Role of mitochondrial dysfunction in cancer progression

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Abstract

Deregulated cellular energetics was one of the cancer hallmarks. Several underlying mechanisms of deregulated cellular energetics are associated with mitochondrial dysfunction caused by mitochondrial DNA mutations, mitochondrial enzyme defects, or altered oncogenes/tumor suppressors. In this review, we summarize the current understanding about the role of mitochondrial dysfunction in cancer progression. Point mutations and copy number changes are the two most common mitochondrial DNA alterations in cancers, and mitochondrial dysfunction induced by chemical depletion of mitochondrial DNA or impairment of mitochondrial respiratory chain in cancer cells promotes cancer progression to a chemoresistance or invasive phenotype. Moreover, defects in mitochondrial enzymes, such as succinate dehydrogenase, fumarate hydratase, and isocitrate dehydrogenase, are associated with both familial and sporadic forms of cancer. Deregulated mitochondrial deacetylase sirtuin 3 might modulate cancer progression by regulating cellular metabolism and oxidative stress. These mitochondrial defects during oncogenesis and tumor progression activate cytosolic signaling pathways that ultimately alter nuclear gene expression, a process called retrograde signaling. Changes in the intracellular level of reactive oxygen species, Ca^{2+}, or oncometabolites are important in the mitochondrial retrograde signaling for neoplastic transformation and cancer progression. In addition, altered oncogenes/tumor suppressors including hypoxia-inducible factor 1 and tumor suppressor p53 regulate mitochondrial respiration and cellular metabolism by modulating the expression of their target genes. We thus suggest that mitochondrial dysfunction plays a critical role in cancer progression and that targeting mitochondrial alterations and mitochondrial retrograde signaling might be a promising strategy for the development of selective anticancer therapy.

Keywords: Cancer, carcinogenesis, medicine/oncology, metabolism, mitochondrial, DNA

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Introduction

Based on the increased understanding in the past few decades, deregulated cellular energetics was recently added as one of the cancer hallmarks.[1] Otto Warburg first proposed that tumor cells, unlike normal cells, exhibit increased glycolytic activity and reduced mitochondrial respiration even in the presence of oxygen. This phenomenon is known as the ''Warburg effect.'' Several underlying mechanisms of deregulated cellular energetics are associated with mitochondrial dysfunction caused by mitochondrial DNA (mtDNA) mutations, mitochondrial enzyme defects, or altered oncogenes/tumor suppressors.[2–5]

Mitochondria are intracellular organelles in eukaryotic cells that participate in bioenergetic metabolism and cellular homeostasis, including the generation of ATP through electron transport and oxidative phosphorylation in conjunction with the oxidation of metabolites by tricarboxylic acid (TCA) cycle and catabolism of fatty acids by β-oxidation, the production of reactive oxygen species (ROS), and the initiation and execution of apoptosis.[6,7] Mitochondria contain multiple copies of mtDNA. Human mtDNA is a 16.6-kb double-stranded, circular DNA molecule that encodes 13 respiratory enzyme complex polypeptides, 22 transfer RNAs, and 2 ribosomal RNAs required for mitochondrial protein synthesis.[8] Because mtDNA is essential for the maintenance of functionally competent organelles, the accumulation of mtDNA mutations or decreased mtDNA copy number is expected to affect energy production and enhance ROS generation and cell survival, and these processes may be involved in aging, mitochondrial diseases, or cancer.[4,9–11]

Although mitochondria have their own genome, most of the proteins and enzymes that reside in mitochondria are nuclear gene products. The enzymes of the TCA cycle are encoded by nuclear DNA and are located in the mitochondrial matrix or embedded in the inner mitochondrial membrane. Sirtuin 3 (SIRT3) is a nuclear-encoded mitochondrial

protein deacetylase that regulates the function of several mitochondrial proteins involved in oxidative phosphorylation and intermediate metabolism.[12] Mitochondrial dysfunction, caused by mtDNA mutations or mitochondrial enzyme defects, not only perturbs cellular bioenergetics, supporting the metabolic reprogramming of cancer cells, but also triggers tumor-promoting changes mediated by the ROS, Ca^{2+}, or small molecule metabolites released by mitochondria. Moreover, some oncogenes or tumor suppressors including hypoxia-inducible factor 1 (HIF-1) and tumor suppressor p53 (TP53) have been shown to regulate mitochondrial respiration and cellular metabolism. Altered oncogenes/tumor suppressors thus provide a direct link between mitochondrial dysfunction and tumorigenesis. In this review, we discuss the role of mitochondrial dysfunction caused by either mtDNA mutations, mitochondrial enzyme defects, or altered oncogenes/tumor suppressors in initiating a complex cellular reprogramming that supports the formation and progression of cancers.

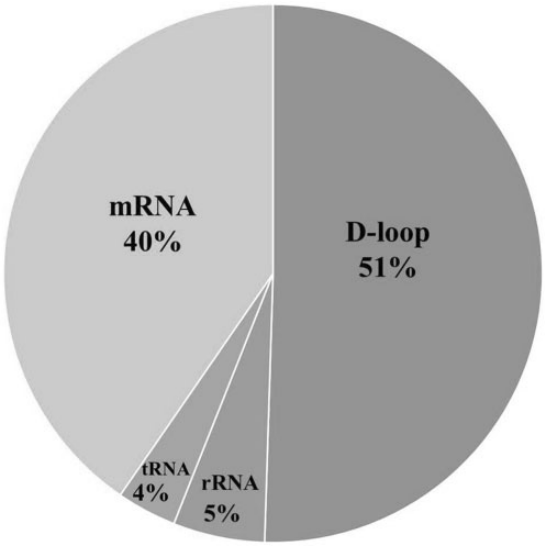

Figure 1 The location distribution of somatic mutations in mtDNA of human cancers analyzed in a total 859 patients with 20 different types of cancer. Source: Data adapted from Lee et al.[14]

