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Review

The role of mitochondrial electron transport in tumorigenesis and metastasis☆

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ABSTRACT

Background: Tumor formation and spread via the circulatory and lymphatic drainage systems is associated with metabolic reprogramming that often includes increased glycolytic metabolism relative to mitochondrial energy production. However, cells within a tumor are not identical due to genetic change, clonal evolution and layers of epigenetic reprogramming. In addition, cell hierarchy impinges on metabolic status while tumor cell phenotype and metabolic status will be influenced by the local microenvironment including stromal cells, developing blood and lymphatic vessels and innate and adaptive immune cells. Mitochondrial mutations and changes in mitochondrial electron transport contribute to metabolic remodeling in cancer in ways that are poorly understood.
Scope of Review: This review concerns the role of mitochondria, mitochondrial mutations and mitochondrial electron transport function in tumorigenesis and metastasis.
Major Conclusions: It is concluded that mitochondrial electron transport is required for tumor initiation, growth and metastasis. Nevertheless, defects in mitochondrial electron transport that compromise mitochondrial energy metabolism can contribute to tumor formation and spread. These apparently contradictory phenomena can be reconciled by cells in individual tumors in a particular environment adapting dynamically to optimally balance mitochondrial genome changes and bioenergetic status.
General Significance: Tumors are complex evolving biological systems characterized by genetic and adaptive epigenetic changes. Understanding the complexity of these changes in terms of bioenergetics and metabolic changes will permit the development of better combination anticancer therapies. This article is part of a Special Issue entitled Frontiers of Mitochondrial Research.

1. Introduction

Tumor formation and progression to metastasis is associated with metabolic remodeling that is contributed by both genetic and epigenetic processes. Genetic changes include both nuclear and mitochondrial mutations while epigenetic changes are driven by the environment in which the tumor develops, and by definition occur independently of, but often in concert with genetic alteration. Together, these reprogramming events effectively rebalance tumor bioenergetics (that branch of metabolism concerned with ATP production) in favor of glycolytic energy metabolism over mitochondrial energy production, changes that are associated with a diverse spectrum of metabolic adjustments commensurate with tissue of origin and residence, increased cell proliferation and self-renewal, and poorly differentiated cell function. At the level of individual tumor cells, cells are not identical due to genetic change, clonal evolution and extinction and layers of epigenetic modification. Thus, cell hierarchy impinges on metabolic status in a complex and dynamic manner that is influenced by the local microenvironment including stromal cells, developing blood and lymphatic vessels as well as cells of the innate and adaptive immune system. Mitochondrial mutations that alter mitochondrial electron transport function contribute to metabolic remodeling in ways that are poorly understood, but these mutations are not considered to drive tumorigenesis in the same way that oncogenes complement tumor suppressors to initiate tumor formation. This review primarily concerns the role of mitochondria, mitochondrial mutations and mitochondrial electron transport function in tumor development and metastasis.

2. Cancer as a metabolic disease

The view that cancer is basically a metabolic disease was developed in the early decades of the 20th century during which time this view of tumorigenesis was promoted primarily by Otto Warburg [1–4]. The emergence of molecular biology in the following decades, and the subsequent discovery of the genetic origins of cancer muted this classical biochemical explanation of cancer, not before some robust debate that continues today [5]. Nevertheless, the last decade has seen a renaissance of the view that cancer is a metabolic disease, although the concept promoted stridently by Warburg that mitochondrial damage is the cause of cancer is no longer literally tenable: rather

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tumor-specific reprogramming of cellular metabolism by oncogenes, tumor suppressors and other metabolic regulators better explain the etiology of cancer [2,6]. In addition, epigenetic changes and metabolic flexibility [2,7–11] contribute to an emerging 21st century consensus of dynamic complex metabolic remodeling in cancer. Similar considerations apply as in non-tumor situations where stem cell homeostasis, committed progenitor cell expansion and stem cell differentiation all involve metabolic reprogramming and redirection of bioenergetic function [6,12,13] that includes metabolic plasticity, transcriptional remodeling and higher level epigenetic changes [14]. Mutations have been reported in several nuclear-encoded mitochondrial enzymes involved in metabolism including fumarate hydratase [15,16], succinate dehydrogenase (SDH) A-D [17] and isocitrate dehydrogenase (IDH) 2 [18,19], while cytosolic phosphoglycerate dehydrogenase (PHGDH) [20,21] and IDH1 [18,19] are also directly involved. A simplified map that locates most of these metabolic enzymes is shown in Fig. 1 together with other metabolic and bioenergetic points of interest mentioned in this review. Many other classical oncogenes and tumor suppressors are indirectly involved in controlling metabolism through key regulatory nodes including mTOR and PI3K/AKT [22]. In addition, MYC, mutant RAS and RAF, PDH and its phosphorylation regulators, PDK1 and PDP2 [23], hexokinase (HK) II relocation to mitochondria [24] and the pyruvate kinase (PK) splice variant, PKM2 [25–27] are all involved to varying degrees in this reprogramming, some more related to cell proliferation than to cancer *per se*.

Recently, the sirtuins, a family of NAD^+ deacetylases and ADP ribosyltransferases, have emerged as key players in regulating metabolic adaptation and genomic stability in cancer [28,29]. Although several studies support a role for SIRT1 as a tumor suppressor through mechanisms that include MYC deacetylation [30], tumor-promoting roles have also been reported for the sirtuins depending on tumor type and signaling pathways involved. For example, SIRT6 has been shown to control glucose homeostasis through histone H3K9 deacetylation via HIF-1α [31] and to suppress aerobic glycolysis [29] while SIRT2 prevents chromosomal instability [32] and mitochondrial SIRT4 is involved in the DNA damage response (DDR) through anapleurotic blockade of glutamine catabolism [33]. Additionally, SIRT7 has recently been shown to stabilize tumor cell

