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Role of mitochondrial DNA alterations in the development and progression of Oral cancer

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Abstract
Distinct genetic and metabolic differences have been identified between the mitochondria of healthy and cancerous cells. These differences encompass changes in the expression and activity of enzymes that play roles in both aerobic and anaerobic respiration, the oxidation-reduction pathways, and the processes of mitochondrial DNA (mtDNA) translation and transcription. Although these phenomena have been extensively documented, the underlying mechanisms that lead to mtDNA mutations and their implications in cancer, drug resistance, and disease progression remain unclear.
In this review, we present an overview of the known alterations in mitochondrial DNA associated with human oral cancer and precancerous conditions, while also discussing the potential mechanisms behind their emergence and their clinical significance. The objective of this review is to elucidate the role of mtDNA mutations in oral carcinogenesis and to investigate the potential of these mitochondrial mutations as biomarkers for predicting oral precancer and cancer, as well as their viability as targets for anti-cancer therapies.

Keywords: DNA, Oral cancer, alterations, OSCC

Introduction

Cancer is a genetic disease caused by changes to genes that control the way our cells function, especially how they grow and divide. Characterized by the uncontrolled proliferation of certain cells within the body, which can subsequently disseminate to various regions. Malignant tumors can infiltrate adjacent tissues and may also migrate to remote sites, leading to the formation of secondary tumors, a phenomenon known as metastasis [1].

Oral squamous cell carcinoma (OSCC) represents the predominant form of oral malignancy, resulting from the detrimental effects on oral epithelial cells caused by the accumulation of numerous genetic mutations.[1] OSCC continues to be a significant contributor to both morbidity and mortality among individuals diagnosed with head and neck cancers.

Cancer causing genetic changes can happen because of

- Damage that occur in the process of cell division.
- DNA damage caused by harmful substances in the environment, like chemicals in tobacco smoke, ultraviolet rays from the sun, hazardous chemicals, pesticides and preservatives from food.
- Mutations inherited from parents.

Oral cancer is the sixth most prevalent cancer type worldwide. India accounts for the highest incidence of oral cancer cases, representing one-third of the global burden. The condition typically manifests as a small, unusual, and unexplained growth or sore in various oral regions, including the lips, cheeks, sinuses, tongue, hard and soft palate, and the base of the mouth extending into the oropharynx. The pathogenesis of oral cancer involves the damage to oral epithelial cells, which results from the accumulation of numerous genetic mutations. Oral squamous cell carcinoma (OSCC) is a significant contributor to morbidity and mortality among patients diagnosed with head and neck cancers. Key risk factors for the elevated prevalence of OSCC include the use of tobacco, smoking, alcohol consumption—either independently or in conjunction with chewing tobacco—and the use of betel quid. Current treatment modalities for OSCC like chemotherapy, radiotherapy, surgery, EGFR inhibitors and COX-2 inhibitors, and photodynamic therapy have led to the major problems related to non-specific cell death. However, the five-year overall survival rate of OSCC remains approximately 60% due to therapy resistance and side effects. More effective therapies are needed for OSCC.[2]

Mitochondria and mitochondrial DNA

Mitochondria, the vital powerhouse of the cell under electron micrographs have been visualized as static, 'cigar-shaped' organelles, bounded by two distinct membranes, outer and inner separated by intermembrane space as in Fig1.

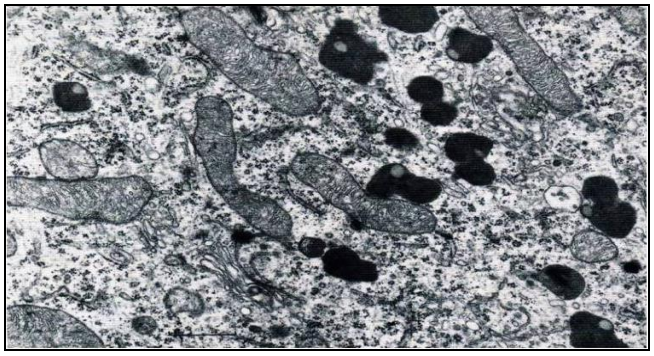

Fig 1: Mitochondrial profile and polymorphic pigment granule scale 0.5 um

Visualized by fluorescence microscopy after staining with rhodamine123 dye, mitochondria appear as a long filamentous dynamic network that shows changes in size, form, and location. They have double-stranded circular 16.6

