Somatic alterations in mitochondrial DNA and mitochondrial dysfunction in gastric cancer progression

Hsin-Chen Lee, Kuo-Hung Huang, Tien-Shun Yeh, Chin-Wen Chi

Hsin-Chen Lee, Chin-Wen Chi, Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei 112, Taiwan
Kuo-Hung Huang, Division of General Surgery, Department of Surgery, Taipei Veterans General Hospital, Taipei 112, Taiwan
Kuo-Hung Huang, Institute of Clinical Medicine, School of Medicine, National Yang-Ming University, Taipei 112, Taiwan
Tien-Shun Yeh, Department of Anatomy and Cell Biology, School of Medicine, National Yang-Ming University, Taipei 112, Taiwan
Chin-Wen Chi, Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei 112, Taiwan

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Correspondence to: Hsin-Chen Lee, Professor, Institute of Pharmacology, School of Medicine, National Yang-Ming University, No. 155, Li-Nong St., Sec. 2, Taipei 112, Taiwan. hclee2@ym.edu.tw

Telephone: +886-2-28267327 Fax: +886-2-28264372
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Abstract

Energy metabolism reprogramming was recently identified as one of the cancer hallmarks. One of the underlying mechanisms of energy metabolism reprogramming is mitochondrial dysfunction caused by mutations in nuclear genes or mitochondrial DNA (mtDNA). In the past decades, several types of somatic mtDNA alterations have been identified in gastric cancer. However, the role of these mtDNA alterations in gastric cancer progression remains unclear. In this review, we summarize recently identified somatic mtDNA alterations in gastric cancers as well as the relationship between these alterations and the clinicopathological features of gastric cancer. The causative factors and potential roles of the somatic mtDNA alterations in cancer progression are also discussed. We suggest that point mutations and mtDNA copy number decreases are the two most common mtDNA alterations that result in mitochondrial dysfunction in gastric cancers. The two primary mutation types (transition mutations and mononucleotide or dinucleotide repeat instability) imply potential causative factors. Mitochondrial dysfunction-generated reactive oxygen species may be involved in the malignant changes of gastric cancer. The search for strategies to prevent mtDNA alterations and inhibit the mitochondrial retrograde signaling will benefit the development of novel treatments for gastric cancer and other malignancies.

Key words: Gastric cancer; Somatic mitochondrial DNA mutations; Mitochondrial dysfunction

Core tip: In this review, we summarize recent somatic mitochondrial DNA (mtDNA) alterations identified in gastric cancer, and the relationship between these alterations and the clinicopathological features of gastric cancer. We suggest that point mutations and mtDNA copy number decreases are the two most common mtDNA alterations that potentially result in mitochondrial dysfunction in gastric cancer. Mitochondrial dysfunction-generated reactive oxygen species may be involved in the malignant changes of gastric cancer. The search for strategies to prevent the mtDNA alterations and inhibit the mitochondrial retrograde signaling will benefit the development of novel treatments for gastric cancer and other malignancies.

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# INTRODUCTION

Gastric cancer is one of the most common causes of death in cancer patients throughout the world. Surgical resection with radical lymph nodes dissection is the primary therapy for gastric cancer\(^2\). Chemotherapy is an alternative treatment for unresectable gastric cancer or tumor recurrence after surgical resection. However, the response to chemotherapy remains unsatisfactory. Thus, it is important to identify novel drug targets and develop effective treatments for gastric cancer.

Based on the conceptual progress of the past decades, energy metabolism reprogramming was recently included as one of the cancer hallmarks\(^2\). Warburg\(^3,4\) first proposed that tumor cells, unlike normal cells, exhibit increased glycolytic activity and reduced mitochondrial respiration even in the presence of oxygen. This phenomenon is known as the “Warburg effect”. Increasing lines of evidence suggest that various molecular mechanisms generate the Warburg effect\(^5,6\). One of these mechanisms is mitochondrial dysfunction resulting from mutations in nuclear genes or mitochondrial DNA (mtDNA)\(^6,7\).

Mitochondria are intracellular organelles in eukaryotic cells that participate in bioenergetics metabolism and cellular homeostasis, including the generation of ATP through respiration and oxidative phosphorylation (OXPHOS), the production of reactive oxygen species (ROS), and the initiation and execution of apoptosis\(^8\). Mitochondria contain multiple copies of mitochondrial DNA (mtDNA). Human mtDNA is a 16.6-kb double-stranded, circular DNA molecule that encodes 13 respiratory enzyme complex polypeptides, 22 transfer RNAs and 2 ribosomal RNAs required for mitochondrial protein synthesis\(^8\). Because mtDNA is essential for the maintenance of functionally competent organelles, the accumulation of mtDNA mutations or decreased mtDNA copy number is expected to affect energy production as well as enhance ROS generation and cell survival, and these processes may be involved in aging, mitochondrial diseases or cancer\(^9,10\).

In the past decade, somatic mtDNA alterations have been identified in several types of cancer\(^8,9\), including gastric cancer\(^11-15\). However, the role of these mtDNA alterations in tumorigenesis and cancer progression remains unclear. In this article, we review recent findings on somatic mtDNA alterations in gastric cancer. In addition, we discuss the potential factors that may lead to mtDNA mutations and propose a role of mtDNA alterations and mitochondrial dysfunction in the progression of gastric cancer.

# SOMATIC MITOCHONDRIAL DNA ALTERATIONS IN GASTRIC CANCER

Several studies have identified various types of mtDNA alterations in gastric cancer\(^13-15\), including point mutations, large-scale deletions, insertions, and copy number changes.

In one of our studies\(^18\), 65% of the examined gastric cancer patients carried at least one mtDNA somatic point mutation. Among the identified point mutations, 69% occur in the D-loop region of mtDNA, 27% are found in the protein-coding region, and 4% are located in rRNA genes. Compared with other cancers, these mutations are similar in their incidence and distribution (Table 1)\(^13,38\). The D-loop region of mtDNA is the most frequent site of somatic mutation in cancers. Because the D-loop region contains the major regulatory sites for mtDNA replication and transcription, mutations near these sites might affect mtDNA copy number in cancers.

