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Review Article

A Review on the Role of Mitochondrial DNA Mutations in Cancer

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Abstract

Mitochondria implement various cellular functions, including energy production through the electron transport chain by oxidative phosphorylation mechanism. These respiratory chains consist of several complexes and protein subunits which are encoded by nuclear and mitochondrial genes. Due to mutation susceptibility and repair limitation, more aberrations have occurred in mitochondrial DNA in comparison to nuclear DNA. Given the fact that mitochondrial DNA lacks introns, mutations almost occur in the coding sequence, which comprises a direct impact on its functions. Emerging evidence indicates that mutations in the mitochondrial DNA led to the production of reactive oxygen species, disrupted apoptosis, and tumor development. Studies reported various somatic and germline variants in mitochondrial DNA related to tumorigenesis. The D-loop region which is the starting point for replication and transcription of mitochondrial DNA is the most prevalent site of somatic mutations in solid tumors. The D-loop mutations also cause copy number variations which are gaining interest in studies of solid tumors including breast cancer, colon cancer, hepatocellular carcinomas, and prostate cancer. Most studies have reported a mitochondrial DNA reduction which subsequently prevents apoptosis and promotes metastasis. The mitochondrial DNA region-specific haplogroups are also involved in the sequence variations due to processes such as genetic drift and adaptive selection.

This review article discusses the biology and function of mitochondria and related genes. By explanation of mitochondrial dysfunction caused by different kinds of alterations, we attempt to elucidate the role of mitochondria in tumorigenesis. Prominently published articles in this field were reviewed and the role of germline and somatic mutations of mitochondrial DNA have been investigated in common cancers.

Keywords: Mitochondria; mtDNA Copy Number; Tumorigenesis; Polymorphisms.

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Introduction

Mitochondria are the main center of energy production in the cell that also regulate reactive oxygen species (ROS) and apoptosis. In addition to these activities, mitochondria are involved in signaling, survival, immunity, and cell homeostasis. Healthy cells are made up of hundreds of mitochondria that supply energy by oxidative phosphorylation (OXPHOS) through their electron transport chain (ETC) (1, 2). Each mitochondrion contains several copies of the mitochondrial genome that encode proteins involved in the respiratory chain. Mitochondria have their specific and independent transcription, translation, and also protein assembly machinery (1). Diseases caused by mitochondrial dysfunction are either due to mutations in the nuclear genome (nDNA) or in the mitochondrial genome (mtDNA). Both somatic and germline mutations of the mtDNA have been detected in a variety of cancers including breast, prostate, and colorectal tumors. Cancer cells get energy from aerobic glycolysis and the accumulation of ROS due to mitochondrial dysfunction is their common phenomenon (3, 4). Mitochondrial dysfunctions also prevent apoptosis and further lead to cancer cell growth (5).

The study of mitochondrial function in the pathogenesis of cancer is of particular importance. In this review, we aimed to delineate the role of mitochondria in the development of cancer from different perspectives.

1. Mitochondria

1.1. Structure and function

Structurally, mitochondria are double-membrane organelles. The inner membrane has a greater surface in comparison to the outer membrane, which can be diverse based on the tissue and cell types. Enzymes involved in the OXPHOS are located in the inner membrane (6).

Adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) are produced through the mechanism of OXPHOS in the ETC. Besides OXPHOS, mitochondria direct several other activities including fatty acid oxidation (FAO) and tricarboxylic acid cycle (TCA cycle). A variety of amino acids, lipids, carbohydrates, and nucleotides are produced by these organelles (7).

The mitochondrial OXPHOS is controlled by four respiratory and one ATP synthase complexes including complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (ubiquinone-cytochrome c oxidoreductase), complex IV (cytochrome C oxidase (COX)) and complex V (ATP synthase). All these complexes are located in the inner mitochondrial membrane. In addition to these complexes, two-electron carriers called coenzyme Q (CoQ or ubiquinone) and Cyt c is involved in OXPHOS (8).

Defects in the activity of the complex I due to mtDNA mutations play a role in cancer progression by increased ROS production. Since only 7 of 46 polypeptides of complex I are encoded by mtDNA and the rest are encoded by nuclear genes, the defect in this complex could be due to the nDNA mutation, as well (6, 9). In addition to complex I, some subunits of the complexes III (1 mtDNA+ 10 nDNA), IV (3 mtDNA+ 10 nDNA) and V (2 mtDNA+ 14 nDNA) are encoded by both the mtDNA and nDNA, while all 4 subunits of complex II are encoded by the nDNA. As a result, alterations in the mitochondrial genome disrupt the complexes of the mitochondrial respiratory chain except for complex II (10).

Table 1 indicates the nDNA encoded genes related to each OXPHOS complex. As presented, they are scattered throughout the human genome and vary in size and number of amino acids.

Mitocheckpoint controls mitochondrial function and regulates apoptosis and anti-apoptosis signals. In the case of mitochondrial dysfunction, mitocheckpoint maintains normal mitochondrial function but prevents the production of defective mitochondria (11).

The mitocheckpoint proteins interact synergically with cell cycle checkpoint proteins. It has been shown that in oxidative stress, cyclin B1 translocated from the nucleus to the cytoplasm and mitochondria (12). One of the key mitocheckpoint proteins is p53, which plays an important role in mtDNA copy number (CN) and mitochondrial biogenesis (13). By RNA sequencing Nagano et al showed that p53 regulated dihydropyrimidinase-like 4 (DPYSL4) which is involved in cancer invasion and tumor growth. DPYSL4 is linked to mitochondrial supercomplexes and is localized in mitochondria (14).

1.2. Human mtDNA

Exclusively transmitted maternally, the mtDNA genome is a circular double-stranded molecule. It comprises 16,569 bp and has 37 genes that encode 12S and 16S ribosomal RNAs (rRNAs), 22 transfer RNAs tRNAs), and 13 essential proteins for respiration. As shown in Figure 1, 13 essential protein-coding genes are; 7 subunits of complex I (ND1, ND2, ND3, ND4L, ND4, ND5, and ND6), 1 complex III subunits (cytochrome b (Cyt b)), 3 subunits of complex IV (Cyt c oxidase, COXI, COXII and COXIII) and 2 subunits of complex V (ATP6 and ATP8). All genes encoding rRNAs and proteins involved in the OXPHOS system are located on the H-strand except the ND6 gene (15).

