REVIEW

Mitochondrial DNA alteration and pathological progression in colorectal cancer

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Mitochondrion, the major organelle involved in cell energy metabolism, plays important roles in many human diseases. Nowadays, its dysfunction during initation and progression of cancers is calling more and more attention. Since Otto Warburg brought out his hypothesis, mitochondrial DNA (mtDNA) may affect on cancer development by regulating oxidative phosphorylation, many important findings have been discovered. It is widely recognized that research the potential correlations between mitochondrion and mtDNA in cancers are of great importance. And the trends of change of mtDNA content are different in different cancers. Thus leaves us numerous works to do. Although some cancers have already been reported their relation with mtDNA alteration, different experimental methods or evidence may lead to different conclusions. This article presents a brief in summarizing the mitochondrion dysfunction in human disease and the alteration of mtDNA in cancers. Moreover, the recent highlighting achievements on colorectal cancer and mtDNA are also summarized to provide a discussion on possible pathological mechanism and clinical usage.

Keywords: mitochondrion; mtDNA alteration; colorectal cancer; energy metabolism; D-loop region

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Introduction

As early as in the mid19th century, the intracellular organelles have been described by cytologists [1]. However, the term of mitochondrion was created until 1898. But a long time after that the function of mitochondrion remained undetermined. Till the mid20th century, the function of mitochondrion was firstly systemically described, its role in oxidative energy metabolism [2]. By discovering the function of mitochondria, it is very easy for researchers to link it with the cancer cell. It was almost the same time as people identifying the function of mitochondrion that some researchers started the field of role of mitochondrion in cancer research [3-5].

Otto Warburg, the Noble laureate of 1931, brought out his hypothesis which is also called “Warburg effect” to explain the significant high energy metabolism of cancer cell.

Following Warburg's research, an increasing number of researchers have focused on the metabolic activity of cancer cells. By studying the respiratory tumor, Burk and Schade published their article on Science in 1956, saying "Respiratory impairment in living cancer cells, first described by Otto Warburg in 1923, is an experimental fact, and not, as described by Weinhouse" [6]. After that, Weinhouse who disagreed with Warburg's hypothesis at first, also declared that the high activity of pyruvate kinase might be the primary factor leading to high glycolysis in given tumors, as well as Weinhouse's collaborators [7-9]. But Warburg's researches didn't truly prove that the increased energy metabolism in cancer cell is caused by up-regulated glycolysis or oxidative phosphorylation. At first, many researchers believed that the energy of cancer cell is supplied by glycolysis, moreover, such energy supply is limited in glycolysis, and the mitochondrion is not involved.

Basing on the development of biochemical test methods, NADH level monitoring has been used in vitro and in vivo during the past half century [10, 11]. This provides us a method to investigate the energy metabolism level of cancer tissues. In 2004, Zu and Guppy reviewed the experimental data in 40 years and declared that "there is no evidence that cancer cells are inherently glycolytic, but that some tumors might indeed be glycolytic in vivo as a result of their hypoxic environment" [12]. Furthermore, in 2006, Shaw and colleagues concluded that a change in cellular glucose metabolism is the crucial biochemical hallmark in cancer cells [13].

The aims of this paper are to present a general review on mitochondrial energy metabolism and cancer energy metabolism. And also provide a proposal that could be investigated and used as a clinical diagnostic method in the future.

Mitochondrial DNA (mtDNA) and mitochondrial function

To breakdown glucose to supply ATP consists of two procedures, named glycolysis (occurs in cytoplasm) and oxidative phosphorylation (occurs in mitochondrion). The mitochondrion plays an important role in ATP production as well as many other biochemical processes such as iron and calcium homeostasis and cell apoptosis. Human mtDNA exists inside the cytoplasm of mitochondrion. It is the only genetic material outside the cellular nucleus. mtDNA is a 16.6-kb closed circular double-stranded DNA molecule, which contains no introns and has no protective histones. It encodes 13 respiratory chain polypeptides, and 2 ribosomal and 22 transfer RNAs [14].

