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Mitochondrial DNA alterations and mitochondrial dysfunction in the progression of hepatocellular carcinoma

Hsu CC *et al.* MtDNA alterations in HCC progression

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**Abstract**

Hepatocellular carcinoma (HCC) is one of the most common malignancies and is ranked third in mortality among cancer-related diseases. Mitochondria are intracellular organelles that are responsible for energy metabolism and cellular homeostasis, and mitochondrial dysfunction has been regarded as a hallmark of cancer. Over the past decades, several types of mitochondrial DNA (mtDNA) alterations have been identified in human cancers, including HCC. However, the role of these mtDNA alterations in cancer progression is unclear. In this review, we summarize the recent findings on the somatic mtDNA alterations identified in HCC and their relationships with the clinicopathological features of HCC. Recent advances in understanding the potential roles of somatic mtDNA alterations in the progression of HCC are also discussed. We suggest that somatic mtDNA mutations and a decrease in the mtDNA copy number are common events in HCC and that a mitochondrial dysfunction-activated signaling cascade may play an important role in the progression of HCC. Elucidation of the retrograde signaling pathways in HCC and the quest for strategies to block some of these pathways will be instrumental for the development of novel treatments for this and other malignancies.

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**Key words:** Hepatocellular carcinoma; Somatic mitochondrial DNA mutations; Mitochondrial dysfunction

**Core tip:** In this review, we summarize the recent findings on the somatic mtDNA alterations identified in hepatocellular carcinoma (HCC) and their relationships with the clinicopathological features of HCC. We suggest that somatic mtDNA mutations and a decrease in the mtDNA copy number are common events in HCC and that a mitochondrial dysfunction-activated signaling cascade may play an important role in the progression of HCC. Elucidation of the retrograde signaling pathways in HCC and the quest for strategies to block some of these pathways will be instrumental for the development of novel treatments for this and other malignancies.

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**INTRODUCTION**

Hepatocellular carcinoma (HCC) is the one of most common cancers worldwide and is ranked third with respect to mortality[1, 2]. There are approximately 434000 new cases of HCC per year[3]. Several risk factors have been suggested to be involved in the development of HCC, including aflatoxin exposure, alcohol consumption, chronic inﬂammation associated with viral hepatitis and familial tendency[4-8]. Moreover, inflammation and oxidative stress have been suggested to contribute to the carcinogenesis of HCC[9-11].

In the 1930s, the German biochemist Otto Warburg proposed that tumor cells prefer to utilize glycolysis rather than respiration as a primary energy source, even in the presence of abundant oxygen[12]. This phenomenon was termed “aerobic glycolysis” or the “Warburg effect”. He further proposed that defects in energy metabolism, especially in the mitochondria, are involved in the initiation or progression of cancer[13].

Mitochondria are cytoplasmic organelles that play multiple roles in energy metabolism and cellular homeostasis, including the generation of ATP *via* respiration and oxidative phosphorylation (OXPHOS), the production of reactive oxygen species (ROS), metabolic homeostasis, and the initiation and execution of apoptosis[14, 15]. These roles are executed by proteins that are encoded by genes in the nucleus and mitochondria. Mitochondrial DNA (mtDNA) is a 16.6-kb, double-stranded circular DNA that contains genes for 22 transfer RNAs, 2 ribosomal RNAs and 13 polypeptides that comprise the respiratory enzyme complexes[16]. In addition to the coding region, mtDNA contains a non-coding region called the “D-loop”, which is approximately 1.1 kb, encompasses nucleotide position (np) 16024-16576, and controls the replication and transcription of the mtDNA[17].

Due to its lack of protective histone proteins, a limited DNA repair system and its spatial proximity to a high level of ROS, mtDNA sustains a 10-fold higher level of damage than that of nuclear DNA (nDNA)[18-20]. Somatic mutation and damage to mtDNA can lead to impairment of the OXPHOS system and enhanced ROS generation, which in turn accelerates the occurrence of DNA mutations. This scenario has been proposed to contribute to the initiation and progression of tumors[21, 22].

Over the past decade, somatic mtDNA mutations have been identified in several types of cancer, including HCC[23-27]. Some of the acquired mtDNA mutations have been suggested to cause mitochondrial dysfunction, increase the production of ROS, and promote tumor growth[28, 29].

In this article, we review the recent findings on somatic mtDNA alterations in HCC. In addition, we discuss the potential roles of mtDNA alterations and mitochondrial dysfunction in the progression and metastasis of HCC.

**SOMATIC MITOCHONDRIAL DNA ALTERATIONS IN HCC**

Over the past decade, several types of somatic mtDNA alterations have been identified in human HCC. These mtDNA alterations include point mutations, deletions, insertions and copy number changes.

