Chapter 10
Mitochondrial Mutations in Cancer Progression: Causative, Bystanders, or Modifiers of Tumorigenesis?

Ivana Kurelac, Michele Vidone, Giulia Girolimetti, Claudia Calabrese, and Giuseppe Gasparre

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10.1 Introduction

Mitochondria are semiautonomous organelles in the way that they possess their own small double-stranded circular chromosome, which in humans is on average 16,565 bp long. With a compact structure, the mitochondrial DNA (mtDNA) encodes 13 subunits of the respiratory chain complexes I, III, IV, and V, 22 transfer

I. Kurelac • M. Vidone • G. Girolimetti • C. Calabrese
Department of Medical and Surgical Sciences, Medical Genetics, University Hospital S.Orsola-Malpighi, University of Bologna, via Massarenti 9, 40138 Bologna, Italy

G. Gasparre (✉)
Department of Medical and Surgical Sciences, Medical Genetics, University Hospital S.Orsola-Malpighi, University of Bologna, via Massarenti 9, 40138 Bologna, Italy

Dipartimento di Scienze Mediche e Chirurgiche, University of Bologna, Via Massarenti 11, Bologna, Italy
e-mail: giuseppe.gasparre@gmail.com; giuseppe.gasparre3@unibo.it

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RNAs, and 2 ribosomal RNAs for *in organello* translation. The latter is a necessary process since mtDNA does not follow the universal code for protein translation and codon usage is different from that occurring within the cytosol. The remaining subunits of the respiratory complexes, especially those forming the large complex I holoenzyme, are encoded by nucleus-residing genes and subsequently imported within mitochondria with the aid of chaperones. The mtDNA also contains a 1Kb promoter-like region called displacement loop (D-loop) where replication and transcription starting sites are mapped.

Mitochondria constitute a network that harbors a number of mtDNA molecules ranging from several hundreds to nearly 100,000. Therefore, the mitochondrial genome of an organism is polyploid, which gives rise to the possibility of the coexistence of different genetic variants within a cell or a tissue, a condition known as heteroplasmy. Homoplasmy, on the other hand, is referred to as a genetically homogeneous mtDNA content. As a consequence of mitochondrial polyplasmy, the phenotypic effect of a variant depends on the mutant load. Usually, a certain critical portion of mutated molecules needs to be reached before functional consequences begin to arise, which depends on the type of variant and on the context in which it occurs (Rossignol et al. 2003). Threshold values have been determined for some of the most common mtDNA mutations (Carelli et al. 2002; Laloi-Michelin et al. 2009), but the lack of appropriate and standardized methods for heteroplasmy investigation has held back a complete understanding of the mtDNA genotype–phenotype correlation (Wong and Boles 2005).

The mitochondrial genome is maternally inherited. Oocyte mitochondria exclusively contribute to gamete development, whereas sperm mtDNA is eliminated after fertilization (St John et al. 2010). As a consequence, the human lineage carries mtDNA molecules derived by descent from the same ancestral mitochondrial genome (Torroni et al. 2006). Since mtDNA displays high genetic variability due to an elevated mutagenesis rate (see below), the ancestral mitochondrial chromosome has over time acquired diverse polymorphisms which currently define more than 30 mitochondrial haplogroups (van Oven and Kayser 2009). On the other hand, non-polymorphic mtDNA variants, which cause defects in the electron transport chain (ETC) and oxidative phosphorylation (OXPHOS), are related to mitochondrial diseases, such as Leber's hereditary optic neuropathy (LHON); neuropathy, ataxia, and retinitis pigmentosa (NARP); myoclonic epilepsy with ragged red fibers (MERRF); chronic progressive external ophthalmoplegia (CPEO); and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) (Wallace 1999). Moreover, with the emerging roles of mitochondria in many vital cellular processes, it is now recognized that OXPHOS damage may also contribute to the development of other pathologies, such as diabetes, neurodegenerative diseases, and cancer (Wallace 2005).

The first large study reporting mtDNA variants in cancer was performed in 1998 on colorectal cancer cell lines (Polyak et al. 1998). Ever since, a plethora of reports have described the occurrence of mtDNA mutations in virtually all cancer types (Brandon et al. 2006; Chatterjee et al. 2006; Yu 2012). Nevertheless, no consensus has been reached to date to standardize approaches for mtDNA mutations

recognition, often resulting in erroneous interpretations (Yao et al. 2009; Liu et al. 2012). A good practice would be to evaluate the actual population frequency of a specific mtDNA mutation through the use of carefully curated and comprehensive databases, such as the Human Mitochondrial Database (HmtDB) (Lascaro et al. 2008; Rubino et al. 2012), when attempting to infer a role in disease. Another recommendable approach to reduce misleading analyses would be to assess if the mtDNA alleles observed in a sample are coherent with the corresponding haplogroup (Salas et al. 2005; Rubino et al. 2012). Furthermore, during mtDNA sequencing, it is important to avoid the co-amplification of nuclear mitochondrial sequences (NumtS), i.e., noncoding mitochondrial pseudogenes abundantly incorporated in the nuclear chromosomes (Hazkani-Covo et al. 2010; Simone et al. 2011; Lang et al. 2012; Petruzzella et al. 2012).

mtDNA mutations in cancer have been initially associated only with highly glycolytic neoplastic cells, which were observed in 1924 by German biochemist Otto Warburg, who suggested such a phenotype to be a consequence of mitochondrial damage (Warburg 1956; Warburg et al. 1924). However, considering the vast spectrum of mitochondrial roles in metabolism, as well as in apoptosis and hypoxic adaptation, it is not surprising that mtDNA mutations may have a greater impact on tumor development and progression (Galluzzi et al. 2010).

A complete functional effect of mtDNA mutations in cancer is difficult to assess, mainly due to the peculiarities of mitochondrial genetics and to the heterogeneous and ever-changing tumor microenvironment. The most widely used approach for analysis of mtDNA functional effects involves the generation of *trans*-mitochondrial hybrids (cybrids), which allow to distinguish a mitochondria-specific contribution from that of the nuclear genome (Moraes et al. 2001). On the other hand, introduction of allotopically expressed mtDNA genes recoded for translation in the cytoplasm is used to investigate the complementation of damaging mtDNA mutations effects (Bonnet et al. 2007). It is important to note that functional studies on mtDNA mutations in molecular oncology ought to be preferentially performed in vivo, in order to take into account the dynamic cancer microenvironment and selective pressures such as aglycemia and hypoxia, conditions that are difficult to reproduce in vitro.

In this review, the central role of the mitochondrion within cancer progression will be revised, with particular focus on the consequences of genetic lesions and functional impairment occurring in oxidative metabolism enzymes, both encoded by the mitochondrial chromosome and by the nucleus. Reference will be made to the pleiotropic effects of diverse mtDNA mutations and to the triggering of the Warburg effect, especially in hypoxic conditions. Finally, an excursus on the peculiarities of oncocytic tumors, a subset of neoplasms well characterized in terms of mitochondrial aberrations, will provide ground for several general considerations on the importance of these organelles in determining a cancer cell fate.

10.2 Sources of mtDNA Mutations in Cancer

It has been estimated that mtDNA has a 10- to 17-fold higher mutation rate than the nuclear DNA (Tuppen et al. 2010), a feature that has been attributed to elevated oxidative damage to which the mitochondrial chromosomes are exposed due to their proximity to the electron transport chain (ETC) (Chatterjee et al. 2006). Moreover, mitochondria do not contain the full range of DNA repair mechanisms that operate in the nucleus (Boesch et al. 2010). The currently known repertoire of mtDNA repair includes single-nucleotide base excision repair (BER), long-patch BER, single-strand break repair, YB-1-mediated mismatch repair, removal of adenine opposite 8-oxo-dG, and MTH1 removal of 8-oxo-dGTP and 8-oxo-2-′-dATP from the mitochondrial nucleotide pool (Cline 2012).

Damage to mtDNA bases in both normal population and during cancer progression may be caused either by endogenously produced metabolic products or by exogenous sources.

The main endogenous determinants are represented by reactive oxygen species (ROS), which are generated subsequently to the leakage of electrons during OXPHOS, and may physiologically serve as signaling molecules in the cell (Ray et al. 2012). Anomalous high ROS levels are known to augment the mutation rate of both mitochondrial and nuclear DNA through mechanisms that include base modifications, sugar breakdown products, base-free sites, and strand breaks (Cline 2012). The type of ROS-derived damage usually regards 8-OH-GTP, which may cause a transversion of G:C to T:A (Wallace 2005). Furthermore, ROS may be responsible for changes in the cellular dGTP pool and 8-OH-dGTP generation, which in turn leads to transversion from A:T to C:G (Wiseman and Halliwell 1996). It is important to note that, on these bases, the expected spectrum of mutations should provide an excess of A:T to C:G and G:C to T:A transversions in the mtDNA sequence. However, the current spectrum of mtDNA mutations in human populations and in cancer does not correspond to the view of a damage induced prevalently by ROS, as observed, for instance, in colorectal cancer (Skonieczna et al. 2012), suggesting the existence of different predominant mutagenic mechanisms during tumor progression.

In fact, other endogenous cell metabolites such as estrogens may cause formation of adducts such as S-adenosylmethionine, which are able to methylate DNA in a nonenzymatic manner (alkylation damage) (Alexeyev et al. 2013). The mtDNA may also react with the products of endogenous fatty acid peroxidation, resulting in adduct formation (Nair et al. 2005). Moreover, reactive nitrogen species (RNS) can promote mutational events since they react with the superoxide anion $O_2^{\bullet -}$ and cause DNA base oxidation and sugar fragmentation, which may induce mtDNA strand breaks (Burney et al. 1999).

Random mtDNA mutations may also arise as a result of an erroneous DNA replication caused by polymerase errors, particularly plausible at the homopolymeric stretches that frequently occur in mtDNA regions (Denver et al. 2000). Mutations in either the polymerase or the exonuclease domain of POL-γ, the

functional DNA-dependent DNA polymerase in mitochondria, have been in fact associated with increased occurrence of mtDNA mutations (Copeland et al. 2003). In cancer, elevated mtDNA mutagenesis and frequent mtDNA copy number alterations have often been attributed to dysfunction of proteins involved in mtDNA integrity maintenance. For example, *POLG*, the gene encoding POL-γ, has been found mutated in 63 % of breast tumors, with a consequent mtDNA depletion and increased tumorigenicity (Singh et al. 2009). The knockdown of nuclear-encoded RNA helicase, SUV3, results in reduced mtDNA copy number and elevated somatic mtDNA mutation frequency in murine models (Chen et al. 2012). Similar consequences have been observed in colorectal cancers carrying mutations in the mitochondrial transcription factor (TFAM), which also plays a histone-like function within the nucleoids (Campbell et al. 2012).

Moreover, given that tumor suppressor p53 is known to enter mitochondria and play a role in mtDNA maintenance, it is not surprising that p53 disruption has been associated with occurrence of mtDNA alterations (Lebedeva et al. 2009). In this context, it is tempting to speculate that mutations in other genes involved in the mitochondrial genome organization and maintenance may contribute to the development of cancer-associated mtDNA alterations. Among these, mutations in *OPA1*, responsible for a dominant form of optic atrophy, have been shown to induce the accumulation of mtDNA deletions in the skeletal muscle of patients (Amati-Bonneau et al. 2008; Elachouri et al. 2011). Although OPA1 has not yet been linked to cancer, it is possible that it might contribute to mitochondrial alterations in certain contexts of tumorigenesis.

Furthermore, it is likely that the inherited genetic background of mtDNA variants may also contribute to the spectrum of mtDNA mutations in cancer. For instance, the co-occurrence of germline and somatic mtDNA mutations in cancer has led to the hypothesis that inherited mutations may predispose to a facilitated acquisition of additional mutations (Petros et al. 2005; Gochhait et al. 2008). This aspect is still largely unexplored in cancer, since the scenario of "universal" variability in the form of inherited low heteroplasmy mtDNA variants has only recently been discovered in healthy individuals (He et al. 2010; Payne et al. 2013).

Exogenous sources of mtDNA mutations include ultraviolet light, ozone, ionizing radiations, metals, pesticides, air pollutants or pharmaceutical drugs, asbestos, and arsenic (Partridge et al. 2009; Boesch et al. 2010). The benzo[a]pyrene and acrolein components of cigarette smoke, the fungal toxin aflatoxin B1, platinum-based chemotherapy agents, and antiviral nucleoside analogs all induce DNA adducts that may interfere with the POL-γ function (Cline 2012). These mutagens are also exogenous sources of ROS; therefore, it is difficult to assess whether mutations are a direct consequence of exogenous agents or if the latter increase of free radicals generation leads to oxidative damage and mtDNA mutagenesis. It is quite possible that both scenarios occur in vivo (Chatterjee et al. 2011).

