



Roles of Mitochondrial DNA Changes on Cancer Initiation and Progression

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Mitochondrial DNA

Mitochondria are essential organelles that generate ATP through oxidative phosphorylation. This process is accomplished by a series of protein complexes and mitochondrial respiratory chains (MRC) encoded by nuclear DNA and mitochondrial DNA (mtDNA). Human mtDNA is remarkably small (16,569 bp) compared with nuclear DNA (approximately 10⁹ bp) and encodes only a few but important proteins in the MRC: 13 polypeptides, 7 in complex I, cytochrome b in complex III, 3 in complex IV and 2 in complex V (F₀-F₁ ATPase), 22 transfer RNAs, and 2 ribosomal RNAs (Anderson, 1981 #1323). The majority of mitochondrial respiratory proteins (at least 74) are encoded by nuclear DNA that are translated in the cytoplasm and then imported into mitochondria.

Cancer and mtDNA Changes

Inhibition of the repair system for damages to mtDNA, detoxification of ROS, or increase in ROS encoded in nuclear DNA induces a lot of point mutations of mtDNA, accumulation of large-deletion mutant mtDNA, and the depletion of mtDNA. In addition, nuclear DNA mutations of the mitochondrial proteins, such as adenine nucleotide translocator 1 (ANT1) [1], Twinkle [2], and polymerase γ [3], can cause depletion and multiple deletions of mtDNA.

Several studies demonstrated that mtDNA mutation is common in cancer [4,5]. Mutation of mtDNA was observed in all of the 13 coding regions, two ribosomal RNA regions, D-loop region and transfer RNA regions [5]. Frequency of mtDNA mutation is highest in D-loop region. D-loop region is non-coding displacement (D)-loop (~ 1,122 base pairs) that harbors the main promoter for the transcription of the heavy strand and the light strand of the genome. Mutation to the D-loop region causes reduction of mtDNA content [6]. Frequency of mutation is also higher in complex I region as compared with other regions. Deletion of mtDNA was also observed in several cancers [7]. Thus, association of mtDNA changes to cancer is well accepted. However, Palanichamy and Zhang indicated that more care should be taken to eliminate artifacts and mix-ups especially for clinical samples [8].

Next, I would like to demonstrate how and whether mtDNA

changes regulate cancer. I will talk about the roles of mtDNA changes on two phenotypes "cancer initiation" (cancer occurrence) and "progression to aggressive phenotype" (cancer development) which are sometimes considered equal.

mtDNA Change and Cancer Initiation

Rasmussen et al. showed that mtDNA mutation increases in the frequency of the mutation of nuclear DNA dependent or independent of reactive oxygen species [9]. They hypothesized that these events may lead to cancer initiation and progression. Regarding cancer initiation, however, a contradictory report was published by Akimoto et al. using xenographic tumor formation model [10]. They showed that genome chimera cells (Cybrids) carrying nuclear DNA from tumor cells and mtDNA from normal cells formed tumor, whereas those carrying nuclear DNA from normal cells and mtDNA from tumor cells did not. These observations provided direct evidence that nuclear DNA, but not mtDNA, is responsible for cancer initiation at least in a short term. Human 8-oxoguanine DNA glycosylase 1 (hOGG1) gene is a repair enzyme of mtDNA. Zang et al. showed that hOGG1 overexpression in mitochondria increased mutation in mtDNA [11] and that generated *hOGG1 transgenic mouse* showed obesity and increased frequencies of malignant lymphoma [12]. This report clearly demonstrates that mtDNA damages can induce cancer initiation.

mtDNA Changes and Cancer Progression to an Aggressive Phenotype

mtDNA damage generally induces reduction of oxidative phosphorylation leading to the reduction of ATP synthesis and oxygen consumption. Another potential change induced by mtDNA damage is ROS generation. Specific inhibition of MRC complex I [13] and complex III [14] generates ROS, and therefore, specific mutation of mtDNA may generate dysfunctional complex I and/or complex III to generate ROS.