As mentioned above human mtDNA contains 37 genes without any introns, but it contains a non-coding region (D-loop region) which consists of the elements involved in replication and transcription [15]. The function of mtDNA is relatively independent, so far no strong evidence indicates that nuclear DNA(nDNA) is controlling or regulating the replication and transcription of mtDNA. The mtDNA can replicate regardless to cell cycle [16, 17]. Its replication is executed by mtDNA polymerase gamma (PolG) [18].

Unlike nDNA, mtDNA is presenting at extremely high levels, usually 10^3-10^4 copies per cell [19]. But this number can be different in different tissue or cell. Moreover, this number can also be changed during aging process or under different physiological and/or pathological states. Furthermore, each mitochondrion contains several copies of mtDNA. And the number of mitochondrion in different cells can be different basing on the requirement of energy of the cell. For example, a brain cell may contain over 2000 mitochondria [20], while a white blood cell may only contain less than 100 [21]. Even to a special cell type, such number also can be different depending on the stage of the cell cycle, the stage of differentiation, the extracellular environment and many cell signaling mechanisms [22, 23]. Such statements above explained the mtDNA content varies.

The mtDNA damage and mitochondrial function during aging process has been detected for almost 60 years already. The mainstream theory in this area is the mitochondrial free radical theory of aging (MFRTA), which is brought out by Harman [24, 25]. This theory base on the structural difference between nDNA and mtDNA, which is the mtDNA is not protected by histones. And Harman believes that the reactive oxygen species (ROS) is particularly rich in mitochondrion. Under the attack of ROS, the unprotected mtDNA and other cellular macromolecules are very easy being damaged, eventually, result in mitochondrion breakdown, even cell death [26]. Truly, some other researchers hold different opinions. For example, Rissignol *et al*. published their opinion in 2003, they believe that mtDNA mutation frequency cannot reach a critical threshold to lead mitochondrial dysfunction during aging [27]. And a similar opinion is also published by Larsson in 2010 [28]. Still and all, one thing in common, aging process does increase the mutation frequency of mtDNA and the number of dysfunctional mitochondrion. Some researchers also reported that the mtDNA content increases in brain and lung during the aging process by using quantitative PCR analysis [29, 30].

Furthermore, numerous studies have showed that the mutational rate of non-coding D-loop region is about 10 times higher than that of coding region [31]. After studied 5140 human genomes, Pereira *et al*. reported that the polymorphic positions within the coding region are only 6%

of all polymorphic positions [32, 33]. More important, by analyzing of the coding region of global population researchers found that the most polymorphic positions were genes encoding two subunits of ATP synthetase, NADH dehydrogenase subunit 6 and cytochrome b [32, 34]. Such recent reports indicate that it is possible for mtDNA lost its function by alterations.

Nowadays, many researches involve in detecting the mitochondrial function, the mtDNA content is a commonly used stander. It is measured as Mt/N (mitochondrial to nuclear genome ratio) by real time quantitative PCR (qPCR) [35, 36]. Normally, this ratio is within a certain range to a particular type of cell. But under the condition of increased oxidative stress, the ratio would increase beyond the normal range. If oxidative stress keeps a long time, it could present as a mixture of functional mtDNA and damaged dysfunctional mtDNA. And the damaged mtDNA could not be cleaned by the mitochondrion or cell.