kb DNA, with self-transcription, translation, and protein assembly machinery, providing them genomic independence. MtDNA is less than 1% of total cellular DNA, but its gene products are essential for normal cell function. The inner mitochondrial membrane harbours the enzymes forming the respiratory chain in oxidative phosphorylation. The complete respiratory chain inside mitochondria contains 87 polypeptides of which 13 are mitochondria coded. Each mitochondrion contains generally less than 10 copies of its genome. Approximately 1500 genes constitute the mitochondrial genome. The mtDNA genes code a 12S and 16S rRNA, 22 tRNAs, and 13 essential OXPHOS polypeptide subunits. Mt DNA is tightly organized and clever in arrangement such that it performs duty as a spacer between the genes for proteins, and code tRNAs [2, 3].

Mitochondrial DNA mutations: Mechanism

Mitochondrial DNA is subjected to mutations at a rate that is 100 times greater than that of nuclear DNA, primarily due to insufficient histone protection, a limited capacity for repair, and its close association with the electron transport chain, which is a site for the generation of superoxide radicals. Although mutations and deletions in mitochondrial DNA are observed at a frequency of less than 1%, this frequency tends to rise with age and is particularly susceptible to large-scale deletions in the regions adjacent to the repeats [4]. The following theories have been proposed for the origin of mitochondrial mutations:

1. Slip mismatching.
2. Illegitimate elongation of the D-loop strand.
3. Free radical-induced deletions and mutations.
4. Clonal expansion of mutations in the stem cells.

Mitochondria & carcinogenesis

Mitochondrial functions significantly influence cellular physiology, extending beyond the mere production of ATP. Researchers propose that oxidative stress plays a critical role in the development of oral cancer, as illustrated in Fig 2. Oxidative stress arises from an imbalance between the generation and elimination of reactive oxygen species (ROS), leading to an accumulation of these harmful molecules [5]. Mitochondria serve as a primary source of ROS due to their participation in oxidative phosphorylation. By their very nature, ROS are inherently unstable and can cause damage to both nuclear and mitochondrial DNA [6].

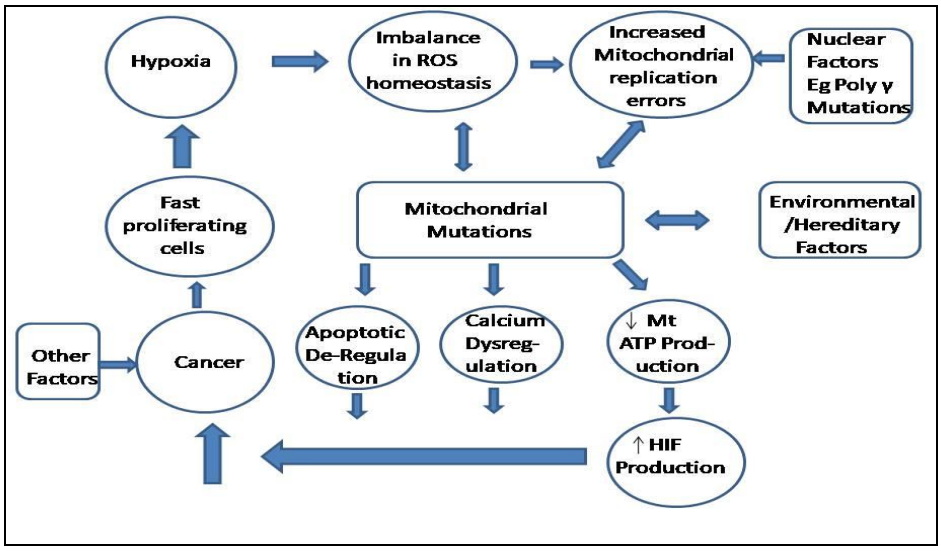

Fig 2: Mitochondria events in carcinogenesis.

The mitochondrial genome is susceptible to such damage that can lead to mitochondrial dysfunction. Such damage not only results in mutations but may also alter the expression levels of genes responsible for cell growth, including proliferation and differentiation, while also impacting lipid peroxidation, protein modification, and membrane integrity.