Somatic mutations and decreased copy number of mtDNA in tumor cells may lead to mitochondrial dysfunction and cancer progression

Several types of mtDNA alterations, such as point mutations, large-scale deletions, insertions, and copy number changes, have been identified in human cancers.[13] Point mutations and copy number alterations are the two most common mtDNA alterations in cancers.[9]

Point mutations

According to an analysis based on a total 859 patients with 20 different types of cancer, 66% of cancers carried at least one somatic point mutation of mtDNA,[14] suggesting that somatic point mutation in mtDNA is a common event in human cancer progress. Among these identified mutations, 51% occurred in the D-loop region of mtDNA, 40% were found in the protein-coding region, 5% were located in the rRNA genes, and 4% were observed in the tRNA genes (Figure 1).[14]

The D-loop region of mtDNA is the most frequent site of somatic mutation in cancers. The mononucleotide repeat region of the poly-cytosine (poly-C) sequence at nucleotide positions (np) 303–309 (D310) in mtDNA is a hot spot for somatic mutation.[15] Because the D-loop region contains the major regulatory sites for mtDNA replication and transcription, mutations near these sites might affect mtDNA copy number and its transcription in cancer cells.[13,16] Importantly, clinical correlation analyses in various cancers showed that cancer patients with mtDNA D-loop mutation in their cancer tissues exhibited poorer prognoses than those who were free of the mtDNA mutations.[17–19]

Among the identified mutations in the protein-coding regions, 25% were missense, nonsense, or frame-shift mutations that have high potential to cause mitochondrial dysfunction. Several mutations identified in the mtDNA protein-coding region and tRNA genes of cancer tissues were pathogenic or have been reported to be associated with mitochondrial diseases (Table 1). These mutations include frame-shift mutations, e.g. 11032delA in the NADH dehydrogenase subunit 4 (ND4) gene[20,28,29] and 12418insA in the ND5 gene,[20,21,26,27,30] which result in truncated polypeptides, and tRNA mutations (e.g. T1659C in the $tRNA^{Val}$ gene,[20] G5650A in the $tRNA^{Ala}$ gene,[20] and 7472insC the $tRNA^{Ser(UCN)}$ gene[27] that potentially alter tRNA structure).

The 11032delA mutation was reported to be identified in prostate cancer,[28] renal oncocytoma tissues,[29] and hepatocellular carcinoma (HCC).[20] The 11032delA mutation is an ''A'' nucleotide deletion in the mononucleotide repeat of a poly-adenosine (poly-A) sequence at np 11032–11038 in mtDNA. The mutation causes a frame-shift and premature termination of the ND4 gene, thereby resulting in a truncated ND4 subunit protein. Moreover, the 11032delA mutation is correlated with a loss of Complex I activity in renal oncocytomas.[29]

The 12418insA mutation was also identified in several types of cancer, including the rotenone-resistant VA2B cell line,[30] colorectal cancer,[26] HCC,[20] gastric cancer,[27] and breast cancer specimens.[21] The 12418insA mutation is an ''A'' nucleotide insertion in the mononucleotide repeat of a poly-A sequence at np 12418–12425 in mtDNA. The mutation causes a frame-shift and premature termination of the ND5 gene, thereby resulting in a truncated ND5 subunit protein. Using a cybrid cell model, a heteroplasmic 12418insA mutation was demonstrated to reduce oxidative phosphorylation and increase ROS production in human cancer cells and promotes tumor growth in nude mice, suggesting that this mtDNA mutation contributes to tumorigenesis.[22]

The T1659C transition in the $tRNA^{Val}$ gene and the G5650A transition in the $tRNA^{Ala}$ gene were identified in two independent HCC tissues.[20] Clinically, the two

Table 1 Somatic mutations of mitochondrial DNA in human cancers

Nucleotide position at mtDNA	Cancer type	Mutation	Gene	Amino acid change	Correlated function	References
956	Hepatocellular carcinoma	Poly-C	12s rRNA			20
1499	Breast cancer	T → C/T	12S rRNA			21
1659	Hepatocellular carcinoma	T → C/T	$tRNA^{Val}$			20
1913	Breast cancer	G → A	16S rRNA			21
3409	Breast cancer	A3 → A3/2	ND1	Frame shift	Potential to cause mitochondrial Complex I dysfunction (truncated ND1)	21
3697	Gastric cancer	G → A	ND1	Gly (GGC) → Ser (AGC)	Potential to cause mitochondrial Complex I dysfunction (mutation in highly conserved residue)	22
3842	Hepatocellular carcinoma	G → A/G	ND1	Trp (TGA) → Stop (TAA)	Potential to cause mitochondrial Complex I dysfunction (truncated ND1)	20
3894–3960/3901–3967	Hepatocellular carcinoma	66 bp del	ND1	Frame shift	Potential to cause mitochondrial Complex I dysfunction (truncated ND1)	20
4561	Breast cancer	T → TT/T	ND2	Frame shift	Potential to cause mitochondrial Complex I dysfunction (truncated ND2)	21
4605	Breast cancer	A7 → A7/8	ND1	Frame shift	Potential to cause mitochondrial Complex I dysfunction (truncated ND2)	21
4996	Gastric cancer	G → A	ND2	Arg (CGC) → His (CAC)	Potential to cause mitochondrial Complex I dysfunction (mutation in highly conserved residue)	22
5112	Breast cancer	G → A/G	ND2	Ala (GCA) → Thr (ACA)		21
5522	Breast cancer	G → A/G	$tRNA^{Trp}$			21
5650	Hepatocellular carcinoma	G → A/G	$tRNA^{Ala}$		Decreased Complex I and IV activity	20, 23
5809	Breast cancer	G/A → A/G	$tRNA^{Cys}$			21
5895	Gastric cancer	$C_{19/n}$ → $C_{18/n}$	Non-coding nucleotides			22
6384	Breast cancer	G → A/G	COI	Ala (GCC) → Thr (ACC)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)	21
6768	Breast cancer	G → A/G	COI	Ala (GCA) → Thr (ACA)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)	21
6787	Hepatocellular carcinoma	T → C	COI	Val (GTA) → Ala (GCA)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)	20