Change of mtDNA content

As early as 1999, some researchers have already reviewed that numerous diseases such as neurodegenerative disease, diabetes, cancer and aging process are associated with acquired mutations in mtDNA [37-39]. While reviewing these papers and some other studies, we noticed that not only in disease and aging process, but also in development and exercise are involved in mtDNA content change.

mtDNA content and development

To fetal development many researched pointed out the change of mtDNA content is related to many developmental events. In 2004, Kao *et al*. reported that poor quality sperm that could be responsible for infertility is related to reduce mtDNA content [40]. But 3 years after, Amaral *et al*. published their opinion that high quality sperm has lower mtDNA content but with high levels of transcription factor [41]. On the other hand, to the oocyte lower mtDNA content was found in ovarian insufficiency [42], and it is responsible for lower developmental potential [43]. While during the pregnancy, the mtDNA content in mother's circulating blood is significantly reduced during the fetal development, however, the cases of intrauterine growth restricted pregnancies mtDNA content showed marked increase comparing to normal pregnancies [44]. Furthermore, in human cord blood, mtDNA content also showed close relation to child's birth weight [45].

mtDNA content and human diseases

Numerous reports showed that the mtDNA content is related to many human diseases, not only the sick organ, such changed mtDNA content even can be measured in circulating blood. Biliary atresua, a liver disease associate with high oxidative stress, has been tested to be associate with marked decreases of mtDNA content in liver [46]. Such similarly down-regulated mtDNA content trend was also identified in non-alcoholic fatty liver [47]. In fact such liver diseases all accompany with a high level of oxidative stress, which indicates that the change of mtDNA content is caused by the high extracellular oxidative stress environment. While in chronic renal failure cases the increased mtDNA content was identified in white blood cell. Although this disease is accompanied with high oxidative stress too, the inflammation is also involved [48]. The similarly increased mtDNA content also showed in septic shock, while testing the blood of patients [49]. The degree of abnormal mtDNA content is believed related to disease severity [50]. Another example of inflammation can cause increased mtDNA content is nasal polyps, which is believed being caused by inflammation mainly. In 2009, Park *et al*. tested the inflammatory tissue of nasal polyps and non-polyp tissue found that the mtDNA content is markedly increased [51]. The data above indicates that unlike high oxidative stress the inflammation regulates mtDNA content downwards. But the situation of diseases related to nerve tissues is very complex. Autosomal dominant optic atrophy associated with decreased mtDNA content [52]. Blokhin tested neurons from multiple sclerosis found decreased mtDNA content [53]. While studying multiple sclerosis lesions Witte found enhanced numbers of mitochondrion [54].

Mitochondrion and cancers

Mitochondrial oxidative phosphorylation was first present by Louis Pasteur in 1857. His study found that under anaerobic extracellular microenvironment the glucose metabolism produces lactic acid, and this progress can be depressed by increasing oxygen (the Pasterur Effect). This theory was being put in explaining the cancer cell energy metabolism in a long time. But as early as in the 1920th, Warburg described "aerobic glycolysis" in cancer cells, which stand on the opposite side to the Pasterur Effect. Moreover, in recent decades the researches on cancer focusing on signal transmitting and apoptosis all based on Warburg's theory that mitochondrial defects may result in cancer, although these research areas do not directly relate to energetic metabolism. In 2006, Brandon reviewed the previous studies and concluded that "mitochondrial dysfunction does appear to be a factor in cancer etiology, an insight that may suggest new approaches for diagnosis and treatment" [55]. The renal cancer metabolism basing on a large number of experimental and clinical studies, by Simonnet in 2002 and Godinot in 2007, presents six fields related to mitochondrial function or dysfunction, which shows us the

complex relation between mitochondrion and cancer [56, 57].