***Point mutations***

Screening for somatic point mutations in the whole mitochondrial genomes of HCC samples[29, 30] revealed that approximately 52% of HCC patients carry at least one homoplasmic or heteroplasmic point mutation in their tumor tissue mtDNA. Of the identified point mutations, 76% are located in the D-loop region, 2% are located in rRNA genes, 3% are located in tRNA genes and 19% are located in mRNA genes (Figure 1A). The incidence and location distribution of the point mutations are consistent with those observed in other cancer types[25].

The D-loop region is a hot spot for somatic mtDNA mutations in HCC and other cancers. It was reported that the D-loop region of mtDNA, especially the mononucleotide repeat in the np 303-309 poly-C sequence, is the most susceptible site to oxidative damage compared with the other regions of the mtDNA, implying that oxidative damage contributes to point mutations in the D-loop and/or the instability of the mononucleotide or dinucleotide repeats in the mtDNA. However, the unique G-to-T transversion caused by oxidative DNA damage is not detected in HCC[29, 30]. Among the mtDNA mutations that have been identified in HCC, approximately 59% are transition mutations (G/A-to-A/G or C/T-to-T/C) and 38% are mono- or di-nucleotide instabilities (Figure 1B), suggesting that oxidative damage *per se* is not the major factor responsible for point mutations in HCC. The presence of hepatitis B infection, liver cirrhosis, alcohol abuse or their combination may affect the qualitative changes in the mtDNA in HCC[30].

Because the D-loop region controls the replication and transcription of the mtDNA, mutations in the D-loop region may influence the mtDNA copy number and the expression of the mitochondrial genome[31]. It has been shown that the occurrence of point mutations in the D-loop, especially near the replication origin of the heavy-strand (OH) of the mtDNA, affects the mtDNA copy number in HCC[32]. In addition, Nishikawa and colleagues reported that the number of mtDNA mutations in the D-loop region is positively correlated with a poor HCC differentiation grade[33]. These findings suggest that the somatic mutations in the mtDNA D-loop region may affect mitochondrial function by decreasing the mtDNA copy number and/or transcription in HCC, thereby leading to HCC progression.

Among the mutations in the coding region, the nonsense mutation G3842A creates a premature stop codon and the missense mutations T6787C, G7976A, G9267A, and A11708G result in amino acid substitutions in the highly evolutionally conserved regions of the affected mitochondrial genes. Moreover, the base-pair deletion and insertion 11032delA and 12418insA may lead to a frame-shift mutation, and the tRNA mutations T1659C in tRNAVal and G5650A in tRNAAla may alter the tRNA structure and were shown to associate with mitochondrial disorders[29]. Therefore, these mtDNA point mutations may result in mitochondrial dysfunction in HCC.

***Deletions***

Among the large-scale deletions identified in the mtDNA in different cancer types[32, 34-37], the 4977-bp deletion is the most common mtDNA deletion in tumors[23, 38-43]. Consistent with findings in other types of cancer, the incidence of the 4977-bp deletion and its accumulation level are lower in the malignant tissues than the non-tumor tissues of HCC patients[23, 39]. Moreover, gender and a long-term history of alcohol consumption in HCC patients may affect the accumulation of the 4977-bp-deleted mtDNA[23]. Although the role of mtDNA deletion in HCC is unclear, it has been suggested that the observed decrease in mtDNA with a deletion is the result of the tumor cells adapting to a new microenvironment during hepatocarcinogenesis[25, 44].

In addition, a 50-bp deletion was previously reported in one HCC patient[32]. This deletion is flanked by a 9-bp direct repeat in the D-loop region of the mtDNA. The mtDNA deletion appeared to be homoplasmic in the HCC tissue but was not detected in the corresponding non-tumor liver tissue. The tumor-specific accumulation of this deletion does not seem to be similar to that of the 4977-bp deletion in cancers. Because this deletion partly truncates the regulatory region of the mtDNA, the mtDNA copy number in the HCC tissue was found to be significantly reduced compared with that in the non-tumor liver tissue[32]. This mtDNA deletion may lead to mitochondrial dysfunction *via* mtDNA depletion and/or impairment of the transcription of mitochondrial genes.

***Insertions***

Two small insertions (approximately 260 bp and approximately 520 bp) have been identified as a tandem duplication and a tandem triplication and are flanked by two poly-cytosine (poly-C) sequences at np 303-309 and np 568-573 in the D-loop region of the mtDNA in various human cancers, including HCC[35]. This tandem duplication or triplication was detected in approximately 4% of HCCs and is highly correlated with the presence of length variation in the poly-C at np 568[44]. However, these insertions have also been found in somatic tissues in elderly subjects and, thus, are not specific for cancer tissues.

