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Mitochondrial DNA Somatic Mutation in Cancer

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Cancer cells are known to drastically alter cellular energy metabolism. The Warburg effect has been known for over 80 years as pertaining cancer-specific aerobic glycolysis. As underlying molecular mechanisms are elucidated so that cancer cells alter the cellular energy metabolism for their advantage, the significance of the modulation of metabolic profiles is gaining attention. Now, metabolic reprogramming is becoming an emerging hallmark of cancer. Therapeutic agents that target cancer energy metabolism are under intensive investigation, but these investigations are mostly focused on the cytosolic glycolytic processes. Although mitochondrial oxidative phosphorylation is an integral part of cellular energy metabolism, until recently, it has been regarded as an auxiliary to cytosolic glycolytic processes in cancer energy metabolism. In this review, we will discuss the importance of mitochondrial respiration in the metabolic reprogramming of cancer, in addition to discussing the justification for using mitochondrial DNA somatic mutation as metabolic determinants for cancer sensitivity in glucose limitation.

Key words: Mitochondrial DNA, Somatic mutation, Cancer, Aerobic glycolysis, Warburg effect, Oxidative phosphorylation, Oncobiguanide, Efficacy, Toxicity

INTRODUCTION

We are accumulating ever-growing experimental evidence that helps us to better understand cancer's biology, and thereby, also helping to develop the therapeutic magic bullet that once and for all eradicates cancer cells from patients. The American Cancer Society recently announced that there was a 20% decline in cancer's mortality in the United States during last two decades (1). The authors credited this overall decrease to improved cancer prevention, early detection, and various treatment options. Tumor bioenergetics is one of the emerging hallmarks of cancer (2), and it is unraveling itself, mainly through cancer genomics and cancer biology. Without a doubt, tumor specific bioenergetics serves as a promising therapeutic target for cancer. Several excellent reviews on tumor energy metabolism have recently been published (3-5). Therefore, the focus of this review will mainly be on the importance of mitochondria genomics and mitochondria biology in tumor bioenergetics.

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AN EMERGING HALLMARK OF CANCER: METABOLIC REPROGRAMMING OF CANCER CELLS

Our knowledge regarding cancer is rapidly expanding. Readers may easily appreciate the amounts of knowledge we have gained during the last decade by simply comparing two reviews published by Hanahan and Weinberg (2,6). The authors have proposed six hallmarks of cancer, and every one of them seems to serve as a promising therapeutic target for cancer drug development.

The distinctive aerobic glycolytic metabolism utilized by cancer cells is called the Warburg effect, first reported by Otto Warburg in 1930 (7). Energy production via aerobic glycolysis (2 ATP molecules per glucose) is largely inefficient when compared to mitochondrial oxidative phosphorylation (OxPhos) (36~38 ATP molecules per glucose). Warburg proposed that mitochondrial dysfunction drove cancer cells to employ inefficient glycolysis as an alternative mechanism (8) (Fig. 1A and 1B).

Over past eight decades, the seemingly counter-intuitive Warburg effect greatly puzzled scientists, but the theory holds strong even under excruciating and extensive studies in the field of cancer biology. It is only recently that the eight decades-old concept started receiving some much-