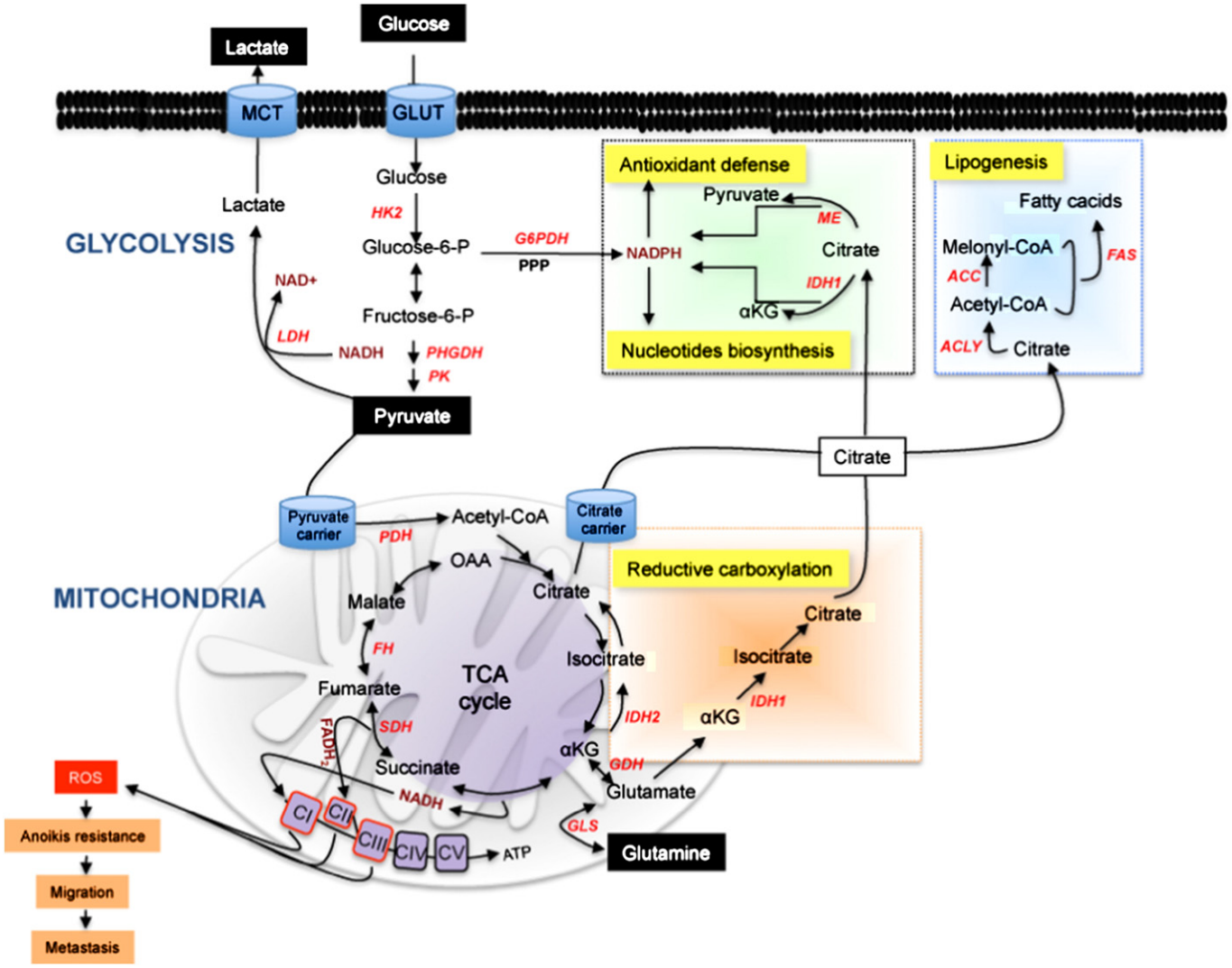

Fig. 1. Major metabolic and bioenergetic pathways involved in tumor cell reprogramming. The enzymes involved in regulating energy metabolism in tumor cells are depicted in red. Most are referred to in Section 2. Abbreviations used: HK2, hexokinase 2; LDH, lactate dehydrogenase; PHGDH, phosphoglycerate dehydrogenase; PK, pyruvate kinase; GAPDH, glucose-6-phosphate dehydrogenase; ME, malic enzyme; IDH, isocitrate dehydrogenase; PDH, pyruvate dehydrogenase; SDH, succinate dehydrogenase; FH, fumarate dehydratase; GLS, glutaminase; GDH, glutamate dehydrogenase; ACLY, ATP citrate lyase; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; PPP, pentose phosphate pathway; TCA, tricarboxylic acid cycle; CI-V, mitochondrial respiratory complexes I, II, III, IV, V; OAA, oxaloacetate; αKG, α-ketoglutarate, ROS, reactive oxygen species.

phenotype by deacetylating H3K18 [34]. The transcription factors MYC and HIF-1α, which is stabilized via the tumor suppressor, von-Hippel Lindau factor, coordinate metabolic remodeling including up-regulation of the facilitative glucose transporter, GLUT-1, and many enzymes involved in glycolysis and metabolism [2,35]. Thus, cancer is now recognized as a complex disease in which metabolic reprogramming plays a key role in tumorigenesis and metastasis. These changes are integrated through genetic and epigenetic events that reset cellular signaling pathways. Although discussion of the evolution of metabolic remodeling in cancer has often focused on rebalancing bioenergetics towards glycolysis, increased anaplerotic use of amino acids such as glutamine that feed into the TCA cycle [8,36–39] and elevated fatty acid metabolism [8,36] are also involved in this remodeling, particularly where glucose is limiting. Importantly, changes in mitochondrial respiration also contribute to metabolic remodeling. However, although reduced mitochondrial electron transport function can support tumor cell proliferation, mitochondrial electron transport function is also required for tumor formation [40] and has been shown to be favored by some tumor cells [41–45]. In particular, although highly aggressive tumors can be highly glycolytic, this is not always associated with reduced oxygen consumption [5]. Alternatively, slowly growing more differentiated tumors that are well vascularized would be expected to favor mitochondrial energy metabolism. This concept is represented diagrammatically in Fig. 2 where the bioenergetic status of a tumor, as measured by the ratio of ATP produced by glycolysis relative to mitochondrial oxidative phosphorylation (OXPHOS), is related to tumor frequency (and also to tumor mass and progression). This temporally integrated frequency distribution will define thresholds of cell survival within the tumor and within cell subpopulations of cells within the tumor, be they rapidly proliferating, quiescent or slowly self-renewing.

3. Cancer metabolism and the microenvironment

Tumors contain many non-cancer cells including stromal cells, immune cells and cells involved in angiogenesis. These cells contribute to tumor volume to varying extents and have the potential to grossly distort metabolic analysis depending on the tumor type. Stromal cells lay down connective tissue matrix that adds considerably to tumor bulk and structure while disorganized blood vessels develop in tumors above a few millimeters in diameter. Those tumor cells close to blood vessels will experience high oxygen and nutrient concentrations compared with cells further removed from these vessels [10,46,47]. Tumor cells in hypoxic regions will favor glycolysis for their energy needs and consequently generate lactate and lower pH in the local environment, but sustaining glycolysis when oxygen is present will require metabolic reprogramming including epigenetic and genetic changes [48]. Increased production of reactive oxygen species (ROS) under hypoxic conditions will generate mutations while lactate production and acidosis will impact on both glycolysis and mitochondrial function. Thus, throughout growth and metastasis, tumors accumulate DNA mutations giving rise to distinct clones of cells [49] that populate different locations within primary tumors and different metastatic sites [50–52]. This genetic heterogeneity complicates metabolic analysis of tumors although the overall bias towards elevated glycolytic metabolism remains an overarching property of most metastatic tumors and is the basis of increased [^{18}F]-2-fluoro-2-deoxy-D-glucose uptake as determined by PET (positron emission tomography) scanning that is often used to determine the extent of metastatic spread of a tumor. Tissue of origin and site of metastasis also dictate the outcome of individual oncogene and tumor suppressor lesions [53].

In another twist to the story of tumor cell metabolic heterogeneity, stromal cells in the local microenvironment can be hijacked by cancer

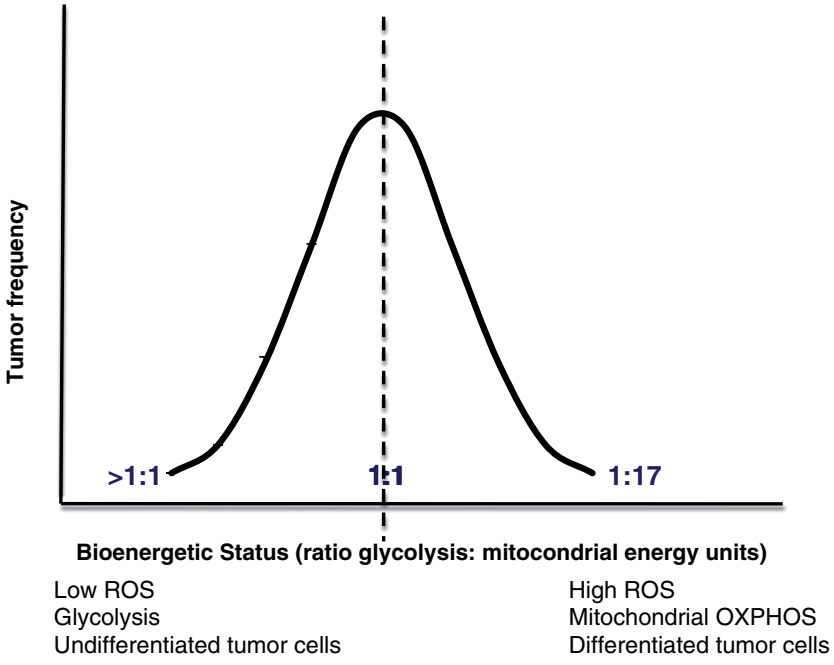

Fig. 2. Tumor formation and progression depends on bioenergetics status. The relationship between bioenergetic status, as defined by the ratio of ATP generated by glycolysis relative to mitochondrial respiration, is related to tumor forming ability. Tumor cells that are devoid of mtDNA and are therefore purely glycolytic fail to form tumors. In contrast, cells that exhibit efficient mitochondrial energy production, as depicted in most classical biochemistry textbooks (2 ATP from glycolysis and 34 from mitochondrial respiration), are strongly biased towards a non-proliferative differentiated phenotype that is not conducive to tumor formation. Inefficient mitochondrial respiration will lead to high ROS production, excessive mutational load and compromised tumor formation while cells that are glycolytic will have ROS levels that are suboptimal for self-renewal and proliferation. In between these extremes, there is a bioenergetic and metabolic sweet spot, ideal for tumor initiation and progression. Bioenergetic and metabolic flexibility around this sweet spot, which we have arbitrarily set at equal ATP produced by glycolysis and mitochondrial respiration will provide a window that facilitates tumor formation and progression.

cells to provide essential nutrients such as lactate to promote tumor growth, a phenomenon referred to as the "reverse Warburg effect" [41,54–60].

4. Mutations that modify mitochondrial electron transport in cancer

Having discussed briefly the extent to which cancer can be considered as a metabolic disease, we now turn our attention to mitochondrial electron transport, the central respiratory pathway within mitochondria that uses reducing equivalents from the mitochondrial TCA cycle, and to a lesser extent from glycolysis, to fuel ATP production (Fig. 1). As mitochondrial respiration is the sole metabolic pathway in mammalian cells that is directly dependent on proteins encoded by the mitochondrial genome, and these proteins are essential for mitochondrial respiratory function, it is particularly important to understand how mutations in mitochondrial DNA (mtDNA) affect the machinery of mitochondrial respiration and metabolism in cancer.

