A comprehensive overview of mitochondrial DNA 4977-bp deletion in cancer studies

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Abstract

Mitochondria are cellular machines essential for energy production. The biogenesis of mitochondria is a highly complex and it depends on the coordination of the nuclear and mitochondrial genome. Mitochondrial DNA (mtDNA) mutations and deletions are suspected to be associated with carcinogenesis. The most described mtDNA deletion in various human cancers is called the 4977-bp common deletion (mDNA4977) and it has been explored since two decades. In spite of that, its implication in carcinogenesis still unknown and its predictive and prognostic impact remains controversial. This review article provides an overview of some of the cellular and molecular mechanisms underlying mDNA4977 formation and a detailed summary about mDNA4977 reported in various types of cancers. The current knowledge of mDNA4977 as a prognostic and predictive marker are also discussed.

Introduction

Cancer can be defined as an ailment characterized by progressive genomic changes, and it remains the second leading cause of death globally after cardiovascular diseases. According to an estimate by GLOBOCAN, in 2015 there were almost 17.5 million cancer cases worldwide and approximately 8.7 million people died due to cancer. It is predicted that the global cancer burden will increase by 2030, with ~26 million new cases and 17 million deaths.

Cancer results from uncontrolled proliferation of abnormal cells due to instability of the genome. Genomic instability is a fundamental indicator of human cancer that can lead to an excessive accumulation in genetic alterations, including from single DNA sequence mutations to entire chromosome abnormalities.

Although extensive efforts have been made by researchers to strengthen our understanding on the etiology of cancer but there is still considerable uncertainty over the precise molecular mechanism underlying cancer tumorigenesis. Because of the complexity of genomic changes within cancer cells, some researchers have shifted their focus to another genome. In addition to the nuclear genome (nDNA), it is worth to consider there is another genome that needs to be investigated.

Human mitochondrial genome (mtDNA), our other genome serves as a separate genome, which is present from several hundreds to thousands of copies per cell and their replication occur independently of the nDNA. Decades ago, Otto Warburg was the first scientist that proposed the relevance of mitochondria with cancer. Warburg’s view suggested that mitochondrial alterations in function may enhance tumor growth or promote cancer progression. Since then, diverse molecular aberrations in mtDNA include point mutations, deletions, insertions, microsatellite instability, polymorphisms, and changes in mtDNA content have been identified and characterized in human cancers.

Because mtDNA is a primary target for oxidative stress, it seems that mtDNA is susceptible to such damage. In addition to DNA damage, oxidative damage to mtDNA can also result in mtDNA deletions by causing double-strand breaks in the DNA. Indeed, large scale deletions in mtDNA were among the first mtDNA alterations detected to cause human diseases. So far, as stated by Mitomap (http://www.mitomap.org), a mitochondrial genome database, over 150 deletions in mtDNA associated with various diseases have been reported. Among mtDNA deletions, one of the most vital that causes a huge destruction of almost one-third length of the mitochondrial genome is the 4977-bp mtDNA deletion (mDNA4977). The mDNA4977 is previously reported to be involved in myopathies, Alzheimer disease, Kearns-Sayre syndrome (KSS), chronic progressive external ophthalmoplegia (CPEO) and photoaging of the skin. Furthermore, this deletion has also been found to increase with aging in many post-mitotic human tissues.

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Key words: Mitochondrial DNA (mtDNA) deletion; 4977-bp mtDNA; cancer.

Funding: the authors thanks the Universiti Sains Malaysia for financial support (Research University Grant: 1001/PPSP/8012242 & 1001/PPSP/8012224).

Contributions: AAMY, WSWA wrote the manuscript; SZNMK, SMAR designed the figures and prepared the tables. AAMY, conceived and revised the manuscript; WSWA, SZNMK, SMAR helped in reference search.

Conflict of interest: the author declares no potential conflict of interest.

Received for publication: 26 December 2018.
Revision received: 14 February 2019.
Accepted for publication: 19 February 2019.

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Oncology Reviews 2019; 13:409
doi:10.4081/oncol.2019.409
In this review, we summarize the current progress in understanding the cellular and molecular mechanisms underlying the formation of mtDNA4977 and discuss its potential role in tumorigenesis. We then review recent discoveries about the reported mtDNA4977 in various human neoplasias.

Mitochondrial DNA (mtDNA)

Mitochondria are recognized as the energy powerhouses of cells and are extraordinary organelles which contain their own DNA and independent biogenesis system. The structure, organization and function of mtDNA should be considered in order to understand the impact of mtDNA in cancer.

The human mtDNA is a 16,569 base-pair circular molecule that encodes for the 13 most essential genes for mitochondrial oxidative phosphorylation (OXPHOS), the primary cellular energy production system, together with the 2 ribosomal RNAs and 22 transfer RNAs for mtDNA gene expression.21,22 The entire sequence of the human mtDNA can be obtained at www.mitomap.org. It should be noted that, the only region in mtDNA where the mostly non-coding is the control region - also called the displacement-loop (D-loop) that consists of 1123 base-pair sequences. D-loop is the major control site for mtDNA expression and replication.

