HHS Public Access

Author manuscript

Nat Rev Cancer. Author manuscript; available in PMC 2015 March 24.

Published in final edited form as:

Nat Rev Cancer. 2012 October ; 12(10): 685–698. doi:10.1038/nrc3365.

Mitochondria and cancer

Douglas C. Wallace

Children's Hospital of Philadelphia, Director, Center for Mitochondrial and Epigenomic Medicine (CMEM), Colket Translational Research Building, Room 6060, 3501 Civic Center Boulevard, Philadelphia, Pennsylvania 19104, USA. wallacedl@email.chop.edu

Abstract

Contrary to conventional wisdom, functional mitochondria are essential for the cancer cell. Although mutations in mitochondrial genes are common in cancer cells, they do not inactivate mitochondrial energy metabolism but rather alter the mitochondrial bioenergetic and biosynthetic state. These states communicate with the nucleus through mitochondrial 'retrograde signalling' to modulate signal transduction pathways, transcriptional circuits and chromatin structure to meet the perceived mitochondrial and nuclear requirements of the cancer cell. Cancer cells then reprogramme adjacent stromal cells to optimize the cancer cell environment. These alterations activate out-of-context programmes that are important in development, stress response, wound healing and nutritional status.

Over 70 years ago Otto Warburg observed that cancer cells produce excessive lactate in the presence of oxygen, a state he termed 'aerobic glycolysis'[1–3]. Today, this high rate of glucose uptake by solid tumours is used as a diagnostic through ^{18}F-2-deoxyglucose (FDG) accumulation detected by positron emission tomography[4]. During the twentieth century considerable effort was expended to determine whether aerobic glycolysis was due to defects in mitochondrial oxidative phosphorylation (OXPHOS), with generally negative results[5]. However, this failure was more a reflection of a lack of fundamental knowledge about mitochondrial biology than a lack of relevance of mitochondria to cancer.

The mitochondrial bacterium is the 2 billion year symbiotic partner of the nuclear–cytosolic organism[6,7]. Today, the mitochondrial genome encompasses between one and two thousand nuclear DNA (nDNA) genes plus thousands of copies of mitochondrial DNA (mtDNA) that reside within the mitochondrion. The mtDNA retains the 13 most important OXPHOS genes, while the nDNA contains all of the remaining OXPHOS genes, as well as the genes for mitochondrial metabolism and biogenesis (FIGS 1,2). The mitochondrion is a highly evolved system for coordinating energy production and distribution based on the availability of calories and oxygen and the demands for cellular maintenance and reproduction. In many

Competing interests statement

The authors declare no competing financial interests.

DATABASES

National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary Pathway Interaction Database: http://pid.nci.nih.gov

The MITOMAP curated human mitochondrial genome database: http://www.mitomap.org/MITOMAP

cancer cells, bioenergetic reprogramming involves switching from the maximal ATP production by OXPHOS in quiescent, differentiated cells to the requirement for balancing energy needs with substrate generation for cellular biogenesis and reproduction in rapidly growing cells.

Many vital cellular parameters are controlled by mitochondria. These include regulation of energy production, modulation of oxidation–reduction (redox) status, generation of reactive oxygen species (ROS), control of cytosolic calcium (Ca^{2+}) levels, contribution to cytosolic biosynthetic precursors such as acetyl-CoA and pyrimidines, and initiation of apoptosis through the activation of the mitochondrial permeability transition pore (mtPTP) (FIG. 3). Changes in these parameters can impinge on biosynthetic pathways, cellular signal transduction pathways, transcription factors and chromatin structure to shift the cell from a quiescent, differentiated state to an actively proliferating one.

Although mutations in the mtDNA in cancer cells have been recognized for more than two decades[8], interest in the role of mitochondrial alterations in cancer came to general attention with the discovery of mitochondrial tricarboxylic acid (TCA) cycle gene mutations in cancer cells. Cancer cell defects are now well established in the genes for succinate dehydrogenase (SDH), fumarate hydratase (*FH*), and isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* (FIG. 2).

Analysis of the pathophysiological consequences of mitochondrial gene mutations is providing broad new insights into the importance and complexity of mitochondrial alterations in cancer. In this Review I discuss these observations in the context of how alterations in mitochondrial function can signal to the nucleus to change its developmental and replicative status (‘retrograde signalling’) and influence the metabolic status of the surrounding stromal cells (the ‘reverse Warburg effect’).

Mutations that affect the expression of mtDNA

ρ^o cells and tumorigenesis

The requirement of cancer cells for functional mitochondria has been confirmed by the elimination of mtDNA from various cancer cells through growth in ethidium bromide (ρ^o cells). The resulting ρ^o cancer cells have reduced growth rates, decreased colony formation in soft agar and markedly reduced tumour formation in nude mice[9–15]. The importance of functional mitochondria has been further demonstrated by the transmissible tumours of Tazmanian devils and dogs. These tumours have been transferred from animal to animal for tens of thousands of years[16] and should have died out owing to the decay of their mtDNAs. However, this limitation has been circumvented by the periodic acquisition by tumours of normal mtDNA from host cells[17,18].

mtDNA mutations and cancer

Somatic and germline mtDNA mutations have been reported for a wide variety of cancers. These include renal adenocarcinoma, colon cancer cells, head and neck tumours, astrocytic tumours, thyroid tumours, breast tumours, ovarian tumours, prostate and bladder cancer, neuroblastomas and oncocytomas[19–24]. Although technical and interpretive errors were common in early publications on mtDNA mutations in cancer[25–27], the identification of

clearly deleterious mtDNA mutations in cancer tissues — such as an intragenic deletion[8] or the common tRNA$^{Leu(UUR)}$ A3243G MELAS mutation[26] — validate the relevance of pathogenic mutations in neoplastic transformation. The importance of the mtDNA in cancer has been confirmed by the exchange of cancer cell mtDNA with pathogenic or normal mtDNA, resulting in alterations of cancer cell phenotypes[28–31].

Ancient mtDNA population variants have also been correlated with cancer risk. For example, the macro-haplogroup N variant in the complex I, subunit ND3 gene (*ND3*; also known as *MTND3*) at nucleotide G10398A (resulting in a T114A amino acid change) has been associated with breast cancer risk in African American women[32], and the 16519 T to C mtDNA control region variant is associated with endometrial cancer[33]. A mtDNA cytochrome *c* oxidase subunit 1 (*CO1*; also known as *MTCO1*) T6777C nucleotide variant has been linked with epithelial ovarian cancer, along with variants in several nDNA mitochondrial genes[34]. Furthermore, the mtDNA control region variant C150 (REF. 35) has been associated with an increased risk of human papilloma virus (HPV) infection and cervical cancer in Chinese women[36].

A meta-analysis of many cancer-associated mtDNA mutations revealed that many cancer cell mtDNA mutations clearly inhibit OXPHOS. However, a significant proportion of the reported variants are the same nucleotide changes that have previously been reported as ancient adaptive mtDNA variants in human populations[37–40]. Although some of these cancer ‘associations’ are clear misinterpretations of ancient polymorphisms, others may be valid cancer cell mutations[41]. Consequently, there may be two classes of mutations in cancer cell mtDNA: mutations that impair OXPHOS and serve to stimulate neo-plastic transformation, and those that facilitate cancer cell adaption to changing bioenergetic environments[19].

The importance of mtDNA mutations in neoplastic transformation is best illustrated by example. In one prostate tumour a *de novo CO1* chain termination mutation was homoplasmic (pure) mutant in the tumour but homoplasmic wild-type in adjacent normal epithelial tissue. This abrupt change in the thousands of copies of mtDNA implies that the *CO1* nonsense mutation was under intense positive selection in the cancer cell[29]. In an oncocytoma, the tumour cells were homoplasmic for an *ND5* gene frame-shift mutation, and the normal tissues of the patient and his two sisters harboured the same mutation, but at low frequency (low heteroplasmy). Hence, an *ND5* frame-shift mutation transmitted through the maternal lineage at low heteroplasmy switched to high heteroplasmy in the tumour, again demonstrating positive selection for the mutant mtDNA[22].

The mtDNA is condensed into nucleoids through the binding of mitochondrial transcription factor A (TFAM; also known as mtTFA). Some colorectal cancers harbour heterozygous *TFAM* mutations that are associated with mtDNA depletion[42]. TFAM has also been observed to be translocated into the nucleus in prostate cancer cells, and overexpression of TFAM can stimulate cell proliferation[43]. In addition to TFAM, mtDNA nucleoids harbour multiple additional proteins that are involved in mtDNA replication and transcription[44,45], one of which is the nuclear-encoded RNA helicase, SUV3 (also known as SUPV3L1). Knockdown of SUV3 results in reduced mtDNA copy number, a shift in mitochondrial

morphology, downregulation of OXPHOS enzymes, increased ROS generation, reduced membrane potential and reduced ATP production[46]. Mice heterozygous for a *Suv3* null mutation have a reduced lifespan and increased tumour incidence, both of which are associated with increased mtDNA somatic mutation levels and decreased mtDNA copy number. The reduced lifespan and increased tumorigenesis that is evident in a $Suv3^{+/-}$ mother can be transmitted to her $Suv3^{+/+}$ (wild-type) daughters. Hence, reduced longevity and cancer predilection are the result of *Suv3*-induced maternally transmitted mtDNA alterations[47].

Although functional mitochondria and mtDNAs are essential for cancer cell growth and tumori genesis, mtDNA mutations and/or reductions in mtDNA copy number that alter the OXPHOS physiology are common features of cancer. This implies that alterations in mitochondrial bioenergetics and metabolism have a role in initiating and/or sustaining the transformed state.

Mitochondrial enzyme defects in cancer

In addition to mutations that directly affect mtDNA, mutations in nDNA-encoded mitochondrial enzymes have been found in specific cancers. Analysis of the physiological consequences of these mutations is providing insight into how changes in mitochondrial metabolism can reprogramme the nucleus.

SDH mutations

Homozygous null mutations in the genes of the various SDH subunits have been observed in paragangliomas and pheochromocytomas[48]. SDH (also known as respiratory complex II) is an integral mitochondrial inner membrane protein complex that oxidizes succi-nate to fumarate and transfers two electrons to coenzyme Q_{10} (CoQ) (FIG. 2). SDH is composed of four subunits: SDHA–SDHD. SDHA contains an FAD cofactor that binds the substrates succinate and fumarate; SDHB contains the iron–sulphur centre electron carriers; and SDHC and SDHD constitute the inner membrane cytochrome *b* and form the CoQ binding site. The assembly of SDH requires two factors, SDH assembly factor 1 (SDHAF1) and SDHAF2 (REF. 48). Initial reports of SDH mutations in paragangliomas involved *SDHD*[49], *SDHC*[50] and *SDHB*[51]. Subsequent research revealed that cancerous mutations can also occur in *SDHA* and *SDHAF2*, with SDH defects accounting for 10–30% of sporadic paragangliomas and 10–70% of familial paragangliomas[48].

Inhibition of SDH increases mitochondrial and cytosolic succinate levels, which inhibits α-ketoglutarate-dependent prolyl hydroxylases (PHDs), thus causing stabilization of hypoxia-inducible factor 1α (HIF1α)[52]. The stabilized HIF1α is then translocated into the nucleus[48] and causes a shift in energy metabolism from oxidative to glycolytic[53]. HIF1α may also be stabilized by the inactivation of PHDs by ROS that have been generated by mitochondrial complex III[54–56], as SDH-deficient cells have increased mitochondrial ROS production and oxidative stress[48]. Succinate and fumarate also inhibit other α-ketoglutarate-dependent dioxygenases, including the Jumonji-C histone demethylases (JHDMs) and the TET family of 5-methylcytosine hydroxylases, resulting in genome-wide alterations of histone and DNA methylation and epigenetic dysregulation[48,57].

FH mutation

Homozygous null mutations in the *FH* gene are associated with multiple cutaneous and uterine leiomyomatas and aggressive forms of renal cell cancer[58]. FH converts fumarate to malate, and cells harbouring *FH* mutations produce up to 100-fold more fumarate, sevenfold more succinate, and have a marked decrease in malate and citrate levels[59] (FIG. 2). Fumarate has also been hypothesized to inhibit PHDs and stabilize HIF1α. However, in $Fh^{-/-}$ mice the additional knockout of *Hif1a* increased, rather than decreased, the formation of renal cysts, which are progenitors of papillary renal cell carcinoma. Assuming that the $Fh^{-/-}$ mouse approximates the situation in human tumours, this implies that the stabilization of HIF1α is not required for $FH^{-/-}$ tumorigenesis[60].