Given that the mtDNA D-loop region is a hot spot for somatic mutations in gastric cancer as well as other cancers, numerous studies focus on somatic mutations in this region\(^13,39-43\). The incidence of somatic mtDNA point mutations in the D-loop of gastric cancer patients ranges from 4% to 48%. The most common mutations in this region are mononucleotide repeat variants of the poly-cytosine (poly-C) sequence at nucleotide positions (np) 303-309 (D310) in mtDNA\(^9\). The variants were also identified in normal subjects\(^44\) and patients with neurodegenerative diseases\(^45\). The effect of these variants is not clearly defined.

Moreover, several somatic point mutations identified in the mtDNA protein-coding region and tRNA genes in gastric cancer patients are potentially harmful\(^45\). These mutations include missense mutations (e.g., G3697A and G4996A) that cause amino acid substitutions at the highly evolutionarily conserved amino acid residues, frame-shift mutations (e.g., 12418insA) that result in truncated polypeptides, and tRNA mutations (e.g., 7472insC) that potentially alter tRNA structure. Moreover, studies have demonstrated that these mutations are pathogenic and associated with mitochondrial diseases\(^46,47\). tRNA gene mutations as well as missense and frame-shift mutations in the mitochondrial genome may promote mitochondrial dysfunction in gastric cancer cells.

A common 4977-bp mtDNA deletion occur less frequently in gastric cancers compared with the corresponding noncancerous stomach tissues\(^41,46,47\), though large-scale mtDNA deletions are the most common mutation in the somatic tissues of aged human subjects\(^39\). This finding is consistent with observations in other types of cancer\(^39\). The low accumulation of large-scale mtDNA deletions in cancer could result because an increased frequency of these mutations may cause severe mitochondrial dysfunction and sensitize the cells to apoptosis. Any cells harboring high levels of large-scale mtDNA deletions could be eliminated during tumorigenesis\(^39\).

Unlike large-scale deletions, a 50-bp deletion flanked
Lee HC et al. MitDNA alterations in gastric cancer progression

Table 1 The distribution of somatic mitochondrial DNA mutations in human cancers a (%)

| Cancer                  | Cases | No. of cancers with mutation | No. of mutations | D-loop | tRNA | rRNA | mRNA | Ref. |
|-------------------------|-------|-----------------------------|------------------|--------|------|------|------|------|
| Adult leukemia          | 24    | 9 (37.5)                    | 9                | 2 (22.2)| 1 (11.1) | 0    | 6 (66.7) | [16] |
| Bladder cancer          | 14    | 9 (64.3)                    | 20               | 6 (30.0)| 3 (15.0) | 0    | 11 (55.0) | [17] |
| Breast cancer           | 18    | 11 (61.1)                   | 12               | 7 (58.3)| 0    | 0    | 5 (41.7) | [18] |
| Esophageal cancer       | 19    | 14 (73.7)                   | 27               | 22 (81.5)| 1 (3.7) | 0    | 4 (14.8) | [19] |
| Follicular thyroid cancer | 15   | 14 (93.3)                   | 45               | 17 (37.8)| 3 (6.7) | 2 (4.4)| 23 (51.1) | [20] |
| Gastric cancer          | 58    | 27 (46.6)                   | 40               | 21 (52.5)| 2 (5.0) | 2 (5.0)| 15 (37.5) | [21] |
| Head-and-neck cancer    | 20    | 11 (55.0)                   | 14               | 9 (46.3)| 1 (7.1) | 0    | 4 (28.6) | [22] |
| Hepatocellular cancer   | 3     | 2 (100.0)                   | 4                | 2 (50.0)| 2 (50.0)| 0    | 0     | [23] |
| Head and-neck cancer    | 31    | 20 (64.5)                   | 26               | 18 (69.2)| 0    | 1 (3.8)| 7 (26.9) | [15] |
| Lung cancer             | 10    | 5 (50.0)                    | 24               | 23 (95.8)| 0    | 0    | 1 (4.2)  | [24] |
| Medullary blastoma      | 44    | 23 (52.3)                   | 34               | 21 (61.8)| 1 (2.9) | 2 (5.9)| 10 (29.4) | [25] |
| Medullary blastoma      | 14    | 6 (47.1)                    | 10               | 7 (70.0)| 1 (10.0) | 2 (20.0)| 0     | [17] |
| Medullary blastoma      | 55    | 33 (60.0)                   | 56               | 18 (32.1)| 1 (1.8) | 3 (5.4)| 34 (60.7) | [26] |
| Oncocytic head-and-neck tumor | 25  | 16 (64.0)                   | 18               | 11 (61.1)| 0    | 3 (16.7)| 4 (22.2) | [27] |
| Oncocytic pituitary adenoma | 25  | 18 (72.0)                   | 20               | 3 (15.0)| 0    | 2 (10.0)| 15 (75.0) | [28] |
| Oncocytic thyroid tumor | 45    | 26 (57.8)                   | 30               | 0      | 0    | 0    | 30 (100.0) | [29] |
| Oral cancer             | 300   | 240 (80.0)                  | 645              | 355 (55.0)| 36 (5.6)| 21 (3.3)| 233 (36.1) | [31] |
| Ovarian cancer          | 10    | 6 (60.0)                    | 15               | 11 (73.3)| 3 (20.0)| 0 | 1 (6.7)  | [32] |
| Pancreatic cancer       | 5     | 4 (80.0)                    | 4                | 0      | 1 (25.0)| 1 (25.0)| 2 (50.0) | [33] |
| Papillary thyroid cancer | 7    | 3 (42.9)                    | 4                | 1 (25.0)| 0    | 0    | 4 (100.0) | [23] |
| Parathyroid adenoma     | 30    | 15 (50.0)                   | 27               | 6 (22.2)| 1 (3.3)| 1 (3.3)| 19 (70.4) | [34] |
| Renal cell cancer       | 8     | 5 (62.5)                    | 6                | 1 (16.7)| 2 (33.3)| 0 | 3 (50.0) | [35] |
| Renal oncocytomas       | 9     | 9 (100.0)                   | 14               | 1 (7.1) | 0 | 0 | 13 (29.2) | [38] |
| Total                   | 859   | 567 (66.0)                  | 1180             | 595 (50.5)| 65 (5.5)| 42 (3.6)| 478 (40.4) |             |

by a 9-bp direct repeat at nps 298-306 and 348-356 of the mtDNA D-loop region was reportedly found at high levels in four gastric cancers[40]. This deletion is associated with decreased mtDNA copy number in cancer[19].

