MINI-REVIEW

Mitochondrial DNA instability in human cancers

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Summary  Recently, a metabolic shift between the increased cytoplasmic glycolysis and decreased mitochondrial respiration in cellular energy production has been regarded as a biological hallmark for human cancers. As speculated by Warburg in the 1950s, respiratory complexes (i.e., mitochondria in modern terminology) were impaired or suppressed in human cancers, and such a defect may play an important role in the carcinogenesis and progression of human cancer. To appraise the mitochondrial defect, several researchers paid considerable attention to the role of mitochondrial DNA (mtDNA) alterations in multiple human cancers, and some results showed clinical significance. Generally, the way to analyze mtDNA alterations is mainly focused on the quantitative copy number changes and the qualitative displacement loop (D-loop) mutations. Based on the heteroplasmic to homoplasmic D310 mutation of D-loop and progressive increase of mtDNA copy number, the theory of cancer clonal expansion can be well explained from the viewpoint of mtDNA alteration. In conclusion, the role of mtDNA instability might be a good biological marker to correlate with carcinogenesis, disease progression, and drug resistance in several human cancers.

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1. Introduction

Mitochondria, located in the cytoplasm, are the main organelles responsible for adenosine triphosphate (ATP) production through the Krebs cycle (also known as citric acid cycle or tricarboxylic acid cycle), electron transport chain, and oxidative phosphorylation, to meet the energy demands of human cells. As early as in the 1930s, Otto Warburg, a German biochemist, found that human cancer cells displayed an avid glucose uptake and modified glucose metabolism to generate ATP, including increased lactate fermentation in the cytoplasm and decreased respiration in the "respiration complexes" (known in modern terminology as the mitochondria) even if the oxygen supply is sufficient. The theory of respiratory impairment (i.e., the mitochondrial dysfunction in human cancers) has been adopted since the 1950s by Otto Warburg. Although the vast majority of mitochondrial proteins (about 900) are encoded in the nuclear genome and imported into the mitochondria to maintain the mitochondrial function, there are 13 polypeptides necessary for constitution of the electron transport chain, and 22 tRNAs plus two rRNAs essential for the translation of mitochondrial DNA (mtDNA) transcripts encoded in the mitochondrial genome. To appraise the role of mitochondrial dysfunction in human cancers, the analysis of mtDNA alterations is indispensable. Herein, we will discuss the role of mtDNA instability in human cancers.

2. Mitochondria and Warburg effect in human cancers

Generally, 1 mol of glucose can generate approximately 36–38 mol of ATP, including 2 mol ATP from glycolysis in the cytoplasm (oxygen independent) and 34–36 mol ATP from oxidative phosphorylation in the mitochondrion (oxygen dependent), after a complete glucose metabolism. During glycolysis, 1 mol glucose can be converted into 2 mol pyruvate, which then enters the Krebs cycle through acetyl-CoA for subsequent electron transport and oxidative phosphorylation in the mitochondria. However, under specific conditions of either insufficient oxygen supply (e.g., muscles undergoing prolonged exercise) or impaired mitochondria (e.g., sepsis), pyruvate is transiently reduced to lactate by lactate dehydrogenase in the cytoplasm instead of entering the mitochondria. This kind of modified glucose metabolism is termed anaerobic glycolysis or lactate fermentation, and only 2 mol ATP can be generated.\(^1\)

In sharp contrast to the normal human cells, in addition to the mitochondrial respiration, human cancer cells also exhibit a profound glucose uptake to generate ATP through lactate fermentation, even if the surrounding oxygen supply is sufficient. This special phenomenon, was first observed as early as in 1930s by a German biochemist, Otto Warburg, and reported in the 1950s, it is now called the Warburg effect.\(^2\)–\(^4\) He contended that human cancer cells, unlike normal human cells, can obtain ATP from both oxidative phosphorylation in the mitochondria and lactate fermentation in the cytoplasm with a shifting of decreased ratio in oxidative phosphorylation and increased ratio in lactate fermentation. This kind of metabolic shift prompted Warburg to speculate that the respiratory enzyme complexes (i.e., the mitochondria) in human cancer cells might be impaired or suppressed.\(^2\)–\(^5\)

3. Human mtDNA copy number

In human cells, mitochondria are distributed in the cellular cytoplasm to form a mitochondrial network. The majority of these mitochondria are connected to one another most of the time.\(^6\) Generally speaking, each human cell contains several hundreds to 1000 connected mitochondria, and each mitochondrion has approximately 2–10 copies of mtDNA. As a result, there are several thousand copies of mtDNA molecules distributed in the cytoplasmic mitochondria in a human cell. The number of mtDNA copies in normal human tissues is highly dynamic and varies widely with different cell types and various physiological conditions. Theoretically, the higher the energy demand, the more mtDNA copies are present in the human cells.\(^7\)