Increased fumarate levels have been found to activate the stress response pathway that is regulated by NFE2-related factor 2 (NRF2; also known as NFE2L2). NRF2 is normally kept at low levels though degradation by the kelch-like ECH-associated protein 1 (KEAP1) and the ubiquitin E3 ligase cullin 3 complex. However, excess fumarate can inactivate KEAP1 through succination of cysteines 151 and 288, thereby stabilizing NRF2. NRF2 binds to the antioxidant response elements (AREs) and turns on nDNA stress-response genes, one of which is haem oxygenase 1 (*HMOX1*)[60]. The induction of HMOX1 must be important to tumorigenesis because silencing or inhibiting HMOX1 in $Fh^{-/-}$ cells reduces colony-forming capacity[59].

Inactivation of FH would not only increase fumarate and succinate levels but would also increase succinyl-CoA levels. The induction of HMOX1 could reduce the levels of these metabolites as haem biosynthesis involves combining succinyl-CoA with glycine to generate δ-aminolevulinic acid (ALA) by ALA synthetase (ALAS) to initiate porphyrin synthesis. Porphyrins are converted to haem, which is degraded by HMOX1 to result in the production and excretion of bilirubin (FIG. 2). This raises the question of why might it be beneficial to reduce succinyl-CoA levels? One possibility could be that when succinyl-CoA is converted to succinate by succinyl-CoA synthetase, GTP is generated. GTP hydrolysis is in turn coupled to the conversion of oxaloacetate (OAA) to phosphoenolpyruvate (PEP) by mitochondrial PEP carboxykinase (PEPCK-M), and mitochondrial PEP is exported to the cytosol where it can be converted to pyruvate and ATP[61,62]. If succinyl-CoA were in excess, this reaction could deplete the mitochondrion of OAA that is required for citrate synthesis and lipid biogenesis. Moreover, excess GTP might adversely affect the homeostasis of the mitochondrial nucleotide pool and compromise mtDNA transcription and replication.

IDH1 and IDH2

Heterozygous missense mutations in the two $NADP^+$-dependent IDH enzymes, cytosolic IDH1 and mitochondrial IDH2, have been observed in gliomas, astrocytomas, chondromas and acute myeloid leukaemia (AML). A third IDH, NAD^+-dependent IDH3, is the primary TCA cycle enzyme for decarboxylating isocitrate to α-ketoglutarate (FIG. 2), yet this enzyme has not been found to be mutated in cancer. In gliomas, the common IDH mutations are IDH1-R132, IDH2-R172 (the analogous amino acid to IDH1-R132) and IDH2-R140. IDH2-R140 is also the most common IDH mutation in AML. Additional rare IDH1 and IDH2 mutations have also been reported[63–65].

IDHs are homodimer enzymes, and the cancer cell mutations identified to date create a neomorphic function[66]. Like IDH3, IDH1 and IDH2 can oxidatively decarboxylate isocitrate to α-ketoglutarate. However, IDH1 and IDH2 reduce $NADP^+$ instead of NAD^+, and the $NADP^+$-dependent reaction is reversible because NADPH can provide sufficient energy to drive the reductive carboxylation of α-ketoglutarate to isocitrate (FIG. 4). However, the neomorphic IDH1-R132 and IDH2-R172 mutants use NADPH to reduce α-ketoglutarate to *R*(-)-2-hydroxyglutarate ((*R*)-2HG). As a result, IDH1 and IDH2 mutant cancers produce 10–100-fold increased levels of (*R*)-2HG[64], which has been hypothesized to be an 'oncometabolite'. Contrary to expectations, (*R*)-2HG has been shown to activate PHD1 (also known as EGLN2) and PHD2 (also known as EGLN1) resulting in decreased HIF1α, unlike the *S*-enantiomer, which inhibits PHD1 and PHD2. Furthermore, in IDH1-R132H-transformed astrocytes, knockdown of HIF1α increases cellular proliferation and cloning in soft agar, indicating that in this context HIF1α may act as a tumour suppressor gene[67]. (*R*)-2HG is associated with alterations in cellular genomic methylation and transcription patterns and is a potent inhibitor of the α-ketoglutarate-dependent Jumonji-C domain histone N^{ε}-lysine demethylases (JMJD2A, JMJD2C and JMJD2D (also known as KDM4D)). Hence, (*R*)-2HG may act by altering chromatin modifications[67–69]. As human astrocytes transformed with IDH-R132H exhibit heightened proliferation at confluence after approximately 14 passages[67], this suggests that (*R*)-2HG causes a progressive change in the cellular epigenome. In fact, transduction of mutant *IDH1* results in a rapid increase in trimethylation of histone H3 at lysine 9 (H3K9me3) and a subsequent increase in DNA CpG island methylation[70]. Excessive CpG island methylation (the CpG island methylator phenotype (CIMP)) is seen in a subset of glioblastomas, and CIMP correlates almost perfectly with IDH1 and IDH2 mutations. Glioblastomas with IDH1 and IDH2 mutations and CIMP exhibit striking changes in gene expression profile, with differential methylation of over 3,000 genes. The target genes showing marked changes include transforming growth factor-β (TGFβ), RAS, epidermal growth factor receptor (EGFR), WNT and genes in angiogensis pathways[71]. As the WNT pathway, among others, has been implicated in the regulation of mitochondrial energy metabolism[72], such global changes in chromatin structure could accompany alterations in bioenergetics[53,73–75]. Indeed, rendering cancer cells mtDNA-deficient (ρ^o) also results in alterations in CpG island methylation patterns[76,77].

IDH1 and IDH2 mutations may also alter the cellular redox state. Mitochondria produce NADPH by the transfer of reducing equivalents from NADH to $NADP^+$. This reaction is mediated by the mitochondrial inner membrane enzyme nicotinamide nucleotide transhydrogenase (NNT), with the energy differential between NADH at –250 mV and NADPH at –405 mV being supplied by the mitochondrial inner membrane electrochemical potential (FIG. 4). The importance of the mitochondrial production of NADPH is demonstrated by the multiple metabolic abnormalities that are observed in $Nnt^{-/-}$ mice[78–82]. Whereas NADH is oxidized to produce energy, NADPH is used as a reductant for biosynthetic reactions, the glutathione-based detoxification of peroxides, and the regulation of thiol-disulphide enzymes and transcription factors[53]. Using NNT-generated NADPH, mitochondrial α-ketoglutarate can be reductively carboxylated to isocitrate by IDH2 (REF. 83). Isocitrate can then be converted to citrate by mitochondrial aconitase. Citrate can be

exported to the cytosol where it can be converted to isocitrate by cytosolic aconitase and to α-ketoglutarate by IDH1, generating cytosolic NADPH. α-ketoglutarate or glutamate can then be returned to the mitochondrion to complete the cycle[63,84] (FIG. 4).

As both the mitochondria and the cytosol require NADPH for antioxidant defences, the flux of NADPH from the mitochondrion to the cytosol must be regulated to retain NADPH when the mitochondrion is under increased oxidative stress. This may be accomplished by the acute sensitivity of the iron-sulphur centre of the mitochondrial aconitase to inactivation by oxidative stress[85–87], inactivation of which would stop the cycle and block the export of mitochondrial NADPH (FIG. 4).

In heterozygous *IDH1* and *IDH2* cancer cells the altered IDH activity could generate a futile cycle, which wastes NADPH and α-ketoglutarate. The wild-type IDH1 or IDH2 proteins would generate NADPH from the conversion of isocitrate to α-ketoglutarate but then the mutant IDH1 and IDH2 proteins would expend the NADPH to convert α-ketoglutarate to (*R*)-2HG (FIG. 4). The resulting decrease in NADPH levels would inhibit glutathione peroxidase, thus increasing H_2O_2 levels with heightened signalling effects. Decreased NADPH production would also inhibit the thioredoxins and the bifunctional apurinic/apyrimidinic endonuclease 1 (APE1; also known as redox factor 1 (REF1)), thus perturbing the redox status of crucial transcription factors and favouring proliferation and tumorigenesis.

Recently, a transgenic mouse has been reported in which an IDH1-R132H allele is expressed in haematopoietic cells. The NAD^+, $NADP^+$, NADH and NADPH levels in bone-marrow-derived macrophages of these animals were reported to be unchanged[88]. However, these experiments seem to have been performed on the Jackson Laboratory C57BL/6j background, which is homozygous for deletion of *Nnt*[78,82]. Hence, differential effects on the NADPH/$NADP^+$ ratio may not have been apparent in this experimental system.

Mitochondrial ROS production and redox biology

NADPH is essential for the reduction of mitochondri-ally generated H_2O_2 and other peroxides by glutathione and glutathione peroxidases. When mitochondrial ROS production is too high, it is toxic to the cell and can induce apoptosis or necrosis. However, mitochondrial ROS are also important signalling molecules and potent mitogens[84,89–91], so if apoptosis is inhibited increased ROS production can contribute to neoplastic transformation.

NADPH is also essential for the regulation of the protein function through reduction of thiols (-SH) (FIG 4). For example, when cysteine thiols in the FOS–JUN heterodimer, which constitute an AP-1 transcription factor, are oxidized to sulphenic acids, the affinity of AP-1 for binding sites in target genes is blocked. However, the FOS–JUN sulphenic acids can be reduced by the bifunctional APE1 protein, the reducing equivalents from NADPH being transmitted through the nuclear–cytosolic thioredoxin 1 (TRX1). APE1 also regulates NRF2, nuclear factor-κB (NF-κB), p53, glucocorticoid receptor (GR), oestrogen receptor (ER) and HIF1α by reducing cysteine residues in these proteins that are required for DNA

binding[92–95]. The mitochondrion has its own thioredoxin, TRX2, and also imports APE1, so protein redox regulation is also crucial for mitochondrial function[84,96–98] (FIG. 4).

Mitochondrial ROS production and redox balance are modulated by the mitochondrial inner membrane electrochemical gradient. The mitochondrial H^+-translocating ATP synthase (known as ATP synthase or complex V) uses the proton gradient to produce ATP from ADP and inorganic phosphate (P_i) (FIGS 2,3). Inhibition of the ATP synthase by oligomycin results in the maximization of the mitochondrial inner membrane polarization, which stalls the electron transport chain (ETC) and reduces the electron carriers in complexes I and III and CoQ. The excess electrons can then be transferred directly to O_2 to generate superoxide ($O_2^{\bullet-}$). Mitochondrial matrix superoxide can be dismutated into H_2O_2 by Mn superoxide dismutase (MnSOD; encoded by *SOD2*) while intermembrane-space $O_2^{\bullet-}$, which is primarily generated by complex III[15,99], is dismutated by intermembrane and cytosolic Cu/ZnSOD (also known as SOD1) (FIG. 3). H_2O_2 at increased levels then functions as a diffusible signalling molecule[53,84].

Inhibition of the ATP synthase has been observed in various carcinomas. This can result from increased levels of the ATP synthase inhibitor protein IF1[100] or inhibition of the transcription or translation of the ATP synthase catalytic β-subunit[5,101]. Knockdown of the increased levels of IF1 in carcinomas results in a decline in glycolysis and an increase in OXPHOS[100]. As observed with oligo mycin, inhibition of the ATP synthase increases the mitochondrial membrane potential, stalls the ETC and increases ROS production.

Alterations in bioenergetic metabolism in cancer

The PI3K pathway

The shift from oxidative to more glycolytic metabolism commonly involves activation of the PI3K–PTEN–AKT signal transduction pathway, which is one of the most common changes found in cancer[102]. PI3K–PTEN–AKT pathway activation increases the cell surface expression of transporters for glucose and other nutrients, enhances the expression of glycolytic and lipogenesis genes, stimulates hexokinase and phosphofructokinase to drive glycolysis, and activates mTOR. Consistent with the importance of the PI3K–PTEN–AKT pathway in cancer, cancer cells use not only high levels of glucose but also convert over 90% of the resulting pyruvate into lactate by lactate dehydrogenase A (LDHA)[102,103].

The PI3K–PTEN–AKT pathway also inhibits expression of carnitine palmitoyltransferase IA, the rate limiting step for fatty acid oxidation[104], and phosphorylates hexokinase II, enabling it to bind to the mitochondrial outer membrane voltage-dependent anion channel (VDAC; also known as porin) (FIG. 3). There, hexokinase II captures mitochondrial ATP and uses it to phosphorylate glucose to glucose-6-phosphate, thus driving carbon flow into glycolysis or the pentose phosphate pathway[105,106].