Although heredity of nuclear gene follows a Mendelian inheritance pattern, the transmission of mtDNA is thought to be strictly maternally inherited, due to elimination paternal mtDNA from the fertilized oocyte.23

Alterations in mtDNA

Alterations in the mitochondrial genome have been implicated as playing a role in diverse forms of human disease and aging. It is believed that various mtDNA alterations increase with advancing age in human tissues, such as point mutations and deletions in mtDNA have been identified to accumulate in the aged human brain, skeletal muscles, heart and colon tissues.15,25 Also, mtDNA alterations have been reported as a frequent event in many human cancer studies in the types of point mutations, multiple insertions and deletions and microsatellite instability (MSI).26,27

Among the mtDNA alterations, large scale deletions in mtDNA are one of the essential mtDNA mutations that related to human diseases.28 One of the best-described large scale mtDNA deletion is the specific 4977-bp deletion in mitochondrial genome, which has been accumulated in various disorders, including mitochondrial diseases and many different types of cancer.29 The mtDNA4977 has been broadly studied and has been observed to exist with increasing age in normal human brain, heart and skeletal muscle.20 In addition, the mtDNA4977 has also been exhibited to occur more frequently with increasing sun exposure in human skin, as compared with sun-protected skin.30,32

mtDNA4977 common deletion

mtDNA4977 was initially spotted in the muscle of a patient with neuromuscular diseases - Kearns-Sayre/progressive external ophthalmoplegia plus syndrome in 1989,13 and later frequently discovered to accumulate in various human tissues with aging.13-15 This genetic alteration also known as common deletion eliminates between nucleotides 8470 and 13447 of the human mitochondrial genome. mtDNA4977 removes all 5 tRNA genes (tRNA(Leu), tRNA(Ile), tRNA(Thr), tRNA(Asp) and tRNA(Lys)) and 7 genes encoding 4 Complex I subunits (ND3, ND4, ND4L, partial ND5), 1 Complex IV subunit (COX III), 2 Complex V subunits (ATP6 and partial ATP8), that are crucial for supporting normal mitochondrial OXPHOS function (Figure 1).

mtDNA4977 has been categorized in class I deletions as these deletions almost take place within two 13-bp perfect direct repeats (ACCTCCCTCACCA) flanking the 5′- and 3′-end breakpoints, located at nucleotide positions 8470-8482 within the ATPase 8 gene) and 13447-13459 (within the ND5 gene).36,37 One of these repeats is removed with the deleted fragment while the other is retained in mtDNA4977. Commonly, the nucleotide sequence from position 8470 to 13446 will be deleted.

The mtDNA4977 has been considered as the pathogenic mutation in human. The loss of some tRNA genes and coding regions within the deleted region disables mitochondrial protein synthesis as well as the entire mitochondrial biogenesis process. The major effect of this large deletion could cause a complete failure of ATP production and subsequently impair mitochondrial functions.38 mtDNA4977 exists in nature of heteroplasmic, with clinical signs only become apparent when the amount of deleted molecules exceeds a critical threshold.39 There has been much agreement that the generation of this common deletion because of replication error that related to the slipped mispairing between these two perfect repeats.40,41

Generation of mtDNA4977

For a long time, the sources on how deletions are generated within mtDNA genome are not well established, despite the prevalence of mtDNA deletions in human patients with mitochondrial disease and the precise molecular mechanisms underlying the formation are still under debate.

Based on the studies of deletion generation, it has been proposed that mtDNA deletions might arise either through spontaneous errors during mtDNA replication (replication slippage) or aberrancy of double-strand break repair (DBS).40,41 One possible
mechanism behind this mtDNA deletion generation is replication as considered by most researchers.

Over the years, the exact mechanism behind human mtDNA replication is not completely understood and remains controversial. However, at least three major mechanisms of mtDNA replication have been proposed: i) a strand-asynchronous replication;\textsuperscript{44} ii) a strand-synchronous replication;\textsuperscript{45} and iii) a RITOLS (RNA incorporation throughout the lagging strand) model.\textsuperscript{46}

The traditional strand-asynchronous (or strand-displacement) model of mammalian mtDNA replication primarily recommended by Clayton is directly based on the electron microscopic observation of replicating mtDNA intermediates.\textsuperscript{44} As stated in this model, mtDNA replication begins with the heavy strand synthesis at the origin of the heavy strand (O\textsubscript{H}) and proceeds two-thirds of the mtDNA circle prior to exposing the origin of the light strand (O\textsubscript{L}) and initiating replication of the second strand in the opposite direction.\textsuperscript{44} The Clayton model has been challenged by Holt and colleagues mainly using data generated from the classical method of neutral two-dimensional DNA electrophoresis. The Holt model proposes that coupled mtDNA replication starts from a single origin or zone and that the leading and lagging strands are synthesized simultaneously, or synchronously.\textsuperscript{45} Later, a third model which describes long single stranded regions of mtDNA covered entirely in RNA as replication intermediate (RITOLS), has also been described.\textsuperscript{46}

Most mtDNA deletions are major arc deletions with breakpoints commonly placed between O\textsubscript{L} and O\textsubscript{H} replication within the major arc. It has been previously postulated that the mechanism for mtDNA\textsuperscript{4977} formation occurs throughout an intragenomic recombination event during the slipped strand mispairing between two 13-bp perfect direct repeats.\textsuperscript{40,42,43,47} The slipped mispairing action mediates the generation of mtDNA common deletion via asynchronous replication. Asynchronous replication synthesizes a single strand to give the two repeats a chance to mispair that flank the deletion. A whole mtDNA replication initiates at the O\textsubscript{H}. Synthesis of the H-strand proceeds until the O\textsubscript{L} becomes exposed, after replication has passed two-thirds of the way around the mitochondrial genome. In the opposite direction, the L-strand can then begin to synthesize. When the upstream repeat in the parent H-strand anneals with the L-strand downstream repeat, a single-strand loop is formed. After this loop breaks followed by degradation, the H-strand will be ligated and utilized as a template to generate a deleted mtDNA.\textsuperscript{43}