***Copy number changes***

A decrease in the mtDNA copy number is a common event in HCC[23, 32, 45, 46]. Over 60% of HCCs have a lower mtDNA copy number than their corresponding non-tumor liver tissues. As mentioned above, it was observed that the reduction in the mtDNA copy number is associated with point mutations located near the replication origin in the D-loop region of the mtDNA[32]. Moreover, it was suggested that the decrease in the mtDNA copy number in HCC may be related to or result from the altered expression of genes involved in mitochondrial biogenesis, such as peroxisome proliferator-activated receptactivator-1 (PGC-1) and mitochondrial single-stranded DNA binding protein (mtSSB)[23]. These results suggest that the mtDNA mutations in the D-loop region and the impairment of mitochondrial biogenesis contribute to the decrease in the mtDNA copy number in HCC[34].

The reduction in the mtDNA copy number seems to be more frequently observed in female patients with HCC compared with male patients with HCC[23]. This difference between male and female HCC patients could be a result of clinical manifestation, progression and/or mortality rate[23]. Yamada *et al*[46] showed that the low mtDNA copy number in HCC is significantly correlated with large tumor size and liver cirrhosis. In addition, HCC patients with a lower mtDNA copy number in their tumors tend to show poorer 5-year survival compared with patients with a higher mtDNA copy number[46]. It was also suggested that hepatitis B infection, liver cirrhosis, and alcohol abuse affect quantitative changes in the mtDNA in HCC[29]. Recently, it was reported that there is an association between the mtDNA content in the peripheral blood leukocytes and hepatitis B virus-related hepatocellular carcinoma[47], which suggests that the mtDNA copy number in the peripheral blood leukocytes could be used as a predictor of HCC occurrence.

**POTENTIAL ROLES OF MITOCHONDRIAL DNA MUTATIONS AND MITOCHONDRIAL DYSFUNCTION IN HCC PROGRESSION**

Several types of somatic mtDNA alterations have been identified in HCC, but the roles of these mtDNA alterations in HCC progression are unclear. Evidence from several lines of research has substantiated the pathological role of mtDNA mutation and mitochondrial dysfunction in HCC.

The majority of the somatic point mutations in the mitochondrial coding region and the decrease in the mtDNA copy number may cause mitochondrial dysfunction in HCC. These findings provide a molecular basis for the Warburg effect. In addition, it has been shown that the low mtDNA copy number in HCC is significantly correlated with large tumor size, liver cirrhosis, and poor 5-year survival[46]. Therefore, it is possible that mtDNA mutations and a decrease in the mtDNA copy number and, thereby, mitochondrial dysfunction modify the progression of HCC.

In the human SK-Hep1 hepatoma cell line, mtDNA depletion was demonstrated to induce resistance to oxidative stress and chemotherapeutic agents through an adaptive increase in the expression of manganese superoxide dismutase (MnSOD) and other antioxidant enzymes[48]. Moreover, chloramphenicol was found to inhibit mitochondrial protein synthesis in human hepatoma HepG2 cells and to render these cancer cells resistant to mitomycin-induced apoptosis[49]. Using similar approaches, respiratory inhibitors and an uncoupler of mitochondrial respiration as well as inhibitors of mtDNA replication or protein synthesis in the mitochondria were found to induce mitochondrial dysfunction and cisplatin resistance in human hepatoma HepG2 cells and to promote cell migration in other hepatoma cells *via* a paracrine signaling pathway[50]. It was further demonstrated that the mitochondrial dysfunction-induced upregulation of amphiregulin contributes to the cisplatin resistance and cell migration of hepatoma cells[50]. In addition, these treatments also induced changes in the expression of genes that affect the metastatic ability of cancers, including the integrin pathway, the PDGF signaling pathway and the cadherin signaling pathway[51]. On the other hand, the overexpression of PGC-1 in HepG2 cells was found to elevate mitochondrial protein expression and to reduce cell mobility *via* increased E-cadherin expression[52, 53]. These findings support the hypothesis that mtDNA mutations and mitochondrial dysfunction contribute to the malignant progression of HCC.

 Mitochondrial dysfunction increases ROS production and Ca2+ mobilization and reduces ATP generation, which may be involved in the malignant changes induced by mtDNA mutations and mitochondrial dysfunction in HCC. It has been demonstrated that antioxidants and calcium chelators can block mitochondrial dysfunction-induced amphiregulin expression and prevent cisplatin resistance and cell migration[50]. In addition, it was recently demonstrated that mitochondrial dysfunction-reduced intracellular ATP content represses the protein expression of hypoxia-inducible factor-1 (HIF-1) through the activation of the AMP-activated protein kinase (AMPK)-mTOR pathways in HepG2 cells[54]. HIF-1 is a nuclear transcription factor that plays a crucial role in cancer progression, including angiogenesis, invasion and metastasis[55]. These findings suggest that mitochondrial dysfunction regulates nuclear gene expression and phenotypic changes to face the different microenvironments of HCC. Therefore, the activation of retrograde signaling from the mitochondria to the nucleus may play an important role in the malignant progression of HCC.