First of all, to demonstrate the role of mtDNA damage on cancer progression, mitochondrial genomic knock-out ($\rho 0$) with deficient in respiration has been utilized. Amuthan et al. showed that depletion of mtDNA induces aggressive phenotype leading to invasion [15]. We previously showed that TNF and serum starvation could not induce TNF-induced apoptosis in $\rho 0$ cells, whereas they induced apoptosis in parental cells and cells reconstituted with normal mtDNA [16]. We also showed that AKT activation is responsible for the inhibition of apoptosis in $\rho 0$ cells [17]. These results suggest that mtDNA change is associated with apoptosis-resistance phenotype of cancer. Additionally, Reduction of mtDNA content shifted androgen-dependent prostate cancer cells to an androgen-independent phenotype *in vitro* and *in vivo* [18], induced epithelial to mesenchymal transition changes [19] and silencing of putative tumor suppressor genes by hyper methylation of CpG islands [20]. Several signaling associated with cancer progression such as NF- κ B [21,22], AKT [17-23,24], AP-1 [25], ERK [19], JNK [19] and Calcineurin [26] can be induced in $\rho 0$ cells.

In addition to the inhibition of oxidative phosphorylation, mutation to the specific coding site of mtDNA may induce ROS

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generation. To investigate the roles of specific mtDNA mutation on cancer progression, several researchers utilized cybrid cells. In these experiments, cybrid cells have nuclear DNA from cancer cells with specific mtDNA mutation. Cybrid cells with specific mutant mtDNA in ND6 region in an *in vivo* mouse model system induces metastatic phenotype, suggesting that specific mutations ND6 in complex I give a survival advantage and induce metastasis [27]. Additionally, several investigators showed enhanced growth of tumor size of cybrids with mutant mtDNA *in vivo* [28,29].

Regarding similarity of $\rho 0$ and cybrids with specific mtDNA mutation, both cell types share the phenotype induced by the inhibition of respiratory function leading to reduction of ATP synthesis and inhibition of oxygen consumption. In contrast, ROS generation may be only induced in cybrid with mtDNA mutation since superoxide generation in $\rho 0$ cells are generally inhibited [9,22] although contradictory results was reported [30]. When investigating cybrid with mutated mtDNA, the copy number of mtDNA needs to be investigated since reduction of mtDNA content may occur in cybrid with specific mtDNA mutation [31] and such cybrid will show $\rho 0$ phenotype.

Mechanisms How mtDNA Damage Induces Aggressive Phenotype

Since ROS can activate the receptor tyrosine kinase [32,33], Ras [33], phosphatidylinositol 3'kinase pathways [32], NF- κ B [34] and JNK [35], ROS from the cells with mutated mtDNA may activate these signaling leading to an aggressive phenotype. It is generally believed that ROS is generated in the cells with dysfunctional respiratory chain by donating electron from MRC to oxygen molecules. However, Lu et al. showed new pathways that cells with damaged mtDNA activates NADPH oxidase and induces ROS generation [36]. This alternative pathway should be considered for the roles of ROS in cells with damaged mtDNA.

Pelicano et al. showed how AKT is activated in the cells with damaged mtDNA [37]. They showed that increase in NADH by respiratory deficiency inactivates phosphatase and tensin homolog (PTEN) through redox modification mechanism, leading to Akt activation.

Regulation of Ras Pathway by Intracellular Oxygen Concentration Regulated by mtDNA

Ras signaling is one of the most important signaling to induce cancer initiation and progression [38]. Cook et al. showed that intracellular oxygen concentration regulated by mtDNA is responsible for cancer progression [39]. Loss of mtDNA content reversibly induced activation of proto-oncogenic Ras responsible for the activation of AKT and ERK leading to cancer progression. Ras activation was mediated by high farnesylation induced by the overexpression of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR), the rate-limiting enzyme of the mevalonate pathway. Hypoxia is known to induce proteasomal degradation of HMGR [40,41]. Well-differentiated cancer cells had high mtDNA content, consumed a large amount of oxygen and induced intracellular hypoxia. Loss of mtDNA reduced oxygen consumption and increased in oxygen concentration in the cells. The hypoxic-to-normoxic shift led to the overexpression of HMGR through inhibiting proteasomal degradation. Therefore, damage to mtDNA induced overexpression of HMGR through hypoxic to normoxic shift. Subsequently, the

endogenous induction of the mevalonate pathway activated Ras that mediates advanced phenotype. The results elucidate a coherent mechanism that directly links the mitochondrial genome with the advanced progression of the disease.

Taken together, several mechanisms are generated by mtDNA damage(s) and may work cooperatively to induce cancer progression. A confirmation and an understanding of the underlying mechanisms for cancer initiation and progression induced by mtDNA damage will lead to early detection of the potential aggressive cancer, novel approaches for targeted prevention, or therapy for prostate cancer.

