Chapter 15
Oxidative Stress in Cancer

Peter Storz

Abstract Increased oxidative stress is a common feature observed in many different types of cancer. Depending on the radical formed, its concentration, and cellular location where its generation occurs, reactive oxygen species (ROS) have multiple functions within tumor cells. ROS-induced macromolecule damage can contribute to tumor initiation. Low levels of ROS can initiate cellular signaling pathways that mediate tumor cell proliferation, survival and tumor progression to a metastatic phenotype. High levels of ROS initiate signaling pathways that mediate tumor cell death, but also contribute to formation of cancer stem cells that induce tumor recurrence. Understanding the multitude and complexity of ROS-regulated pathways in cancer cells and targeted modulation of intracellular ROS levels using antioxidants or chemotherapy at different stages of tumor progression may be an effective strategy for combination therapy.

Keywords Oxidative stress • Reactive oxygen species • Antioxidants • Tumor • Cancer

Abbreviations

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2DG	2-deoxyglucose
4-HNE	4-Hydroxy-noneal
Ask1	apoptosis signal-regulating kinase-1
ATM	ataxia telangiectasia mutated
BSO	L-S, R-buthionine sulphoximine
CSC	cancer stem cells

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DR   death receptor
DRL   death receptor ligand
EGF   epidermal growth factor
EGF-R   epidermal growth factor-receptor
Erk1/2   extracellular-regulated kinases 1/2
FOXO   forkhead homeobox type O
GF   growth factor
GF-R   growth factor receptor
GSH   glutathione
GSSG   glutathione disulphide
GPX   glutathione peroxidase
GST   Glutathione S-transferase
H_2O_2 hydrogen peroxide
HIF-1   hypoxia-inducible factor-1
JNK   c-Jun N-terminal Kinase
MAPK   mitogen-activated protein kinase
MMP   matrix metalloproteinase
MPTP   mitochondrial permeability transition pore
mROS   mitochondrial ROS
NF-κB   nuclear factor κ-B
NOX   NADPH oxidase
PDK1   phosphoinositide-dependent kinase 1
PDGF   platelet-derived growth factor
PDGF-R   platelet-derived growth factor-receptor
PI3-K   phosphatidylinositol 3-kinase
PKC   protein kinase C
PKD   protein kinase D
ROS   reactive oxygen species
Prdx   peroxiredoxin
PTEN   phosphatase and tensin homologue
SOD   superoxide dismutase
TGFβ   transforming growth factor β
TIMP   tissue inhibitor of metalloproteinases
TNFα   tumor necrosis factor α
VEGF   vascular epithelial growth factor.

15.1 Oxidative Stress in Cancer Cells

Oxidative stress occurs when an imbalance between pro-oxidant and anti-oxidant molecules alters the redox potential in a cell with a net effect of increasing ROS levels. Accumulation of intracellular oxidative stress can lead to the conversion of normal cells to cancer cells, and increased levels of oxidative stress are hallmarks of many cancers (Storz 2005; Trachootham et al. 2006). Oxidative stress-induced
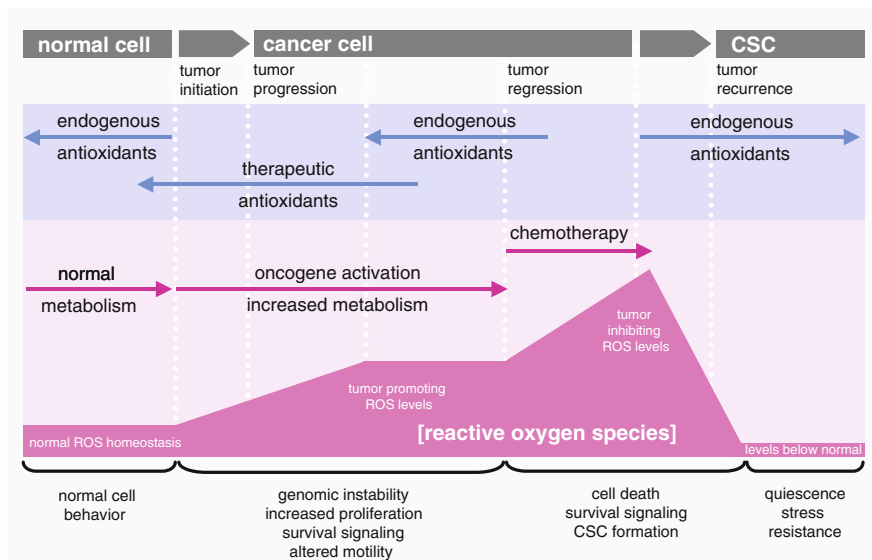
Fig. 15.1 Relations between cellular or therapy-induced oxidative stress and cellular or therapeutic antioxidants at different stages of tumor biology. While in normal cells antioxidants and ROS generation are in balance (normal homeostasis), increased ROS levels are hallmarks of tumor cells. In tumors increased ROS generation can lead to genomic instability, increased cell proliferation, survival signaling and increased cell motility. At this stage, lowering intracellular ROS levels by therapeutic antioxidants may have regulatory effects on tumor progression. Exuberant increase in ROS can be achieved by chemotherapy and can mediate tumor regression by inducing irreparable cell damage and tumor cell death. However, some tumor cells can develop stress resistance by upregulating antioxidant systems. Such cancer stem cells (*CSCs*) are highly resistant to stresses and after clonal expansion can be responsible for tumor recurrence

macromolecule damage and ROS-regulated signaling events have been reported to affect all aspects of tumor formation and progression, including genomic instability and mutagenesis, energy production, cell proliferation, survival and chemoresistance, increased cell motility and metastasis, stemness and recurrence, and angiogenesis (Fig. 15.1).

However, when produced in excess or when cancer cell antioxidant systems fail, they can contribute to tumor cell death. Tumor cells react to increases in oxidative stress by upregulating antioxidant systems, with the outcome that a balance is established that allows beneficiary effects of ROS, but avoids damaging effects that induce cell death. The cellular signaling pathways, by which such fine tuning is accomplished are little known, and some of the ROS-sensing proteins or molecules that contribute to such signaling are discussed in this chapter. Moreover, ROS-sensitive signaling pathways are constitutively switched on in many cancer cells and they participate in all aspects of tumor biology.

The sources for intracellular oxidative stress in cancer cells are manifold and different reactive oxygen species are generated. These include radicals such as

superoxide ($O_2 \cdot^-$), nitric oxide (NO·), or hydroxyl radicals (·OH), as well as non-radicals such as hydrogen peroxide (H_2O_2). Among the variety of cellular ROS that can play roles in cancer, hydrogen peroxide, superoxide and hydroxyl radicals are the best studied in cancer cells (Liou and Storz 2010). Hydroxyl ions are macromolecule damaging molecules and can contribute to DNA damage and genomic instability. Superoxide and hydrogen peroxide have roles in cell signaling (Finkel 2000; Sundaresan et al. 1995).

15.1.1   Sources for ROS in Cancer Cells

In cancer cells, several sources can be responsible for increased oxidative stress levels. Conditions leading to increased ROS production include increased oncogenic signaling, increased activity of mitochondria or peroxisomes, or increased metabolic and enzymatic activity (Liou and Storz 2010; Babior 1999; Szatrowski and Nathan 1991). Additional inducers of oxidative stress in tumor tissue are infiltrating immune cells such as macrophages (Storz 2005).

Growth factors and cytokines such as tumor necrosis factor α (TNFα), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor β (TGFβ), or insulin can induce intracellular ROS production in cancer cells. Most of them induce the formation of superoxide, which then is converted to hydrogen peroxide (Storz 2005; Sundaresan et al. 1995; Bae et al. 2000; Lo and Cruz 1995; Meier et al. 1989; Ohba et al. 1994; Tiku et al. 1990). Additionally, oncogenic mutations of molecules downstream of growth factor receptors such as K-ras lead to increased superoxide production (Minamoto et al. 2000, 2002). Another downstream effector of many growth factor receptors, including epidermal growth factor-receptor (EGF-R) and c-Met, is the small RhoGTPase Rac-1 (Ferraro et al. 2006). Both, oncogenic K-ras and Rac-1 have been shown to induce superoxide production either via NADPH oxidases (NOX) or at the mitochondria (Liou and Storz 2010; Chiarugi and Fiaschi 2007).