 Consistent findings were observed in other types of cancer. It has been reported that in some cancer cell lines, a pathogenic mtDNA mutation (*e.g*., the T8993G transversion) promotes tumor growth in nude mice by preventing apoptosis[56-58]. Moreover, it was shown that the heteroplasmic 12418insA mutation, which has been identified in HCC[29] and other cancers[24, 26, 59], impairs mitochondrial respiratory function and promotes tumorigenesis by enhancing ROS production[60]. In addition, ROS-generating mtDNA mutations have been demonstrated to regulate tumor cell metastasis[61]. It was also shown that mtDNA depletion or mitochondrial dysfunction can enhance invasive phenotype changes[62-64] or chemo-resistance in some specific types of cancer[65]. The underlying mechanisms have been suggested to involve the communication between the mitochondria and the nucleus called “retrograde signaling”[66, 67]. Several biomolecules have been identified to be involved in this signal transduction, including calcineurin, NFAT, ATF2, Akt, and NFkB/Rel, which then affect the expression of an array of nuclear genes[68, 69]. The detailed mechanisms by which mtDNA mutations and mitochondrial dysfunction affect HCC progression await further investigation.

Although mtDNA alterations have been identified in HCC, it remains controversial whether mtDNA alterations are correlated with the initiation and progression of HCC. To dissect the role of mtDNA alterations in HCC, a larger sample size of HCC is required in future research. Moreover, some lines of evidence suggest that mitochondrial dysfunction and the dysfunctions caused by mtDNA alterations have the potential to contribute to tumor progression. However, whether a specific mtDNA mutation plays a driving force or is an indirect consequence of HCC progression requires further evaluation. Therefore, it is important to develop a strategy to dissect the role of specific mtDNA mutations in cancer progression and/or to exclude non-causal epiphenomena.

Because the coordination between the nDNA and mtDNA is important for the maintenance of mitochondrial structure and function[14, 17], mutations in the nDNA-encoded genes that are responsible for mtDNA integrity and/or mitochondrial function may play an important role in tumorigenesis and cancer progression. For example, it was recently reported that defects in P53[70], mitochondrial DNA polymerase[71], and mitochondrial deacetylase SIRT3[72] may affect mtDNA integrity and promote tumorigenesis. In addition, not only mtDNA mutations but also mitochondrial dysfunction caused by nDNA mutations, oncogenes, and tumor microenvironments (hypoxia and inflammation) are suggested to underlie energy metabolism reprogramming (or the Warburg effect)[73, 74]. In summary, the interaction between mtDNA and nDNA may play an important role in the initiation and progression of HCC.

**CONCLUSION**

Several types of mtDNA alterations, including point mutations, deletions, insertions and copy number changes, have been identified in HCC. Somatic point mutations and depletions are the two most common of these mtDNA alterations in HCC. The low mtDNA copy number in HCC has been shown to be significantly correlated with large tumor size, liver cirrhosis, and poor 5-year survival[46]. However, the presence of somatic mtDNA point mutations in HCC does not seem to correlate with the patient’s age or sex, the tumor size or grade, hepatitis virus infection, or the patient’s survival[29, 30]. This finding may result from the possibility that mtDNA point mutations do not always play a similar role in HCC progression. In addition, the heteroplasmic or homoplasmic level of the same mtDNA mutation may result in different consequences in tumorigenesis[60]. Therefore, the role of the specific mtDNA mutation and its level during HCC progression warrant further study.

 The majority of the point mutations in the coding region of the mtDNA and the decrease in the mtDNA copy number likely cause mitochondrial dysfunction in HCC. These findings have provided solid evidence to substantiate the mechanism by which mitochondrial dysfunction is involved in metabolic reprogramming or the “Warburg effect” in cancer. Several lines of evidence have important implications in the pathological role of mtDNA mutation and mitochondrial dysfunction in HCC. Pharmacologic approaches to induce mitochondrial dysfunction can enhance chemo-resistance and promote metastasis, which may contribute to the malignant progression of HCC. Thus, the increased ROS production and Ca2+ mobilization and the reduced ATP generation induced by mitochondrial dysfunction may be involved in the malignant changes of HCC. However, the detailed mechanism by which mtDNA mutation and mitochondrial dysfunction affect HCC progression remains unclear. Elucidation of the retrograde signaling pathways in HCC and the search for strategies to block these pathways will be important for the development of novel treatments for this and other malignancies.

**REFERENCES**

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

2 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]

3 World Health Organisation (WHO) Databank. WHO statistical information system[Internet]. Geneva: WHO. Available at http: //www.who.int/whosis.