4. Structure of human mtDNA

Human mtDNA\(^5\) is located in the inner membrane of the mitochondrion, and is a closed circular and double-stranded DNA structure that contains a total of 16,569 base pairs (bp). The inner strand is called the light (L) strand, and the outer strand is called the heavy (H) strand.\(^7\) As regards the entire human mtDNA, it consists of a non-coding (also called D-loop) and a coding region, and has been sequenced completely (L-strand, revised Cambridge Reference Sequence, rCRS).\(^8\) The D-loop is located from np 16024 through 16569, 1, and then 576 (16024→16569→1→576) with 1124 bp in size and is essential for the regulation of mtDNA transcription and replication. The replication origin of the H-strand and transcription promoters of the L- and H-strands are all located in the D-loop. However, the replication origin of the L-strand is located in the coding region.\(^7,9\) The coding region is located between np 577 and np 16023 and is coding for 13 polypeptides (12 in the H-strand and 1 in the L-strand) that participate in the electron transport and oxidative phosphorylation. Furthermore, the mtDNA is also coding for two rRNAs (in H-strand) and a set of 22 tRNAs (14 in the H-strand and 8 in the L-strand) that are required for protein synthesis in the mitochondria. All 13 mtDNA-encoded polypeptides are required for the assembly of respiratory enzyme complexes, including seven (ND1, ND2, ND3, ND4, ND4L, ND5, and ND6, where ND denotes NADH dehydrogenase) in Complex I, one (cytochrome b) in Complex III, three (COX I, COX II, and COX III, where COX denotes cytochrome c oxidase) in Complex IV, and two (ATPase 6 and ATPase 8) in Complex V.\(^8\) Only ND6 is encoded in the L-strand, and the other 12 polypeptides are encoded in the H-strand. With the exception of 13 polypeptides, approximately 90 polypeptides constituting the respiratory enzyme complexes are encoded in nuclear DNA (nDNA). All subunits of Complex II (succinate-coenzyme Q oxidoreductase) are totally nDNA-encoded. Although mitochondria have their own genomes, all proteins required for the regulation of mtDNA replication and transcription are
encoded in nDNA. Among these regulators, the mitochondrial transcription factor (TFAM) plays a pivotal role because TFAM can bind to the D-loop to regulate the replication and transcription of mtDNA for mitochondrial biogenesis.

5. Hypothesis of mtDNA instability in Warburg effect

One of the most commonly adopted hypotheses to explain the association between human mtDNA instability and Warburg effect in human cancers is oxidative damage and its vicious cycle. As we know, oxidative DNA damage has been demonstrated to play an important role in the carcinogenesis of human cancers. Such an oxidative damage in human mtDNA may cause a dysregulation of human mtDNA with subsequent mitochondrial dysfunction. Impaired mitochondrial function further initiates an improper electron transport and an electron leak. The leaking electron may cause another course of oxidative mtDNA damage. Then, the vicious cycle is sustained in human cancers and the Warburg effect follows. When discussing the mtDNA instability in human cancers, most researchers paid attention to the quantitative mtDNA copy number changes and the qualitative mtDNA D-loop mutations, especially the D310 mutation, with their association to clinicopathological parameters.

6. Human mtDNA damage and mutation

In general, nDNA is inherited from parents with equal contributions, and it is termed as heterozygotic condition. On the contrary, human mtDNA is transmitted exclusively through the maternal lineage with a single origin. Moreover, the majority of mtDNA molecules in the human cells of the postmitotic tissues of an individual are assumed to be identical at birth, and such a situation is termed as "homoplasmy or homoplasmic condition." Because of the following characteristics—absence of introns, lack of histone protection, insufficient DNA repair systems, lower fidelity of DNA polymerase gamma, and increased exposure to reactive oxygen species inside the mitochondria—human mtDNA molecules are far more susceptible to oxidative damage compared to nDNA molecules. When the mutant or damaged mtDNA variants coexist with the wild-type inborn mtDNA, the pattern of homoplasy is disrupted and is shifted to the so-called "heteroplasmy or heteroplasmic condition." With regard to the entire mtDNA structure, damage occurs much more easily in the D-loop region, especially in the two hypervariable regions (HV1 at np 16024 and HV2 at np 57713). Among various kinds of D-loop damage, the D310 mutation (a hot spot in HV2) is the most common one. Within np 303 and np 316 of the D-loop, there is a poly-cytidine (PCT) tract with a thymidine inserted at np 310 (5'-C303CCCCCCCT310CCCCCC316-3'). As a rule, the cytidine number after thymidine remains constant as 6. However, the cytidine number prior to thymidine is highly variable. Generally, the cytidine number prior to the thymidine is seven (7-C, based on the rCRS), but 6-C, 8-C, and 9-C variants have also been reported. Variations of the cytidine number in the D-loop of mtDNA between np 303 and np 309 is termed as D310 polymorphism or D310 sequence variations.