Increased superoxide generation at the mitochondria is also induced by mitochondrial dysfunction (Cadenas 2004; Dayal et al. 2009; Pelicano et al. 2009). Mitochondrial respiration produces superoxide as a byproduct of oxidative phosphorylation at complexes I (NADH-ubiquinone oxidoreductase) and III (ubiquinol-cytochrome c oxidoreductase). Superoxide is released into the mitochondrial matrix and the inter-membrane space, but can be released into the cytoplasm via the mitochondrial permeability transition pore (MPTP) (Crompton 1999; Storz 2006). Superoxide usually has a very short half-life and rapidly is dismutated into hydrogen peroxide. This step is mediated by superoxide dismutase (SOD) enzymes. Its breakdown product hydrogen peroxide is a *bona fide* second messenger since it is highly diffusible and can regulate the activity of signaling molecules by direct and reversible oxidation (Liou and Storz 2010; Chiarugi and Fiaschi 2007; Rhee et al. 2000).

Other respiratory organelles that can contribute to increased superoxide and hydrogen peroxide levels in cancer cells are the peroxisomes (Dansen and Wirtz 2001). Peroxisomes generate ROS through acyl-CoA oxidase and xanthine oxidase (del Rio et al. 1992; Singh 1996). Additionally contributing to increased intracellular ROS levels in cancer cells are increased activities of metabolic enzymes such as oxidases, cyclo-oxygenases, lipoxygenases, or thymidine phosphorylase (reviewed in Liou and Storz 2010).

Macrophages or other inflammatory cells (i.e. neutrophils, eosinophils) infiltrating the tumor tissue can be a source of external oxidative stress. Macrophages infiltration into normal tissues may contribute to tumor initiation since infections or inflammation (i.e. pancreatitis) have been identified as a critical risk factor for tumor development (Balkwill 2009). Macrophages can produce and release reactive oxygen species such a superoxide, nitric oxide or hydrogen peroxide (respiratory burst), and released components such as nitric oxide and superoxide can react with each other to generate the highly toxic peroxinitrite radicals (Babior 1999; Cui et al. 1994). Moreover, macrophages can secrete cytokines such as TNFα to induce ROS production in target cells (Babior 1999; Balkwill 2009).

15.1.2 Endogenous Antioxidants in Cancer Cells

Increases in oxidative stress in normal cells can lead to mutations and initiate cancer. In proliferating cancer cells increased ROS levels that are induced by above described mechanisms need to be kept in check to facilitate optimal growth conditions. Cancer stem cells (CSC) often have upregulated antioxidant systems that make them unresponsive to chemotherapy. The detoxification from ROS in cancer cells can be mediated by several antioxidant enzymes.

Depletion of cancer cells from superoxide is occurs through upregulation of superoxide dismutase enzymes. These include MnSOD, Cu/ZnSOD and FeSOD. All are metallo-enzymes that utilize divalent metal ions as cofactors to dismutate superoxide to hydrogen peroxide. Different isoforms of SODs are located at distinct compartments in the cell. For example, MnSOD is located in the mitochondria matrix and Cu/ZnSOD in the cytosol (Liou and Storz 2010). SOD enzymes are encoded by nuclear genes and signaling pathways exist by which increases in mitochondrial oxidative stress are sensed and translated to nuclear gene induction (Storz 2006, 2007; Storz et al. 2005a). The breakdown product of superoxide in SOD-catalyzed dismutase reactions is hydrogen peroxide.

Multiple enzymes detoxify cells from hydrogen peroxide by generating water and oxygen. Most prominent are catalase enzymes located in the cytosol and peroxisomes (Bendayan and Reddy 1982; Hashimoto and Hayashi 1990; Litwin et al. 1987). Peroxiredoxins (Prdx) are thioredoxin peroxidases that catalyze the reduction of hydrogen peroxide, organic hydroperoxides and peroxynitrite (Hofmann et al. 2002; Rhee et al. 1999; Wood et al. 2003). Disregulation of enzymes that detoxify

cells from hydrogen peroxide can have dramatic effects on cancer development or progression. For example, the knockout of the Prdx1 gene in mice leads to dramatically increased levels of oxidative stress and animals prematurely die of cancer (Neumann et al. 2003).

Glutathione peroxidases (GPX) mediate the breakdown of hydrogen peroxide and organic hydroperoxides (Brigelius-Flohe 1999). Glutathione (GSH) protects cellular proteins from oxidative stress by reducing disulphide bonds to cysteins. During this process GSH gets oxidized to glutathione disulphide (GSSG). Glutathione reductase enzymes then recycle GSSG to GSH (Beutler 1969). These enzymes are constitutively active in many tumor cells. Glutathione S-transferases (GST) are overexpressed in a variety of tumors and catalyze the conjunction of GSH to electrophilic compounds (Sharma et al. 2004; Townsend and Tew 2003). Glutathione S-transferases have been shown to contribute to chemoresistance, as well as to regulate cellular signaling pathways (Townsend and Tew 2003).

15.2 Altered ROS Levels – Consequences for Cancer Cells

15.2.1 ROS in Macromolecule Damage and Tumor Initiation

Increased oxidative stress in cells can induce the damage of macromolecules including DNA, proteins and lipids. This can have a role in tumor initiation (DNA damage and mutation). Additionally, altered proteins and lipids could serve as biomarkers.

DNA damaging effects of ROS are mostly induced by hydroxyl ions since hydrogen peroxide for example is not very reactive towards DNA. Hydroxyl ions are radicals that are highly diffusible. They can initiate the formation of DNA lesions through mediating single-strand or double-strand breaks or by oxidation of DNA bases and formation of DNA adducts (Maynard et al. 2009; Wiseman and Halliwell 1996). If cells harboring such DNA modifications escape programmed cell death they can continue to proliferate, raising the likelihood for cancerous growth.

Oxidation of proteins can alter their function, including inactivation or constitutive activation, which can contribute to oncogenic growth. Examples are direct oxidation of signaling molecules that lead to their activation (i.e. K-ras, Src) (Lander et al. 1997; Nakashima et al. 2002; Sun and Kemble 2009) or their inactivation (i.e. phosphatases, DNA repair enzymes) (Meng et al. 2002). In contrast to such specific ROS-regulated mechanisms, oxidative stress can induce less-specific modifications including formation of crosslinked and glycated proteins, increased protein carbonylation, nitration of amino-acid residues and protein degradation (Levine 2002; Squier and Bigelow 2000; Wells-Knecht et al. 1997).

Finally, by reacting with polyunsatureated or polydesaturated fatty acids ROS can induce lipid peroxidation (Gardner 1989; North et al. 1994). Lipid peroxidation generates several genotoxic molecules such as reactive aldehydes that can

modify proteins and DNA. As an example, 4-Hydroxy-noneal (4-HNE) is the most cytotoxic lipid peroxidation product. 4-HNE can diffuse away from its membrane productions site and covalently modify proteins to alter their function. Clinical studies show that lipid peroxidation events induced by ROS can be used as tumor markers in the serum of patients (Lauschke et al. 2002).

15.2.2   ROS in Tumor Cell Proliferation

Tumor cell proliferation can be induced by increased production of superoxide at the mitochondria (Burdon 1995; Burdon et al. 1990; Parkash et al. 2006). The increased expression of MnSOD depletes superoxide levels and mediates quiescence (Wang et al. 2005). For example, stable expression of MnSOD in pancreatic cancer cells, which are known for high intracellular oxidative stress levels, reduced their proliferation rate (Cullen et al. 2003). This led to the hypothesis that mitochondrial superoxide drives proliferation of cells, whereas SOD-induced conversion into hydrogen peroxide drives quiescence, identifying MnSOD as a mitochondrial ROS switch that determines cell fate (Sarsour et al. 2008).