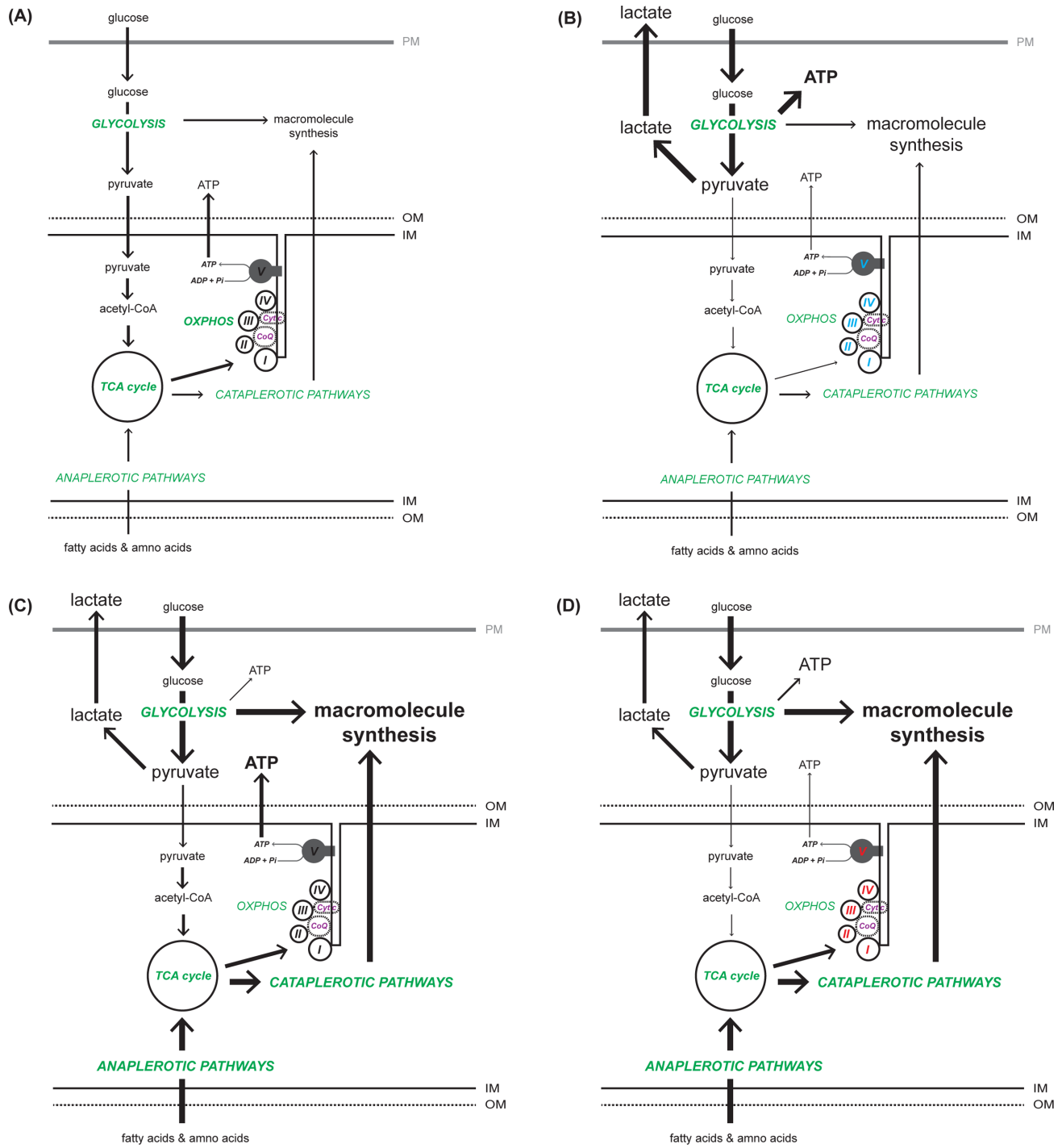

Fig. 1. Tumor bioenergetics. (A) non-proliferating normal cells utilize glycolysis and oxidative phosphorylation to fully oxidize glucose. Most of ATP are generated through glucose oxidation. (B) In a classical view on the Warburg effect, cancer cells enhance glycolysis to compensate the reduced ATP production due to dysfunctional mitochondria. Incomplete oxidation of glucose leads to production of lactate. Causal role of dysfunctional mitochondria in the enhanced glycolysis in cancer cells is yet to be empirically confirmed. (C) In a current view on cancer energy metabolism, cancer cells generate most of ATP through mitochondrial oxidative phosphorylation. Enhanced glycolysis as well as anaplerosis/cataplerosis support anabolic metabolism of cancer cells for the rapid proliferation. (D) Targeted drugs for TAC cycle or oxidative phosphorylation lead to drastic decrease in ATP generation. Impact on the rapidly proliferating cancer cells would be more drastic than slowly growing cancer cells or non-proliferating normal cells when ATP generation is reduced. Mitochondrial DNA somatic mutations will potentiate susceptibility of cancer cells to the targeted drugs for TCA cycle or oxidative phosphorylation, whereas drug toxicity on normal cells will be reduced.