Table 1. Nuclear encoded genes related to each OXPHOS complexes.

Complex	Nuclear Genes (Chromosomal location, Amino acid number)

I	NDUFS1(2q33.3, 727aa) ; NDUFS2(1q23.3 , 463aa) ; NDUFS3(11p11.2 , 264aa) ; NDUFS4(5q11.2 , 175aa) ; NDUFS5(1p34.3 , 106aa); NDUFS6(5p15.33 , 124aa) ; NDUFS7(19p13.3 , 213aa) ; NDUFS8(11q13.2 , 210aa) ; NDUFV1(11q13.2 , 464aa) ; NDUFV2(18p11.22 , 249aa) ; NDUFV3(21q22.3 , 473aa) ; NDUFA1(Xq24 , 70aa) ; NDUFA2(5q31.3 , 99aa) ; NDUFA3(19q13.42 , 84aa) ; NDUFA4(7p21.3 , 81aa) ; NDUFA5(7q31.32, 116aa) ; NDUFA6(22q13.2 , 128aa) ; NDUFA7(19p13.2 , 113aa) ; NDUFA8(9q33.2 , 172aa) ; NDUFA9(12p13.32 , 377aa) ; NDUFA10(2q37.3 , 355aa) ; NDUFA11(19p13.3 , 141aa) ; NDUFA12(12q22 , 145aa) ; NDUFA13(19p13.11 , 144aa) ; NDUFB1(14q32.12 , 58aa) ; NDUFB2(7q34 , 105aa) ; NDUFB3(2q33.1 , 98aa) ; NDUFB4(3q13.33 , 129aa) ; NDUFB5(3q26.33 , 189aa) ; NDUFB6(9p21.1 , 128aa) ; NDUFB7(19p13.12 , 137aa) ; NDUFB8(10q24.31 , 186aa) ; NDUFB9(8q24.13 , 179aa) ; NDUFB10(16p13.3 , 172aa) ; NDUFB11(Xp11.3 , 163aa) ; NDUFAB1(16p12.2 , 156aa) ; NDUFC1(4q31.1 , 76aa) ; NDUFC2(11q14.1 , 119aa)
II	SDHA(5p15.33 , 664aa) ; SDHB(1p36.13 , 280aa) ; SDHC(1q23.3 , 169aa) ; SDHD(11q23.1 ,159aa)
III	UQCRB(8q22.1 , 111aa) ; UQCRH(1p33 , 91aa) ; UQCRQ(5q31.1 , 82aa) ; UQCRC1(3p21.31 , 480aa) ; UQCRC2(16p12.2 , 453aa) ; UQCR10(22q12.2 , 63aa); UQCR11(19p13.3 , 56aa) ; UQCRFS1(19q12 , 274aa) ; CYC1(8q24.3 , 325aa) ; CYCS(7p15.3 , 105aa)
IV	COX4-1(16q24.1 , 169aa) ; COX5a(15q24.2 , 150aa) ; COX5b(2q11.2 , 129aa) ; COX6a(12q24.31 , 109aa) ; COX6b(19q13.12 , 86aa) ; COX6c(8q22.2 , 75aa) ; COX7b(Xq21.1 , 80aa) ; COX7C(5q14.3 , 63aa) ; COX8a(11q13.1 , 69aa) ; COX8c(14q32.12 , 72aa)
V	ATP5F1A(18q21.1 , 553aa) ; ATP5F1B(12q13.3 , 529aa) ; ATP8A2(13q12.13 , 1188aa) ; ATP5F1D(19p13.3 , 168aa) ; ATP5F1E(20q13.32 , 51aa) ; ATP5PB(1p13.2 , 256aa) ; ATP5MC1(17q21.32 , 136aa) ; ATP5PD(17q25.1 , 161aa) ; ATP5ME(4p16.3 , 69aa) ; ATP5MF(7q22.1 , 94aa) ; ATP5MG(11q23.3 , 103aa) ; ATP5PF(21q21.3 , 108aa) ; ATP5IF1(1p35.3 , 106aa) ; ATP5PO(21q22.11 , 213aa)

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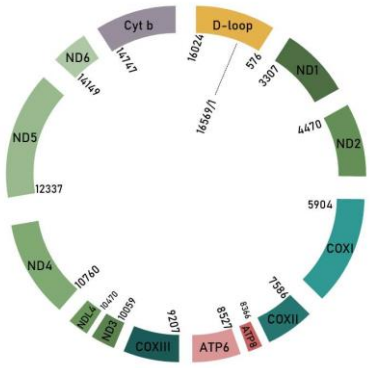

Complex	Mitochondrial Genes (location, Amino acid number)
I	ND1 (3307-4262 , 318aa) ; ND2 (4470-5511 , 347aa) ; ND3 (10059-10404 , 115aa) ; ND4L (10470-10766 , 98aa) ; ND4 (10760-12137 , 459aa) ; ND5 (12337-14148 , 603aa) ; ND6 (14149-14673 , 174aa)
III	cyt b (14747-15887 , 380aa)
IV	COXI (5904-7445 , 513aa) ; COXII (7586-8269 , 227aa) ; COXIII (9207-9990 , 261aa)
V	ATP6 (8527-9207 , 226aa) ; ATP8 (8366-8572 , 68aa)

Figure 1: The mtDNA genes related to OXPHOS complexes. Numbers represent the region in the mitochondrial genome where the gene begins. The D-loop region is located at 16024–16569 and 1–576. The below table indicates the size of the encoded proteins.

The occurrence of mtDNA replication is completely independent of the cell cycle and nuclear DNA replication. DNA polymerase γ (POLG1 and POLG2) and mitochondrial transcription factor A (TFAM) are proteins that are encoded by nuclear genes and are required for mtDNA replication. Therefore, mutations in these genes can alter mtDNA content and ultimately disrupt their mitochondrial function (16, 17). However, mtDNA has a higher rate of mutation frequency due to the lack of repair system and/or histone proteins. The number and type of the mutations can influence various aspects of cellular bioenergetics (18).

