvation. Proc Natl Acad Sci U S A 107:18511–18516

Schonhoff CM, Daou MC, Jones SN, Schiffer CA, Ross AH (2002) Nitric oxide-mediated inhibition of Hdm2-p53 binding. Biochemistry 41:13570–13574

Schwartzenberg-Bar-Yoseph F, Armoni M, Karnieli E (2004) The tumour suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. Cancer Res 64:2627–2633

Sen N, Satija YK, Das S (2011) PGC-1alpha, a key modulator of p53, promotes cell survival upon metabolic stress. Mol Cell 44:621–634

Stambolsky P, Weisz L, Shats I, Klein Y, Goldfinger N, Oren M, Rotter V (2006) Regulation of AIF expression by p53. Cell Death Differ 13:2140–2149

Sung HJ, Ma W, Wang PY, Hynes J, O'Riordan TC, Combs CA, McCoy JP Jr, Bunz F, Kang JG, Hwang PM (2010) Mitochondrial respiration protects against oxygen-associated DNA damage. Nat Commun 1:5

Suzuki S, Tanaka T, Poyurovsky MV, Nagano H, Mayama T, Ohkubo S, Lokshin M, Hosokawa H, Nakayama T, Suzuki Y, Sugano S, Sato E, Nagao T, Yokote K, Tatsuno I, Prives C (2010) Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species. Proc Natl Acad Sci U S A 107:7461–7466

Tan M, Li S, Swaroop M, Guan K, Oberley LW, Sun Y (1999) Transcriptional activation of the human glutathione peroxidase promoter by p53. J Biol Chem 274:12061–12066

Tanaka H, Arakawa H, Yamaguchi T, Shiraishi K, Fukuda S, Matsui K, Takei Y, Nakamura Y (2000) A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. Nature 404:42–49

Tasdemir E, Maiuri MC, Galluzzi L, Vitale I, Djavaheri-Mergny M, D'Amelio M, Criollo A, Morselli E, Zhu C, Harper F, Nannmark U, Samara C, Pinton P, Vicencio JM, Carnuccio R, Moll UM, Madeo F, Paterlini-Brechot P, Rizzuto R, Szabadkai G, Pierron G, Blomgren K, Tavernarakis N, Codogno P, Cecconi F, Kroemer G (2008a) Regulation of autophagy by cytoplasmic p53. Nat Cell Biol 10:676–687

Tasdemir E, Maiuri MC, Orhon I, Kepp O, Morselli E, Criollo A, Kroemer G (2008b) p53 represses autophagy in a cell cycle-dependent fashion. Cell Cycle 7:3006–3011

Tavernarakis N, Pasparaki A, Tasdemir E, Maiuri MC, Kroemer G (2008) The effects of p53 on whole organism longevity are mediated by autophagy. Autophagy 4:870–873

Tong X, Zhao F, Mancuso A, Gruber JJ, Thompson CB (2009) The glucose-responsive transcription factor ChREBP contributes to glucose-dependent anabolic synthesis and cell proliferation. Proc Natl Acad Sci U S A 106:21660–21665

Trinei M, Giorgio M, Cicalese A, Barozzi S, Ventura A, Migliaccio E, Milia E, Padura IM, Raker VA, Maccarana M, Petronilli V, Minucci S, Bernardi P, Lanfrancone L, Pelicci PG (2002) A p53-p66Shc signalling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis. Oncogene 21:3872–3878

Tu HC, Ren D, Wang GX, Chen DY, Westergard TD, Kim H, Sasagawa S, Hsieh JJ, Cheng EH (2009) The p53-cathepsin axis cooperates with ROS to activate programmed necrotic death upon DNA damage. Proc Natl Acad Sci U S A 106:1093–1098

Ueno M, Masutani H, Arai RJ, Yamauchi A, Hirota K, Sakai T, Inamoto T, Yamaoka Y, Yodoi J, Nikaido T (1999) Thioredoxin-dependent redox regulation of p53-mediated p21 activation. J Biol Chem 274:35809–35815

Vahsen N, Cande C, Briere JJ, Benit P, Joza N, Larochette N, Mastroberardino PG, Pequignot MO, Casares N, Lazar V, Feraud O, Debili N, Wissing S, Engelhardt S, Madeo F, Piacentini M, Penninger JM, Schagger H, Rustin P, Kroemer G (2004) AIF deficiency compromises oxidative phosphorylation. EMBO J 23:4679–4689

Velu CS, Niture SK, Doneanu CE, Pattabiraman N, Srivenugopal KS (2007) Human p53 is inhibited by glutathionylation of cysteines present in the proximal DNA-binding domain during oxidative stress. Biochemistry 46:7765–7780

Webster NJ, Resnik JL, Reichart DB, Strauss B, Haas M, Seely BL (1996) Repression of the insulin receptor promoter by the tumour suppressor gene product p53: a possible mechanism for receptor overexpression in breast cancer. Cancer Res 56:2781–2788

Wong TS, Rajagopalan S, Townsley FM, Freund SM, Petrovich M, Loakes D, Fersht AR (2009) Physical and functional interactions between human mitochondrial single-stranded DNA-binding protein and tumour suppressor p53. Nucleic Acids Res 37:568–581

Yakovlev VA, Bayden AS, Graves PR, Kellogg GE, Mikkelsen RB (2010) Nitration of the tumour suppressor protein p53 at tyrosine 327 promotes p53 oligomerization and activation. Biochemistry 49:5331–5339

Yamaoka A, Kuwabara I, Frigeri LG, Liu FT (1995) A human lectin, galectin-3 (epsilon bp/Mac-2), stimulates superoxide production by neutrophils. J Immunol 154:3479–3487

Yee KS, Wilkinson S, James J, Ryan KM, Vousden KH (2009) PUMA- and Bax-induced autophagy contributes to apoptosis. Cell Death Differ 16:1135–1145

Yoon KA, Nakamura Y, Arakawa H (2004) Identification of ALDH4 as a p53-inducible gene and its protective role in cellular stresses. J Hum Genet 49:134–140

Yoshida Y, Izumi H, Torigoe T, Ishiguchi H, Itoh H, Kang D, Kohno K (2003) P53 physically interacts with mitochondrial transcription factor A and differentially regulates binding to damaged DNA. Cancer Res 63:3729–3734

Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL (2008) Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem 283:10892–10903

Zhang C, Lin M, Wu R, Wang X, Yang B, Levine AJ, Hu W, Feng Z (2011) Parkin, a p53 target gene, mediates the role of p53 in glucose metabolism and the Warburg effect. Proc Natl Acad Sci U S A 108:16259–16264

Zhao Y, Chaiswing L, Velez JM, Batinic-Haberle I, Colburn NH, Oberley TD, St Clair DK (2005) p53 translocation to mitochondria precedes its nuclear translocation and targets mitochondrial oxidative defense protein-manganese superoxide dismutase. Cancer Res 65:3745–3750

Zhou S, Kachhap S, Singh KK (2003) Mitochondrial impairment in p53-deficient human cancer cells. Mutagenesis 18:287–292

Chapter 13
The Progression of Cardiomyopathy in the Mitochondrial Disease, Friedreich's Ataxia

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Contents

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Abstract Friedreich's ataxia (FA) is a debilitating mitochondrial disorder leading to neural and cardiac degeneration that is caused by a mutation in the frataxin gene. The most common cause of death in these patients is heart failure, although it is not known how the deficiency in frataxin potentiates the observed cardiomyopathy. However, there have been a number of proposed mechanisms involving the function of frataxin, and the origins of heart failure in FA that will be discussed in this chapter. It is hoped that the elucidation of these potential mechanisms will lead to a comprehensive understanding of the pathogenesis of FA, and contribute to the development of treatments and therapeutics.

Keywords Autophagy • Friedreich's ataxia • Autophagy • Mitophagy • Cardiac hypertrophy • Iron • Mitochondria • Frataxin • Oxidative stress • Hypoxia-inducible factor • Apoptosis • Heart failure

1 Introduction

Friedreich's ataxia (FA) is an autosomal recessive neuro- and cardio-degenerative disorder that is caused by the deficient expression of the nuclear-encoded mitochondrial protein, frataxin (Campuzano et al. 1997). It is characterised by gait and limb ataxia, dysarthria, absent muscle stretch reflexes in the lower limbs, sensory loss, and pyramidal signs (Dürr et al. 1996; Babcock et al. 1997). In addition, patients generally suffer from skeletal deformities, diabetes and cardiomyopathy (Babcock et al. 1997). The disorder has an estimated prevalence of 1 in 50,000 in European populations, which makes it the most common hereditary ataxia among Caucasians, though it is rare among Asians, sub-Saharan Africans and American Indians (Dürr et al. 1996; Santos et al. 2010).

Although a small proportion of FA patients have been found to possess a point or missense mutation in an allele, frataxin-deficiency is primarily due to a homozygous GAA triplet repeat expansion on the first intron of the corresponding gene (Dürr et al. 1996; Santos et al. 2010). These repeats can range from 66 to 1,700, as compared to a normal allele which contains between 6 and 36 triplets (Pilch et al. 2002). Expansions in the gene cause an abnormal conformation in the DNA, resulting in decreased transcription, and in a subsequent reduced expression of the frataxin protein (Bidichandani et al. 1998). Importantly, the sizes of the GAA repeats have been found to positively correlate with the severity of the disease, and negatively correlate with the age of onset (Dürr et al. 1996). There are a number of atypical variants of FA such as: **(1)** late-onset FA (LOFA) where disease progression is slower, and onset is after 25 years of age (Pilch et al. 2002); **(2)** Acadian- type FA (where the symptoms are less severe); and **(3)** FA with retained reflexes, where tendon reflexes in the lower limbs are preserved, and clinical features are less pronounced (Klockgether et al. 1991; Filla et al. 1996; Santos et al. 2010). These atypical variants with less severe symptoms such as LOFA typically have shorter GAA expansions (Filla et al. 1996). The pathogenesis of the typical form

of FA has a mean age of onset at 10.52 ± 7.4 years, and death at 37.54 ± 14.35 years of age (Harding 1981).

The association of FA with mitochondrial dysfunction is supported by a large body of evidence. The mitochondrion is not only a vital organelle for energy production, but it is also important in iron metabolism as it is the primary site for haem and iron-sulphur cluster (ISC) synthesis (Richardson et al. 2010). These processes are significantly affected in FA (Huang et al. 2011). Hence, as a result of the disruption of normal mitochondrial function, some of the notable features that are observed in the disorder include mitochondrial iron accumulation in the heart and ISC deficiency (Babcock et al. 1997; Mühlenhoff et al. 2002). In addition, FA primarily affects mitochondria-rich tissues that are largely dependent on oxidative energy metabolism such as the heart, pancreas and neurons, resulting in the selective loss of dorsal root ganglia neurons, cardiomyocytes and pancreatic beta cells (Mühlenhoff et al. 2002; Gakh et al. 2006; Santos et al. 2010). However, it is not known why certain neuronal types such as spinal cord and brainstem motor neurons are exempted in the pathogenesis of the disorder (Babcock et al. 1997; Santos et al. 2010).

While there is no known cure for FA, some forms of treatment are available. This includes anti-oxidant therapy which is only targeted to cardiomyopathy, physical therapy, and standard treatment for patients suffering from diabetes, scoliosis, and arrhythmias (Lodi et al. 2001; Maring and Croarkin 2007; Schulz et al. 2009). It is therefore hoped that the elucidation of frataxin's role in iron metabolism will provide insight into its function and into the pathogenesis of the disease, so as to work towards a more effective treatment for FA.

2 Frataxin

Frataxin is a 210 amino acid nuclear-encoded protein, and it is predominantly found in the mitochondrion, either associated with the mitochondrial membrane and crests, or as a free soluble protein in the matrix (Gibson et al. 1996; Babcock et al. 1997; Campuzano et al. 1997). Although frataxin is ubiquitous, its highest levels of expression are in the heart and spinal cord – the main sites affected by the disease (Santos et al. 2010). While it is known that frataxin is linked to neuro- and cardio-degeneration, the function of the protein is still unclear. However, frataxin is likely to play a role in iron metabolism, as it has been proposed to interact with proteins such as ferrochelatase and the scaffold protein, ISU, which are markedly involved in iron utilisation pathways such as haem synthesis, and iron-sulphur cluster (ISC) synthesis respectively (Mühlenhoff et al. 2002; Yoon and Cowan 2003; Yoon and Cowan 2004). It has also been shown that iron chelators such as desferrioxamine (DFO) can reduce the steady state levels of frataxin, although it should be noted that this is an isolated study, and that contradicting results have been reported (Becker et al. 2002; Li et al. 2008). Considering this, there have been a number of hypotheses regarding frataxin's role in cellular iron homeostasis and these are discussed below.