4 **Yuen MF**, Hou JL, Chutaputti A. Hepatocellular carcinoma in the Asia pacific region. *J Gastroenterol Hepatol* 2009; **24**: 346-353 [PMID: 19220670 DOI: 10.1111/]

5 **Schütte K**, Bornschein J, Malfertheiner P. Hepatocellular carcinoma--epidemiological trends and risk factors. *Dig Dis* 2009; **27**: 80-92 [PMID: 19546545 DOI: 10.1159/000218339]

6 **Hassan MM**, Spitz MR, Thomas MB, Curley SA, Patt YZ, Vauthey JN, Glover KY, Kaseb A, Lozano RD, El-Deeb AS, Nguyen NT, Wei SH, Chan W, Abbruzzese JL, Li D. The association of family history of liver cancer with hepatocellular carcinoma: a case-control study in the United States. *J Hepatol* 2009; **50**: 334-341 [PMID: 19070394 DOI: 10.1016/j.jhep.2008.08.016]

7 **Blonski W**, Kotlyar DS, Forde KA. Non-viral causes of hepatocellular carcinoma. *World J Gastroenterol* 2010; **16**: 3603-3615 [PMID: 20677332 DOI: 10.3748/wjg.v16.i29.3603]

8 **Asim M**, Sarma MP, Thayumanavan L, Kar P. Role of aflatoxin B1 as a risk for primary liver cancer in north Indian population. *Clin Biochem* 2011; **44**: 1235-1240 [PMID: 21854762 DOI: 10.1016/j.clinbiochem.2011.07.017]

9 **Klaunig JE**, Kamendulis LM. The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicol* 2004; **44**: 239-267 [PMID: 14744246 DOI: 10.1146/annurev.pharmtox.44.101802.121851]

10 **Kawanishi S**, Hiraku Y, Pinlaor S, Ma N. Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. *Biol Chem* 2006; **387**: 365-372 [PMID: 16606333 DOI: 10.1515/BC.2006.049]

11 **Kumar M**, Zhao X, Wang XW. Molecular carcinogenesis of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: one step closer to personalized medicine? *Cell Biosci* 2011; **1**: 5 [PMID: 21711594 DOI: 10.1186/2045-3701-1]

12 **Warburg O**. The metabolism of tumors. Arnold Constable: London 1930; pp. 254-270

13 **Warburg O**. On the origin of cancer cells. *Science* 1956; **123**: 309-314 [PMID: 13298683 DOI: 10.1126/science]

14 **Wallace DC**, Fan W, Procaccio V. Mitochondrial energetics and therapeutics. *Annu Rev Pathol* 2010; **5**: 297-348 [PMID: 20078222 DOI: 10.1146/annurev.]

15 **Galluzzi L**, Kepp O, Kroemer G. Mitochondria: master regulators of danger signalling. *Nat Rev Mol Cell Biol* 2012; **13**: 780-788 [PMID: 23175281 DOI: 10.1038/nrm3479]

16 **Anderson S**, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG. Sequence and organization of the human mitochondrial genome. *Nature* 1981; **290**: 457-465 [PMID: 7219534 DOI:10.1038/290457a0]

17 **Attardi G**, Schatz G. Biogenesis of mitochondria. *Annu Rev Cell Biol* 1988; **4**: 289-333 [PMID: 2461720 DOI: 10.1146/annurev.cb.04.110188.001445]

18 **Bianchi NO**, Bianchi MS, Richard SM. Mitochondrial genome instability in human cancers. *Mutat Res* 2001; **488**: 9-23 [PMID: 11223402]

19 **Haag-Liautard C**, Dorris M, Maside X, Macaskill S, Halligan DL, Houle D, Charlesworth B, Keightley PD. Direct estimation of per nucleotide and genomic deleterious mutation rates in Drosophila. *Nature* 2007; **445**: 82-85 [PMID: 17203060 DOI: 10.1038/nature05388]

20 **Larsen NB**, Rasmussen M, Rasmussen LJ. Nuclear and mitochondrial DNA repair: similar pathways? *Mitochondrion* 2005; **5**: 89-108 [PMID: 16050976 DOI: 10.1016/j.mito.2005.02.002]

21 **Brandon M**, Baldi P, Wallace DC. Mitochondrial mutations in cancer. *Oncogene* 2006; **25**: 4647-4662 [PMID: 16892079 DOI: 10.1038/sj.onc.1209607]

22 **Lee HC**, Wei YH. Mitochondrial DNA instability and metabolic shift in human cancers. *Int J Mol Sci* 2009; **10**: 674-701 [PMID: 19333428 DOI: 10.3390/]

23 **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: 15150555 DOI: 10.1038/sj.bjc.6601838]

24 **Hung WY**, Wu CW, Yin PH, Chang CJ, Li AF, Chi CW, Wei YH, Lee HC. Somatic mutations in mitochondrial genome and their potential roles in the progression of human gastric cancer. *Biochim Biophys Acta* 2010; **1800**: 264-270 [PMID: 19527772 DOI: 10.1016/j.bbagen.2009.06.006]

25 **Lee HC**, Chang CM, Chi CW. Somatic mutations of mitochondrial DNA in aging and cancer progression. *Ageing Res Rev* 2010; **9** Suppl 1: S47-S58 [PMID: 20816876 DOI: 10.1016/j.arr.2010.08.009]