A study by Birch-Machin et al. supported the above theory of the common deletion formation mechanism in human skin photoaging through forming a single-strand loop.\textsuperscript{30} The human skin may either be affected directly from prolonged exposure to ultraviolet radiation by promoting base substitutions or indirectly by introducing free radicals which consequently increasing the chances of common deletion occurrences.\textsuperscript{30}

Misrepair of DSBs has been theorized as a possible cause of deletion formation including the mtDNA\textsuperscript{4977}.\textsuperscript{42} The generation of mtDNA\textsuperscript{4977} has also been considered to be linked with the activity of microhomology-mediated end joining (MMEJ), which typically requires less than 12 bp of homology.\textsuperscript{48} In more recent study, Phillips et al. performed a single molecule assay of mtDNA replication based on DNA combing technique to define the underlying molecular mechanism of mtDNA\textsuperscript{4977} formation.\textsuperscript{49} According to their observations, mito-TALEN induces the breaks adjacent to the 5’ repeat and hence triggers formation of the common deletion. Finally, they clarified that mtDNA\textsuperscript{4977} was generated as a consequence of frequent fork stalling, a process which was mediated by the mitochondrial replisome, but independent of canonical DSB repair. They concluded that mtDNA\textsuperscript{4977} was not due to MMEJ or homologous recombination.\textsuperscript{49}

The endogenous and exogenous source of the mtDNA\textsuperscript{4977} formation

DNA can be easily damage by endogenous (cellular metabolic

Figure 1. The 4977-bp human mtDNA deletion. Also known as common deletion that eliminates all 5 tRNA genes (L: tRNA\textsubscript{Leu}, S: tRNA\textsubscript{Ser}, H: tRNA\textsubscript{His}, R: tRNA\textsubscript{Arg}, G: tRNA\textsubscript{Gly}) and 7 genes encoding 4 Complex I subunits (ND3, ND4, ND4L, partial ND5), 1 Complex IV subunit (COX III), 2 Complex V subunits (ATPase6 & partial ATPase8).
pathways, ROS and errors in DNA replication) or exogenous sources (environmental factors including ionizing radiations and ultraviolet (UV) radiations from the sun). It has been believed that the increased oxidative stress in the cells results in mtDNA alterations. Indeed, the hypoxic microenvironment of cancer cells triggers the production of mitochondrial ROS that can induce DNA damages and lead to genomic instability in both mitochondria and nucleus. As mtDNA is in close proximity to the respiratory chain, it is also permanently exposed to ROS produced as a byproduct of OXPHOS that can damage DNA and trigger accumulation of deletions. Thus, there is considerable evidence from the published literature supporting the idea. Interruption of ANT1 (adenine nucleotide translocator isoform 1) function is associated with increased ROS production, which leads to accumulation of mtDNA deletions. In the same manner, a partial loss of SOD2 (manganese superoxide dismutase) results in raised ROS levels and mitochondrial oxidative stress, which link to elevated mtDNA deletions.

Researchers have discovered several important elements that are potentially linked to mtDNA formation via the experimental models in vitro. In one particular pioneering study, Porteous’s group published that the bioenergetic function of cybrids containing less than 50-55% of mtDNA was comparable to those cybrids with wild type mtDNA molecules. But, when the proportion of mtDNA exceeded this threshold, resulting in decreased mitochondrial membrane potential, ATP synthesis rate and cellular ATP/ADP ratio. A similar approach using a series of cybrids containing different proportions of mtDNA associated with a chronic progressive external ophthalmoplegia (CPEO) patient has been conducted by Wei and colleagues. In this case, they determined that mitochondrial oxidative stress and increased mitochondrial mass and mtDNA in response to mtDNA correlate with impaired respiratory function. In a subsequent paper from the same group, Wei’s team observed that a feed-forward, self-accelerating vicious cycle of mitochondrial ROS production could be triggered following brief, intense oxidative stress treatment in cybrids harboring mtDNA. External environmental factors are likely to influence mtDNA formation. It has been considered that ROS can also be exogenously generated, mainly by ultraviolet (UV) radiations, playing a role in the formation of mtDNA. Accordingly, it has been previously reported that UV exposure-induced mtDNA accumulation is related to photo-aging of skin.

The sensitivity of mtDNA to UV-generated oxidative stress has been demonstrated for skin cells in vitro and in vivo as previously published. Accumulation of mtDNA is intimately associated with ROS in the dermis of both normally and photoaged human skin in vivo. Quan et al. determined that the magnitude of the mtDNA is up to 10-fold higher in photoaged skin than in normally aged skin.

There has been reported that singlet oxygen as generated by UVA-irradiation can cause mtDNA, which also detected at higher rate in photoaged skin. Berneburg et al. revealed that repetitive doses of non-lethal UVA irradiation promotes the mtDNA in normal human fibroblasts. In the study of Koch et al. on human keratinocytes, the level of the mtDNA had a significant increased following 2 weeks of UVA irradiation. They also observed that the level of the mtDNA decreased by 90% after prolonged culture of these irradiated cells. This situation could be either the replication is reduced or blocked in deleted mtDNA genomes to permit degradation of mitochondria carrying the mtDNA.