mtDNA content and cancer

Detecting the mtDNA content in cancers is a new researching field that appeared after the year of 2000. The researchers depending on measuring the mtDNA content of the tumor tissue or cell directly showed different trends in different cancers. Kim *et al*. studied the premalignant and malignant lesions in head and neck, and found that the quantity of mtDNA increases with histopathologic grade [58]. By using micro-dissection method to obtain the endometrial adenocarcinoma cell, Wang *et al*. reported that the mtDNA content showed a marked increase, compared to normal endometrial glandular epithelial cell [59]. In 2008, Mizumachi *et al*. tested the mtDNA content in prostate cancer cells as well as in prostate cancer cell lines, also reported that the abnormally increased mtDNA content was tested, and they believed the increased mtDNA content can be considered as a typic feature of prostate cancer cell [60]. In esophageal squamous cell carcinomas, the high level of mtDNA content is also reported in 2010. And the authors conjectured that the up-regulated mtDNA content is related to the fast growth of cancer cells [61]. All these studies above may show us the potential interconnection between increased mtDNA and cancer cells, but, other reports also showed the converse trend. As early as the year of 2004, one research on renal carcinoma tumor showed that the significant decreased mtDNA content was tested in 34 out of 37 cases, which indicates that mtDNA is more likely to decrease rather than to increase its content in renal cancer [62]. Another report on hepatocellular carcinoma also pointed out the decreased mtDNA content can occur in cancer cells in 2006 [63]. At the same year a report on ovarian cancer also showed mtDNA content could be down-regulated, furthermore, such a down-regulated pattern is associated with the progression of ovarian cancer [64]. In recent years, many researchers focus on breast cancer and its possible relation with mtDNA, Yu *et al*. reported an inverse correlation in 2007. This report showed the mtDNA content decreases during the tumor progression of breast cancer [65]. Later in 2009, Fan *et al*. reported that basing on clinical case analysis 82% of malignant breast tumor accompany with lower mtDNA level compared to normal breast tissue [66]. In 2011, the mtDNA content in breast cancer was tested again in 302 cancerous breast tissues and in their corresponding surrounding normal tissues, the result also showed the cancerous tissue contains lower mtDNA content than normal ones [67].

On the other hand, almost at the same time, many researchers aim at monitoring the mtDNA content in circulating cells or body fluid showed us another correlation between cancer and mtDNA content. In 2005, Jiang *et al*. reported that high level of mtDNA content can be tested in the saliva of head and neck cancer patients and the increased mtDNA content correlated with the tumor diagnosis [68]. In Lan's research, increased mtDNA content of peripheral white blood cell was proved to be a risk factor that related to non-Hodgkin lymphoma [69]. The high content of cell free mtDNA in serum is involved in testicular cancer [70]. Interestingly, the mtDNA content in peripheral blood was reported decreasing in stage 1 breast cancer [71], while it is also reported out its increase can be considered as a significant risk of breast cancer [72]. The down-regulated mtDNA level in peripheral white blood cell was also reported accompanying with Hepatitis B virus related hepatic cancer [73].

mtDNA and colorectal cancer

The most studied region of mtDNA was the mononucleotide repeat region which is located between nucleotide 303 and 315 in revised Cambridge Reference Sequence (rCRS) also is called D310. This fragment may consist of 12 cytosines disrupted by thymine. Just because this region is extremely unstable, so usually accounting the phylogenetic classification it is not included. Mutations in this fragment usually show as one nucleotide insertion or deletion, such mutations can be identified around 23% to 44% of colorectal cancer patients [74, 75]. On the other hand, no other clinical features such as gender, age, cancer stage were reported involved in colorectal cancer studies [76].

Another worth mentioning DNA alteration is microsatellite sequence instability. It occurs in the nuclear genome and related to polymerase slippage during the cell or DNA replications and also has great influence on DNA repair genes, such as hMSH2, hMLH1, hPMS1 and hPMS2. At first, some researchers conjectured that the abnormally expressing of such DNA repair genes plays important roles in mtDNA replication as well as its mutations. But no evidence appears supporting the correlation between mutations of mtDNA and microsatellite instability [33, 77-79]. One important gene that is believed directly involved in mtDNA replication that is mtDNA polymerase gamma (POLG), which encodes the DNA polymerase in human mitochondria. Singh *et al*. recently reported that the dysfunctional POLG can lead to down-regulated oxidative phosphorylation level in breast cancer as well as to raise the tumor risk.