26 **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]

27 **Wallace DC**. Mitochondria and cancer. *Nat Rev Cancer* 2012; **12**: 685-698 [PMID: 23001348 DOI: 10.1038/nrc3365]

28 **Penta JS**, Johnson FM, Wachsman JT, Copeland WC. Mitochondrial DNA in human malignancy. *Mutat Res* 2001; **488**: 119-133 [PMID: 11344040]

29 **Yin PH**, Wu CC, Lin JC, Chi CW, Wei YH, Lee HC. Somatic mutations of mitochondrial genome in hepatocellular carcinoma. *Mitochondrion* 2010; **10**: 174-182 [PMID: 20006738 DOI: 10.1016/j.mito.2009.12.147]

30 **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]

31 **Shadel GS**, Clayton DA. Mitochondrial DNA maintenance in vertebrates. *Annu Rev Biochem* 1997; **66**: 409-435 [PMID: 9242913 DOI: 10.1146/annurev.biochem.66.1.409]

32 **Lee HC**, Li SH, Lin JC, Wu CC, Yeh DC, Wei YH. Somatic mutations in the D-loop and decrease in the copy number of mitochondrial DNA in human hepatocellular carcinoma. *Mutat Res* 2004; **547**: 71-78 [PMID: 15013701 DOI: 10.1016/j.mrfmmm.2003.12.011]

33 **Nishikawa M**, Nishiguchi S, Shiomi S, Tamori A, Koh N, Takeda T, Kubo S, Hirohashi K, Kinoshita H, Sato E, Inoue M. Somatic mutation of mitochondrial DNA in cancerous and noncancerous liver tissue in individuals with hepatocellular carcinoma. *Cancer Res* 2001; **61**: 1843-1845 [PMID: 11280735]

34 **Lee HC**, Yin PH, Lin JC, Wu CC, Chen CY, Wu CW, Chi CW, Tam TN, Wei YH. Mitochondrial genome instability and mtDNA depletion in human cancers. *Ann N Y Acad Sci* 2005; **1042**: 109-122 [PMID: 15965052 DOI: 10.1196/annals.]

35 **Wu CW**, Yin PH, Hung WY, Li AF, Li SH, Chi CW, Wei YH, Lee HC. Mitochondrial DNA mutations and mitochondrial DNA depletion in gastric cancer. *Genes Chromosomes Cancer* 2005; **44**: 19-28 [PMID: 15892105 DOI: 10.1002/gcc.20213]

36 **Tseng LM**, Yin PH, Chi CW, Hsu CY, Wu CW, 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]

37 **Lin CS**, Lee HT, Lee SY, Shen YA, Wang LS, Chen YJ, Wei YH. High mitochondrial DNA copy number and bioenergetic function are associated with tumor invasion of esophageal squamous cell carcinoma cell lines. *Int J Mol Sci* 2012; **13**: 11228-11246 [PMID: 23109849 DOI: 10.3390/ijms130911228]

38 **Fukushima S**, Honda K, Awane M, Yamamoto E, Takeda R, Kaneko I, Tanaka A, Morimoto T, Tanaka K, Yamaoka Y. The frequency of 4977 base pair deletion of mitochondrial DNA in various types of liver disease and in normal liver. *Hepatology* 1995; **21**: 1547-1551 [PMID: 7768499]

39 **Kotake K**, Nonami T, Kurokawa T, Nakao A, Murakami T, Shimomura Y. Human livers with cirrhosis and hepatocellular carcinoma have less mitochondrial DNA deletion than normal human livers. *Life Sci* 1999; **64**: 1785-1791 [PMID: 10353633]

40 **Lee HC**, Yin PH, Yu TN, Chang YD, Hsu WC, Kao SY, Chi CW, Liu TY, Wei YH. Accumulation of mitochondrial DNA deletions in human oral tissues -- effects of betel quid chewing and oral cancer. *Mutat Res* 2001; **493**: 67-74 [PMID: 11516716]

41 **Yang JH**, Lee HC, Chung JG, Wei YH. Mitochondrial DNA mutations in light-associated skin tumors. *Anticancer Res* 2004; **24**: 1753-1758 [PMID: 15274351]

42 **Wheelhouse NM**, Lai PB, Wigmore SJ, Ross JA, Harrison DJ. Mitochondrial D-loop mutations and deletion profiles of cancerous and noncancerous liver tissue in hepatitis B virus-infected liver. *Br J Cancer* 2005; **92**: 1268-1272 [PMID: 15785740 DOI: 10.1038/sj.bjc.6602496]

43 **Chen T**, He J, Shen L, Fang H, Nie H, Jin T, Wei X, Xin Y, Jiang Y, Li H, Chen G, Lu J, Bai Y. The mitochondrial DNA 4,977-bp deletion and its implication in copy number alteration in colorectal cancer. *BMC Med Genet* 2011; **12**: 8 [PMID: 21232124 DOI: 10.1186/1471-2350-12-8]