deserved appreciation. Hanahan and Weinberg even proposed that 'major reprogramming of cellular energy metabolism' is one of two emerging hallmarks of cancer (2).

Regarding cancer treatment, the majority of drugs being tested in clinical trials are targeted therapeutics. For example, with the success of imatinib in 2003, numerous kinase targeting drugs are currently under development or in clinical trials (9). However, Herceptin, once considered as the magic bullet for HER2-positive breast cancer, has been associated with side effects, such as patients not being responsive to the drug, as well as acquired drug resistance among once-responsive patients (10). Because the drug's initial efficacy was so dramatic among HER2-positive breast cancer patients, as the complexity of resistance against targeted therapies is revealed (11), the limitation of Herceptin seems to be equally, if not more, distressful news to patients who are waiting for the magic bullet to cure their cancer.

In 2006, oncologists started actively discussing bioenergetics as the seventh hallmark of cancer (12). Thompson's group proposed that proliferating cells such as cancer cells enhance glycolysis in order to rapidly assimilate nutrients into cellular 'building blocks', synthesizing biomass for new cells (13) (Fig. 1C). Over the past five years or so, agents of tumor energy metabolism, such as the glycolysis, tricarboxylic acid (TCA) cycle and OxPhos, have been intensively tested as possible therapeutic targets for cancer. Many studies are focusing on the glycolytic pathway in cytosol (4,14,15), and some are also investigating the TCA cycle and OxPhos (15). However, from the start of the discussion on tumor bioenergetics (12), scientists clearly were aware of the complexity of targeting tumor bioenergetics and are yet to deliver the best target for drugs to act on, effectively blocking cancer energy metabolism.

Regardless of the pathway targeted to inhibit tumor bioenergetics, mitochondria seem to be considered a passive organelle whose functions are mainly governed by cytosolic events as well as nuclear genes. On the contrary, mitochondrion is an integral part of tumor biology (16) and the complete oxidation of nutrients is only accomplished via OxPhos. Therefore, one must sufficiently consider mitochondrial genomics and mitochondrial biology to thoroughly understand tumor bioenergetics. Without insights into the mitochondrial role in cancer energy metabolism, targeted therapeutics for cancer energy metabolism are likely to encounter unforeseen complications during clinical applications.

CLINICAL SIGNIFICANCE OF METABOLIC REPROGRAMMING OF CANCER

A rather provocative question is arising in the field of oncology: Is somatic mutation the origin of cancer? (17). Alternatively, mitochondrial respiratory dysfunction is openly proposed as the origin of cancer (18). Without solid insight into how cancer arises, our efforts to prevent, control and cure cancers often run into major impediments during clinical application. In 2005, Evan and colleagues first reported that type 2 diabetic patients on metformin had a reduced risk of cancer (19). This report brought metformin, a widely used anti-diabetic drug, into the center of tumor bioenergetics. Several hundred clinical trials that tested the anti-cancer effects of metformin were immediately initiated (20). Putative mechanisms for the anti-cancer effects of metformin were suggested, and the term 'oncobiguanide' was coined to describe the unconventional use of metformin as an anti-cancer drug (20,21).