In *Caenorhabditis elegans*, insulin-like ligands bind to the DAF-2 receptor (the insulin and insulin-like growth factor 1 receptor analogue), which activates AKT. AKT phosphorylates DAF-16 (the FOXO analogue), resulting in the exclusion of DAF-16 from the nucleus. Inactivation of DAF-2 permits DAF-16 to enter the nucleus and upregulate antioxidant and

stress defences, thus extending lifespan[107,108]. In mammals, there are four FOXO proteins (FOXO1 (also known as FKBR), FOXO3A, FOXO4 and FOXO6) that bind to insulin-response elements (IREs). The mitochondrial transcriptional co-activator, peroxisome proliferation-activated receptor-γ (PPARγ)-co-activator 1α (PGC1α; encoded by *PPARGC1A*), has three IREs. These are bound by unphosphorylated FOXO1 and this increases *PPARGC1A* transcription[53,109]. Expression of PGC1α induces many nDNA-encoded mitochondrial genes, including those for OXPHOS and antioxidant defences[110]. Therefore, activation of the PI3K–PTEN–AKT pathway should remove the FOXOs from the nucleus and downregulate mitochondrial function and antioxidant defences. However, overexpression of FOXO3A has been reported to downregulate mitochondrial function[111]. This occurs through the induction of MAX-interacting protein 1 (MXI1), which inhibits the transcriptional activity of MYC by binding the MYC partner protein MAX. As MYC can induce mitochondrial biogenesis by promoting the transcription of relevant genes[112], FOXO3A activation must decrease mitochondrial OXPHOS. FOXO3A has also been shown to induce *SOD2,* which can decrease mitochondrial ROS production. Furthermore, FOXO3A lowered ROS levels in the presence of hypoxia and prevented the stabilization of HIF1α[111]. As HIF1 can repress mitochondrial biogenesis, its degradation would be expected to increase mitochondrial biogenesis. Hence, the bioenergetic effects of FOXO inactivation are complex and must be context-specific.

Although glycolysis can rapidly produce ATP under low oxygen tension, it is also important for generating the precursors and reducing equivalents for cellular biogenesis and antioxidant defences[102]. In glycolysis, pyruvate kinase (PK), which converts PEP to pyruvate, is the rate-limiting step for pyruvate synthesis. In cancer cells it is frequently argued that the common tissue isoform PKM1 is switched to PKM2. However, a recent proteomic analysis concluded that both PKM1 and PKM2 are expressed in normal and cancerous tissues, and that PKM2 is consistently the predominant isoform[113]. PKM2 can be inhibited by Tyr105 phosphorylation by various tyrosine kinases including fibroblast growth factor receptor type 1. Phosphorylation disrupts the active tetramer form, thus releasing the cofactor fructose-1,6-bisphosphate[114]. PKM2 can also be inhibited by oxidative stress through modification of Cys358 (REF. 115). Inhibition of PKM2 redirects carbon from oxidation to the anabolic pathways that emanate from glucose-6-phosphate. These include the pentose phosphate pathway, which generates both ribose-5-phosphate for nucleotide synthesis and NADPH for redox control, and glycolysis, which generates glycerol-3-phosphate for lipid synthesis[116]. When oxidative stress is low, PKM2 is active and glucose is metabolized to pyruvate and lactate to generate ATP, but when oxidative stress is high PMK2 is inactive and glucose- 6-phosphate is shunted into the pentose phosphate pathway to generate NADPH for antioxidant defences[117].

Acetyl-CoA regulation

Mitochondrial and cytosolic acetyl-CoA can be hydrolysed by the mitochondria to generate ATP. Acetyl-CoA is also required for cytosolic lipid synthesis[104] and is the co-reactant in the acetylation of proteins that modulate the signal transduction pathways and epigenome[53]. Much of the cellular acetyl-CoA is generated within the mitochondrion from pyruvate and fatty acid oxidation. Cytosolic pyruvate is imported into the mitochondrion by the pyruvate

carrier[118] and converted to acetyl-CoA by mitochondrial pyruvate dehydrogenase (PDH). PDH is inactivated through phosphorylation by PDH kinase (PDHK) and is reactivated by PDH phosphatases. When reducing equivalents in the ETC are limited, the mitochondrial inter membrane space contains a signalosome complex encompassing protein kinase Cδ (PKCδ), retinol, cytochrome *c* and $p66^{SHC}$ that increases acetyl-CoA flux into the mitochondrion from pyruvate by inhibiting PDHK2 (REF. 119).

Acetyl-CoA is also generated within the mitochondrion by the oxidation of fatty acids. Fatty acyl-CoA molecules are imported into the mitochondrion via carnitine: the transfer from CoA to carnitine is mediated by carnitine palmitoyltransferases. The carnitine palmitoyltransferase isoform 1C is upregulated in lung cancer, indicating the importance of mitochondrial energy and precursor production for this type of cancer cell[120].

Cytosolic acetyl-CoA is essential for fatty acid bio-genesis. One important source of cytosolic acetyl-CoA is the export from the mitochondrion of citrate. In the cytosol citrate is then cleaved by the cytosolic ATP-dependent lyase to OAA and acetyl-CoA[104]. The acetyl-CoA can then be used to synthesize fatty acids, sphingomyelin, cholesterol and isoprenoids[102,119,121], and can also be used in other acetylation reactions.

MYC and glutaminolysis

Citrate production requires not only acetyl-CoA but also OAA. However, the appropriation of citrate for macromolecular synthesis depletes mitochondrial OAA, thus impeding the TCA cycle. This deficiency can be compensated by the expression of the *MYC* oncogene, which induces glutaminolysis to feed α-ketoglutarate into the TCA cycle to regenerate OAA, a process known as anaplerosis[121]. Increased expression levels of MYC are seen in many aggressive tumours and have been found to upregulate mitochondrial oxygen consumption and mitochondrial biogenesis. This is associated with transcriptional induction of *TFAM*, as well as the genes for subunits of complex I, uncoupling proteins, mitochondrial membrane proteins and genes involved in intermediary metabolism[112].

MYC upregulation of mitochondrial function is associated with induction of glutaminase, which converts glutamine to glutamate. MYC also inhibits the expression of the microRNAs miR-23a and miR-23b, which are translational inhibitors of glutaminase. Glutamate is converted to α-ketoglutarate by mitochondrial glutamate dehydrogenase, and α-ketoglutarate progresses around the TCA cycle to OAA[103,121,122]. Hence, MYC activation of mitochondrial function and glutaminolysis renders cancer cells glutamine-dependent[122].

p53 and mitochondria

The tumour suppressor p53 (encoded by *TP53*) can mediate growth arrest and initiate apoptosis[102]. However, it can also be phosphorylated by AMP-activated protein kinase (AMPK) in response to energy limitation, thus activating cell cycle checkpoints. p53 also favours ATP production by OXPHOS and the decrease of cellular ROS production by inducing TP53-induced glycolysis and apoptosis regulator (TIGAR). TIGAR negatively regulates glycolysis by degrading fructose-2,6-bisphosphate, which is an allosteric activator of phosphofructokinase 1 (REF. 102). This shifts carbon flux away from glycolysis and into

the pentose phosphate pathway, which increases NADPH production and thus heightens antioxidant defences[123,124]. p53 also negatively regulates phosphoglycerate mutase and AKT, thus further inhibiting glycolysis and upregulating OXPHOS complex IV by the induction of the cytochrome *c* oxidase (COX) Cu^{2+} chaperone, SCO2 (REF. 125). Because the inhibition of glycolysis can redirect glucose-6-phosphate into the pentose phosphate pathway, this could increase antioxidant defences in conjunction with increased OXPHOS. Thus, the inactivation of p53 should decrease OXPHOS in favour of glycolysis, increase ROS production and inhibit apoptosis.

In other contexts, p53 activation can also induce cellular senescence. Excessive shortening of chromosomal telomeres activates p53, which then inhibits the transcription of PGC1α and PGC1β. This results in diminished mitochondrial function, increased ROS levels and senescence[126].

HIF and mitochondria

The transcription factor HIF1 induces glycolysis under low oxygen tension through the upregulation of genes encoding glucose transporters, glycolytic proteins and angiogenic factors (such as erythropoietin and vascular endothelial growth factor (VEGF)), and the inhibition of mitochondrial function. HIF1 affects mitochondria by various mechanisms: it induces PDHK1, thus inhibiting PDH and retarding the conversion of pyruvate to mitochondrial acetyl-CoA; it induces the low oxygen tension subunit of complex IV, COX4-2; it upregulates the mitochondrial LON protease to degrade the normoxic subunit, COX4-1; it activates mitophagy to degrade existing mitochondria; and it inhibits MYC signalling[127–131]. HIF1 also upregulates the transcription of miR-210, which downregulates mitochondrial metabolism by inhibiting expression of the *ISCU1* and *ISCU2* genes — which encode proteins involved in iron sulphur centre synthesis — and genes for subunits of complex I (*NDUFA4*), complex II (*SDHD*) and complex IV (*COX10*)[132].

Mitochondria also regulate HIF1. Mitochondrial ROS from complex III has been shown to inactivate PHD2 and thus to stabilize HIF1α[54,99]. Mitochondrial sirtuin 3 (SIRT3) also modulates HIF1α through mitochondrially generated ROS[133]. Additionally, mitochondria can stabilize HIF1α through the action of the coiled-coil–helix–coiled-coil–helix (CHCH) domain protein CHCHD4 isoforms, which are part of the mitochondrial protein disulphide relay system involving cytochrome *c* and complex IV[134].

Finally, HIF1 mediates the transcription of PKM2, but not PKM1, and PKM2 also serves as a co-transcriptional activator of HIF1. This is mediated by PHD3, which hydroxylates prolines 403 and 408 of PKM2, thus enhancing the binding of PKM2 to HIF1α[135,136].

Calcium, mitochondria and apoptosis

Mitochondrial physiology is strongly regulated by Ca^{2+} (REFS 137,138). Mitochondria import Ca^{2+} through the Ca^{2+} uniporter, which is energized by an electrochemical gradient. Mitochondrially associated endoplasmic reticulum membranes (MAMs) bring the endoplasmic reticulum type 3 inositol triphosphate receptor (IP3R) Ca^{2+} release channels into juxtaposition with the mitochondrial Ca^{2+} uniporter[139–141]. The promyelocytic

leukaemia (PML) protein resides in MAMs, and its inactivation leads to the AKT-mediated hyperphosphorylation of the IP3R3 channels and reduced Ca^{2+} flux[141]. Because excessive Ca^{2+} uptake by mitochondria can activate the mtPTP and initiate apoptosis[142–144], inactivation of PML may limit apoptosis and increase cancer cell survival.

Mitochondrial retrograde signalling

As mitochondrial gene mutations are common in cancer, but mtDNA-encoded OXPHOS genes are essential for cancer cell survival and growth, mutations in mitochondrial genes must therefore be altering cellular bioenergetics and metabolism in ways that are important for neoplastic transformation. For SDH defects this involves the activation of HIF1 signalling; for FH defects, alteration of NRF2 signalling; for IDH1 and IDH2 mutations, alteration of redox signalling; and for all four genes, alteration of chromatin methylation and of the epigenome. Thus, mitochondrial cancer mutations must serve to reprogramme the nucleus, a process called retrograde signalling.

The importance of retrograde signalling has been clearly demonstrated for changes in mitochondrial Ca^{2+} signalling and in mitochondrial ROS production and redox control. The importance of mitochondrial Ca^{2+} regulation in retrograde signalling has been shown by transiently treating cells, such as mouse myoblast C2C12 cells, with either ethidium bromide (to reduce the mtDNA content 50–80%) or with the mitochondrial uncoupler carbonyl cyanide m-chlorophenylhydrazone (CCCP). Both treatments decrease the mitochondrial inner membrane electrochemical potential. These treatments convert the C2C12 cells from being nontumorigenic to having invasive growth patterns in transplantation experiments[145]. A decrease in the mitochondrial membrane potential reduces the energetic drive for mitochondria to import Ca^{2+}, thus increasing the cytosolic Ca^{2+} concentration. Cytosolic Ca^{2+} activates calcineurin, which activates IκBβ-dependent NF-κB to cause the nuclear translocation of the REL–p50 heterodimer. REL–p50 then associates with C/EBPδ, CREB and NFAT in a nuclear enhance-osome[146]. Calcineurin also activates insulin-like growth factor 1 receptor (IGF1R), which acts through PI3K to activate AKT. Phospho-AKT enters the nucleus where it phosphorylates and activates heterogeneous nuclear ribonucleoprotein A2 (HNRNPA2), which acts as a transcriptional co-activator of the enhanceosome. These changes result in the transcriptional upregulation of over 120 nDNA genes. Notably, affected genes include those for maintaining Ca^{2+} regulation and homeostasis (such as ryanodine receptor 1 (RYR1), calsequestrin and calreticulin), glucose metabolism (such as IGF1R, insulin receptor substrate 1 (IRS1), GLUT4 and hexokinase), apoptosis (such as AKT1, PI3K, BCL-X_S, BID, BAD and BAX), and tissue invasiveness and tumorigenesis (such as cathepsin L, AKT1, transforming growth factor-β (TGFβ), p53 and mouse melanoma antigen)[147–150].