(continued)

Table 1 Continued

Nucleotide position at mtDNA	Cancer type	Mutation	Gene	Amino acid change	Correlated function	References
7293	Breast cancer	G → A	COI	Ala (GCA) → Thr (ACA)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)	21
7472	Gastric cancer	insC	$tRNA^{Ser(UCN)}$		Decreased Complex I activity, lower oxygen consumption rate, and higher lactic acid production	22, 24, 25
7976	Hepatocellular carcinoma	G → A	COII	Gly (GGC) → Ser (AGC)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)	20
9263	Hepatocellular carcinoma	A → G	COIII	Thr (ACA) → Thr (ACG)		20
9267	Hepatocellular carcinoma	G → A	COIII	Ala (GCC) → Thr (ACC)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)	20
9412	Breast cancer	G → A/G	COIII	Gly (GGC) → Asp (GAC)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)	21
9545	Hepatocellular carcinoma	A/G → G	COIII	Gly (GGA) → Gly (GGG)		20
9774	Breast cancer	G → A/G	COIII	Asp (GAC) → Asn (AAC)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)	21
9901	Breast cancer	A → C/A	COIII	His (CAC) → Pro (CCC)	Potential to cause mitochondrial Complex IV dysfunction (mutation in highly conserved residue)	21
9986	Gastric cancer	C → A	COIII	Gly (GGG) → Gly (GGA)		22
10599	Breast cancer	G → A/G	ND4L	Ala (GCT) → Thr (ACT)		21
11032	Prostate cancer Renal oncocytomas Hepatocellular carcinoma	A7 → A6/7	ND4	Frame shift	Loss of Complex I activity	20, 28, 29
11708	Hepatocellular carcinoma	A → G	ND4	Ile (ATC) → Val (GTC)		20
12405	Gastric cancer	C → T	ND5	Leu (CTC) → Leu (CTT)		22
12418	Colorectal cancer Hepatocellular carcinoma Breast cancer Gastric cancer	A8 → A8/9	ND5	Frame shift	Defective mitochondrial respiratory function, higher lactate production and increased tumorigenesis	20, 21, 22, 26, 27
13015	Gastric cancer	T → C	ND5	Leu (TTA) → Leu (CTA)		22
13878	Breast cancer	A → G/A	ND5	Lys (AAA) → Lys (AAG)		21
13980	Breast cancer	G → C/G	ND5	Leu (CTG) → Leu (CTC)		21
15416	Breast cancer	T → C/T	CytB	Tyr (TAC) → His (CAC)	Potential to cause mitochondrial Complex III dysfunction (mutation in highly conserved residue)	21

mutations have been previously reported to be associated with distinct mitochondrial disorders.[23,31,32] The T1659C transition was found at a very high level of heteroplasmy in the blood, and skeletal muscle from a young girl with learning difficulties, hemiplegia, and a movement disorder, as well as an increased lactate level in the cerebrospinal fluid.[32] The G5650A transition was reported at a high level of heteroplasmy in the muscle and blood of a patient with a stereotypic clinical presentation of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and myopathy with ragged-red fibers.[31] The mtDNA mutation was also found in a family with a predominantly proximal myopathy and was associated with a large number of cytochrome *c* oxidase-deficient fibers and a marked decrease in the activities of both Complex I and Complex IV.[23]

The 7472insC was identified in a gastric cancer patient[27] and was a ''C'' nucleotide insertion in the mononucleotide repeat of a poly-C sequence at np 7466–7472 in mtDNA, which could alter the structure of the TψC loop in the clover leaf secondary structure of $tRNA^{Ser(UCN)}$. The mutation was reported to be associated with maternally inherited hearing loss, ataxia and myoclonus syndrome,[24,25] rapidly progressive neurodegeneration,[33] and progressive myoclonus epilepsy with ragged-red fibers (MERRF) and a MERRF-like phenotype.[34,35] It has been shown that the cybrids carrying homoplasmic 7472insC mutant mtDNA exhibited decreased Complex I activity, low oxygen consumption rate and high lactic acid production.[24,25]

Another pathogenic mutation T8993G in the ATP synthase subunit 6 (ATPase 6) gene of mtDNA was introduced into the PC3 prostate cancer cell line by a cybrid transfer technique, and the mtDNA mutation exhibited enhanced tumorigenesis in nude mice.[36] Moreover, using cybrids containing the T8993G or T9176C mutation in the ATPase 6 gene in the HeLa cell line, these mtDNA mutations were also shown to confer an advantage in tumor growth in nude mice by preventing apoptosis.[37]

These findings suggest that most mtDNA point mutations identified in cancer tissues have a high potential to result in mitochondrial dysfunction. Some point mutations were shown to contribute to tumorigenesis. However, it is unclear whether the different mtDNA point mutations play similar role in cancer progression. The cybrid transfer technique provides a strategy to approach this issue for specific mtDNA mutation.