Contrary to metformin's rapid acceptance in the area of clinical research, the lack of a specific molecular target has been major hurdle for studying the anti-cancer effects of metformin and other biguanides. The Hirst group (22) recently overcame this impediment by demonstrating the direct interaction between metformin and mitochondrial complex I. According to the study, metformin accumulates in the mitochondrial matrix, and binds to complex I via two separate sites, both leading to the decreased complex I activity and the inhibition of ATPase activity of complex V (22). As suggested by the authors, impaired mitochondrial respiration leads to decreased ATP production. Cancer cells with high metabolic demands may be more vulnerable to energy depletion resulting in cell death.

In a toxicological point of view, however, targeting mitochondrial respiration by directly inhibiting the electron transport chain (ETC) inevitably influences the bioenergetics of healthy normal cells. The central role of mitochondrial dysfunction in cellular toxicity is undisputable (23). Furthermore, a normal cell relies on its ability to modulate mitochondrial respiration for cytoprotection under pathological conditions (24). Therefore, the therapeutic window for 'oncobiguanides' may be too narrow to be clinically applicable. Such a concern regarding 'oncobiguanides' from the toxicological aspect may be circumvented through the use of mitochondrial DNA somatic mutations as metabolic determinants of tumor bioenergetics.

Clinically, primary as well as metastatic solid tumors often show severe hypoxia; it is also often heterogeneous within the same tumor, but not regulated according to metabolic demand unlike normal tissue (25). Cancers often show several distinctive metabolic profiles *in vivo*: (1) extremely low glucose concentrations, (2) high lactate and glycolytic intermediate concentrations, and (3) high amino acid accumulations with the exception of glutamine (26). These observations imply that the solid tumor endures poor metabolic environments to proliferate, as well as to metastasize *in vivo*. More importantly, the inadequacy of the *in vitro* culture condition (high glucose and glutamine supplements under 20% oxygen concentration) becomes apparent under the condition that most of oncological experiments are conducted.

Birsoy and colleagues (27) recapitulated the sustained low glucose status in the tumor microenvironment *in vitro.* Under glucose limitation, drastic changes in tumor metabolism occurred: decreases in the glucose consumption and lactate production, and increases in the NAD^+/NADH ratio, with a slight but statistically significant decrease in the ATP production. Under unlimited glucose supply, cancer cells up-regulate glycolytic genes, whereas when glucose becomes scarce, they turn to mitochondrial OxPhos by up-regulating the nucleus-encoded mitochondrial OxPhos genes.

Importantly, the cellular doubling time of some cancer cell lines remained relatively unchanged, but others showed drastically prolonged doubling times under glucose limitation (27). They systemically analyzed the possible metabolic determinants that result in drastically different responses to a low glucose supply among the various cancer cell lines. As expected, the cell lines with LKB1 deficiency or impaired glucose utilization (mutation in GLUT genes) showed decreased proliferation capacity under glucose limitation. In addition, many had mutations in the mitochondrial complex I genes. MT-ND genes acquired point mutations or insertions, resulting in a frame-shift or alteration in the amino acid. Also, severe heteroplasmy was identified in mitochondrial DNA among cancer cell lines with impaired proliferation capacities under glucose limitation. Consequently, phenformin, another 'oncobiguanide', dramatically suppressed proliferation of these cancer cells in xenograft mouse models. Therefore, it seems highly feasible to develop anticancer drugs targeting energy metabolism of cancer cells, particularly mitochondrial OxPhos.

MITOCHONDRIA IN METABOLIC REPROGRAMMING OF CANCER

In general, impaired mitochondrial ETC generates reactive oxygen species, and produces less ATP. Mitochondrial reactive oxygen species cause decrease in mitochondrial membrane potential, activate mitophagy/autophagy, and trigger mitochondria-driven apoptosis (28). Dysfunctional mitochondria are implicated in various pathogenic processes. Oxidative stress due to mitochondrial reactive oxygen species, mitophagy/autophagy as well as inflammasome activation are strongly associated with aging and aging-related diseases (29). However, experimental evidence is currently very limited that dysfunctional mitochondria are solely responsible for the onset of aforementioned pathophysiological conditions. One of the best experimental model is thyroid oncocytic cells carrying mutations in MT-ND1 and CYTB (30). These cells showed defective complex I and III activities. They consumed less oxygen, generated more mitochondrial reactive oxygen species, and produced fewer ATP. Consequently, these cells become susceptible for metabolic inhibition (Fig. 1D).