The importance of ROS and redox signalling in cancer has been demonstrated for HIF1α stabilization[54], FOS–JUN activation[93], and activation of proliferation through regulation of the ERK1 and ERK2 MAPK pathway[15]. Direct evidence that mitochondrial ROS are important in neoplastic transformation has come from the analysis of mtDNA mutations found in cultured cancer cells. For example, analyses of mtDNA in mouse L cells has shown that each cell harbours a functional mtDNA mutation and that these mutated mtDNAs

increase mitochondrial ROS production and cellular proliferation[151]. The mtDNA mutation *ATP6* T8993G — which is found in patients with neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP) and Leigh syndrome — results in increased ROS production and tumorigenicity when expressed in the prostate cancer cell line PC3 and HeLa cells. The growth stimulation of the ATP6 mutation can be inhibited by targeting normal ATP6 protein into the mutant mitochondria through allotropic gene expression[29,30]. A human cell line with an *ND5* frameshift mutation and low ROS production had low tumorigenicity, but when the frameshift mtDNA was mixed with wild-type mtDNA ROS production and tumorigenicity increased[152]. Over 30 years ago, it was observed that the fusion of a nontumorigenic hamster cytoplast (an enucleated cell) with a more cancerous hamster cell could partially suppress tumorigenicity[28]. Cytoplasts harbouring mtDNAs with either an *ND6* missense or frameshift mutation produce high ROS levels in association with high tumorigenicity when fused with cells with low tumorigenicity, indicating that the constituent mitochondria can transfer these tumorigenic traits. The high tumorigenicity of the original tumour cells and their derived cytoplasmic hybrids (cybrids) is associated with increased HIF1α and MCL1 expression[31]. KRAS-transformed cells produce increased ROS from mitochondrial complex III and this is required for maintaining anchorage-independent growth. These effects of KRAS are inhibited in ρ^o cells, in cells in which TFAM has been knocked down, and in cells treated with mitochondri-ally targeted antioxidants, all of which inhibit mitochondrial ROS production. Carbon flow through glycolysis is also diminished in these cells, which redirect the carbon into the pentose phosphate pathway to generate NADPH and ribose[15]. Although ROS are required for anchorage-independent growth, it follows that chronically increased mitochondrial ROS levels would severely damage mitochondria and mtDNAs. Perhaps to compensate for this effect, RAS-transformed cancer cells are addicted to autophagy, and inhibition of this function results in a decline of mitochondrial function[153]. Proof of the importance of mitochondrial ROS production comes from the suppression of tumorigenesis by the genetic introduction of a mitochondrially targeted catalase (mCAT) into mice harbouring the polyoma middle T oncoprotein[154] or the $Apc^{Min/+}$;$Tfam^{+/-}$ genotype[155]. Catalase is a peroxisomal enzyme that rapidly degrades H_2O_2 to O_2 and H_2O. In the *mCAT* transgene the catalase cDNA is modified to remove the carboxy-terminal peroxisomal-targeting sequence, which is replaced by an amino-terminal mitochondrial targeting sequence[156]. Hence, removal of mitochondrial H_2O_2 can inhibit nDNA-oncogene-induced tumorigenesis.

The reverse Warburg effect

From the discussions above, it is clear that different types of tumours undergo different bioenergetic adjustments; tumours do not consistently inhibit mitochondrial bioenergetics as suggested by Warburg. Indeed, one study of the energy budget of various cancer cells under normoxic conditions concluded that most of the ATP in cancer cells comes from mitochondria, as is true for normal cells[157]. If this is the case, then why can cancers be detected using FDG accumulation[4]? The answer may lie with the interaction between the tumour cells and the adjacent stromal cells.

It has been observed that stromal fibroblasts that are associated with breast cancer epithelial cells are glycolytic. The conversion of these fibroblasts from oxidative to glycolytic

metabolism is induced by the H_2O_2 that is secreted from the adjacent cancer cells[158]. This results in loss of caveolin 1 from the fibroblasts, and this in turn causes induction of mitophagy. Mitophagy reduces the number of mitochondria in the stromal fibroblasts, thus reducing mitochondrial function and switching the fibroblast metabolism over to glycolysis. The increased glycolysis due to mitochondrial turnover generates excessive stromal cell lactate and ketones, which are secreted into the intracellular space. The cancer cells then take up the lactate and ketones and use them to feed cancer cell mitochondrial energy production and to generate mitochondrial precursors for cancer cell biogenesis. This metabolic coupling between breast cancer epithelial cells and the stromal fibroblasts renders much of the surrounding tissue glycolytic, which would enhance an FDG signal. This intercellular cooperation has been designated ‘stromal–epithelial metabolic coupling’ or the ‘reverse Warburg effect’ and is analogous to the ‘neuron–glia metabolic coupling’ that is seen in normal brain tissue and in brain tissue from patients with Alzheimer's disease[158–162].

Conclusion

The findings discussed throughout this Review indicate that mitochondrial function is essential for cancer cells. However, different cancer cell types undergo different bioenergetic alterations, some to more glycolytic and others to more oxidative, depending in part on the developmental state of the cell undergoing neoplastic transformation. This tissue-specific contextual basis of cancer precludes there being a single bioenergetic transition that is common to all cancer cell types as proposed by Warburg. The resulting diversity probably explains why HIF1α can either be a tumour promoter or suppressor, FOXO and MYC can either increase or decrease mitochondrial biogenesis, and p53 can either enhance or inhibit OXPHOS in cancer.

A primary reason for this somewhat confusing picture could be our lack of knowledge of basic mitochondrial biology and genetics, investigations that have been severely constrained over the past 30 years. As a result, our understanding is quite immature regarding various aspects of mitochondrial biology. These include: the cataloguing of proteins that reside in mitochondria[163]; the regulation of the nuclear-coded mitochondrial genes and the relation of bioenergetics to the epigenome[53]; the mechanisms by which mitochondrial redox and Ca^{2+} are regulated and their effect on the nucleus and cytosol[53,84]; the characterization of mitochondrial biogenesis within the mitochondrion[164]; the consequences of mtDNA heteroplasmy and mtDNA–nDNA interactions[108]; the definition of systems for tissue-specific bioenergetic regulation[53,84,165]; and the elucidation of the interactions between the cancer cell and the stromal tissue milieu[158–162]. Progress in all of these areas will require moving cancer studies from isolated cancer cells and tumours into genetically engineered animals in which intercellular interactions can be explored. This will necessitate the introduction of a wide range of mitochondrial gene mutations, particularly mtDNA mutations, into mice[47,166,167]. Using such mice, different combinations of mtDNA mutations and nuclear oncogene and tumour suppressor mutations can be generated and studied. Therefore, to move forward we must expand our understanding of the biology and genetics of mitochondria, the neglected cellular symbiont that is so central to our cells, our health and our lives[37].

Acknowledgements

The author would like to thank L. Adang and M. Lott for their assistance in preparing this manuscript. This work was supported by the US National Institutes of Health (NIH) grants NS21328, NS070298, AG24373 and DK73691, and a Simons Foundation Grant 205844.

References

1. Warburg O. Smith RR. The Metabolism of Tumors. 1931
2. Warburg O. On the origin of cancer cells. Science. 1956; 123:309–314. [PubMed: 13298683]
3. Wallace DC. Mitochondria and cancer: Warburg addressed. Cold Spring Harb. Symp. Quant. Biol. 2005; 70:363–374. [PubMed: 16869773]
4. Lin M. Molecular imaging using positron emission tomography in colorectal cancer. Discov. Med. 2011; 11:435–447. [PubMed: 21616042]
5. Pedersen PL. Tumor mitochondria and the bioenergetics of cancer cells. Prog. Exp. Tumor Res. 1978; 22:190–274. [PubMed: 149996]
6. Lane N, Martin W. The energetics of genome complexity. Nature. 2010; 467:929–934. [PubMed: 20962839]
7. Wallace DC. Why do we have a maternally inherited mitochondrial DNA? Insights from evolutionary medicine. Annu. Rev. Biochem. 2007; 76:781–821. [PubMed: 17506638]
8. Horton TM, et al. Novel mitochondrial DNA deletion found in a renal cell carcinoma. Genes Chromosomes Cancer. 1996; 15:95–101. [PubMed: 8834172]
9. Desjardins P, Frost E, Morais R. Ethidium bromide-induced loss of mitochondrial DNA from primary chicken embryo fibroblasts. Mol. Cell. Biol. 1985; 5:1163–1169. [PubMed: 2987677]
10. Desjardins P, de Muys JM, Morais R. An established avian fibroblast cell line without mitochondrial DNA. Somat. Cell Genet. 1986; 12:133–139.
11. King MP, Attardi G. Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. Science. 1989; 246:500–503. [PubMed: 2814477]
12. Magda D, et al. mtDNA depletion confers specific gene expression profiles in human cells grown in culture and in xenograft. BMC Genomics. 2008; 9:521. [PubMed: 18980691]
13. Morais R, et al. Tumor-forming ability in athymic nude mice of human cell lines devoid of mitochondrial DNA. Cancer Res. 1994; 54:3889–3896. [PubMed: 8033112]
14. Cavalli LR, Varella-Garcia M, Liang BC. Diminished tumorigenic phenotype after depletion of mitochondrial DNA. Cell Growth Differ. 1997; 8:1189–1198. [PubMed: 9372242]
15. Weinberg F, et al. Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. Proc. Natl Acad. Sci. USA. 2010; 107:8788–8793. [PubMed: 20421486]
16. Murgia M, Giorgi C, Pinton P, Rizzuto R. Controlling metabolism and cell death: at the heart of mitochondrial calcium signalling. J. Mol. Cell. Cardiol. 2009; 46:781–788. [PubMed: 19285982]
17. Aanen DK, Maas MF. Recruitment of healthy mitochondria fuels transmissible cancers. Trends Genet. 2012; 28:1–6. [PubMed: 22030339]
18. Rebbeck CA, Leroi AM, Burt A. Mitochondrial capture by a transmissible cancer. Science. 2011; 331:303. [PubMed: 21252340]
19. Brandon M, Baldi P, Wallace DC. Mitochondrial mutations in cancer. Oncogene. 2006; 25:4647–4662. [PubMed: 16892079]
20. Chinnery PF, Samuels DC, Elson J, Turnbull DM. Accumulation of mitochondrial DNA mutations in ageing, cancer, and mitochondrial disease: is there a common mechanism? Lancet. 2002; 360:1323–1325. [PubMed: 12414225]
21. Copeland WC, Wachsman JT, Johnson FM, Penta JS. Mitochondrial DNA alterations in cancer. Cancer Invest. 2002; 20:557–569. [PubMed: 12094550]
22. Gasparre G, et al. Clonal expansion of mutated mitochondrial DNA is associated with tumor formation and complex I deficiency in the benign renal oncocytoma. Hum. Mol. Genet. 2008; 17:986–995. [PubMed: 18156159]