Somatic mutations have been shown to occur across the mitochondrial genome with some affecting amino acid sequence of the 13 mitochondrially encoded protein subunits of mitochondrial respiratory complexes I (7 mitochondrially-encoded subunits), III (1 mitochondrially-encoded subunit), IV (3 mitochondrially-encoded subunits) and V (2 mitochondrially-encoded subunits). Mutations in other mitochondrial genes that encode the 2 rRNAs and 22 tRNAs can also affect respiratory function through indirect effects on mitochondrial protein synthesis. Finally, mutations in the regulatory displacement (D)-loop and adjacent control regions that are involved in mtDNA replication and transcription can affect respiratory function through effects on mtDNA copy number and mRNA production [50]. Some cancer-related mtDNA mutations are known population variants and may facilitate bioenergetic adaptation to altered environment while others may promote transformation and metastasis [40]. The presence of mtDNA sequences in the nucleus of mammalian cells can also complicate analysis of mitochondrial sequence data and introduce unwanted artefacts [61] and these need to be carefully controlled for. In a recent study, somatic mtDNA mutations occurred at a frequency of 13–63% across 5 tumor types [62] supporting previous evidence of a high somatic mutation rate, mostly homoplastic, in colorectal cancers [63].

Analysis of mtDNA mutations in 921 tumors where the entire genome had been sequenced showed 56% of all tumors contained at least one mutation with 28% being in Complex I and 35% in the D-loop. Differences between the various functional regions of the mitochondrial genome (Complexes I, III, IV and V, and the tRNAs and rRNAs) disappeared when normalized to size of the region [64]. Similar survey results of mutations in mitochondrially encoded Complex I genes have been reported by Lu and colleagues [65] for a wide range of cancers.

Because genetic tools are not available to specifically manipulate mitochondrial genes, the effects of mutations in individual mitochondrial genes, other than those that occur in nature, cannot be evaluated experimentally. Heteroplasmy also complicates interpretation of the effects of mtDNA mutations on cancer, raising the issue of the extent to which threshold effects may be relevant.

In addition to mitochondrially encoded subunits, mammalian mitochondrial respiratory complexes are also composed of about 77 nuclear-encoded proteins that contribute to the structure and function of Complexes I–V [61] which are organized into supramolecular structures collectively referred to as the respirasome [66,67]. Many hundreds of nuclear genes also affect mitochondrial integrity and function, but a comprehensive review of this topic, though overdue, is well beyond the scope of this article.

4.1. Complex I

Most investigations have focused on human Complex I which comprises 45 subunits. Seven of the 14 evolutionarily conserved core subunits of Complex I are encoded by the mitochondrial genome and constitute the hydrophobic arm as revealed in the recently elucidated 3D structure of Complex I from *Thermus thermophilus* [68]. Loss of function mutations in Complex I have been reported in most tumor types and are major players in metabolic remodeling that characterizes cancer [64,69]. In general, Complex I mutations are thought to promote tumorigenesis through production of reactive oxygen species (ROS), thus increasing DNA damage and activating oncogenic signaling pathways, while increased lactate production from glycolytic metabolism promotes metastasis [48,70–72]. On the other hand, severe disassembly-inducing Complex I mutations such as those in the benign oncocytomas appear to have negative effects on tumor progression depending on the degree of heteroplasmy [73,74]. These results suggest that a threshold level of mitochondrial electron transport function is necessary to support tumor progression as depicted in Fig. 2.

4.2. Complex II

Complex II functions jointly in the TCA cycle and in the mitochondrial electron transport chain and its subunits are all nuclear encoded. Mutations in SDH subunits B, C and D are associated with paragangliomas [17,75–79] and pheochromocytoma [75]. Mutations in subunits B–D which are associated with mitochondrial electron transport function increase ROS in contrast to mutations in the TCA cycle subunit, SDH A, which lowers ROS and is associated with the pediatric neurodegenerative disease, Leigh's syndrome [80].

4.3. Complexes III–V

Mutations in the *COI* gene that encodes the cytochrome oxidase I subunit of Complex IV have been linked with epithelial ovarian cancer along with variants in nuclear genes encoding mitochondrial proteins [81]. In addition, mutations in *COI* have been found to be associated with 11–12% of prostate cancers [82]. Of the 921 cancers analyzed by Iommarini et al. [64], 5.8% had somatic mtDNA mutations in Complex III, 12.7% in Complex IV and 3.8% in Complex V.

4.4. TCA cycle

The TCA cycle generates reducing equivalents that fuel mitochondrial electron transport primarily at the level of Complex I, although oxidation of succinate at Complex II is also an entry point for electrons in the respiratory chain (Fig. 1). Mutations in TCA cycle enzymes, all of which are nuclear encoded, are associated with various cancers. For example, oncogenic fumarate hydratase (*FH*) mutations occur in uterine leiomyomas and in renal carcinomas [83] and *SDH* mutations in paragangliomas (see above). In contrast, mutations in *IDH1* and *IDH2* that generate (D)-2-hydroxyglutarate from a-ketoglutarate are tumor suppressive and occur in gliomas [8,19,84,85] and in AML [18].

4.5. Mitochondrial protein synthesis

The mitochondrial genome encodes 2 rRNAs and 22 tRNAs that are required for mitochondrial protein synthesis, without which the 13 essential respiratory complex proteins also encoded by the mitochondrial genome would not be translated. Cancer-associated mutations in these rRNA and tRNA genes have been documented [64,65,77], although many of these mutations have also been identified as population variants [77] raising questions about possible adaptive roles in cancer. These mutations can be either neutral or functional, affecting mitochondrial protein synthesis and consequently respiration.

4.6. D-loop

The displacement loop or D-loop of the mitochondrial genome that is in the non-coding region, includes replication and transcriptional start sites. This region contains the highest frequency of somatic mutations [64,65], 6–7.5 times higher than that of other regions. The vast majority (85%) of these mutations are also observed as population variants [77] suggesting functional significance in tumor development or adaptation to environment.

5. Mitochondrial genome involvement in tumorigenesis and metastasis: cybrid approaches

The role of somatic mtDNA mutations in tumor formation and metastasis remains controversial [61,86–88]. Although tumor-associated mutations in mtDNA have been shown on numerous occasions (see above) [63,89,90] the frequency of these mutations varies widely between different tumor types being particularly high in melanomas [91] and lower than non-tumor tissue in colorectal cancer [87]. Many of these mtDNA mutations appear to be neutral polymorphisms [40] while others may affect OXPHOS function. Failure to uncover maternally inherited mtDNA mutations in cancer however, argues against a causative role of these mutations in tumorigenesis [40,61,92].

5.1. Tumorigenesis

Early studies used nuclear-cytoplasm transfer approaches to explore the relative roles of the nucleus and cytoplasm (containing mitochondria) in tumor formation. In these studies, some of which crossed species boundaries, enucleated normal cells, a source of mitochondrial genes, were fused with nucleated tumor cells or karyoblasts and the tumor-forming ability of the resulting cybrids determined in recipient animals including immunocompromised mice. Reduced tumorigenicity was observed in some studies and was more profound when karyoplasts were employed [93–97], leading to mitochondria being referred to as "the ultimate tumor suppressor" in support of Warburg's theory of carcinogenesis [5]. However, reduced tumorigenicity did not always track with non-tumor cytoplasm [93]. In these quite varied experiments, cytoplasmic dosage, time of passaging prior to tumor testing, the nature of the mutagenic stimulus, the cell combinations used particularly in interspecific hybrids and the nature of the test for tumorigenicity were identified as important factors and these were not always well controlled. In reciprocal fusions, tumorigenicity was not cytoplasmically transmitted [92].

Experiments by Hayashi and colleagues [98,99] used human tumor cell lines and more recently a murine genetic model involving cybrid formation between carcinogen-treated skin fibroblast lines and enucleated cells or platelets not exposed to carcinogen [100]. Taken together, their results showed that tumorigenicity tracked with the nuclear genome and *not* with mitochondria from 3-methylcolanthrene-treated fibroblast cell lines. Although these results do not exclude mitochondrial genome involvement in tumorigenesis, they do show that in this carcinogen-induced tumor model, the mtDNA mutations generated were not major players in tumor formation in nude mice. These results, when considered together with the lack of maternal inheritance [40,92] and the nature of the mtDNA mutations (see above) argue against the mitochondrial theory of cancer, at least from the perspective of mtDNA mutations driving tumorigenesis.

More recent results from Hayashi's group provide evidence that mice containing mitochondria with a mutation (G13997A) in the *ND6* mtDNA gene from highly metastatic lung carcinoma cells (mito-mice) promote B cell lymphoma formation and metastasis on the lymphoma-prone B6 background with about 46% penetrance [101]. Cell type-specific tumor formation and subsequent metastasis suggest that this mtDNA mutation is tissue context specific and complements B-lymphoma predisposing nuclear mutations. Using different mito-mice containing mitochondria with a mutation (T6589C) in the *CO1* mtDNA gene that is only weakly associated with tumor formation, tumor incidence was similar to the background observed in B6 mice.