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References

1. Van Goethem G, Martin JJ, Van Broeckhoven C (2002) Progressive external ophthalmoplegia and multiple mitochondrial DNA deletions. *Acta Neurol Belg* 102: 39-42.
2. Spelbrink JN, Li FY, Tiranti V, Nikali K, Yuan QP, et al. (2001) Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nat Genet* 28: 223-231.
3. Van Goethem G, Dermaut B, Lofgren A, Martin JJ, Van Broeckhoven C (2001) Mutation of POLG is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. *Nat Genet* 28: 211-212.
4. Cook CC, Higuchi M (2011) The awakening of an advanced malignant cancer: An insult to the mitochondrial genome. *Biochim Biophys Acta* 1820: 652-662.
5. Lu J, Sharma LK, Bai Y (2009) Implications of mitochondrial DNA mutations and mitochondrial dysfunction in tumorigenesis. *Cell Res* 19: 802-815.
6. Barthelemy C, de Baulny HO, Lombes A (2002) D-loop mutations in mitochondrial DNA: link with mitochondrial DNA depletion? *Hum Genet* 110: 479-487.
7. Lee HC, Hsu LS, Yin PH, Lee LM, Chi CW (2007) Heteroplasmic mutation of mitochondrial DNA D-loop and 4977-bp deletion in human cancer cells during mitochondrial DNA depletion. *Mitochondrion* 7: 157-163.
8. Palanichamy MG, Zhang YP (2010) Potential pitfalls in MitoChip detected tumor-specific somatic mutations: a call for caution when interpreting patient data. *BMC Cancer* 10: 597.
9. Rasmussen AK, Chatterjee A, Rasmussen LJ, Singh KK (2003) Mitochondria-mediated nuclear mutator phenotype in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 31: 3909-3917.
10. Akimoto M, Niikura M, Ichikawa M, Yonekawa H, Nakada K, et al. (2005) Nuclear DNA but not mtDNA controls tumor phenotypes in mouse cells. *Biochem Biophys Res Commun* 327: 1028-1035.
11. Zhang H, Mizumachi T, Carcel-Trullols J, Li L, Naito A, et al. (2007) Targeting human 8-oxoguanine DNA glycosylase (hOGG1) to mitochondria enhances cisplatin cytotoxicity in hepatoma cells. *Carcinogenesis* 28: 1629-1637.
12. Zhang H, Xie C, Spencer HJ, Zuo C, Higuchi M, et al. (2011) Obesity and hepatosteatosis in mice with enhanced oxidative DNA damage processing in mitochondria. *Am J Pathol* 178: 1715-1727.
13. Turrens JF, Boveris A (1980) Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem J* 191: 421-427.
14. Ksenzenko M, Konstantinov AA, Khomutov GB, Tikhonov AN, Ruuge EK (1983) Effect of electron transfer inhibitors on superoxide generation in the cytochrome bc1 site of the mitochondrial respiratory chain. *FEBS Lett* 155: 19-24.
15. Amuthan G, Biswas G, Zhang S, Klein-Szanto A, Vijayasathy C, et al. (2001) Mitochondria-to-nucleus stress signaling induces phenotypic changes, tumor progression and cell invasion. *Embo J* 20: 1910-1920.
16. Higuchi M, Aggarwal BB, Yeh ET (1997) Activation of CPP32-like protease in tumor necrosis factor-induced apoptosis is dependent on mitochondrial function. *J Clin Invest* 99: 1751-1758.