It is important to note that certain chemotherapeutic approaches employed in cancer therapies may have off-target effects on mitochondria, causing both direct and indirect damage to mtDNA. These include cisplatinum (Chatterjee et al. 2011), bleomycin, and neocarzinostatin (Cline 2012). For example, platinum-based

chemotherapy drugs, such as cisplatin, carboplatin, and oxaliplatin, bind directly to DNA to form single base adducts and intra- and interstrand cross-links between guanine bases (Cline 2012).

In summary, the high occurrence of mtDNA mutations in cancer is thought to be a result of (1) proximity of mtDNA to ROS production sites, (2) an inadequate set of mtDNA repair mechanisms, (3) potential dysfunction of proteins involved in mtDNA integrity maintenance, (4) diverse exogenous mutagens including chemotherapics, and (5) accumulation of inherited germline mutations.

10.3 Selection of mtDNA Mutations in Cancer

Whether an mtDNA mutation will be expanded and stabilized in a tissue mainly depends on putative selective pressures which in cancer are heterogeneous and change over time (Aanen and Maas 2012). By analyzing mtDNA mutational hot spots, their mutant load, and the type of damage they induce, it is possible to characterize the selective forces which drive their accumulation in cancer, providing at the same time information about the complex mechanisms which operate during tumor progression in general.

In healthy individuals, inherited mitochondrial mutations in protein-coding genes are subjected to negative selection and are preferentially eliminated within a few generations (Stafford and Chen-Quin 2010; Freyer et al. 2012). Inheritance of tRNA and rRNA mutations, whose functional effects are more difficult to ascertain, is considered to be either subjected to random genetic drift or to negative selection (Schon et al. 2012). The regulatory D-loop region particularly displays a high variability rate (Pereira et al. 2009). Among the mtDNA coding regions, the most evolutionary conserved genes are *MT-COI*, *MT-COII*, *MT-ND4*, and *MT-ND4L*, whereas the most polymorphic regions include *MT-ATP6*, *MT-ND6*, and *MT-CYTB* (Pereira et al. 2009).

In cancer tissues, the D-loop appears to be the region that is more prone to acquire somatic mutations, but it seems that there are no preferential hot spots among rRNA, tRNA, or coding genes, since cancer-specific mutations are distributed uniformly across the genome (Iommarini et al. 2012; Liu et al. 2012). However, in colon cancer, most somatic mtDNA mutations were shown to accumulate in *MT-ND4L*, while genes encoding subunits of complex V remained mutation free (Skonieczna et al. 2012), indicating that a selective bias might exist, at least in some types of cancer. Whatever the case, it is certain that selection of mtDNA mutations in cancer varies from the purifying forces observed in mitochondrial genome evolution of healthy individuals.

Certain somatic mtDNA mutations may positively contribute to cancer cell development, as suggested from the observation of mitochondrial genotypes pattern in some tumor tissues. For example, in hepatocellular carcinoma, renal, breast, gastric, rectal, and ovarian cancer, variable heteroplasmic levels of an ND5-truncating mitochondrial mutation were recurrently found (Larman

et al. 2012), suggesting a role in conferring a selective advantage to neoplastic cells. It is interesting to note that the same mutation was found in the germline of a patient presenting a nasopharyngeal oncocytoma (Gasparre et al. 2009). Albeit heteroplasmic in all the tissues analyzed, the mutation shifted to homoplasmy only in the oncocytic areas of the tumor (Gasparre et al. 2009). Along this line, since the majority of mtDNA mutations in cancer have long been reported only as homoplasmic, a positive selection during tumor progression was one of the most recognized hypotheses. However, mathematical modelings suggest that a shift to homoplasmy might be the result of a random event, i.e., achieved without the action of selective forces (Coller et al. 2001). In fact, studies that have more accurately taken into account the load of mtDNA mutations, such as the most recent next-generation sequencing (NGS) approaches (He et al. 2010; Larman et al. 2012; Guha and Avadhani 2013), suggest that mutant load in cancer tissues may vary and include also an extremely low-level heteroplasmy, which was up to date neglected due to technical limitations (Kurelac et al. 2011). Therefore, the exclusively positive selection of mtDNA mutations in cancer has recently become questioned: a large number of studies, together with advances in mitochondrial genome analyses and a better understanding of pathogenic effects, suggested that they are not only positively selected during tumor progression as initially recognized but that a relaxed negative selection is the most plausible mechanism that shapes mtDNA variation in cancer cells (Stafford and Chen-Quin 2010; Liu et al. 2012). Overall, mtDNA mutations in cancer may be likely subjected to the negative selection observed also in the evolution of healthy individuals, albeit at certain points of cancer progression, purifying effects may be lost due to metabolic reprogramming and to the onset of diverse microenvironment conditions, leading to a shift to homoplasmy (Fig. 10.1).

Prediction of functional effects is particularly important in this context to understand selective forces in cancer. It has been shown that non-synonymous mutations occur with a higher frequency in cancer than in the general population and that these changes are usually predicted to influence protein function (Larman et al. 2012; Liu et al. 2012). However, mtDNA mutations with substantially high pathogenicity scores are not often found in human neoplasms and are mainly restricted to oncocytomas, a peculiar type of less aggressive tumors (Pereira et al. 2012). In fact, highly severe mutations, such as stop/frameshift, which may completely inhibit OXPHOS, are usually heteroplasmic or purified in cancers (Brandon et al. 2006; Larman et al. 2012) (Fig. 10.2a). Similarly, the analysis of mutations affecting complex I genes implied a strong negative selection of stop and frameshift mutations in most human tumors (Iommarini et al. 2012). Investigation of the same dataset showed that this holds true also for mutations affecting other respiratory chain complexes whose subunits are encoded by the mtDNA (Fig. 10.2b). Moreover, an analysis of colon cancer datasets indicated only 7 % of stop-inducing mutations (Skonieczna et al. 2012).

The degree of mitochondrial function necessary for cell survival may depend on the tumor stage and microenvironment and may range from completely attenuated OXPHOS activity during early glycolytic cancer metabolism to even enhanced

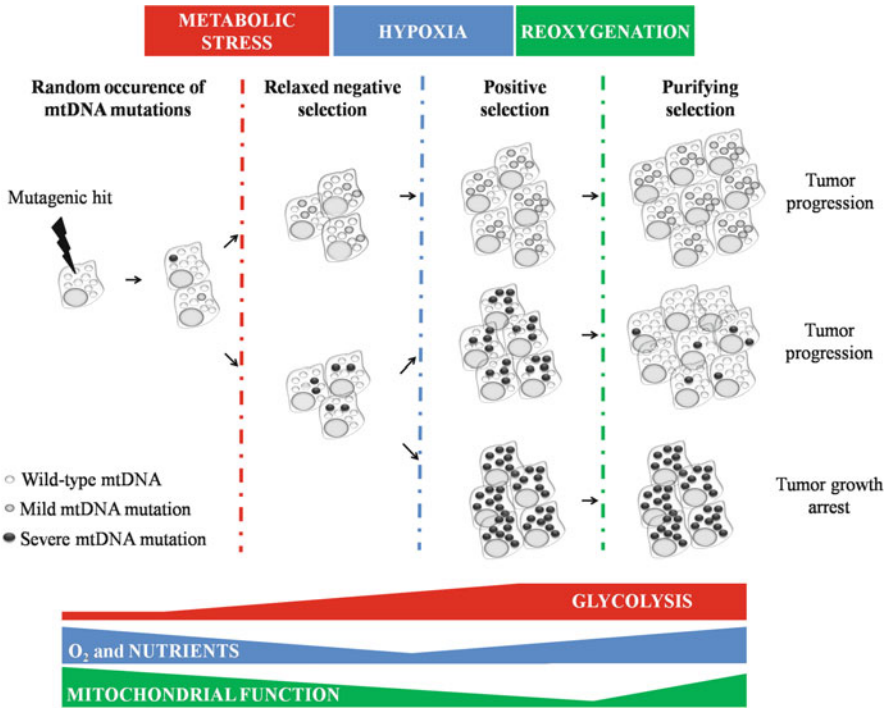

Fig. 10.1 Selection of mtDNA mutations in cancer. At the initial stages of tumorigenesis, uncontrolled cellular proliferation causes metabolic stress and promotes glycolysis, during which mtDNA mutations may accumulate randomly under a relaxed negative selection, since mitochondrial function is not essential. In hypoxic conditions, downregulation of mitochondrial function lowers cell requirements for oxygen and thus mtDNA mutations may be positively selected. On the other hand, in a reoxygenized environment, mtDNA mutations may become subjected to a negative selection, since mitochondrial function may regain importance, i.e., due to higher energy requirements. In this context, if a severe mtDNA mutation shifts to homoplasmy during the hypoxic stage, this irreversible mitochondrial damage may lead to tumor growth arrest in reoxygenized conditions, conversely from mild mtDNA mutations which may instead escape negative selection

OXPHOS activity observed in several tumor types (Smolkova et al. 2011). For example, in hypoxic conditions, where lack of oxygen is the main selective pressure, cancer cells carrying damaging mtDNA mutations may be preferentially selected since downregulation of mitochondrial function becomes advantageous. On the other hand, in tumor areas that have gone through hypoxic adaptation, the elevated anabolic metabolism of macromolecule biosynthesis requires functional mitochondria, such as during the process of non-anoxic glutaminolysis (Wise et al. 2011). Therefore, it is most probable that the early shift to glycolysis, shared by most solid tumors, allows for a relaxed negative selection of pathogenic mtDNA mutations in cancer (Liu et al. 2012), while the same mutations may become disadvantageous, for instance, in reoxygenated metastatic cancer tissue (Horton

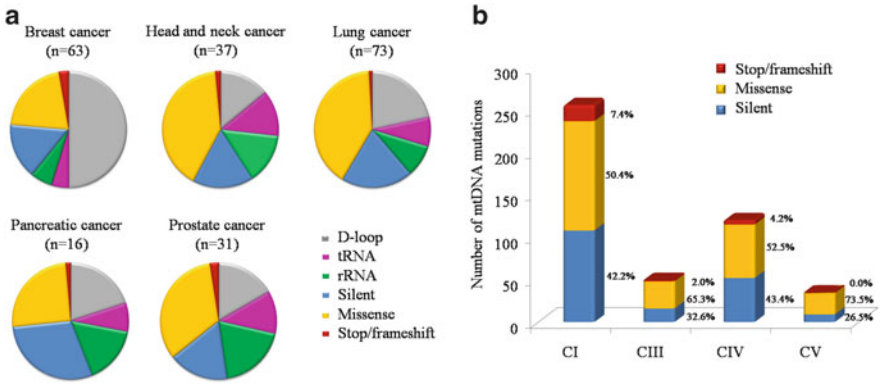

Fig. 10.2 Somatic mtDNA mutations in cancer. (**a**) Somatic mtDNA mutation frequency in different cancers, with distinction between mtDNA regions and mutation types for protein-coding genes. (**b**) Distribution of protein-coding mtDNA mutations in cancers of different origin, with distinction between mitochondrial respiratory complexes. Data for this analyses were extracted from reports published between 1998 and 2011, in which the entire mtDNA was sequenced and for which the somatic status of the mutations was indicated (Iommarini et al. 2012). Oncocytic tumors were excluded from the dataset

et al. 1996; Brandon et al. 2006) (Fig. 10.1). In the event of accumulation of disadvantageous mutations to homoplasmy during the initial permissive glycolytic phases, it is plausible that the consequence would be the inability for tumors to progress to malignancy, as deduced from the study of oncocytomas, which remain mostly confined as low proliferative lesions (Iommarini et al. 2012; Pereira et al. 2012).

Taken together, mtDNA mutations in cancer are subjected to ever-changing selective pressures which may be neutral, positive, or purifying, depending on the mutation type and on the specific phases of tumor progression in which they occur (Fig. 10.1). As a consequence, mtDNA mutations may be impartial bystanders, but also positively selected or eliminated, depending on whether their overall functional effect is advantageous during the waves of tumor reprogramming and adaptation (Smolkova et al. 2011).

It is important to note that cancer-specific changes in mtDNA copy number have also been reported as means of regulating mitochondrial function, which may be altered by defects in mitochondrial biogenesis or may be a consequence of mutations within the D-loop origin of replication. In particular, increase in mtDNA copy number has been observed in endometrial and esophageal squamous cell carcinoma, prostate cancer, ovarian cancer, papillary thyroid carcinoma, and in oncocytic tumors, while advanced gastric cancer, breast cancer, Ewing's sarcoma, and renal cell cancer appear to display a low number of mtDNA copies (Yu 2012). It is most likely that similarly to mtDNA selection, mtDNA copy number in cancer also depends on the tumor stage and metabolic requirements (Cook and Higuchi 2012).