These results show that mtDNA mutations can contribute qualitatively or quantitatively to tumor development depending on the genetic background of the mouse strain. At present, genetic tools to investigate the contribution of changes in mtDNA to tumorigenesis in mitochondrial replacement experiments are severely constrained to known or randomly generated carcinogenic alterations, with little control over the specific genetic change under investigation. New genetic tools for targeted mutations and mitochondrial genome replacement are urgently needed to address these constraints and progress knowledge about the role of mtDNA mutations in tumorigenesis and metastasis.

5.2. Metastasis

To address the question of whether mtDNA mutations are involved in metastasis, Ishikawa et al. [102] used the same cybrid technology to show that mtDNA can regulate metastatic potential. Thus, cybrids between low metastatic Lewis lung carcinoma P29 cells depleted of their mitochondrial DNA (P29ρ^o) and enucleated highly metastatic A11 cells with the mtDNA deletion mutation ΔmtDNA4609 exhibited high metastatic potential whereas A11ρ^o cells fused with enucleated low metastatic potential P29 cells exhibited low metastatic potential. Thus, metastatic potential was linked to the mitochondrial genotype. In these studies, metastatic potential was associated with deficient Complex I activity and elevated ROS production, while the ROS scavenger, N-acetylcysteine, suppressed metastatic potential. The mechanism of mtDNA-mediated metastasis involved Complex I defects and elevated ROS production, and was shown to involve up-regulation of HIF-1α, VEGF and MCL-1. These results and others [103,104] show that metastatic phenotype can be affected by mtDNA mutations that affect the function of Complex I, and that both ROS-dependent and ROS-independent mechanisms can be involved.

Recently, Santidrian and colleagues [104] showed that Complex I activity was critical in defining the aggressive phenotype of MDA-MB-435 and MDA-MB-231 cells stably transfected with the yeast NADH dehydrogenase gene, *Ndi1*. Enhancing Complex I activity inhibited tumor growth and metastasis whereas non-lethal reduction of NAD^+ levels resulted in tumors that were more aggressive and exhibited increased metastasis.

6. Mitochondrial genome involvement in tumorigenesis and metastasis: genome deletion approaches

Mitochondrial genome deletion has been used as a model to investigate the role of mitochondrial electron transport function in tumor formation in a number of different studies. One rationale behind these experiments was to determine whether tumor cells with imposed glycolytic metabolism were capable of forming tumors. On the one hand, increasing glycolytic metabolism/aerobic glycolysis might be predicted to enhance tumorigenesis. Thus, if mitochondrial function is indeed tumor suppressive as has been proposed on a number of occasions [5], lifting this suppression might be expected to enhance tumor growth. Alternatively, if components of the mitochondrial respiratory chain that are encoded by the mitochondrial genome are necessary to realize the full tumorigenic potential of tumor cells, and perhaps also their metastatic potential, then deleting the mitochondrial genome will stall tumor formation until such time as compensatory reprogramming of gene expression is achieved or a replacement genome can be sourced.

6.1. Tumorigenesis

Using the EB8 clone of HeLa cells extensively depleted of mitochondrial DNA by long-term treatment with ethidium bromide,

Hayashi and colleagues [98] found no tumor growth following subcutaneous injection of cells into the backs of nude mice. Furthermore, loss of tumorigenicity correlated with loss of mtDNA and poor growth in culture, which were restored, as was drug resistance, by polyethylene glycol-induced fusion with enucleated fibroblasts. In contrast to this early study, Morais and colleagues [105] showed that clones of the human osteosarcoma cell line, 143B, the serous ovarian carcinoma, 2008, and HeLa cells devoid of mtDNA were poorly- or non-tumorigenic following subcutaneous injection into nude mice. However, all but one HeLa clone grew when cells were injected intramuscularly into nude mice. Reduced tumor growth was also reported by Cavelli [106] for human DBTRGρ^o cells and MCF-7ρ^o breast carcinoma cells injected subcutaneously into nude mice, by Yu and colleagues [90] for human T47Dρ^o breast carcinoma cells injected subcutaneously into estrogen-treated SCID mice and by Magda and colleagues [107] using human A549ρ^o lung cancer cells injected subcutaneously into nude mice. Others have reported that mtDNA deletion promotes the growth of non-tumorigenic (MCF-12A) epithelial breast cell line and tumorigenic MDA-MB-435 breast carcinoma cells injected subcutaneously into SCID mice [108], and that SK-Hep-1 hepatoma cells with deleted or mutated mtDNA have increased angiogenic and invasive properties. In summary, mitochondrial genome deletion can result in loss of tumorigenicity, reduced tumorigenicity or enhanced tumorigenicity depending on the tumor type, the site of tumor cell injection and perhaps, the transplantation recipient.

In the examples discussed above, human tumor cell lines devoid of mtDNA or with mtDNA mutations were transplanted into immunocompromised nude or SCID mice raising questions about whether human to mouse tumor transplantation models reflect autologous tumor transplantation settings. In particular, questions arise as to whether similar mechanisms of regulation of tumor cell growth apply in these diverse models, and whether the immune system, particularly innate mechanisms may distinguish between mitochondrially competent and mitochondrially compromised tumor cells.

Further evidence that functional mitochondrial electron transport is required for tumorigenesis comes from an oncogenic KRAS-driven mouse model of lung cancer involving floxxed alleles of the nuclear-encoded mitochondrial transcription factor A (TFAM) [109]. In this model, lung tumor formation and lesion size was reduced and this was associated with reduced mitochondrial metabolism, lowered ROS generation and loss of anchorage-independent growth.

6.2. Metastasis

We have investigated the effects of mitochondrial genome deletion on tumor growth and metastasis in autologous mouse tumor models. Using B16 metastatic melanoma cells depleted of their mitochondrial DNA (B16ρ^o) and maintained as a stable line in continuous culture for several years in the presence of uridine for which these cells are auxotrophic, a long 20–30 day delay in tumor growth was observed when these cells were injected subcutaneously into C57BL/6 or NOD/scid mice [110] (Tan & Berridge, unpublished results). When injected intravenously, these cells failed to seed and grow in the lung. Lag to initiation of tumor growth was found to be dependent on the number of cells injected and, surprisingly, was associated with cells acquiring the mitochondrially-encoded *cytb* gene (unpublished data). Subsequently, we have used a mtDNA polymorphism to show that the acquired mtDNA is derived from the recipient mouse and not from latent mtDNA of B16 cell origin. These results indicate that mtDNA moves from cells in the microenvironment to ρ^o tumor cells lacking mtDNA and that this acquisition is required for tumor growth (see schematic). Repeated acquisition of mtDNA by tumor cells has previously been implicated in a phylogenetic study of a canine transmissible venereal tumor [111], indicating a novel mechanism of escaping mtDNA mutations that compromise mitochondrial respiratory function. Mitochondrial transfer between cells in culture has been shown to occur via membrane nanotubes [112–115], but whether this mechanism of mtDNA replacement occurs in tumors is not known.

7. Mitochondrial ROS production and anchorage-independent growth are essential for tumorigenesis and metastasis

Warburg postulated that tumor cells have defective mitochondrial oxidative phosphorylation and therefore rely more on aerobic glycolysis as a major source of ATP to fuel cellular proliferation. Nevertheless, Warburg and others have shown that mitochondrial respiration remains an important component of tumor bioenergetic balance, and that mitochondrial electron transport is required for tumor formation [116,117]. Furthermore, oncogenic activation depends on increased oxidative phosphorylation [118] and this correlates with anchorage-independent cell growth and metastatic potential [119]. Normal epithelial cells undergo cell death following detachment from their basement membrane or substrate (Fig. 3). This phenomenon, referred to as anoikis, was first described by Frisch and Francis [120]. However, some detached cells can overcome anoikis (Fig. 3) and survive in the absence of signals from the extracellular matrix, an emerging hallmark of metastatic malignancies. The underlying mechanisms allowing cancer cells to withstand anoikis are not fully understood, but it has been suggested that stimulation of pro-survival signals and inhibition of apoptotic pathways may be involved [121].

Loss of cell attachment triggers metabolic and oxidative stress. Mitochondria are the main generators of ROS that when overproduced results in oxidative stress, a condition associated with many pathologies [122,123]. On the other hand, ROS production has also been detected during metastasis [121] suggesting that these reactive oxygen moieties can also act as signaling messengers within cells to promote survival, migration and metastasis depending on the cell type and oncogenes involved.