Tumor cell proliferation is regulated by ROS-mediated alterations in cell cycle control and induction of signaling pathways. Cellular signaling mechanisms that are used by superoxide to induce cell proliferation are not well defined. One *bona fide* signaling pathway that can regulate tumor cell proliferation and may be activated by superoxide, but also its breakdown product hydrogen peroxide, is the mitogen-activated protein kinase (MAPK) cascade (Irani et al. 1997; Kumar et al. 2008; McCubrey et al. 2007; Roberts and Der 2007). Extracellular-regulated kinases 1/2 (Erk1/2) signaling is activated by growth factors and K-ras activating mutations (Roberts and Der 2007; Khavari and Rinn 2007). Mechanisms of how Erk1/2 is activated by oxidative stress are either direct oxidation and activation of Ras (Lander et al. 1997), ROS-induced inactivation of negatively-regulatory phosphatases (Chan et al. 2008), or activation of other upstream kinases such as p90[RSK] (McCubrey et al. 2007). Other ROS-sensing signaling proteins are Akt, protein kinase C (PKC) or protein kinase D (PKD) enzymes, and the phosphatidylinositol 3-kinase (PI3-K)/Akt as well as the PKC-PKD pathways can induce cell proliferation in various cancer cell lines (Storz 2005; Rozengurt 2011). However, above signaling molecules were shown to be activated by hydrogen peroxide rather than superoxide (Liou and Storz 2010; Song et al. 2009; Storz and Toker 2003; Prasad et al. 2000).

Loss of redox control or increased oxidative stress can also mediate cell cycle progression by upregulating gene transcription of cyclins (Felty et al. 2005; Menon et al. 2005; Ruiz-Ramos et al. 2009). Another cell cycle-related protein activated by ROS is ataxia telangiectasia mutated (ATM) (Ditch and Paull 2012). Animal models lacking ATM have high levels of ROS and increased oxidative stress-induced damage (Browne et al. 2004). Similar is found in patients lacking ATM (Reichenbach et al. 2002).
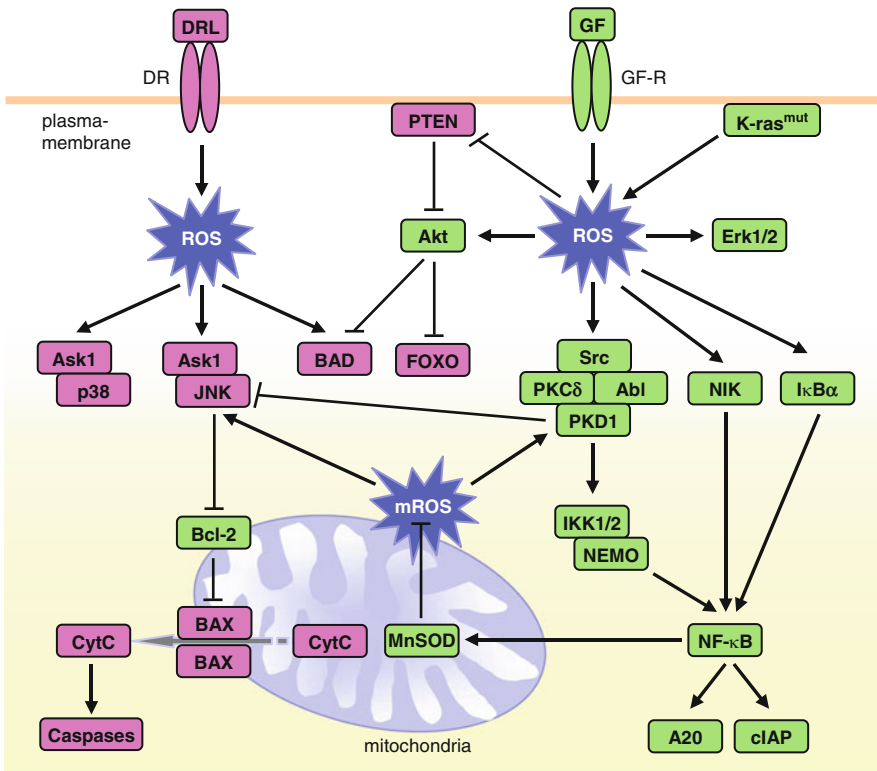
Fig. 15.2 ROS signaling in tumor cell survival and apoptosis. Reactive oxygen species (*ROS*) either generated at the plasma membrane through growth factor receptors (*GF-R*) or death receptors (*DR*) or at the mitochondria (*mROS*) can initiate both protective and cell death signaling pathways. The activation of these pathways is also dependent on radical formed and its concentration. Shown in *green* are protective signaling molecules and their pathways of activation. Shown in *red* are signaling molecules that contribute to cell death in response to ROS

15.2.3 ROS in Tumor Cell Survival and Apoptosis

Dependent on the radical formed, cellular location where this occurs, as well as its concentration, reactive oxygen species can have divergent effects on cell survival and cell death pathways (Fig. 15.2).

Mitochondria-generated oxidative stress and treatment of cells with hydrogen peroxide can lead to the recruitment and activation of a signaling complex consisting of Src, Abl, PKCδ, and PKD1 that initiates activation of the transcription factor nuclear factor-κB (NF-κB) via the canonical IKK complex and phosphorylation and degradation of IκBα, an inhibitory protein for NF-κB (Storz 2006; Storz et al. 2004a, b, 2005a; Song et al. 2009; Storz and Toker 2003; Chiu et al. 2007; Mihailovic et al. 2004). Target genes for NF-κB in this signaling pathway encode

anti-apoptotic proteins such as cIAP and A20, as well as antioxidant proteins including MnSOD (Storz 2007; Storz et al. 2005a, b). Inhibition of this pathway in tumor cells renders them susceptible to ROS-mediated cell death (Storz and Toker 2003). This survival pathway is also activated when cells loose their anchorage (Cowell et al. 2009). Loss of anchorage in non-transformed cells induces an apoptotic cell death program called anoikis (Chiarugi and Fiaschi 2007). In tumor cells survival to this mechanism allows tumor metastasis to distant sites.

NF-κB in cancer cells is a redox sensor and many cancers show increased expression and activity of NF-κB (Rayet and Gelinas 1999; Li and Karin 1999; Schreck et al. 1992). Besides the PKC/PKD pathway, its activation by ROS can be caused through several other signaling mechanisms. These include hydrogen peroxide-mediated activation of NIK (Wang et al. 2007) through oxidative inhibition of NIK-regulating phosphatases, resulting in downstream signaling of NIK to the IKK complex (Li and Engelhardt 2006). Finally, NF-κB activation in response to oxidative stress can occur in an IKK-independent fashion. For example, it was shown that ROS can lead to tyrosine phosphorylation of IκBα. Tyrosine phosphorylation of IκBα can lead to its release from NF-κB, without inducing its degradation (Imbert et al. 1996). Besides mediating tumor cell survival, NF-κB was implicated in tumor cell proliferation, increased tumor cell motility, and development of drug resistance during therapy (chemoresistance) (Ahmed et al. 2006).

Mitochondrially-generated ROS are increased by the tumor suppressor protein p53 and p66[shc] through various pathways (reviewed in Pani and Galeotti 2011). p53-induced ROS production and activation of p53 target genes participate in the induction of cell growth arrest, apoptosis and senescence (Johnson et al. 1996). Dependent on its acetylation status, p53 also promotes the expression of antioxidant genes, which decreases its effects on senescence (Lu and Finkel 2008).

Growth factor receptors such as EGF-R or platelet-derived growth factor-receptor (PDGF-R) as well as activating mutations of K-ras can lead to oxidative stress-mediated activation of Akt. Akt is activated by hydrogen peroxide, either directly or through ROS-induced activation of its upstream kinase phosphoinositide-dependent kinase 1 (PDK1) (Prasad et al. 2000; Higaki et al. 2008). Moreover, the tumor suppressor phosphatase and tensin homologue (PTEN), a negative regulator of Akt signaling, can be inactivated by hydrogen peroxide, further potentiating Akt functions (Lee et al. 2002). Inactivation of PTEN additionally-increases cellular ROS levels due to deregulation of antioxidant enzymes (Huo et al. 2008). Akt can increase tumor cell survival through phosphorylation and inactivation of pro-apoptotic proteins including forkhead homeobox type O (FOXO) transcription factors, Bad, BimEL and Bax (Pastorino et al. 1999; Qi et al. 2006; Xin and Deng 2005). FOXO transcription factors are redox-sensing proteins and oxidative stress can mediate their activation (Storz 2011). This leads to induction of genes that mediate cell cycle arrest, induce apoptosis (i.e. FasL), or encode antioxidant genes (Storz 2011).