10.4 Consequences of mtDNA Mutations on Tumorigenesis and Tumor Progression

Mitochondrial dysfunction has been associated with promotion of tumor growth ever since Warburg observed a higher glucose uptake and increased lactate production in cancer cells (Warburg et al. 1924). The first genetic evidence for mitochondria-induced cancer development was found in two nuclear metabolic genes encoding tricarboxylic acid (TCA) cycle enzymes, namely, succinate dehydrogenase (SDH) and fumarate hydratase (FH), in which loss of function mutations has been reported to promote tumorigenesis by stabilizing the hypoxia-inducible factor 1-alpha (HIF1α) in normoxic conditions, in patients with hereditary paragangliomas (HPGL) (Baysal et al. 2000; Astuti et al. 2001) and hereditary leiomyomatosis and renal cell cancer (HLRCC) (Tomlinson et al. 2002). Along this line, loss of function mtDNA mutations have initially been considered as pro-tumorigenic events (Polyak et al. 1998; Petros et al. 2005) and even sometimes as the initiators of tumorigenesis (Shay and Werbin 1987). However, considering the complexity of mitochondrial genetics, the vastness of cellular and metabolic reactions in which mitochondria are involved, and the extremely high tumor heterogeneity, this definition has been corrected, particularly in the context of a recent revision of the Warburg hypothesis, by which now it is recognized that a certain, at least minimal, degree of mitochondrial function is mandatory for cancer cell survival (Wallace 2012).

A classification of mtDNA mutations based on their effect on tumor progression may be complex, since it ought to depend both on the type of damage they induce and on the context in which they arise. For example, mtDNA variants causing mild complex I dysfunctions, such as missense mutations, are indeed usually associated with pro-tumorigenic effects, whereas mutations leading to complete mitochondrial complex I disassembly have been shown to reduce tumorigenic potential (Gasparre et al. 2011; Iommarini et al. 2014). Furthermore, a 21 bp deletion in *MT-CYTB* was appointed to cause an increase in the tumorigenic potential of bladder cancer cells (Dasgupta et al. 2008), whereas a similar *MT-CYTB* mutation decreased tumor growth of *KRAS*-mutated osteosarcoma cells (Weinberg et al. 2010), indicating that *KRAS*-induced tumorigenicity may be more reliant on mitochondrial function. Despite both mutations causing increased ROS production, the nuclear background presumably set the frame for the final outcome of the mtDNA mutation, just as the tumor cell microenvironment sets pressures for mtDNA mutation selection. It is important to note that even the same mutation may exhibit different, even opposite, effects on tumorigenesis, depending on the mutant load.

Nowadays, mtDNA mutations in cancer are generally rather considered as modifiers of tumor progression, and three groups of mtDNA mutations may be distinguished with respect to their effects on tumorigenesis: neutral, pro-tumorigenic/pro-metastatic, and antitumorigenic. A difficult task concerns the positioning of D-loop mutations within these categories. Although frequently described in cancer, a clear indication on their functional consequences has been

lacking to date. In fact, the D-loop appears to be hostile for functional investigation, and only a few works indicate an indirect positive contribution of D-loop mutations to mitochondrial dysfunction and cancer progression (Gasparre et al. 2008; Li et al. 2008), suggesting that the bulk of data on D-loop cancer variants ought to be interpreted with caution, especially since this is a highly polymorphic region.

mtDNA mutations exhibiting a neutral effect on tumorigenesis are the most common somatic changes observed in cancer, as suggested by several revisions on the currently available analyses on cancer-specific mtDNA mutations (Lu et al. 2009; Iommarini et al. 2012; Yu 2012). It has been estimated that synonymous changes represent 75 % of all cancer-related coding mtDNA mutations (Yu 2012), indicating that many of such randomly occurring genetic events are mere bystanders that may not affect tumor progression. It is important, however, not to neglect the potential effects of such variants since a few have been shown to alter protein structure (Kimchi-Sarfaty et al. 2007; Komar 2007). Moreover, some studies implicated a haplogroup-related predisposition to cancer development, indicating that mtDNA polymorphisms may have a role in cancer progression, a hypothesis that warrants further investigation in larger cohorts (Liu et al. 2003; Canter et al. 2005). It has been indeed hypothesized that haplogroups may favor a looser coupling between oxidation and phosphorylation, thereby generating a surplus of heat at the expenses of ATP and in turn a better survival of human populations in colder climates (Ruiz-Pesini et al. 2004). Although controversial, this hypothesis suggests that polymorphic germline mtDNA variants may exert a phenotypic effect, when working synergistically. On the other hand, even functionally active (pathogenic/phenotype changing) mtDNA mutations may be neutral, as it has been observed for the m.6930G > A/*MT-COI*, which causes decrease in ATP synthesis and reduces respiration of osteosarcoma-derived cybrids, but shows no influence on tumor growth (Sharma et al. 2011). Similarly, the heteroplasmic m.3571insC/*MT-ND1* induces no modifying effects on tumorigenic potential compared to wild-type mitochondria with completely functional complex I (Gasparre et al. 2011). However, the same mutation in mutant loads higher than 83 % exhibits antitumorigenic properties in the same cells.

Pro-tumorigenic mtDNA mutations are also commonly found in cancer tissues and mostly include point mutations inducing amino acid changes. The first functional studies on the role of mtDNA mutations in cancer demonstrated that an *MT-ATP6* mutation increased tumorigenesis in vivo by causing elevated ROS production and downregulation of apoptotic mechanisms in prostate and cervix cancer cells (Petros et al. 2005; Shidara et al. 2005). Although this mutation is not usually found in cancer, but is etiological for the NARP syndrome, these experiments confirmed the Warburg hypothesis and were in line with the suggested bioenergetic profile of decreased ATP synthase expression described in most solid tumors (Cuezva et al. 2009). It is important to note that such a mutation may not be an initiator of tumorigenesis since otherwise NARP patients would be more prone to cancer development. A subsequent series of studies aimed at identifying diverse mechanisms by which mtDNA mutations may exhibit pro-tumorigenic properties. Taken together, a dozen pro-tumorigenic mtDNA

mutations were functionally characterized up to date, which include different mtDNA loci, including the D-loop (Li et al. 2008), and affect function of diverse respiratory chain complexes. For instance, the m.6124T > C/*MT-COI* induced a decrease in cytochrome c oxidase (COX) activity and elevated tumorigenic potential of prostate cancer cells (Arnold et al. 2013); reduced complex III activity caused by a deletion in *MT-CYTB* increased bladder cancer growth (Dasgupta et al. 2008) and mtDNA mutations in genes encoding different complex I subunits led to increased tumorigenic potentials of head-and-neck (Zhou et al. 2007; Sun et al. 2009), colon (Park et al. 2009), lung (Dasgupta et al. 2008; Ishikawa et al. 2008), and breast (Kulawiec et al. 2009b) cancer cells. Particularly interesting are the mtDNA mutations that have been shown to increase metastatic potential of cancer cells in vivo. These were mainly associated with increased ROS production (Ishikawa et al. 2008; Kulawiec et al. 2009a), although mtDNA mutations that promote metastatic events through ROS-independent mechanisms have been described (Imanishi et al. 2011).

Certain mutations may induce neutral effects on tumorigenesis and yet confer resistance to therapeutic approaches, which substantially renders them pro-tumorigenic. Association of mtDNA mutations with therapy resistance is a particularly interesting field of research since their identification may be helpful in the prediction of therapy response in patients. Pancreatic cancer-specific mtDNA mutations showed to confer resistance to staurosporine-induced apoptosis (Mizutani et al. 2009); mtDNA-depleted HeLa (Shidara et al. 2005) and osteosarcoma (Yen et al. 2005) cells showed resistance to cisplatin, and chronic lymphocytic leukemia patients refractory to conventional therapeutic agents tended to have higher mtDNA mutation rates than patients who responded to treatment (Carew et al. 2003). Moreover, a case of ovarian carcinoma harboring a pathogenic mtDNA mutation, probably induced by carboplatin chemotherapy, displayed resistance to paclitaxel combined treatment (Guerra et al. 2012). Albeit in a small number of studies, the functional status of mtDNA mutations has been shown to play an essential role also in radiation induced cytotoxicity, in the way that radiosensitivity of cells bearing such mutations was reduced (Alsbeih et al. 2009; Cloos et al. 2009). Interestingly, gamma radiation commonly used in anticancer therapy was shown to increase mtDNA copy number, which may further prompt accumulation of mitochondrial mutations (Bartoletti-Stella et al. 2013).

Finally, mtDNA mutations that lead to a reduced tumorigenic potential have only recently been recognized, along with a thorough revision of the essential function of mitochondria in cancer and of the Warburg hypothesis (Wallace 2012). The first evidence came from experiments with mtDNA-depleted cancer cells (Rho zero), characterized by a severe mitochondrial respiration damage and displaying low proliferative and low invasive potentials, often failing to form tumors in vivo, unlike their nuclearly isogenic counterparts carrying normal mtDNA pool (Morais et al. 1994; Cavalli et al. 1997; Shidara et al. 2005; Imanishi et al. 2011). Likewise, several functional studies demonstrated how clearly pathogenic mtDNA mutations reduce or inhibit tumor growth. These mainly include mutations affecting complex I subunits (Park et al. 2009; Gasparre et al. 2011) and

tRNA loci (Arnould et al. 2002; Iommarini et al. 2014), which lead to such an extent of complex I deficiency and respiratory chain defects to cause lethality. In fact, highly severe mutations are negatively selected in cancer tissue and are usually found heteroplasmic (Brandon et al. 2006; Larman et al. 2012). In case such mutations do cross the threshold value for their disruptive phenotypic effect, the concordant low aggressive phenotype is developed, as observed in oncocytomas (Iommarini et al. 2012; Pereira et al. 2012) (see Sect. 10.6).

10.5 Mitochondrial Mutations and Cancer Metabolism

Apart from being genetically determined, cancer is also a metabolic disease. Metabolic reprogramming has been recently introduced as one of the hallmarks of cancer cells (Hanahan and Weinberg 2011; Ward and Thompson 2012), since a high proliferation rate requires rapid energy production, a shift toward biosynthetic reactions and parallel maintenance of the redox homeostasis (Cairns et al. 2011). It has been extensively demonstrated that oncogene-driven tumorigenesis is always accompanied by adequate metabolic adaptations. Oncogenes promoting phosphatidylinositide 3-kinases (PI3K) signaling was shown to lead to increased glucose uptake and glycolysis (Elstrom et al. 2004). Myc-induced transformation promotes glutaminolysis, the second major carbon source in cancer metabolism necessary for TCA cycle maintenance and anaplerosis (DeBerardinis et al. 2007; Wise et al. 2008). On the other hand, loss of p53 function is associated with downregulation of mitochondrial metabolism (Matoba et al. 2006; Wang et al. 2013) and redirection of glucose to the pentose phosphate pathway, which ensures NADPH-dependent redox maintenance (Bensaad et al. 2006).

In the context of genetically regulated metabolic reprogramming (DeBerardinis et al. 2008; Ward and Thompson 2012), mtDNA mutations represent a powerful tool for modulation, since mitochondria play a substantial role in a number of metabolic reactions. Moreover, due to heteroplasmy and threshold effect, the reversibility and the array of mtDNA mutation-induced phenotypes, ranging from mild to severe functional consequences, allow an efficient fine-tuning of cancer cell metabolism depending on the ever-changing selective pressures during tumor progression. The following key elements of cellular metabolism have been shown to be altered in response to the occurrence of mtDNA mutations in cancer cells:

ROS The most investigated metabolic consequence of cancer mtDNA mutations is related to ROS signaling, since complex I and complex III are two main sources of ROS, and they are both partly encoded by mtDNA genes. Increased ROS levels have been mainly associated with promotion of tumor progression and metastases (Ishikawa et al. 2008). Their mitogenic properties are exhibited through the interaction with various regulatory factors such as MAP kinases, PI3Ks, PTEN, and protein tyrosine phosphatases (Ray et al. 2012), and their pro-metastatic capacity has been suggested to be related to HIF1α stabilization (Chandel et al. 2000;

Ishikawa et al. 2008). However, although ROS appear to be required for anchorage-independent growth, chronically increased levels severely damage mitochondria. At high levels, they are toxic, and, depending on their type and concentration, they may mediate both pro- and antiapoptotic events (Shen et al. 1998). It has been shown that functionally mild mtDNA mutations, such as the heteroplasmic m.12417insA/*MT-ND5*, lead to pro-tumorigenic signaling by increasing superoxide in mitochondria. Elevated peroxide levels in cytosol, which are induced by the homoplasmic version of the same mutation, may instead lead to apoptosis (Park et al. 2009). In that study, an increased expression of anti-oxidative enzymes levels was detected in heteroplasmic cells. Similar compensatory effect in the form of upregulation of detoxifying enzymes was observed in osteosarcoma and thyroid cancer cells carrying the nearly homoplasmic m.3571insC/*MT-ND1* mutation (Porcelli et al. 2010), suggesting that the effect of a mutation on tumor progression will depend also on the activity of detoxifying mechanisms which may protect from harmful ROS effects. It is interesting to note that it remains to be explained how such truncative mutations that likely abolish function of the main ROS-generating sites may lead to an increased ROS production.