Birch-Machin’s group study suggests that mtDNA deletions may be useful as a biomarker of cumulative UV radiation exposure in human skin with the major species have been the mtDNA and a 3895-bp deletion. In addition, they also identified a higher frequency of tandem mtDNA duplications in sun-exposed human skin.

Additionally, some exogenous chemicals or their metabolic intermediaries also have the ability to trigger either directly or indirectly oxidative DNA damage. Acrolein is a β,β-unaturated aldehyde, and is highly toxic to mitochondria. The mechanisms of acrolein-induced cytotoxicity have been proposed to be related to oxidative stress and mitochondrial impairment. According to Luo et al. acrolein exposure causes dysfunction of mitochondria, indicated by reduced mitochondrial membrane potential, ATP levels and decreased oxygen consumption. More recently, Wang et al. revealed that acrolein promotes mitochondrial ROS over-production, resulting in the formation of the mtDNA and also depletion of mtDNA content in human lung cells.

Involvement of the mtDNA in human cancers

Lee et al. detected the mtDNA in oral tumors. They also observed a positive correlation between this large-scale deletion and human non-tumor oral tissues with betel quid chewing history. Since then, diverse association studies have been conducted in different populations, seeking a correlation between the mtDNA and various types of cancer (Table 1).

Breast cancer

Through a literature search of the Pubmed, Google Scholar and other databases, the majority of mtDNA studies in cancer research have been focused on breast cancer. The mtDNA of 60 breast cancer and paired non-tumorous breast tissues from 60 Taiwanese patients were analyzed by Tseng et al. using regular PCR analysis. It was found that the mtDNA was found to accumulate in non-tumorous tissues (28/60, 47%) rather than in tumor tissues (3/60, 5%). In addition, the author demonstrated that the mtDNA was associated with NAD(P)H:quinone oxidoreductase 1 (NQO1) enzyme deficiency in carcinogenesis of breast cancer.

In another study that involved a quantitative real-time PCR assay to examine the level of the mtDNA in 55 primary breast cancer patients and 21 patients with benign breast disease, all of the cases were detected to be deleted in mtDNA. However, the mtDNA level was lower in tumor tissues compared to adjacent normal tissues in all cases. On the other hand, Pavicic and Richard also reported that the appearance of mtDNA at higher rates in non-tumorous tissue (70/95, 73.7%) than in corresponding tumoral (43/95, 45.3%) counterpart of breast cancer patients.

In a different study, Dimberg et al. analyzed this large-scale deletion of mtDNA in 106 Vietnamese breast cancer patients by sequencing PCR products. They noticed that the mtDNA was significantly more frequent in normal tissue in comparison with paired cancer tissue. This is also in accordance with the previous study of Dani et al. which demonstrated the lower proportion deletion in tumors than in adjacent non-tumoral tissues.

In almost all of the previous studies of the mtDNA in breast cancer have reported a lower frequency in cancerous tissues as compared with corresponding non-cancerous paired tissues. The lower incidence of the mtDNA in cancerous tissue than in non-cancerous tissue has been also reported in other different cancers. The clarification of the lower incidence of the
Table 1. Study of the mtDNA<sup>4977</sup> in selected cancer sites from different populations.