Another kinase that was implicated in ROS-induced cell survival and resistance to oxidative stress is Erk1/2, but in some cancers Erk1/2 seems to sensitize to ROS-induced apoptosis (Chan et al. 2008; Lee et al. 2005; Ostrakhovitch and

Cherian 2005). Besides Erk1/2, other members of the MAPK family such as p38 and c-Jun N-terminal Kinase (JNK), when activated by oxidative stress, are pro-apoptotic. Both p38 and JNK are activated by apoptosis signal-regulating kinase-1 (Ask1). Ask1 is regulated in its activity by interacting with thioredoxin, a redox-regulated protein (Saitoh et al. 1998; Takeda et al. 2003). JNK activation, for example, can occur in response to mitochondrial, as well as NADPH oxidase-induced oxidative stress (Storz 2005, 2006, 2007; Simon et al. 2000; Xu et al. 2002). When activated by ROS, JNK mediates phosphorylation and downregulation of Bcl-XL and Bcl-2, two proteins that protect from ROS-mediated apoptosis (Cadenas 2004; Gottlieb et al. 2000). JNK also increases the expression of pro-apoptotic proteins such as Bax to facilitate mitochondrial cytochrome c release (Shim et al. 2007; Zhang et al. 2008).

15.2.4 ROS in Tumor Cell Motility

The intracellular ROS state in cells governs all crucial mechanisms contributing to tumor cell metastasis, including degradation of extracellular matrix, increased potential to migrate and invade, intravasation and protection from anoikis.

Increased oxidative stress can contribute to increased cell motility, resulting in tumor expansion and metastasis. For example, subpopulations of breast cancer cell lines that show higher levels of endogenous ROS production than their origin counterpart, showed increased motility. Orthotopic tumors generated with these cells were metastatic, whereas parental cells were not (Pelicano et al. 2009). Another example is that carcinoma cells, when treated with hydrogen peroxide, prior to intravenous injection into mice show enhanced metastasis (Kundu et al. 1995). On the cellular levels it was shown that highly invasive tumor cell lines express rather high levels of intracellular ROS, probably due to downregulation of MnSOD (Hitchler et al. 2006, 2008).

Above we discussed the importance of the PI3-K/Akt pathway for tumor cell survival and the transcription factor FOXO3a as one of the primary targets of Akt to mediate this. Dependent on the tumor cell type and isoform expressed Akt also can decrease cell motility (Yoeli-Lerner and Toker 2006; Yoeli-Lerner et al. 2005). It was shown that stress conditions such as increased oxidative stress or nutrient depletion can activate the transcription factor FOXO3a (Storz et al. 2009). When activated in cells that grow under suboptimal conditions (where Akt is not active), FOXO3a induces an increase in their motility, potentially allowing their escape from stress or nutrient depletion (Storz et al. 2009). This is facilitated by upregulation of the matrix metalloproteinases (MMP) MMP-9 and MMP-13 (Storz et al. 2009). MMPs reorganize the extracellular matrix, degrade proteins that compose the basal membrane and have been implicated in many aspects of tumor progression (Radisky et al. 2005). Besides FOXO3a other ROS-induced transcription factors such as NF-κB have been implicated as regulators of MMP expression (Brenneisen et al. 2002; Kheradmand et al. 1998). Moreover, it was shown that hydrogen peroxide

and not superoxide is responsible for MMP induction (Wenk et al. 1999). Hydrogen peroxide induces MMP expression via activation of the MAP kinases Erk1/2, p38 and JNK (Nelson and Melendez 2004). Increases in ROS can also lead to activation of MMPs by direct oxidation or downregulate tissue inhibitor of metalloproteinases (TIMP), proteins that inactivate MMPs (Rajagopalan et al. 1996). Treatment of tumor cells with MMP-3 can induce mitochondrial ROS production, initiating epithelial-to-mesenchymal transition (EMT) (Radisky et al. 2005). One of the drivers of this process is the RhoGTPase Rac1b (Radisky et al. 2005).

Another member of the Rac family, Rac-1, activates the NADPH oxidase NOX1 and increases superoxide production at cellular membranes (Werner and Werb 2002). NOX1 expression not only can lead to cell transformation, it also can maintain the transformed state (Mochizuki et al. 2006; Tobar et al. 2008). Further it is necessary for formation of invadopodia, which are actin-based structures needed for tumor cells to invade tissue and blood vessels (Diaz et al. 2009). In this context Rac-1/NOX signaling can mediate actin cytoskeleton rearrangements. For example, Rac-1 can activate the actin severing enzyme cofilin to increase cell migration (Kim et al. 2009; Sundaresan et al. 1996). Similar to Rac-1, Src can regulate NOX1-mediated induction of ROS to mediate cell migration (Gianni et al. 2008). Moreover, ROS can regulate the transcriptional repressor Snail to decrease expression of E-cadherin, contributing to EMT and increased cell motility (Wu 2006).

The Rac-1/Nox pathway can act through NF-κB to modulate cell adhesion (Tobar et al. 2008). In endothelial cells this can lead to a loss of cell-cell adhesions, loosen the integrity of the endothelium, and result in increased vascular permeability and intravasation of cancer cells (Cheng et al. 2004; van Wetering et al. 2002). Besides NOX-induced ROS, mitochondrially-induced ROS have been shown to regulate cell adhesion (Chiarugi and Fiaschi 2007). This is probably mediated through Src and FAK, both kinases located to focal contacts, actin-based structures that contribute to cell spreading, cell migration and prevention of anoikis. Interestingly, Rac-1 may be involved in this process too, since growth factors and integrins can induce a Rac-1-caused increase in mitochondrial oxidative stress, leading to Src activation, cell adhesion and cell spreading (Bae et al. 2000; Werner and Werb 2002).

15.2.5 ROS in Cancer Stem Cells

Following radiotherapy or chemotherapy, recurrence of tumors can be initiated by a small subpopulation of surviving cancer cells. These cells are highly stress- and drug-resistant, express markers of stem cells, and therefore were named cancer stem cells (Diehn et al. 2009). CSCs are capable of self-renewal and differentiation and therefore have features to initiate tumors. Their adaption to increased ROS and promotion of cell survival and stress resistance is mediated by an increase in their antioxidant capacity (Tanno and Matsui 2011). For example, mammary epithelial CSCs contain low levels of superoxide when compared to the more mature progeny or even normal epithelia cells (Diehn et al. 2009). This is caused by increased

expression of a variety of genes encoding ROS scavenging enzymes or regulating glutathione synthesis (Diehn et al. 2009; Trachootham et al. 2009). Further, keeping ROS at such low levels is critical for maintaining their stem cell phenotype.

Increased levels of ROS are critical mediators of many chemotherapeutics and ionizing radiation therapy leading to DNA damage and tumor cell death (Shackleton et al. 2006; Ward 1985). The expression of antioxidants in CSCs prevents oxidative damage of DNA and cell death, and CSC-enriched tumor cell populations accumulate fewer DNA strand breaks or mutations after irradiation.

Important regulators of stress resistance in CSCs are FOXO transcription factors (Storz 2011). These transcription factors in cancer have been implicated in regulation of migration, proliferation, DNA repair, cell cycle arrest and cell death. They are less expressed in the bulk of tumor cells, but are critical for the maintenance of leukemic stem cells. For example FOXO1 confers stress resistance by upregulating catalase and superoxide dismutase genes (SOD) (Tothova et al. 2007). Consequently, treatment of CSCs with L-S, R-buthionine sulphoximine (BSO), a compound that pharmacologically depletes the ROS scavenger glutathione significantly sensitizes them to radiotherapy (Diehn et al. 2009).