Sources of intracellular ROS include the mitochondrial electron transport chain, NADPH oxidases, cyclooxygenases and lipoxygenases [124]. With the mitochondrial electron transport chain, ROS are produced predominantly at Complex I and Complex III [125] and to some extent, Complex II [126]. It is still not clear how mitochondria, by means of ROS, mediate changes in cellular fates. One possibility is that ROS generated by mitochondria react with redox-sensitive proteins that signal to the nucleus and to other parts of the cell.

Mitochondria are organelles that may play a role in the cytoplasmic support of malignancy [98]. Mitochondrial dysfunction can be caused by mutations in mtDNA or nuclear gene-encoded mitochondrial proteins [77,127], consequently inhibiting OXPHOS, increasing ROS, promoting uncontrolled growth and supporting a metastatic phenotype [77,102]. ROS production generated via Complex I [102] and Complex III [109] has been reported to contribute to tumorigenesis and metastasis. On the other hand, treatment of tumor cells with ethidium bromide to deplete mtDNA (ρ^o cells) reduces ROS production [128] and diminishes the ability to grow in an anchorage-independent fashion [129] as well as *in situ* [130]. Experiments in our laboratory with B16ρ^o melanoma, 4T1ρ^o breast carcinoma and other mtDNA-depleted tumor cells show reduced ROS production, anchorage-dependent survival, delayed tumor growth and failure to form lung tumors *in vivo* [7] (see Fig. 3).

Genes such as *Bcl-2* and mutant *TP53* that are important in tumorigenicity [131] and anchorage independence [132] through mitochondrial mechanisms [133], are not differentially expressed between wild type and ρ^o cells [134]. A variety of anaplerotic pathways are activated in respiration-deficient cells to maintain the synthesis of α-ketoglutarate, a precursor to glutamate and glutamine (Fig. 1), thus preventing amino acid starvation [135]. However, we have observed that B16ρ^o and 4T1ρ^o cells are highly dependent on glucose but not glutamine in addition to their auxotrophy for pyruvate and uridine for growth. This may indicate that glutamine conversion to glutamate via glutaminase in these cells may be defective. Glutamine is a

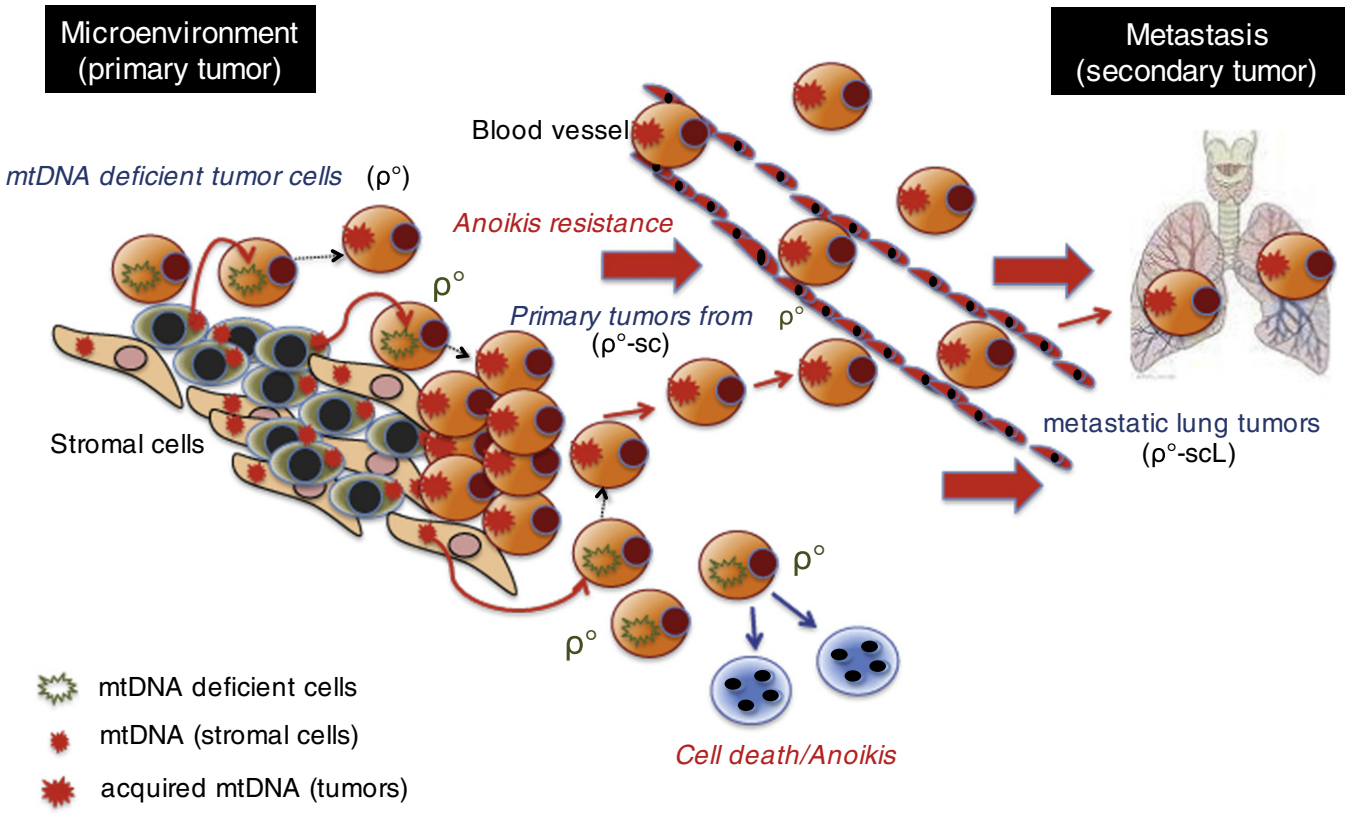

Fig. 3. Mitochondrial DNA acquisition by ρ° tumor cells facilitates tumor growth and metastasis. Schematic diagram indicating primary tumor formation and metastasis to the lung following mtDNA acquisition by ρ° tumor cells lacking mtDNA. The mitochondrial genotype follows that of the recipient mouse and is unrelated to that of the parent tumor.

major respiratory substrate in cancer cells, providing energy and carbon via the TCA cycle to generate ***α***-ketoglutarate for growth [8,136]. Glutamine-dependent pathways have been shown to be the dominant metabolism in malignant cells containing mutations in Complex I and Complex III [137]. α-ketoglutarate which is derived from glutamine through glutaminase and glutamate dehydrogenase has also been shown to be essential for KRAS-induced anchorage-independent growth [109], a prerequisite for tumor metastasis [138] as previously discussed.

A better understanding of the molecular mechanisms involved in mitochondrial respiratory defects in anoikis resistance and metastasis would assist in the development of anticancer drugs to prevent tumor metastases, the major cause of death from cancer.

8. Conclusion and perspectives

The role of mitochondrial respiratory function in tumor formation and metastasis is explored in this review. It is concluded that mitochondrial electron transport is required for tumor formation and metastasis, but that mtDNA mutations that compromise mitochondrial electron transport function can also contribute to tumor formation and spread by optimizing the balance between glycolytic and mitochondrial energy production. In order to reconcile these apparently contradictory requirements for mitochondrial respiratory function in tumorigenesis and metastasis, we propose that rapidly proliferating and self-renewing cells within each tumor are characterized by a "bioenergetic and metabolic sweet spot" (Fig. 2) that is determined not only by mitochondrial mutations and retrograde signaling to the nucleus but also by somatic nuclear mutations, by layers of epigenetic remodelling and by the microenvironment that contributes essential nutrients, bioenergetic fuels and oxygen to the developing tumor (see Graphical Abstract). The fact that tumors as they develop and evolve are genetically and metabolically heterogeneous creates a moving target that can be further complicated by intercellular transfer of mitochondria along with their genomic cargo. Metabolic flexibility will generate a bioenergetic window within which tumor cells can survive and propagate: outside of this window, survival will be compromised either because effects on mitochondrial electron transport function are so severe that ATP production becomes suboptimal for survival, or because the combined genetic and epigenetic effects result in inefficient electron transport, excessive ROS production and escalating mitochondrial and nuclear damage that exceeds repair capability (Fig. 2). In either case, cell death by apoptosis or autophagy will result. The reason why ρ° tumor cells devoid of their mitochondrial genome are capable of growing in tissue culture, albeit with uridine and to a lesser extent, pyruvate supplementation, but fail to grow as tumors in a syngeneic setting until they have acquired a mitochondrial genome may relate as much to their high requirement for glucose and conversely, sensitivity to glucose deprivation, as to their auxotrophic requirement for uridine. In this context, the local microenvironment may well be the limiting factor that determines the survival of developing tumor cells with mitochondrial mutations that compromise mitochondrial electron transport.