A 260-bp tandem duplication/triplication mtDNA mutation in the D-loop region was identified in approximately 13% of the examined gastric cancers[14]. The duplicate/triplicate insertion of an 260-bp fragment is flanked by two poly-C sequences at nps 303-309 and 568-573[13,14,44]. The insertion was also detected in other types of cancer[14]. However, the occurrence of this mutation does not appear to be specific to cancer cells[14,44,50-52].

Decreased mtDNA copy number was frequently detected in gastric cancer patient tissues compared with corresponding noncancerous stomach tissue[13,33]. Alterations in mtDNA copy number change (increase or decrease) appear to be tissue specific[14,33,44]. A decreased mtDNA copy number is also found in the majority of hepatocellular carcinomas[39] and breast cancers[19].

These findings reveal that somatic point mutations and a decreased mtDNA copy number are two common mtDNA alterations in gastric cancer. The increased rate of somatic mtDNA alterations in gastric cancer is also observed in other cancers, suggesting that these two types of somatic mtDNA alterations are common events in human cancer progression. These mtDNA alterations may result from similar factor(s) and/or play a consistent role in the tumorigenesis of gastric cancer and other malignancies.

SEVERAL POTENTIAL FACTORS MAY CAUSE TO SOMATIC mtDNA ALTERATIONS IN GASTRIC CANCER

The mutation type could provide clues regarding factors that potentially contributing to somatic mtDNA alterations in gastric cancer. Among the mtDNA mutations identified in gastric cancer, 46% of the somatic point mutations are transition mutations (e.g., T-to-C or G-to-A), and another 46% result from mononucleotide or dinucleotide repeat instability (e.g., poly-C or poly-A)[15]. Compared with other types of cancer, 60% of the mutations are transition mutations, 31% are mononucleotide or dinucleotide repeat instability, and 4% are transversion mutations (e.g., T-to-A or G-to-C) (Table 2)[15,30]. These findings indicate that transition mutations and mononucleotide or dinucleotide repeat instability are two major types of somatic mtDNA mutations in cancers.

Given that the mitochondrial electron transport chain is a major site for intracellular ROS formation, oxidative mtDNA damage is predicted to be an important factor promoting mtDNA mutations and genome instability in cancers. However, whether steady-state levels of oxidative mtDNA damage are increased in gastric cancer compared with corresponding noncancerous stomach tissue remains unknown.

The main pyrimidine and purine product of oxidative DNA base damage is thymine glycol and 7,8-dihydro-
8-oxo-2’-deoxyguanosine (8-oxodG), respectively.[56-59] Thymine glycol is poorly mutagenic, but 8-oxodG can result in G-to-T transversion mutations during replication because unrepaired 8-oxodG can pair with adenine.[60]. However, the most common mtDNA mutations in cancer are transition mutations rather than the mutational consequences specific to 8-oxodG (G-to-T transversion). Therefore, DNA lesions other than 8-oxodG could be primarily responsible for mtDNA transition mutations in cancer. Some studies indicated that oxidative lesion 8-oxodG can be efficiently repaired in mtDNA.[61]. In addition, oxidative DNA damage can produce a range of base lesions, and the mutagenic potential of these lesions has not been fully elucidated.[62]. In fact, some of these lesions may be responsible for ROS-mediated mtDNA mutagenesis. Moreover, reactive nitrogen species (RNS) can deaminate adenine to hypoxanthine, cytosine to uracil, and guanine to xanthine, thereby causing transition mutations.[63,64]. Thus, it is possible that mtDNA transition mutations in cancer could result from the deamination of adenine, cytosine, or guanine by RNS. Alternatively, factors other than oxidative damage are primarily responsible for the formation of mtDNA mutations, such as defects in mtDNA polymerase or repair systems.[65,66].

Oxidative damage could also contribute to mononucleotide or dinucleotide repeat instability in mtDNA.[67]. The mononucleotide repeat in the D310 poly-C sequence of the D-loop region, the most common site of somatic mtDNA mutations in cancer, is the site most susceptible to oxidative damage in mtDNA.[68]. Moreover, extensive oxidative damage to the mononucleotide repeats may result in slippage and/or misincorporation of nucleotides during mtDNA replication or repair by mtDNA polymerase (POLG). Importantly, it has been reported that POLG is a target of oxidative damage[69] and frequently harbors mutations in cancerous tissues.[68]. Specifically, mutations were identified in all three domains of the POLG protein, including the exonuclease domain, the linker region and the polymerase domain.[65]. In addition, increased mtDNA mutations are observed in Polg−/+ mice[69-74]. Therefore, defects in the polymerase and repair activities of POLG might enhance the generation of mtDNA mutations and genome instability in cancer. However, whether a general defect in POLG per se leads to increased mutations or genome instability in the D-loop region compared with other region in the mitochondrial genome and the mechanisms governing this action remains unknown.

Some studies indicated that Helicobacter pylori (H. pylori) infection can affect mitochondrial function and impair DNA repair mechanisms, thereby inducing genetic instability of nuclear and mitochondrial DNA in gastric cells[71-73]. Therefore, H. pylori infection may promote mtDNA instability and contribute to gastric carcinogenesis in infected individuals.