15.2.6 Hypoxia, Angiogenesis and HIF-1

In primary tumors an increasing tumor mass faces cycles of oxygen depletion (hypoxia) and reoxygenation (Dewhirst et al. 2008; Hockel and Vaupel 2001). If prolonged, such limitations in oxygen supply can be damaging to the tumor cells. An escape mechanism for tumor cells to such conditions is their metabolic switch to anaerobic glycolysis (Pani et al. 2009). While normal cells only switch to glycolysis when adequate oxygen supply is not ensured or when mitochondria function is suppressed (Ganapathy et al. 2009), tumor cells can constantly use glycolysis as an energy source under normoxia (Warburg effect) to become independent of such conditions (Dang and Semenza 1999). This switch can be induced by mitochondrial dysfunction, oncogenic transformation or loss of tumor suppressor genes (Hsu and Sabatini 2008). The transcription factor hypoxia-inducible factor-1 (HIF-1) regulates glycolysis-related genes and inhibits mitochondrial respiration (Pani et al. 2009), which in sum results in adaption of tumor cells to hypoxic conditions (Pouyssegur et al. 2006) and the development of an aggressive tumor phenotype (Harris 2002).

With increased tumor mass, nutrient and oxygen support is limited to cells in the tumor center. Therefore, more nascent blood vessels are required to ensure supply to the tumor center (Claffey et al. 1996; Senger et al. 1994). Hypoxic conditions stimulate blood vessel development (angiogenesis) through induction of intracellular oxidative stress. For example, an increase in intracellular oxidative stress levels can be mediated by hypoxia-induced expression of VEGF (Jo et al. 2011). Antioxidants effectively inhibit or decrease angiogenesis since they can modify densitometry of microvessels and proliferation of endothelial cells (Rabbani

et al. 2009). Blood flow in newly developed vessels often is instable, leading to periods of high oxygen and hypoxia, and causing additional oxidative stress (Brown and Bicknell 2001). Nutrient deprivation and hypoxia both can increase expression of vascular epithelial growth factor (VEGF) (Spitz et al. 2000). This is mediated through HIF-1 and its co-factor p300 (Liou and Storz 2010).

HIF-1 is composed of the two subunits HIF-1α and HIF-1β (Harris 2002). Under normoxia conditions HIF-1α is proteasomally degraded (Kaelin and Ratcliffe 2008), whereas under conditions of hypoxia, formation of superoxide and hydrogen peroxide lead to accumulation of HIF-1α (Wang et al. 2005). HIF can be induced by increased production of both reactive oxygen species in response to oncogenic signaling through the PTEN/PI3-K/Akt signaling pathway, or to mitochondria-generated oxidative stress (Huo et al. 2008; Liu et al. 2006; Xia et al. 2007). Due to its function as an inducer of VEGF signaling and contribution to angiogenesis, increased HIF-1 expression was shown to correlate with poor prognosis and increased metastasis. HIF-1 also impacts tumor cell metastasis by impacting cell motility and EMT (Erler et al. 2006). This is mediated through HIF-1α regulation of metastasis-related genes including lysyl oxidase and MMPs. For example, MMP-9 and MMP-13 can directly mediate tumor cell invasion and metastasis when expressed by tumor cells (Storz et al. 2009). As a consequence of its effects on preventing cellular acidification, HIF-1 can contribute to cell invasion by increasing the formation of lactate and CO_2, both of which favor the degradation of extracellular matrix (Pouyssegur et al. 2006; Rofstad et al. 2006). MMPs are important mediators of vessel growth within the tumor microenvironment and MMP-induced formation of capillary-like structures occurs through upregulation of ROS.

15.3   Modulation of ROS Levels as Therapeutic Strategy

ROS can be beneficial for tumor cells allowing acquisition and maintenance of tumorigenic characteristics, but can be damaging when in excess. Many tumor tissues have elevated levels of oxidative stress, which in most normal tissues would induce cell death (Storz 2005). The upregulation of intracellular antioxidant systems allows tumor cells not only to keep ROS levels in check, but also to keep them at levels where they contribute to beneficiary signaling. Therefore, tumor cells can thrive under oxidative stress, inducing increased tumor cell proliferation, anti-apoptotic signaling and increasing cell motility (Liou and Storz 2010).

There are several therapeutic strategies aiming to increase intracellular ROS levels in cancer cells, with the goal that oxidative stress reaches a level that induces senescence or even cell death. This may be achieved in cancer cells by compounds that inhibit antioxidant systems or downregulate cellular pathways that mediate expression of antioxidants. Decreasing their antioxidant capacity can dramatically increase intracellular ROS levels in cancer cells, leading to irreparable damage. Normal cells may not be affected by such a strategy since they generally have lower

basal ROS levels than cancer cells and are less dependent on antioxidant systems. A caveat with this approach is that a threshold of cytotoxicity may not be reached in cancer cells. An advantage is that cancer stem cells may also be targeted by this approach, since they show increased expression of antioxidant systems to keep their intracellular ROS levels low (Trachootham et al. 2009).

Another strategy is to exuberantly-increase oxidative stress in tumor cells to levels at which they induce cell death. This can be achieved by radiation or chemotherapy (Trachootham et al. 2009). Increased ROS levels in tumor cells can be obtained by blocking glucose metabolism, which is increased in tumor cells and compensates excess metabolic production of ROS. It was suggested that inhibition of glucose metabolism may provide a mechanism by which cancer cells can be specifically targeted. This can be achieved with glucose analogues that can not be metabolized such as 2-deoxyglucose (2DG) and is even more pronounced when combined with mitochondrial electron chain blockers that mediate additional induction of ROS (Aykin-Burns et al. 2009; Coleman et al. 2008). Strategies to increase intracellular ROS generation in tumor cells may be even more effective when antioxidant systems are depleted at the same time (Trachootham et al. 2009). However, high levels of ROS can drive tumor cells into a stem cell-like phenotype, which is stress resistant and responsible for recurrence of tumors. Thus, therapies that increase intracellular ROS in cancer cells may represent a good strategy to target the bulk of proliferating tumor cells, but may drive a subpopulation of tumor cells into a dormant state or a CSC phenotype. These cells then need to be targeted with a separate strategy that considers their unique redox status.

Depleting ROS levels with therapeutic antioxidants may have tumor preventive functions. For example, oncogenic mutations in K-ras have been linked to ROS formation leading to premalignant lesions in pancreatic cancer (Li et al. 2002). Moreover, ROS generated by macrophages have been implicated in the initiation and progression of many tumor types (Keibel et al. 2009; Qian and Pollard 2010). Depleting oxidative stress at early stages therefore may be effective in prevention or delaying tumor development. Examples of antioxidants that are tested for tumor therapy are NOV-002, a mimetic of glutathione disulfide, or EUK-134, a mimetic of superoxide dismutase (SOD) (Townsend and Tew 2003; Bechtel and Bauer 2009). It becomes obvious that depending on the strategy used for therapy, the use of antioxidants in combination therapy could have adverse effects on chemotherapeutic drugs that kill cells by increasing intracellular ROS levels. Therefore, combination therapy with antioxidants and apoptosis-inducing agents may only be effective when these compounds can mediate cell death via pathways independent of ROS. Another explanation of why many tumor therapies in which antioxidants were used were quite ineffective is that at treatment the tumor cells may already have acquired a mutational state in which they are independent of ROS as a tumorigenic factor.

In summary, specific modulation of oxidative stress levels or regulation of specific production of certain reactive oxygen species in tumor cells could be a powerful tool to enhance therapeutic outcomes. However, it may require combination therapy with inhibitors of other pathways, specifically tailored to the signaling pathway or tumor type that needs to be targeted. One example where this type of therapy may be

effective is the use of chemotherapy in combination with a specific strategy to target antioxidant systems of CSCs, with the goal to kill the bulk of tumor and additionally prevent recurrence.