44 **Hung WY**, Lin JC, Lee LM, Wu CW, Tseng LM, Yin PH, Chi CW, Lee HC. Tandem duplication/triplication correlated with poly-cytosine stretch variation in human mitochondrial DNA D-loop region. *Mutagenesis* 2008; **23**: 137-142 [PMID: 18252697 DOI: 10.1093/mutage/gen002]

45 **Cuezva JM**, Krajewska M, de Heredia ML, Krajewski S, Santamaría G, Kim H, Zapata JM, Marusawa H, Chamorro M, Reed JC. The bioenergetic signature of cancer: a marker of tumor progression. *Cancer Res* 2002; **62**: 6674-6681 [PMID: 12438266]

46 **Yamada S**, Nomoto S, Fujii T, Kaneko T, Takeda S, Inoue S, Kanazumi N, Nakao A. Correlation between copy number of mitochondrial DNA and clinico-pathologic parameters of hepatocellular carcinoma. *Eur J Surg Oncol* 2006; **32**: 303-307 [PMID: 16478656 DOI: 10.1016/j.ejso.2006.01.002]

47 **Zhao S**, Yang Y, Liu J, Liu H, Ge N, Yang H, Zhang H, Xing J. Association of mitochondrial DNA content in peripheral blood leukocyte with hepatitis B virus-related hepatocellular carcinoma in a Chinese Han population. *Cancer Sci* 2011; **102**: 1553-1558 [PMID: 21521418 DOI: 10.1111/j.1349-7006.2011.01968]

48 **Park SY**, Chang I, Kim JY, Kang SW, Park SH, Singh K, Lee MS. Resistance of mitochondrial DNA-depleted cells against cell death: role of mitochondrial superoxide dismutase. *J Biol Chem* 2004; **279**: 7512-7520 [PMID: 14660625 DOI: 10.1074/jbc.M307677200]

49 **Li CH**, Tzeng SL, Cheng YW, Kang JJ. Chloramphenicol-induced mitochondrial stress increases p21 expression and prevents cell apoptosis through a p21-dependent pathway. *J Biol Chem* 2005; **280**: 26193-26199 [PMID: 15905168 DOI: 10.1074/jbc.M501371200]

50 **Chang CJ**, Yin PH, Yang DM, Wang CH, Hung WY, Chi CW, Wei YH, Lee HC. Mitochondrial dysfunction-induced amphiregulin upregulation mediates chemo-resistance and cell migration in HepG2 cells. *Cell Mol Life Sci* 2009; **66**: 1755-1765 [PMID: 19337692 DOI: 10.1007/s00018-009-8767-5]

51 **Chang CJ,** Yin PH, Wang CH, Chi CW, Wei YH, Lee HC. Mitochondrial stress-induced genes and pathways changes in human hepatoma cells. In: BIOCOMP 2008; 43-49

52 **Lee HJ**, Su Y, Lui WY, Chau GY, Yin PH, Lee HC, Chi CW. Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1alpha) upregulated E-cadherin expression in HepG2 cells. *FEBS Lett* 2008; **582**: 627-634 [PMID: 18242180 DOI: 10.1016/j.febslet.2008.01.033]

53 **Lee HJ**, Su Y, Yin PH, Lee HC, Chi CW. PPAR(gamma)/PGC-1(alpha) pathway in E-cadherin expression and motility of HepG2 cells. *Anticancer Res* 2009; **29**: 5057-5063 [PMID: 20044617]

54 **Hsu CC**, Wang CH, Wu LC, Hsia CY, Chi CW, Yin PH, Chang CJ, Sung MT, Wei YH, Lu SH, Lee HC. Mitochondrial dysfunction represses HIF-1α protein synthesis through AMPK activation in human hepatoma HepG2 cells. *Biochim Biophys Acta* 2013; **1830**: 4743-4751 [PMID: 23791554 DOI: 10.1016/j.bbagen.2013.06.004]

55 **Wilson WR**, Hay MP. Targeting hypoxia in cancer therapy. *Nat Rev Cancer* 2011; **11**: 393-410 [PMID: 21606941 DOI: 10.1038/nrc3064]

56 **Petros JA**, Baumann AK, Ruiz-Pesini E, Amin MB, Sun CQ, Hall J, Lim S, Issa MM, Flanders WD, Hosseini SH, Marshall FF, Wallace DC. mtDNA mutations increase tumorigenicity in prostate cancer. *Proc Natl Acad Sci U S A* 2005; **102**: 719-724 [PMID: 15647368 DOI: 10.1073/pnas.0408894102]