Decreased mtDNA copy number could result from

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**Table 2 The types of somatic mitochondrial DNA mutations in human cancers a (%)**

| Cancer | Cases | No. of cancers with mutation (%) | No. of mutations | Transitions | Transversions | Mono-/di-nucleotide repeat instability | Others | Ref. |
|--------|-------|---------------------------------|-----------------|-------------|--------------|---------------------------------------|--------|------|
| Adult leukemia | 24 | 9 (37.5) | 9 | 9 (100.0) | 0 | 0 | 0 | [16] |
| Bladder cancer | 14 | 9 (64.3) | 20 | 14 (70.0) | 3 (15.0) | 1 (5.0) | 2 (10.0) | [17] |
| Breast cancer | 18 | 11 (61.1) | 12 | 6 (50.0) | 1 (8.3) | 5 (41.7) | 0 | [18] |
| 19 | 14 (73.7) | 27 | 22 (81.5) | 1 (3.7) | 4 (14.8) | 0 | [19] |
| 15 | 14 (93.3) | 45 | 33 (73.3) | 7 (15.6) | 5 (11.1) | 0 | [20] |
| 58 | 27 (50.0) | 40 | 20 (50.0) | 2 (5.0) | 17 (42.5) | 1 (2.5) | [21] |
| Esophageal cancer | 20 | 11 (55.0) | 14 | 3 (21.4) | 1 (7.1) | 9 (64.3) | 1 (7.1) | [22] |
| Follicular thyroid cancer | 3 | 3 (100.0) | 4 | 3 (75.0) | 0 | 1 (25.0) | 0 | [23] |
| Gastric cancer | 31 | 20 (64.5) | 26 | 12 (46.2) | 0 | 12 (46.2) | 2 (7.2) | [15] |
| Head-and-neck cancer | 13 | 6 (46.2) | 9 | 7 (77.8) | 0 | 2 (22.2) | 0 | [17] |
| Hepatocellular cancer | 10 | 5 (50.0) | 24 | 15 (62.5) | 0 | 9 (37.5) | 0 | [24] |
| 44 | 23 (52.3) | 34 | 19 (55.9) | 0 | 13 (38.2) | 2 (5.9) | [25] |
| Lung cancer | 14 | 6 (47.1) | 10 | 8 (80.0) | 1 (10.0) | 1 (10.0) | 0 | [17] |
| 55 | 33 (60.0) | 56 | 47 (83.9) | 1 (1.8) | 8 (14.3) | 0 | [26] |
| Medulloblastoma | 15 | 6 (40.0) | 18 | 13 (72.2) | 0 | 5 (27.8) | 0 | [27] |
| Oncocytic head-and-neck tumor | 25 | 16 (64.0) | 25 | 13 (72.2) | 1 (5.6) | 1 (5.6) | 3 (16.7) | [28] |
| Oncocytic pituitary adenoma | 25 | 18 (72.0) | 20 | 10 (50.0) | 0 | 9 (45.0) | 1 (5.0) | [28] |
| Oncocytic thyroid tumor | 45 | 26 (57.8) | 30 | 22 (73.3) | 1 (3.3) | 5 (15.2) | 2 (6.7) | [29] |
| Oral cancer | 18 | 14 (77.8) | 26 | 12 (46.2) | 4 (15.4) | 8 (30.8) | 2 (14.3) | [30] |
| 300 | 240 (80.0) | 645 | 356 (55.2) | 20 (3.1) | 237 (29.7) | 32 (5.0) | [31] |
| Ovarian cancer | 10 | 6 (60.0) | 15 | 10 (66.7) | 0 | 4 (26.7) | 1 (6.7) | [32] |
| Pancreatic cancer | 5 | 4 (80.0) | 4 | 3 (75.0) | 1 (25.0) | 0 | 0 | [33] |
| Papillary thyroid cancer | 7 | 3 (42.9) | 4 | 4 (100.0) | 0 | 0 | 0 | [23] |
| Parathyroid adenoma | 30 | 15 (50.0) | 27 | 18 (66.7) | 1 (3.7) | 6 (22.2) | 2 (7.4) | [34] |
| Renal cell cancer | 8 | 5 (62.5) | 6 | 2 (33.3) | 1 (16.7) | 1 (16.7) | 2 (33.3) | [35] |
| 9 | 7 (77.8) | 9 | 6 (66.7) | 0 | 3 (33.3) | 0 | [36] |
| 15 | 7 (46.7) | 14 | 13 (92.9) | 1 (7.1) | 0 | 0 | 0 | [37] |
| Renal oncocytoma | 9 | 9 (100.0) | 14 | 7 (50.0) | 2 (14.3) | 4 (28.6) | 1 (7.1) | [38] |
| Total | 859 | 567 (66.0) | 1180 | 707 (59.9) | 49 (4.2) | 370 (31.4) | 54 (4.6) | |
mutations in the D-loop region. Because this region is the control site for mtDNA replication and transcription, mutations in the region could repress the rates of primer synthesis and mtDNA replication. This hypothesis is supported by the observation that decreased mtDNA copy number is associated with the mutations in the D-loop region \[1\].

In addition, decreased mtDNA copy number in cancer could be attributed to defects in mitochondrial biogenesis or other proteins localized to the mitochondria (e.g., p53 or SIRT3). Defects or decreased expression in several factors involved in mtDNA replication and maintenance as well as mitochondrial biogenesis, such as POLG \[2\], peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1) \[3\], mitochondrial single-strand DNA binding protein (mtSSB) \[4\], and mitochondrial transcription factor A (mtTFA) \[5\], have been observed in cancer. Decreased mtDNA copy number correlates with reduced expression of PGC-1 in HCC \[6\] and mtTFA in colorectal cancer \[7\]. These findings suggest that reduced mitochondrial biogenesis may lead to decreased mtDNA copy number in cancers. Moreover, the tumor suppressor p53 can localize to mitochondria, and contribute to the maintenance of mtDNA stability through interactions with POLG \[8\]. Thus, the loss of p53 in cancer may lead to decreased mtDNA copy number. In addition, the mitochondrial deacetylase SIRT3 is down-regulated and acts as a tumor suppressor in several cancers, including gastric cancer \[9,10\]. The loss of SIRT3 expression is an independent prognostic marker for reduced disease-free survival and overall survival in gastric cancer \[11,12\]. The loss of SIRT3 is correlated with decreased mtDNA integrity and mtDNA copy number \[13\].

Therefore, enhanced mtDNA damages and/or reduced efficiency in the mtDNA replication and repair activities as well as the loss of mitochondrial-localized proteins may contribute to mtDNA somatic mutations and decreased copy number in gastric cancer.