References

Ahmed KM, Cao N, Li JJ (2006) HER-2 and NF-kappaB as the targets for therapy-resistant breast cancer. Anticancer Res 26(6B):4235–4243

Aykin-Burns N et al (2009) Increased levels of superoxide and H_2O_2 mediate the differential susceptibility of cancer cells versus normal cells to glucose deprivation. Biochem J 418(1):29–37

Babior BM (1999) NADPH oxidase: an update. Blood 93(5):1464–1476

Bae YS et al (2000) Platelet-derived growth factor-induced H(2)O(2) production requires the activation of phosphatidylinositol 3-kinase. J Biol Chem 275(14):10527–10531

Balkwill F (2009) Tumour necrosis factor and cancer. Nat Rev Cancer 9(5):361–371

Bechtel W, Bauer G (2009) Modulation of intercellular ROS signaling of human tumor cells. Anticancer Res 29(11):4559–4570

Bendayan M, Reddy JK (1982) Immunocytochemical localization of catalase and heat-labile enoyl-CoA hydratase in the livers of normal and peroxisome proliferator-treated rats. Lab Invest 47(4):364–369

Beutler E (1969) Effect of flavin compounds on glutathione reductase activity: in vivo and in vitro studies. J Clin Invest 48(10):1957–1966

Brenneisen P, Sies H, Scharffetter-Kochanek K (2002) Ultraviolet-B irradiation and matrix metalloproteinases: from induction via signaling to initial events. Ann N Y Acad Sci 973:31–43

Brigelius-Flohe R (1999) Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med 27(9–10):951–965

Brown NS, Bicknell R (2001) Hypoxia and oxidative stress in breast cancer. Oxidative stress: its effects on the growth, metastatic potential and response to therapy of breast cancer. Breast Cancer Res 3(5):323–327

Browne SE et al (2004) Treatment with a catalytic antioxidant corrects the neurobehavioral defect in ataxia-telangiectasia mice. Free Radic Biol Med 36(7):938–942

Burdon RH (1995) Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radic Biol Med 18(4):775–794

Burdon RH, Gill V, Rice-Evans C (1990) Oxidative stress and tumour cell proliferation. Free Radic Res Commun 11(1–3):65–76

Cadenas E (2004) Mitochondrial free radical production and cell signaling. Mol Aspects Med 25(1–2):17–26

Chan DW et al (2008) Loss of MKP3 mediated by oxidative stress enhances tumorigenicity and chemoresistance of ovarian cancer cells. Carcinogenesis 29(9):1742–1750

Cheng GC et al (2004) Oxidative stress and thioredoxin-interacting protein promote intravasation of melanoma cells. Exp Cell Res 300(2):297–307

Chiarugi P, Fiaschi T (2007) Redox signalling in anchorage-dependent cell growth. Cell Signal 19(4):672–682

Chiu TT et al (2007) Protein kinase D2 mediates lysophosphatidic acid-induced interleukin 8 production in nontransformed human colonic epithelial cells through NF-kappaB. Am J Physiol Cell Physiol 292(2):C767–C777

Claffey KP et al (1996) Expression of vascular permeability factor/vascular endothelial growth factor by melanoma cells increases tumor growth, angiogenesis, and experimental metastasis. Cancer Res 56(1):172–181

Coleman MC et al (2008) 2-deoxy-D-glucose causes cytotoxicity, oxidative stress, and radiosensitization in pancreatic cancer. Free Radic Biol Med 44(3):322–331

Cowell CF et al (2009) Loss of cell-cell contacts induces NF-kappaB via RhoA-mediated activation of protein kinase D1. J Cell Biochem 106(4):714–728

Crompton M (1999) The mitochondrial permeability transition pore and its role in cell death. Biochem J 341(Pt 2):233–249

Cui S et al (1994) Activated murine macrophages induce apoptosis in tumor cells through nitric oxide-dependent or -independent mechanisms. Cancer Res 54(9):2462–2467

Cullen JJ et al (2003) The role of manganese superoxide dismutase in the growth of pancreatic adenocarcinoma. Cancer Res 63(6):1297–1303

Dang CV, Semenza GL (1999) Oncogenic alterations of metabolism. Trends Biochem Sci 24(2):68–72

Dansen TB, Wirtz KW (2001) The peroxisome in oxidative stress. IUBMB Life 51(4):223–230

Dayal D et al (2009) Mitochondrial complex II dysfunction can contribute significantly to genomic instability after exposure to ionizing radiation. Radiat Res 172(6):737–745

del Rio LA et al (1992) Metabolism of oxygen radicals in peroxisomes and cellular implications. Free Radic Biol Med 13(5):557–580

Dewhirst MW, Cao Y, Moeller B (2008) Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Nat Rev Cancer 8(6):425–437

Diaz B et al (2009) Tks5-dependent, nox-mediated generation of reactive oxygen species is necessary for invadopodia formation. Sci Signal 2(88):ra53

Diehn M et al (2009) Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 458(7239):780–783

Ditch S, Paull TT (2012) The ATM protein kinase and cellular redox signaling: beyond the DNA damage response. Trends Biochem Sci 37(1):15–22

Erler JT et al (2006) Lysyl oxidase is essential for hypoxia-induced metastasis. Nature 440(7088):1222–1226

Felty Q, Singh KP, Roy D (2005) Estrogen-induced G1/S transition of G0-arrested estrogen-dependent breast cancer cells is regulated by mitochondrial oxidant signaling. Oncogene 24(31):4883–4893

Ferraro D et al (2006) Pro-metastatic signaling by c-Met through RAC-1 and reactive oxygen species (ROS). Oncogene 25(26):3689–3698

Finkel T (2000) Redox-dependent signal transduction. FEBS Lett 476(1–2):52–54

Ganapathy V, Thangaraju M, Prasad PD (2009) Nutrient transporters in cancer: relevance to Warburg hypothesis and beyond. Pharmacol Ther 121(1):29–40

Gardner HW (1989) Oxygen radical chemistry of polyunsaturated fatty acids. Free Radic Biol Med 7(1):65–86

Gianni D et al (2008) The involvement of the tyrosine kinase c-Src in the regulation of reactive oxygen species generation mediated by NADPH oxidase-1. Mol Biol Cell 19(7):2984–2994

Gottlieb E, Vander Heiden MG, Thompson CB (2000) Bcl-x(L) prevents the initial decrease in mitochondrial membrane potential and subsequent reactive oxygen species production during tumor necrosis factor alpha-induced apoptosis. Mol Cell Biol 20(15):5680–5689

Harris AL (2002) Hypoxia – a key regulatory factor in tumour growth. Nat Rev Cancer 2(1):38–47

Hashimoto F, Hayashi H (1990) Significance of catalase in peroxisomal fatty acyl-CoA beta-oxidation: NADH oxidation by acetoacetyl-CoA and H_2O_2. J Biochem 108(3):426–431

Higaki Y et al (2008) Oxidative stress stimulates skeletal muscle glucose uptake through a phosphatidylinositol 3-kinase-dependent pathway. Am J Physiol Endocrinol Metab 294(5):E889–E897

Hitchler MJ et al (2006) Epigenetic regulation of manganese superoxide dismutase expression in human breast cancer cells. Epigenetics 1(4):163–171

Hitchler MJ, Oberley LW, Domann FE (2008) Epigenetic silencing of SOD2 by histone modifications in human breast cancer cells. Free Radic Biol Med 45(11):1573–1580

Hockel M, Vaupel P (2001) Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 93(4):266–276

Hofmann B, Hecht HJ, Flohe L (2002) Peroxiredoxins. Biol Chem 383(3–4):347–364

Hsu PP, Sabatini DM (2008) Cancer cell metabolism: Warburg and beyond. Cell 134(5):703–707

Huo YY et al (2008) PTEN deletion leads to deregulation of antioxidants and increased oxidative damage in mouse embryonic fibroblasts. Free Radic Biol Med 44(8):1578–1591

Imbert V et al (1996) Tyrosine phosphorylation of I kappa B-alpha activates NF-kappa B without proteolytic degradation of I kappa B-alpha. Cell 86(5):787–798

Irani K et al (1997) Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. Science 275(5306):1649–1652

Jo M et al (2011) Oxidative stress is closely associated with tumor angiogenesis of hepatocellular carcinoma. J Gastroenterol 46(6):809–821