ATP Production of energy in the form of ATP is the main task of OXPHOS and thus mtDNA mutations are very often associated with a decrease in OXPHOS-derived energy production (Park et al. 2009; Gasparre et al. 2011; Sharma et al. 2011; Jandova et al. 2012a). However, this is recuperated through increased glycolysis rate, and thus an alteration in ATP concentrations has rarely been brought into a direct association with a modification of hyperproliferative processes. Nevertheless, it has been demonstrated that despite the reduced OXPHOS in cancer, a significant amount of cancer cell energy still derives from at least partially functional ETC (Zu and Guppy 2004). In fact, severe mtDNA mutations, such as the m.3243A > G/*MT-TL1*, were shown to induce energetic crisis, alteration in AMP/ADP/ATP levels, and subsequent decrease of tumor growth of cybrids in vivo (Iommarini et al. 2014), indicating that substantial damage to OXPHOS-mediated energy production may be fatal for the cancer cell. In fact, such mutations are typical of neuromuscular mitochondrial diseases and rarely found in human cancers (Iommarini et al. 2012). The mechanism by which these severe mutations modulate tumor progression involves activation of AMP-activated protein kinase (AMPK) (Iommarini et al. 2014), the main cellular energy sensor which promotes catabolic ATP-generating reactions, while downregulating ATP-consuming biosynthetic pathways (Hardie 2011), for which a tumor suppressor role has been inferred (Faubert et al. 2013). It is interesting to note that AMPK also upregulates mitochondrial biogenesis and regulates disposal of damaged mitochondria (mitophagy) to preserve the overall cellular ATP-generating capacity (Hardie 2011), suggesting that AMPK status may contribute to mtDNA selection in cancer.

NAD^+/NADH NAD homeostasis is vital for cellular signaling reactions, such as protein ribosylation and deacetylation reactions, and generation of Ca^{2+}-mobilizing messenger molecules (Chiarugi et al. 2012). In comparison to normal cells, the NAD^+/NADH ratio is somewhat reduced in cancer due to the high glycolysis rate

accompanied with lower ETC reducing activity and due to elevated biosynthetic reactions for which NAD^+-derived NADP is required (Chiarugi et al. 2012). An additional drop in this ratio would be fatal for a cell and thus targeting NAD^+ synthesis has been proposed as a cancer therapy (Hasmann and Schemainda 2003). Along this line, since mitochondrial complex I is the main regenerator of cellular NAD^+, mtDNA mutations causing defects in NADH dehydrogenase activity may compromise NAD^+ homeostasis and prevent tumor growth. In fact, severe mtDNA mutations leading to substantial reduction in the NAD^+/NADH ratio were shown to decrease the tumorigenic potential of cancer cells (Calabrese et al. 2013; Iommarini et al. 2014). It must be noted, however, that non-lethal reduction in NAD^+ levels induced a more aggressive, pro-metastatic phenotype in breast cancer models (Santidrian et al. 2013) and some studies indicate that the decrease in NAD^+/NADH ratio promotes tumor growth through NADH-regulated PTEN inactivation and promotion of AKT survival signaling (Pelicano et al. 2006; Sharma et al. 2011). Again, the specific tumor microenvironment, the genetic background, and the degree of imbalance in NAD^+ homeostasis probably explain the pleiotropic effects of mtDNA mutations on cancer progression.

Lactate High rate of aerobic glycolysis in cancer leads to elevated lactate production which compromises intracellular pH homeostasis but promotes invasiveness by acidification of tumor stroma (Warburg et al. 1924; Chiche et al. 2010). HIF1α-signaling contributes to lactate increase in cancer cells by inhibiting pyruvate dehydrogenase and redirecting pyruvate from the TCA cycle to fermentation and, at the same time, activating pH maintenance pathways which ensure that cell survival is not jeopardized by lactate-induced acidosis. Several complex I mtDNA mutations have been shown to contribute to elevated lactate levels in cancer cells and to promote tumor growth by inducing HIF1α-dependent mechanisms (Zhou et al. 2007; Sun et al. 2009). On the other hand, the increase in lactate concentration described in breast cancer cell lines carrying the m.12084C > T/*MT-ND4* and the m.13966A > G/*MT-ND5* mutations was not associated with elevated HIF1α expression but was nonetheless sufficient to confer a higher metastatic potential (Imanishi et al. 2011). The latter study suggests that mtDNA mutations may influence tumor progression by regulating cancer cell pH homeostasis, also in an HIF1-α-independent manner. Interestingly, the same breast cancer cells deprived of their mtDNA produced extremely high lactate levels, which were not associated with metastasis development, indicating that cells that have not gone through HIF1α-mediated adaptation may not sustain the extreme acidosis caused by severe mitochondrial defects (Imanishi et al. 2011).

Although lactate acidosis is mainly associated with hypoxia, it has also been observed in normoxic cancer cell environment (Icard and Lincet 2012). It has been suggested that lactate produced by hypoxic cells is not merely a waste product, but may be used as an energy fuel for ATP production through OXPHOS in normoxic cells (Feron 2009). Such metabolic symbiosis spares glucose which may then reach hypoxic and anoxic cells where it is used for anaerobic glycolysis. It is important to

note that in this context, different selective pressures would affect accumulation of mtDNA mutations between normoxic and anoxic cancer cell.

TCA Cycle Intermediates The TCA or Krebs cycle is a central pathway in the metabolism of sugar, lipids, and amino acids. Far from being a closed pathway, the TCA cycle rather integrates several metabolic reactions in a cell, such as the metabolism of amino acids, fatty acids, and heme, via anaplerotic reactions. The cytoplasmic and mitochondrial pools of TCA cycle intermediates are in tight connection, and metabolite accumulation in one of the pools is immediately reflected in the other.

Alteration of TCA cycle as a mechanism of tumorigenesis has been initially associated to mutations in two key enzymes that catalyze essential steps within the cycle, namely, SDH and FH, whose loss of function leads to the accumulation of fumarate and succinate, causing abnormal normoxic stabilization of HIF1α, since high levels of these metabolites inhibit the prolyl-hydroxylase reaction which mediates HIF1α degradation (Isaacs et al. 2005; Selak et al. 2005). Moreover, high concentrations of fumarate and succinate have been shown to induce aberrant patterns of gene expression by inhibiting enzymes involved in DNA and histone demethylation (Cervera et al. 2009) or by causing protein impairment via succinylation (Alderson et al. 2006).

Imbalance in TCA cycle intermediates concentrations has also been observed as a consequence of mtDNA mutations. Cancer cells harboring nearly homoplasmic levels of the m.3571insC/*MT-ND1* mutation, which leads to a dysfunctional complex I, display an increased α-ketoglutarate/succinate ratio as a direct consequence of NADH accumulation. In this case, conversely from what is observed in SDH and FH mutated tumors, HIF1α is chronically destabilized even in hypoxic conditions (pseudonormoxia), since α-ketoglutarate feeds and promotes the prolyl-hydroxylase reaction (Gasparre et al. 2011). Therefore, the m.3571insC/*MT-ND1* may hamper tumor growth by introducing imbalance in TCA cycle metabolites. It is very likely that other mtDNA mutations may modify tumor progression by similarly changing the cellular metabolite concentrations.

The stalling of the TCA cycle due to FH or SDH deficiency may prevent cells from generating TCA cycle intermediates such as malate, oxaloacetate, and citrate by conventional oxidative metabolism. It has been shown that in such conditions, a cell turns to alternative pathways. For instance, in vitro studies on cancer cell lines with FH mutations or defective complex I or III have demonstrated a switch to anoxic glutaminolysis as a dominant mode of metabolism for supporting cell proliferation and generating citrate (Mullen et al. 2011). However, according to the model suggested by Smolkova and colleagues (Smolkova et al. 2011), this process would not be sufficient to support proliferation for a long period due to energetic deficiency, and increase in glycolysis or oxidative carboxylation would have to be established. In this context, it is important to note that because of its essential dependence on complex II (Smolkova and Jezek 2012), oxidative glutaminolysis may be sustained only in cancer cells with at least partially functional ETC. For instance, severely dysfunctional complex I cells should be able to

sustain oxidative glutaminolysis, whereas cancer cells with a downstream damage in ETC would compromise this metabolic pathway and possibly hold back tumor progression.

10.6 Cell-Systemic Consequences of mtDNA Mutations That Modify Tumor Progression

Apart from their role in metabolic reactions, mitochondria are involved in many other pivotal cellular processes, such as apoptosis, hypoxic adaptation, or autophagy. The ways in which mtDNA mutations may influence tumor progression by altering proper functions of such pathways are here described.

Apoptosis The most common form of cell death in mammalian cells involves the mitochondrial apoptotic pathway, in which the outer mitochondrial membrane is permeabilized, releasing caspase-activating molecules and caspase-independent death effectors (Green and Kroemer 2004). This process is regulated by cytosolic p53 and Bcl-2 family of pro- and antiapoptotic proteins, but may also be a consequence of mitochondrial damage, such as dissipation of mitochondrial membrane potential (Green and Kroemer 2009). Evasion of apoptosis and, in particular, a related resistance to mitochondrial outer membrane permeabilization is a hallmark of cancer cells. In this context, mtDNA mutations are often associated with modulation of apoptotic mechanisms in cancer. Decrease in apoptosis was associated with elevated ROS production in m.6124T > C/*MT-COI* mutant prostate cancer cells (Arnold et al. 2013), m.10398G > A/*MT-ND3* mutant breast cancer cells (Kulawiec et al. 2009b), and colorectal cancer cell heteroplasmic for the m.12417insA/*MT-ND5* (Park et al. 2009). On the other hand, certain mutations have been shown to boost apoptotic mechanisms and prevent tumor growth, such as the homoplasmic version of the m.12417insA/*MT-ND5* (Park et al. 2009).

Interestingly, mtDNA mutations appear to have the ability to influence expression of apoptotic genes. Depending on the degree of respiratory chain damage induced by different mtDNA mutations, osteosarcoma-derived cybrids displayed diverse levels of Bcl-2 family protein expression and exhibited either pro- or antiapoptotic signaling, despite their isogenic nuclear background (Kwong et al. 2007). In particular, COX- and CYTB-deficient cells showed lower expression of Bcl-XL and Bcl-2, respectively, resulting in resistance to staurosporine-induced apoptosis. On the other hand, NARP mutants that maintain all respiratory chain complexes, albeit at reduced amounts and activities, displayed staurosporine sensitivity followed by low Bcl-2 and high Bcl-XL expression levels. Moreover, thapsigargin, which mimics stress derived from the endoplasmic reticulum, was shown to induce apoptosis in all of these cells, while Rho zero cells and MERRF mutants, which completely lack functional and structural integrity of respiratory chain complexes, were resistant to this type of programmed death (Kwong et al. 2007).

Interestingly, it has been shown that mitochondrial damage may cause alterations in pyrimidine synthesis and folate metabolism and thus result in less efficient DNA repair and chromosomal instability (Desler et al. 2007; Minocherhomji et al. 2012). In this context, since p53 responds to DNA damage, it is interesting to speculate that mtDNA mutations might influence p53 activity by regulating the nucleotide pool of a cancer cell (Naviaux 2008).

Taken together, the apoptotic responses in mtDNA-mutated cancer cells may depend on the type of cellular stress, their effective consequence on mitochondrial membrane potential, and on the expression of pro- and antiapoptotic proteins.