Less than 3% of the mtDNA is non-coding which mainly located in the displacement loop (D-loop) at 16024–16569 and 1–576 (Figure 1). This area is 1.1 kb in size and is associated with the replication and transcription of mtDNA which is regulated by transferred nuclear-encoded proteins (19). D-loop region is composed of two hyper-variable areas (HV1 and HV2) which are positioned at 16024-16383 and 57-372, respectively (15, 20). Single nucleotide polymorphisms (SNPs) of the D-loop region including HV1 and HV2 were connected to the development of various cancers (20, 21).

2. Variation aspects of mtDNA

Polyplasmia is a fundamental characteristic of mtDNA which arises from its high CN compared to nDNA in the cell (10-10000 copies). Based on the distribution of normal or abnormal mtDNA between daughter cells, it results in homoplasmy (identical mtDNA variants) or heteroplasmy (different mtDNA variants) (18, 22).

The ultimate dominant phenotype of the cell depends on both features of the mutation and the proportion of the heteroplasmy (23, 24). The heteroplasmy explains the diverse phenotypes between members of a family bearing an identical pathogenic mutation. Not only homoplasmic but also heteroplasmic mutations have been contributed to cancer cell progression (25, 26). Notably, pathogenic mtDNA mutations are mainly heteroplasmic, while benign polymorphisms usually represent homoplasmic status (27, 28).

In addition to heteroplasmy, other essential variations are seen in mtDNA. Haplogroups are genetic groups with one or more specific mutations in mtDNA. They involve funder mutations inherited from a common ancestor (29). As it is presented in Figure 2, haplogroups M and N are derived from an African haplogroup called L3 and are known as the founders of all Eurasians. The most common haplogroups in modern African are L0-6, while HV and X are dominantly found in modern European. In the modern Asian populations, B, F, C, M, D and CZ haplogroups are mostly reported (30, 31).

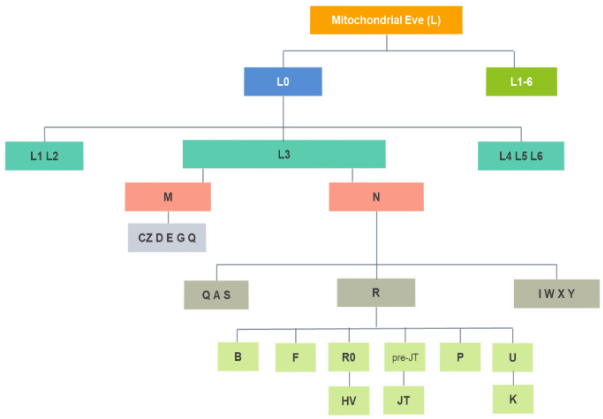

Figure 2: Human mtDNA haplogroups. Each haplogroup originates from a preceding haplogroup and remains part of it. Mitochondrial Eve is the matrilineal most recent common ancestor of all humans.

3. mtDNA mutations and copy number variation in cancer

Based on previous researches, mtDNA is ten times more susceptible to mutations than nDNA. Lack of the introns, histone proteins, and repair machinery in mtDNA could explain this finding. Both somatic and germline mtDNA substitutions are involved in a variety of cancers. Mutational mtDNA evaluation in 1675 tumor samples revealed that C > T and A > G largely occur on the H-strand (3).

The mtDNA mutations are either point mutations (deletions, insertions, substitutions) or mtDNA copy number variations (CNV) (32). The mutations that occur in the D-loop sequence cause changes in the CN of mtDNA. mtDNA CNV has been seen in many cancers, including breast cancer, colon cancer, hepatocellular carcinomas, and prostate cancer (33-35). Factors that reduce mtDNA content include mutations in the D-loop region of the mtDNA and

mutations in the POLG which is encoded by the nuclear gene. These proteins are essential for the replication of the mitochondrial genome (36). Most cancers have some form of mtDNA-depleted or reduced status, although an increase in mtDNA CNV has also been reported (32). Environmental interfering factors can be the cause of this deviation. A recent study revealed that mtDNA CNV was significantly higher in HPV-positive cervical cancer patients than controls. HPV stimulates chronic ROS and mtDNA damage that implicate mitochondrial replication and CN in cervical cells (37). Using an array-based digital polymerase chain reaction (PCR) in ulcerative colitis-associated colorectal cancer (CRC), an increased mtDNA CNV was reported (38).

The mtDNA variations have been studied from different aspects in tumorigenesis. A new study examined the response to chemotherapy medication in different mtDNA haplogroups by using transmitochondrial cybrids. Transmitochondrial cybrids are cellular models to study the role of mitochondria in the same nuclear genome background with different mtDNA. They showed that following cisplatin treatment haplogroup J had lower levels of ROS than haplogroup H. In addition, haplogroup J was more sensitive to cisplatin, indicating that mtDNA is involved in the expression of genes influencing the resistance and side effects of cancer drugs (39).

3.1. Breast cancer

Breast cancer is one of the most prevalent cancers and the second factor of cancer mortality among women (40, 41). Estrogen receptor-negative (ER-) and progesterone receptor-negative (PR-) breast tumors exhibited more somatic mutations in the D-loop region than estrogen receptor-positive (ER+) and progesterone-receptor positive (PR+) tumors (42).

Earlier studies linked several large deletions of mtDNA including 4977bp and 4576bp deletions, to breast cancer pathogenesis (43). It has been shown that the majority of the mutations related to breast cancer are somatic mutations located in the D-loop region. The other reported mutations were in the ND1, ND4, ND5, and Cyt b genes (44).

In 2011, a research study showed that two types of mtDNA mutations, the 12084C > T in the ND4 and the 13966 A > G in the ND5 gene, reduce the activity of the complex I in the highly invasive MDA-MB-231 cell line of breast cancer and ultimately impair mitochondrial function (9).

Canter et al. reported that 10398A germline SNP located at ND3 gene along with other risk factors can be important in the case of breast cancer (45). Some additional studies confirmed 10398A > G SNP as a risk factor, while others have found no significant association between this SNP and breast cancer. A study by Darvishi et al. reported the mitochondrial allele 10398A as a risk factor for sporadic breast cancer as it elevates electron leakage rate and the overproduction of ROS (46). Czarnecka et al. found a higher frequency of the mitochondrial allele 10398G in the Polish breast cancer patients than the control population and identified it as a risk factor (47). Contrary to these studies, Pezzotti et al. found no link between 10398A > G SNP and breast cancer susceptibility (48).