| Cancer type | Country       | No. of patients (N) | mtDNA<sup>4977</sup> in cancerous tissue/blood, n (%) | mtDNA<sup>4977</sup> in adjacent non-cancerous tissue, n (%) | mtDNA<sup>4977</sup> in healthy normal tissue/blood, n/N (%) | Author |
|-------------|---------------|---------------------|------------------------------------------------------|-------------------------------------------------|------------------------------------------------------|--------|
| Breast      | Taiwan        | 60                  | 3 (5%)                                               | 28 (47%)                                        |                                                      | Tseng et al.74 |
|             | Taiwan        | 60                  | 3 (5%)                                               | 29 (48.3%)                                      |                                                      | Tseng et al.77 |
|             | China         | 76                  | 76 (100%)                                            | 76 (100%)                                       |                                                      | Ye et al.73    |
|             | Argentina     | 85                  | 43 (45.3%)                                           | 70 (73.7%)                                      | 78/199 (39.2%)                                     | Pavicic and Richard79 |
|             | Vietnam       | 106                 | 73 (68.8%)                                           | 89 (84%)                                        | 10/113 (8.9%)                                      | Dimberg et al.80 |
|             | China         | 107 (BC); 118 (BBD) | 50 (46.7%) (BC)<sup>a</sup> | 44 (41.1%)                                      |                                                      | Nie et al.85   |
|             | Iraq          | 26 (BC) 33 (BBD)    | 0 (0%)                                               | 0 (0%)                                          |                                                      | Dahi et al.86  |
|             | Turkey        | 25                  | 0 (0%)                                               | 13 (26.3%)                                      | 6/23 (26.1%)                                      | Aral et al.80  |
|             | USA           | 19                  | 0 (0%)                                               | 13 (76.9%)                                      | 2/17 (11.8%)                                      | Tan et al.87   |
|             | USA           | 39                  | 18 (46.2%)                                           | 14 (100%)                                       |                                                      | Zhu et al.88   |
|             | Brazil        | 17                  | 4 (23.5%)                                            | 14 (100%)                                       |                                                      | Dani et al.89  |
| Gastric     | Portugal      | 32                  | 17 (53.1%)                                           | N/A                                             |                                                      | Máximo et al.90 |
|             | China         | 108                 | 86 (79.6%)                                           | 73 (67.6%)                                      | 29/56 (51.8%)                                     | Wang and Liu71  |
|             | Taiwan        | 31                  | 3 (9.7%)                                             | 17 (55%)                                        |                                                      | Wu et al.92    |
|             | Iran          | 107                 | 6 (5.6%)                                             | 18 (16.8%)                                      |                                                      | Kamalidehghan et al.93 |
|             | Brazil        | 14                  | 11 (78.6%)                                           | 14 (100%)                                       |                                                      | Dani et al.94  |
| Colorectal  | Taiwan        | 46                  | 24 (52.2%)                                           | 38 (82.6%)                                      | 2/17 (11.8%)                                      | Dani et al.95  |
|             | China         | 104                 | 17 (16.3%)                                           | 13 (12.5%)                                      |                                                      | Chen et al.96  |
|             | Sweden        | 105                 | 71 (67.9%)                                           | 97 (92.4%)                                      |                                                      | Dimberg et al.97 |
|             | Vietnam       | 88                  | 71 (80.7%)                                           | 81 (92.0%)                                      |                                                      | Dimberg et al.98 |
|             | China         | 27b                 | 27 (100%)                                            | 18 (67.9%)                                      |                                                      | Li et al.99    |
|             | Turkey        | 25                  | 0 (0%)                                               | 14 (100%)                                       |                                                      | Aral et al.100 |
| Hepatocellular | Japan       | 28                  | 7 (25%)                                              | 23 (82.1%)                                      | 23/35 (65.7%)                                     | Fukushima et al.101 |
|             | China         | 27                  | 19 (70.4%)                                           | 12 (44.4%)                                      |                                                      | Shao et al.102  |
|             | Taiwan        | 18                  | 16 (100%)                                            | 18 (100%)                                       |                                                      | Yin et al.103  |
|             | China         | 62                  | 17 (28%)                                             | 39 (65%)                                        | 6/10 (60%)                                        | Wheelhouse et al.104 |
|             | Korea         | 27                  | 3 (11.1%)                                            | 23 (88.9%)                                      | 8/10 (80%)                                        | Gwak et al.105  |
|             | China         | 105                 | 10 (9.52%)                                           | 6 (52.4%)                                       | 0/6 (0%)                                         | Guo et al.106  |
| Esophageal  | China         | 19                  | 17 (80%)                                             | 18 (95%)                                        | 23 (85%)                                         | Abnet et al.107 |
|             | India         | 39                  | 2 (5.1%)                                             | 1 (2.6%)                                        | 9/10 (90%)                                       | Upadhyay et al.108 |
|             | UK            | 12                  | 2 (16.7%)                                            | 9/10 (90%)                                      | 1/12 (8.3%)                                      | Tan et al.109  |
| Oral        | Taiwan        | 53                  | 24 (46.1%)                                           | 36 (67.9%)                                      |                                                      | Lee et al.110  |
|             | India         | 18                  | 2 (11.1%)                                            | 5 (27.8%)                                       |                                                      | Tan et al.111  |
|             | Taiwan        | 12                  | 12 (100%)                                            | 12 (100%)                                       |                                                      | Shieh et al.112 |
|             | India         | 50                  | 42 (84%)                                             | 18 (50%)                                        |                                                      | Pandey et al.113 |
| Skin        | Taiwan        | 10 (SCC) 7 (BCC)    | 7 (70.0%)                                             | -                                               | 26/53 (49.1%)                                     | Yang et al.114  |
|             | Germany       | 20 (SCC) 21 (BCC)   | 19 (95.0%)                                           | 40/41 (97.6%)                                   |                                                      | Kamenisch et al.115 |
| Thyroid     | Portugal      | 5 (FC) 13 (HFC)     | 0 (0%)                                               | 0/5 (0%) (AP)                                   |                                                      | Máximo et al.116 |
|             |                | 16 (PC) 10 (HPC)    | 5 (38.8%)                                             | 2/16 (12.5%)                                    |                                                      |                   |
|             | Turkey        | 50                  | 4 (8%)                                               | 3/8 (33.3%)                                     | 0/9 (0%)                                         | Aral et al.117  |
| Lung        | China         | 37                  | 20 (54.1%)                                           | 22 (59.5%)                                      | 6/20 (30%)                                       | Dai et al.118   |
| Endometrial | Poland        | 37                  | 30 (81.1%)                                           | 32 (88.6%)                                      |                                                      | Futyma et al.119 |
| Cervix      | Turkey        | 21                  | 4 (19%)                                              | -                                               | 5/16 (31.3%)                                     | Kara et al.120  |
| Prostate    | China         | 130                 | 98 (75.4%)                                           | 14 (10.8%)                                      |                                                      | Yu and Yan121   |