Hypoxic Adaptation The ability to stabilize HIF1α is considered a feature of malignant tumors. During the oxygen deprivation that follows the increase in size of a cancer mass before neovascularization sets in, HIF1α stabilization is pivotal to adapt to hypoxic conditions, mainly by upregulating the expression of glycolytic genes (Semenza et al. 1994). Moreover, it has been shown that hypoxic adaptation also requires HIF1α-mediated suppression of TCA cycle and OXPHOS (Kim et al. 2006; Papandreou et al. 2006). As mentioned earlier, *FH* and *SDH* mutations have been associated with tumorigenesis due to an aberrant HIF1α stabilization. Indeed, by slowing down oxidative metabolism during hypoxia, tumor cells decrease their oxygen requirements, and in this context, many mtDNA mutations have been shown to contribute to hypoxic adaptation. For instance, cells harboring the m.4776G > A/*MT-ND2* missense mutation showed an increased tumorigenicity and HIF1α accumulation (Zhou et al. 2007). These effects were due to the ROS-mediated upregulation of pyruvate dehydrogenase kinase 2, with a subsequent inhibition of pyruvate dehydrogenase and finally block of pyruvate entrance in the TCA cycle (Sun et al. 2009). Moreover, it has been shown that ROS-mediated HIF1α accumulation may lead to enhanced metastatic potential of cells harboring the m.13997G > A/*MT-ND6* missense mutation (Ishikawa et al. 2008). However, conversely from the hypoxic and anoxic areas of quickly growing primary tumors, it is interesting to note that metastatic cells ought to be well oxygenated. Consequently, revival of mitochondrial function may be profitable in such cells. Indeed, there are single reports of mtDNA mutations present in primary tumor, but purified in putative metastases (Horton et al. 1996). Therefore, it remains to be explained why mitochondrial damage should be an advantageous feature of pre-metastatic clones.

Besides the well-known negative regulation of mitochondrial function by HIF1α, through activation of pyruvate dehydrogenase kinase 1 (Kim et al. 2006; Papandreou et al. 2006), there are indications that HIF1α stabilization actually depends on functional mitochondria. For instance, several studies have reported that loss of respiratory complex III may contribute to ROS-mediated destabilization of HIF1α (Guzy et al. 2005; Mansfield et al. 2005). Moreover, it has been shown that dysfunction in complex I caused by the severe m.3571insC/*MT-ND1* mutation strongly contributes to HIF1α destabilization by alteration of TCA cycle metabolite balance (Gasparre et al. 2011). Allotopic expression of functional ND1 in *MT-ND1* osteosarcoma mutant cells demonstrated that a certain portion of functional

complex I is required for the establishment of the Warburg effect during response to hypoxia (Calabrese et al. 2013). In this context, it is interesting to note that, conversely from *SDH* and *FH* mutated tumors, a novel class of mutations in isocitrate dehydrogenase (*IDH*) 1 and 2 genes was recently reported in gliomas and acute myeloid leukemia (AML) (Koivunen et al. 2012; Losman et al. 2013). These mutations have been shown to induce the accumulation of (R)-2-hydroxyglutarate (R-2HG), an activator of prolyl-hydroxylases, thus causing consequent destabilization of HIF1α. This characteristic has been suggested to be responsible for less malignant behavior in glioma patients (Koivunen et al. 2012).

Therefore, it seems that a two-way relationship between hypoxic adaptation and mitochondrial function exists, in which mtDNA mutations may result as positive or negative modifiers of hypoxic adaptation, since the ETC is de facto a fundamental oxygen sensor as is the HIF1α pathway.

Signaling Pathways In order to maintain cellular homeostasis, mitochondria are capable of communicating with the nucleus through so-called retrograde signaling (Guha and Avadhani 2013). Among others, mitochondria-to-nucleus crosstalk includes Ca^{2+} (Biswas et al. 1999) and a redox-related flow of information (Ray et al. 2012), both of which may be altered due to a mitochondrial damage (Amuthan et al. 2002; Ishikawa et al. 2008). For instance, mtDNA depletion has been shown to induce a drop in membrane potential, which decreases Ca^{2+} uptake into mitochondria, increasing the levels of cytoplasmic Ca^{2+} concentrations, activating retrograde signaling, and promoting invasiveness of lung cancer cells (Amuthan et al. 2002). On the other hand, ROS signaling has been associated with AKT survival pathway. In particular, in cancer cells carrying heteroplasmic complex I damaging *MT-ND5* mutation, ROS- and NAD^+-/NADH-mediated phosphorylation of AKT led to upregulation of HIF1α activity (Sharma et al. 2011). These effects on AKT signaling pathway were specific to complex I damage, since mutations in COX only displayed a decrease in ATP and respiration defects, without affecting the ROS-AKT-HIF axis (Sharma et al. 2011). Similarly, AKT activation was induced by mtDNA depletion in a number of cancer cell lines, in which increases in NADH concentrations and subsequent decreases in NADPH were associated with PTEN inactivation and promotion of AKT signaling (Pelicano et al. 2006). Moreover, a CPEO-associated mtDNA mutation in *MT-TRNA* for leucine was shown to cause ROS-independent AKT activation, resulting in apoptosis resistance and increase in metastatic potential of breast cancer cells (Kulawiec et al. 2009a).

Phosphorylation of AKT leads to mTOR kinase activation, one of the best known pathways regulating cell metabolism (Memmott and Dennis 2009) and the main cellular sensor of nutrients promoting protein and lipid synthesis. Alternatively, mTOR is negatively regulated by AMPK, which switches off biosynthetic reactions in low energy conditions, in order to maintain cellular energy homeostasis (Hardie 2011). In the study by Sharma et al, complex I mutant cybrids displayed dephosphorylation of AMPK, in comparison to wild-type mtDNA cybrids. Conversely, severe mtDNA mutants appear to induce AMPK activation, which ultimately results in suppression of tumor growth, in agreement with the inferred role

of tumor suppressor for this crucial kinase (Iommarini et al. 2014). Therefore, depending on their type and mutant load, mtDNA mutations may influence mTOR signaling through both AKT and AMPK pathways.

Furthermore, it has recently been demonstrated that mtDNA depletion may lead to increased expression of HMGR, a rate-limiting enzyme in the mevalonate pathway, which is an important source of farnesyl moieties (Cook and Higuchi 2012). The latter are used for K-Ras prenylation, a posttranslational modification that allows K-Ras/Raf complex formation and downstream AKT activation. That study provides an interesting example on how mitochondrial function is connected to the cellular signaling pathways and suggests that targeting such metabolic reactions may be potentially exploited in cancer therapy development.

Apart from mTOR, a number of other signaling pathways have been shown to be influenced by mitochondrial damage, such as ERK1/2-MAP kinase (Weinberg et al. 2010) and NFKB2 (Higuchi et al. 2002; Dasgupta et al. 2008; Jandova et al. 2012b).

Taken together, it is emerging that oncogenic signaling pathways may depend on mitochondrial function, among others, through alteration of cellular ROS and Ca^{2+} concentrations and by modulating the availability of metabolites involved in pro-oncogenic signaling.

Epigenetics Oncogenes and tumor suppressors are known to regulate metabolic reprogramming during cancer progression (Ward and Thompson 2012) and thus create selective pressures under which mtDNA mutations may be accumulated or purified. In turn, mitochondrial damage induced by mtDNA mutations may have consequences on oncogenes and tumor suppressors function by regulating their gene expression, indicating that oncogenic properties may be transmitted from mitochondria to the nucleus (Ma et al. 2010; Jandova et al. 2012b). It was shown that certain mtDNA haplogroups may influence nuclear methylation patterns (Bellizzi et al. 2012) and that mtDNA depletion leads to general hypomethylation of nuclear genes, potentially promoting activation of oncogenes (Smiraglia et al. 2008).

Since epigenetic control of gene expression involves a series of enzymatic reactions, including DNA and histone methylation, acetylation, hydroxylation, and phosphorylation, mechanisms through which mitochondrial alterations may regulate nuclear gene expression mainly concern the availability of metabolic substrates or cofactors required for these reactions (Naviaux 2008). For instance, the NAD^+/NADH ratio is important for the activities of sirtuin histone deacetylases (Imai et al. 2000); TCA cycle intermediates have been shown to regulate histones and DNA demethylation reactions by Jumonji C-domain-containing histone lysine demethylases (JHDM) and ten-eleven translocation family of 5-methylcytosine hydroxylases (TET) (Lu and Thompson 2012), and cytosolic citrate is converted to acetyl-CoA required as a donor of acetyl groups for HAT-mediated histone acetylation (Wellen et al. 2009). Moreover AMPK, activated by a low ATP/AMP ratio, is able to phosphorylate histones (Bungard et al. 2010). The abundance of metabolic substrates and cofactors in epigenetic mechanisms is highly dependent

on the metabolic state of a cell and may be regulated by a mitochondrial damage induced both by mtDNA mutations and mutations in nuclear genes encoding mitochondrial proteins. The latter have been particularly often associated with epigenetic reprogramming in cancer, since TCA cycle intermediates may inhibit α-ketoglutarate-dependent dioxygenases involved in DNA and histone demethylation, as observed in tumors harboring dysfunctional FH and SDH where fumarate and succinate have been shown to inhibit JHDM and TET protein function by competitive inhibition (Xiao et al. 2012). Along this line, increase in R-2HG concentrations, caused by *IDH* mutations, has been shown to induce epigenetic alterations that affect cell differentiation in gliomas and AML, eventually leading to cell transformation (Koivunen et al. 2012; Losman et al. 2013). Interestingly, the same metabolite has been associated with inability to stabilize HIF1α, which may at least partly explain the more favorable prognosis in glioma patients carrying *IDH* mutations (Koivunen et al. 2012; Losman et al. 2013).

Furthermore, it was recently discovered that DNA methyltransferase 1 may translocate into mitochondria (Shock et al. 2011) and methylate mtDNA CpG islands. Interestingly, the distribution of CpG islands in mtDNA reveals that they are more common in the regulatory D-loop region, where the control of the whole polycistron resides, and less frequent than expected by chance in mitochondrial tRNA and coding genes (Chinnery et al. 2012). Along this line, it has been shown that mutations in mtDNA may influence its own methylation causing metabolic alterations potentially important for cancer progression (Raimundo et al. 2012).

Autophagy Autophagy is the main cellular route for degradation of bulk cytoplasm and organelles, which may lead either to cell death (Gozuacik and Kimchi 2004) or to the recycling of cellular building blocks for macromolecule synthesis in stress conditions such as hypoxia (Lozy and Karantza 2012). Generally, it is inhibited by high nutrient availability and growth factors, whereas ROS, hypoxia, and starvation promote autophagy (Lozy and Karantza 2012). In normal conditions, autophagy accounts for removal of damaged and permeabilized mitochondria (mitophagy) and thus counteracts proapoptotic effects of mitochondrial outer membrane permeabilization (Hill et al. 2012). In cancer, autophagy seems to be context dependent (Lozy and Karantza 2012). On one hand, the decrease in autophagy often observed in cancer may permit the relaxation of negative selection and promote accumulation of proapoptotic mtDNA mutations. Along this line, complementation of mitochondrial damage caused downregulation of mTOR signaling pathway and subsequent increase in autophagy, inhibiting the metastatic activity of breast cancer cells (Santidrian et al. 2013). Conversely, *KRAS*-driven tumorigenesis was shown to upregulate autophagy in order to maintain functional mitochondrial metabolism, necessary for propagation of such tumors (Guo et al. 2011). In such *KRAS*-driven models, defects in autophagy were shown to mimic the antitumorigenic effects of severe mtDNA mutations by redirecting cancer progression from carcinoma to benign oncocytoma (Guo et al. 2013). The relationship between mitochondria, autophagy, and tumor progression is therefore not straightforward, and further studies are warranted to resolve their connections.

10.7 Oncocytic Tumors

Cells of certain tumor types are characterized by abnormal accumulation of mitochondria in their cytoplasm, a phenotype that in pathology is referred to as oncocytic change (Tallini 1998). Oncocytic tumors are mainly observed in lesions of endocrine tissues such as the thyroid (Gasparre et al. 2010a), kidney (Akhtar and Kott 1979), parotid, or pituitary gland (Silbergeld et al. 1993), but have also been described in cases of breast, colon, lung, glioblastoma, nasopharynx, and endometrial cancers (Damiani et al. 1998; Gasparre et al. 2010b; Guerra et al. 2011; Marucci et al. 2013). Mitochondria of oncocytic cells are often damaged, as observed from electron micrographs showing their swollen morphology and deranged cristae. This has been partly explained by the high frequency of pathogenic mtDNA mutations that is found in oncocytic lesions. Depending on the tissue type, 45–100 % of such neoplasms carry some kind of mtDNA mutations, which is significantly more frequent than what is observed in their non-oncocytic counterparts (Gasparre et al. 2007, 2010b; Porcelli et al. 2010; Pereira et al. 2012; Kurelac et al. 2013). In particular, frameshift and nonsense mtDNA mutations are far more common and are often homoplasmic in oncocytomas, resulting in substantial defects in mitochondrial function.