**CLINICAL CORRELATIONS OF SOMATIC mtDNA ALTERATIONS IN GASTRIC CANCERS**

To understand the roles of somatic mtDNA alterations in gastric cancer progression, the analysis of the clinicopathological features of cancers harboring these mutations may provide insight.

We analyzed the relationships between each somatic mtDNA mutation and the clinicopathological features of gastric cancer. However, no significant correlation was observed between the clinicopathological features of gastric cancer and somatic point mutations in the D-loop \[13,14\] or the mitochondrial genome \[15\], the 4977-bp deletion \[16\], or the tandem duplication/triplication of mtDNA \[17\].

For mutations of a specific mononucleotide repeat (D310) of mtDNA, the mutations are not associated with nuclear microsatellite instability in gastric cancer \[18\], and are more frequent in gastric cancer patients with \(H. pylori\)-associated chronic gastritis compared with cancer-free patients \[19\]. These findings suggest that mtDNA mononucleotide instability may be involved in the early stages of gastric carcinogenesis.

A significant association between decreased mtDNA copy number and ill-defined gastric cancers, including the ill-defined ulcerative and infiltrating (Borrmann’s type III) and diffusely infiltrating (Borrmann’s type IV) types, was observed \[20\]. A recent report further confirmed that mtDNA copy number is significantly decreased in gastric cancer, particularly in ill-defined stage III and IV cases, and suggested that alterations in mtDNA copy number may correlate with DNA methylation \[21\]. Because most patients with Borrmann’s type III and IV gastric cancer have a poorer prognosis and reduced 5-year survival rate after gastrectomy, these findings suggest that decreased mtDNA copy number may modify gastric cancer progression.

**THE POTENTIAL ROLES OF mtDNA MUTATIONS AND MITOCHONDRIAL DYSFUNCTION IN GASTRIC CANCER PROGRESSION**

In gastric cancer, somatic point mutations in the mitochondrial coding region are potentially harmful mutations that may cause mitochondrial dysfunction. These harmful mtDNA mutations along with decreased mtDNA copy number contribute to mitochondrial dysfunction. In addition, decreased mitochondrial aconitase (ACO2) expression, decreased respiratory capacity, and mitochondrial complex I deficiency were observed in gastric cancer \[22,23\]. These findings have been suggested as a mechanism to explain the Warburg effect. However, the role of mtDNA mutations and mitochondrial dysfunction in tumorigenesis and cancer progression remains unclear in gastric cancer.

Among the mtDNA mutations identified in gastric cancers, the role of the 12418insA mutation in tumorigenesis has been examined using a hybrid cell model (though not in gastric cancer cells) \[24\]. The 12418insA mutation is an “A” nucleotide insertion in the mononucleotide repeat of a poly-adenosine (poly-A) sequence at np 12418-12425 in mtDNA. The mutation causes a frameshift and premature termination of the ND5 gene, thereby resulting in a truncated ND5 subunit protein. In addition to gastric cancer \[25\], this mutation was also reported in the retinoblastoma-resistant VAV2 cell line \[26\], colorectal cancer \[27\], HCC \[28\], and breast cancer specimens \[29\]. A study revealed that the heteroplasmic 12418insA mutation contributes to reduced oxidative phosphorylation and increased ROS production in human cancer cells and promotes tumorigenesis in nude mice \[30\]. The report provided evidence suggesting that mtDNA mutation and mitochondrial dysfunction contribute to tumorigenesis.

Additional evidence was obtained from an approach...
using mitochondrial specific inhibitors to suggest that mitochondrial dysfunction enhances chemo-resistance and cell migration in human gastric cancer cells[88,91]. Oligomycin-induced mitochondrial dysfunction promotes cisplatin resistance and enhances cell migration in a human gastric cancer cell line[15]. Moreover, mitochondrial inhibitors (antimycin A and oligomycin) increased intracellular ROS levels, and the antioxidant N-acetyl-cysteine prevents the enhanced cell migration mediated by the mitochondrial inhibitors. These results suggest that ROS generated by defective mitochondria may be involved in the mechanism[15,87]. In addition, the mitochondrial inhibitors increase the expression of the cell adhesion molecule alpha5-integrin via ROS induction[87]. Alpha5-integrin on the cell surface is required for mitochondrial dysfunction-enhanced cell migration[87]. These findings suggest that ROS-mediated increased alpha5-integrin expression might serve as the molecular basis by which mitochondrial dysfunction promotes gastric cancer cell migration.

An addition approach employed a method to select the subpopulation of cancer cells demonstrating enhanced migration. This study indicated that highly migratory gastric cancer cells display reduced oxygen consumption rates, increased intracellular ROS content and increased alpha5-integrin expression compared with the parental cells[87]. Importantly, the evidence from clinicopathological studies with gastric cancer specimens suggest that alpha5-integrin expression is highly correlated with gastric cancer invasion[87]. These results further support the association between mitochondrial dysfunction and cell migration in gastric cancer. Although most of the studies were not focused on gastric cancer, data from several lines of research have substantiated the pathological role of mtDNA mutation or mitochondrial dysfunction in cancer. Using cybrid cell models, pathogenic mtDNA mutation (e.g., the T8993G transversion) have been shown to promote tumor growth in nude mice by preventing apoptosis[88-90]. Moreover, it was reported that the mtDNA mutation-mediated mitochondrial dysfunction contributes to metastatic cancer phenotypes, and ROS induction is mechanistically involved[88,91]. Mitochondrial inhibitors or mtDNA deletion can induce chemo-resistance or enhance the invasive phenotypes of various cancers[92-94]. “Retrograde signaling” signaling from mitochondria to the nucleus[95,96], has been proposed to be mechanistically involved. However, the common biomolecules involved in retrograde signaling remain undefined. The detailed mechanisms by which mtDNA mutation and mitochondrial dysfunction affect gastric cancer progression require further investigations.