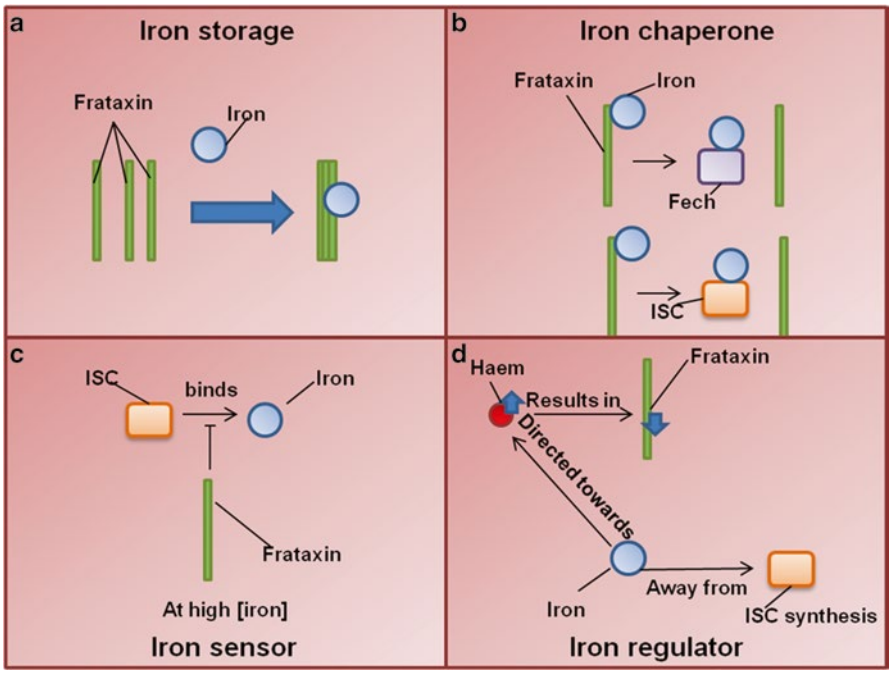

Fig. 13.1 The proposed roles of frataxin in iron metabolism in mammals. Frataxin has been suggested to be involved in a number of iron-related processes such as iron storage, acting as an iron chaperone, iron sensing and iron regulation

2.1 Proposed Roles of Frataxin

2.1.1 Frataxin as an Iron-Binding Protein

In vitro studies have revealed that the yeast and bacterial orthologues of frataxin, Yfh1 and CyaY respectively, homo-oligomerise in the presence of excess iron, and these aggregates have strong iron binding abilities with ferroxidase activity (Adamec et al. 2000; Bencze et al. 2006) (Fig. 13.1a). Similarly, the over-production of human frataxin in *E. coli* has been shown to result in the assembly of a homo-oligomer which is able to bind approximately 10 iron atoms per frataxin molecule (Cavadini et al. 2002). This helps to keep iron in its inert state so as to shield it from redox reactions, and thus limit iron toxicity (Bencze et al. 2006; Gakh et al. 2006). This iron-binding ability through aggregation has also been found in the cytosolic iron storage protein, ferritin, which suggests that frataxin may play a similar role (Gakh et al. 2006). Consistent with this proposal, the structures of three frataxin orthologues were resolved, and it was found that there was a highly conserved *N*-terminal region consisting of the metal-binding Asp and Glu residues (Bencze et al. 2006).

The aggregation of human frataxin does not occur spontaneously *in vitro*, and, in some cases, there was no clear interaction between human frataxin and iron

(Adinolfi et al. 2002). In addition, a study showed that a mutant form of Yfh1, that was unable to oligomerise and store iron, was phenotypically silent *in vivo*, suggesting that iron-binding is not a primary function of frataxin (Aloria et al. 2004). Finally, the discovery of mitochondrial ferritin (Ftmt) in human tissues suggests that the proposed role of frataxin as an iron binding protein in the mitochondrion may be redundant in humans (Levi et al. 2001; Drysdale et al. 2002).

2.1.2 Frataxin as an Iron Chaperone

Based on isothermal titration calorimetry and fluorescence quenching experiments, it was found that holo-frataxin, which is the iron-carrying form of frataxin, has a high binding affinity for ferrochelatase (Yoon and Cowan 2004). Ferrochelatase is the enzyme which is responsible for the insertion of iron into the protoporphyrin ring in the terminal step of haem synthesis (Taketani 1994). Therefore, it has been proposed that frataxin acts as an iron chaperone in haem synthesis which donates an iron atom to ferrochelatase, and consequently limits the toxicity of free iron within the cell (Bencze et al. 2006) (Fig. 13.1b).

There has also been evidence to suggest that frataxin serves as an iron chaperone in the assembly of ISCs (Gerber et al. 2003; Yoon and Cowan 2003; Gakh et al. 2006; Adinolfi et al. 2009). ISCs are essential for a number of biological processes such as the electron transport chain, and other enzymatic and regulatory processes (Rouault and Tong 2005). Biosynthesis begins with the formation of a sulphide bridge between cysteine desulphurase and mitochondrial cysteine, and this leads to the formation of [2Fe-2S] or [4Fe-4S] clusters on scaffold proteins such as ISU (Rouault and Tong 2005).

It was found that holo-frataxin forms a complex with ISU, and, in the absence of external ligands, iron is released from frataxin (Yoon and Cowan 2003). In addition, when high iron ion concentrations were used to mediate iron delivery to ISUs, negligible cluster formation was observed[18]. However, ISCs could be assembled *in vitro* in the absence of accessory proteins (Yoon and Cowan 2003). This implies that accessory proteins such as frataxin act as carriers instead of catalysts in the formation of ISCs. Hence, human frataxin is likely to mediate iron transfer through the interaction of holo-frataxin and nucleation sites for the formation of the [2Fe-2S] cluster on the ISU (Yoon and Cowan 2003). A similar interaction between the ISC-assembly complex and the yeast orthologue of frataxin has been reported, as holo-frataxin has been shown to form a complex with the scaffold protein, ISU (Gerber et al. 2003; Aloria et al. 2004; Li et al. 2008; Huang et al. 2011).

2.1.3 Frataxin as an Iron Regulator

Contrary to frataxin's proposed role as an iron chaperone, it was found that CyaY specifically inhibits [2Fe-2S] cluster synthesis (Adinolfi et al. 2009). In addition, it appeared to attenuate ISC formation in response to varying iron concentrations (Adinolfi et al. 2009) (Fig. 13.1c). It has therefore been proposed that frataxin

regulates ISC formation by controlling the ratio of Fe-S clusters and its apo acceptors (Adinolfi et al. 2009). This would also explain frataxin's increased affinity for ISCs at higher iron concentrations, as it serves to limit iron toxicity that can occur from the breakdown of ISCs when there are insufficient acceptors. Frataxin may therefore act as an iron sensor through its participation in ISC formation (Adinolfi et al. 2002; Gerber et al. 2003; Adinolfi et al. 2009).

Another role that frataxin could play in the regulation of iron metabolism is one where it can act as a metabolic switch in terms of directing iron towards haem synthesis and away from ISC formation (Fig. 13.1d), which is evident from the down-regulation of frataxin expression during erythroid differentiation (Becker et al. 2002). In this latter study, it was found that frataxin was down-regulated at increased levels of the haem synthesis enzyme, protoporphyrin IX, and, correspondingly, iron was directed away from ISC synthesis (Becker et al. 2002). Consistent with this hypothesis, it was found that the rate of haem synthesis was inversely related to the ratio of the levels between frataxin and ferrochelatase (Yoon and Cowan 2004). Higher levels of frataxin were found to lead to decreased rates of haem synthesis, which might have been to allow for the direction of iron towards ISC synthesis.

2.2 Effects of Frataxin Deficiency

2.2.1 Iron Accumulation

The importance of frataxin in iron regulation is further reflected in the observation that mitochondrial iron accumulates in frataxin-deficient cardiomyocytes (Babcock et al. 1997; Foury and Cazzalini 1997). Among human patients, iron accumulation has been detected in the heart, liver, and spleen, but not in the dorsal root ganglion (DRG), spinal cord, skeletal muscle, cerebellum, peripheral nerves, or pancreas (Bradley et al. 2004; Santos et al. 2010). A similar observation was made in the 10-week old muscle creatine kinase (MCK) conditional frataxin knockout mice, where significant iron deposition was detected in the cardiomyocytes (Puccio et al. 2001).

Prior to 10 weeks of age, levels of mitochondrial iron in the 7 week MCK mutants appeared to be either normal or only slightly increased, whereas ISC enzyme activities were already defective at this timepoint (Puccio et al. 2001). In addition, iron deposits were not found in the neuron-specific enolase (Koglin et al. 1999) model, but both models showed signs of cardiac hypertrophy and deficits in Fe-S enzymes (Puccio et al. 2001). This suggests that iron accumulation is likely to occur independently of these other symptoms. A gene array analysis of the MCK mice identified a mechanism of iron redistribution characterised by an up-regulation of genes involved in cellular and mitochondrial iron uptake, and a down-regulation of genes that encode proteins which play roles in cytosolic iron storage and mitochondrial iron-processing (Huang et al. 2009). It is therefore likely that mitochondrial iron accumulation occurs from the increased uptake of iron into the mitochondrion,

coupled with a lack of iron-processing in the mitochondrion (Huang et al. 2009). In support of this view, microarray studies of the yeast frataxin knockout model have shown an up-regulation of genes that are under the influence of the iron-sensing transcription factors, AFT1/AFT2 (Mühlenhoff et al. 2002). Considering the induction of the AFT regulatory system by low levels of cytosolic iron, it is possible that there was a redistribution of cytosolic iron to the mitochondrion in response to frataxin deficiency (Mühlenhoff et al. 2002). Taken together, these observations suggest that frataxin plays a significant role in mitochondrial iron metabolism, and that its deficiency is potentially responsible for the mitochondrial iron accumulation that is observed in FA.

2.2.2 Oxidative Stress

The accumulation of iron within a redox active environment such as the mitochondrion can lead to the production of free radicals through the Fenton reaction (Fenton 1894; Papanikolaou and Pantopoulos 2005) (Eq. 13.1):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH \tag{13.1}$$

Therefore, it is possible that the accumulation of mitochondrial iron observed in FA patients will result in oxidative stress and free radical induced cytotoxicity. In addition, it was shown that in the absence of Yfh1, mitochondrial damage in yeast cells was proportional to the concentration and duration of exposure to extracellular iron, suggesting a potential causative effect that iron accumulation has on cellular damage in FA (Radisky et al. 1999).

Further evidence that supports a role of oxidative stress in FA was reflected in a study where the administration of the anti-oxidant, idebenone, led to a decrease in myocardial hypertrophy, and improved myocardial energy deficiency (Rustin et al. 1999; Papanikolaou and Pantopoulos 2005). Another study showed increased concentrations of markers of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, and of lipid peroxidation, serum malondialdehyde, that were noted in patients (Emond et al. 2000; Schulz et al. 2000; Cavadini et al. 2002; Yoon and Cowan 2004). Finally, it has been shown that there is a higher mitochondrial DNA mutation rate in genes encoding NADH dehydrogenase in FA patients than in controls, indicating the occurrence of reactive oxygen species (ROS) production (Santos et al. 2010). It is therefore likely that these mutations contribute to mitochondrial instability.

However, there have also been contradicting studies to suggest that frataxin deficiency is not linked to oxidative stress. For example, Seznec et al. showed that frataxin deficiency did not induce oxidative stress in neuronal tissues, and that it also led to the eventual down-regulation of the anti-oxidant enzyme, manganese superoxide dismutase (MnSOD) (Seznec et al. 2005). It was thus proposed that the reduced levels of MnSOD was indicative of the lack of endogenous free radical production, instead of as the cause of oxidative damage in FA patients (Seznec et al. 2005).