Copy number changes

A decrease in mtDNA copy number was frequently detected in cancer tissues compared with corresponding noncancerous tissues. Alterations in mtDNA copy number of cancers appear to be tissue specific.[13,15] A decreased mtDNA copy number is frequently found in the majority of HCC, gastric cancers, and breast cancers.[16,19,38]

In HCC, a decrease in mtDNA copy number was found to more frequently occur in females than in males, indicating that the change of mtDNA copy number might contribute to the differences in clinical manifestations including tumorigenesis, cancer progression, metastasis, and prognosis.[39] HCC patients with lower mtDNA content exhibited poorer 5-year survival than patients with higher mtDNA content.[40] In gastric cancer, a decrease in mtDNA copy number was reported to be correlated with patients with ulcerated and infiltrating or diffusely thick types, which were associated with poor prognoses and lower 5-year survival rates after gastric resection.[38] An analysis of a different set of patients revealed that increased mtDNA copy number is associated with worse survival in patients with late-stage tumors.[41] In breast cancer, a decrease in mtDNA copy number was correlated with higher histological grade, poorer disease-free survival, and lower overall survival.[42] In colorectal cancer, patients with lower mtDNA copy number showed higher TNM stages and poorer differentiation.[43]

On the other hand, in head and neck cancers, an increase in mtDNA copy number was positively correlated with the histopathological grade, and mtDNA copy number in saliva from patients with head and neck squamous cell carcinoma was higher than controls and was associated with advanced tumor stage.[44,45] In ovarian cancer, the mtDNA copy number in patients with pathologically high-grade tumors was higher than in patients with low-grade tumors.[46] Similarly, in esophageal squamous cell carcinoma, the mtDNA copy number in the cancers of metastatic lymph nodes is higher than that in noncancerous tissue.[47,48]

The factor involving in the tissue-specific changes of mtDNA copy number in cancers is still unclear. Based on the clinicopathological correlations with changes of mtDNA copy number, these mtDNA copy number changes can potentially be used as a molecular prognostic marker of some types of cancer.

These findings suggest that somatic point mutations or decreased copy number of mtDNA in cancers may result in mitochondrial dysfunction. It needs to further evaluate whether these somatic mtDNA alterations might contribute to cancer progression or might be bystanders during cancer progression.

Mitochondrial dysfunction induced by somatic mtDNA alterations promotes cancer progression

Mitochondrial dysfunction induced by somatic mtDNA alterations in cancers might provide a mechanism to trigger the energy metabolism change of tumor cells from oxidative phosphorylation to glycolysis. It was thus hypothesized that mitochondrial dysfunction might contribute to cancer progression. To examine this hypothesis, several strategies, including chemical depletion of mtDNA, chemical impairment of mitochondrial respiratory chain, and cybrid transfer technique, were adopted to evaluate whether mitochondrial dysfunction promotes cancer progression to an apoptosis-resistant/chemo-resistant and/or invasive phenotype and to dissect the underlying mechanism.

It was demonstrated that depletion of mtDNA in HeLa cells prevents activation of adriamycin in the cells through impairing mitochondrial Complex I activity and subsequently results in resistance to this drug.[49] Moreover, depletion of mtDNA in human hepatoma SK-Hep1 cells results in an adaptive increase in the expression of manganese

superoxide dismutase (MnSOD) and other antioxidant enzymes that enabled the cancer cells to counteract oxidative stress or chemotherapeutic agents.[50] The depletion of mtDNA was also shown to increase the expression of the multidrug resistance 1 (MDR1) gene and hence to exhibit higher tolerance to anti-cancer agents in human colon cancer HCT-8 cells,[51] osteosarcoma 143B cells,[52] and hepatoma cells.[53] Chloramphenicol-induced mitochondrial stress in human hepatoma HepG2 cells and non-small cell lung cancer H1299 cells increases p21 expression and prevents mitomycin-induced apoptosis through a p21-dependent pathway.[54] Down-regulation of the α subunit of ATP synthase and low ATP synthase activity in human colon cancer cells exhibits resistance to 5-fluorouracil.[55] Mitochondrial respiration defects in cancer cells cause activation of the Akt survival pathway and contribute to drug resistance through a redox mediated mechanism.[56] We also found that chemical impairment of the mitochondrial respiratory chain enhances cisplatin-resistance in human HepG2 cells through up-regulated expression and secretion of amphiregulin[57] and in human gastric cancer cells through a ROS-mediated regulation.[58]

In addition, mtDNA copy number might affect hormone dependence in prostate cancer cells[59] and breast cancer cells.[60] It was demonstrated that the depletion of mtDNA in androgen-dependent LNCaP cells results in a loss of androgen dependence.[61] The depletion of mtDNA in MCF-7 cells exhibits resistance to hydroxytamoxifen and ICI182780.[62] These findings suggest that mtDNA defects may play an important role in the development of hormone independence, which may contribute to the progression of these cancers.

It was demonstrated that partial chemical depletion of mtDNA and impairment of mitochondrial respiratory chain in human lung cancer A549 cells induce an invasive phenotype.[63] It was also reported that the mitochondrial dysfunction-modulated invasive phenotype is induced by transcriptional regulation of extracellular matrix-remodeling genes.[61] Moreover, depletion of mtDNA in LNCaP and MCF-7 cells exhibits an invasive phenotype by promoting epithelial-mesenchymal transition.[62] Depletion of mtDNA in breast cancer cells by expressing mutant mitochondrial DNA polymerase γ increases Matrigel invasion.[64] In addition, chemical impairment of the mitochondrial respiratory chain in HepG2 cells enhances their migratory phenotype through the overexpression and secretion of amphiregulin by an autocrine or paracrine loop,[57] and overexpression of peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α) to promote mitochondrial biogenesis in HepG2 cells can markedly repress cell migration through the upregulation of E-cadherin expression.[65,66] We also found that chemical impairment of the mitochondrial respiratory chain in human gastric cancer cells enhances cell migration through ROS-induced integrin β5 expression.[58]

To rule out the toxic effects of chemical treatments, the cybrid transfer technique for replacement of mtDNA in tumor cells was used to demonstrate that mtDNA mutations may promote tumorigenesis and contribute to cancer progression. It was demonstrated that the heteroplasmic 12418insA mutation,[22] the T8993G mutation,[36,37] and the T9176C mutation[37] promotes tumor growth in nude mice. Moreover, ROS-generating mtDNA mutations were found to enhance the metastatic potential of tumor cells.[67]

These findings together suggest that mitochondrial dysfunction may enhance tumor growth or promote cancer progression to an apoptosis-resistant/chemo-resistant and/or invasive phenotype through various mechanisms.