The conditions that promote increased reactive oxygen species (ROS) generation are diverse, encompassing psychological, genetic, and environmental factors. Cigarette smoke contains over 19 pyrolytic carcinogenic substances, which disrupt the delicate equilibrium between ROS production and consumption. This disruption in the homeostasis of free radicals can result in mutations in mitochondrial DNA (mtDNA), affecting the components of the electron transport chain. Consequently, this may hinder normal electron flow, leading to heightened electron leakage and an increase in superoxide radicals, which serve as precursors to other free radicals or ROS. Research indicates a correlation between mtDNA mutations and elevated oxidative stress, both of which have been documented in various types of cancer [7].

A strong association exists between apoptosis and mitochondria. Moreover, it is known that the mitochondria initiate the early apoptotic events evident by the involvement of two members of the Bcl-2 family. Bcl-2, the anti-apoptotic factor, maintains the inner mitochondrial membrane electrochemical gradient by controlling the influx and efflux of Ca2+ into and out of the mitochondria, whereas BAX, the pro-apoptotic member, is involved in the disrupting of the fore mentioned membrane electrochemical gradient [7, 8].

Mitochondria and OSCC

Interestingly, additional functions of mitochondria besides energy production have been reported, including apoptosis induction, reactive oxygen species (ROS) production, mitochondrial fission and mitophagy. Mitochondria play a significant role in the physiological and pathological

processes in cells. Of note is that increasing evidence shows that mitochondria have links to cancer [9].

In recent years, the relationship between mitochondria and OSCC, in terms of therapy, has attracted increasing attention. For example, casticin could induce apoptosis in OSCC by regulating the mitochondrial apoptosis pathway (MAP). Moreover, mitochondrial ROS (mtROS) can also induce cytochrome C (Cyt C) release by opening the mitochondrial permeability transition pore (mPTP), which causes the apoptosis of OSCC cells. Mitochondrial fission can also boost the production of mtROS and Cyt C. However, other characteristics of mitochondria can cause therapeutic resistance. Like, mitophagy could remove mitochondria damaged by ROS, thereby decreasing the effect of ROS-mediated therapy in OSCC. In addition, abnormal nucleic acids in mitochondria can also interfere with treatment by regulating cellular metabolism to meet the need for cancer cell survival, and some constituents of the tumor micro environment (TME) interact with mitochondria in OSCC, which can also lead to resistance. Regulating these mechanisms is beneficial to OSCC therapy [8, 9].

The Warburg hypothesis posits that impaired mitochondrial function plays a significant role in the development of cancer. The Warburg effect suggests that a majority of cancer cells predominantly utilize aerobic glycolysis for energy production. Advocates of the Warburg effect argue that this phenomenon may arise from mitochondrial impairment, cellular adaptation to hypoxic conditions within tumors, or alterations in mitochondrial function induced by cancer cells to evade apoptosis [10].

Mitochondrial DNA (mtDNA) mutations in oral cancer were first documented in 2000, encompassing a variety of alterations, including insertions, deletions, chain-termination mutations, and missense mutations. Numerous studies have reported substantial variations in both the quantity (mitochondrial copy number) and quality (nucleotide alterations) of mtDNA. It has been observed that the mtDNA copy number in certain cancers correlates with specific mutation sites. While mtDNA mutations are distributed throughout the mitochondrial genome, they are particularly prevalent in protein-coding genes, rRNA genes, and the D-loop region.[10] The majority of these mutations involve T to C and G to A base transitions, suggesting that reactive oxygen species (ROS) may be the primary mutagenic factor.

Escape of cancer cells from hypoxia

The characteristic feature of cancer cells is rapid proliferation and growth, which results in hypoxia because local vasculature is unable to supply an adequate amount of oxygen. Strikingly, such hypoxic conditions cause cellular death in non-malignant cells [10].

Cancerous cells escape hypoxia mediated death by:

- Lowering p53 expression or
- p53 mutation or
- Hypoxia Inducible Factor (HIF) dependent mechanisms.