It has been hypothesized that the mitochondrial hyperplasia in oncocytic tumors is a direct consequence of mtDNA mutations, since the resulting mitochondrial damage may provoke increased organelle biogenesis in order to compensate for the bioenergetic defect (Savagner et al. 2001). In fact, introduction of a severe complex I disruptive homoplasmic mtDNA mutation in cybrid cells was shown to lead to the development of osteosarcoma with an oncocytic phenotype, followed by a less efficient tumor settlement and decreased tumor growth, compared to their wild-type or heteroplasmic counterparts (Gasparre et al. 2011). This is in line with the recent revision of the Warburg hypothesis, which suggests that mitochondrial function must not be completely abolished, since it would lead to lethality. In fact, oncocytic tumors are most often low aggressive and are usually considered benign quiescent neoplasias (Gasparre et al. 2010b; Rossi et al. 2013), as supported by a lower genomic instability in pituitary oncocytomas (Kurelac et al. 2013). Functional studies in athymic mice demonstrate that the most common mtDNA mutation found in oncocytomas, namely, the m.3571insC/*MT-ND1*, is capable of abolishing osteosarcoma growth in vivo and that compensation with a functional ND1 reverts the phenotype and reestablishes tumorigenic potential (Gasparre et al. 2009; Calabrese et al. 2013). This mutation, when homoplasmic, leads to abolishment of complex I function and affects efficiency of complex I assembly. In particular, it results in a truncated ND1 subunit which most likely hampers the formation of the binding site between different assembly modules of the complex, potentially affecting the whole respirasome (Vogel et al. 2007; Baradaran et al. 2013). In agreement with the main role of complex I in sustaining mitochondrial membrane potential and cellular redox homeostasis by oxidation of NADH, in the m.3571insC/*MT-ND1* homoplasmic cancer models, both ATP production and

NAD^+/NADH ratio were significantly reduced when compared to the heteroplasmic counterparts and wild-type cells, leading to activation of AMPK (Iommarini et al. 2014) and destabilization of HIF1α (Calabrese et al. 2013), respectively. These studies demonstrate that functional complex I is necessary for the activation of the HIF1α pathway and for the establishment of the Warburg effect (Calabrese et al. 2013), explaining at least in part why oncocytic tumors carrying such damage often do not progress to the malignant state. In addition, the inability to stabilize HIF1α may contribute to increased mitochondrial hyperplasia in oncocytic tumors, since this transcription factor is a known negative regulator of mitochondrial biogenesis (Papandreou et al. 2006; Bartoletti-Stella et al. 2013).

It is interesting to note that an oncocytic change, followed by tumor growth arrest, was recently observed to be triggered during *KRAS*-driven cancer transformation when the pivotal autophagy-regulating gene was knocked out (Guo et al. 2013). This suggests that mtDNA mutations causing inadequate function of autophagic machinery may also contribute to the mechanism of oncocytic phenotype development.

Despite being a relatively rare entity, oncocytic tumors have provided many insights on the role of mtDNA mutations and mitochondrial metabolism in cancer. Most importantly, analyses of mtDNA mutations in oncocytic tumors allowed to identify a novel class of cancer-associated genes, namely, the oncojanus, that may behave in a twofold manner. Depending on the mtDNA type and mutant load, the same gene may behave as both tumor suppressor and as a lethality gene, underlining the importance of the application of appropriate measures when analyzing mtDNA mutations in cancer.

Conclusions

The consequences of mtDNA mutations are numerous and primarily depend on the type of damage, i.e., which part of the ETC is involved, to what extent, and during which phase of tumorigenesis. It is unlikely that mtDNA mutations on their own are sufficient to initiate tumorigenesis, but they may provide a fertile ground for adaptive mechanisms and may modify tumor progression. The general rule of thumb seems to be that mutations which cause severe mitochondrial damage leading to extreme metabolic conditions usually compromise tumor growth, whereas mild mutations which allow fine-tuning of tumor metabolism may be advantageous since they represent a pool of adaptive tools in cancer cell evolution. The most intriguing point is that an mtDNA mutation may in some contexts act as a neutral bystander, whereas in others it becomes advantageous for tumor growth, and thus appropriate analyses must be performed in order to assign a role for mtDNA mutations in tumor progression, especially in the context of using mtDNA mutations as diagnostic and prognostic markers in oncology.

(continued)

In this context, the advent of NGS, which has already revolutionized the field of genomics, is expected to further elucidate the mechanisms of mitochondrial genetics in cancer (He et al. 2010; Tang and Huang 2010; Zaragoza et al. 2010; Payne et al. 2013). It is peculiar to observe that the mtDNA does not represent the direct target in any of the currently used high-throughput sequencing methods, but is paradoxically retained as off-target sequence because of the overlapping nuclear probes designed against NumtS (Pesole et al. 2012; Samuels et al. 2013). As a consequence of this unintended contamination with mtDNA in NGS, a series of bioinformatics tools have been proposed to recover the lost mtDNA information from high-throughput sequencing (Picardi and Pesole 2012; Calabrese et al. 2014). Therefore, large-scale mtDNA genotyping in public cancer studies is actually possible and will inevitably lead to even better understanding of mtDNA selection in cancer and thus to a further elucidation of the roles of mitochondria and metabolic reprogramming in this complex disease.

Overall, the combination of next generation genetic approaches in mitochondrial genomics, sustained by a deeper understanding of the metabolic consequences that revolve around this pivotal organelle, is likely to provide oncology not only with powerful prognostic tools but also with a novel array of strategies and candidates for the development of anticancer therapies.

References

Aanen DK, Maas MF (2012) Recruitment of healthy mitochondria fuels transmissible cancers. Trends Genet 28:1–6

Akhtar M, Kott E (1979) Oncocytoma of kidney. Urology 14:397–400

Alderson NL, Wang Y, Blatnik M, Frizzell N, Walla MD, Lyons TJ et al (2006) S-(2-Succinyl) cysteine: a novel chemical modification of tissue proteins by a Krebs cycle intermediate. Arch Biochem Biophys 450:1–8

Alexeyev M, Shokolenko I, Wilson G, LeDoux S (2013) The maintenance of mitochondrial DNA integrity—critical analysis and update. Cold Spring Harb Perspect Biol 5:a012641

Alsbeih GA, Al-Harbi NM, El-Sebaie MM, Al-Rajhi NM, Al-Hadyan KS, Abu-Amero KK (2009) Involvement of mitochondrial DNA sequence variations and respiratory activity in late complications following radiotherapy. Clin Cancer Res 15:7352–7360

Amati-Bonneau P, Valentino ML, Reynier P, Gallardo ME, Bornstein B, Boissiere A et al (2008) OPA1 mutations induce mitochondrial DNA instability and optic atrophy 'plus' phenotypes. Brain 131:338–351

Amuthan G, Biswas G, Ananadatheerthavarada HK, Vijayasarathy C, Shephard HM, Avadhani NG (2002) Mitochondrial stress-induced calcium signaling, phenotypic changes and invasive behavior in human lung carcinoma A549 cells. Oncogene 21:7839–7849

Arnold RS, Sun Q, Sun CQ, Richards JC, O'Hearn S, Osunkoya AO et al (2013) An inherited heteroplasmic mutation in mitochondrial gene COI in a patient with prostate cancer alters reactive oxygen, reactive nitrogen and proliferation. Biomed Res Int 2013:239257

Arnould T, Vankoningsloo S, Renard P, Houbion A, Ninane N, Demazy C et al (2002) CREB activation induced by mitochondrial dysfunction is a new signaling pathway that impairs cell proliferation. EMBO J 21:53–63

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

Baradaran R, Berrisford JM, Minhas GS, Sazanov LA (2013) Crystal structure of the entire respiratory complex I. Nature 494:443–448

Bartoletti-Stella A, Mariani E, Kurelac I, Maresca A, Caratozzolo MF, Iommarini L et al (2013) Gamma rays induce a p53-independent mitochondrial biogenesis that is counter-regulated by HIF1alpha. Cell Death Dis 4:e663

Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A et al (2000) Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science 287:848–851

Bellizzi D, D'Aquila P, Giordano M, Montesanto A, Passarino G (2012) Global DNA methylation levels are modulated by mitochondrial DNA variants. Epigenomics 4:17–27

Bensaad K, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R et al (2006) TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell 126:107–120

Biswas G, Adebanjo OA, Freedman BD, Anandatheerthavarada HK, Vijayasarathy C, Zaidi M et al (1999) Retrograde Ca2+ signaling in C2C12 skeletal myocytes in response to mitochondrial genetic and metabolic stress: a novel mode of inter-organelle crosstalk. EMBO J 18:522–533

Boesch P, Weber-Lotfi F, Ibrahim N, Tarasenko V, Cosset A, Paulus F et al (2010) DNA repair in organelles: pathways, organization, regulation, relevance in disease and aging. Biochim Biophys Acta 1813:186–200

Bonnet C, Kaltimbacher V, Ellouze S, Augustin S, Benit P, Forster V et al (2007) Allotopic mRNA localization to the mitochondrial surface rescues respiratory chain defects in fibroblasts harboring mitochondrial DNA mutations affecting complex I or v subunits. Rejuvenation Res 10:127–144

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

Bungard D, Fuerth BJ, Zeng PY, Faubert B, Maas NL, Viollet B et al (2010) Signaling kinase AMPK activates stress-promoted transcription via histone H2B phosphorylation. Science 329:1201–1205

Burney S, Caulfield JL, Niles JC, Wishnok JS, Tannenbaum SR (1999) The chemistry of DNA damage from nitric oxide and peroxynitrite. Mutat Res 424:37–49

Cairns RA, Harris IS, Mak TW (2011) Regulation of cancer cell metabolism. Nat Rev Cancer 11:85–95

Calabrese C, Iommarini L, Kurelac I, Calvaruso MA, Capristo M, Lollini PL et al (2013) Respiratory complex I is essential to induce a Warburg profile in mitochondria-defective tumor cells. Cancer Metab 1:11

Calabrese C, Simone D, Diroma MA, Santorsola M, Guttà C, Gasparre G, Picardi E, Pesole G, Attimonelli M (2014) MToolBox: a highly automated pipeline for heteroplasmy annotation and prioritization analysis of human mitochondrial variants in high-throughput sequencing. Bioinformatics pii:btu483

Campbell CT, Kolesar JE, Kaufman BA (2012) Mitochondrial transcription factor A regulates mitochondrial transcription initiation, DNA packaging, and genome copy number. Biochim Biophys Acta 1819:921–929

Canter JA, Kallianpur AR, Parl FF, Millikan RC (2005) Mitochondrial DNA G10398A polymorphism and invasive breast cancer in African-American women. Cancer Res 65:8028–8033

Carelli V, Baracca A, Barogi S, Pallotti F, Valentino ML, Montagna P et al (2002) Biochemical-clinical correlation in patients with different loads of the mitochondrial DNA T8993G mutation. Arch Neurol 59:264–270

Carew JS, Zhou Y, Albitar M, Carew JD, Keating MJ, Huang P (2003) Mitochondrial DNA mutations in primary leukemia cells after chemotherapy: clinical significance and therapeutic implications. Leukemia 17:1437–1447