Defects in mitochondrial enzyme in tumor cells may lead to mitochondrial dysfunction and cancer progression

In addition to somatic mtDNA alterations, deregulated cellular energetics in cancer cells might result from defects in the nuclear-encoded mitochondrial enzymes, including several enzymes of the TCA cycle and mitochondrial deacetylase SIRT3.

Succinate dehydrogenase (SDH)

The SDH (also known as respiratory Complex II) is a heterotetrameric protein complex that is located on the mitochondrial inner membrane, and contains two catalytic subunits (SDHA and SDHB) and two integral subunits (SDHC and SDHD).[68] The four subunits are encoded by nuclear genes and two assembly factors, SDH assembly factor 1 (SDHAF1) and SDHAF2, are essential for the assembly and activity of the SDH protein complex.[69,70] In the TCA cycle, the SDH complex converts succinate into fumarate in a reaction that is coupled to the reduction of flavin adenine dinucleotide (FAD) to $FADH_2$, and electrons are transferred to coenzyme Q. Inactivating mutations in the genes of the SDH subunits and assembly factors have been identified in pheochromocytoma (PCC),[71] paraganglioma (PGL),[71–74] gastrointestinal stromal tumors (GISTs),[75] renal carcinoma,[76,77] thyroid tumors, testicular seminoma, neuroblastomas, and breast cancer.[78]

The oncogenic activity of SDH defects has been attributed to succinate accumulation. Defects in SDH have been shown to result in the accumulation of extra-mitochondrial succinate and are linked to the inhibition of prolyl hydroxylases and hence the stabilization and activation of HIF-1α under normoxic conditions.[79–81] The SDH defects thus establish a tumorigenic ''pseudo-hypoxic'' state. In addition, SDH mutations have been reported to trigger ROS production, which might result in HIF activation through the inactivation of prolyl hydroxylases[68,82] or might lead to genomic instability.[83] Moreover, the accumulation of succinate was found to result in epigenetic changes in gene expression by the inhibition of α-ketoglutarate-dependent histone and DNA demethylases.[68,84,85]

Fumarate hydratase

Fumarate hydratase (also known as fumarase) is a nuclear-encoding mitochondrial matrix enzyme, which is involved in the TCA cycle for converting fumarate to malate. Loss of functional fumarate hydratase lead to low respiratory rate and increased glucose addition and lactate production in a UOK262 kidney cancer cell line derived from a metastasis in

a patient with hereditary leiomyomatosis renal cell carcinoma (HLRCC).[86,87] The altered mitochondrial respiration and cellular metabolism in the fumarase-deficient cells might result from ROS-dependent stabilization and activation of HIF-1α and lower expression of TP53.[86,88]

Fumarate hydratase mutations have been found in uterine leiomyomas and renal cell cancer,[89] dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer,[90] clear cell renal cancer,[91] and PGL/PCC.[92] Similar to SDH, the tumorigenic activity of fumarate hydratase defects is attributed to the abnormal accumulation of fumarate and the stabilization and activation of HIF-1α by inhibition of prolyl hydroxylases.[80,93] Moreover, it was found that fumarate covalently binds to cysteine residues in protein, a process called succination, and modulates the enzyme activity. The accumulation of intracellular fumarate can result in the succination of Kelch-like ECH-associated protein 1 (Keap1) and abrogates Keap1-mediated degradation of the nuclear factor erythroid 2-related factor 2 (NRF2).[94] The increased NRF2 activates several antioxidant genes and supports tumor formation.[94] In addition, the accumulation of fumarate might promote tumorigenesis by inhibiting α-ketoglutarate-dependent genome-wide histones and DNA methylations, hence resulting in epigenetic alterations in gene expression,[84] or by increasing ROS-dependent signaling via glutathione succination.[95]

Isocitrate dehydrogenase (IDH)

There are three isozymes of IDH in mammalian cell: IDH1, IDH2, and IDH3. IDH1 and IDH2 are homodimeric $NADP^+$-dependent enzymes located in the cytosol and mitochondrial matrix, respectively. IDH3 is a heterotetrameric NAD^+-dependent enzyme (consist of two alpha subunits, one beta subunit, and one gamma subunit) located in the mitochondrial matrix. The IDHs can oxidatively decarboxylate isocitrate to α-ketoglutarate. Mutations in IDH1 and IDH2 frequently occur in glioma (>75%)[96] and secondary glioblastoma (GBM) (70–75%) and are rarely detected in primary glioblastoma (5%).[97,98] Mutations in IDH1 and IDH2 have also been found in various human cancers with different frequencies, including acute myeloid leukemia (AML),[99] angioimmunoblastic T-cell lymphoma,[100] cholangiocarcinoma,[101,102] chondrosarcoma,[103] colon cancer,[104] giant cell tumor of bone,[105] melanoma,[106] prostate cancer,[107] and osteosarcoma.[108]

Most IDH1 and IDH2 mutations in cancers are often heterozygous with a wild-type allele,[96,97,109] and the mutations have been found to result in a neomorphic enzyme activity that converts α-ketoglutarate into the R-enantiomer of 2-hydroxyglutarate.[110–113] 2-Hydroxyglutarate inhibits the enzymatic activity of Complex IV (cytochrome *c* oxidase) and Complex V (ATP synthase)[114,115] and alters the gene expression of TCA cycle enzymes in cancer cells.[116] These findings suggest that the accumulation of 2-hydroxyglutarate contributes to the alterations in energy metabolism in IDH-mutant cancer cells.