BC, breast carcinoma; BBD, benign breast disease; SCC, squamous cell carcinoma; BCC, basal cell carcinoma; AP, adjacent parenchyma; FC, follicular carcinoma; HFC, Hurthle cell follicular carcinoma; PC, papillary carcinoma; HPC, Hürthle cell papillary carcinoma. Blood; mesenteric arteries from colorectal cancer.
mtDNA$^{4977}$ in tumors could be either the consequence of a dilution effect because of mitotic clonal expansion throughout cancer progression or an active selection mechanism that eliminates cancer cells harboring the deleted mtDNA.\textsuperscript{75} An increased accumulation of mtDNA$^{4977}$ has also been reported in cancerous breast tissues. In 2004, Zhu et al. found a high mtDNA large scale deletion rate in the breast cancer tissue samples than that in the matched normal tissues nearby to the tumor.\textsuperscript{84} In a more recent study, Nie et al. carried out a comprehensive experiment to detect the mtDNA$^{4977}$ as well as mtDNA copy number alteration in breast carcinoma and benign breast disease patients.\textsuperscript{85} The authors revealed that the mtDNA$^{4977}$ in the blood of breast carcinoma patients was significantly higher (51/107, 48%) than that of benign breast disease patients and healthy controls. This deletion has also been found to be correlated with a lower mtDNA content and higher MnSOD level and oxidative damage in both group patients. These outcomes conclude that the occurrence of mtDNA$^{4977}$ in blood may potentially serve as a biomarker for breast cancer.

There is also a report that shows a negative finding of this mtDNA common deletion in breast cancer patients.\textsuperscript{86-88} In 2016, Dhahi et al. published the results of a study in which mtDNA$^{4977}$ was not detected in any of tumor tissues of breast cancer as well as in adjacent non-tumor tissues.\textsuperscript{86} This finding is in agreement with two of the previous study by Tan et al. and Aral et al. which report the absence of mtDNA$^{4977}$ in all breast tumors and their matched surrounding normal tissues.\textsuperscript{87,88}

**Gastric cancer**

The mtDNA large scale deletions have been first described in gastric carcinomas by Máximo et al.\textsuperscript{89,90} They reported a high frequency of 4977-bp mtDNA deletion (53.1%) in 17/32 primary sporadic gastric carcinomas. Consistent with these data, Wang and Lü also detected a higher mtDNA$^{4977}$ rate in gastric cancer tissues (86/108, 79.6%) than in the adjacent normal tissues (73/108, 67.6%).\textsuperscript{91} However, in contrast to this, another study by Wu et al. only 9.7% of common mtDNA$^{4977}$ was detected in gastric carcinomas compared to 55% in non-cancerous tissue samples.\textsuperscript{83} Kamalidehghan et al. also found a low frequency (6/107, 5.6%) of 4977-bp deletion in tumoral tissues of gastric cancer.\textsuperscript{92} Wu et al.’s and Kamalidehghan et al.’s findings are consistent with those for oral cancer\textsuperscript{76} and breast cancer.\textsuperscript{76}

**Colorectal cancer**

A decreased proportion of mtDNA$^{4977}$ that found in tumor tissues compared to adjacent non-tumor tissue also has been reported in colorectal cancer.\textsuperscript{81} In 2004, Dani et al. investigated the involvement of mtDNA$^{4977}$ in colorectal cancer and other types of tumors with multiplex PCR assay.\textsuperscript{81} They found that the level of the mtDNA$^{4977}$ in tumor tissues of colorectal cancer (52%) was significantly less than that in adjacent non-tumor tissues (83%).\textsuperscript{83} And this observation was further supported by the finding of Chen et al. which analyzed the mtDNA$^{4977}$ level in 104 colorectal cancer of Chinese patients.\textsuperscript{71} The authors revealed that the level of deletion in cancerous tissues appeared lower than those in non-cancerous areas. However, they claimed that decreased this deletion level lead to a significantly increased mtDNA copy number and correlated with cancer stage.

In a separate study, Dimberg et al. also demonstrated that the mtDNA$^{4977}$ was found at a significantly higher frequency in normal tissues compared to paired cancer tissues in colorectal cancer cases of Swedish and Vietnamese patients.\textsuperscript{93} However, this view is in contradiction with the study of mesenteric arteries from colorectal cancer patients. Li et al. reported that the mtDNA$^{4977}$ was significantly increased in cancer-associated mesenteric arteries than healthy controls.\textsuperscript{94} The authors suggested that the presence of this large-scale deletion in blood vessels near-cancer cells may be related to oxidative stress in the tumor microenvironment which may contribute to more mtDNA damage.\textsuperscript{94}

**Hepatocellular cancer**

Overall, 6 published studies and findings that have examined the mtDNA$^{4977}$ in patients with hepatocellular carcinoma (HCC).\textsuperscript{85-100} The large-scale deletion has been first described in human hepatic cancer samples by Fukushima et al. in 1995.\textsuperscript{95} In 2004, Yin et al. analyzed the association of HCC patients with the level of the mtDNA$^{4977}$ and mtDNA content.\textsuperscript{96} They observed that this common deletion accumulated in all of the tumors and the non-tumors liver tissues of HCC patients.\textsuperscript{96} In addition, the authors also found that non-cancerous liver of the HCC patients with a background of long-term alcohol intake have a significantly increased level of the mtDNA$^{4977}$ and decreased mtDNA content.\textsuperscript{96} While in the same year, Shao et al. reported the first quantitative study of frequent occurrence of the mtDNA$^{4977}$ in HCC patients.\textsuperscript{97} The authors suggested that this common deletion may play an essential role in HCC development and progression.