Hypoxia induces an upregulation of the glycolytic pathway in tumor cells, as the mitochondria are unable to meet the heightened energy demands, even following the activation of hypoxia-inducible factor 1 (HIF-1), Phosphoinositide 3-kinase (PI3K), and its downstream effector, Akt (protein kinase B). Consequently, it seems that mitochondrial function plays a minimal role in redirecting metabolic pathways to accommodate the increased ATP requirements of tumor cells. Should any dysfunction or structural abnormalities occur in the mitochondria during subsequent metabolic processes, it appears that even suboptimal mitochondrial performance does not significantly hinder tumor cell proliferation.[10] Recent data indicate that glycolysis contributes to approximately 60% of the total ATP production in most cancer cells. This suggests that while many cancer cells engage in oxidative respiration, the rate of oxidative phosphorylation is diminished due to elevated glycolysis and lactate production. Thus, this metabolic shift serves a crucial function by providing additional substrates necessary for enhanced DNA synthesis and tumor cell growth. Inhibition of lactate dehydrogenase or activation of pyruvate dehydrogenase can prompt tumor cells to oxidize pyruvate within the tricarboxylic acid (TCA) cycle, thereby enhancing mitochondrial respiration. This implies that mitochondrial function is not fundamentally compromised in cancer cells. Nevertheless, the presence of mitochondrial mutations in oral tumors presents a complex issue, and it remains a topic of debate whether these mutations are merely incidental to the tumorigenesis process [11].

It has been observed that the prognosis of tumors is clinically associated with the level of glycolysis. Researchers have characterized tumor aggressiveness as the ratio of the activities of the mitochondrial enzyme ATP synthase to the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase. This characterization is biochemical in nature, indicating that mitochondrial function in cancer cells may be diminished due to factors such as low oxygen availability, fluctuations in metabolic pathways, and alterations in gene expression. Furthermore, it has been suggested that mutations in mitochondrial DNA, along with impairments in respiratory enzyme complexes, contribute to an increase in reactive oxygen species (ROS) production [12]. Supporting this theory, it has been documented that compromised activity of the mitochondrial respiratory chain results in:

1. NADPH oxidase (NOX1) over expression which produces superoxide
2. Increased nuclear DNA damage and hypermutagenesis
3. Apoptosis resistance.

Such cellular phenotype changes contribute to development of cancer. However, mitochondrial dysfunction during metabolic transformation of cancerous cells is still debated and a more detailed analysis of molecular events in the process of tumorigenesis is required to assign characteristic role to mitochondrial mutations. Mutant mtDNA in tumor cells, small in size and more in number, is readily detectable in urine, blood and saliva samples of different cancer patients. Despite this, no single site of mutation or type of mutation has been found common across the wide spectrum of cancer patients [13].

Relevance of mitochondrial DNA mutations

Various sequencing technologies, including microarray-based, next-generation, ion-based, and biochip-based methods, offer a dependable and swift approach to identify all mutations within the complete mitochondrial genome. Although recent advancements have enabled the sequencing of the entire mitochondrial genome using diverse

technologies, further characterization of the multiple deletions linked to tumors is necessary to assess the mutation burden on an individual level. Mutations in the mitochondrial genome occur recurrently in both primary tumor tissues and their corresponding body fluids. Consequently, the analysis of mtDNA mutations may serve as a valuable molecular tool for the early detection and prognosis of cancer [14]. Leveraging the latest technologies, relatively straightforward diagnostic tests for identifying mtDNA mutations hold significant promise for cancer detection and prognosis. The dysfunction of mitochondria can be evaluated through the Warburg effect, which has recently found applications in medicine, particularly in positron emission tomography (PET). This technique visualizes the high rate of aerobic glycolysis in malignant tumors through the uptake of 2-18F-2-deoxyglucose (FDG), a radioactive modified hexokinase substrate. This characteristic is clinically relevant for diagnosing and monitoring the treatment responses of cancers with therapeutic agents [15].