Cavalli LR, Varella-Garcia M, Liang BC (1997) Diminished tumorigenic phenotype after depletion of mitochondrial DNA. Cell Growth Differ 8:1189–1198
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, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM et al (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
Chatterjee A, Dasgupta S, Sidransky D (2011) Mitochondrial subversion in cancer. Cancer Prev Res (Phila) 4:638–654
Chatterjee A, Mambo E, Sidransky D (2006) Mitochondrial DNA mutations in human cancer. Oncogene 25:4663–4674
Chen PL, Chen CF, Chen Y, Guo XE, Huang CK, Shew JY et al (2012) Mitochondrial genome instability resulting from SUV3 haploinsufficiency leads to tumorigenesis and shortened lifespan. Oncogene 32:1193–1201
Chiarugi A, Dolle C, Felici R, Ziegler M (2012) The NAD metabolome—a key determinant of cancer cell biology. Nat Rev Cancer 12:741–752
Chiche J, Brahimi-Horn MC, Pouyssegur J (2010) Tumour hypoxia induces a metabolic shift causing acidosis: a common feature in cancer. J Cell Mol Med 14:771–794
Chinnery PF, Elliott HR, Hudson G, Samuels DC, Relton CL (2012) Epigenetics, epidemiology and mitochondrial DNA diseases. Int J Epidemiol 41:177–187
Cline SD (2012) Mitochondrial DNA damage and its consequences for mitochondrial gene expression. Biochim Biophys Acta 1819:979–991
Cloos CR, Daniels DH, Kalen A, Matthews K, Du J, Goswami PC et al (2009) Mitochondrial DNA depletion induces radioresistance by suppressing G2 checkpoint activation in human pancreatic cancer cells. Radiat Res 171:581–587
Coller HA, Khrapko K, Bodyak ND, Nekhaeva E, Herrero-Jimenez P, Thilly WG (2001) High frequency of homoplasmic mitochondrial DNA mutations in human tumors can be explained without selection. Nat Genet 28:147–150
Cook CC, Higuchi M (2012) The awakening of an advanced malignant cancer: an insult to the mitochondrial genome. Biochim Biophys Acta 1820:652–662
Copeland WC, Ponamarev MV, Nguyen D, Kunkel TA, Longley MJ (2003) Mutations in DNA polymerase gamma cause error prone DNA synthesis in human mitochondrial disorders. Acta Biochim Pol 50:155–167
Cuezva JM, Ortega AD, Willers I, Sanchez-Cenizo L, Aldea M, Sanchez-Arago M (2009) The tumor suppressor function of mitochondria: translation into the clinics. Biochim Biophys Acta 1792:1145–1158
Damiani S, Eusebi V, Losi L, D'Adda T, Rosai J (1998) Oncocytic carcinoma (malignant oncocytoma) of the breast. Am J Surg Pathol 22:221–230
Dasgupta S, Hoque MO, Upadhyay S, Sidransky D (2008) Mitochondrial cytochrome B gene mutation promotes tumor growth in bladder cancer. Cancer Res 68:700–706
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
DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S et al (2007) Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc Natl Acad Sci USA 104:19345–19350
Denver DR, Morris K, Lynch M, Vassilieva LL, Thomas WK (2000) High direct estimate of the mutation rate in the mitochondrial genome of Caenorhabditis elegans. Science 289:2342–2344
Desler C, Munch-Petersen B, Stevnsner T, Matsui S, Kulawiec M, Singh KK et al (2007) Mitochondria as determinant of nucleotide pools and chromosomal stability. Mutat Res 625:112–124
Elachouri G, Vidoni S, Zanna C, Pattyn A, Boukhaddaoui H, Gaget K et al (2011) OPA1 links human mitochondrial genome maintenance to mtDNA replication and distribution. Genome Res 21:12–20

Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR et al (2004) Akt stimulates aerobic glycolysis in cancer cells. Cancer Res 64:3892–3899

Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z et al (2013) AMPK is a negative regulator of the Warburg effect and suppresses tumor growth in vivo. Cell Metab 17:113–124

Feron O (2009) Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells. Radiother Oncol 92:329–333

Freyer C, Cree LM, Mourier A, Stewart JB, Koolmeister C, Milenkovic D et al (2012) Variation in germline mtDNA heteroplasmy is determined prenatally but modified during subsequent transmission. Nat Genet 44:1282–1285

Galluzzi L, Morselli E, Kepp O, Vitale I, Rigoni A, Vacchelli E et al (2010) Mitochondrial gateways to cancer. Mol Aspects Med 31:1–20

Gasparre G, Bonora E, Tallini G, Romeo G (2010a) Molecular features of thyroid oncocytic tumors. Mol Cell Endocrinol 321:67–76

Gasparre G, Hervouet E, de Laplanche E, Demont J, Pennisi LF, Colombel M et al (2008) Clonal expansion of mutated mitochondrial DNA is associated with tumor formation and complex I deficiency in the benign renal oncocytoma. Hum Mol Genet 17:986–995

Gasparre G, Iommarini L, Porcelli AM, Lang M, Ferri GG, Kurelac I et al (2009) An inherited mitochondrial DNA disruptive mutation shifts to homoplasmy in oncocytic tumor cells. Hum Mutat 30:391–396

Gasparre G, Kurelac I, Capristo M, Iommarini L, Ghelli A, Ceccarelli C et al (2011) A mutation threshold distinguishes the antitumorigenic effects of the mitochondrial gene MTND1, an oncojanus function. Cancer Res 71:6220–6229

Gasparre G, Porcelli AM, Bonora E, Pennisi LF, Toller M, Iommarini L et al (2007) Disruptive mitochondrial DNA mutations in complex I subunits are markers of oncocytic phenotype in thyroid tumors. Proc Natl Acad Sci USA 104:9001–9006

Gasparre G, Romeo G, Rugolo M, Porcelli AM (2010b) Learning from oncocytic tumors: why choose inefficient mitochondria? Biochim Biophys Acta 1807:633–642

Gochhait S, Bhatt A, Sharma S, Singh YP, Gupta P, Bamezai RN (2008) Concomitant presence of mutations in mitochondrial genome and p53 in cancer development—a study in north Indian sporadic breast and esophageal cancer patients. Int J Cancer 123:2580–2586

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

Green DR, Kroemer G (2009) Cytoplasmic functions of the tumour suppressor p53. Nature 458:1127–1130

Green DR, Kroemer G (2004) The pathophysiology of mitochondrial cell death. Science 305:626–629

Guerra F, Kurelac I, Cormio A, Zuntini R, Amato LB, Ceccarelli C et al (2011) Placing mitochondrial DNA mutations within the progression model of type I endometrial carcinoma. Hum Mol Genet 20:2394–2405

Guerra F, Perrone AM, Kurelac I, Santini D, Ceccarelli C, Cricca M et al (2012) Mitochondrial DNA mutation in serous ovarian cancer: implications for mitochondria-coded genes in chemoresistance. J Clin Oncol 30:e373–e378

Guha M, Avadhani NG (2013) Mitochondrial retrograde signaling at the crossroads of tumor bioenergetics, genetics and epigenetics. Mitochondrion 13:577–591

Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G et al (2011) Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. Genes Dev 25:460–470

Guo JY, Karsli-Uzunbas G, Mathew R, Aisner SC, Kamphorst JJ, Strohecker AM et al (2013) Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. Genes Dev 27:1447–1461

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

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674
Hardie DG (2011) AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. Genes Dev 25:1895–1908
Hasmann M, Schemainda I (2003) FK866, a highly specific noncompetitive inhibitor of nicotinamide phosphoribosyltransferase, represents a novel mechanism for induction of tumor cell apoptosis. Cancer Res 63:7436–7442
Hazkani-Covo E, Zeller RM, Martin W (2010) Molecular poltergeists: mitochondrial DNA copies (numts) in sequenced nuclear genomes. PLoS Genet 6:e1000834
He Y, Wu J, Dressman DC, Iacobuzio-Donahue C, Markowitz SD, Velculescu VE et al (2010) Heteroplasmic mitochondrial DNA mutations in normal and tumour cells. Nature 464:610–614
Higuchi M, Manna SK, Sasaki R, Aggarwal BB (2002) Regulation of the activation of nuclear factor kappaB by mitochondrial respiratory function: evidence for the reactive oxygen species-dependent and -independent pathways. Antioxid Redox Signal 4:945–955
Hill BG, Benavides GA, Lancaster JR Jr, Ballinger S, Dell'Italia L, Jianhua Z et al (2012) Integration of cellular bioenergetics with mitochondrial quality control and autophagy. Biol Chem 393:1485–1512
Horton TM, Petros JA, Heddi A, Shoffner J, Kaufman AE, Graham SD Jr et al (1996) Novel mitochondrial DNA deletion found in a renal cell carcinoma. Genes Chromosomes Cancer 15:95–101
Icard P, Lincet H (2012) A global view of the biochemical pathways involved in the regulation of the metabolism of cancer cells. Biochim Biophys Acta 1826:423–433
Imai S, Armstrong CM, Kaeberlein M, Guarente L (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403:795–800
Imanishi H, Hattori K, Wada R, Ishikawa K, Fukuda S, Takenaga K et al (2011) Mitochondrial DNA mutations regulate metastasis of human breast cancer cells. PLoS One 6:e23401
Iommarini L, Calvaruso MA, Kurelac I, Gasparre G, Porcelli AM (2012) Complex I impairment in mitochondrial diseases and cancer: parallel roads leading to different outcomes. Int J Biochem Cell Biol 45:47–63
Iommarini L, Kurelac I, Capristo M, Calvaruso MA, Giorgio V, Bergamini C, Ghelli A, Nanni P, De Giovanni C, Carelli V, Fato R, Lollini PL, Rugolo M, Gasparre G, Porcelli AM (2014) Different mtDNA mutations modify tumor progression in dependence of the degree of respiratory complex I impairment. Hum Mol Genet 23(6):1453–1466
Isaacs JS, Jung YJ, Mole DR, Lee S, Torres-Cabala C, Chung YL et al (2005) HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. Cancer Cell 8:143–153
Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H et al (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 320:661–664
Jandova J, Eshaghian A, Shi M, Li M, King LE, Janda J et al (2012a) Identification of an mtDNA mutation hot spot in UV-induced mouse skin tumors producing altered cellular biochemistry. J Invest Dermatol 132:421–428
Jandova J, Janda J, Sligh JE (2012b) Changes in mitochondrial DNA alter expression of nuclear encoded genes associated with tumorigenesis. Exp Cell Res 318:2215–2225
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
Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV et al (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 315:525–528
Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkissoon S et al (2012) Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. Nature 483:484–488
Komar AA (2007) Genetics. SNPs, silent but not invisible. Science 315:466–467
Kulawiec M, Owens KM, Singh KK (2009a) Cancer cell mitochondria confer apoptosis resistance and promote metastasis. Cancer Biol Ther 8:1378–1385

Kulawiec M, Owens KM, Singh KK (2009b) mtDNA G10398A variant in African-American women with breast cancer provides resistance to apoptosis and promotes metastasis in mice. J Hum Genet 54:647–654

Kurelac I, Lang M, Zuntini R, Calabrese C, Simone D, Vicario S et al (2011) Searching for a needle in the haystack: comparing six methods to evaluate heteroplasmy in difficult sequence context. Biotechnol Adv 30:363–371

Kurelac I, MacKay A, Lambros MB, Di Cesare E, Cenacchi G, Ceccarelli C et al (2013) Somatic complex I disruptive mitochondrial DNA mutations are modifiers of tumorigenesis that correlate with low genomic instability in pituitary adenomas. Hum Mol Genet 22:226–238

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

Laloi-Michelin M, Meas T, Ambonville C, Bellanne-Chantelot C, Beaufils S, Massin P et al (2009) The clinical variability of maternally inherited diabetes and deafness is associated with the degree of heteroplasmy in blood leukocytes. J Clin Endocrinol Metab 94:3025–3030

Lang M, Sazzini M, Calabrese FM, Simone D, Boattini A, Romeo G et al (2012) Polymorphic NumtS trace human population relationships. Hum Genet 131:757–771

Larman TC, DePalma SR, Hadjipanayis AG, Protopopov A, Zhang J, Gabriel SB et al (2012) Spectrum of somatic mitochondrial mutations in five cancers. Proc Natl Acad Sci USA 109:14087–14091

Lascaro D, Castellana S, Gasparre G, Romeo G, Saccone C, Attimonelli M (2008) The RHNumtS compilation: features and bioinformatics approaches to locate and quantify Human NumtS. BMC Genomics 9:267

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

Li Y, Weibing S, Liu H, Hongli J, Zhuosheng L, Yadong W et al (2008) Mitochondrial DNA from colorectal cancer cells promotes the malignant phenotype of NIH3T3 cells. Cell Biol Int 32:979–983

Liu J, Wang LD, Sun YB, Li EM, Xu LY, Zhang YP et al (2012) Deciphering the signature of selective constraints on cancerous mitochondrial genome. Mol Biol Evol 29:1255–1261

Liu VW, Wang Y, Yang HJ, Tsang PC, Ng TY, Wong LC et al (2003) Mitochondrial DNA variant 16189T > C is associated with susceptibility to endometrial cancer. Hum Mutat 22:173–174

Losman JA, Looper RE, Koivunen P, Lee S, Schneider RK, McMahon C et al (2013) (R)-2-hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. Science 339:1621–1625

Lozy F, Karantza V (2012) Autophagy and cancer cell metabolism. Semin Cell Dev Biol 23:395–401

Lu C, Thompson CB (2012) Metabolic regulation of epigenetics. Cell Metab 16:9–17

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

Ma Y, Bai RK, Trieu R, Wong LJ (2010) Mitochondrial dysfunction in human breast cancer cells and their transmitochondrial cybrids. Biochim Biophys Acta 1797:29–37

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

Marucci G, Maresca A, Caporali L, Farnedi A, Betts CM, Morandi L et al (2013) Oncocytic glioblastoma: a glioblastoma showing oncocytic changes and increased mitochondrial DNA copy number. Hum Pathol 44:1867–1876

Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O et al (2006) p53 regulates mitochondrial respiration. Science 312:1650–1653

Memmott RM, Dennis PA (2009) Akt-dependent and -independent mechanisms of mTOR regulation in cancer. Cell Signal 21:656–664