Jiang et al. also reported no association between 10398 A > G substitution and breast cancer. However, they observed decreased mtDNA content in premenopausal women with breast cancer (49). It had also been previously reported that a reduction in the mtDNA CN increased the production of ROS and mitochondrial impairment in breast tumors (42). Contrary to these results, in a prospective cohort with 6-years separate blood sampling, higher mtDNA CN was associated with increased breast cancer risk (35).

3.2. Gastric cancer

Various genetic and epigenetic alterations such as microsatellite instability (MSI) are responsible for the development of gastric cancer which is the third cause of cancer mortality (50). It has been identified that the majority of mitochondrial somatic mutations were associated with increased MSI of the nDNA in gastric cancers (51). One of the earliest pieces of evidence indicating mtDNA involvement in gastric cancer was provided in 1995 by demonstrating a somatic 50-bp deletion in the D-loop region (52).

Research study conducted by Wang et al. showed a significant association between gastric cancer and SNPs in D-loop including, 73G > A, 235A > G, 324C

> G, 16362T > C and 16519C > T (53). By analyzing peripheral blood leukocytes (PBL) Liao et al. observed no association between leukocyte mtDNA content and risk of gastric cancer. However, they identified that mtDNA CN can be important in the early stage of cancer progression (54).

In late-stage tumors, increased mtDNA content showed a significant link to poor prognosis and worse survival of gastric cancer patients (55). These results were confirmed by the observation of the association between high mtDNA CN and increased gastric cancer risk in a Chinese population (56).

In Gastroenteropancreatic Neuroendocrine Neoplasm (GEP-NEN), Er et al. identified survival SNPs including 16257C > A, and 150C > T located in the D-loop (57). In a separate previous study by the same team, 73G, 150T, 151T, 492C, 16257A, 16261T, and 16399G were recognized as the risk evaluation markers for GEP-NEN. They revealed the role of SNPs located in the D-loop region including 7C > T in disease susceptibility (58). Moreover, an association between mitochondrial ND3 SNPs such as rs28358278 (10400C > T), rs2853826 (10398A > G), rs41467651 (10310G > A) and elevated risk of gastric cancer in Korean population has been shown (59). Interestingly, it has been identified that haplogroup N has a survival role in gastric cancer patients in comparison to haplogroup M (60).

3.3. Prostate cancer

Numerous studies have been performed to find diagnostic biomarkers in prostate cancer which is the second cause of cancer death in men, worldwide (61). Earlier evidence showed that germline mutations of the COXI, the core catalytic subunit of cytochrome c oxidase (complex IV), were associated with the risk of prostate cancer. They reported COXI conserved amino acid mutations in 12% of prostate cancer samples. They also showed that 8993T > G mutation in ATP6 led to the accumulation of ROS and the progression of prostate cancer (62).

mtDNA whole-genome sequencing (WGS) in prostate tumors revealed more than 40 somatic mutations in COXII, COXIII, ATP6, and ND6 genes. The tRNAs gene alterations were linked to increased Prostate-specific antigen (PSA) (63).

In 2013, Koochekpour and colleagues showed lower mtDNA in African American men compared to Caucasian American men, which could contribute to a higher risk and poor prognosis of prostate cancer (64). Further evidence confirmed that low mtDNA CN in PBL was involved in the invasive form of prostate cancer (65).

The mtDNA WGS of 384 prostate cancer samples revealed at least one mitochondrial single nucleotide variant (mtSNV) for each patient, some of them related to the aggressive form of the tumor. This phenomenon was more detected in older patients. They also reported co-occurrence between mtDNA and nDNA profiles (66).

In a paired benign/malignant prostate cancer survey, Schöpf et al. showed higher mtDNA mutations including complex I-encoding genes. Tumor transcriptome analysis revealed a significant link between mtDNA mutations and shorter patient survival (67).

3.4 Colorectal cancer

Another cancer associated with gene alterations in mtDNA is CRC which is the third most prevalent cancer in the world. One of the earliest proofs of mtDNA involvement in CRC was exhibited by an Iranian research study. They identified that individuals with a higher level of mtDNA variants are more susceptible to CRC (68). In another article published by the same research group, 4216T > C in the ND1 gene has also been linked to CRC pathogenesis (69).

In 2010, Theodoratou et al. reported that 752G > A, 1440G > A variants located on the 12SrRNA gene and 4770G > A located on the ND2 gene were associated with the CRC prognosis and mortality (70).

In a case-control study conducted on CRC, common SNPs of D-loop were investigated. 73G > A, 146T > C, 195T > C, 324C > G, 16261C > T, 16304T > C and 309C/C (insert) were showed significant correlation to the disease susceptibility (71).

The mRNA expression levels of ND1, ND6, Cyt b, COXI, 12SrRNA, and ATP6 were tested by Reverse transcription (RT) PCR in CRC. The results indicate increased mRNA levels of all six genes with a more prominent elevation in COXI in late malignant

tissues (72). By sequencing the entire 1124 bp D-loop region of CRC tumors, Govatati et al. found that most CRC-related SNPs are located in the HV1. They concluded that mutations in the D-loop region disrupt mitochondrial function and lead to increased production of ROS and oxidative stress (73). In 2018, Thyogarajan et al. pointed out that SNPs including 16294C > T, 16296C > T, 16278C > T and 16069C > T which are located in the HV1, were associated with the colorectal adenoma risk (74).

In 2016, the mtDNA CNV association with the expression of P53 and TFAM were examined in CRC, precancerous tissues and 9 colorectal cancer cell lines. A significant correlation was observed between p53 and TFAM co-expression and clinicopathological characteristics of the patients. They also observed that p53 could increase TFAM expression by binding to the TFAM promoter, and their co-expression regulates the mtDNA CN (75).