Johnson TM et al (1996) Reactive oxygen species are downstream mediators of p53-dependent apoptosis. Proc Natl Acad Sci USA 93(21):11848–11852

Kaelin WG Jr, Ratcliffe PJ (2008) Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell 30(4):393–402

Keibel A, Singh V, Sharma MC (2009) Inflammation, microenvironment, and the immune system in cancer progression. Curr Pharm Des 15(17):1949–1955

Khavari TA, Rinn J (2007) Ras/Erk MAPK signaling in epidermal homeostasis and neoplasia. Cell Cycle 6(23):2928–2931

Kheradmand F et al (1998) Role of Rac1 and oxygen radicals in collagenase-1 expression induced by cell shape change. Science 280(5365):898–902

Kim JS, Huang TY, Bokoch GM (2009) Reactive oxygen species regulate a slingshot-cofilin activation pathway. Mol Biol Cell 20(11):2650–2660

Kumar B et al (2008) Oxidative stress is inherent in prostate cancer cells and is required for aggressive phenotype. Cancer Res 68(6):1777–1785

Kundu N, Zhang S, Fulton AM (1995) Sublethal oxidative stress inhibits tumor cell adhesion and enhances experimental metastasis of murine mammary carcinoma. Clin Exp Metastasis 13(1):16–22

Lander HM et al (1997) A molecular redox switch on p21(ras). Structural basis for the nitric oxide-p21(ras) interaction. J Biol Chem 272(7):4323–4326

Lauschke H et al (2002) Lipid peroxidation as additional marker in patients with colorectal cancer. Results of a preliminary study. Eur Surg Res 34(5):346–350

Lee SR et al (2002) Reversible inactivation of the tumor suppressor PTEN by H_2O_2. J Biol Chem 277(23):20336–20342

Lee WC et al (2005) Role of ERK in hydrogen peroxide-induced cell death of human glioma cells. Neurochem Res 30(2):263–270

Levine RL (2002) Carbonyl modified proteins in cellular regulation, aging, and disease. Free Radic Biol Med 32(9):790–796

Li Q, Engelhardt JF (2006) Interleukin-1beta induction of NFkappaB is partially regulated by H_2O_2-mediated activation of NFkappaB-inducing kinase. J Biol Chem 281(3):1495–1505

Li N, Karin M (1999) Is NF-kappaB the sensor of oxidative stress? FASEB J 13(10):1137–1143

Li D et al (2002) DNA adducts, genetic polymorphisms, and K-ras mutation in human pancreatic cancer. Mutat Res 513(1–2):37–48

Liou GY, Storz P (2010) Reactive oxygen species in cancer. Free Radic Res 44(5):479–496

Litwin JA et al (1987) Immunocytochemical localization of peroxisomal enzymes in human liver biopsies. Am J Pathol 128(1):141–150

Liu LZ et al (2006) Reactive oxygen species regulate epidermal growth factor-induced vascular endothelial growth factor and hypoxia-inducible factor-1alpha expression through activation of AKT and P70S6K1 in human ovarian cancer cells. Free Radic Biol Med 41(10):1521–1533

Lo YY, Cruz TF (1995) Involvement of reactive oxygen species in cytokine and growth factor induction of c-fos expression in chondrocytes. J Biol Chem 270(20):11727–11730

Lu T, Finkel T (2008) Free radicals and senescence. Exp Cell Res 314(9):1918–1922

Maynard S et al (2009) Base excision repair of oxidative DNA damage and association with cancer and aging. Carcinogenesis 30(1):2–10

McCubrey JA et al (2007) Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta 1773(8):1263–1284

Meier B et al (1989) Human fibroblasts release reactive oxygen species in response to interleukin-1 or tumour necrosis factor-alpha. Biochem J 263(2):539–545

Meng TC, Fukada T, Tonks NK (2002) Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. Mol Cell 9(2):387–399

Menon SG et al (2005) Differential susceptibility of nonmalignant human breast epithelial cells and breast cancer cells to thiol antioxidant-induced G(1)-delay. Antioxid Redox Signal 7(5–6):711–718

Mihailovic T et al (2004) Protein kinase D2 mediates activation of nuclear factor kappaB by Bcr-Abl in Bcr-Abl + human myeloid leukemia cells. Cancer Res 64(24):8939–8944

Minamoto T, Mai M, Ronai Z (2000) K-ras mutation: early detection in molecular diagnosis and risk assessment of colorectal, pancreas, and lung cancers – a review. Cancer Detect Prev 24(1):1–12

Minamoto T, Ougolkov AV, Mai M (2002) Detection of oncogenes in the diagnosis of cancers with active oncogenic signaling. Expert Rev Mol Diagn 2(6):565–575

Mochizuki T et al (2006) Inhibition of NADPH oxidase 4 activates apoptosis via the AKT/apoptosis signal-regulating kinase 1 pathway in pancreatic cancer PANC-1 cells. Oncogene 25(26):3699–3707

Nakashima I et al (2002) Redox-linked signal transduction pathways for protein tyrosine kinase activation. Antioxid Redox Signal 4(3):517–531

Nelson KK, Melendez JA (2004) Mitochondrial redox control of matrix metalloproteinases. Free Radic Biol Med 37(6):768–784

Neumann CA et al (2003) Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. Nature 424(6948):561–565

North JA, Spector AA, Buettner GR (1994) Cell fatty acid composition affects free radical formation during lipid peroxidation. Am J Physiol 267(1 Pt 1):C177–C188

Ohba M et al (1994) Production of hydrogen peroxide by transforming growth factor-beta 1 and its involvement in induction of egr-1 in mouse osteoblastic cells. J Cell Biol 126(4):1079–1088

Ostrakhovitch EA, Cherian MG (2005) Inhibition of extracellular signal regulated kinase (ERK) leads to apoptosis inducing factor (AIF) mediated apoptosis in epithelial breast cancer cells: the lack of effect of ERK in p53 mediated copper induced apoptosis. J Cell Biochem 95(6):1120–1134

Pani G, Galeotti T (2011) Role of MnSOD and p66shc in mitochondrial response to p53. Antioxid Redox Signal 15(6):1715–1727

Pani G et al (2009) Redox-based escape mechanism from death: the cancer lesson. Antioxid Redox Signal 11(11):2791–2806

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(9):2872–2881

Pastorino JG, Tafani M, Farber JL (1999) Tumor necrosis factor induces phosphorylation and translocation of BAD through a phosphatidylinositide-3-OH kinase-dependent pathway. J Biol Chem 274(27):19411–19416

Pelicano H et al (2009) Mitochondrial dysfunction and reactive oxygen species imbalance promote breast cancer cell motility through a CXCL14-mediated mechanism. Cancer Res 69(6):2375–2383

Pouyssegur J, Dayan F, Mazure NM (2006) Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 441(7092):437–443

Prasad N et al (2000) Oxidative stress and vanadate induce tyrosine phosphorylation of phosphoinositide-dependent kinase 1 (PDK1). Biochemistry 39(23):6929–6935

Qi XJ, Wildey GM, Howe PH (2006) Evidence that Ser87 of BimEL is phosphorylated by Akt and regulates BimEL apoptotic function. J Biol Chem 281(2):813–823

Qian BZ, Pollard JW (2010) Macrophage diversity enhances tumor progression and metastasis. Cell 141(1):39–51

Rabbani ZN et al (2009) Antiangiogenic action of redox-modulating Mn(III) ortho-tetrakis-N-ethylpyridylporphyrin, MnTE-2-PyP(5+), via suppression of oxidative stress in a mouse model of breast tumor. Free Radic Biol Med 47:992–1004

Radisky DC et al (2005) Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 436(7047):123–127

Rajagopalan S et al (1996) Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest 98(11):2572–2579

Rayet B, Gelinas C (1999) Aberrant rel/nfkb genes and activity in human cancer. Oncogene 18(49):6938–6947

Reichenbach J et al (2002) Elevated oxidative stress in patients with ataxia telangiectasia. Antioxid Redox Signal 4(3):465–469

Rhee SG et al (1999) A family of novel peroxidases, peroxiredoxins. Biofactors 10(2–3):207–209