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References

[1] L.M. Ferreira, Cancer metabolism: the Warburg effect today, Exp. Mol. Pathol. 89 (2010) 372–380.
[2] W.H. Koppenol, P.L. Bounds, C.V. Dang, Otto Warburg's contributions to current concepts of cancer metabolism, Nat. Rev. Cancer 11 (2011) 325–337.
[3] O. Warburg, Uber den stoffwechsel der tumoren, Berlin-Dahlem (Julius Springer), Berlin, 1926.
[4] O. Warburg, On the origin of cancer cells, Science 123 (1956) 309–314.
[5] T.N. Seyfried, Cancer as a Metabolic Disease, On the Origin, Management and Prevention of Cancer, John Wiley & Sons, Inc, Hobken, New Jersey, 2012.
[6] P.S. Ward, C.B. Thompson, Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate, Cancer Cell 21 (2012) 297–308.
[7] M.V. Berridge, P.M. Herst, A.S. Tan, Metabolic flexibility and cell hierarchy in metastatic cancer, Mitochondrion 10 (2010) 584–588.
[8] R.J. DeBerardinis, A. Mancuso, E. Daikhin, et al., Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 19345–19350.
[9] P.M. Herst, M.V. Berridge, Cell hierarchy, metabolic flexibity and systems approaches to cancer treatment, Curr. Pharm. Biotechnol. 14 (2013) 289–299.
[10] K. Smolkova, L. Plecita-Hlavata, N. Bellance, et al., Waves of gene regulation suppress and then restore oxidative phosphorylation in cancer cells, Int. J. Biochem. Cell Biol. 43 (2011) 950–968.
[11] V. Chen, E. Shtivelman, CC3/TIP30 regulates metabolic adaptation of tumor cells to glucose limitation, Cell Cycle 9 (2010) 4941–4953.
[12] A. Schulze, A.L. Harris, How cancer metabolism is tuned for proliferation and vulnerable to disruption, Nature 491 (2012) 364–373.
[13] M.G. Vander Heiden, L.C. Cantley, C.B. Thompson, Understanding the Warburg effect: the metabolic requirements of cell proliferation, Science 324 (2009) 1029–1033.
[14] Z. Chen, L. Wang, Q. Wang, et al., Histone modifications and chromatin organization in prostate cancer, Epigenomics 2 (2010) 551–560.
[15] J.S. Isaacs, Y.J. Jung, D.R. Mole, et al., HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability, Cancer Cell 8 (2005) 143–153.
[16] J.R. Toro, M.L. Nickerson, M.H. Wei, et al., Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America, Am. J. Hum. Genet. 73 (2003) 95–106.
[17] J.P. Bayley, H.P. Kunst, A. Cascon, et al., SDHAF2 mutations in familial and sporadic paraganglioma and phaeochromocytoma, Lancet Oncol. 11 (2010) 366–372.
[18] E.R. Mardis, L. Ding, D.J. Dooling, et al., Recurring mutations found by sequencing an acute myeloid leukemia genome, N. Engl. J. Med. 361 (2009) 1058–1066.
[19] D.W. Parsons, S. Jones, X. Zhang, et al., An integrated genomic analysis of human glioblastoma multiforme, Science 321 (2008) 1807–1812.
[20] J.W. Locasale, A.R. Grassian, T. Melman, et al., Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis, Nat. Genet. 43 (2011) 869–874.
[21] R. Possemato, K.M. Marks, Y.D. Shaul, et al., Functional genomics reveal that the serine synthesis pathway is essential in breast cancer, Nature 476 (2011) 346–350.
[22] R.J. Shaw, L.C. Cantley, Decoding key nodes in the metabolism of cancer cells: sugar & spice and all things nice, F1000, Biol. Reprod. 4 (2012) 2.
[23] J. Kaplon, L. Zheng, K. Meissl, et al., A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence, Nature 498 (2013) 109–112.
[24] S.P. Mathupala, Y.H. Ko, P.L. Pedersen, Hexokinase-2 bound to mitochondria: cancer's stygian link to the "Warburg Effect" and a pivotal target for effective therapy, Semin. Cancer Biol. 19 (2009) 17–24.
[25] H.R. Christofk, M.G. Vander Heiden, M.H. Harris, et al., The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth, Nature 452 (2008) 230–233.
[26] D.Y. Gui, C.A. Lewis, M.G. Vander Heiden, Allosteric regulation of PKM2 allows cellular adaptation to different physiological states, Sci. Signal. 6 (2013) pe7.
[27] S. Mazurek, Pyruvate kinase type M2: a key regulator of the metabolic budget system in tumor cells, Int. J. Biochem. Cell Biol. 43 (2011) 969–980.
[28] B. Martinez-Pastor, R. Mostoslavsky, Sirtuins, metabolism, and cancer, Front. Pharmacol. 3 (2012) 22.
[29] C. Sebastian, F.K. Satterstrom, M.C. Haigis, et al., From sirtuin biology to human diseases: an update, J. Biol. Chem. 287 (2012) 42444–42452.
[30] J. Yuan, K. Minter-Dykhouse, Z. Lou, A c-Myc-SIRT1 feedback loop regulates cell growth and transformation, J. Cell Biol. 185 (2009) 203–211.
[31] L. Zhong, A. D'Urso, D. Toiber, et al., The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1alpha, Cell 140 (2010) 280–293.
[32] S.C. Dryden, F.A. Nahhas, J.E. Nowak, et al., Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle, Mol. Cell. Biol. 23 (2003) 3173–3185.
[33] S.M. Jeong, C. Xiao, L.W. Finley, et al., SIRT4 has tumor-suppressive activity and regulates the cellular metabolic response to DNA damage by inhibiting mitochondrial glutamine metabolism, Cancer Cell 23 (2013) 450–463.
[34] M.F. Barber, E. Michishita-Kioi, Y. Xi, et al., SIRT7 links H3K18 deacetylation to maintenance of oncogenic transformation, Nature 487 (2012) 114–118.
[35] C.V. Dang, J.W. Kim, P. Gao, et al., The interplay between MYC and HIF in cancer, Nat. Rev. Cancer 8 (2008) 51–56.
[36] A. Carracedo, L.C. Cantley, P.P. Pandolfi, Cancer metabolism: fatty acid oxidation in the limelight, Nat. Rev. Cancer 13 (2013) 227–232.
[37] P.A. Gameiro, J. Yang, A.M. Metelo, et al., In vivo HIF-mediated reductive carboxylation is regulated by citrate levels and sensitizes VHL-deficient cells to glutamine deprivation, Cell Metab. 17 (2013) 372–385.
[38] J. Son, C.A. Lyssiotis, H. Ying, et al., Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway, Nature 496 (2013) 101–105.
[39] D.R. Wise, C.B. Thompson, Glutamine addiction: a new therapeutic target in cancer, Trends Biochem. Sci. 35 (2010) 427–433.
[40] D.C. Wallace, Mitochondria and cancer, Nat. Rev. Cancer 12 (2012) 685–698.
[41] A. Ertel, A. Tsirigos, D. Whitaker-Menezes, et al., Is cancer a metabolic rebellion against host aging? In the quest for immortality, tumor cells try to save themselves by boosting mitochondrial metabolism, Cell Cycle 11 (2012) 253–263.
[42] R. Haq, J. Shoag, P. Andreu-Perez, et al., Oncogenic BRAF regulates oxidative metabolism via PGC1alpha and MITF, Cancer Cell 23 (2013) 302–315.
[43] R. Moreno-Sanchez, S. Rodriguez-Enriquez, A. Marin-Hernandez, et al., Energy metabolism in tumor cells, FEBS J. 274 (2007) 1393–1418.
[44] F. Vazquez, J.H. Lim, H. Chim, et al., PGC1alpha expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress, Cancer Cell 23 (2013) 287–301.
[45] X.L. Zu, M. Guppy, Cancer metabolism: facts, fantasy, and fiction, Biochem. Biophys. Res. Commun. 313 (2004) 459–465.
[46] L.I. Cardenas-Navia, D. Mace, R.A. Richardson, et al., The pervasive presence of fluctuating oxygenation in tumors, Cancer Res. 68 (2008) 5812–5819.
[47] P. Jezek, L. Plecita-Hlavata, K. Smolkova, et al., Distinctions and similarities of cell bioenergetics and the role of mitochondria in hypoxia, cancer, and embryonic development, Int. J. Biochem. Cell Biol. 42 (2010) 604–622.
[48] F. Hirschhaeuser, U.G. Sattler, W. Mueller-Klieser, Lactate: a metabolic key player in cancer, Cancer Res. 71 (2011) 6921–6925.
[49] P.C. Nowell, The clonal evolution of tumor cell populations, Science 194 (1976) 23–28.
[50] D.J. Burgess, Genomic instability: close-up on cancer copy number alterations, Nat. Rev. Genet. 13 (2012) 5.
[51] M. Greaves, C.C. Maley, Clonal evolution in cancer, Nature 481 (2012) 306–313.
[52] T. Kuukasjarvi, R. Karhu, M. Tanner, et al., Genetic heterogeneity and clonal evolution underlying development of asynchronous metastasis in human breast cancer, Cancer Res. 57 (1997) 1597–1604.
[53] M.O. Yuneva, T.W. Fan, T.D. Allen, et al., The metabolic profile of tumors depends on both the responsible genetic lesion and tissue type, Cell Metab. 15 (2012) 157–170.
[54] G. Bonuccelli, D. Whitaker-Menezes, R. Castello-Cros, et al., The reverse Warburg effect: glycolysis inhibitors prevent the tumor promoting effects of caveolin-1 deficient cancer associated fibroblasts, Cell Cycle 9 (2010) 1960–1971.
[55] R. Castello-Cros, G. Bonuccelli, A. Molchansky, et al., Matrix remodeling stimulates stromal autophagy, "fueling" cancer cell mitochondrial metabolism and metastasis, Cell Cycle 10 (2011) 2021–2034.
[56] U.E. Martinez-Outschoorn, S. Pavlides, A. Howell, et al., Stromal–epithelial metabolic coupling in cancer: integrating autophagy and metabolism in the tumor microenvironment, Int. J. Biochem. Cell Biol. 43 (2011) 1045–1051.
[57] S. Pavlides, A. Tsirigos, I. Vera, et al., Loss of stromal caveolin-1 leads to oxidative stress, mimics hypoxia and drives inflammation in the tumor microenvironment, conferring the "reverse Warburg effect": a transcriptional informatics analysis with validation, Cell Cycle 9 (2010) 2201–2219.
[58] S. Pavlides, A. Tsirigos, I. Vera, et al., Transcriptional evidence for the "Reverse Warburg Effect" in human breast cancer tumor stroma and metastasis: similarities with oxidative stress, inflammation, Alzheimer's disease, and "Neuron-Glia Metabolic Coupling", Aging (Albany NY) 2 (2010) 185–199.
[59] S. Pavlides, D. Whitaker-Menezes, R. Castello-Cros, et al., The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma, Cell Cycle 8 (2009) 3984–4001.
[60] F. Sotgia, D. Whitaker-Menezes, U.E. Martinez-Outschoorn, et al., Mitochondria "fuel" breast cancer metabolism: fifteen markers of mitochondrial biogenesis label epithelial cancer cells, but are excluded from adjacent stromal cells, Cell Cycle 11 (2012) 4390–4401.
[61] E.A. Schon, S. DiMauro, M. Hirano, Human mitochondrial DNA: roles of inherited and somatic mutations, Nat. Rev. Genet. 13 (2012) 878–890.
[62] T.C. Larman, S.R. DePalma, A.G. Hadjipanayis, et al., Spectrum of somatic mitochondrial mutations in five cancers, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 14087–14091.
[63] K. Polyak, Y. Li, H. Zhu, et al., Somatic mutations of the mitochondrial genome in human colorectal tumours, Nat. Genet. 20 (1998) 291–293.
[64] L. Iommarini, M.A. Calvaruso, I. Kurelac, et al., Complex I impairment in mitochondrial diseases and cancer: parallel roads leading to different outcomes, Int. J. Biochem. Cell Biol. 45 (2013) 47–63.
[65] J. Lu, L.K. Sharma, Y. Bai, Implications of mitochondrial DNA mutations and mitochondrial dysfunction in tumorigenesis, Cell Res. 19 (2009) 802–815.
[66] H. Schagger, K. Pfeiffer, Supercomplexes in the respiratory chains of yeast and mammalian mitochondria, EMBO J. 19 (2000) 1777–1783.
[67] D.R. Winge, Sealing the mitochondrial respirasome, Mol. Cell. Biol. 32 (2012) 2647–2652.
[68] R. Baradaran, J.M. Berrisford, G.S. Minhas, et al., Crystal structure of the entire respiratory complex I, Nature 494 (2013) 443–448.