3.5 Renal cell carcinoma

Research on Renal cell carcinoma (RCC) the most prevalent kidney cancer in adults identified the role of mtDNA mutations in cancer for the first time. Studies on Tumor samples of patients showed a heteroplasmic 294 bp deletion in the ND1 gene (76). In 2013, Xu et al. demonstrated that 16293A > G D-loop variant linked to increased risk of early-onset RCC. The minor allele G was significantly correlated with the lower onset of the disease (77). Further investigation suggested some D-loop SNPs such as; 16293A > G, 262A > G, 488T > C, 16298T > C and 16319G > as the potential markers for RCC diagnosis and subgroups identification (78). The same research team further emphasized the role of 262C > T SNP in the HV2 associated with poor RCC outcome (79). They also reported that mtMSI of the CCCCCTCTA at position 8272 was associated with the incidence of RCC (80).

By a quantitative real-time PCR assay on PBL, Purdue et al. found a significant link between lower mtDNA CN and RCC susceptibility (81). They further conducted a nested case-control study and evaluated leukocyte mtDNA up to 13 years before diagnosis of CRC. Interestingly, the results revealed high pre-diagnostic leukocyte mtDNA CN associated with an increased risk of the disease (82).

3.6 Lung cancer

Based on WHO report in 2020, lung cancer, with 2.21 million cases, is the second most common, and with 1.80 million deaths is the most common cause of cancer mortality (https://www.who.int/news-room/fact-sheets/detail/cancer).

By analyzing the early-stage Non-Small Cell Lung Cancer (NSCLC), it has been shown that the haplogroup D/D4 were hotspots for somatic mutations linked to the disease survival (83). Ding et al. identified polymorphisms at 235A> G and 324A/G correlated to increased risk of lung cancer and considered them as informant factors in a high-risk population. They also reported 16298T> C located in the D-loop region is associated with small-cell lung cancer (84).

The Efficiency of plasma mtDNA copy number content as a biomarker for lung cancer was estimated in a recent study. The receiver operating characteristic (ROC) curve analysis revealed that plasma mtDNA content could detect lung cancer with a sensitivity and specificity of 71.1% and 70.1%, respectively (85).

By whole mitochondrial genome sequencing of NSCLC, Kazdal et al. found numerous non-repetitive mtDNA somatic mutations. Largely variable mtDNA CN was also observed in different tumor segments with significantly lower load in solid predominant segments (86). This is while in a simultaneous study in NSCLC, increase and decreased total mtDNA CN was shown in the non-tumor and tumor segments, respectively. They concluded that mtDNA plays a prominent role in the human NSCLC progression (87).

Conclusion

During the last decade, the contribution of mtDNA in cancer has been vastly investigated. Emerging evidence now supports the concept that mtDNA changes result in oxidative stress, free radical generation, and cancer progression. mtDNA mutations were considered the main cause of mitochondrial function abnormalities and inherited susceptibility to the diseases. Subsequent alterations in expressed proteins were noticed in the vast variety of solid tumors. Although cancer cells need mitochondria as the source of energy, the

mitochondrial dysfunction somehow acts in the direction of tumorigenesis. The accurate mechanisms of these manners are not clear yet; however, mtDNA molecular analysis may lead to discovering the driver mutations, functional perspectives and involved pathways.

Future studies on mtDNA in the context of tumor development would be promising in cancer prevention and therapeutic approaches.

Conflict of Interest

The authors declare no conflict of interest.

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Ethics

Ethics code: IR.MODARES.REC.1399.131

Authors Contributions

Conceptualization, S.S., F.D., S.M. and L.G.; methodology, S.S., F.D., S.M. and L.G. and S.S., F.D., S.M. and L.G.; software, S.S., F.D., S.M. and L.G.; validation, S.S., F.D., S.M. and L.G.; investigation, S.S., F.D., S.M. and L.G. ; resources, S.S., F.D., S.M. and L.G.; data curation, S.S., F.D., S.M. and L.G.; writing—original draft preparation, S.S., F.D., S.M. and L.G.; writing—review and editing, S.S., F.D., S.M. and L.G.

References

1. Wallace DC. Mitochondrial DNA mutations in disease and aging. Environmental and Molecular Mutagenesis. 2010;51(5):440-50.

2. Chandra D, Singh KK. Genetic insights into OXPHOS defect and its role in cancer. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 2011;1807(6):620-5.

3. Ju YS, Alexandrov LB, Gerstung M, Martincorena I, Nik-Zainal S, Ramakrishna M, et al. Origins and functional consequences of somatic mitochondrial DNA mutations in human cancer. eLife. 2014;3.

4. Stewart JB, Alaei-Mahabadi B, Sabarinathan R, Samuelsson T, Gorodkin J, Gustafsson CM, et al. Simultaneous DNA and RNA Mapping of Somatic Mitochondrial Mutations across Diverse Human Cancers. PLoS genetics. 2015;11(6):e1005333.

5. Kaipparettu BA, Ma Y, Park JH, Lee TL, Zhang Y, Yotnda P, et al. Crosstalk from non-cancerous mitochondria can inhibit tumor properties of metastatic cells by suppressing oncogenic pathways. PloS one. 2013;8(5):e61747.

6. Kühlbrandt W. Structure and function of mitochondrial membrane protein complexes. BMC Biology. 2015;13(1):89.

7. Chiang SC, Meagher M, Kassouf N, Hafezparast M, McKinnon PJ, Haywood R, et al. Mitochondrial protein-linked DNA breaks perturb mitochondrial gene transcription and trigger free radical-induced DNA damage. Science advances. 2017;3(4):e1602506.

8. Schon EA, DiMauro S, Hirano M. Human mitochondrial DNA: roles of inherited and somatic mutations. Nature reviews Genetics. 2012;13(12):878-90.

9. Imanishi H, Hattori K, Wada R, Ishikawa K, Fukuda S, Takenaga K, et al. Mitochondrial DNA mutations regulate metastasis of human breast cancer cells. PloS one. 2011;6(8):e23401.

10. Pfleger J, He M, Abdellatif M. Mitochondrial complex II is a source of the reserve respiratory capacity that is regulated by metabolic sensors and promotes cell survival. Cell Death & Disease. 2015;6(7):e1835-e.

11. Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. Science (New York, NY). 1989;246(4930):629-34.