By using dideoxy sequencing method, the complete mtDNA genome sequences were determined in 10 colorectal cancer cell lines and non-cancerous corresponding tissues, 1 homoplasmic insertion in mtDNA mononcleotide repeat region were found, and 2 heteroplasmic as well as 9 homoplasmic substitutions in mtDNA coding region were

also found cell line [80]. Another research basing on cancer and non-cancerous tissues of colorectal cancer patients reported 3 homoplasmic substitutions and 7 insertions or deletions in D-loop were identified in 7 out of 11 colorectal cases [76]. Furthermore, Wang *et al*. tested 20 colorectal cancer tissues and their corresponding non-cancerous tissues and found 8 heteroplasmic substitutions [81]. Recent study is showing that by measuring the whole mtDNA sequences in 10 cancerous tissues 21 somatic mutations were found in 9 out of 10 cases. And 90% of the mutations were found in heteroplasmic state [82].

In 2009, Huang P *et al*. test the copy number of the ND1 gene of mtDNA in 50 colorectal cancer tissues. And found that a significant decrease of mtDNA content, further more they also detected the microstatellite instability of mtDNA and conjectured that there might be a potential possibility that the down-regulated mtDNA content is caused by microsatellite instability [83]. But in this research, the authors monitored the ND1 level and used that as the level of mtDNA, which remains a problem that is, the replication of and expression of mtDNA might be different. After this research, in 2011, Shi F *et al*. reported that mtDNA copy number showed significant increase in colorectal cancer, furthermore, by analysis the experimental results and clinicopathological stages found that such increase is associated with the tumor stage but not related to the gender, age or other clinical data [84]. In order to further detect the correlation between increased mtDNA content and oxidative phosphorylation in colorectal cancer, Shi F *et al*. measured the levels of mRNA and protein expressions of ND2 gene, and reported the ND2 expression is also up-regulated, moreover, they also tested the DNA methylation status of D-loop region and found the D-loop region tends to demethylation status [85, 86]. Another report aiming at detecting the mechanism that regulates the mtDNA content in colorectal cancer was published in the year of 2011. The authors detected 104 colorectal cases and found that the mtDNA 4,977-bp deletion may be a reason that causes the alteration of mtDNA content in the early stage of colorectal cancer [87].

It is the same as other cancer researchers, the peripheral circulating cell mtDNA change also showed the association with colorectal cancer. In 2011, Qu F *et al*. tested the white blood cell mtDNA content of 320 colorectal patients and compared that result with 320 controls by real-time PCR. Through a trend analysis, they found that the high mtDNA content in peripheral white blood cell was linked to elevated colorectal cancer risk [88].

Conclusions

It is widely recognized that research the potential correlations between mitochondrion and mtDNA in cancers are of great importance. And the trends of change of mtDNA content are different in different cancers. Thus leaves us numerous works to do. Furthermore, even some cancers have already been reported their relation with mtDNA alteration, but different experimental methods or evidence may lead to different conclusions. That also compels us to detect deeper. On the other hand, the mechanism leads to the alteration of mtDNA in cancer still remains too much undetermined. Exploring these questions might provide us a new way to struggle with cancers. Furthermore, till now, we know that the mtDNA content of peripheral cells does change in many cancers, and it seems to be always increased. Nonetheless, we still lack of researches on detecting the increased peripheral cell mtDNA content. Whether such increases are different in different cancers and what caused such increase? That is also a question required being answered. Many laboratory researchers showed a significant increase or decrease on mtDNA content in cancerous tissues. In many of these researches even declare that such increase or decrease is associated with tumor stage. May measure the mtDNA status can be used as a clinically applicable marker in colorectal and other cancers, still requires more researches.

Conflicting interests

The authors have declared that no competing interests exist.

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Abbreviations

mtDNA: mitochondrial DNA; nDNA: nuclear DNA; qPCR: quantitative PCR; rCRS: revised Cambridge Reference Sequence.

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JH Gao, SL Wen, HY Zhou and S Feng wrote and revised the manuscript. HY Zhou and S Feng designed the concept and structure of this review. S Feng obtained funding.

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