[69] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674.
[70] S. Dasgupta, E. Soudry, N. Mukhopadhyay, et al., Mitochondrial DNA mutations in respiratory complex-I in never-smoker lung cancer patients contribute to lung cancer progression and associated with EGFR gene mutation, J. Cell. Physiol. 227 (2012) 2451–2460.
[71] L.K. Sharma, H. Fang, J. Liu, et al., Mitochondrial respiratory complex I dysfunction promotes tumorigenesis through ROS alteration and AKT activation, Hum. Mol. Genet. 20 (2011) 4605–4616.
[72] W. Sun, S. Zhou, S.S. Chang, et al., 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 (2009) 476–484.
[73] G. Gasparre, I. Kurelac, M. Capristo, et al., A mutation threshold distinguishes the antitumorigenic effects of the mitochondrial gene MTND1, an oncojanus function, Cancer Res. 71 (2011) 6220–6229.
[74] J.S. Park, L.K. Sharma, H. Li, et al., A heteroplasmic, not homoplasmic, mitochondrial DNA mutation promotes tumorigenesis via alteration in reactive oxygen species generation and apoptosis, Hum. Mol. Genet. 18 (2009) 1578–1589.
[75] D. Astuti, F. Latif, A. Dallol, et al., Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma, Am. J. Hum. Genet. 69 (2001) 49–54.
[76] B.E. Baysal, R.E. Ferrell, J.E. Willett-Brozick, et al., Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma, Science 287 (2000) 848–851.
[77] M. Brandon, P. Baldi, D.C. Wallace, Mitochondrial mutations in cancer, Oncogene 25 (2006) 4647–4662.
[78] S. Niemann, U. Muller, Mutations in SDHC cause autosomal dominant paraganglioma, type 3, Nat. Genet. 26 (2000) 268–270.
[79] S. Vanharanta, M. Buchta, S.R. McWhinney, et al., Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma, Am. J. Hum. Genet. 74 (2004) 153–159.
[80] T. Bourgeron, P. Rustin, D. Chretien, et al., Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency, Nat. Genet. 11 (1995) 144–149.
[81] J. Permuth-Wey, Y.A. Chen, Y.Y. Tsai, et al., Inherited variants in mitochondrial biogenesis genes may influence epithelial ovarian cancer risk, Cancer Epidemiol. Biomarkers Prev. 20 (2011) 1131–1145.
[82] J.A. Petros, A.K. Baumann, E. Ruiz-Pesini, et al., mtDNA mutations increase tumorigenicity in prostate cancer, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 719–724.
[83] R. Lehtonen, M. Kiuru, S. Vanharanta, et al., Biallelic inactivation of fumarate hydratase (FH) occurs in nonsyndromic uterine leiomyomas but is rare in other tumors, Am. J. Pathol. 164 (2004) 17–22.
[84] L. Dang, D.W. White, S. Gross, et al., Cancer-associated IDH1 mutations produce 2-hydroxyglutarate, Nature 462 (2009) 739–744.
[85] R.J. DeBerardinis, C.B. Thompson, Cellular metabolism and disease: what do metabolic outliers teach us? Cell 148 (2012) 1132–1144.
[86] I.A. Barbosa, N.G. Machado, A.J. Skildum, et al., Mitochondrial remodeling in cancer metabolism and survival: potential for new therapies, Biochim. Biophys. Acta 1826 (2012) 238–254.
[87] N.G. Ericson, M. Kulawiec, M. Vermulst, et al., Decreased mitochondrial DNA mutagenesis in human colorectal cancer, PLoS Genet. 8 (2012) e1002689.
[88] K. Skonieczna, B.A. Malyarchuk, T. Grzybowski, The landscape of mitochondrial DNA variation in human colorectal cancer on the background of phylogenetic knowledge, Biochim. Biophys. Acta 1825 (2012) 153–159.
[89] R.W. Taylor, D.M. Turnbull, Mitochondrial DNA mutations in human disease, Nat. Rev. Genet. 6 (2005) 389–402.
[90] M. Yu, Y. Shi, X. Wei, et al., Mitochondrial DNA depletion promotes impaired oxidative status and adaptive resistance to apoptosis in T47D breast cancer cells, Eur. J. Cancer Prev. 18 (2009) 445–457.
[91] S.K. Mithani, I.M. Smith, S.L. Topalian, et al., Nonsynonymous somatic mitochondrial mutations occur in the majority of cutaneous melanomas, Melanoma Res. 18 (2008) 214–219.
[92] Y. Guo, Q. Cai, D.C. Samuels, et al., The use of next generation sequencing technology to study the effect of radiation therapy on mitochondrial DNA mutation, Mutat. Res. 744 (2012) 154–160.
[93] A.N. Howell, R. Sager, Tumorigenicity and its suppression in cybrids of mouse and Chinese hamster cell lines, Proc. Natl. Acad. Sci. U. S. A. 75 (1978) 2358–2362.
[94] B.A. Israel, W.I. Schaeffer, Cytoplasmic suppression of malignancy, In Vitro Cell. Dev. Biol. 23 (1987) 627–632.
[95] M. Koura, H. Isaka, M.C. Yoshida, et al., Suppression of tumorigenicity in interspecific reconstituted cells and cybrids, Gann 73 (1982) 574–580.
[96] J.W. Shay, Y.N. Liu, H. Werbin, Cytoplasmic suppression of tumor progression in reconstituted cells, Somat. Cell Mol. Genet. 14 (1988) 345–350.
[97] J.W. Shay, H. Werbin, Cytoplasmic suppression of tumorigenicity in reconstructed mouse cells, Cancer Res. 48 (1988) 830–833.
[98] J. Hayashi, M. Takemitsu, I. Nonaka, Recovery of the missing tumorigenicity in mitochondrial DNA-less HeLa cells by introduction of mitochondrial DNA from normal human cells, Somat. Cell Mol. Genet. 18 (1992) 123–129.
[99] J. Hayashi, H. Werbin, J.W. Shay, Effects of normal human fibroblast mitochondrial DNA on segregation of HeLaTG Mitochondrial DNA and on tumorigenicity of HeLaTG cells, Cancer Res. 46 (1986) 4001–4006.
[100] M. Akimoto, M. Niikura, M. Ichikawa, et al., Nuclear DNA but not mtDNA controls tumor phenotypes in mouse cells, Biochem. Biophys. Res. Commun. 327 (2005) 1028–1035.
[101] O. Hashizume, A. Shimizu, M. Yokota, et al., Specific mitochondrial DNA mutation in mice regulates diabetes and lymphoma development, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 10528–10533.
[102] K. Ishikawa, K. Takenaga, M. Akimoto, et al., ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis, Science 320 (2008) 661–664.
[103] K. Ishikawa, O. Hashizume, N. Koshikawa, et al., Enhanced glycolysis induced by mtDNA mutations does not regulate metastasis, FEBS Lett. 582 (2008) 3525–3530.