2.2.3 Association with Hypoxia Inducible Factors

Another study showed that the mouse frataxin gene is regulated by hypoxia inducible factor (HIF) -2α, as levels of frataxin were reduced in response to a knockout of the HIF2α-encoding *Epas1* (Dürr et al. 1996; Oktay et al. 2007). It was similarly found in human tumour cell lines that frataxin is up-regulated in response to hypoxic stress (Guccini et al. 2011). Namely, frataxin was shown to modulate the expression of the tumour suppressor gene, p53, and it was also found to be dependent on HIFs (Guccini et al. 2011). However, it is still not known how HIFs are activated, although it could possibly be due to a release of mitochondrial reactive oxygen species or other environmental stresses that elicit the hypoxic response. In summary, it is clear that further studies are required to identify the pathways that are responsible for the severe cellular damage found in FA patients.

3 Cardiomyopathy in Friedreich's Ataxia

3.1 Symptoms and Treatment

Cardiomyopathy is the most common cause of death in FA and it affects two-thirds of patients (Sedlak et al. 2004; Santos et al. 2010). Patients are often found with left ventricular hypertrophy which can progress to the more fatal dilated cardiomyopathy, and adequate systolic function is usually maintained until shortly before death (Puccio et al. 2001; Papanikolaou and Pantopoulos 2005; Payne 2011). Both ventricles are affected, and arrhythmias of atrial origin are common and important in determining the prognosis of the patient, as they are indicative of left ventricular dysfunction (Santos et al. 2010; Bourke and Keane 2011). Microscopically, it has been found that cardiomyocyte hypertrophy, focal necrosis and diffuse fibrosis occur in the left ventricle (Payne 2011).

Moreover, a significant decrease in myocardial energy generation was shown to correlate with the degree of hypertrophy (Payne 2011). Accordingly, short term studies have shown that the use of idebenone has been successful in improving myocardial energy deficiency and decreasing hypertrophy (Rustin et al. 1999; Payne 2011). However, longer term studies have failed to show a decrease in arrhythmias or progression of heart failure (Payne 2011; Velasco-Sánchez et al. 2011). Another possible treatment method for cardiomyopathy in FA is cardiac transplants. It was known to be performed in one case, and the patient's ataxia improved considerably, possibly due to the reperfusion of blood to the limbs (Sedlak et al. 2004).

In the experimental MCK model of FA, cardiac hypertrophy was determined by their heart/body weight ratio (Puccio et al. 2001). At the early onset of the disease, the mutants did not display any significant differences to wild-type mice until late onset, where the mass of the MCK hearts increased significantly till death (Puccio et al. 2001) (Fig. 13.2). The cardiac hypertrophy was observed to develop into a dilated cardiomyopathy, which is congruent with the progression of the human

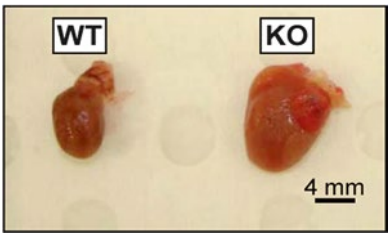

Fig. 13.2 Photographs of hearts from wild-type and knockout MCK mice at 10 weeks of age (Taken from Huang et al. 2013). At 10 weeks of age, the heart of the MCK knockout mouse is significantly enlarged compared to its wild-type counterpart. This is indicative of the cardiac hypertrophy that is evident in the disorder

disease (Puccio et al. 2001; Drysdale et al. 2002; Koeppen 2011). The aforementioned ISC deficiency was not displayed until late onset, which indicates that this process occurs in parallel with the pathogenesis of cardiomyopathy in FA (Puccio et al. 2001). In addition, the application of anti-oxidant therapy using idebenone had a similar effect on the mouse models as it also slowed the progression of cardiomyopathy (Seznec et al. 2004).

3.2 Progression of Cardiomyopathy in Friedreich's Ataxia

The previously mentioned, microarray analysis performed by Huang et al. demonstrated that there was up-regulation of genes involved in the integrated stress response (ISR) (Narula et al. 1999) as early on as 4 weeks, when the mice were phenotypically asymptomatic (Harding et al. 2003; Seznec et al. 2004; Huang et al. 2013). The ISR which will be discussed below, orchestrates different stress pathways in response to cellular damage, and it is also known to be able to lead to autophagy and apoptosis in the heart (Okada et al. 2004; Kroemer et al. 2010). One of the key features of cardiomyopathy is the loss of cardiomyocytes from the deficient regeneration of cells, leading to subsequent structural abnormalities and functional deficiencies (Rustin et al. 1999; Sabbah 1999; Knaapen et al. 2001; Hein et al. 2003). The cause of cardiomyocyte death has not been determined in human FA patients, although studies utilising the animal model of FA have identified the potential involvement of autophagy and apoptosis (Cossée et al. 2000; Santos et al. 2001; Palomo et al. 2011; Huang et al. 2013).

In vitro studies have demonstrated that apoptosis occurs in cultured cells from FA patients in response to oxidative damage (Wong et al. 1999; Santos et al. 2001). However, these observations have yet to be made *in vivo* as apoptotic neuronal cells are readily phagocytosed by neighbouring glial cells and are therefore difficult to detect (Puccio et al. 2001; Drysdale et al. 2002; Palomo et al. 2011). An accumulation of lipofuscin was also found in both the dorsal root ganglia and the cardiomyocytes of FA patients (Lamarche et al. 1980; Larnaout et al. 1997).

Lipofuscin is a protein aggregate that is formed after the degradation of iron-containing proteins by the lysosome, and it is known to be important in the autophagy of mitochondria (Brunk and Terman 2002; Keller et al. 2004). This raises the possibility that autophagy occurs in the hearts of FA patients, and it might therefore be a major contributor to cardiomyopathy in FA. It is also of interest that autophagic cardiomyocytes are observed at a significantly higher frequency in failing hearts than apoptotic markers (Martinet et al. 2007). While it has been shown that autophagic markers were present neurologically in FA patients, it is not known if it occurs in the heart (Seznec et al. 2004).

These findings are important because autophagy and apoptosis can play a vital role in the development of cardiac failure in the disease, and they might also act synergistically to result in heart failure (Eisenberg-Lerner et al. 2009). Hence, these processes and the pathways that trigger them need to be considered in the pathogenesis of FA.

4 The Integrated Stress Response

The ISR (Narula et al. 1999) is a highly conserved adaptive stress response initiated by kinases which results in the phosphorylation of eukaryotic initiation factor (eIF)-2α (Harding et al. 2003). Four known kinases upstream of eIF2α are phosphorylated in response to cellular stressors. These include haem-regulated inhibitor (Hri) which autophosphorylates in response to haem deficiency; Protein kinase R (Pkr), which is activated after viral infection; Protein kinase-like ER kinase (PERK), which is up-regulated following ER stress; and general control non-redepressing 2 (Gcn2) which is active in response to amino acid deficiency (Nanduri et al. 2000; McEwen et al. 2005; Chen 2007; Eisenberg-Lerner et al. 2009; Kilberg et al. 2009).

These divergent stress signals converge at the phosphorylation of eIF2α which leads to a reduced rate of cellular protein synthesis, but a paradoxical translation of selective transcripts, including the activating transcription factor 4 (ATF4) (Harding et al. 2003) (Fig. 13.3). ATF4 is responsible for the activation of different adaptive cellular events by binding and promoting the expression of stress-inducible genes responsible for amino acid synthesis (Kilberg et al. 2009). However, if the adaptive response is unsuccessful, prolonged activation of the ISR can lead to the induction of autophagy and apoptosis (Kilberg et al. 2009; Kroemer et al. 2010) (Fig. 13.3).

It is known that autophagy is induced in response to a number of stress signals such as nutrient stress, ER stress, hypoxia, anoxia and redox stress, as Gcn2 and Pkr have been shown to up-regulate autophagy in response to nutrient deprivation (Tallóczy et al. 2002; Kroemer et al. 2010; Huang et al. 2013). It has also been found that cells that carry a mutation where eIF2α cannot be phosphorylated are unable to induce autophagy in response to starvation (Kroemer et al. 2010). However, it is still not known how the ISR contributes to autophagy. As mentioned previously, a gene array analysis of the MCK mice revealed an up-regulation of ATF4-inducible genes at an early age of onset (Huang et al. 2013).

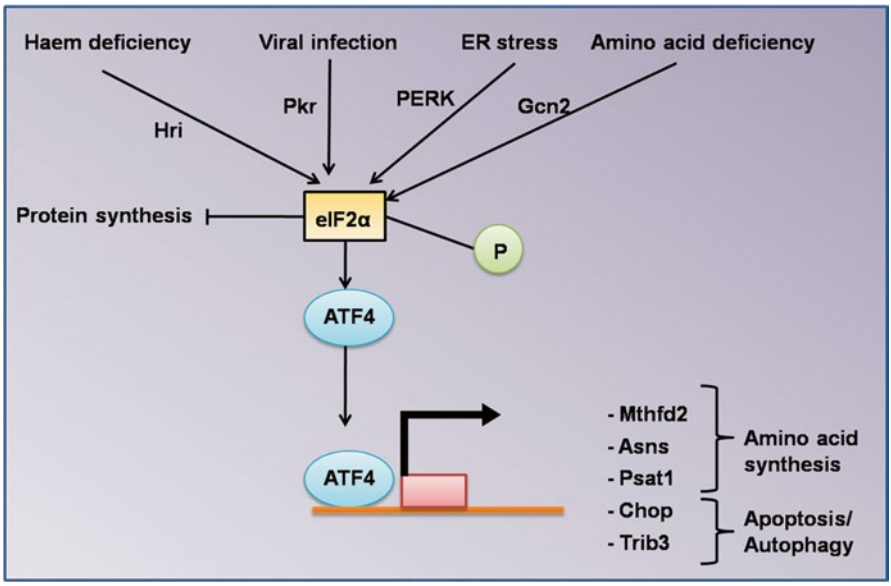

Fig. 13.3 The integrated stress response. Both extra- and intra-cellular stress signals activate the integrated stress response through the phosphorylation of eIF2α by distinct upstream kinases. The subsequent downstream activation of a number of transcription factors leads to either an up-regulation of genes involved in amino acid synthesis, or cell death by either apoptosis or autophagy

These genes included: *asparagine synthase* (*Asns*), *methylenetetrahydrofolate dehydrogenase* (NADP+-dependent) 2 (*Mthfd2*) and *phosphoserine aminotransferase 1* (Psat1) which are associated with amino acid deficiency; *Chop* which has been implicated in different forms of cardiomyopathy leading to heart failure, and which has been observed in apoptotic cardiomyocytes; and *Trib3*, which regulates the ISR *via* negative feedback and inhibits the expression of Asns and Chop (Okada et al. 2004; Fu et al. 2010; Huang et al. 2013). It is also of interest that Chop and Trib3 are necessary for cardiomyocyte apoptosis in the ISR (Okada et al. 2004; Seznec et al. 2004; Ohoka et al. 2005). Although it is not currently known which of the stress signals causes the activation of the ISR in FA, it is known that the activation of the ISR is significant in the progression of cardiomyopathy (Okada et al. 2004). It is therefore important to investigate the significance of the ISR in heart failure.

5 Autophagy

Autophagy is a physiological process that contributes to cell homeostasis, and it occurs in all eukaryotic cells in response to extra- and intra-cellular stress (Yang et al. 2005). The exact role of autophagy is controversial as it is not known if it is a

pro-survival or pro-death response. Most evidence suggests that it is an adaptive response that is responsible for the recycling of long-lived and damaged organelles by lysosomal degradation (Martinet et al. 2007). However, paradoxically, excessive stimulation of the autophagic pathway also appears to result in cell death through the destruction of organelles (Martinet et al. 2007; Nishida et al. 2008).

Some important autophagic markers which are essential for autophagosome formation include Beclin-1 and LC3 (Ohoka et al. 2005; Nishida et al. 2008), which will be discussed below. The mammalian target of rapamycin (mTOR) is a protein kinase that is also essential for the activation of autophagy, as its inhibition by rapamycin or by nutrient stress results in the induction of autophagy (Nemchenko et al. 2011). There is also a very likely interaction between mTOR and UNC-51 like kinase (ULK), which is required for autophagy (Jung et al. 2010). However, it is still not known which phosphorylation sites are targeted by mTOR (Jung et al. 2010).