2-Hydroxyglutarate is considered a major contributor to the oncogenic activity of IDH mutations and is thus identified as an ''onco-metabolite'' that promotes tumorigenesis.[112] The increased 2-hydroxyglutarate levels were shown to be associated with DNA hypermethylation and a broad epigenetic change, which results in epigenetic alterations of gene expression.[113,117,118] The oncogenic activity of 2-hydroxyglutarate has been attributed to its accumulation and the inhibitory effect on various α-ketoglutarate-dependent dioxygenases, including the prolyl hydroxylases, histone demethylase KDM4C, and 5-methylcytosine hydroxylase TET2.[113,117,119,120] However, the effects of 2-hydroxyglutarate on HIF-1α and prolyl hydroxylases could be controversial. Some studies reported that 2-HG leads to inhibition of HIF-1α via activation of prolyl hydroxylases, whereas other studies showed that 2-hydroxyglutarate inhibits prolyl hydroxylases and induces HIF-1α expression.[100,113,121,122] These findings imply that the effects of 2-hydroxyglutarate on HIF-1α signaling might be cell type dependent and the role of IDH mutants in tumorigenesis by HIF-1α signaling needs further investigation.

SIRT3

SIRT3 is one of the nuclear-encoded mitochondrial protein deacetylases. SIRT3 regulates the function of several mitochondrial proteins involved in oxidative phosphorylation, fatty acid oxidation, the urea cycle, and the antioxidant response system.[123–131] SIRT3 is thought to play a tumor suppressor role in various cancers, including breast, colorectal, HCC, lung and gastric cancers.[132–137] Loss of SIRT3 was found to increase intracellular ROS levels and result in the stability and activation of HIF1α and the Warburg effect phenotype, which promote tumorigenesis.[133]

These findings together suggest that mitochondrial dysfunction induced by these defects in several nuclear-encoded enzymes of the TCA cycle, such as SDH, fumarate hydratase, and IDH, and the down-regulation of mitochondrial deacetylase SIRT3 results in mitochondrial dysfunction, and enhances tumor growth, as well as promotes cancer progression.

Altered oncogene/tumor suppressor gene represses mitochondrial respiratory function in cancers

Alterations in oncogene/oncosuppressor including HIF-1 and TP53 have been shown to regulate mitochondrial respiration and cellular metabolism.

HIF-1

HIF-1 is a heterodimeric protein consisting of HIF-1α and HIF-1β subunits, both of which are members of the basic helix-loop-helix family of transcription factors, that modulates the cellular response to hypoxia in normal and tumor cells.[138] Although HIF-1β is a constitutively expressed nuclear protein, HIF-1α is tightly regulated by oxygen availability. Under normoxia, HIF-1α subunit is rapidly hydroxylated on the oxygen-dependent degradation (ODD) domain by prolyl hydroxylases and degraded through von Hippel–Lindau tumor suppressor protein (pVHL)-mediated proteasome degradation pathway.[139,140] In tumor cells, HIF-1α plays a crucial role in

angiogenesis, proliferation, invasion, and metastasis.[138,141] Overexpressed HIF-1α is associated with malignancy in many types of tumor including bladder, brain, breast, colon, oral, liver, lung, pancreas, skin, stomach, uterus, and leukemia.[142] Several factors have been reported to increase HIF-1α expression, including inhibition of SDH and fumarate hydratase, or activation of phosphoinositide 3-kinase (PI3K) and viral transforming genes.[29,143] Moreover, HIF1α plays a critical role in the regulation of mitochondrial respiration and cellular metabolism.[144,145] The activation of HIF1α in tumor can alter energy metabolism from oxidative phosphorylation to glycolysis through the expression of pyruvate dehydrogenase kinase 1 (PDK1), which inhibits the conversion of pyruvate to acetyl-CoA as the substrate for the TCA cycle by phosphorylation of pyruvate dehydrogenase. The inhibition of pyruvate dehydrogenase leads to repression of mitochondrial oxidative metabolism. Moreover, HIF-1α increases glycolysis by up-regulation the gene expressions of the glucose transporters, glycolytic enzymes, and lactate dehydrogenase A (LDHA).[143] HIF-1α is thought to contribute to reprogramming energy metabolism in tumor cells from oxidative phosphorylation to glycolysis. In addition, HIF-1α induces the expression of mitochondrial LON protease, which is important in the degradation of the COX4-1 subunit of the cytochrome *c* oxidase complex (COX), and the increased expression of COX4-2, a downstream target gene of HIF-1α, is used to substitute COX4-1. It was found that the replacement of COX4-1 with COX4-2 in cancer cells increases the efficiency of electron transfer to oxygen and continued respiration and decreases the ROS production under limited oxygen availability.[146] A recent study also showed that the expression of mitochondrial chaperone TRAP1 is increased in cancer cells, and the chaperone interacts with and inhibits SDH. The interaction between TRAP1 and SDH results in the accumulation of succinate and the stabilization of HIF-1α and hence induces metabolic reprogramming and promotes tumorigenesis.[147] These findings suggest that HIF-1α plays a crucial role in the cellular metabolism and tumorigenesis of cancer cells.

TP53

TP53 is well known for the functions in DNA damage response (DDR), cell cycle arrest, and apoptosis. Loss or inactive mutations of p53 were often observed in several types of tumor (approximately 60%) (TP53 website). Moreover, animals lacking TP53 have been shown to exhibit the spontaneous development of tumors.[148,149] In addition, TP53 was found to be important for maintenance of the mitochondrial respiration and regulation of cellular metabolism. TP53 defects are thought to contribute to the Warburg effect in cancer cells.

TP53 can repress the transcription of glucose transporter (GLUT) isoform 1 (GLUT1) and GLUT4 and reduce the expression of GLUT3 through an IKK/nuclear factor-κB (NFκB)-dependent manner.[150,151] These glucose transporters are important in glucose uptake, and the loss of TP53 can increase glucose consumption in cancer cells. Moreover, TP53 was found to modulate glycolytic enzymes through several mechanisms.[149] TP53 can stimulate the expression of the TP53-induced glycolysis and the apoptosis regulator (TIGAR) and repress glycolysis by degrading fructose-2,6-bisphosphate, which is an activator of phosphofructokinase 1.[152] In addition, TP53 can promote the degradation of phosphoglycerate mutase and hence can repress glycolysis.[153] TP53 defects can result in an increase in glycolytic flux by reduced TIGAR and increased phosphoglycerate mutase.