23. Bartoletti-Stella A, et al. Mitochondrial DNA mutations in oncocytic adnexal lacrimal glands of the conjunctiva. Arch. Ophthalmol. 2011; 129:664–666. [PubMed: 21555623]
24. Pereira L, Soares P, Maximo V, Samuels DC. Somatic mitochondrial DNA mutations in cancer escape purifying selection and high pathogenicity mutations lead to the oncocytic phenotype: pathogenicity analysis of reported somatic mtDNA mutations in tumors. BMC Cancer. 2012; 12:53. [PubMed: 22299657]
25. Salas A, et al. A critical reassessment of the role of mitochondria in tumorigenesis. PLoS Med. 2005; 2:e296. [PubMed: 16187796]
26. Meierhofer D, et al. Mitochondrial DNA mutations in renal cell carcinomas revealed no general impact on energy metabolism. Br. J. Cancer. 2006; 94:268–274. [PubMed: 16404428]
27. Czarnecka AM, et al. Molecular oncology focus - is carcinogenesis a 'mitochondriopathy'? J. Biomed. Sci. 2010; 17:31. [PubMed: 20416110]
28. Howell AN, Sager R. Tumorigenicity and its suppression in cybrids of mouse and Chinese hamster cell lines. Proc. Natl Acad. Sci. USA. 1978; 75:2358–2362. [PubMed: 276880]
29. Petros JA, et al. mtDNA mutations increase tumorigenicity in prostate cancer. Proc. Natl Acad. Sci. USA. 2005; 102:719–724. [PubMed: 15647368] [A demonstration that human mtDNA mutations that increase ROS production enhance tumorigenesis, whereas normal mtDNAs suppress tumorigenesis.]
30. Shidara Y, et al. Positive contribution of pathogenic mutations in the mitochondrial genome to the promotion of cancer by prevention from apoptosis. Cancer Res. 2005; 65:1655–1663. [PubMed: 15753359]
31. Ishikawa K, et al. ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science. 2008; 320:661–664. [PubMed: 18388260] [This study shows that mouse mtDNA mutations that increase mitochondrial ROS levels also increase tumorigenesis.]
32. Canter JA, Kallianpur AR, Parl FF, Millikan RC. Mitochondrial DNA G10398A polymorphism and invasive breast cancer in African-American women. Cancer Res. 2005; 65:8028–8033. [PubMed: 16140977]
33. Liu VW, et al. Mitochondrial DNA variant 16189T>C is associated with susceptibility to endometrial cancer. Hum. Mut. 2003; 22:173–174. [PubMed: 12872259]
34. Permuth-Wey J, et al. Inherited variants in mitochondrial biogenesis genes may influence epithelial ovarian cancer risk. Cancer Epidemiol. Biomarkers Prev. 2011; 20:1131–1145. [PubMed: 21447778]
35. Zhang J, et al. Strikingly higher frequency in centenarians and twins of mtDNA mutation causing remodeling of replication origin in leukocytes. Proc. Natl Acad. Sci. USA. 2003; 100:1116–1121. [PubMed: 12538859]
36. Zhai K, Chang L, Zhang Q, Liu B, Wu Y. Mitochondrial C150T polymorphism increases the risk of cervical cancer and HPV infection. Mitochondrion. 2011; 11:559–563. [PubMed: 21385627]
37. Wallace DC. Bioenergetic origins of complexity and diseases. Cold Spring Harb. Symp. Quant. Biol. 2011; 76:1–16. [PubMed: 22194359]
38. Ruiz-Pesini E, Wallace DC. Evidence for adaptive selection acting on the tRNA and rRNA genes of the human mitochondrial DNA. Hum. Mut. 2006; 27:1072–1081. [PubMed: 16947981]
39. Mishmar D, et al. Natural selection shaped regional mtDNA variation in humans. Proc. Natl Acad. Sci. USA. 2003; 100:171–176. [PubMed: 12509511]
40. Wallace DC. Colloquium paper: bioenergetics, the origins of complexity, and the ascent of man. Proc. Natl Acad. Sci. USA. 2010; 107:8947–8953. [PubMed: 20445102]
41. Parrella P, et al. Detection of mitochondrial DNA mutations in primary breast cancer and fine-needle aspirates. Cancer Res. 2001; 61:7623–7626. [PubMed: 11606403]
42. Guo J, et al. Frequent truncating mutation of TFAM induces mitochondrial DNA depletion and apoptotic resistance in microsatellite-unstable colorectal cancer. Cancer Res. 2011; 71:2978–2987. [PubMed: 21467167]
43. Han B, et al. Human mitochondrial transcription factor A functions in both nuclei and mitochondria and regulates cancer cell growth. Biochem. Biophys. Res. Commun. 2011; 408:45–51. [PubMed: 21453679]

44. Bogenhagen DF, Rousseau D, Burke S. The layered structure of human mitochondrial DNA nucleoids. J. Biol. Chem. 2008; 283:3665–3675. [PubMed: 18063578]
45. Bogenhagen DF. Mitochondrial DNA nucleoid structure. Biochim. Biophys. Acta. 2011; 1819:914–920. [PubMed: 22142616]
46. Khidr L, et al. Role of SUV3 helicase in maintaining mitochondrial homeostasis in human cells. J. Biol. Chem. 2008; 283:27064–27073. [PubMed: 18678873]
47. Chen P-L, et al. Mitochondrial genome instability resulting from SUV3 haploinsufficiency leads to tumorigenesis and shortened lifespan. Oncogene. May 7.2012 doi:10.1038/onc.2012.120.
48. Bardella C, Pollard PJ, Tomlinson I. SDH mutations in cancer. Biochim. Biophys. Acta. 2011; 1807:1432–1443. [PubMed: 21771581]
49. Baysal BE, et al. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science. 2000; 287:848–851. [PubMed: 10657297] [The first report that inactivation of SDHD can cause paragangliosis.]
50. Niemann S, Muller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. Nature Genet. 2000; 26:268–270. [PubMed: 11062460]
51. Astuti D, et al. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am. J. Hum. Genet. 2001; 69:49–54. [PubMed: 11404820]
52. Kurelac I, Romeo G, Gasparre G. Mitochondrial metabolism and cancer. Mitochondrion. 2011; 11:635–637. [PubMed: 21447406]
53. Wallace DC, Fan W. Energetics, epigenetics, mitochondrial genetics. Mitochondrion. 2010; 10:12–31. [PubMed: 19796712]
54. Chandel NS, et al. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1α during hypoxia: a mechanism of O2 sensing. J. Biol. Chem. 2000; 275:25130–25138. [PubMed: 10833514] [Evidence that increased mitochondrial ROS levels can inactivate PHDs and activate HIF1α.]
55. Guzy RD, Sharma B, Bell E, Chandel NS, Schumacker PT. Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. Mol. Cell. Biochem. 2008; 28:718–731.
56. Selak MA, Duran RV, Gottlieb E. Redox stress is not essential for the pseudo-hypoxic phenotype of succinate dehydrogenase deficient cells. Biochim. Biophys. Acta. 2006; 1757:567–572. [PubMed: 16797480]
57. Xiao M, et al. Inhibition of α-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. Genes Dev. 2012; 26:1326–1338. [PubMed: 22677546] [A demonstration that excessive dicarboxylic acids from SDH and FH inactivation inhibit α-ketoglutarate-dependent dioxygenases, thus altering chromatin structure and gene expression.]
58. Picaud S, et al. Structural basis of fumarate hydratase deficiency. J. Inherit. Metab. Dis. 2011; 34:671–676. [PubMed: 21445611]
59. Frezza C, et al. Haem oxygenase is synthetically lethal with the tumour suppressor fumarate hydratase. Nature. 2011; 477:225–228. [PubMed: 21849978] [A report that increased fumarate levels activates the NRF2 stress response, inducing HMOX1 and haem degradation.]
60. Adam J, et al. Renal cyst formation in Fh1-deficient mice is independent of the Hif/Phd pathway: roles for fumarate in KEAP1 succination and Nrf2 signaling. Cancer Cell. 2011; 20:524–537. [PubMed: 22014577]
61. Stark R, et al. Phosphoenolpyruvate cycling via mitochondrial phosphoenolpyruvate carboxykinase links anaplerosis and mitochondrial GTP with insulin secretion. J. Biol. Chem. 2009; 284:26578–26590. [PubMed: 19635791]
62. Kibbey RG, et al. Mitochondrial GTP regulates glucose-stimulated insulin secretion. Cell Metab. 2007; 5:253–264. [PubMed: 17403370]
63. Thompson CB. Metabolic enzymes as oncogenes or tumor suppressors. New Engl. J. Med. 2009; 360:813–815. [PubMed: 19228626]

64. Ward PS, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting α-ketoglutarate to 2-hydroxyglutarate. Cancer Cell. 2010; 17:225–234. [PubMed: 20171147]
65. Ward PS, et al. Identification of additional IDH mutations associated with oncometabolite R(–)-2-hydroxyglutarate production. Oncogene. 2012; 31:2491–2498. [PubMed: 21996744]
66. Dang L, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature. 2009; 462:739–744. [PubMed: 19935646] [This study reports that heterozygous IDH1mutation creates a neomorphic enzyme that generates the novel metabolite (R)-2HG.]
67. Koivunen P, et al. Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. Nature. 2012; 483:484–488. [PubMed: 22343896] [This study shows that in contrast to succinate and fumarate, (R)-2HG does not inactivate PHDs and activate HIF1α, implying that tumorigenesis involves an alternative pathway.]
68. Figueroa ME, et al. DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. Cancer Cell. 2010; 17:13–27. [PubMed: 20060365]
69. Chowdhury R, et al. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. EMBO Rep. 2011; 12:463–469. [PubMed: 21460794]
70. Lu C, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature. 2012; 483:474–478. [PubMed: 22343901]
71. Turcan S, et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature. 2012; 483:479–483. [PubMed: 22343889]
72. Yoon JC, et al. Wnt signaling regulates mitochondrial physiology and insulin sensitivity. Genes Dev. 2010; 24:1507–1518. [PubMed: 20634317]
73. Bjornsson HT, et al. Epigenetic specificity of loss of imprinting of the IGF2 gene in Wilms tumors. J. Natl Cancer Inst. 2007; 99:1270–1273. [PubMed: 17686827]
74. Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. Nature. 2007; 447:433–440. [PubMed: 17522677]
75. Feinberg AP. Epigenetics at the epicenter of modern medicine. JAMA. 2008; 299:1345–1350. [PubMed: 18349095]
76. Smiraglia DJ, Kulawiec M, Bistulfi GL, Gupta SG, Singh KK. A novel role for mitochondria in regulating epigenetic modification in the nucleus. Cancer Biol. Ther. 2008; 7:1182–1190. [PubMed: 18458531]
77. Naviaux RK. Mitochondrial control of epigenetics. Cancer Biol. Ther. 2008; 7:1191–1193. [PubMed: 18719362]
78. Toye AA, et al. A genetic and physiological study of impaired glucose homeostasis control in C57BL/6J mice. Diabetologia. 2005; 48:675–686. [PubMed: 15729571]
79. Freeman HC, Hugill A, Dear NT, Ashcroft FM, Cox RD. Deletion of nicotinamide nucleotide transhydrogenase: a new quantitive trait locus accounting for glucose intolerance in C57BL/6J mice. Diabetes. 2006; 55:2153–2156. [PubMed: 16804088]
80. Collins S, Martin TL, Surwit RS, Robidoux J. Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. Physiol. Behav. 2004; 81:243–248. [PubMed: 15159170]
81. Nicholson A, et al. Diet-induced obesity in two C57BL/6 substrains with intact or mutant nicotinamide nucleotide transhydrogenase (Nnt) gene. Obesity. 2010; 18:1902–1905. [PubMed: 20057372]
82. Huang TT, et al. Genetic modifiers of the phenotype of mice deficient in mitochondrial superoxide dismutase. Hum. Mol. Genet. 2006; 15:1187–1194. [PubMed: 16497723]
83. Mullen AR, et al. Reductive carboxylation supports growth in tumour cells with defective mitochondria. Nature. 2012; 481:385–388. [PubMed: 22101431] [This study shows that mitochondrial α-ketoglutarate can be reductively carboxylated using mitochondrial NADPH to increase citrate production.]
84. Wallace DC, Fan W, Procaccio V. Mitochondrial energetics and therapeutics. Annu. Rev. Pathol. 2010; 5:297–348. [PubMed: 20078222]
85. Melov S, et al. Mitochondrial disease in superoxide dismutase 2 mutant mice. Proc. Natl Acad. Sci. USA. 1999; 96:846–851. [PubMed: 9927656]

86. Yan LJ, Levine RL, Sohal RS. Oxidative damage during aging targets mitochondrial aconitase. Proc. Natl Acad. Sci. USA. 1997; 94:11168–11172. [PubMed: 9326580]

87. Tong J, Schriner SE, McCleary D, Day BJ, Wallace DC. Life extension through neurofibromin mitochondrial regulation and antioxidant therapy for Neurofibromatosis-1 in Drosophila melanogaster. Nature Genet. 2007; 39:476–485. [PubMed: 17369827]

88. Sasaki M, et al. IDH1(R132H) mutation increases murine haematopoietic progenitors and alters epigenetics. Nature. 2012; 488:656–659. [PubMed: 22763442]

89. Gupta SC, et al. Upsides and downsides of ROS for cancer: the roles of ROS in tumorigenesis, prevention, and therapy. Antioxid. Redox Signal. 2012; 16:1295–1322. [PubMed: 22117137]

90. Burdon RH. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Rad. Biol. Med. 1995; 18:775–794. [PubMed: 7750801]

91. Lander HM. An essential role for free radicals and derived species in signal transduction. FASEB J. 1997; 11:118–124. [PubMed: 9039953]

92. Abate C, Patel L, Rauscher FJ, Curran T. Redox regulation of fos and jun DNA-binding activity in vitro. Science. 1990; 249:1157–1161. [PubMed: 2118682] [The first demonstration that FOS and JUN are regulated by cysteine oxidation–reduction.]