Autophagy has also been implicated in a number of human neuro-degenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases (Yang et al. 2005). It is also of great interest that autophagy occurs at a high rate in failing human hearts (Elsässer et al. 2004). Hence, a greater understanding of the role of autophagy will enable us to gain more insight into new therapeutic approaches that can be useful for the treatment of many diseases.

5.1 Metabolic Process

There are three pathways in autophagy which include: macroautophagy, microautophagy and chaperone-mediated autophagy, although macroautophagy is the most common form of autophagy (Yang et al. 2005). During macroautophagy, an isolation membrane grows into a double-membrane structure that engulfs cytosolic organelles, thus forming the autophagosome. The autophagosome is targeted to the lysosome, and these fuse to form an autolysosome, where its contents are degraded (Gomes and Scorrano 2013) (Fig. 13.4).

Autophagy is controlled by autophagy-related genes (Atgs), which are largely involved in the formation of the autophagosome (Okada et al. 2004; Yang et al. 2005; Nishida et al. 2008). Beclin-1 (Atg6) and class III phosphatidylinositol-3-kinase are important proteins in the process that form a complex which is needed for the nucleation of the isolation membrane (Yang et al. 2005). The elongation of the isolation membrane then involves one of two conjugation systems. One pathway involves the conjugation of Atg12 to Atg5, and the second pathway involves the conjugation of microtubule-associated protein 1 light chain 3 (LC3). In the second pathway, newly synthesised LC3 is cleaved to form LC3-I, which is found in the cytoplasm (Yang et al. 2005). With the aid of Atg7 and Atg3, LC3-I is then converted to LC3-II which tightly bound to the autophagosome, and it does not dissociate after autophagosome formation (Nishida et al. 2008) (Fig. 13.5). Thus, the conversion of LC3 from LC3-I to LC3-II is used as a marker of autophagy (Yang et al. 2005).

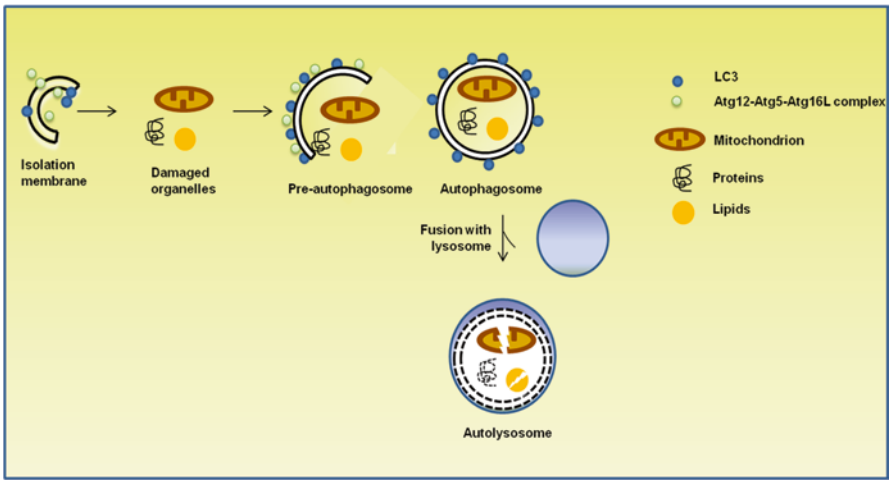

Fig. 13.4 The autophagic pathway (Adapted from Martinet et al. 2007). The Atg12-Atg5-Atg16L complex interacts with the isolation membrane and localises asymmetrically as it elongates in response to stress signals sent from damaged organelles (Yang et al. 2005). The pre-autophagosome engulfs these organelles along with a bulk of cytoplasm, and the Atg-complex dissociates from the membrane upon autophagosome formation (Yang et al. 2005). Thereafter, the autophagosome fuses with a lysosome. The resultant autolysosome is thus responsible for the degradation of its contents

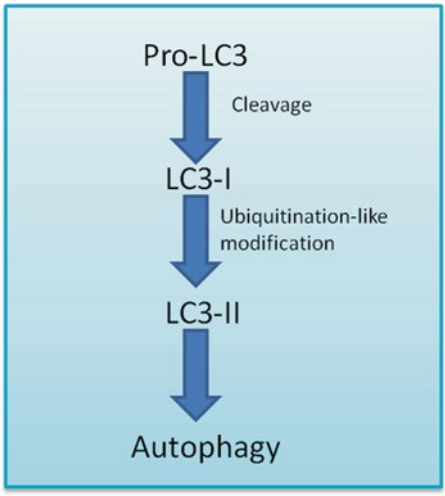

Fig. 13.5 The formation of LC3-II. The carboxyl terminus of pro-LC3 is cleaved by a cysteine protease known as autophagin to form LC3-I (Yang et al. 2005). LC3-I then undergoes a number of ubiquitination-like reactions to form LC3-II, which is tightly bound to the autophagosomal membrane (Yang et al. 2005)

5.1.1 Mitophagy

Autophagy is generally a non-selective process where portions of the cytoplasm are randomly sequestered into the autophagosome (Yang et al. 2005). However, selective forms of autophagy have been found such as mitophagy where the mitochondrion is selectively targeted for degradation in the autophagosome (Gomes and Scorrano 2013).

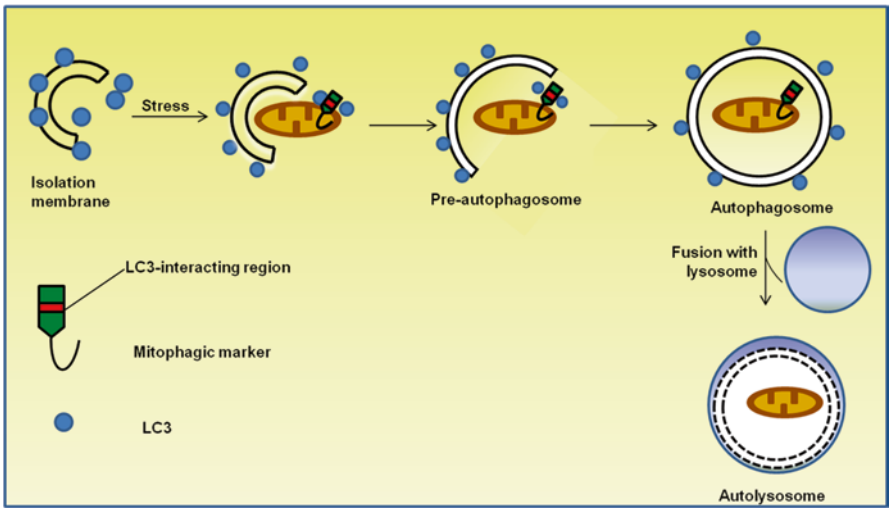

Fig. 13.6 The process of mitophagy (Adapted from Liu et al. 2012). A similar mechanism to the second pathway described in autophagy occurs in mitophagy. Here, a mitochondrial protein with an LC3-interacting region (*LIR*) that is specific for mitophagy is expressed at the outer-membrane. This results in the incorporation of the mitochondrion into the autophagosome, and its subsequent degradation

This has been observed in several different processes such as erythroid differentiation, the metamorphosis of silk moth muscles, and in the development of T-lymphocytes (Beaulaton and Lockshin 1977; Sandoval et al. 2008; Pua et al. 2009).

While mitochondria are essential for energy transduction and cell signalling, damaged and dysfunctional mitochondria can be detrimental to the cell leading to the release of pro-apoptotic factors such as cytochrome *c* (Saelens et al. 2004). Hence, the selective removal of defective mitochondria by mitophagy might be a mechanism to prevent cell death by apoptosis. This selectivity is mediated by the interaction between autophagy modifiers expressed on the surface of the autophagosome such as LC3/GABARAP proteins, and selective autophagic receptors (Gomes and Scorrano 2013) (Fig. 13.6).

A number of markers specific for mitophagy have been identified. This includes Bcl-2/adenovirus E1B 19 kDa-interacting protein (BNIP3) and FUN14 domain containing protein 1 (FUNDC1) which appear to be autophagy receptors that interact with LC3 under hypoxic conditions (Kubli et al. 2007; Schweers et al. 2007; Liu et al. 2012). It has also been found that BNIP3 is up-regulated in cardiomyocytes that suffer ischemic injury (Hamacher-Brady et al. 2007). Indeed, BNIP3 appears to stimulate apoptotic signalling during ischemia-reperfusion injury by inducing the fragmentation of mitochondria that leads to the production of superoxide and pro-apoptotic factors (Hamacher-Brady et al. 2007). It has also been established that BNIP3 is involved in mitophagy, although the mechanism by which this occurs remains unknown (Ohoka et al. 2005; Quinsay et al. 2010; Rikka et al. 2011; Hanna et al. 2012).

It is of significance to this discussion that electron microscopy of hearts from both the MCK and NSE mutant mouse lines revealed the presence of myofibrils with abnormal alignment and disruption, as well as disintegrating mitochondria, which is suggestive of mitochondrial dysfunction (Puccio et al. 2001). It is therefore possible that mitophagy contributes to heart failure in FA, as damaged mitochondria in the disease might induce mitophagy through the expression of selective autophagic receptors like BNIP3 or FUNDC1. However, this is an area of study that has yet to be explored.

5.2 Autophagy in Heart Failure

Heart failure occurs after a process known as cardiac remodelling, where the heart undergoes an alteration in structure and function in response to cardiac load and injury, thus resulting in an increased rate of cardiomyocyte death (Cohn et al. 2000; Nishida et al. 2008). Multiple forms of cell death mechanisms including apoptosis and necrosis have been observed in heart failure (Kostin et al. 2003). However, signs of autophagy from dead and dying cardiomyocytes have also been reported to occur in heart failure caused by dilated cardiomyopathy, hypertensive disease, chronic ischemia, and stunned myocardium, but have not been found in healthy human hearts (Knaapen et al. 2001; Shimomura et al. 2001; Kostin et al. 2003; Elsässer et al. 2004; Yan et al. 2005). In addition, it was found that there is a higher incidence of autophagic cardiomyocytes in heart failure than apoptotic cells (Kostin et al. 2003; Elsässer et al. 2004). It is therefore clear that autophagy is important in the progression of heart failure. However, it is not known if autophagy plays a protective role, or if it in fact contributes to the pathogenesis of heart failure. This is because while autophagy is often associated with heart failure, it is also essential for the survival of cardiomyocytes through the production of free amino acids and fatty acids from the breakdown of proteins and organelles, by the autophagic-lysosomal pathway, which is beneficial to cells (Gustafsson and Gottlieb 2008). It has been reported that autophagy might be beneficial in the progression of cardiomyopathy up to a point, but when it reaches a certain threshold, autophagy could lead to heart failure (Gustafsson and Gottlieb 2008).

Autophagy has been shown to protect against β-adrenergic stimulation which enhances apoptosis, cardiac hypertrophy and heart failure (Shizukuda and Buttrick 2002; Xiang and Kobilka 2003; Nakai et al. 2007). This was observed from a study where autophagy-deficient mice displayed increased sensitivity to isoproterenol, a synthetic β-adrenergic agent, leading to left ventricular dilatation and cardiac dysfunction (Nakai et al. 2007). In addition, cardiac hypertrophy from thyroid hormone treatment was prevented upon treatment of rapamycin, an activator of autophagy (Kuzman et al. 2007). Rapamycin treatment was also able to reduce ischemia-reperfusion injury on Langendorff perfused rat hearts (Beugnet et al. 2003; Ravikumar et al. 2004). Furthermore, it was shown that glucose deprivation, which is important in ischemia, resulted in the up-regulation of autophagy in isolated cardiac myocytes (Gustafsson and Gottlieb 2009). The inhibition of autophagy was correspondingly found to enhance glucose deprivation-mediated death (Matsui et al. 2007).

Even though autophagy appears to play a protective role in ischemic cardiac injury, it was shown from the same study that autophagy had an opposite effect on myocytes during reperfusion (Matsui et al. 2007). In this study, Matsui et al. demonstrated that autophagy was regulated by AMP-activated protein kinase (AMPK) in ischemia, and by Beclin-1 in reperfusion (Matsui et al. 2007). Although AMPK-activated autophagy appeared to be protective in ischemia, the knockdown of Beclin-1 proved to increase cell viability, thus showing that autophagy in the reperfusion phase was more detrimental (Matsui et al. 2007). This reflects the dual nature of the effects autophagy.