CONCLUSION

Several types of somatic mtDNA alterations have been identified in human gastric cancers. The point mutation and decreased mtDNA copy number are the two most common mtDNA alterations, and these alterations might result in mitochondrial dysfunction in gastric cancers. These findings provide a molecular basis for the metabolic reprogramming or the “Warburg effect” in gastric cancers. Clinical correlative analyses reveal that decreased mtDNA copy number is associated with the ill-defined ulcerated and infiltrating types as well as the diffusely infiltrating types of gastric cancer, which might correlate with poorer patient prognosis[15,87]. However, the presence of somatic mtDNA point mutations in gastric cancers does not correlate with tumor size and grade, or patient survival[15]. This finding might be attributed to the possibility that these mtDNA point mutations do not always affect mitochondrial function but contribute to gastric cancer progression. In addition, different heteroplasmic levels of the same mtDNA mutation might produce varying results for tumorigenesis and cancer progression. The results are consistent with in vitro studies using mitochondrial inhibitors, suggesting that mitochondrial dysfunction might induce chemo-resistance and enhance cell migration in part in gastric cancer cells[15,84,86]. Thus, the role of specific mtDNA point mutation in mitochondrial function and gastric cancer progression warrants further study.

Among the somatic mtDNA mutations identified in gastric cancer, transition mutations and mononucleotide or dinucleotide repeat instability, not transversion mutations, are the two most common types of mutation. Transition mutations may not result from oxidative DNA damage; rather, these mutations may result from specific types of DNA damage and/or reduced efficiency in mtDNA replication and repair activities as well as other undefined mechanisms.

Increasing lines of evidence have important implications in the pathological role of mtDNA mutation or mitochondrial dysfunction in gastric cancer. Increased ROS production induced by mitochondrial dysfunction may be involved in the malignant changes of gastric cancer. However, the detailed mechanism by which mtDNA mutation and mitochondrial dysfunction affect gastric cancer progression remains unclear. Elucidation of the factors causing mtDNA mutations and activating retrograde signaling pathways in gastric cancer will be important for understanding the role of mitochondria and mtDNA in gastric cancer. The search for strategies to prevent mtDNA alterations and inhibit these pathways will aid in the development of novel treatments for gastric cancers.

REFERENCES

1. Wu CW, Hsiung CA, Lo SS, Hsieh MC, Chen JH, Li AF, Lui WY, Whang-Peng J. Nodal dissection for patients with gastric cancer: a randomised controlled trial. Lancet Oncol 2006; 7: 309-315 [PMID: 16574546 DOI: 10.1016/S1470-2045(06)70623-4]
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]
3. Warburg O. The metabolism of tumours. London: Arnold Constable, 1930: 254-270
4. Warburg O. On the origin of cancer cells. Science 1956; 123: 309-314 [PMID: 13298685]
5. Chen Z, Lu W, García-Prieto C, Huang P. The Warburg effect and its cancer therapeutic implications. J Bioenerg
Mitochondrial DNA (mtDNA) abnormalities have been implicated in various diseases, including cancer. Recent studies have highlighted the significance of somatic mutations and content alteration in mitochondrial DNA in carcinogenesis. This review aims to summarize the current knowledge on mitochondrial DNA mutations in cancer, focusing on their implications and potential clinical applications.

Mitochondrial DNA mutations in cancer have been extensively studied, and they play a crucial role in cancer progression. Somatic mutations in mitochondrial DNA have been found in various human cancers, including esophageal, breast, and lung cancers. These mutations, often involving the D-loop region, are associated with increased proliferation, cell survival, and resistance to apoptosis.

One of the key findings is the presence of informative mtDNA signatures in cancer tissues, which can be used for diagnostic and prognostic purposes. For instance, the D-loop mutations in breast cancer have been associated with poor prognosis, while mitochondrial DNA deletions in esophageal cancer have been linked to advanced tumor stages.

Moreover, mitochondria are known to contribute to the Warburg effect, a metabolic shift in cancer cells that favors aerobic glycolysis over oxidative phosphorylation. This shift is driven, in part, by mitochondrial dysfunction, leading to increased production of lactate, which can serve as an energetic substrate for cancer cells.

Additionally, mitochondrial DNA mutations have been linked to the stabilization of hypoxia-inducible factor (HIF) alpha, a key regulator of the Warburg effect. HIF stabilization leads to the upregulation of genes involved in glucose metabolism and angiogenesis, further supporting the Warburg effect.

In conclusion, mitochondrial DNA mutations play a critical role in cancer progression by mediating metabolic, proliferative, and survival advantages in cancer cells. Further research is needed to elucidate the complex interactions between mitochondrial DNA alterations and cancer, with the potential to develop targeted therapeutics.

**References**

1. Parrella P, Yao X, Fliss M, Sanchez-Cespedes M, Mazzaferri P, Rinaldi N, Micali N, Gabrielsson E, Cuomo C, Cohen D, Pandit S, Spencer M, Rabitti C, Fazio VM, Sidransky D. Detection of mitochondrial DNA mutations in primary breast cancer and fine-needle aspirates. *Cancer Res* 2001; 61: 7623-7626 [PMID: 11606403]

2. Tan DJ, Bai RK, Wong LJ. Comprehensive scanning of somatic mitochondrial DNA mutations in breast cancer. *Cancer Res* 2002; 62: 972-976 [PMID: 11861366]

3. Zhu W, Qin W, Bradley P, Wessel A, Puckett CL, Sauter ER. Mitochondrial DNA mutations in breast cancer tissues and in matched nipple aspirate fluid. *Carcinogenesis* 2005; 26: 145-152 [PMID: 15375511 DOI: 10.1093/carcin/bqi282]

4. Tseng LM, Yin PH, Yang CW, Tsai YF, Hsu CY, Chi CW, Lee HC. Somatic mutations of the mitochondrial genome in human breast cancers. *Genes Chromosomes Cancer* 2011; 50: 800-811 [PMID: 21748819 DOI: 10.1002/gcc.20901]

5. Witte J. Lehrmann S, Wulfert M, Yang Q, Röher HD. Mitochondrial DNA mutations in differentiated thyroid cancer with respect to the age factor. *World J Surg* 2007; 31: 51-59 [PMID: 17171498 DOI: 10.1007/s00268-005-0447-5]