Experimental evidence is emerging that mitochondrial dysfunction, particularly due to mitochondrial DNA somatic mutations, could be a determining factor for cancer cells' susceptibility of anti-cancer drugs targeting energy metabolism. The Birsoy and colleagues suggested that defects in glucose utilization and mitochondrial DNA somatic mutation would be the metabolic determinants of cancer cell's sensitivity to glucose limitation (27). However, it is very unlikely that the defects in glucose utilization may serve as a proper determinant *in vivo* since GLUTs are known to be up-regulated in human cancers (31). Recent studies strongly support mitochondrial DNA somatic mutations as putative metabolic determinants for glucose-sensitivity of cancer cells (32-34).

When mitochondrial respiration is inhibited without limitation of glucose supply, non-proliferating normal cells with low energy demands stabilize the hypoxia inducible factor 1α (HIF1α) which is responsible for turning on the glycolytic pathway to compensate for the reduced ATP production, ensuring survival (32). Highly proliferative tumor cells also turn on autophagic pathways as an additional source for nutrients, which ensures the cell's survival (26). High glucose promotes HIF1α stabilization under hypoxic conditions (35). Tumor cells in microenvironments where both oxygen and glucose concentrations are low are likely to enhance autophagic pathways and turn to OxPhos for APT generation. Under such circumstances, tumor cells are likely to be more sensitive to mitochondrial respiration inhibition. Indeed, cancer cells in the core of the multicellular spheroid were vulnerable to mitochondrial respiration inhibition and showed reduced clonogenicity *in vitro* (32).

Clinical relevance of the metabolic profile of cancer cells was further emphasized in a KRas-expressing pancreatic ductal adenocarcinoma mouse model (33). When the KRas-driven signaling pathway was blocked through genetic or pharmacological means, the surviving cells showed enhanced tumorigenic potency. These cells seem to represent 'cancer stem cells', which are responsible for tumor relapse after cancer treatment. Authors analyzed the metabolic profile of surviving cells from the KRas ablation, demonstrating that they enhanced the autophagic pathway, mitochondrial respiration and peroxisomal β-oxidation (33). Importantly, KRas-expressing cells were resistant to oligomycin treatment, whereas the surviving cells from the KRas ablation became sensitized to oligomycin treatment, because their metabolic profiles were reprogrammed to utilize OxPhos. Oligomycin treatment also effectively blocked the spherogenic potential of the tumor cells when the oncogenic signaling pathway was blocked via pharmacological means (33).

Le and colleagues demonstrated the heterogeneous metabolic profiles of cancer cells *in vivo* (34). According to their study, some cancer cells were non-cycling without HIF1α activation under hypoxic conditions. However, these cells were fully tumorigenic and up-regulated nuclear encoded mitochondrial genes. Consequently, they showed elevated

resting oxygen consumption rates as well as an enhanced mitochondrial respiration capacity, suggesting that they have fully functional mitochondria (Fig. 1C). The authors named these cells as 'non-Warburg' cells to emphasize their distinctive metabolic profiles.

Both studies (33,34) suggested that cancer treatments targeting tumor metabolism take the metabolic heterogeneity of the cancer cells into consideration: some prefer aerobic glycolysis, while others prefer oxidative phosphorylation. In this regard, 'oncobiguanides' will be an excellent therapeutic choice to target cancer cells with respiring mitochondria. Furthermore, the therapeutic window for 'oncobiguanides' will dramatically improve *in vivo* when tumor metabolic profiles are carefully assessed and properly targeted.