Support for the role of autophagy in cardiomyocyte death was found in human patients suffering from idiopathic dilated cardiomyopathy where Beclin-1 was up-regulated during cardiac injury by ischemia-reperfusion (Vigliano et al. 2011). Consistent with these findings, Akazawa et al. demonstrated that cardiomyocyte degeneration was induced in transgenic mice expressing the human diphtheria-toxin receptor in the heart, which led to heart failure and subsequent death in 80 % of the animals (Akazawa et al. 2004). Thereafter, it was found that there were signs of autophagic cell death such as an accumulation of autophagosomes and an up-regulation of lysosomal markers, thus highlighting the potential role of autophagy in cell death (Akazawa et al. 2004). However, it is not known if the presence of autophagic markers signifies a failed protective mechanism or a pathway for cell death. Regardless, it is clear that cardiomyocytes with a loss of organelles will be unable to produce functional contractile power, thus exacerbating the effect of heart failure (Takemura et al. 2006).

In addition to the role that autophagy might play in cell death, under extreme conditions of stress, the loss of trans-membrane potential of the mitochondrial inner membrane can result in the exhaustion of the intracellular supply of ATP, and lead to necrotic cell death (Nishida et al. 2008). Damaged mitochondria can also release of pro-apoptotic factors that lead to apoptotic cell death (Nishida et al. 2008). Multiple forms of cell death are therefore often observed in the pathogenesis of heart failure, and these can be distinguished by a number of characteristic morphological features (Table 13.1) (Kostin et al. 2003). However, it should also be noted that cross-talk exists between these cell death mechanisms, as autophagy and apoptosis have been shown to both counter each other and act synergistically, and they also share many of the same molecular regulators (Eisenberg-Lerner et al. 2009).

6 Apoptosis

Apoptosis is a process of programmed cell death, and it is essential for cell turnover and development (Elmore 2007). It occurs normally as a means of maintaining cell populations in tissues, and it also acts as a defence mechanism in response to cell damage and mutations (Norbury and Hickson 2001). The death process is mediated by three pathways: the extrinsic pathway, the intrinsic pathway and the immune pathway, which can be separated into the granzyme A and granzyme B pathways (Tang et al. 2011).

Table 13.1 Morphological features of different forms of cell death

Cell death features	Apoptosis	Autophagy	Necrosis
Cell shrinkage	++	+	–
Cellular swelling	–	–	++
Chromatin condensation	++	+	–
Nuclear fragmentation	++	–	–
Loss of plasma-membrane integrity	–	–	++
Vacuolization	–	++	+
Caspase activation	++	–	–
Oligonucleosomal DNA fragmentation	++	–	–
Random DNA degradation	–	–	+
Processing of LC3	–	++	–

Autophagy differs from apoptosis in that apoptosis is characterised by cell shrinkage, chromatin condensation and nuclear fragmentation. In addition, caspases are activated in apoptosis, whereas caspase activation is not needed in autophagy (Martinet et al. 2007). The distinction between apoptotic cell death and autophagy can be done through monitoring caspase activity, chromatin degradation and other apoptotic markers, as well as through the observation of the relationship between the specific inhibition of autophagy, and cell death (Aloria et al. 2004; Martinet et al. 2007; Eisenberg-Lerner et al. 2009). Table adapted from Martinet et al. 2007

The extrinsic pathway is initiated by transmembrane death receptors that engage with members of the tumour necrosis factor (TNF) family, and it is responsible for transmitting signals from the cell surface to intracellular signalling pathways by recruiting caspase-8 (Locksley et al. 2001; Saelens et al. 2004; Elmore 2007). The intrinsic pathway, in contrast, acts through intracellular signals and involves non-receptor-mediated stimuli that leads to the activation of caspase-9 (Elmore 2007). Finally, the granzyme A and B pathways are immune pathways that involve T-cell-mediated cytotoxicity and perforin-granzyme-dependent killing of the cell (Elmore 2007).

The extrinsic, intrinsic and granzyme B pathways are initiated by the cleavage of caspase-3 and they have been found to converge into the execution pathway, and ultimately result in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors, and the uptake of phagocytic cells (Elmore 2007). The granzyme A pathway activates a caspase-independent cell death pathway through DNA damage (Elmore 2007). From here on, the focus of the discussion on apoptosis will be on the extrinsic and intrinsic pathways.

6.1 The Intrinsic Pathway

The intrinsic pathway is regulated by positive and negative stimuli, and it is the main mitochondrial pathway in cell death. Some examples of positive stimuli include, radiation, toxins, hypoxia, hyperthermia, viral infection and free radicals

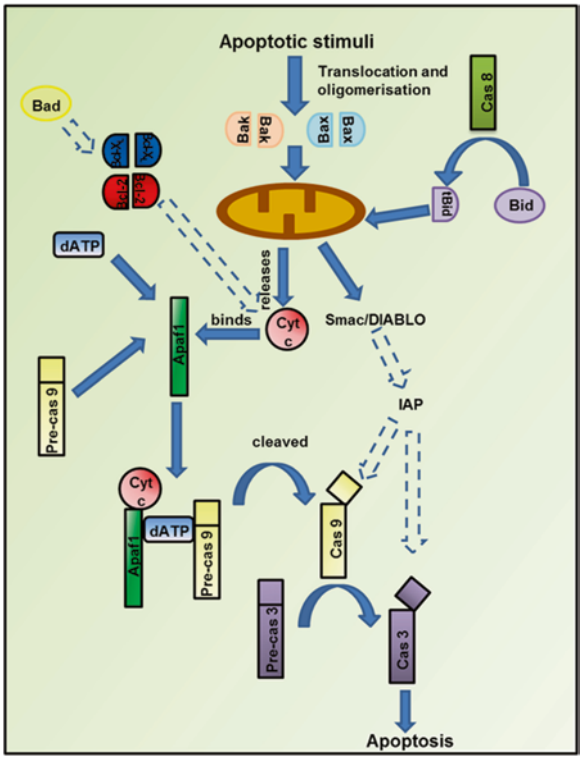

Fig. 13.7 The intrinsic pathway of apoptosis. Apoptotic stimuli that can be either extra- or intracellular, results in the translocation and homo-oligomerisation of Bax and Bak on the mitochondrion, as well as the cleavage of Bid to tBid. This leads to the release of cytochrome c, which binds to Apaf1 and pre-caspase 9 in the presence of dATP. This leads to the cleavage and activation of caspase 9, which cleaves pre-caspase 3. Caspase 3 is thus responsible for execution pathway in apoptosis. The release of Smac/DIABLO inhibits the inhibitors of apoptosis (*IAP*), thus allowing apoptosis to proceed. Members of the Bcl-2 family are able to inhibit cytochrome c release. However, in the presence of Bad, Bcl-2 members are sequestered and the intrinsic pathway is allowed to progress

(Elmore 2007). This can cause changes in the inner mitochondrial membrane that leads to the loss of potential in the mitochondrial permeability transition pore, as well as the release of pro-apoptotic proteins such as cytochrome *c* and Smac/DIABLO. This results in the downstream activation of caspases, and eventual cell death (Saelens et al. 2004) (Fig. 13.7).

This process is mediated by the B-cell lymphoma 2 (Bcl-2) family of proteins which are responsible for the mitochondrial initiation of the cell death programme (Adams and Cory 1998). Members of this family consist of both pro- and anti-apoptotic proteins that share up to four conserved Bcl-2 homology domains (Kubli et al. 2007). Some of the anti-apoptotic proteins include Bcl-2 and Bcl-X_L, and

some of the pro-apoptotic proteins include members of the Bax family which includes Bax and Bak, and the BH3-only proteins such as, Bad, Bid, and BNIP3 (Elmore 2007).

With the exception of Bid, the BH3-only proteins appear to bind to and possibly neutralize the anti-apoptotic proteins (Cory and Adams 2002). For example, Bad heterodimerises with Bcl-X_L or Bcl-2, thus promoting cell death (Yang et al. 1995). When not bound to Bad, Bcl-X_L and Bcl-2 inhibit the release of cytochrome *c* from the mitochondrion (Elmore 2007). Bid, the pro-apoptotic cytosolic protein, is cleaved by caspase 8 and converted to tBid after a death signal from the extrinsic pathway (Kroemer et al. 2007). The tBid protein is then targeted to the mitochondrion and it accelerates apoptosis and triggers cytochrome *c* release in the presence of either Bak or Bax (Kroemer et al. 2007).

Under normal conditions, Bax and Bak are stable monomers. However, they appear to change conformations and homo-oligomerise during apoptosis (Cory and Adams 2002). These oligomers are believed to contribute to the permeabilisation of the outer mitochondrial membrane and allow the efflux of pro-apoptotic proteins, such as cytochrome *c* (Cory and Adams 2002). The mitochondrion is important in apoptosis as reflected by a study whereby spontaneous nuclear condensation that could be inhibited by Bcl-2, and DNA fragmentation, were found to be dependent on the presence of the mitochondrion (Green and Reed 1998). It was also found that cytochrome *c*, which is released from damaged mitochondria, is needed to induce caspase activation (Liu et al. 1996). Cytochrome *c* forms an activation complex with the apoptotic protease-activating factor 1 (Apaf1) and caspase-9 to activate downstream caspase activity (Cory and Adams 2002). It is currently believed that once cytochrome *c* is released, the cell is committed to cell death through either the rapid apoptotic pathway, or a slow necrotic pathway due to the collapse of the electron transport chain, as free radicals are generated and levels of ATP fall (Green and Reed 1998).

6.2 The Extrinsic Pathway

The extrinsic pathway is distinct from the intrinsic pathway in that it involves trans-membrane receptor mediation. These receptors are part of the TNF receptor gene superfamily, and they contain a 'death domain' in the cytoplasmic region (Ashkenazi and Dixit 1998; Locksley et al. 2001). This death domain is important in carrying extracellular signals to intracellular signalling pathways (Cory and Adams 2002). Upon ligand binding to trans-membrane receptors, cytoplasmic adapter proteins bind to these receptors on their death domains (Cory and Adams 2002). The ligation of receptors such as the binding of the Fas ligand to the Fas receptor or the binding of the TNF ligand to the TNF receptor, results in the recruitment of the adapter protein, Fas-associated protein with death domain (FADD), and in the binding of the adapter protein, tumour necrosis factor type 1-associated DEATH domain protein (TRADD) with recruitment of FADD (Hsu et al. 1995). FADD

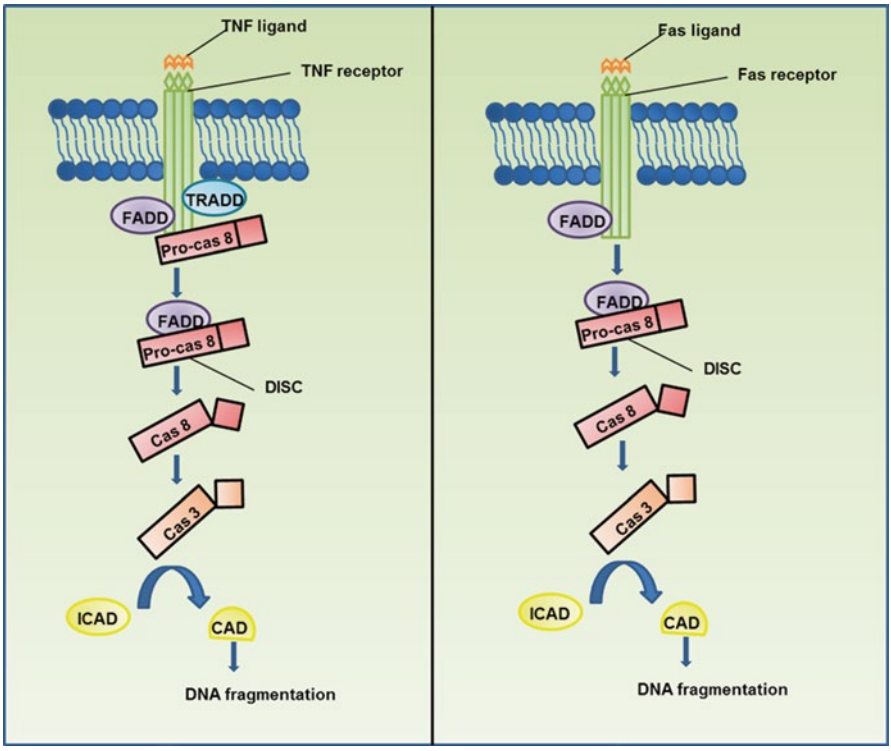

Fig. 13.8 The extrinsic pathway leading to the execution phase of apoptosis. The binding of the TNF ligand to its receptor results in the recruitment of FADD and TRADD, and the eventual activation of caspase 3. A similar process occurs in the binding of Fas to the Fas receptor

then associates with pro-caspase-8 by dimerising with the death effector domain. At this point, a death-inducing signalling complex (DISC) is formed, resulting in the auto-catalytic activation of pro-caspase-8. Once caspase-8 is activated, the execution phase of apoptosis is triggered (Elmore 2007) (Fig. 13.8).