Minocherhomji S, Tollefsbol TO, Singh KK (2012) Mitochondrial regulation of epigenetics and its role in human diseases. Epigenetics 7:326–334

Mizutani S, Miyato Y, Shidara Y, Asoh S, Tokunaga A, Tajiri T et al (2009) Mutations in the mitochondrial genome confer resistance of cancer cells to anticancer drugs. Cancer Sci 100:1680–1687

Moraes CT, Dey R, Barrientos A (2001) Transmitochondrial technology in animal cells. Methods Cell Biol 65:397–412

Morais R, Zinkewich-Peotti K, Parent M, Wang H, Babai F, Zollinger M (1994) Tumor-forming ability in athymic nude mice of human cell lines devoid of mitochondrial DNA. Cancer Res 54:3889–3896

Mullen AR, Wheaton WW, Jin ES, Chen PH, Sullivan LB, Cheng T et al (2011) Reductive carboxylation supports growth in tumour cells with defective mitochondria. Nature 481:385–388

Nair J, Strand S, Frank N, Knauft J, Wesch H, Galle PR et al (2005) Apoptosis and age-dependant induction of nuclear and mitochondrial etheno-DNA adducts in Long-Evans Cinnamon (LEC) rats: enhanced DNA damage by dietary curcumin upon copper accumulation. Carcinogenesis 26:1307–1315

Naviaux RK (2008) Mitochondrial control of epigenetics. Cancer Biol Ther 7:1191–1193

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 et al (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

Partridge MA, Huang SX, Kibriya MG, Ahsan H, Davidson MM, Hei TK (2009) Environmental mutagens induced transversions but not transitions in regulatory region of mitochondrial DNA. J Toxicol Environ Health A 72:301–304

Payne BA, Wilson IJ, Yu-Wai-Man P, Coxhead J, Deehan D, Horvath R et al (2013) Universal heteroplasmy of human mitochondrial DNA. Hum Mol Genet 22:384–390

Pelicano H, Xu RH, Du M, Feng L, Sasaki R, Carew JS et al (2006) Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism. J Cell Biol 175:913–923

Pereira L, Freitas F, Fernandes V, Pereira JB, Costa MD, Costa S et al (2009) The diversity present in 5140 human mitochondrial genomes. Am J Hum Genet 84:628–640

Pereira L, Soares P, Maximo V, Samuels DC (2012) Somatic mitochondrial DNA mutations in cancer escape negative selection and high pathogenicity mutations lead to the oncocytic phenotype: pathogenicity analysis of reported somatic mtDNA mutations in tumors. BMC Cancer 12:53

Pesole G, Allen JF, Lane N, Martin W, Rand DM, Schatz G et al (2012) The neglected genome. EMBO Rep 13:473–474

Petros JA, Baumann AK, Ruiz-Pesini E, Amin MB, Sun CQ, Hall J et al (2005) mtDNA mutations increase tumorigenicity in prostate cancer. Proc Natl Acad Sci USA 102:719–724

Petruzzella V, Carrozzo R, Calabrese C, Dell'Aglio R, Trentadue R, Piredda R et al (2012) Deep sequencing unearths nuclear mitochondrial sequences under Leber's hereditary optic neuropathy-associated false heteroplasmic mitochondrial DNA variants. Hum Mol Genet 21:3753–3764

Picardi E, Pesole G (2012) Mitochondrial genomes gleaned from human whole-exome sequencing. Nat Methods 9:523–524

Polyak K, Li Y, Zhu H, Lengauer C, Willson JK, Markowitz SD et al (1998) Somatic mutations of the mitochondrial genome in human colorectal tumours. Nat Genet 20:291–293

Porcelli AM, Ghelli A, Ceccarelli C, Lang M, Cenacchi G, Capristo M et al (2010) The genetic and metabolic signature of oncocytic transformation implicates HIF1alpha destabilization. Hum Mol Genet 19:1019–1032

Raimundo N, Song L, Shutt TE, McKay SE, Cotney J, Guan MX et al (2012) Mitochondrial stress engages E2F1 apoptotic signaling to cause deafness. Cell 148:716–726

Ray PD, Huang BW, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24:981–990

Rossi ED, Martini M, Straccia P, Raffaelli M, Pennacchia I, Marrucci E et al (2013) The cytologic category of oncocytic (Hurthle) cell neoplasm mostly includes low-risk lesions at histology: an institutional experience. Eur J Endocrinol 169:649–655

Rossignol R, Faustin B, Rocher C, Malgat M, Mazat JP, Letellier T (2003) Mitochondrial threshold effects. Biochem J 370:751–762

Rubino F, Piredda R, Calabrese FM, Simone D, Lang M, Calabrese C et al (2012) HmtDB, a genomic resource for mitochondrion-based human variability studies. Nucleic Acids Res 40: D1150–D1159

Ruiz-Pesini E, Mishmar D, Brandon M, Procaccio V, Wallace DC (2004) Effects of purifying and adaptive selection on regional variation in human mtDNA. Science 303:223–226

Salas A, Yao YG, Macaulay V, Vega A, Carracedo A, Bandelt HJ (2005) A critical reassessment of the role of mitochondria in tumorigenesis. PLoS Med 2:e296

Samuels DC, Han L, Li J, Quanghu S, Clark TA, Shyr Y et al (2013) Finding the lost treasures in exome sequencing data. Trends Genet 29:593–599

Santidrian AF, Matsuno-Yagi A, Ritland M, Seo BB, LeBoeuf SE, Gay LJ et al (2013) Mitochondrial complex I activity and NAD+/NADH balance regulate breast cancer progression. J Clin Invest 123:1068–1081

Savagner F, Franc B, Guyetant S, Rodien P, Reynier P, Malthiery Y (2001) Defective mitochondrial ATP synthesis in oxyphilic thyroid tumors. J Clin Endocrinol Metab 86:4920–4925

Schon EA, DiMauro S, Hirano M (2012) Human mitochondrial DNA: roles of inherited and somatic mutations. Nat Rev Genet 13:878–890

Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD et al (2005) Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell 7:77–85

Semenza GL, Roth PH, Fang HM, Wang GL (1994) Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. J Biol Chem 269:23757–23763

Sharma LK, Fang H, Liu J, Vartak R, Deng J, Bai Y (2011) Mitochondrial respiratory complex I dysfunction promotes tumorigenesis through ROS alteration and AKT activation. Hum Mol Genet 20:4605–4616

Shay JW, Werbin H (1987) Are mitochondrial DNA mutations involved in the carcinogenic process? Mutat Res 186:149–160

Shen YH, Wang XL, Wilcken DE (1998) Nitric oxide induces and inhibits apoptosis through different pathways. FEBS Lett 433:125–131

Shidara Y, Yamagata K, Kanamori T, Nakano K, Kwong JQ, Manfredi G et al (2005) Positive contribution of pathogenic mutations in the mitochondrial genome to the promotion of cancer by prevention from apoptosis. Cancer Res 65:1655–1663

Shock LS, Thakkar PV, Peterson EJ, Moran RG, Taylor SM (2011) DNA methyltransferase 1, cytosine methylation, and cytosine hydroxymethylation in mammalian mitochondria. Proc Natl Acad Sci USA 108:3630–3635

Silbergeld DL, Mayberg MR, Berger MS, Ali-Osman F, Kelly WA, Shaw CM (1993) Pituitary oncocytomas: clinical features, characteristics in cell culture, and treatment recommendations. J Neurooncol 16:39–46

Simone D, Calabrese FM, Lang M, Gasparre G, Attimonelli M (2011) The reference human nuclear mitochondrial sequences compilation validated and implemented on the UCSC genome browser. BMC Genomics 12:517

Singh KK, Ayyasamy V, Owens KM, Koul MS, Vujcic M (2009) Mutations in mitochondrial DNA polymerase-gamma promote breast tumorigenesis. J Hum Genet 54:516–524

Skonieczna K, Malyarchuk BA, Grzybowski T (2012) The landscape of mitochondrial DNA variation in human colorectal cancer on the background of phylogenetic knowledge. Biochim Biophys Acta 1825:153–159

Smiraglia DJ, Kulawiec M, Bistulfi GL, Gupta SG, Singh KK (2008) A novel role for mitochondria in regulating epigenetic modification in the nucleus. Cancer Biol Ther 7:1182–1190

Smolkova K, Jezek P (2012) The role of mitochondrial NADPH-dependent isocitrate dehydrogenase in cancer cells. Int J Cell Biol 2012:273947

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

St John JC, Facucho-Oliveira J, Jiang Y, Kelly R, Salah R (2010) Mitochondrial DNA transmission, replication and inheritance: a journey from the gamete through the embryo and into offspring and embryonic stem cells. Hum Reprod Update 16:488–509

Stafford P, Chen-Quin EB (2010) The pattern of natural selection in somatic cancer mutations of human mtDNA. J Hum Genet 55:605–612

Sun W, Zhou S, Chang SS, McFate T, Verma A, Califano JA (2009) Mitochondrial mutations contribute to HIF1alpha accumulation via increased reactive oxygen species and up-regulated pyruvate dehydrogenease kinase 2 in head and neck squamous cell carcinoma. Clin Cancer Res 15:476–484

Tallini G (1998) Oncocytic tumours. Virchows Arch 433:5–12

Tang S, Huang T (2010) Characterization of mitochondrial DNA heteroplasmy using a parallel sequencing system. Biotechniques 48:287–296

Tomlinson IP, Alam NA, Rowan AJ, Barclay E, Jaeger EE, Kelsell D et al (2002) Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nat Genet 30:406–410

Torroni A, Achilli A, Macaulay V, Richards M, Bandelt HJ (2006) Harvesting the fruit of the human mtDNA tree. Trends Genet 22:339–345

Tuppen HA, Blakely EL, Turnbull DM, Taylor RW (2010) Mitochondrial DNA mutations and human disease. Biochim Biophys Acta 1797:113–128

van Oven M, Kayser M (2009) Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 30:E386–E394

Vogel RO, Smeitink JA, Nijtmans LG (2007) Human mitochondrial complex I assembly: a dynamic and versatile process. Biochim Biophys Acta 1767:1215–1227

Wallace DC (2012) Mitochondria and cancer. Nat Rev Cancer 12:685–698

Wallace DC (1999) Mitochondrial diseases in man and mouse. Science 283:1482–1488

Wallace DC (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 39:359–407

Wang PY, Ma W, Park JY, Celi FS, Arena R, Choi JW et al (2013) Increased oxidative metabolism in the Li-Fraumeni syndrome. N Engl J Med 368:1027–1032

Warburg O (1956) On respiratory impairment in cancer cells. Science 124:269–270

Warburg O, Posener K, Negelein E (1924) Uber den Stoffwechsel der Carcinomzelle. Biochem Zeitschr 152:309–344

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

Weinberg F, Hamanaka R, Wheaton WW, Weinberg S, Joseph J, Lopez M et al (2010) Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. Proc Natl Acad Sci USA 107:8788–8793

Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB (2009) ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324:1076–1080

Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK et al (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci USA 105:18782–18787

Wise DR, Ward PS, Shay JE, Cross JR, Gruber JJ, Sachdeva UM et al (2011) Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of alpha-ketoglutarate to citrate to support cell growth and viability. Proc Natl Acad Sci USA 108:19611–19616

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

Wong LJ, Boles RG (2005) Mitochondrial DNA analysis in clinical laboratory diagnostics. Clin Chim Acta 354:1–20

Xiao M, Yang H, Xu W, Ma S, Lin H, Zhu H et al (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

Yao YG, Salas A, Logan I, Bandelt HJ (2009) mtDNA data mining in GenBank needs surveying. Am J Hum Genet 85:929–933, author reply 33

Yen HC, Tang YC, Chen FY, Chen SW, Majima HJ (2005) Enhancement of cisplatin-induced apoptosis and caspase 3 activation by depletion of mitochondrial DNA in a human osteosarcoma cell line. Ann N Y Acad Sci 1042:516–522

Yu M (2012) Somatic mitochondrial DNA mutations in human cancers. Adv Clin Chem 57:99–138

Zaragoza MV, Fass J, Diegoli M, Lin D, Arbustini E (2010) Mitochondrial DNA variant discovery and evaluation in human Cardiomyopathies through next-generation sequencing. PLoS One 5: e12295

Zhou S, Kachhap S, Sun W, Wu G, Chuang A, Poeta L et al (2007) Frequency and phenotypic implications of mitochondrial DNA mutations in human squamous cell cancers of the head and neck. Proc Natl Acad Sci USA 104:7540–7545

Zu XL, Guppy M (2004) Cancer metabolism: facts, fantasy, and fiction. Biochem Biophys Res Commun 313:459–465