MITOCHONDRIAL DNA SOMATIC MUTATION: A METABOLIC DETERMINANT OF CANCER SENSITIVITY TO GLUCOSE LIMITATION

As cancer progresses, cells with genetic and epigenetic alterations that render greater advantages for survival and proliferation are likely to be selected and propagate. These alterations probably vary considerably in a temporal as well as spatial aspect of cancer progression. When accurately assessed, these genetic and epigenetic alterations will serve as excellent targets for targeted therapy. Aerobic glycolysis in cancer cells is well documented; hypoxia-mediated stabilization or signaling pathway-mediated enhanced protein synthesis of HIF1α in cancer cells is thoroughly investigated (36). On the contrary, despite mitochondrial respiration being an integral part of cellular energy metabolism, the direct measurement of mitochondrial respiration in cancer cells has been rare (37). Owing to technical progression, it is now possible to systemically measure alterations in glycolytic, as well as in oxidative, substrate flux in cancer cells (38). Except in some cases, such as with oncocytic tumors (30), the direct causal and effecter relationship between mitochondrial DNA mutations and decreased mitochondrial respiration has been infrequently reported (37). With a thorough investigation on mitochondrial DNA mutation and mitochondrial respiration during carcinogenesis, we may reconcile disputes on the origin of cancer: somatic mutation versus dysfunctional mitochondrial respiration (17,18).

There have been several studies in which clinical human cancer samples were analyzed for abnormalities in mitochondrial genome (39-46). Most studies are based on the partial mitochondrial genome sequences, as well as on a limited number of samples or patients. Recently, there has been substantial improvement in mitochondrial DNA sequencing techniques. We are now able to study the whole mitochondrial genome in a reliable and cost-effective manner. Additionally, mitochondrial DNA sequences can be extracted from other sequencing data, such as exome and whole genome sequencing data. The Cancer Genome Atlas (TCGA) is an excellent example extracting mitochondrial DNA somatic mutations (47). Compared to nuclear DNA sequencing, there are special considerations required for mitochondrial DNA sequencing: (1) enrichment of mitochondrial DNA ensuring whole mitochondrial genome coverage, (2) decisions regarding single nucleotide polymorphism and heteroplasmy of mitochondrial DNA, (3) precise measurement of the copy number of mitochondrial DNA, and (4) structural variations due to large deletion or breakage of mitochondrial genomes (47).

Although whole mitochondrial DNA could be subjected to somatic mutations, the displacement loop (D-loop) is known as a mutational hot spot for human cancer (48). It is the strongest deletion breakpoint mapped to position 16,071 (49). The D-loop is a non-coding sequence where mitochondrial DNA replication starts and promoters for the transcription of mitochondrial genes are present. Therefore, mutations in the D-loop are likely to significantly influence the copy number, as well as the heteroplasmy of mitochondrial DNA. However, direct consequences of the D-loop mutation have not yet been empirically examined (48). Contrary to the widely accepted theory that mitochondrial reactive oxygen species are causing mitochondrial DNA mutation (48), Ju and colleagues (50) reported that the patterns of mitochondrial DNA mutations in cancers seemed to be derived from replication errors caused by the mitochondria themselves during the cell cycle. Furthermore, the researchers systematically analyzed mitochondrial DNA mutations in over 1,600 tumors and identified over 1,900 mitochondrial somatic DNA mutations, but found no evidence for the strong correlation between mitochondrial DNA mutations and carcinogenesis (50). It seemed that most missense DNA mutations in the mitochondrial genome develop early in carcinogenesis with no physiological advantage to tumor cells. Consequently, as cancer progresses, these mutations become homoplasmic. On the other hand, in the case of mitochondrial DNA somatic mutations resulting in the alteration of proteins, tumor cells cannot allow heteroplasmy of mutated mitochondrial DNA to propagate without compromising the selective fitness during cancer cell evolution (50).