93. Liu H, Colavitti R, Rovira II, Finkel T. Redox-dependent transcriptional regulation. Circul. Res. 2005; 97:967–974.

94. Ordway JM, Eberhart D, Curran T. Cysteine 64 of Ref-1 is not essential for redox regulation of AP-1 DNA binding. Mol. Cell. Biol. 2003; 23:4257–4266. [PubMed: 12773568]

95. Xanthoudakis S, Miao GG, Curran T. The redox and DNA-repair activities of Ref-1 are encoded by nonoverlapping domains. Proc. Natl Acad. Sci. USA. 1994; 91:23–27. [PubMed: 7506414]

96. Go YM, Jones DP. Redox compartmentalization in eukaryotic cells. Biochim. Biophys. Acta. 2008; 1780:1273–1290. [PubMed: 18267127]

97. Jones DP. Radical-free biology of oxidative stress. Am. J. Physiol. Cell Physiol. 2008; 295:C849–C868. [PubMed: 18684987]

98. Kemp M, Go YM, Jones DP. Nonequilibrium thermodynamics of thiol/disulfide redox systems: a perspective on redox systems biology. Free Rad. Biol. Med. 2008; 44:921–937. [PubMed: 18155672]

99. Guzy RD, Mack MM, Schumacker PT. Mitochondrial complex III is required for hypoxia-induced ROS production and gene transcription in yeast. Antioxid. Redox Signal. 2007; 9:1317–1328. [PubMed: 17627464]

100. Sanchez-Cenizo L, et al. Up-regulation of the ATPase inhibitory factor 1 (IF1) of the mitochondrial H+-ATP synthase in human tumors mediates the metabolic shift of cancer cells to a Warburg phenotype. J. Biol. Chem. 2010; 285:25308–25313. [PubMed: 20538613]

101. Willers IM, Cuezva JM. Post-transcriptional regulation of the mitochondrial H+-ATP synthase: a key regulator of the metabolic phenotype in cancer. Biochim. Biophys. Acta. 2011; 1807:543–551. [PubMed: 21035425]

102. Jones RG, Thompson CB. Tumor suppressors and cell metabolism: a recipe for cancer growth. Genes Dev. 2009; 23:537–548. [PubMed: 19270154]

103. Wise DR, Thompson CB. Glutamine addiction: a new therapeutic target in cancer. Trends Biochem. Sci. 2010; 35:427–433. [PubMed: 20570523]

104. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab. 2008; 7:11–20. [PubMed: 18177721] [A report that activation of the PI3K–PTEN–AKT pathway redirects cellular metabolism from oxidative catabolism to glycolytic anabolism, thus enhancing cancer cell biogenesis.]

105. Pedersen PL, Mathupala S, Rempel A, Geschwind JF, Ko YH. Mitochondrial bound type II hexokinase: a key player in the growth and survival of many cancers and an ideal prospect for therapeutic intervention. Biochim. Biophys. Acta. 2002; 1555:14–20. [PubMed: 12206885]

106. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nature Rev. Cancer. 2004; 4:891–899. [PubMed: 15516961]

107. Nemoto S, Finkel T. Ageing and the mystery at Arles. Nature. 2004; 429:149–152. [PubMed: 15141200]

108. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu. Rev. Genet. 2005; 39:359–407. [PubMed: 16285865] [An overview of mitochondrial biology and genetics and their relation to disease.]
109. Daitoku H, Yamagata K, Matsuzaki H, Hatta M, Fukamizu A. Regulation of PGC-1 promoter activity by protein kinase B and the forkhead transcription factor FKHR. Diabetes. 2003; 52:642–649. [PubMed: 12606503]
110. Spiegelman BM, Heinrich R. Biological control through regulated transcriptional coactivators. Cell. 2004; 119:157–167. [PubMed: 15479634]
111. Ferber EC, et al. FOXO3a regulates reactive oxygen metabolism by inhibiting mitochondrial gene expression. Cell Death Differ. 2012; 19:968–979. [PubMed: 22139133]
112. Li F, et al. Myc stimulates nuclearly encoded mitochondrial genes and mitochondrial biogenesis. Mol. Cell. Biol. 2005; 25:6225–6234. [PubMed: 15988031]
113. Bluemlein K, et al. No evidence for a shift in pyruvate kinase PKM1 to PKM2 expression during tumorigenesis. Oncotarget. 2011; 2:393–400. [PubMed: 21789790]
114. Hitosugi T, et al. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. Sci. Signal. 2009; 2:ra73. [PubMed: 19920251]
115. Anastasiou D, et al. Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. Science. 2011; 334:1278–1283. [PubMed: 22052977]
116. Gruning NM, et al. Pyruvate kinase triggers a metabolic feedback loop that controls redox metabolism in respiring cells. Cell Metab. 2011; 14:415–427. [PubMed: 21907146] [A demonstration that oxidation of PKM2 inhibits glycolysis and redirects substrates into the pentose phosphate pathway to synthesize NADPH and enhance antioxidant defences.]
117. Hamanaka RB, Chandel NS. Warburg effect and redox balance. Science. 2011; 334:1219–1220. [PubMed: 22144609]
118. Bricker DK, et al. A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, Drosophila, and humans. Science. 2012; 337:96–100. [PubMed: 22628558]
119. Hoyos B, Acin-Perez R, Fischman DA, Manfredi G, Hammerling U. Hiding in plain sight: uncovering a new function of vitamin A in redox signaling. Biochim. Biophys. Acta. 2012; 1821:241–247. [PubMed: 21763457]
120. Zaugg K, et al. Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress. Genes Dev. 2011; 25:1041–1051. [PubMed: 21576264]
121. DeBerardinis RJ, 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. USA. 2007; 104:19345–19350. [PubMed: 18032601] [This study shows that MYC induction of glutaminolysis provides anaplerotic TCA cycle intermediates to generate citrate and sustain cytosolic fatty acid synthesis.]
122. Wise DR, et al. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc. Natl Acad. Sci. USA. 2008; 105:18782–18787. [PubMed: 19033189]
123. Bensaad K, Cheung EC, Vousden KH. Modulation of intracellular ROS levels by TIGAR controls autophagy. EMBO J. 2009; 28:3015–3026. [PubMed: 19713938]
124. Bensaad K, et al. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell. 2006; 126:107–120. [PubMed: 16839880]
125. Matoba S, et al. p53 regulates mitochondrial respiration. Science. 2006; 312:1650–1653. [PubMed: 16728594]
126. Sahin E, Depinho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature. 2010; 464:520–528. [PubMed: 20336134]
127. Fukuda R, et al. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. Cell. 2007; 129:111–122. [PubMed: 17418790]
128. Semenza GL. Oxygen-dependent regulation of mitochondrial respiration by hypoxia-inducible factor 1. Biochem. J. 2007; 405:1–9. [PubMed: 17555402]
129. Zhang H, et al. HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. Cancer Cell. 2007; 11:407–420. [PubMed: 17482131]

130. Zhang H, et al. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J. Biol. Chem. 2008; 283:10892–10903. [PubMed: 18281291]

131. Semenza GL. Mitochondrial autophagy: life and breath of the cell. Autophagy. 2008; 4:534–536. [PubMed: 18376135]

132. Devlin C, Greco S, Martelli F, Ivan M. miR-210: more than a silent player in hypoxia. IUBMB Life. 2011; 63:94–100. [PubMed: 21360638]

133. Bell EL, Emerling BM, Ricoult SJ, Guarente L. SirT3 suppresses hypoxia inducible factor 1α and tumor growth by inhibiting mitochondrial ROS production. Oncogene. 2011; 30:2986–2996. [PubMed: 21358671]

134. Yang J, et al. Human CHCHD4 mitochondrial proteins regulate cellular oxygen consumption rate and metabolism and provide a critical role in hypoxia signaling and tumor progression. J. Clin. Invest. 2012; 122:600–611. [PubMed: 22214851]

135. Luo W, et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell. 2011; 145:732–744. [PubMed: 21620138]

136. Luo W, Semenza GL. Pyruvate kinase M2 regulates glucose metabolism by functioning as a coactivator for hypoxia-inducible factor 1 in cancer cells. Oncotarget. 2011; 2:551–556. [PubMed: 21709315]

137. McCormack JG, Denton RM. A comparative study of the regulation of Ca2+ of the activities of the 2-oxoglutarate dehydrogenase complex and NAD+-isocitrate dehydrogenase from a variety of sources. Biochem. J. 1981; 196:619–624. [PubMed: 7032511]

138. McCormack JG, Halestrap AP, Denton RM. Role of calcium ions in regulation of mammalian intramitochondrial metabolism. Physiol. Rev. 1990; 70:391–425. [PubMed: 2157230]

139. Baughman JM, et al. Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature. 2011; 476:341–345. [PubMed: 21685886]

140. De Stefani D, Raffaello A, Teardo E, Szabo I, Rizzuto R. A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature. 2011; 476:336–340. [PubMed: 21685888]

141. Pinton P, Giorgi C, Pandolfi PP. The role of PML in the control of apoptotic cell fate: a new key player at ER-mitochondria sites. Cell Death Differ. 2011; 18:1450–1456. [PubMed: 21475307]

142. Martinez-Caballero S, et al. Assembly of the mitochondrial apoptosis-induced channel, MAC. J. Biol. Chem. 2009; 284:12235–12245. [PubMed: 19261612]

143. Peixoto PM, Ryu SY, Bombrun A, Antonsson B, Kinnally KW. MAC inhibitors suppress mitochondrial apoptosis. Biochem. J. 2009; 423:381–387. [PubMed: 19691447]

144. Dejean LM, et al. MAC and Bcl-2 family proteins conspire in a deadly plot. Biochim. Biophys. Acta. 2010; 1797:1231–1238. [PubMed: 20083086]

145. Amuthan G, et al. Mitochondria-to-nucleus stress signaling induces phenotypic changes, tumor progression and cell invasion. EMBO J. 2001; 20:1910–1920. [PubMed: 11296224] [A demonstration that a decrease in cancer cell mtDNA content and membrane potential increases cytosolic Ca2+, activates 'retrograde signalling', and increases epithelial–mesenchymal transition and cellular invasiveness.]

146. Biswas G, et al. A distinctive physiological role for IκBβ in the propagation of mitochondrial respiratory stress signaling. J. Biol. Chem. 2008; 283:12586–12594. [PubMed: 18272519]

147. Guha M, Srinivasan S, Biswas G, Avadhani NG. Activation of a novel calcineurin-mediated insulin-like growth factor-1 receptor pathway, altered metabolism, and tumor cell invasion in cells subjected to mitochondrial respiratory stress. J. Biol. Chem. 2007; 282:14536–14546. [PubMed: 17355970]

148. Guha M, Pan H, Fang JK, Avadhani NG. Heterogeneous nuclear ribonucleoprotein A2 is a common transcriptional coactivator in the nuclear transcription response to mitochondrial respiratory stress. Mol. Biol. Cell. 2009; 20:4107–4119. [PubMed: 19641020]

149. Guha M, Fang JK, Monks R, Birnbaum MJ, Avadhani NG. Activation of Akt is essential for the propagation of mitochondrial respiratory stress signaling and activation of the transcriptional coactivator heterogeneous ribonucleoprotein A2. Mol. Biol. Cell. 2010; 21:3578–3589. [PubMed: 20719961]

150. Guha M, Tang W, Sondheimer N, Avadhani NG. Role of calcineurin, hnRNPA2 and Akt in mitochondrial respiratory stress-mediated transcription activation of nuclear gene targets. Biochim. Biophys. Acta. 2010; 1797:1055–1065. [PubMed: 20153290]

151. Fan W, Lin CS, Potluri P, Procaccio V, Wallace DC. MtDNA lineage analysis of mouse L cell lines reveals the accumulation of multiple mtDNA mutants and intermolecular recombination. Genes Dev. 2012; 26:384–394. [PubMed: 22345519]

152. Park JS, et al. A heteroplasmic, not homoplasmic, mitochondrial DNA mutation promotes tumorigenesis via alteration in reactive oxygen species generation and apoptosis. Hum. Mol. Genet. 2009; 18:1578–1589. [PubMed: 19208652]

153. Guo JY, et al. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. Genes Dev. 2011; 25:460–470. [PubMed: 21317241]

154. Goh J, et al. Mitochondrial targeted catalase suppresses invasive breast cancer in mice. BMC Cancer. 2011; 11:191. [PubMed: 21605372]

155. Woo DK, et al. Mitochondrial genome instability and ROS enhance intestinal tumorigenesis in APCMin./+ mice. Am. J. Pathol. 2012; 180:24–31. [PubMed: 22056359]

156. Schriner SE, et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science. 2005; 308:1909–1911. [PubMed: 15879174]

157. Zu XL, Guppy M. Cancer metabolism: facts, fantasy, and fiction. Biochem. Biophys. Res. Commun. 2004; 313:459–465. [PubMed: 14697210]

158. Bonuccelli G, et al. The reverse Warburg effect: glycolysis inhibitors prevent the tumor promoting effects of caveolin-1 deficient cancer associated fibroblasts. Cell Cycle. 2010; 9:1960–1971. [PubMed: 20495363]

159. Pavlides S, 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. 2010; 2:185–199. [PubMed: 20442453]

160. Castello-Cros R, et al. Matrix remodeling stimulates stromal autophagy, “fueling” cancer cell mitochondrial metabolism and metastasis. Cell Cycle. 2011; 10:2021–2034. [PubMed: 21646868] [This study shows that cancer cell ROS production inactivates stromal cell caveolin 1, thus inducing stromal lactate production that feeds cancer cell oxidative metabolism and growth, a process known as the ‘reverse Warburg effect’.]