On the other hand, T53 was found to transcriptionally induce the expression of subunit I of cytochrome *c* oxidase[154] and the apoptosis-inducing factor (AIF, which is important in the maintenance of mitochondrial Complex I)[155,156] and the synthesis of cytochrome *c* oxidase 2 (SCO2, which is essential to the assembly of the cytochrome *c* oxidase complex)[157] and glutaminase 2 (GLS2, which catalyzes glutamine to glutamate for fuel for the TCA cycle).[158,159] These findings suggest that TP53 defects can result in down-regulation of mitochondrial respiration and oxidative metabolism.

In addition, TP53 could transcriptionally inactivate the expression of malic enzymes ME1 and ME2, which are involved in recycling of malate to pyruvate, and hence inhibits the intermediates of the TCA cycle entering biosynthetic pathways.[160] Moreover, TP53 was reported to increase the expression of ribonucleotide reductase subunit TP53R2 for maintaining mtDNA integrity.[161] TP53 was also shown to interact with mitochondrial DNA polymerase γ and play an important role in mtDNA maintenance in response to oxidative damage.[162] These findings indicate that TP53 is involved in the regulation of mitochondrial respiration and cellular metabolism, and a loss function of TP53 can contribute to the Warburg effect in tumor cells.

Activation of mitochondrial retrograde signaling contributes to mitochondrial dysfunction-induced tumorigenesis and cancer progression

Because of the roles of HIF-1α and TP53 in both mitochondrial function and tumorigenesis, they might directly link between mitochondrial dysfunction and the formation and progression of cancer. Unlike HIF-1α and TP53, mitochondrial dysfunction caused by mtDNA mutations and the nuclear-encoded mitochondrial enzyme defects might contribute to the formation and progression of cancer by triggering cytosolic signaling pathways that ultimately alter nuclear gene expression, a process called retrograde signaling. Recent studies revealed that changes in the intracellular levels of ROS, Ca^{2+}, and oncometabolites that are released from mitochondria might be important in the mitochondrial retrograde signaling for neoplastic transformation.

Increased low levels of mitochondria-derived ROS can function as signaling messengers by reversibly oxidizing protein thiol groups, thereby modifying protein structure and function. Higher levels of ROS can nonspecifically damaging DNA, proteins, and lipids, which could result in disruption of mitochondrial electron transfer chain and

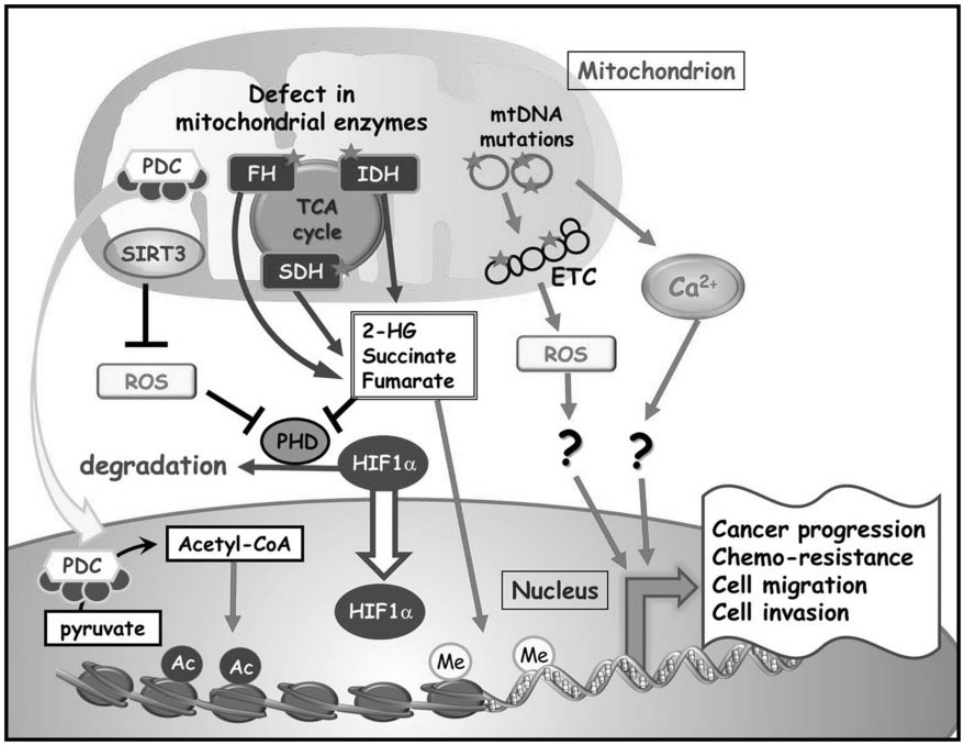

Figure 2 Mitochondrial dysfunction caused by mitochondrial DNA (mtDNA) mutations, mitochondrial enzyme defects, or altered oncogenes/tumor suppressors might contribute to the formation and progression of cancer. Somatic alterations in mtDNA and defects in mitochondrial enzymes, such as succinate dehydrogenase (SDH), fumarate hydratase (FH), and isocitrate dehydrogenase (IDH) were found to be associated with both familial and sporadic forms of cancer. Deregulated mitochondrial deacetylase sirtuin 3 (SIRT3) might modulate cancer progression by regulating cellular metabolism and oxidative stress. These mitochondrial alterations during oncogenesis and tumor progression activate cytosolic signaling pathways that ultimately alter nuclear gene expression, a process called retrograde signaling. Changes in reactive oxygen species (ROS), Ca^{2+}, or oncometabolites are involved in the mitochondrial retrograde signaling for neoplastic transformation by inhibiting prolyl hydroxylases and stabilizing hypoxia-inducible factor 1α (HIF-1α) or by modulating α-ketoglutarate-dependent genome-wide histone and DNA methylations, resulting in epigenetic alterations of gene expression. In addition, altered oncogene/oncosuppressor including HIF-1 and tumor suppressor p53 (TP53) not only regulates mitochondrial respiration and cellular metabolism but also promote the formation and progression of cancer