Although Ju and colleagues concluded that there was no correlation between mitochondrial DNA somatic mutations and carcinogenesis (50), one must consider followings during the interpretation of the data on mitochondrial DNA mutations. First, it may be more appropriate to interpret the importance of mitochondrial DNA somatic mutations according to the association with the disease of interest. As the authors pointed out, mitochondrial DNA itself seems to accumulate diverse mutations through replication-associated processes, and not all mitochondrial DNA mutations are correlated with the disease of interest (50). As an example, among 1,900 mitochondrial DNA somatic mutations in cancers reported by Ju and colleagues (50), only14 cases

Table 1. Mitochondrial DNA somatic mutation as examples for metabolic determinants for cancer sensitivity to glucose limitation in human cancers

Gene[1)]	Mutation[1)]	Protein alteration[1)]	Comments	Reference
MT-ND1	A3467G	K54X	Serve as truncating mutation	(27)
MT-ND1	3571insC	Frameshift/Stop codon	Generate truncated peptide	(27,39)
			Reported in human colorectal carcinomas	(39)
MT-ND4	T11703C	Leu >> Pro	Reported in human pancreatic cancer	(51)
MT-ND4	11872insC	Frameshift/Stop codon	No change in amino acid	(46)
			Reported in human pituitary adenoma	
MT-ND4	T11982C	Leu >> Pro	Reported in human oral squamous cell carcinoma	(52)
MT-ND4	C11240T	Leu >> Phe	No report from human cancer	-
MT-ND5	C12992T	Ala >> Val	No report from human cancer	-
MT-ND5	C13453T	Leu >> Phe	No report from human cancer	-
MT-ND5	12425insA	Frameshift/Stop codon	Generate truncated peptide	(27,39)
			Reported in human colorectal carcinomas	(39)

[1)]Modified from reference (27).

were confirmed for specific disease-association. On the other hand, among the nine mutations in the MT-ND genes in complex I reported by Birsoy and colleagues (27), five mutations were independently reported to be associated with human cancers (Table 1). Second, the significance of mitochondrial DNA somatic mutations in the disease of interest must be evaluated according to the physiological and functional changes in the protein produced from the mutated mitochondrial DNA. In the case of thyroid oncocytic cells carrying mutations in MT-ND1 and CYTB, these pathogenic mutations were able to transfer impaired OxPhos phenotypes to another cell, independent of its nuclear genome profile (30). The importance of analyzing the functional consequence of the mitochondrial DNA somatic mutation is further demonstrated through the report by Birsoy and colleagues (27). Therefore, with proper considerations regarding the functional significance, mitochondrial DNA somatic mutations, especially those of ETC genes, will be excellent metabolic determinants of cancer sensitivity to glucose limitation. When cancer cells with respiring mitochondria are systemically evaluated based on the functional profile of mitochondrial DNA somatic mutations, the therapeutic window for 'oncobiguanides' could be optimized for maximal efficacy, as well as, for minimal toxicity.

CONCLUSION

As an emerging hallmark of cancer, metabolic reprogramming of cancer cells appears to be a promising target for anti-cancer drugs. Cytosolic glycolytic pathway is altered by various oncogenes (33). Many studies have shown the feasibility of targeting cytosolic glycolytic pathway, whereas relatively less attention has been paid to the mitochondrial TCA cycle and OxPhos. Although there is limited direct experimental evidence, recent studies suggest that the metabolic reprogramming in cancer cells through mitochondrial TAC cycle as well as OxPhos could be the better targets developing anti-cancer drugs. As discussed in this review, mitochondrial DNA somatic mutations, if they are evaluated in a disease-specific manner as well as according to functional alterations, may serve as an excellent biomarker for which therapeutic window of targeted drugs is optimized. In addition, it may be possible to circumvent unforeseen complications, often associated with targeted drugs, during clinical applications of anti-cancer drugs targeting tumor energy metabolism.

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