57 **Shidara Y**, Yamagata K, Kanamori T, Nakano K, Kwong JQ, Manfredi G, Oda H, Ohta S. Positive contribution of pathogenic mutations in the mitochondrial genome to the promotion of cancer by prevention from apoptosis. *Cancer Res* 2005; **65**: 1655-1663 [PMID: 15753359]

58 **Ohta S**. Contribution of somatic 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.1209602]

59 **Polyak K**, Li Y, Zhu H, Lengauer C, Willson JK, Markowitz SD, Trush MA, Kinzler KW, Vogelstein B. Somatic mutations of the mitochondrial genome in human colorectal tumours. *Nat Genet* 1998; **20**: 291-293 [PMID: 9806551 DOI: 10.1038/3108]

60 **Park JS**, Sharma LK, Li H, Xiang R, Holstein D, Wu J, Lechleiter J, Naylor SL, Deng JJ, Lu J, Bai Y. A heteroplasmic, not homoplasmic, mitochondrial DNA mutation promotes tumorigenesis via alteration in reactive oxygen species generation and apoptosis. *Hum Mol Genet* 2009; **18**: 1578-1589 [PMID: 19208652 DOI: 10.1093/hmg/ddp069]

61 **Ishikawa K**, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi 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]

62 **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]

63 **Amuthan G**, Biswas G, Ananadatheerthavarada HK, Vijayasarathy C, Shephard HM, Avadhani NG. Mitochondrial stress-induced calcium signaling, phenotypic changes and invasive behavior in human lung carcinoma A549 cells. *Oncogene* 2002; **21**: 7839-7849 [PMID: 12420221 DOI: 10.1038/sj.onc.1205983]

64 **van Waveren C**, Sun Y, Cheung HS, Moraes CT. Oxidative phosphorylation dysfunction modulates expression of extracellular matrix--remodeling genes and invasion. *Carcinogenesis* 2006; **27**: 409-418 [PMID: 16221732]

65 **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]

66 **Butow RA**, Avadhani NG. Mitochondrial signaling: the retrograde response. *Mol Cell* 2004; **14**: 1-15 [PMID: 15068799]

67 **Liu Z**, Butow RA. Mitochondrial retrograde signaling. *Annu Rev Genet* 2006; **40**: 159-185 [PMID: 16771627 DOI: 10.1146/annurev.genet.40.110405.090613]

68 **Biswas G**, Anandatheerthavarada HK, Zaidi M, Avadhani NG. Mitochondria to nucleus stress signaling: a distinctive mechanism of NFkappaB/Rel activation through calcineurin-mediated inactivation of IkappaBbeta. *J Cell Biol* 2003; **161**: 507-519 [PMID: 12732617 DOI: 10.1083/jcb.200211104]

69 **Pelicano H**, Xu RH, Du M, Feng L, Sasaki R, Carew JS, Hu Y, Ramdas L, Hu L, Keating MJ, Zhang W, Plunkett W, Huang P. Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism. *J Cell Biol* 2006; **175**: 913-923 [PMID: 17158952]

70 **Achanta G**, Sasaki R, Feng L, Carew JS, Lu W, Pelicano H, Keating MJ, Huang P. Novel role of p53 in maintaining mitochondrial genetic stability through interaction with DNA Pol gamma. *EMBO J* 2005; **24**: 3482-3492 [PMID: 16163384 DOI: 10.1038/sj.emboj.7600819]

71 **Singh KK**, Ayyasamy V, Owens KM, Koul MS, Vujcic M. Mutations in mitochondrial DNA polymerase-gamma promote breast tumorigenesis. *J Hum Genet* 2009; **54**: 516-524 [PMID: 19629138 DOI: 10.1038/jhg.2009.71]

72 **Kim HS**, Patel K, Muldoon-Jacobs K, Bisht KS, Aykin-Burns N, Pennington JD, van der Meer R, Nguyen P, Savage J, Owens KM, Vassilopoulos A, Ozden O, Park SH, Singh KK, Abdulkadir SA, Spitz DR, Deng CX, Gius D. SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress. *Cancer Cell* 2010; **17**: 41-52 [PMID: 20129246 DOI: 10.1016/j.ccr.2009.11.023]

73 **Chen Z**, Lu W, Garcia-Prieto C, Huang P. The Warburg effect and its cancer therapeutic implications. *J Bioenerg Biomembr* 2007; **39**: 267-274 [PMID: 17551814 DOI: 10.1007/s10863-007-9086-x]

74 **Upadhyay M**, Samal J, Kandpal M, Singh OV, Vivekanandan P. The Warburg effect: insights from the past decade. *Pharmacol Ther* 2013; **137**: 318-330 [PMID: 23159371 DOI: 10.1016/j.pharmthera.2012.11.003]

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**A**



**B**

**Figure 1 The location distribution of the identified somatic point mutations (A) and the types of somatic point mutations (B) in the mtDNA in hepatocellular carcinoma**. Data adapted from Yin *et al*[29] and Wong *et al*[30].