161. Capparelli C, et al. Autophagy and senescence in cancer-associated fibroblasts metabolically supports tumor growth and metastasis via glycolysis and ketone production. Cell Cycle. 2012; 11:2285–2302. [PubMed: 22684298]

162. Pavlides S, et al. Warburg meets autophagy: cancer-associated fibroblasts accelerate tumor growth and metastasis via oxidative stress, mitophagy, and aerobic glycolysis. Antioxid. Redox Signal. 2012; 16:1264–1284. [PubMed: 21883043]

163. Pagliarini DJ, et al. A mitochondrial protein compendium elucidates complex I disease biology. Cell. 2008; 134:112–123. [PubMed: 18614015]

164. Phillips D, et al. Regulation of oxidative phosphorylation complex activity: effects of tissue-specific metabolic stress within an allometric series and acute changes in workload. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2012; 302:R1034–R1048. [PubMed: 22378775]

165. Wallace DC. The epigenome and the mitochondrion: bioenergetics and the environment. Genes Dev. 2010; 24:1571–1573. [PubMed: 20679390]

166. Fan W, et al. A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations. Science. 2008; 319:958–962. [PubMed: 18276892]

167. Hashizume O, et al. Specific mitochondrial DNA mutation in mice regulates diabetes and lymphoma development. Proc. Natl Acad. Sci. USA. 2012; 109:10528–10533. [PubMed: 22689997]

168. Oliver NA, Wallace DC. Assignment of two mitochondrially synthesized polypeptides to human mitochondrial DNA and their use in the study of intracellular mitochondrial interaction. Mol. Cell. Biol. 1982; 2:30–41. [PubMed: 6955589]

At a glance

- Warburg observed, 70 years ago, that tumours produce excess lactate in the presence of oxygen. This became known as aerobic glycolysis or the 'Warburg effect' which he interpreted as mitochondrial dysfunction. However, it is now clear that mitochondrial function is essential for cancer cell viability, because elimination of cancer cell mitochondrial DNAs (mtDNAs) reduces their growth rate and tumorigenicity.
- The mitochondrial genome encompasses thousands of copies of the mtDNA and more than one thousand nuclear DNA (nDNA)-encoded genes. mtDNA mutations have been found in various cancers and seem to alter mitochondrial metabolism, enhance tumorigenesis and permit cancer cell adaptation to changing environments.
- Mutations in nDNA genes involved in mitochondrial metabolism, including succinate dehydrogenase (SDH), fumarate hydratase (*FH*), isocitrate dehydrogenase 1 (*IDH1*) and *IDH2*, result in increased succinate, fumarate, or *R*(-)-2-hydroxyglutarate levels. These metabolic alterations can inhibit various α-ketoglutarate-dependent dioxygenases; they can also activate the NFE2-related factor 2 (NRF2) stress response pathway. All of these effects can contribute to tumorigenesis.
- Activation of signalling pathways and oncogenes that are known to be important in tumorigenesis also affect mitochondrial function. The PI3K–PTEN–AKT pathway shifts metabolism from oxidative to glycolytic, thus permitting the redistribution of glycolytic nutrients from catabolism to anabolism. Activation of MYC induces glutaminolysis, which provides anaplerotic substrates to the mitochondrial tricarboxylic acid cycle, thus enhancing citrate production and its export to the cytosol to provide acetyl-CoA for lipid biogenesis and protein modifications.
- Altered mitochondrial metabolism can increase the production of mitochondrial reactive oxygen species (ROS) and change the cellular redox status, thus altering the activities of transcription factors such as HIF1α and FOS–JUN to change gene expression and stimulate cancer cell proliferation.
- A decrease of the mitochondrial membrane potential or mutation of the promyelocytic leukaemia (*PML*) gene reduces mitochondrial Ca^{2+} uptake, thus decreasing the activation of the mitochondrial intrinsic apoptosis pathway.
- Reduced mitochondrial Ca^{2+} retention increases the cytosolic Ca^{2+} concentration. This activates mitochondrial retrograde signalling through stimulation of calcineurin and IκBβ-dependent NF-κB, activation of enhanceosome-driven transcription and increased metastatic potential.
- Cancer cell ROS production inactivates caveolin 1 in adjacent stromal fibroblasts. This increases mitophagy, reduces mitochondrial function and increases lactate production in these fibroblasts. Secreted stromal cell lactate

then fuels cancer cell oxidative metabolism, which drives tumour growth and proliferation. This is known as the ‘reverse Warburg effect’.

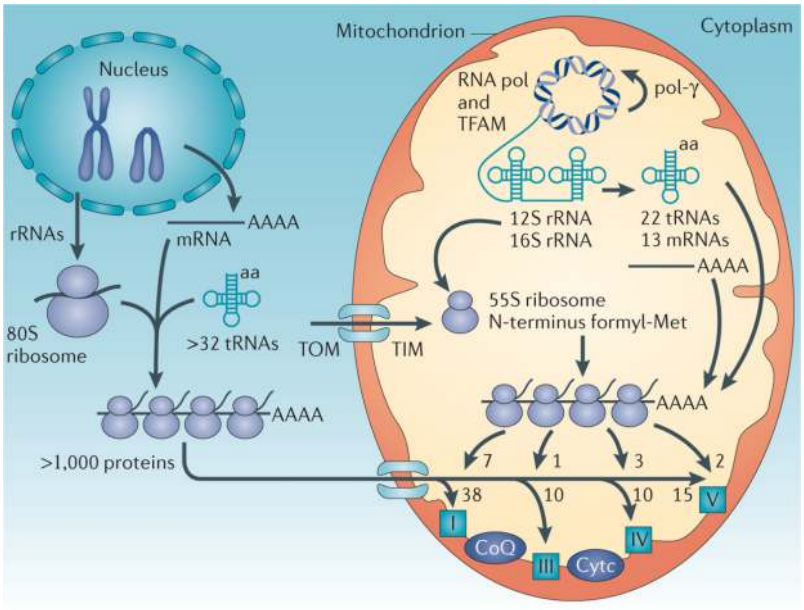

Figure 1. Mitochondrial genome and mitochondrial biogenesis

The mitochondrial genome encompasses between one and two thousand nuclear-DNA-encoded mitochondrial genes and thousands of copies of the mitochondrial DNA (mtDNA). mtDNA has a high mutation rate, and *de novo* mtDNA mutations create a mixture of mutant and normal mtDNAs in cells, a state known as heteroplasmy. As the proportion of mutant mtDNAs increases, the energy output capacity of the cell declines until there is insufficient energy to sustain cellular function, termed the bioenergetic threshold. Mitochondria also constantly undergo fusion and fission, which permits complementation of mtDNAs in *trans*[7,53,84,108,168]. The mtDNA encodes 13 proteins, 22 tRNAs, and 12S and 16S rRNAs. The mtDNA is packaged in the nucleoid and is replicated by DNA polymerase-γ (pol-γ). It is transcribed by mitochondrial RNA polymerase (RNA pol) symmetrically from both stands as large polycistron transcripts in which the larger transcripts are punctuated by the tRNAs. Cleavage of the tRNAs out of the polycistron transcripts creates the mature rRNAs and mRNAs, which are then translated on mitochondrial-specific chloramphenicol- sensitive ribosomes, in which the polypeptides are initiated by *N*-formyl methionine. The mtDNA encodes seven (ND1, ND2, ND3, ND4, ND4L, ND5 and ND6) of the 45 polypeptides of complex I; cytochrome *b* from the 11 polypeptides of complex III; three (cytochrome oxidase I (COI), COII and COIII) of the 13 polypeptides of complex IV; and two (ATP6 and ATP8) of the approximately 17 polypeptides of complex V. These proteins are central electron and proton carriers of the proton-transporting complexes and thus form the wiring diagram for oxidative phosphorylation (OXPHOS). All of the remaining mitochondrial proteins, including approximately 80 OXPHOS subunits and all four subunits of the non-proton-pumping complex II, are encoded by nuclear DNA (nDNA). The mRNAs from the nDNA-encoded subunits are translated on cytosolic ribosomes and the proteins are imported into the mitochondrion by transport through the outer (TOM) and inner (TIM) membrane complexes. TFAM, mitochondrial transcription factor A.

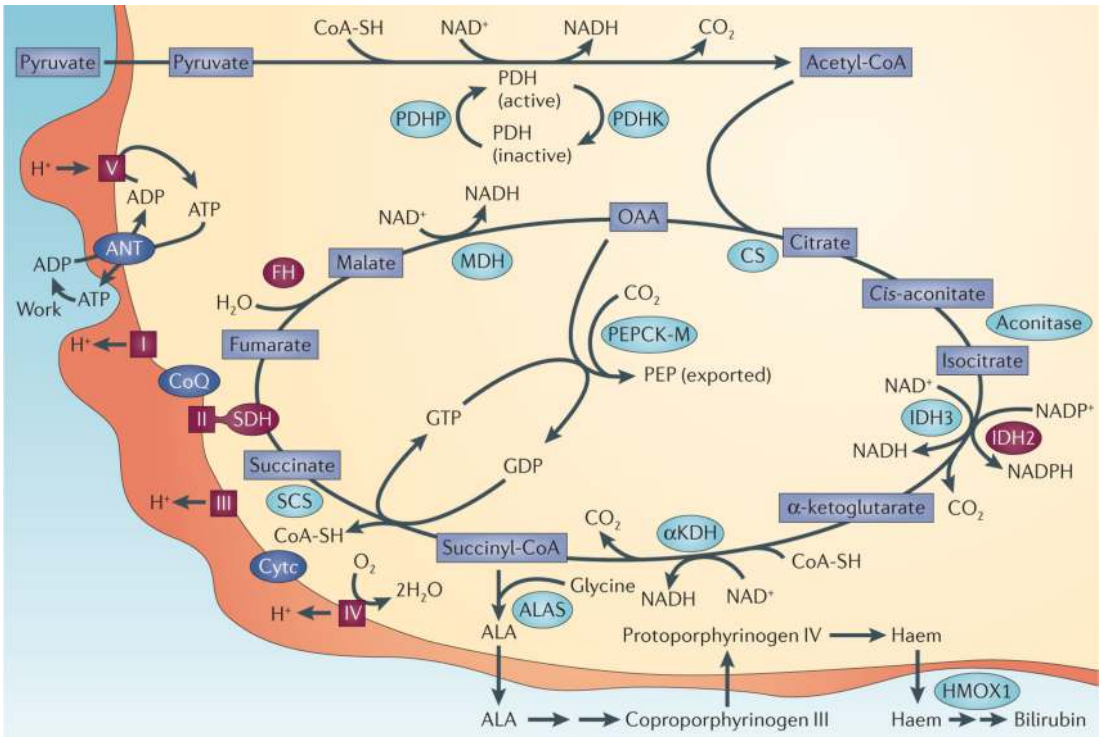

Figure 2. Mitochondrial bioenergetics and cancer cell mutations

Pyruvate from glycolysis is converted to acetyl-CoA, CO_2 and NADH by pyruvate dehydrogenase (PDH). PDH can be inactivated through phosphorylation by PDH kinase (PDHK) and reactivated through dephosphorylation by PDH phosphatase (PDHP). Acetyl-CoA enters the tricarboxylic acid (TCA) cycle by the citrate synthase (CS)-mediated reaction with oxaloacetate (OAA) to generate citrate. Citrate carbons then pass through the TCA cycle via the enzymes aconitase, isocitrate dehydrogenase (IDH) isoforms (IDH2 or IDH3), α-ketoglutarate dehydrogenase (αKDH), succinyl-CoA synthetase (SCS), succinate dehydrogenase (SDH; also known as complex II), fumarate hydratase (FH) and malate dehydrogenase (MDH). Cancer mutations have been identified in IDH2, SDH, and FH (shown as red ovals). FH defects are associated with induction of the haemoxygenase 1 (HMOX1), which degrades haem. Haem is synthesized by the condensation of succinyl-CoA and glycine by δ-aminolevulinic acid (ALA) synthetase (ALAS) to generate ALA. Succinyl-CoA conversion to succinate generates GTP, which can drive the condensation of OAA and CO_2 to phosphoenolpyruvate (PEP) by mitochondrial PEP carboxykinase (PEPCK-M). PEP can then be exported to the cytosol. NADH is generated by PDH, IDH3, αKDH and MDH, and can be oxidized by the electron transport chain (ETC). The ETC encompasses five multi-subunit complexes I–IV. NADH is oxidized by complex I (NADH dehydrogenase), and electrons from complexes I and II are transferred to coenzyme Q_{10} (CoQ), then passed on to complex III (also known as the *b*-*c*1 complex), cytochrome *c* (cyt*c*), complex IV (also known as cytochrome *c* oxidase (COX)), and finally to O_2 (half a molecule of O_2 per electron pair) to generate H_2O. As the electrons traverse complexes I, III and V, protons are pumped out across the mitochondrial inner membrane to generate the electrochemical gradient ($\Delta P = \Delta\Psi + \Delta\mu^{H+}$). ΔP is then used by complex V (H^+-translocating ATP synthase) to condense ADP and inorganic phosphate (P_i) to ATP. The

ADP and ATP are exchanged across the mitochondrial inner membrane by adenine nucleotide translocators (ANTs)[7,53,84,108]. Cancer cell mitochondrial DNA (mtDNA) mutations have been reported in genes for complexes I, III, IV and V (shown as red squares). Hence, many of the mitochondrial gene mutations in cancer are intimately associated with oxidative phosphorylation (OXPHOS) and the redox regulation of reactive oxygen species (ROS).