6. Wong LJ, Tan DJ, Bai RK, Yeh KT, Chang J. Molecular alterations in mitochondrial DNA of hepatocellular carcinomas: is there a correlation with clinicopathological profile? *J Med Genet* 2004; 41: e65 [PMID: 15121793 DOI: 10.1136/jmg.2003.013532]

7. Sinh PH, Wu CC, Lin JC, Chi CW, Wei YH, Lee HC. Somatic mutations of mitochondrial genome in human colorectal cancer. *Chin Med J* 2013; 140: 1405-1412 [PMID: 23543062 DOI: 10.1172/JCI61398]

8. Wang LC, Ngan HY. High incidence of somatic mitochondrial DNA mutations in oral cancer of betel quid chewers. *Ann N Y Acad Sci* 2004; 1011: 301-316 [PMID: 15126307 DOI: 10.1196/annals.1293.030]

9. Parrella P, Jhunjhunwala M, Pambagia A, Hruban RH, Kern SE. Detection of mitochondrial DNA mutations in pancreatic cancer. *Nat Rev Clin Oncol* 2013; 10: 437-448 [PMID: 23568786 DOI: 10.1038/nrclinonc.2013.111]

10. Meierhofer D, Porcelli AM, Ghelini A, Cecarelli C, Maga G, Cenciacci G, Caprio M, Pambagia A, Mazzini E, Capruni S, Fabbri F, Calabrese M, Calabrese L. Mitochondrial DNA mutations in human pancreatic adenocarcinoma. *Nat Genet* 2007; 39: 1286-1288 [PMID: 17682441 DOI: 10.1038/ng.1925]

11. Tan DJ, Chang J, Chen WL, Agress LJ, Fink K, Schmoller N, Kofler B, Schon EA, Meierhofer D. Somatic mitochondrial DNA mutations in adult-onset leukaemia. *Leukemia* 2008; 22: 1231-1236 [PMID: 18377404 DOI: 10.1038/leu.2008.61]

12. Parrella P, Yao X, Fliss M, Sanchez-Cespedes M, Mazzaferri P, Rinaldi N, Micali N, Gabrielsson E, Cuomo C, Cohen D, Pandit S, Spencer M, Rabitti C, Fazio VM, Sidransky D. Detection of mitochondrial DNA mutations in primary breast cancer and fine-needle aspirates. *Cancer Res* 2001; 61: 7623-7626 [PMID: 11606403]
Manfredi G, Servidei S, Bonilla E, Shanske S, Schon EA, DiMauro S, Moraes CT. High levels of mitochondrial DNA with an unstable 260-bp duplication in a patient with mitochondrial myopathy. Neurology 1995; 45: 762-768 [PMID: 7723967 DOI: 10.1212/WNL.45.4.762]

Wen SL, Zhang J, Fong D. Decoded copy number of mitochondrial DNA: A potential diagnostic criterion for gastric cancer. Oncol Lett 2013; 6: 1098-1102 [PMID: 24137470 DOI: 10.3892/ol.2013.1492]

Manmo E, Chatterjee A, Xing M, Tallini G, Haugen BR, Yeung SC, Sukumar S, Sidransky D. Tumor-specific changes in mtDNA content in human cancer. Int J Cancer 2005; 116: 920-924 [PMID: 15856430 DOI: 10.1002/ijc.21116]

Tseng LM, Yin PH, Chi CW, Wu CY, Lee LM, Wei YH, Lee HC. Mitochondrial DNA mutations and mitochondrial DNA depletion in breast cancer. Genes Chromosomes Cancer 2006; 45: 629-638 [PMID: 16568452 DOI: 10.1002/gcc.20326]

Wang D, Kretzter DA, Essigmann JM. Mutagenicity and repair of oxidative DNA damage: insights from studies using defined lesions. Mutat Res 1996; 400: 99-115 [PMID: 9685598 DOI: 10.1016/S0078-5849(96)00066-9]

Bohr VA. Repair of oxidative DNA damage in nuclear and mitochondrial DNA, and some changes with aging in mammalian cells. Free Radic Biol Med 2002; 32: 804-812 [PMID: 11978482 DOI: 10.1016/S0891-5849(02)00787-6]

Christmann M, Tomicic MT, Roos WP, Kaina B. Mechanisms of human DNA repair: an update. Toxicology 2003; 193: 3-34 [PMID: 14599765 DOI: 10.1016/S0300-483X(03)00287-7]

De Bont R, van Larebeke N. Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis 2004; 19: 169-185 [PMID: 15123782 DOI: 10.1093/mutage/geh025]

Hanes JW, Thal DM, Johnson KA. Incorporation and replication of 8-oxo-deoxyguanosine by the human mitochondrial DNA polymerase. Nucleic Acids Res 2003. 31: 2817-2824 [PMID: 12087165 DOI: 10.1093/nar/gkf392]

DiMauro S, Moraes CT. High levels of mitochondrial DNA with an unstable 260-bp duplication in a patient with mitochondrial myopathy. Neurology 1995; 45: 762-768 [PMID: 7723967 DOI: 10.1212/WNL.45.4.762]

Wen SL, Zhang J, Fong D. Decoded copy number of mitochondrial DNA: A potential diagnostic criterion for gastric cancer. Oncol Lett 2013; 6: 1098-1102 [PMID: 24137470 DOI: 10.3892/ol.2013.1492]

Manmo E, Chatterjee A, Xing M, Tallini G, Haugen BR, Yeung SC, Sukumar S, Sidransky D. Tumor-specific changes in mtDNA content in human cancer. Int J Cancer 2005; 116: 920-924 [PMID: 15856430 DOI: 10.1002/ijc.21116]

Tseng LM, Yin PH, Chi CW, Wu CY, Lee LM, Wei YH, Lee HC. Mitochondrial DNA mutations and mitochondrial DNA depletion in breast cancer. Genes Chromosomes Cancer 2006; 45: 629-638 [PMID: 16568452 DOI: 10.1002/gcc.20326]
Lee HC et al. MtDNA alterations in gastric cancer progression