12. Kalen AL, Ahmad IM, Abdalla MY, O'Malley YQ, Goswami PC, Sarsour EH. MnSOD and Cyclin B1 Coordinate a Mito-Checkpoint during Cell Cycle Response to Oxidative Stress. Antioxidants. 2017;6(4):92.

13. Kulawiec M, Ayyasamy V, Singh KK. p53 regulates mtDNA copy number and mitocheckpoint pathway. Journal of carcinogenesis. 2009;8:8.

14. Nagano H, Hashimoto N, Nakayama A, Suzuki S, Miyabayashi Y, Yamato A, et al. p53-inducible DPYSL4 associates with mitochondrial supercomplexes and regulates energy metabolism in adipocytes and cancer cells. Proceedings of the National Academy of Sciences. 2018;115(33):8370-5.

15. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, et al. Sequence and organization of the human mitochondrial genome. Nature. 1981;290(5806):457-65.

16. Kang I, Chu CT, Kaufman BA. The mitochondrial transcription factor TFAM in neurodegeneration: emerging evidence and mechanisms. FEBS letters. 2018;592(5):793-811.

17. Kunkel GH, Chaturvedi P, Tyagi SC. Mitochondrial pathways to cardiac recovery: TFAM. Heart failure reviews. 2016;21(5):499-517.

18. D'Souza AR, Minczuk M. Mitochondrial transcription and translation: overview. Essays in biochemistry. 2018;62(3):309-20.

19. Jemt E, Persson Ö, Shi Y, Mehmedovic M, Uhler JP, Dávila López M, et al. Regulation of DNA replication at the end of the mitochondrial D-loop involves the helicase TWINKLE and a conserved sequence element. Nucleic Acids Research. 2015;43(19):9262-75.

20. Mercer TR, Neph S, Dinger ME, Crawford J, Smith MA, Shearwood AM, et al. The human mitochondrial transcriptome. Cell. 2011;146(4):645-58.

21. Williams SB, Ye Y, Huang M, Chang DW, Kamat AM, Pu X, et al. Mitochondrial DNA Content as Risk Factor for Bladder Cancer and Its Association with Mitochondrial DNA Polymorphisms. Cancer prevention research (Philadelphia, Pa). 2015;8(7):607-13.

22. Lightowlers RN, Chinnery PF, Turnbull DM, Howell N. Mammalian mitochondrial genetics: heredity, heteroplasmy and disease. Trends in genetics : TIG. 1997;13(11):450-5.

23. Stewart JB, Chinnery PF. The dynamics of mitochondrial DNA heteroplasmy: implications for human health and disease. Nature Reviews Genetics. 2015;16(9):530-42.

24. Floros VI, Pyle A, Dietmann S, Wei W, Tang WCW, Irie N, et al. Segregation of mitochondrial DNA heteroplasmy through a developmental genetic bottleneck in human embryos. Nature Cell Biology. 2018;20(2):144-51.

25. Beadnell TC, Scheid AD, Vivian CJ, Welch DR. Roles of the mitochondrial genetics in cancer metastasis: not to be ignored any longer. Cancer metastasis reviews. 2018;37(4):615-32.

26. Chatterjee A, Mambo E, Sidransky D. Mitochondrial DNA mutations in human cancer. Oncogene. 2006;25(34):4663-74.

27. Yuan Y, Ju YS, Kim Y, Li J, Wang Y, Yoon CJ, et al. Comprehensive molecular characterization of mitochondrial genomes in human cancers. Nature Genetics. 2020;52(3):342-52.

28. Tan DJ, Bai RK, Wong LJ. Comprehensive scanning of somatic mitochondrial DNA mutations in breast cancer. Cancer research. 2002;62(4):972-6.

29. Weissensteiner H, Pacher D, Kloss-Brandstätter A, Forer L, Specht G, Bandelt HJ, et al. HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. Nucleic Acids Res. 2016;44(W1):W58-63.

30. Quintana-Murci L, Semino O, Bandelt H-J, Passarino G, McElreavey K, Santachiara-Benerecetti AS. Genetic evidence of an early exit of Homo sapiens sapiens from Africa through eastern Africa. Nature Genetics. 1999;23(4):437-41.

31. Loogväli EL, Roostalu U, Malyarchuk BA, Derenko MV, Kivisild T, Metspalu E, et al. Disuniting uniformity: a pied cladistic canvas of mtDNA haplogroup H in Eurasia. Molecular biology and evolution. 2004;21(11):2012-21.

32. Reznik E, Miller ML, Şenbabaoğlu Y, Riaz N, Sarungbam J, Tickoo SK, et al. Mitochondrial DNA copy number variation across human cancers. eLife. 2016;5.

33. Hu L, Yao X, Shen Y. Altered mitochondrial DNA copy number contributes to human cancer risk: evidence from an updated meta-analysis. Scientific Reports. 2016;6(1):35859.

34. Kalsbeek AMF, Chan EKF, Corcoran NM, Hovens CM, Hayes VM. Mitochondrial genome variation and prostate cancer: a review of the mutational landscape and application to clinical management. Oncotarget. 2017;8(41):71342-57.

35. Lemnrau A, Brook MN, Fletcher O, Coulson P, Tomczyk K, Jones M, et al. Mitochondrial DNA Copy Number in Peripheral Blood Cells and Risk of Developing Breast Cancer. Cancer research. 2015;75(14):2844-50.

36. Achanta G, Sasaki R, Feng L, Carew JS, Lu W, Pelicano H, et al. Novel role of p53 in maintaining mitochondrial genetic stability through interaction with DNA Pol gamma. The EMBO journal. 2005;24(19):3482-92.

37. Sun W, Qin X, Zhou J, Xu M, Lyu Z, Li X, et al. Mitochondrial DNA copy number in cervical exfoliated cells and risk of cervical cancer among HPV-positive women. BMC Women's Health. 2020;20(1):139.

38. Tanaka T, Kobunai T, Yamamoto Y, Murono K, Otani K, Yasuda K, et al. Increased Copy Number Variation of mtDNA in an Array-based Digital PCR Assay Predicts Ulcerative Colitis-associated Colorectal Cancer. In vivo (Athens, Greece). 2017;31(4):713-8.