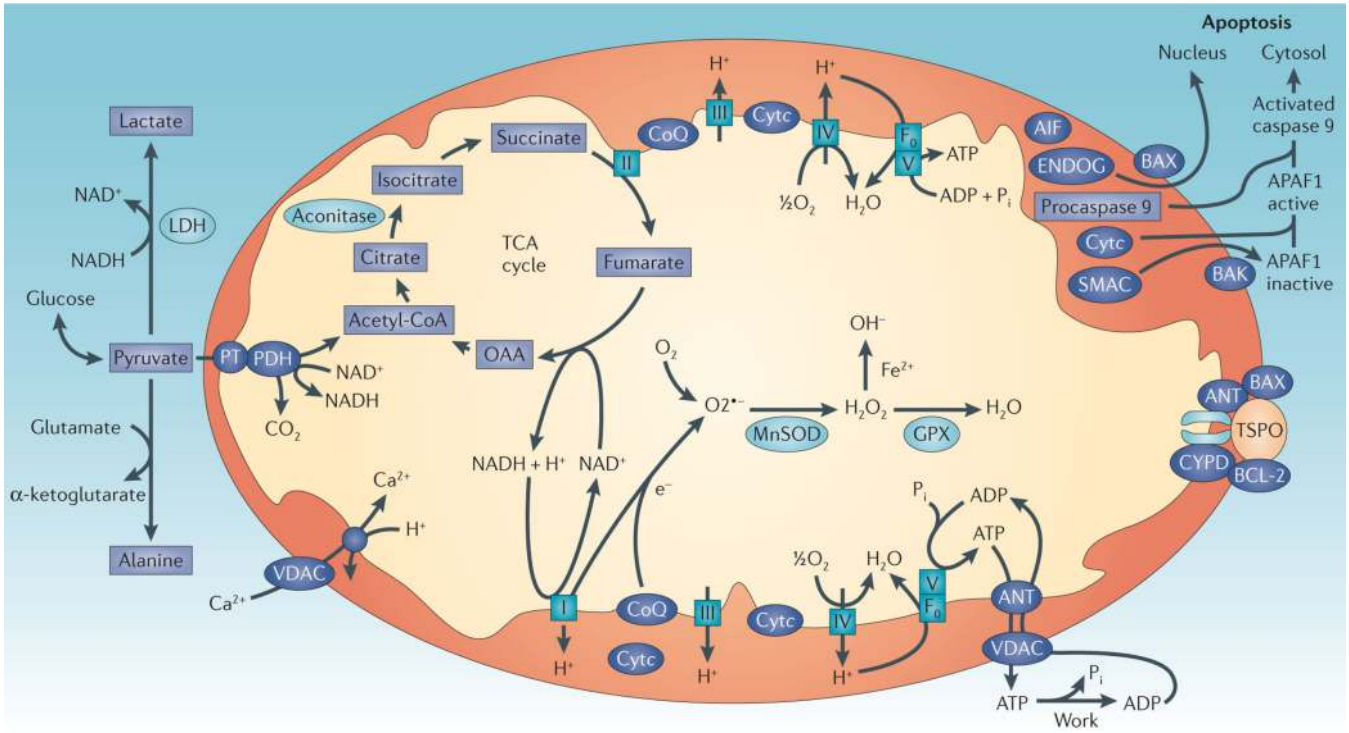

Figure 3. Mitochondrial physiology

Mitochondria lie at the nexus of most biosynthetic pathways, produce much of the cellular energy through oxidative phosphorylation (OXPHOS), regulate mitochondrial and cellular redox status, generate most of the reactive oxygen species (ROS), regulate Ca^{2+} concentrations and can initiate apoptosis by the activation of the mitochondrial permeability transition pore (mtPTP). The mtPTP can be activated by a decreased membrane potential, high-energy phosphates (such as ADP), a more-oxidized redox status, and/or increased mitochondrial matrix Ca^{2+} and ROS concentrations. Reducing equivalents and acetyl-CoA enter the mitochondrion via pyruvate and fatty acids. Pyruvate is transported through the mitochondrial inner membrane by the pyruvate transporter (PT)[118], binds to pyruvate dehydrogenase (PDH), which may be membrane-associated, and is oxidatively decarboxylated to produce acetyl-CoA. Inhibition of mitochondrial function results in pyruvate accumulation in the cytosol, where it can be reduced to lactate. Fatty acids are imported into the mitochondrion bound to carnitine. In the cytosol, fatty acids bound to CoA are transferred to carnitine, transported through the outer and inner mitochondrial membranes, and then transferred back to CoA for β-oxidation. The transfer of fatty acyl groups between CoA and carnitine is mediated by the carnitine palmitoyltransferases (not shown). As a by-product of OXPHOS — the substrates and products of which are transported through the outer membrane by the voltage-dependent anion channels (VDACs) — mitochondria generate ROS by the donation of excess electrons from complexes I and III directly to O_2 to generate superoxide anions ($O_2^{\bullet-}$). Matrix $O_2^{\bullet-}$, primarily from complex I, is dismutated to H_2O_2 by the mitochondrial matrix Mn superoxide dismutase (MnSOD; also known as SOD2), while intermembrane-space $O_2^{\bullet-}$, which is primarily from complex III, is dismutated by Cu/Zn superoxide dismutase (Cu/ZnSOD; also known as SOD1). H_2O_2 can be reduced to water by glutathione peroxidase using reduced glutathione as an electron

donor. Oxidized glutathione is reduced by glutathione reductase using NADPH as a reductant. In the presence of reduced transition metals, H_2O_2 can be reduced to hydroxyl radicals ($^{\bullet}OH$), which are the most reactive ROS. The mtPTP is a protein complex that is thought to include the translocator protein (TSPO; also known as PBR), an unknown inner-membrane channel, adenine nucleotide translocators (ANTs) and the cyclosporine-A-sensitive cyclophilin D (CYPD; also known as PPID), which are regulatory, in association with the BCL-2 pro- and anti-apoptotic family members. When activated, the mtPTP forms a channel between the inner and outer membranes, which short-circuits ΔP. This is associated with the aggregation of BAX and BAD in the mitochondrial outer membrane to form a megachannel. The megachannel releases pro-apoptotic proteins from the intermembrane space into the cytosol to initiate the degradation of the cellular proteins and DNA[7,53,84,108]. AIF, apoptosis-inducing factor; CoA-SH, coenzyme A with a free sulphydryl group; CoQ, coenzyme Q_{10}; ENDOG, mitochondrial endonuclease G; GPX, glutathione peroxidase; LDH, lactate dehydrogenase; OAA, oxaloacetate; SMAC, second mitochondria-derived activator of caspase; TCA, tricarboxylic acid. Modified, with permission, from REF. 3 © (2005) Cold Spring Harbor Laboratory Press.

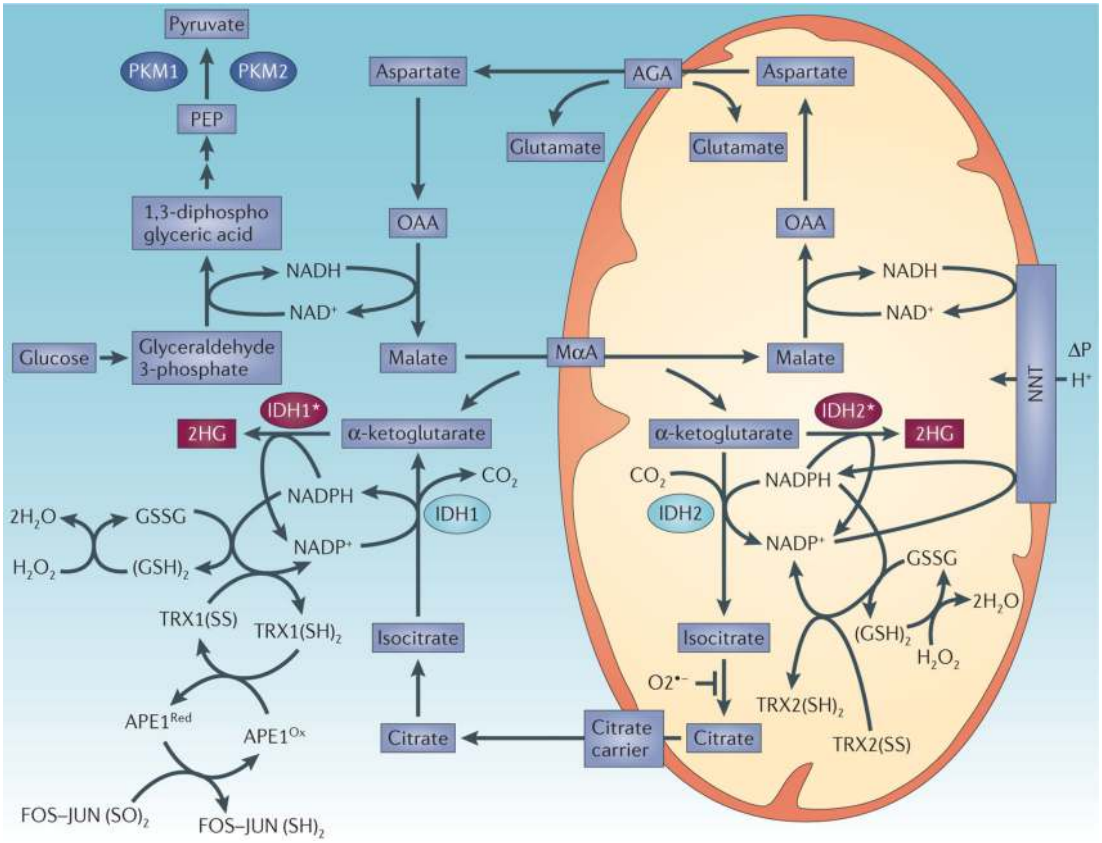

Figure 4. The mitochondrial NADPH shuttle system, and IDH 1 and IDH2 mutations

The mitochondrion can generate NADPH by the transfer of reducing equivalents from NADH to $NADP^+$. This process is mediated by the mitochondrial inner membrane nicotinamide nucleoside transhydrogenase (NNT), which exploits ΔP to provide the additional reducing potential energy. NADH can either be generated within the mitochondrion or can be imported from the cytosol by the aspartate–glutamate and malate–α-ketoglutarate (αKG) shuttle system. Within the mitochondrion, NADPH can be used to reduce glutathione and thus to control mitochondrial reactive oxygen species (ROS) signalling. Alternatively, NADPH can be used to reduce mitochondrial thioredoxin 2 (TRX2), which regulates the thio-disulphide redox state of mitochondrial proteins. However, NADPH can energize the mitochondrial $NADP^+$-dependent isocitrate dehydrogenase 2 (IDH2) to reductively carboxylate αKG to isocitrate. Isocitrate can then be converted to *cis*-aconitate and then citrate by mitochondrial aconitase. Citrate can be exported across the mitochondrial inner membrane into the cytosol by the citrate carrier where it can be converted to *cis*-aconitate and isocitrate by cytosolic aconitase. Cytosolic isocitrate can then be oxidatively decarboxylated by cytosolic $NADP^+$-linked IDH1, producing cytosolic NADPH. The resulting αKG or its aminated derivative glutamate can then be recycled back into the mitochondrion. In the cytosol, the NADPH can be used to reduce glutathione for antioxidant defences, or the reducing equivalents can be funnelled through the nuclear–cytosol TRX1 (for which the reduced (SH_2) and oxidized (SS) forms are shown) and then through the bifunctional apurinic/apyrimidinic endonuclease 1 (APE1; also known as redox factor 1(REF1)) protein to reduce thiols in cytosolic and nuclear proteins, including the FOS

and JUN transcription factors. Oncogenic mutations in IDH1 or IDH2 (shown as the red ovals with the asterisk) can result in a neomorphic function such that the αKG and NADPH generated by the wild-type IDH1 and IDH2 enzymes (shown as blue ovals) is converted to *R*(-)-2-hydroxyglutarate ((*R*)-2HG) and $NADP^+$. This would deplete NADPH, thus increasing ROS production and altering the regulation of nuclear transcription factors[53,84]. AGA, aspartate–glutamate antiporter; GSH, reduced glutathione monomer; GSSG, oxidized glutathione dimer; MαA, malate–αKG antiporter; OAA, oxaloacetate; PEP, phosphoenolpyruvate; PKM, pyruvate kinase isoform M.