The incidence of the mtDNA$^{4977}$ in HCC was much lower than that in non-cancerous hepatic tissues and this had also been reported in two previous studies by Wheelhouse et al.\textsuperscript{98} and Gwak et al.\textsuperscript{99}

In a more recent research, Guo et al. used nested PCR assay to screen a common deletion of the mtDNA$^{4977}$ in blood and tissues of 105 HCC patients and 69 unrelated healthy subjects.\textsuperscript{100} They detected 9.52% of HCC patients contained this deletion, while it was absent in nearby normal tissues and healthy subjects. In addition, this deletion has also been determined to be related with elevated ROS level and mtDNA copy number.\textsuperscript{100}

**Esophageal cancer**

In 2004, Abnet et al. conducted an analysis among a high-risk population of esophageal squamous cell carcinoma in China and discovered that 89% of tumors and 95% of the adjacent normal tissues carried the mtDNA$^{4977}$.\textsuperscript{101} However, in a low-risk population of esophageal cancer in northern India, Upadhyay et al. identified a low frequency of the mtDNA$^{4977}$.\textsuperscript{102} In another study, Tan et al. carried out an experiment to investigate the presence of this common deletion in the progression of Barrett’s esophagus to esophageal adenocarcinoma.\textsuperscript{103} They claimed that the frequency of specimens with the mtDNA$^{4977}$ increased in association with the degree of dysplasia, thus the mtDNA$^{4977}$ may be a potential biomarker to predict the severity of dysplasia.\textsuperscript{103}

**Oral cancer**

There have been very few studies conducted on the connection
of this mtDNA\textsuperscript{4977} in oral carcinogenesis.\textsuperscript{75,104-106} Apart from the earliest study by Lee et al.\textsuperscript{75} that reported very impressive data on the accumulation of large-scale deletions of mtDNA in human oral tissues, similar results were also reported by Tan et al.,\textsuperscript{104} in a study of Taiwanese patients with primary oral squamous cell carcinoma by the same detection approach. Another interesting study also involved Taiwanese patients carried out by Shieh et al. on paired oral cancer and precancerous lesions using the combination techniques of laser microdissection and qPCR, revealed a higher level of the mtDNA\textsuperscript{4977} in stromal non-tumor tissue compared with tumor tissue.\textsuperscript{105}

In another previous study, Pandley et al. reported an interesting finding on the correlation between mtDNA\textsuperscript{4977} and polymorphism in cytochrome P450 2E1 (CYP2E1) gene in oral cancer in the North Indian population.\textsuperscript{106} Their outcomes suggested that CYP2E1 gene polymorphisms coexist with mtDNA\textsuperscript{4977} can contribute to a high risk factor for oral cancer.\textsuperscript{106}

Other cancers

The mtDNA\textsuperscript{4977} has also previously been investigated in several other cancers including skin cancer,\textsuperscript{82,107,108} thyroid cancer,\textsuperscript{88,109} lung cancer,\textsuperscript{110} endometrial cancer,\textsuperscript{111} cervix cancer,\textsuperscript{112} as well as prostate cancer.\textsuperscript{113}

There are a few interesting studies that show a positive association between this mtDNA common deletion and cancer cells. A 2010 study from China investigated this common deletion in prostate cancer patients.\textsuperscript{113} The study showed that there was a significantly higher prevalence of the mtDNA\textsuperscript{4977} in prostate cancer patients compared to benign prostatic hyperplasia patients. Given such a higher frequency of mtDNA\textsuperscript{4977}, the author concluded that their finding is likely to be a useful biomarker for assessing the degree of malignancy in prostate cancer patients.

A 2016 study by Shen et al. examined the relationship between the levels of mtDNA\textsuperscript{4977} in blood samples from 206 melanoma patients and 219 healthy controls.\textsuperscript{108} The study revealed that the mtDNA\textsuperscript{4977} levels in melanoma cases were significantly higher than healthy controls. Furthermore, they reported elevated levels of mtDNA\textsuperscript{4977} were associated with increased risk of melanoma and suggested that mtDNA deletions may mediate the connection between sun exposure and melanoma risk.

Can mtDNA\textsuperscript{4977} be a diagnostic and prognostic biomarker for cancer?

Nowadays there are still no useful or specific tumor biomarkers that can be used for detecting cancer at the early stage, as well as disease recurrence and determining a prognosis. Thus, the exploration of novel tumor biomarkers for early diagnosis, prognostic prediction and effective therapies will positively benefit cancer patients. The most well-established and widely used biomarkers in cancer diagnosis include prostate specific antigen (PSA), carcinoembryonic antigen (CEA), serum cancer antigen (CA 125, CA 15-3 and CA 19-9), human epidermal growth factor receptor-2 (HER2/neu), alpha-fetoprotein (AFP),\textsuperscript{114} In addition, several biomarkers including isocitrate dehydrogenase 1 or 2 (IDH1/2), estrogen receptor (ER), p53, Ki-67, and O\textsuperscript{6}-methylguanine-DNA-methyltransferase (MGMT) are also used in cancer as prognostic and predictive biomarkers.\textsuperscript{115} Improving the accuracy for the detection of the malignancy at an early stage remains a great challenge concerning biomarkers in diagnosing cancer.