6.3 The Execution Pathway

The extrinsic and intrinsic pathways converge at the execution phase, which is the pathway that carries out cell death (Elmore 2007). The members of the execution phase are the execution caspases which activate cytoplasmic endonuclease, which is responsible for the degradation of nuclear material and proteases that break down nuclear and cytoskeletal proteins.

Caspases 3, 6, and 7 are the effector caspases that are responsible for the activation of proteins that are responsible for the morphological and biochemical changes

that are observed in apoptotic cells (Slee et al. 2001). Caspase 3 is activated by caspases 9, 8 and 10 (Elmore 2007). It is also responsible for the activation of an endonuclease known as CAD which acts to degrade chromosomal DNA and cause chromatin condensation (Sakahira et al. 1998). The last step of apoptosis is characterised by phagocytic uptake. In this phase, phosphatidylserine is externalised on the surface of apoptotic cells and this allows for phagocytic recognition so the apoptotic cell can be cleared (Fadok et al. 2001).

6.4 Apoptosis in Heart Failure

It is generally accepted that heart failure is caused by the loss of cardiomyocytes. Although cells such as neurons and cardiomyocytes have a limited ability to proliferate, they still retain the genes and signal transduction pathways necessary to induce apoptosis (Barr and Tomei 1994). It is also of relevance to this discussion that apoptosis has been reported to occur in several frataxin-deficient cell lines, raising the possibility that apoptosis occurs in the progression of cardiomyopathy in FA (Santos et al. 2010). The participation of apoptosis in heart failure has therefore been a subject of great interest. Signs of apoptosis in advanced heart failure have been found in explanted human hearts (Sabbah 1999). Apoptosis leading to heart failure has also been most commonly identified in idiopathic dilated cardiomyopathy and ischemic cardiomyopathy, and it has also been proposed to contribute to the wall thinning observed in cardiac hypertrophy (Bennett 2002).

Studies have shown a low rate of apoptosis in heart failure (<1 % TUNEL-positive cells), but it must be noted that the detection of apoptosis could be dependent on the stage of the disease (Kang and Izumo 2000). Most of the studies on human patients have been performed at an advanced stage of heart failure, and it is possible that signs of apoptosis were diminished at that point (Kang and Izumo 2000). In addition, higher rates of apoptosis have been observed in rabbits following acute ischemia and reperfusion (Yue et al. 1998). Hence, it appears that the observed occurrence of apoptosis is dependent on the models used, and on the conditions under which cardiomyopathy occurs.

Apoptosis in cardiomyocytes can be induced by stimuli such as hypoxia and ischemia which are associated with the activation of Fas (Bennett 2002). The Fas ligand as well as TNF-α were found to be up-regulated in patients with end-stage heart failure, and this extrinsic pathway also appears to be implicated in immune-mediated cardiomyopathy (Nishigaki et al. 1997; Yue et al. 1998; Kang and Izumo 2000). This suggests the importance of the death receptor-mediated pathway in apoptotic heart failure, although it appears to be more important in immune-mediated cardiomyopathy than in the more prevalent ischemic or dilated cardiomyopathy (Bennett 2002). It has also been observed that lowered serum glucose concentrations trigger the release of cytochrome *c* from the mitochondria in cardiomyocytes, suggesting that ischemic injury might be mediated by the mitochondrial pathway in apoptosis (Bialik et al. 1999; Bennett 2002; Quinsay et al. 2010). It has indeed been shown that cytosolic cytochrome *c* and the release of caspases occurs in different models of

heart failure, and there has also been further evidence to suggest that cardiomyocytes utilise a mitochondrial-dependent apoptotic pathway (Koglin et al. 1999; Narula et al. 1999; Adams et al. 2000; Kang et al. 2000; Yan et al. 2005). Higher levels of Bcl-2 have also been observed after acute coronary occlusion, but they are decreased after chronic heart failure by pressure overload (Misao et al. 1996; Liu et al. 1998; Condorelli et al. 1999). In addition, the over-expression of Bcl-2 in the heart appears to reduce myocardial reperfusion injury by reducing the occurrence of myocyte apoptosis (Brocheriou et al. 2000).

Analysis of hearts from the FA knockout animals have also shown higher apoptosis counts than the age-matched wild-type mice, which is evidence for ongoing cardiomyocyte loss (Payne 2011). In addition, the use of apoptosis inhibitors is able to improve the viability of frataxin-deficient cells *in vitro* (Wong et al. 1999). In all, these studies indicate that apoptosis is an important pathway that is worth investigating in the pathogenesis of cardiomyopathy in FA.

7 Therapeutic Implications

Considering the potential roles of autophagy and apoptosis in the progression of cardiomyopathy in FA, a number of strategies can be employed to inhibit or manipulate these pathways. A number of therapeutics has been found to be effective in experimental models of apoptosis. For example, the administration of caspase inhibitors in areas of risk resulted in the reduction of infarct sizes in rat hearts during ischemia and reperfusion (Yaoita et al. 1998; Holly et al. 1999). However, while it is possible to target apoptotic pathways, it still needs to be shown that inhibition of apoptosis will delay or prevent heart failure. It is possible that the inhibition of apoptosis will result in other forms of cell death such as necrosis, which could be even more damaging (Bennett 2002). In addition, it is not known what the long term effects of inhibiting apoptosis will be, because apoptosis is beneficial in that it is needed for cell renewal and development. It should also be noted that excessive inhibition of apoptosis is known to be associated with autoimmune disorders and lymphoma (Müllauer et al. 2001). Finally, as discussed earlier, the detection of apoptotic markers is dependent on the stage of the disease, as well as other factors. Hence, the inhibition of apoptosis may not be effective for all forms of heart failure, and it may in fact not be beneficial in the treatment of FA.

Autophagic markers were found to be elevated during cardiac injury induced by ischemia-reperfusion (Matsui et al. 2007). In addition, considering the correlation between cardiac hypertrophy and the prognosis in FA, it might be helpful to target players in the autophagic pathway (Vigliano et al. 2011). However, as it is still not known if autophagy has a protective or deleterious effect in cardiac remodelling, the inhibition of autophagy as a therapeutic has to be approached with caution. Moreover, as in apoptosis, much is left to be known about the cross-talk between autophagy, apoptosis and necrosis, and inhibiting one pathway may lead to the stimulation of another.

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References

Adamec J, Rusnak F, Owen WG, Naylor S, Benson LM, Gacy AM, Isaya G (2000) Iron-dependent self-assembly of recombinant yeast frataxin: implications for Friedreich ataxia. Am J Hum Genet 67:549–562

Adams JM, Cory S (1998) The Bcl-2 protein family: arbiters of cell survival. Science 281:1322–1326

Adams JW, Pagel AL, Means CK, Oksenberg D, Armstrong RC, Brown JH (2000) Cardiomyocyte apoptosis induced by Gαq signaling is mediated by permeability transition pore formation and activation of the mitochondrial death pathway. Circ Res 87:1180–1187

Adinolfi S, Trifuoggi M, Politou AS, Martin S, Pastore A (2002) A structural approach to understanding the iron-binding properties of phylogenetically different frataxins. Hum Mol Genet 11:1865–1877

Adinolfi S, Iannuzzi C, Prischi F, Pastore C, Iametti S, Martin SR, Bonomi F, Pastore A (2009) Bacterial frataxin CyaY is the gatekeeper of iron-sulfur cluster formation catalyzed by IscS. Nat Struct Mol Biol 16:390–396

Akazawa H, Komazaki S, Shimomura H, Terasaki F, Zou Y, Takano H, Nagai T, Komuro I (2004) Diphtheria toxin-induced autophagic cardiomyocyte death plays a pathogenic role in mouse model of heart failure. J Biol Chem 279:41095–41103

Aloria K, Schilke B, Andrew A, Craig EA (2004) Iron-induced oligomerization of yeast frataxin homologue Yfh1 is dispensable in vivo. EMBO Rep 5:1096–1101

Ashkenazi A, Dixit VM (1998) Death receptors: signaling and modulation. Science 281:1305–1308

Babcock M, de Silva D, Oaks R, Davis-Kaplan S, Jiralerspong S, Montermini L, Pandolfo M, Kaplan J (1997) Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. Science 276:1709–1712

Barr P, Tomei LD (1994) Apoptosis and its role in human disease. Biotechnology 12:487–493

Beaulaton J, Lockshin RA (1977) Ultrastructural study of the normal degeneration of the intersegmental muscles of Antheraea polyphemus and Manduca sexta (Insecta, Lepidoptera) with particular reference to cellular autophagy. J Morphol 154:39–57

Becker EM, Greer JM, Ponka P, Richardson DR (2002) Erythroid differentiation and protoporphyrin IX down-regulate frataxin expression in Friend cells: characterization of frataxin expression compared to molecules involved in iron metabolism and hemoglobinization. Blood 99:3813–3822

Bencze KZ, Kondapalli KC, Cook JD, McMahon S, Millán-Pacheco C, Pastor N, Stemmler TL (2006) The structure and function of frataxin. Crit Rev Biochem Mol Biol 41:269–291

Bennett MR (2002) Apoptosis in the cardiovascular system. Heart 87:480–487

Beugnet A, Tee AR, Taylor PM, Proud CG (2003) Regulation of targets of mTOR (mammalian target of rapamycin) signalling by intracellular amino acid availability. Biochem J 372:555–566

Bialik S, Cryns VL, Drincic A, Miyata S, Wollowick AL, Srinivasan A, Kitsis RN (1999) The mitochondrial apoptotic pathway is activated by serum and glucose deprivation in cardiac myocytes. Circ Res 85:403–414

Bidichandani SI, Ashizawa T, Patel PI (1998) The GAA triplet-repeat expansion in Friedreich ataxia interferes with transcription and may be associated with an unusual DNA structure. Am J Hum Genet 62:111–121

Bourke T, Keane D (2011) Friedreich's Ataxia: a review from a cardiology perspective. Ir J Med Sci 180:799–805

Bradley JL, Homayoun S, Hart PE, Schapira AHV, Cooper JM (2004) Role of oxidative damage in Friedreich's ataxia. Neurochem Res 29:561–567

Brocheriou V, Hagège AA, Oubenaïssa A, Lambert M, Mallet VO, Duriez M, Wassef M, Kahn A, Menasché P, Gilgenkrantz H (2000) Cardiac functional improvement by a human Bcl-2 transgene in a mouse model of ischemia/reperfusion injury. J Gene Med 2:326–333

Brunk UT, Terman A (2002) Lipofuscin: mechanisms of age-related accumulation and influence on cell function. Free Radic Biol Med 33:611–619

Campuzano V, Montermini L, Lutz Y, Cova L, Hindelang C, Jiralerspong S, Trottier Y, Kish SJ, Faucheux B, Trouillas P, Authier FJ, Dürr A, Mandel JL, Vescovi A, Pandolfo M, Koenig M (1997) Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. Hum Mol Genet 6:1771–1780

Cavadini P, O'Neill HA, Benada O, Isaya G (2002) Assembly and iron-binding properties of human frataxin, the protein deficient in Friedreich ataxia. Hum Mol Genet 11:217–227

Chen JJ (2007) Regulation of protein synthesis by the heme-regulated eIF2α kinase: relevance to anemias. Blood 109:2693–2699

Cohn JN, Ferrari R, Sharpe N (2000) Cardiac remodeling–concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. J Am Coll Cardiol 35:569–582

Condorelli G, Morisco C, Stassi G, Notte A, Farina F, Sgaramella G, de Rienzo A, Roncarati R, Trimarco B, Lembo G (1999) Increased cardiomyocyte apoptosis and changes in proapoptotic and antiapoptotic genes bax and bcl-2 during left ventricular adaptations to chronic pressure overload in the rat. Circulation 99:3071–3078