39. Patel TH, Norman L, Chang S, Abedi S, Liu C, Chwa M, et al. European mtDNA Variants Are Associated With Differential Responses to Cisplatin, an Anticancer Drug: Implications for Drug Resistance and Side Effects. Frontiers in oncology. 2019;9:640.

40. Shahbazi S, Alavi S, Majidzadeh AK, Ghaffarpour M, Soleimani A, Mahdian R. BsmI but not FokI polymorphism of VDR gene is contributed in breast cancer. Medical oncology (Northwood, London, England). 2013;30(1):393.

41. Mohamadalizadeh-Hanjani Z, Shahbazi S, Geranpayeh L. Investigation of the SPAG5 gene expression and amplification related to the NuMA mRNA levels in breast ductal carcinoma. World Journal of Surgical Oncology. 2020;18(1):225.

42. Tseng LM, Yin PH, Chi CW, Hsu CY, Wu CW, Lee LM, et al. Mitochondrial DNA mutations and mitochondrial DNA depletion in breast cancer. Genes, chromosomes & cancer. 2006;45(7):629-38.

43. Zhu W, Qin W, Sauter ER. Large-scale mitochondrial DNA deletion mutations and nuclear genome instability in human breast cancer. Cancer Detect Prev. 2004;28(2):119-26.

44. Parrella P, Xiao Y, Fliss M, Sanchez-Cespedes M, Mazzarelli P, Rinaldi M, et al. Detection of mitochondrial DNA mutations in primary breast cancer and fine-needle aspirates. Cancer research. 2001;61(20):7623-6.

45. Canter JA, Kallianpur AR, Parl FF, Millikan RC. Mitochondrial DNA G10398A polymorphism and invasive breast cancer in African-American women. Cancer research. 2005;65(17):8028-33.

46. Darvishi K, Sharma S, Bhat AK, Rai E, Bamezai RN. Mitochondrial DNA G10398A polymorphism imparts maternal Haplogroup N a risk for breast and esophageal cancer. Cancer letters. 2007;249(2):249-55.

47. Grzybowska-Szatkowska L, Slaska B. Mitochondrial NADH dehydrogenase polymorphisms are associated with breast cancer in Poland. Journal of applied genetics. 2014;55(2):173-81.

48. Pezzotti A, Kraft P, Hankinson SE, Hunter DJ, Buring J, Cox DG. The mitochondrial A10398G polymorphism, interaction with alcohol consumption, and breast cancer risk. PloS one. 2009;4(4):e5356.

49. Jiang H, Zhao H, Xu H, Hu L, Wang W, Wei Y, et al. Peripheral blood mitochondrial DNA content, A10398G polymorphism, and risk of breast cancer in a Han Chinese population. Cancer science. 2014;105(6):639-45.

50. Behroozi J, Shahbazi S, Bakhtiarizadeh MR, Mahmoodzadeh H. ADAR expression and copy number variation in patients with advanced gastric cancer. BMC gastroenterology. 2020;20(1):152.

51. Hiyama T, Tanaka S, Shima H, Kose K, Tuncel H, Ito M, et al. Somatic mutation in mitochondrial DNA and nuclear microsatellite instability in gastric cancer. Oncology reports. 2003;10(6):1837-41.

52. Burgart LJ, Zheng J, Shu Q, Strickler JG, Shibata D. Somatic mitochondrial mutation in gastric cancer. The American journal of pathology. 1995;147(4):1105-11.

53. Wang H, Wang Y, Zhao Q, Guo Z, Zhang F, Zhao Y, et al. Identification of sequence polymorphisms in the D-Loop region of mitochondrial DNA as a risk factor for gastric cancer. Mitochondrial DNA Part A, DNA mapping, sequencing, and analysis. 2016;27(2):1045-7.

54. Liao LM, Baccarelli A, Shu XO, Gao YT, Ji BT, Yang G, et al. Mitochondrial DNA copy number and risk of gastric cancer: a report from the Shanghai Women's Health Study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2011;20(9):1944-9.

55. Zhang G, Qu Y, Dang S, Yang Q, Shi B, Hou P. Variable copy number of mitochondrial DNA (mtDNA) predicts worse prognosis in advanced gastric cancer patients. Diagnostic pathology. 2013;8:173.

56. Zhu X, Mao Y, Huang T, Yan C, Yu F, Du J, et al. High mitochondrial DNA copy number was associated with an increased gastric cancer risk in a Chinese population. Molecular carcinogenesis. 2017;56(12):2593-600.

57. Er LM, Li Y, Er L, Wu M, Zheng XL, Guo ZJ, et al. Associations between Single Nucleotide Polymorphisms in the Mitochondrial DNA D-Loop Region and Outcome of Gastroenteropancreatic Neuroendocrine Neoplasm. Annals of clinical and laboratory science. 2018;48(3):333-7.

58. Er LM, Wu ML, Gao Y, Wang SJ, Li Y. Identification of sequence polymorphisms in the displacement loop region of mitochondrial DNA as a risk factor for gastroenteropancreatic neuroendocrine neoplasm. Journal of clinical laboratory analysis. 2017;31(5).

59. Jin EH, Sung JK, Lee SI, Hong JH. Mitochondrial NADH Dehydrogenase Subunit 3 (MTND3) Polymorphisms are Associated with Gastric Cancer Susceptibility. International journal of medical sciences. 2018;15(12):1329-33.

60. Wang C, Wang Y, Wang H, Zhang R, Guo Z. Mitochondrial DNA haplogroup N is associated good

outcome of gastric cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(12):12555-9.

61. Shahbazi S, Khorasani M, Mahdian R. Gene expression profile of FVII and AR in primary prostate cancer. Cancer biomarkers : section A of Disease markers. 2016;17(3):353-8.

62. Petros JA, Baumann AK, Ruiz-Pesini E, Amin MB, Sun CQ, Hall J, et al. mtDNA mutations increase tumorigenicity in prostate cancer. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(3):719-24.

63. Kloss-Brandstätter A, Schäfer G, Erhart G, Hüttenhofer A, Coassin S, Seifarth C, et al. Somatic mutations throughout the entire mitochondrial genome are associated with elevated PSA levels in prostate cancer patients. American journal of human genetics. 2010;87(6):802-12.