consequently collapse of mitochondrial function and threaten cell survival. Accumulating evidence has shown that hypoxia, activation of oncogenes, loss of tumor suppressors, and mitochondrial dysfunction induced by mtDNA mutations or mitochondrial enzyme defects increase production of mitochondrial ROS.[163] Recent evidence revealed that tumor cells can enhance glutathione and thioredoxin antioxidant systems to drive cancer initiation and progression by preventing increased ROS from reaching cytotoxic levels.[164] This range of ROS might be capable of increasing tumorigenesis and/or promoting cancer progression by activating signaling pathways that regulate cellular proliferation, metabolic adaptation, antioxidant systems, apoptosis-resistance, chemoresistance, and cellular migration/invasion.[163]

In cytoplasmic hybrids for replacement of mtDNA in tumor cells, ROS-generating mtDNA mutations have been found to increase tumorigenesis[22] and to enhance the metastatic potential of tumor cells.[67] Loss of SDHB[81] and mutations in SDHC[165] were also shown to increase tumorigenesis by enhancing mitochondrial ROS levels. Fumarase-deficient cancer cells were found to increase mitochondrial ROS and HIF-1a stabilization.[86] Moreover, mitochondrial dysfunctions caused by various respiration inhibitors have been shown to promote cell migration via ROS-enhanced β5-integrin expression in gastric cancer SC-M1 cells[58] or ROS-mediated upregulation of amphiregulin in human hepatoma HepG2 cells.[57]

In addition to ROS, Ca^{2+} was found to up-regulate amphiregulin and induce chemo-resistance and migration of human hepatoma HepG2 cells.[57] Moreover, elevated cytosolic Ca^{2+} was found in cancer cells with mtDNA depletion by ethidium bromide[166] and was involved in the mtDNA depletion-induced expression of the invasive markers, cathepsin L and TGFβ1 as well as the invasive phenotype.[61] Activation of protein kinase C (PKC) and calcineurin-induced signaling might mediate the mtDNA depletion-induced invasive phenotype.[166] It was further demonstrated that calcineurin can stimulate IκBβ-dependent NFκB and nuclear translocation of cRel-p50, which results in the changes in the malignant characteristics including metabolic reprogramming, invasive behavior, and resistance to apoptosis.[167] These findings suggest that increased cytosolic Ca^{2+} induced by mitochondrial dysfunction can activate signaling pathways that regulate metabolic alterations, cell invasion, and apoptosis-resistance to promote cancer progression.

As mentioned above, defects in SDH, fumarate hydratase, and IDH can result in the accumulation of metabolites that are released from mitochondria; these metabolites,

such as succinate, fumarate, and 2-hydroxyglutarate, have been found to be important for tumorigenesis activity of these mitochondrial enzyme defects. These metabolites are thus considered oncometabolites. The accumulation of oncometabolites can promote tumorigenesis by inhibiting prolyl hydroxylases and stabilizing HIF-1α.[92,168] Moreover, the accumulation of these oncometabolites might affect α-ketoglutarate-dependent genome-wide histone and DNA methylations and hence might result in epigenetic alterations of gene expression, which contribute to tumorigenesis.[83]

In addition, mitochondrial dysfunctions were found to repress the protein synthesis, trans-activation activity, and targeting gene expressions of HIF-1α through an energy sensor, the AMP-activated protein kinase (AMPK)-dependent pathway.[169] This provides another mechanism for the communication between the mitochondria and the nucleus. We further found that loss of this communication renders HCC cancer cells to exhibit drug resistance.[170] These findings suggest that the mitochondrial retrograde signaling pathway might regulate the sensitivity of cancer cells to anti-cancer agents.

Interestingly, a recent study found that pyruvate dehydrogenase complex (PDC), which is originally found to be located in mitochondria and catalyzes the pyruvate to acetyl-CoA reaction, can be translocated from the mitochondria to the nucleus and can generate acetyl-CoA in the nucleus to be used for histone acetylation under conditions of mitochondrial dysfunction. The nucleus translocation of PDC was found to be important for cell cycle G1-S phase progression in response to exposure of serum and growth factor (such as EGF), suggesting a potential role in cancer with proliferative signals or mitochondrial dysfunction.[171] These findings suggest that the communication between the nucleus and the mitochondria might play an important role in tumorigenesis.

Summary

We have shown several lines of evidence that suggest that the underlying mechanisms of deregulated cellular energetics are associated with mitochondrial dysfunction caused by mtDNA mutations, mitochondrial enzyme defects, or altered oncogenes/tumor suppressors. Mitochondrial dysfunction can promote cancer progression to an apoptosis-resistant/chemo-resistant and/or invasive phenotype through various mechanisms. These mitochondrial alterations during oncogenesis and tumor progression can activate cytosolic signaling pathways from mitochondria to the nucleus and ultimately alter nuclear gene expression for neoplastic transformation (Figure 2). However, it is still largely unclear how specific mtDNA mutations regulate the formation and progression of cancer. Elucidation of the detailed molecular mechanisms for the mitochondrial retrograde signaling still requires further investigation. Based on these findings that mitochondrial dysfunction may contribute to cancer progression, we suggest that targeting mitochondrial alterations and mitochondrial retrograde signaling might be a promising strategy for the development of selective anticancer therapy.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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