Cory S, Adams JM (2002) The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer 2:647–656

Cossée M, Puccio H, Gansmuller A, Koutnikova H, Dierich A, LeMeur M, Fischbeck K, Dollé P, Koenig M (2000) Inactivation of the Friedreich ataxia mouse gene leads to early embryonic lethality without iron accumulation. Hum Mol Genet 9:1219–1226

Drysdale J, Arosio P, Invernizzi R, Cazzola M, Volz A, Corsi B, Biasiotto G, Levi S (2002) Mitochondrial ferritin: a new player in iron metabolism. Blood Cells Mol Dis 29:376–383

Dürr A, Cossee M, Agid Y, Campuzano V, Mignard C, Penet C, Mandel JL, Brice A, Koenig M (1996) Clinical and genetic abnormalities in patients with Friedreich's ataxia. N Engl J Med 335:1169–1175

Eisenberg-Lerner A, Bialik S, Simon HU, Kimchi A (2009) Life and death partners: apoptosis, autophagy and the cross-talk between them. Cell Death Differ 16:966–975

Elmore S (2007) Apoptosis: a review of programmed cell death. Toxicol Pathol 35:495–516

Elsässer A, Vogt AM, Nef H, Kostin S, Möllmann H, Skwara W, Bode C, Hamm C, Schaper J (2004) Human hibernating myocardium is jeopardized by apoptotic and autophagic cell death. J Am Coll Cardiol 43:2191–2199

Emond M, Lepage G, Vanasse M, Pandolfo M (2000) Increased levels of plasma malondialdehyde in Friedreich ataxia. Neurology 55:1752–1753

Fadok VA, de Cathelineau A, Daleke DL, Henson PM, Bratton DL (2001) Loss of phospholipid asymmetry and surface exposure of phosphatidylserine is required for phagocytosis of apoptotic cells by macrophages and fibroblasts. J Biol Chem 276:1071–1077

Fenton HJH (1894) LXXIII.-Oxidation of tartaric acid in presence of iron. J Chem Soc Trans 65:899–910

Filla A, De Michele G, Cavalcanti F, Pianese L, Monticelli A, Campanella G, Cocozza S (1996) The relationship between trinucleotide (GAA) repeat length and clinical features in Friedreich ataxia. Am J Hum Genet 59:554–560

Foury F, Cazzalini O (1997) Deletion of the yeast homologue of the human gene associated with Friedreich's ataxia elicits iron accumulation in mitochondria. FEBS Lett 411:373–377

Fu HY, Okada K, Liao Y, Tsukamoto O, Isomura T, Asai M, Sawada T, Okuda K, Asano Y, Sanada S, Asanuma H, Asakura M, Takashima S, Komuro I, Kitakaze M, Minamino T (2010) Ablation of C/EBP homologous protein attenuates endoplasmic reticulum–mediated apoptosis and cardiac dysfunction induced by pressure overload. Circulation 122:361–369

Gakh O, Park S, Liu G, Macomber L, Imlay JA, Ferreira GC, Isaya G (2006) Mitochondrial iron detoxification is a primary function of frataxin that limits oxidative damage and preserves cell longevity. Hum Mol Genet 15:467–479

Gerber J, Mühlenhoff U, Lill R (2003) An interaction between frataxin and Isu1/Nfs1 that is crucial for Fe/Scluster synthesis on Isu1. EMBO Rep 4:906–911

Gibson TJ, Koonin EV, Musco G, Pastore A, Bork P (1996) Friedreich's ataxia protein: phylogenetic evidence for mitochondrial dysfunction. Trends Neurosci 19:465–468

Gomes LC, Scorrano L (2013) Mitochondrial morphology in mitophagy and macroautophagy. Biochim Biophys Acta 1833:205–212

Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281:1309–1312

Guccini I, Serio D, Condò I, Rufini A, Tomassini B, Mangiola A, Maira G, Anile C, Fina D, Pallone F, Mongiardi MP, Levi A, Ventura N, Testi R, Malisan F (2011) Frataxin participates to the hypoxia-induced response in tumors. Cell Death Dis 2:1–7

Gustafsson ÅB, Gottlieb RA (2008) Recycle or die: the role of autophagy in cardioprotection. J Mol Cell Cardiol 44:654–661

Gustafsson ÅB, Gottlieb RA (2009) Autophagy in ischemic heart disease. Circ Res 104:150–158

Hamacher-Brady A, Brady NR, Logue SE, Sayen MR, Jinno M, Kirshenbaum LA, Gottlieb RA, Gustafsson AB (2007) Response to myocardial ischemia/reperfusion injury involves Bnip3 and autophagy. Cell Death Differ 14:146–157

Hanna RA, Quinsay MN, Orogo AM, Giang K, Rikka S, Gustafsson ÅB (2012) Microtubule-associated protein 1 light chain 3 (LC3) interacts with Bnip3 to selectively remove endoplasmic reticulum and mitochondria via autophagy. J Biol Chem 287:19094–19104

Harding AE (1981) Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. Brain 104:589–620

Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, Ron D (2003) An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell 11:619–633

Hein S, Arnon E, Kostin S, Schönburg M, Elsässer A, Polyakova V, Bauer EP, Klövekorn WP, Schaper J (2003) Progression from compensated hypertrophy to failure in the pressure-overloaded human heart. Circulation 107:984–991

Holly TA, Drincic A, Byun Y, Nakamura S, Harris K, Klocke FJ, Cryns VL (1999) Caspase inhibition reduces myocyte cell death induced by myocardial ischemia and reperfusion in vivo. J Mol Cell Cardiol 31:1709–1715

Hsu H, Xiong J, Goeddel DV (1995) The TNF receptor 1-associated protein TRADD signals cell death and NF-κB activation. Cell 81:495–504

Huang ML, Becker EM, Whitnall M, Suryo Rahmanto Y, Ponka P, Richardson DR (2009) Elucidation of the mechanism of mitochondrial iron loading in Friedreich's ataxia by analysis of a mouse mutant. Proc Natl Acad Sci U S A 106:16381–16386

Huang ML, Lane DJ, Richardson DR (2011) Mitochondrial mayhem: the mitochondrion as a modulator of iron metabolism and its role in disease. Antioxid Redox Signal 15:3003–3019

Huang ML-H, Sivagurnathan S, Ting S, Jansson PJ, Austin CJD, Kelly M, Semsarian C, Zhang D, Richardson DR (2013) Molecular and functional alterations in a mouse cardiac model of Friedreich's Ataxia: induction of the eukaryotic stress response via eIF2α phosphorylation and its downstream targets. Am J Pathol 183(3):745–757

Jung CH, Ro SH, Cao J, Otto NM, Kim DH (2010) mTOR regulation of autophagy. FEBS Lett 584:1287–1295

Kang PM, Izumo S (2000) Apoptosis and heart failure: a critical review of the literature. Circ Res 86:1107–1113

Kang PM, Haunstetter A, Aoki H, Usheva A, Izumo S (2000) Morphological and molecular characterization of adult cardiomyocyte apoptosis during hypoxia and reoxygenation. Circ Res 87:118–125

Keller JN, Dimayuga E, Chen Q, Thorpe J, Gee J, Ding Q (2004) Autophagy, proteasomes, lipofuscin, and oxidative stress in the aging brain. Int J Biochem Cell Biol 36:2376–2391

Kilberg MS, Shan J, Su N (2009) ATF4-dependent transcription mediates signaling of amino acid limitation. Trends Endocrinol Metab 20:436–443
Klockgether T, Petersen D, Grodd W, Dichgans J (1991) Early onset cerebellar ataxia with retained tendon reflexes. Brain 114:1559–1573
Knaapen MW, Davies MJ, De Bie M, Haven AJ, Martinet W, Kockx MM (2001) Apoptotic versus autophagic cell death in heart failure. Cardiovasc Res 51:304–312
Koeppen AH (2011) Friedreich's ataxia: pathology, pathogenesis, and molecular genetics. J Neurol Sci 303:1–12
Koglin J, Granville DJ, Glysing-Jensen T, Mudgett JS, Carthy CM, McManus BM, Russell ME (1999) Attenuated acute cardiac rejection in NOS2 -/- recipients correlates with reduced apoptosis. Circulation 99:836–842
Kostin S, Pool L, Elsässer A, Hein S, Drexler HC, Arnon E, Hayakawa Y, Zimmermann R, Bauer E, Klövekorn WP, Schaper J (2003) Myocytes die by multiple mechanisms in failing human hearts. Circ Res 92:715–724
Kroemer G, Galluzzi L, Brenner C (2007) Mitochondrial membrane permeabilization in cell death. Physiol Rev 87:99–163
Kroemer G, Mariño G, Levine B (2010) Autophagy and the integrated stress response. Mol Cell 40:280–293
Kubli DA, Ycaza JE, Gustafsson AB (2007) Bnip3 mediates mitochondrial dysfunction and cell death through Bax and Bak. Biochem J 405:407–415
Kuzman JA, O'Connell TD, Gerdes AM (2007) Rapamycin prevents thyroid hormone-induced cardiac hypertrophy. Endocrinology 148:3477–3484
Lamarche JB, Côté M, Lemieux B (1980) The cardiomyopathy of Friedreich's ataxia morphological observations in 3 cases. Can J Neurol Sci 7:389–396
Larnaout A, Belal S, Zouari M, Fki M, Ben Hamida C, Goebel HH, Ben Hamida M, Hentati F (1997) Friedreich's ataxia with isolated vitamin E deficiency: a neuropathological study of a Tunisian patient. Acta Neuropathol 93:633–637
Levi S, Corsi B, Bosisio M, Invernizzi R, Volz A, Sanford D, Arosio P, Drysdale J (2001) A human mitochondrial ferritin encoded by an intronless gene. J Biol Chem 276:24437–24440
Li K, Besse EK, Ha D, Kovtunovych G, Rouault TA (2008) Iron-dependent regulation of frataxin expression: implications for treatment of Friedreich ataxia. Hum Mol Genet 17:2265–2273
Liu X, Kim CN, Yang J, Jemmerson R, Wang X (1996) Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell 86:147–157
Liu L, Azhar G, Gao W, Zhang X, Wei JY (1998) Bcl-2 and Bax expression in adult rat hearts after coronary occlusion: age-associated differences. Am J Physiol 275:R315–R322
Liu L, Feng D, Chen G, Chen M, Zheng Q, Song P, Ma Q, Zhu C, Wang R, Qi W, Huang L, Xue P, Li B, Wang X, Jin H, Wang J, Yang F, Liu P, Zhu Y, Sui S, Chen Q (2012) Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. Nat Cell Biol 14:177–185
Locksley RM, Killeen N, Lenardo MJ (2001) The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell 104:487–501
Lodi R, Hart PE, Rajagopalan B, Taylor DJ, Crilley JG, Bradley JL, Blamire AM, Manners D, Styles P, Schapira AH, Cooper JM (2001) Antioxidant treatment improves in vivo cardiac and skeletal muscle bioenergetics in patients with Friedreich's ataxia. Ann Neurol 49:590–596
Maring JR, Croarkin E (2007) Presentation and progression of Friedfreich ataxia and implications for physical therapist examination. Phys Ther 87:1687–1696
Martinet W, Knaapen MWM, Kockx MM, De Meyer GRY (2007) Autophagy in cardiovascular disease. Trends Mol Med 13:482–491
Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, Levine B, Sadoshima J (2007) Distinct roles of autophagy in the heart during ischemia and reperfusion. Circ Res 100:914–922
McEwen E, Kedersha N, Song B, Scheuner D, Gilks N, Han A, Chen JJ, Anderson P, Kaufman RJ (2005) Heme-regulated inhibitor kinase-mediated phosphorylation of eukaryotic translation initiation factor 2 inhibits translation, induces stress granule formation, and mediates survival upon arsenite exposure. J Biol Chem 280:16925–16933

Misao J, Hayakawa Y, Ohno M, Kato S, Fujiwara T, Fujiwara H (1996) Expression of bcl-2 protein, an inhibitor of apoptosis, and Bax, an accelerator of apoptosis, in ventricular myocytes of human hearts with myocardial infarction. Circulation 94:1506–1512

Mühlenhoff U, Richhardt N, Ristow M, Kispal G, Lill R (2002) The yeast frataxin homolog Yfh1p plays a specific role in the maturation of cellular Fe/S proteins. Hum Mol Genet 11:2025–2036