64. Koochekpour S, Marlowe T, Singh KK, Attwood K, Chandra D. Reduced mitochondrial DNA content associates with poor prognosis of prostate cancer in African American men. PloS one. 2013;8(9):e74688.

65. Tu H, Gu J, Meng QH, Kim J, Davis JW, He Y, et al. Mitochondrial DNA copy number in peripheral blood leukocytes and the aggressiveness of localized prostate cancer. Oncotarget. 2015;6(39):41988-96.

66. Hopkins JF, Sabelnykova VY, Weischenfeldt J, Simon R, Aguiar JA, Alkallas R, et al. Mitochondrial mutations drive prostate cancer aggression. Nature Communications. 2017;8(1):656.

67. Schöpf B, Weissensteiner H, Schäfer G, Fazzini F, Charoentong P, Naschberger A, et al. OXPHOS remodeling in high-grade prostate cancer involves mtDNA mutations and increased succinate oxidation. Nature Communications. 2020;11(1):1487.

68. Akouchekian M, Houshmand M, Hemati S, Ansaripour M, Shafa M. High rate of mutation in mitochondrial DNA displacement loop region in human colorectal cancer. Diseases of the colon and rectum. 2009;52(3):526-30.

69. Akouchekian M, Houshmand M, Akbari MH, Kamalidehghan B, Dehghan M. Analysis of mitochondrial ND1 gene in human colorectal cancer. Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences. 2011;16(1):50-5.

70. Theodoratou E, Din FV, Farrington SM, Cetnarskyj R, Barnetson RA, Porteous ME, et al. Association between common mtDNA variants and all-cause or colorectal cancer mortality. Carcinogenesis. 2010;31(2):296-301.

71. Guo Z, Zhao S, Fan H, Du Y, Zhao Y, Wang G. Identification of sequence polymorphisms in the D-Loop region of mitochondrial DNA as a risk factor for colon cancer. Mitochondrial DNA Part A, DNA mapping, sequencing, and analysis. 2016;27(6):4244-5.

72. Wallace L, Mehrabi S, Bacanamwo M, Yao X, Aikhionbare FO. Expression of mitochondrial genes MT-ND1, MT-ND6, MT-CYB, MT-COI, MT-ATP6, and 12S/MT-RNR1 in colorectal adenopolyps. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2016;37(9):12465-75.

73. Govatati S, Saradamma B, Malempati S, Dasi D, Thupurani MK, Nagesh N, et al. Association of mitochondrial displacement loop polymorphisms with risk of colorectal cancer in south Indian population. Mitochondrial DNA Part A, DNA mapping, sequencing, and analysis. 2017;28(5):632-7.

74. Thyagarajan B, Guan W, Fedirko V, Barcelo H, Ramasubramaian R, Gross M, et al. Associations of mitochondrial polymorphisms with sporadic colorectal adenoma. Molecular carcinogenesis. 2018;57(5):598-605.

75. Wen S, Gao J, Zhang L, Zhou H, Fang D, Feng S. p53 increase mitochondrial copy number via up-regulation of mitochondrial transcription factor A in colorectal cancer. Oncotarget. 2016;7(46).

76. Horton TM, Petros JA, Heddi A, Shoffner J, Kaufman AE, Graham SD, Jr., et al. Novel mitochondrial DNA deletion found in a renal cell carcinoma. Genes, chromosomes & cancer. 1996;15(2):95-101.

77. Xu J, Guo Z, Zhang J, Cui L, Zhang S, Bai Y. Single nucleotide polymorphisms in the mitochondrial displacement loop and age-at-onset of renal cell carcinoma. Sci Rep. 2013;3:2408.

78. Zhang J, Guo Z, Bai Y, Cui L, Zhang S, Xu J. Identification of sequence polymorphisms in the displacement loop region of mitochondrial DNA as a risk factor for renal cell carcinoma. Biomedical reports. 2013;1(4):563-6.

79. Bai Y, Guo Z, Xu J, Liu S, Zhang J, Cui L, et al. Single nucleotide polymorphisms in the D-loop region of mitochondrial DNA is associated with renal cell carcinoma outcome. Mitochondrial DNA. 2015;26(2):224-6.

80. Bai Y, Guo Z, Xu J, Zhang J, Cui L, Zhang H, et al. The 9-bp deletion at position 8272 in region V of mitochondrial DNA is associated with renal cell carcinoma outcome. Mitochondrial DNA Part A, DNA mapping, sequencing, and analysis. 2016;27(3):1973-5.

81. Purdue MP, Hofmann JN, Colt JS, Hoxha M, Ruterbusch JJ, Davis FG, et al. A case-control study of

peripheral blood mitochondrial DNA copy number and risk of renal cell carcinoma. PloS one. 2012;7(8):e43149.

82. Hofmann JN, Hosgood HD, 3rd, Liu CS, Chow WH, Shuch B, Cheng WL, et al. A nested case-control study of leukocyte mitochondrial DNA copy number and renal cell carcinoma in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Carcinogenesis. 2014;35(5):1028-31.

83. Wang Z, Choi S, Lee J, Huang YT, Chen F, Zhao Y, et al. Mitochondrial Variations in Non-Small Cell Lung Cancer (NSCLC) Survival. Cancer informatics. 2015;14(Suppl 1):1-9.

84. Ding C, Li R, Wang P, Jin P, Li S, Guo Z. Identification of sequence polymorphisms in the D-loop region of mitochondrial DNA as a risk factor for lung cancer. Mitochondrial DNA. 2012;23(4):251-4.

85. Chen J, Zhang L, Yu X, Zhou H, Luo Y, Wang W, et al. Clinical application of plasma mitochondrial DNA content in patients with lung cancer. Oncology letters. 2018;16(6):7074-81.

86. Kazdal D, Harms A, Endris V, Penzel R, Kriegsmann M, Eichhorn F, et al. Prevalence of somatic mitochondrial mutations and spatial distribution of mitochondria in non-small cell lung cancer. British Journal of Cancer. 2017;117(2):220-6.

87. Lin CS, Lee MC, Hsu JC, Pan SC, Wei YH. AB001. Low mitochondrial DNA copy number is associated with low mitochondrial DNA integrity and advanced T-status in non-small cell lung cancer. Journal of Thoracic Disease. 2017;9(Suppl 14).