[104] A.F. Santidrian, A. Matsuno-Yagi, M. Ritland, et al., Mitochondrial complex I activity and NAD+/NADH balance regulate breast cancer progression, J. Clin. Invest. 123 (2013) 1068–1081.
[105] R. Morais, K. Zinkewich-Peotti, M. Parent, et al., Tumor-forming ability in athymic nude mice of human cell lines devoid of mitochondrial DNA, Cancer Res. 54 (1994) 3889–3896.
[106] L.R. Cavalli, M. Varella-Garcia, B.C. Liang, Diminished tumorigenic phenotype after depletion of mitochondrial DNA, Cell Growth Differ. 8 (1997) 1189–1198.
[107] D. Magda, P. Lecane, J. Prescott, et al., mtDNA depletion confers specific gene expression profiles in human cells grown in culture and in xenograft, BMC Genomics 9 (2008) 521.
[108] M. Kulawiec, A. Safina, M.M. Desouki, et al., Tumorigenic transformation of human breast epithelial cells induced by mitochondrial DNA depletion, Cancer Biol. Ther. 7 (2008) 1732–1743.
[109] F. Weinberg, R. Hamanaka, W.W. Wheaton, et al., Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 8788–8793.
[110] M.V. Berridge, A.S. Tan, Effects of mitochondrial gene deletion on tumorigenicity of metastatic melanoma: reassessing the Warburg effect, Rejuvenation Res. 13 (2010) 139–141.
[111] C.A. Rebbeck, A.M. Leroi, A. Burt, Mitochondrial capture by a transmissible cancer, Science 331 (2011) 303.
[112] Y.M. Cho, J.H. Kim, M. Kim, et al., Mesenchymal stem cells transfer mitochondria to the cells with virtually no mitochondrial function but not with pathogenic mtDNA mutations, PLoS One 7 (2012) e32778.
[113] H.H. Gerdes, R.N. Carvalho, Intercellular transfer mediated by tunneling nanotubes, Curr. Opin. Cell Biol. 20 (2008) 470–475.
[114] J.L. Spees, S.D. Olson, M.J. Whitney, et al., Mitochondrial transfer between cells can rescue aerobic respiration, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 1283–1288.
[115] B. Onfelt, S. Nedvetzki, R.K. Benninger, et al., Structurally distinct membrane nanotubes between human macrophages support long-distance vesicular traffic or surfing of bacteria, J. Immunol. 177 (2006) 8476–8483.
[116] S. Weinhouse, On respiratory impairment in cancer cells, Science 124 (1956) 267–269.
[117] N.C. Denko, Hypoxia, HIF1 and glucose metabolism in the solid tumour, Nat. Rev. Cancer 8 (2008) 705–713.
[118] J.M. Funes, M. Quintero, S. Henderson, et al., Transformation of human mesenchymal stem cells increases their dependency on oxidative phosphorylation for energy production, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 6223–6228.
[119] S. Mori, J.T. Chang, E.R. Andrechek, et al., Anchorage-independent cell growth signature identifies tumors with metastatic potential, Oncogene 28 (2009) 2796–2805.
[120] S.M. Frisch, H. Francis, Disruption of epithelial cell–matrix interactions induces apoptosis, J. Cell Biol. 124 (1994) 619–626.
[121] E. Giannoni, F. Buricchi, G. Grimaldi, et al., Redox regulation of anoikis: reactive oxygen species as essential mediators of cell survival, Cell Death Differ. 15 (2008) 867–878.
[122] J. Rehman, Empowering self-renewal and differentiation: the role of mitochondria in stem cells, J. Mol. Med. (Berl.) 88 (2010) 981–986.
[123] E.H. Sarsour, M.G. Kumar, L. Chaudhuri, et al., Redox control of the cell cycle in health and disease, Antioxid. Redox Signal. 11 (2009) 2985–3011.
[124] G. Gloire, S. Legrand-Poels, J. Piette, NF-kappaB activation by reactive oxygen species: fifteen years later, Biochem. Pharmacol. 72 (2006) 1493–1505.
[125] Y.S. Bae, H. Oh, S.G. Rhee, et al., Regulation of reactive oxygen species generation in cell signaling, Mol. Cell 32 (2011) 491–509.
[126] C.L. Quinlan, A.L. Orr, I.V. Perevoshchikova, et al., Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions, J. Biol. Chem. 287 (2012) 27255–27264.
[127] D.C. Wallace, A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine, Annu. Rev. Genet. 39 (2005) 359–407.
[128] M. Schauen, D. Spitkovsky, J. Schubert, et al., Respiratory chain deficiency slows down cell-cycle progression via reduced ROS generation and is associated with a reduction of p21CIP1/WAF1, J. Cell. Physiol. 209 (2006) 103–112.
[129] K. Zinkewich-Peotti, M. Parent, R. Morais, Mitochondrial DNA modulation of the anchorage-independent phenotype of transformed avian cells, Cancer Res. 50 (1990) 6675–6682.
[130] K. Zinkewich-Peotti, M. Parent, R. Morais, On the tumorigenicity of mitochondrial DNA-depleted avian cells, Cancer Lett. 59 (1991) 119–124.
[131] J.A. Hickman, Apoptosis and chemotherapy resistance, Eur. J. Cancer 32A (1996) 921–926.
[132] M.A. Nikiforov, K. Hagen, V.S. Ossovskaya, et al., p53 modulation of anchorage independent growth and experimental metastasis, Oncogene 13 (1996) 1709–1719.
[133] S.A. Susin, N. Zamzami, M. Castedo, et al., Bcl-2 inhibits the mitochondrial release of an apoptogenic protease, J. Exp. Med. 184 (1996) 1331–1341.

[134] C.A. Kruse, D.H. Mitchell, B.K. Kleinschmidt-DeMasters, et al., Characterization of a continuous human glioma cell line DBTRG-05MG: growth kinetics, karyotype, receptor expression, and tumor suppressor gene analyses, In Vitro Cell. Dev. Biol. 28A (1992) 609–614.
[135] Z. Liu, R.A. Butow, A transcriptional switch in the expression of yeast tricarboxylic acid cycle genes in response to a reduction or loss of respiratory function, Mol. Cell. Biol. 19 (1999) 6720–6728.
[136] L.J. Reitzer, B.M. Wice, D. Kennell, Evidence that glutamine, not sugar, is the major energy source for cultured HeLa cells, J. Biol. Chem. 254 (1979) 2669–2676.
[137] A.R. Mullen, W.W. Wheaton, E.S. Jin, et al., Reductive carboxylation supports growth in tumour cells with defective mitochondria, Nature 481 (2012) 385–388.
[138] Y.N. Kim, K.H. Koo, J.Y. Sung, et al., Anoikis resistance: an essential prerequisite for tumor metastasis, Int. J. Cell Biol. 2012 (2012) 306879.