Wibom R, Tornell J, Jacobs HT, Larsson NG. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 2004; 429: 417-423 [PMID: 15164064 DOI: 10.1038/nature02517]

Kujoth GC. Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, See AY, Sullivan R, Jobling WA, Morrow JD, Van Remmen H, Sedivy JM, Yamasota T, Tanokura M, Weindruch R, Leeuwenburgh C, Prota TA. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science 2005; 309: 481-484 [PMID: 16020738]

Machado AM, Figueiredo C, Touati E, Máximo V, Sousa S, Michel V, Carneiro F, Nielsen PC, Seruca R, Rasmussen LJ. Helicobacter pylori infection induces genetic instability of nuclear and mitochondrial DNA in gastric cells. Clin Cancer Res 2009; 15: 2995-3002 [PMID: 19383819 DOI: 10.1186/1078-0432.CCR-08-2686]

Machado AM, Figueiredo C, Seruca R, Rasmussen LJ. Helicobacter pylori infection generates genetic instability in gastric cells. Biochim Biophys Acta 2010; 1806: 58-65 [PMID: 20122996 DOI: 10.1016/j.bbcan.2010.01.007]

Machado AM, Desler C, Beggild S, Strickertson JA, Friis-Hansen L, Figueiredo C, Seruca R, Rasmussen LJ. Helicobacter pylori infection affects mitochondrial function and DNA repair, thus, mediating genetic instability in gastric cells. Mech Ageing Dev 2013; 134: 460-466 [PMID: 24012633 DOI: 10.1016/j.mad.2013]

Yin PH, Lee HC, Chau GY, Wu YT, Li SH, Lui WY, Wei YH, Liu TY, Chi CW. Alteration of the copy number and deletion of mitochondrial DNA in human hepatocellular carcinoma. Br J Cancer 2004; 90: 2390-2396 [PMID: 15193555 DOI: 10.1038/sj.bjc.6601838]

Lin PC, Lin JK, Yang SH, Wang HS, Li AF, Chang SC. Expression of beta-F1-ATPase and mitochondrial transcription factor A and the change in mitochondrial DNA content in colorectal cancer: clinical data analysis and evidence from an in vitro study. Int J Colorectal Dis 2008; 23: 1223-1232 [PMID: 18569884 DOI: 10.1007/s00068-008-0359-4]

Achanta G, Sasaki R, Feng L, Carew JS, Lu W, Pelicano H, Keating MJ, Huang P. Novel role of p53 in maintaining mitochondrial DNA content in human gastric cancer SC-M1 cells. Mol Cancer 2012; 11: 79-74 [PMID: 22734268 DOI: 10.1186/1476-4598-11-79]

Huang WH, Huang KH, Wu CW, Chi CW, Kao HL, Li AF, Yin PH, Lee HC. Mitochondrial dysfunction promotes cell migration via reactive oxygen species-enhanced β5-integrin expression in human gastric cancer SC-M1 cells. Biochim Biophys Acta 2012; 1820: 1102-1110 [PMID: 22561002 DOI: 10.1016/j.bbamem.2012.04.016]

Petros JA, Baumann AK, Ruiz-Pesini E, Amin MB, Sun CQ, Hall J, Lim S, Isa MM, Planders WD, Hosseini SH, Marshall FF, Wallace DC. mtDNA mutations increase tumorigenicity in prostate cancer. Proc Natl Acad Sci USA 2005; 102: 719-724 [PMID: 15647368 DOI: 10.1073/pnas.0408891102]

Shidara Y, Yamagata K, Kanamori T, Nakano K, Kwong JQ, Manfredi G, Oda H, Ohts S. Positive contribution of pathogenic mutations in the mitochondrial genome to the development of cancer and tolerance against anticancer drugs. Oncogene 2006; 25: 4768-4776 [PMID: 16892089 DOI: 10.1038/sj.onc.1209622]

Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yama-guchi A, Imanishi H, Nakada K, Honma Y, Hayashi J. ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 2008; 320: 661-664 [PMID: 18388260 DOI: 10.1126/science.1156906]

Amuthan G, Biswas G, Zhang SY, Klein-Szanto A, Vijayasarathy C, Avadhani NG. Mitochondria-to-nucleus stress signaling induces phenotypic changes, tumor progression and cell invasion. EMBO J 2001; 20: 1910-1920 [PMID: 11296224 DOI: 10.1093/emboj/20.8.1910]

Amuthan G, Biswas G, Anandadheethavara DA, Vijayasarathy C, Sheehan HM, Avadhani NG. Mitochondria-stress-induced calcium signaling, phenotypic changes and invasive behavior in human lung carcinoma A549 cells. Oncogene 2002; 21: 7839-7849 [PMID: 12402201 DOI: 10.1038/sj.onc.1205983]

van Waveren C, Sun Y, Cheung HS, Moraes CT. Oxidative phosphorylation dysfunction modulates expression of extracellular matrix--remodeling genes and invasion. Cancer genomics 2006; 27: 409-418 [PMID: 16221732 DOI: 10.1093/carcin/bgl079]
Biswas G, Guha M, Avadhani NG. Mitochondria-to-nucleus stress signaling in mammalian cells: nature of nuclear gene targets, transcription regulation, and induced resistance to apoptosis. *Gene* 2005; 354: 132-139 [PMID: 15978749 DOI: 10.1016/j.gene.2005.03.028]

Hsu CC, Lee HC, Wei YH. Mitochondrial DNA alterations and mitochondrial dysfunction in the progression of hepatocellular carcinoma. *World J Gastroenterol* 2013; 19: 8880-8886 [PMID: 24379611 DOI: 10.3748/wjg.v19.i47.8880]

Butow RA, Avadhani NG. Mitochondrial signaling: the retrograde response. *Mol Cell* 2004; 14: 1-15 [PMID: 15068799 DOI: 10.1016/S1097-2765(04)00179-0]

Liu Z, Butow RA. Mitochondrial retrograde signaling. *Annu Rev Genet* 2006; 40: 159-185 [PMID: 16771627 DOI: 10.1146/annurev.genet.40.110405]

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