Rhee SG et al (2000) Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. Sci STKE 2000(53):pe1

Roberts PJ, Der CJ (2007) Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 26(22):3291–3310

Rofstad EK et al (2006) Acidic extracellular pH promotes experimental metastasis of human melanoma cells in athymic nude mice. Cancer Res 66(13):6699–6707

Rozengurt E (2011) Protein kinase D signaling: multiple biological functions in health and disease. Physiology (Bethesda) 26(1):23–33

Ruiz-Ramos R et al (2009) Sodium arsenite induces ROS generation, DNA oxidative damage, HO-1 and c-Myc proteins, NF-kappaB activation and cell proliferation in human breast cancer MCF-7 cells. Mutat Res 674(1–2):109–115

Saitoh M et al (1998) Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J 17(9):2596–2606

Sarsour EH et al (2008) Manganese superoxide dismutase activity regulates transitions between quiescent and proliferative growth. Aging Cell 7(3):405–417

Schreck R, Albermann K, Baeuerle PA (1992) Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). Free Radic Res Commun 17(4):221–237

Senger DR et al (1994) Vascular permeability factor, tumor angiogenesis and stroma generation. Invasion Metastasis 14(1–6):385–394

Shackleton M et al (2006) Generation of a functional mammary gland from a single stem cell. Nature 439(7072):84–88

Sharma R et al (2004) Antioxidant role of glutathione S-transferases: protection against oxidant toxicity and regulation of stress-mediated apoptosis. Antioxid Redox Signal 6(2):289–300

Shim HY et al (2007) Acacetin-induced apoptosis of human breast cancer MCF-7 cells involves caspase cascade, mitochondria-mediated death signaling and SAPK/JNK1/2-c-Jun activation. Mol Cells 24(1):95–104

Simon HU, Haj-Yehia A, Levi-Schaffer F (2000) Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis 5(5):415–418

Singh I (1996) Mammalian peroxisomes: metabolism of oxygen and reactive oxygen species. Ann N Y Acad Sci 804:612–627

Song J et al (2009) PKD prevents H_2O_2-induced apoptosis via NF-kappaB and p38 MAPK in RIE-1 cells. Biochem Biophys Res Commun 378(3):610–614

Spitz DR et al (2000) Glucose deprivation-induced oxidative stress in human tumor cells. A fundamental defect in metabolism? Ann N Y Acad Sci 899:349–362

Squier TC, Bigelow DJ (2000) Protein oxidation and age-dependent alterations in calcium homeostasis. Front Biosci 5:D504–D526

Storz P (2005) Reactive oxygen species in tumor progression. Front Biosci 10:1881–1896

Storz P (2006) Reactive oxygen species-mediated mitochondria-to-nucleus signaling: a key to aging and radical-caused diseases. Sci STKE 2006(332):re3

Storz P (2007) Mitochondrial ROS–radical detoxification, mediated by protein kinase D. Trends Cell Biol 17(1):13–18

Storz P (2011) Forkhead homeobox type O transcription factors in the responses to oxidative stress. Antioxid Redox Signal 14(4):593–605

Storz P, Toker A (2003) Protein kinase D mediates a stress-induced NF-kappaB activation and survival pathway. EMBO J 22(1):109–120

Storz P, Doppler H, Toker A (2004a) Protein kinase Cdelta selectively regulates protein kinase D-dependent activation of NF-kappaB in oxidative stress signaling. Mol Cell Biol 24(7):2614–2626

Storz P, Doppler H, Toker A (2004b) Activation loop phosphorylation controls protein kinase D-dependent activation of nuclear factor kappaB. Mol Pharmacol 66(4):870–879

Storz P, Doppler H, Toker A (2005a) Protein kinase D mediates mitochondrion-to-nucleus signaling and detoxification from mitochondrial reactive oxygen species. Mol Cell Biol 25(19):8520–8530

Storz P et al (2005b) Functional dichotomy of A20 in apoptotic and necrotic cell death. Biochem J 387(Pt 1):47–55

Storz P et al (2009) FOXO3a promotes tumor cell invasion through the induction of matrix metalloproteinases. Mol Cell Biol 29(18):4906–4917

Sun G, Kemble DJ (2009) To C or not to C: direct and indirect redox regulation of Src protein tyrosine kinase. Cell Cycle 8(15):2353–2355

Sundaresan M et al (1995) Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. Science 270(5234):296–299

Sundaresan M et al (1996) Regulation of reactive-oxygen-species generation in fibroblasts by Rac1. Biochem J 318(Pt 2):379–382

Szatrowski TP, Nathan CF (1991) Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res 51(3):794–798

Takeda K et al (2003) Roles of MAPKKK ASK1 in stress-induced cell death. Cell Struct Funct 28(1):23–29

Tanno T, Matsui W (2011) Development and maintenance of cancer stem cells under chronic inflammation. J Nippon Med Sch 78(3):138–145

Tiku ML, Liesch JB, Robertson FM (1990) Production of hydrogen peroxide by rabbit articular chondrocytes. Enhancement by cytokines. J Immunol 145(2):690–696

Tobar N et al (2008) RAC1 activity and intracellular ROS modulate the migratory potential of MCF-7 cells through a NADPH oxidase and NFkappaB-dependent mechanism. Cancer Lett 267(1):125–132

Tothova Z et al (2007) FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell 128(2):325–339

Townsend DM, Tew KD (2003) The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene 22(47):7369–7375

Trachootham D et al (2006) Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. Cancer Cell 10(3):241–252

Trachootham D, Alexandre J, Huang P (2009) Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov 8(7):579–591

van Wetering S et al (2002) Reactive oxygen species mediate Rac-induced loss of cell-cell adhesion in primary human endothelial cells. J Cell Sci 115(Pt 9):1837–1846

Wang M et al (2005) Manganese superoxide dismutase suppresses hypoxic induction of hypoxia-inducible factor-1alpha and vascular endothelial growth factor. Oncogene 24(55):8154–8166

Wang Y et al (2007) The endogenous reactive oxygen species promote NF-kappaB activation by targeting on activation of NF-kappaB-inducing kinase in oral squamous carcinoma cells. Free Radic Res 41(9):963–971

Ward JF (1985) Biochemistry of DNA lesions. Radiat Res Suppl 8:S103–S111

Wells-Knecht MC et al (1997) Age-dependent increase in ortho-tyrosine and methionine sulfoxide in human skin collagen is not accelerated in diabetes. Evidence against a generalized increase in oxidative stress in diabetes. J Clin Invest 100(4):839–846

Wenk J et al (1999) Stable overexpression of manganese superoxide dismutase in mitochondria identifies hydrogen peroxide as a major oxidant in the AP-1-mediated induction of matrix-degrading metalloprotease-1. J Biol Chem 274(36):25869–25876

Werner E, Werb Z (2002) Integrins engage mitochondrial function for signal transduction by a mechanism dependent on Rho GTPases. J Cell Biol 158(2):357–368

Wiseman H, Halliwell B (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J 313(Pt 1):17–29

Wood ZA et al (2003) Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci 28(1):32–40

Wu WS (2006) The signaling mechanism of ROS in tumor progression. Cancer Metastasis Rev 25(4):695–705

Xia C et al (2007) Reactive oxygen species regulate angiogenesis and tumor growth through vascular endothelial growth factor. Cancer Res 67(22):10823–10830

Xin M, Deng X (2005) Nicotine inactivation of the proapoptotic function of Bax through phosphorylation. J Biol Chem 280(11):10781–10789

Xu YC et al (2002) Involvement of TRAF4 in oxidative activation of c-Jun N-terminal kinase. J Biol Chem 277(31):28051–28057

Yoeli-Lerner M, Toker A (2006) Akt/PKB signaling in cancer: a function in cell motility and invasion. Cell Cycle 5(6):603–605

Yoeli-Lerner M et al (2005) Akt blocks breast cancer cell motility and invasion through the transcription factor NFAT. Mol Cell 20(4):539–550

Zhang R et al (2008) In vitro and in vivo induction of apoptosis by capsaicin in pancreatic cancer cells is mediated through ROS generation and mitochondrial death pathway. Apoptosis 13(12):1465–1478