The use of mtDNA as a potential biomarker to assess the risk and prognosis of cancer remains controversial and is still under investigation. The most well-studied of all mtDNA cancer alterations are in the displacement loop (D-loop) region of mtDNA non-coding part. This D-loop region acquires high mutation rates to determine the proliferation and progression of cell lineages.\textsuperscript{116} Previous studies have reported that D-loop mutations were associated with poor prognosis in some types of cancer.\textsuperscript{117,118} The mtDNA\textsuperscript{4977} has been reported to accumulate in many tissues during aging\textsuperscript{119} and has also been widely utilized as a biomarker of mtDNA damage.\textsuperscript{20}

Up to now, the most studied mitochondrial alteration in skin was the mtDNA\textsuperscript{4977}. It has been proposed that the frequency of mtDNA\textsuperscript{4977} contributes a potential biomarker of cumulative UV exposure in human skin.\textsuperscript{30,31} In 2006, Eshaghian et al. detected numerous mtDNA deletions from photoaged, tumor-free skin of multiple individuals which include the mtDNA\textsuperscript{4977}. They concluded that mtDNA deletions may potentially be used as biomarkers of photoaging in the skin. In 2016, Powers et al. quantified the mtDNA\textsuperscript{4977} in the sun and non-sun exposed skin of five cohorts of patients and revealed abnormally high levels of mtDNA\textsuperscript{4977} in sun exposed skin samples. They claimed that mtDNA\textsuperscript{4977} could be indicative of sun exposure.\textsuperscript{120} One year later, the same group extended the study and discovered there was a possible synergistic relationship that caused the localised high levels of mtDNA deletions, presumably due to sun exposure and the prescribed drugs side effects.\textsuperscript{121}

On the cancer-based research, Tan and coworkers reported a 70 specimen-study, ranging from normal to malignant esophageal tissue and proposed that this large deletion might be useful biomarker in detecting the severity of dysplasia in Barret’s esophagus.\textsuperscript{122} In a Chinese study for HCC, the authors proposed that mtDNA\textsuperscript{4977} might be served as a potential biomarker, possibly via the mtDNA copy number and oxidative stress alteration.\textsuperscript{100} In another study of three human tumor cell lines (liver, esophageal and breast), Li et al. suggested that mtDNA\textsuperscript{4977} could be one of the markers for radiation-induced damage due to the occurrences of this common deletion in all the tumor cells after γ-ray irradiation at any doses.

The mtDNA\textsuperscript{4977} has been broadly explored in various cancer types, unfortunately its predictive or prognostic role in cancer is still not well understood. Until now, not much is known about the relationship between mtDNA\textsuperscript{4977} and clinical outcome/prognosis in cancer. Cancer cell proliferation needs a continuous and plentiful supply of energy to fulfill their cell growth and division. This one-third length deletion of mtDNA impairs the function of the respiratory chain, leading to inhibition of cell proliferation. In previous studies, mtDNA\textsuperscript{4977} has been predominantly reported to be detected in normal tissues adjacent to cancerous tissues.\textsuperscript{75,80,82,83,93}

Consistent with these findings, a recently published meta-analysis of 38 studies by Nie et al. also noticed a significantly decreased proportions of mtDNA\textsuperscript{4977} in cancerous tissue compared to adjacent non-cancerous tissue.\textsuperscript{122,123} The authors hypothesized that a greater mutational burden might be needed to initiate tumor growth at the early stages. Through clonal expansion, the cells with mutations resume proliferation which will result in a larger frequency of alterations in adjacent normal tissue than cancerous tissue. Moreover, both factors of genetic and environmental influences should also be taken into consideration.

The prognostic impact of mtDNA\textsuperscript{4977} remains debated and inconsistent. Upadhyay et al. believed that minor frequency of mtDNA\textsuperscript{4977} might have some role in tumor progression and prediction of survival outcome in esophageal cancer.\textsuperscript{125} Badie et al. observed that the mtDNA\textsuperscript{4977} was obviously not correlated with...
prognostic indicators among Sudanese with oral lesions.\textsuperscript{124}

Recently, not on the cancer-based study, Huang\textit{ et al.} reported that mtDNA\textsuperscript{4977} status had no effect on the prognosis of stroke patients.\textsuperscript{125} However, they concluded that undetectable mtDNA\textsuperscript{4977} might be a marker or risk factor for ischemic stroke.\textsuperscript{125} In contrast, in more recent an Italian cohort study, about 515 patients with stable coronary artery disease, the authors demonstrated that the mtDNA\textsuperscript{4977} were highly observed and had prognostic implications in patients with major adverse cardiac events.\textsuperscript{126}

It is widely accepted that deletion of mtDNA\textsuperscript{4977} participates in the pathogenesis of cancer, but there still remains some issue as to whether a mtDNA\textsuperscript{4977} contributes to cancer initiation and progression, and this remains an active area of investigation. Results in the previous studies have been inconsistent and many factors may influence the proportion of the deletion in cancer tissues. Although it is an encouraging finding across many studies, active investigations are needed to discover whether the deletion can potentially be applied as an effective biomarker for cancer. It is believed that early detection of cancer at an earlier stage may lead to improved outcomes, including can increase chances of survival.

Conclusions

This review summarizes the most studies of mtDNA\textsuperscript{4977} that have been reported in different kinds of human cancer. At the moment, significant extensive research efforts have been made to better understand the role of mtDNA\textsuperscript{4977} on the initiation, growth, and progression of cancer. The existence of different proportion of the mtDNA\textsuperscript{4977} in tumor tissue as compared with corresponding non-tumorous tissue could be classified as alterations in connection with the presence of various environmental and genetic factors. More studies and attention should be given before a clear conclusion could be achieved regarding the effect and role of this large mtDNA deletion in carcinogenesis.

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