Müllauer L, Gruber P, Sebinger D, Buch J, Wohlfart S, Chott A (2001) Mutations in apoptosis genes: a pathogenetic factor for human disease. Mutat Res 488:211–231

Nakai A, Yamaguchi O, Takeda T, Higuchi Y, Hikoso S, Taniike M, Omiya S, Mizote I, Matsumura Y, Asahi M, Nishida K, Hori M, Mizushima N, Otsu K (2007) The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. Nat Med 13:619–624

Nanduri S, Rahman F, Williams BRG, Qin J (2000) A dynamically tuned double-stranded RNA binding mechanism for the activation of antiviral kinase PKR. EMBO J 19:5567–5574

Narula J, Pandey P, Arbustini E, Haider N, Narula N, Kolodgie FD, Dal Bello B, Semigran MJ, Bielsa-Masdeu A, Dec GW, Israels S, Ballester M, Virmani R, Saxena S, Kharbanda S (1999) Apoptosis in heart failure: release of cytochrome c from mitochondria and activation of caspase-3 in human cardiomyopathy. Proc Natl Acad Sci U S A 96:8144–8149

Nemchenko A, Chiong M, Turer A, Lavandero S, Hill JA (2011) Autophagy as a therapeutic target in cardiovascular disease. J Mol Cell Cardiol 51:584–593

Nishida K, Kyoi S, Yamaguchi O, Sadoshima J, Otsu K (2008) The role of autophagy in the heart. Cell Death Differ 16:31–38

Nishigaki K, Minatoguchi S, Seishima M, Asano K, Noda T, Yasuda N, Sano H, Kumada H, Takemura M, Noma A, Tanaka T, Watanabe S, Fujiwara H (1997) Plasma Fas ligand, an inducer of apoptosis, and plasma soluble Fas, an inhibitor of apoptosis, in patients with chronic congestive heart failure. J Am Coll Cardiol 29:1214–1220

Norbury CJ, Hickson ID (2001) Cellular responses to DNA damage. Annu Rev Pharmacol Toxicol 41:367–401

Ohoka N, Yoshii S, Hattori T, Onozaki K, Hayashi H (2005) TRB3, a novel ER stress-inducible gene, is induced via ATF4-CHOP pathway and is involved in cell death. EMBO J 24:1243–1255

Okada K, Minamino T, Tsukamoto Y, Liao Y, Tsukamoto O, Takashima S, Hirata A, Fujita M, Nagamachi Y, Nakatani T, Yutani C, Ozawa K, Ogawa S, Tomoike H, Hori M, Kitakaze M (2004) Prolonged endoplasmic reticulum stress in hypertrophic and failing heart after aortic constriction. Circulation 110:705–712

Oktay Y, Dioum E, Matsuzaki S, Ding K, Yan LJ, Haller RG, Szweda LI, Garcia JA (2007) Hypoxia-inducible factor 2α regulates expression of the mitochondrial aconitase chaperone protein frataxin. J Biol Chem 287:11750–11756

Palomo GM, Cerrato T, Gargini R, Diaz-Nido J (2011) Silencing of frataxin gene expression triggers p53-dependent apoptosis in human neuron-like cells. Hum Mol Genet 20:2807–2822

Papanikolaou G, Pantopoulos K (2005) Iron metabolism and toxicity. Toxicol Appl Pharmacol 202:199–211

Payne MR (2011) The heart in Friedreich's ataxia: basic findings and clinical implications. Prog Pediatr Cardiol 31:103–109

Pilch J, Jamroz E, Marsza E (2002) Topical review: Friedreich's ataxia. J Child Neurol 17:315

Pua HH, Guo J, Komatsu M, He YW (2009) Autophagy is essential for mitochondrial clearance in mature T lymphocytes. J Immunol 182:4046–4055

Puccio H, Simon D, Cossée M, Criqui-Filipe P, Tiziano F, Melki J, Hindelang C, Matyas R, Rustin P, Koenig M (2001) Mouse models for Friedreich ataxia exhibit cardiomyopathy, sensory nerve defect and Fe-S enzyme deficiency followed by intramitochondrial iron deposits. Nat Genet 27:181–186

Quinsay MN, Thomas RL, Lee Y, Gustafsson ÅB (2010) Bnip3-mediated mitochondrial autophagy is independent of the mitochondrial permeability transition pore. Autophagy 6:855–862

Radisky DC, Babcock MC, Kaplan J (1999) The yeast frataxin homologue mediates mitochondrial iron efflux. J Biol Chem 274:4497–4499

Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, Scaravilli F, Easton DF, Duden R, O'Kane CJ, Rubinsztein DC (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat Genet 36:585–595

Richardson DR, Lane DJ, Becker EM, Huang ML, Whitnall M, Suryo Rahmanto Y, Sheftel AD, Ponka P (2010) Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion and cytosol. Proc Natl Acad Sci U S A 107:10775–10782

Rikka S, Quinsay MN, Thomas RL, Kubli DA, Zhang X, Murphy AN, Gustafsson ÅB (2011) Bnip3 impairs mitochondrial bioenergetics and stimulates mitochondrial turnover. Cell Death Differ 18:721–731

Rouault TA, Tong WH (2005) Iron-sulphur cluster biogenesis and mitochondrial iron homeostasis. Nat Rev Mol Cell Biol 6:345–351

Rustin P, von Kleist-Retzow JC, Chantrel-Groussard K, Sidi D, Munnich A, Rötig A (1999) Effect of idebenone on cardiomyopathy in Friedreich's ataxia: a preliminary study. Lancet 354:477–479

Sabbah HN (1999) Apoptotic cell death in heart failure. Cardiovasc Res 45:704–712

Saelens X, Festjens N, Vande Walle L, van Gurp M, van Loo G, Vandenabeele P (2004) Toxic proteins released from mitochondria in cell death. Oncogene 23:2861–2874

Sakahira H, Enari M, Nagata S (1998) Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature 391:96–99

Sandoval H, Thiagarajan P, Dasgupta SK, Schumacher A, Prchal JT, Chen M, Wang J (2008) Essential role for Nix in autophagic maturation of erythroid cells. Nature 454:232–235

Santos MM, Ohshima K, Pandolfo M (2001) Frataxin deficiency enhances apoptosis in cells differentiating into neuroectoderm. Hum Mol Genet 10:1935–1944

Santos R, Lefevre S, Sliwa D, Seguin A, Camadro JM, Lesuisse E (2010) Friedreich ataxia: molecular mechanisms, redox considerations, and therapeutic opportunities Antiox Redox. Signal 13:651–690

Schulz JB, Dehmer T, Schöls L, Mende H, Hardt C, Vorgerd M, Bürk K, Matson W, Dichgans J, Beal MF, Bogdanov MB (2000) Oxidative stress in patients with Friedreich ataxia. Neurology 55:1719–1721

Schulz JB, Boesch S, Bürk K, Dürr A, Giunti P, Mariotti C, Pousset F, Schöls L, Vankan P, Pandolfo M (2009) Diagnosis and treatment of Friedreich ataxia: a European perspective. Nat Rev Neurol 5:222–234

Schweers RL, Zhang J, Randall MS, Loyd MR, Li W, Dorsey FC, Kundu M, Opferman JT, Cleveland JL, Miller JL, Ney PA (2007) NIX is required for programmed mitochondrial clearance during reticulocyte maturation. Proc Natl Acad Sci U S A 104:19500–19505

Sedlak TL, Chandavimol M, Straatman L (2004) Cardiac transplantation: a temporary solution for Friedreich's ataxia-induced dilated cardiomyopathy. J Heart Lung Transplant 23:1304–1306

Seznec H, Simon D, Monassier L, Criqui-Filipe P, Gansmuller A, Rustin P, Koenig M, Puccio H (2004) Idebenone delays the onset of cardiac functional alteration without correction of Fe-S enzymes deficit in a mouse model for Friedreich ataxia. Hum Mol Genet 13:1017–1024

Seznec H, Simon D, Bouton C, Reutenauer L, Hertzog A, Golik P, Procaccio V, Patel M, Drapier JC, Koenig M, Puccio H (2005) Friedreich ataxia: the oxidative stress paradox. Hum Mol Genet 14:463–474

Shimomura H, Terasaki F, Hayashi T, Kitaura Y, Isomura T, Suma H (2001) Autophagic degeneration as a possible mechanism of myocardial cell death in dilated cardiomyopathy. Jpn Circ J 65:965–968

Shizukuda Y, Buttrick PM (2002) Subtype specific roles of β-adrenergic receptors in apoptosis of adult rat ventricular myocytes. J Mol Cell Cardiol 34:823–831

Slee EA, Adrain C, Martin SJ (2001) Executioner caspase-3, -6, and -7 perform distinct, non-redundant roles during the demolition phase of apoptosis. J Biol Chem 276:7320–7326

Takemura G, Miyata S, Kawase Y, Okada H, Maruyama R, Fujiwara H (2006) Autophagic degeneration and death of cardiomyocytes in heart failure. Autophagy 2:212–214

Taketani S (1994) Molecular and genetic characterization of ferrochelatase. Stem Cells 12:41–54
Tallóczy Z, Jiang W, Virgin HW 4th, Leib DA, Scheuner D, Kaufman RJ, Eskelinen EL, Levine B (2002) Regulation of starvation- and virus-induced autophagy by the eIF2α kinase signaling pathway. Proc Natl Acad Sci U S A 99:190–195
Tang D, Lotze MT, Kang R, Zeh HJ (2011) Apoptosis promotes early tumorigenesis. Oncogene 30:1851–1854
Velasco-Sánchez D, Aracil A, Montero R, Mas A, Jiménez L, O'Callaghan M, Tondo M, Capdevila A, Blanch J, Artuch R, Pineda M (2011) Combined therapy with idebenone and deferiprone in patients with Friedreich's ataxia. Cerebellum 10:1–8
Vigliano CA, Cabeza Meckert PM, Diez M, Favaloro LE, Cortés C, Fazzi L, Favaloro RR, Laguens RP (2011) Cardiomyocyte hypertrophy, oncosis, and autophagic vacuolization predict mortality in idiopathic dilated cardiomyopathy with advanced heart failure. J Am Coll Cardiol 57:1523–1531
Wong A, Yang J, Cavadini P, Gellera C, Lonnerdal B, Taroni F, Cortopassi G (1999) The Friedreich's ataxia mutation confers cellular sensitivity to oxidant stress which is rescued by chelators of iron and calcium and inhibitors of apoptosis. Hum Mol Genet 8:425–430
Xiang Y, Kobilka BK (2003) Myocyte adrenoceptor signaling pathways. Science 300:1530–1532
Yan L, Vatner DE, Kim SJ, Ge H, Masurekar M, Massover WH, Yang G, Matsui Y, Sadoshima J, Vatner SF (2005) Autophagy in chronically ischemic myocardium. Proc Natl Acad Sci U S A 102:13807–13812
Yang E, Zha J, Jockel J, Boise LH, Thompson CB, Korsmeyer SJ (1995) Bad, a heterodimeric partner for Bcl-xL and Bcl-2, displaces bax and promotes cell death. Cell 80:285–291
Yang YP, Liang ZQ, Gu ZL, Qin ZH (2005) Molecular mechanism and regulation of autophagy. Acta Pharmacol Sin 26:1421–1434
Yaoita H, Ogawa K, Maehara K, Maruyama Y (1998) Attenuation of ischemia/reperfusion injury in rats by a caspase inhibitor. Circulation 97:276–281
Yoon T, Cowan JA (2003) Iron-sulfur cluster biosynthesis. Characterization of frataxin as an iron donor for assembly of [2Fe-2S] clusters in ISU-type proteins. J Am Chem Soc 125:6078–6084
Yoon T, Cowan JA (2004) Frataxin-mediated iron delivery to ferrochelatase in the final step of heme biosynthesis. J Biol Chem 279:25943–25946
Yue TL, Ma XL, Wang X, Romanic AM, Liu GL, Louden C, Gu JL, Kumar S, Poste G, Ruffolo RR Jr, Feuerstein GZ (1998) Possible involvement of stress-activated protein kinase signaling pathway and Fas receptor expression in prevention of ischemia/reperfusion-induced cardiomyocyte apoptosis by Carvedilol. Circ Res 82:166–174

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