17. Suzuki S, Naito A, Asano T, Evans TT, Reddy SA, et al. (2008) Constitutive Activation of AKT Pathway Inhibits TNF-induced Apoptosis in Mitochondrial DNA-Deficient human myelogenous leukemia ML-1a. *Cancer Lett* 268: 31-37.
18. Higuchi M, Kudo T, Suzuki S, Evans TT, Sasaki R, et al. (2006) Mitochondrial DNA determines androgen dependence in prostate cancer cell lines. *Oncogene* 25: 1437-1445.
19. Akihiro Naito, Takatsugu Mizumachi, Mian Wang, Cheng-hui Xie, Teresa T Evans, et al. (2008) Progressive tumor features accompany epithelial-mesenchymal transition induced in mitochondrial DNA depleted cells. *Cancer Sci* 99: 1584-1588.
20. Xie C, Naito A, Mizumachi T, Evans TT, Douglas MG, et al. (2007) Mitochondrial regulation of Cancer Associated Nuclear DNA Methylation. *Biochem Biophys Res Commun* 364: 656-661.
21. Biswas G, Anandatheerthavarada HK, Zaidi M, Avadhani NG (2003) Mitochondria to nucleus stress signaling: a distinctive mechanism of NFkappaB/Rel activation through calcineurin-mediated inactivation of IkkappaBbeta. *J Cell Biol* 161: 507-519.
22. Higuchi M, Manna SK, Sasaki R, Aggarwal BB (2002) Regulation of the Activation of Nuclear Factor kappaB by Mitochondrial Respiratory Function: Evidence for the Reactive Oxygen Species-Dependent and -Independent Pathways. *Antioxid Redox Signal* 4: 945-955.
23. Guha M, Fang JK, Monks R, Birnbaum MJ, Avadhani NG (2010) Activation of Akt is essential for the propagation of mitochondrial respiratory stress signaling and activation of the transcriptional coactivator heterogeneous ribonucleoprotein A2. *Mol Biol Cell* 21: 3578-3589.
24. Pelicano H, Xu RH, Du M, Feng L, Sasaki R, et al. (2006) Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism. *J Cell Biol* 175: 913-923.
25. Nishimura G, Proske RJ, Doyama H, Higuchi M (2001) Regulation of apoptosis by respiration: cytochrome c release by respiratory substrates. *FEBS Lett* 505: 399-404.
26. Guha M, Tang W, Sondheimer N, Avadhani NG (2010) Role of calcineurin, hnRNPA2 and Akt in mitochondrial respiratory stress-mediated transcription activation of nuclear gene targets. *Biochim Biophys Acta* 1797: 1055-1065.
27. Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, et al. (2008) ROS-Generating Mitochondrial DNA Mutations Can Regulate Tumor Cell Metastasis. *Science* 320: 661-664.
28. Petros JA, Baumann AK, Ruiz-Pesini E, Amin MB, Sun CQ, et al. (2005) mtDNA mutations increase tumorigenicity in prostate cancer. *Proc Natl Acad Sci U S A*. 102: 719-724.
29. Shidara Y, Yamagata K, Kanamori T, Nakano K, Kwong JQ, et al. (2005) Positive contribution of pathogenic mutations in the mitochondrial genome to the promotion of cancer by prevention from apoptosis. *Cancer Res* 65: 1655-1663.
30. Indo HP, Davidson M, Yen HC, Suenaga S, Tomita K, et al. (2007) Evidence of ROS generation by mitochondria in cells with impaired electron transport chain and mitochondrial DNA damage. *Mitochondrion* 7: 106-118.
31. Turner CJ, Granycome C, Hurst R, Pohler E, Juhola MK, et al. (2005) Systematic segregation to mutant Mitochondrial DNA and Accompanying Loss of Mitochondrial DNA in Human NT2 Teratocarcinoma Cybrids. *Genetics* 170: 1879-1885.
32. Kamata H, Hirata H (1999) Redox regulation of cellular signalling. *Cell Signal* 11: 1-14.
33. Sundaresan M, Yu ZX, Ferrans VJ, Irani K, Finkel T (1995) Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. *Science* 270: 296-299.
34. Mercurio F, Manning AM (1999) NF-kappaB as a primary regulator of the stress response. *Oncogene* 18: 6163-6171.
35. Adler V, Yin Z, Fuchs S Y, Benezra M, Rosario L, et al. (1999) Regulation of JNK signaling by GSTp. *EMBO J* 18: 1321-1334.
36. Lu W, Hu Y, Chen G, Chen Z, Zhang H, et al. (2012) Novel Role of NOX in Supporting Aerobic Glycolysis in Cancer Cells with Mitochondrial Dysfunction and as a Potential Target for Cancer Therapy. *PLoS biology* 10: e1001326.
37. Pelicano H, Xu RH, Du M, Feng L, Sasaki R, et al. (2006) Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism. *J Cell Biol* 175: 913-923.
38. Downward J (2003) Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 3: 11-22.
39. Cook CC, Kim A, Terao S, Gotoh A, Higuchi M, et al. (2012) Consumption of oxygen: a mitochondrial-generated progression signal of advanced cancer. *Cell Death Dis* 3: e258.
40. Nguyen AD, McDonald JG, Bruick RK, DeBose-Boyd RA (2007) Hypoxia stimulates degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase through accumulation of lanosterol and hypoxia-inducible factor-mediated induction of insigs. *J Biol Chem* 282: 27436-27446.
41. Song BL, Javitt NB, DeBose-Boyd RA, (2005) Insig-mediated degradation of HMG CoA reductase stimulated by lanosterol, an intermediate in the synthesis of cholesterol. *Cell Metab* 1: 179-189.

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