7. Changes of mtDNA copy number in human cancers

The alterations of mtDNA copy number have been evaluated in several human cancers. When compared to the noncancerous counterparts, a decrease in mtDNA copy numbers has been detected in lung cancer, hepatocellular carcinoma, gastric cancer, and breast cancer. In an analysis of 29 lung cancer tissues after neoadjuvant chemotherapy, a decrease of mtDNA copy number was associated with the progression of lung cancer. Furthermore, Wu et al. demonstrated that the decrease of the mtDNA copy number was related to an advanced-stage gastric cancer. All the above findings indicated that the decrease of the mtDNA copy number in human cancers might cause a decrease of mitochondrial function. On the contrary, a progressive increases of the mtDNA copy number was noted in carcinogenesis, a spectrum among normal mucosa, mild dysplasia, moderate dysplasia, severe dysplasia, and invasive carcinoma of head and neck cancers in smokers. Also, when compared to the noncancerous counterpart, an increase of mtDNA copy number was found in thoracic esophageal squamous cell carcinoma (TESSC) and head and neck cancers, especially in those who smoked cigarettes. In TESSC, the increase of the mtDNA copy number from noncancerous esophageal mucosa to TESSC and then metastatic lymph nodes were compatible with the increase of 8-OHdG (8-hydroxyl-2-deoxyguanosine, a marker of oxidative damage on guanine) accumulation in mtDNA of the above tissues. Such an increase was supposed to compensate for the damaged mtDNA to maintain mitochondrial function in proper order. In an in vitro cell line study, it was demonstrated that the high mtDNA copy numbers were associated with a higher invasive activity in TESSC cell lines. Using the knockdown technique to decrease the expression of TFAM, a pivotal protein involved in mtDNA replication and transcription, the mtDNA copy number of TESSC was significantly decreased and the invasive activity was also suppressed. Regardless of the increase or decrease of mtDNA copy number, these mixed results among different human cancers suggested an alteration in mitochondrial function.

8. mtDNA mutation in human cancers

Several authors have evaluated not only the quantitative change in mtDNA copy number, but also the qualitative mtDNA D-loop mutations in human cancers. D-loop mutations, including the D310 region, have been reported in prostate cancer, head and neck cancer, lung cancer, breast cancer, etc. Furthermore, the D-loop mutations were reported to harbor clinicopathological significance in lung cancer and breast cancer. As the D310 region is located near the H-strand replication origin and binding site of TFAM, whether D310 mutation is related to the change of mtDNA copy number and mitochondrial biogenesis in human
cancer remains obscured. However, several studies have demonstrated that D310 is a good marker to trace cancer cell clonal expansion.\(^\text{33,40,42,44}\) An analysis of 72 pairs of TESCC revealed a homoplasmic to heteroplasmic mtDNA D310 distribution from esophageal muscle to esophageal mucosa, and a heteroplasmic to homoplasmic mtDNA D310 redistribution from esophageal nontumor mucosa to TESCC, and then the metastatic lymph node, combined with an increase of mtDNA copy number. This specific finding was compatible with the theory of cancer clonal expansion.\(^\text{33,45}\) As a consequence, the effect of D310 mutations on bioenergetic function of mitochondria in tumor tissues deserves to be further studied in the future.

9. Circulating mtDNA in human cancers

Recently, several investigators have also made great efforts to appraise the alteration of circulating plasma or serum mtDNA in several human cancers and evaluate their clinical implications.\(^\text{46}\) An elevation of circulating mtDNA copy number was noted in patients with urological malignancy,\(^\text{47}\) testicular germ cell cancer,\(^\text{48}\) and lung cancer after radiotherapy.\(^\text{49}\) On the contrary, a decrease of circulating mtDNA copy number was noted in breast cancer.\(^\text{50}\) At present, it is difficult to investigate the sources of these circulating mtDNA copies, and it is still unclear whether these alterations are related to the shifting of cancer metabolism.

In conclusion, because of high susceptibility to oxidative damage and participation in the mitochondrial respiration, the analysis of mtDNA instability is a strategy of choice to evaluate disease progression and drug resistance in human cancers.

References


Web references