Mitochondrial DNA is devoid of introns, resulting in all mutations occurring within the coding region, which have biological significance. These mtDNA mutations can be classified as pathogenic, neutral, or beneficial. In the germ line, mtDNA variants exist in a state of heteroplasmy, and once they reach a 'critical threshold,' they can influence the cellular phenotype. In mature cells, somatic mtDNA mutations accumulate over time, leading to a gradual decline in cellular function. Upon surpassing a critical, yet undetermined, threshold, these mutations can trigger apoptotic cell death. Consequently, the accumulation of mtDNA mutations serves as a marker for biological ageing. The interplay between the accumulated somatic mtDNA mutations and the partially inherited mitochondrial defects amplifies the impact of these defects, which contributes to the late-onset and progressive nature of mitochondrial-associated diseases [16].

The selection of a beneficial mitochondrial DNA (mtDNA) mutation leads to an increase in its prevalence and the number of copies within a population. Due to the absence of recombination in mitochondria, all sequence variants associated with a specific mutation become enriched, resulting in a phenomenon referred to as 'Hitchhiking.' The associated variants on a single mtDNA molecule accumulate and give rise to an mtDNA haplotype. Over time, the descendants of the original mutant mtDNA acquire additional variations, leading to the formation of a group of related haplotypes known as a haplogroup. Haplogroups represent region-specific branches within the mtDNA phylogenetic tree. It can be posited that mitochondrial DNA mutations in oral cancer may arise through two primary mechanisms: first, through oncogenic germline mutations in the female germ line, or second, through tumor-specific somatic mutations in the mtDNAs of affected tissues. Consequently, it can be suggested that mitochondrial mutations might provide a selective advantage, a notion that remains contentious and necessitates a thorough examination of Warburg's hypothesis and its implications. This feature is relevant clinically to diagnose and monitor treatment responses of cancers with drugs [15, 16].

The advantage of oral cancer diagnostics lies in the straightforward anatomical access, allowing for the collection of samples from developing premalignant and malignant lesions to assess DNA adduct levels and evaluate the risk of oral cancer progression. Monitoring free radical levels within cells serves as a potential biomarker for mitochondrial DNA (mtDNA) damage and mitochondrial function. Although a significant correlation exists between free radical levels and mtDNA deletions, it remains unclear whether mitochondrial mutations or altered metabolic conditions are the initial cause. Furthermore, the accuracy of free radical measurement techniques varies, necessitating that association study data be presented only after thorough verification and consensus [18]. Research has indicated distinct differences in the molecular composition of the mitochondrial inner membrane between normal and cancerous cells, with cancer cells exhibiting elevated cholesterol levels and variations in total and individual phospholipid levels. Additionally, notable differences in the presence and abundance of various proteins have been observed between cancerous and control cells, alongside divergent gene expression profiles. Key genes include Bcl-2, Bcl-XL, the peripheral benzodiazepine receptor (PBR), the PBR-associated protein Prax-1, mitochondrial creatine kinase, and BAX, a pro-apoptotic protein located in the inner mitochondrial membrane [19]. The impaired expression of these proteins is attributed to defective mitochondrial DNA, highlighting its functional significance. Consequently, proteomic-based biomarkers related to mitochondrial function may serve as valuable tools for diagnosis.

Conclusions

The inherent dynamism of mitochondria, positions them as a compelling molecular marker for cancer. Mitochondrial DNA (mtDNA), comprising a mere 16 kilobases, can be readily analysed through high-throughput sequencing techniques. A comprehensive investigation is essential to elucidate the functional implications of specific mtDNA modifications in the context of oral carcinogenesis. It is plausible that numerous mutations disrupt apoptotic pathways and energy balance within the cell, serving as both causative and facilitating factors in cancer development. Additionally, certain mutations may influence the cell's autophagic processes. Conducting proteomic analyses to uncover the biochemical ramifications of mtDNA mutations presents challenges, particularly due to the complexities associated with regulating gene expression *in vivo*. The prevalence of mtDNA mutations in cancer is well-established. Future research should focus on cataloging the types and locations of these mutations across various pathological stages of oral cancer, assessing the resulting functional impairments, and developing therapeutic strategies aimed at mitochondrial targets.

References

1. Meng X, Lou Q, Yang W, Wang Y, Chen R, Wang L, *et al*. The role of non-coding RNAs in drug resistance of oral squamous cell carcinoma and therapeutic potential. Cancer Commun,2021:41:981–1006. doi: 10.1002/cac2.12194.
2. Chai AWY, Lim KP, Cheong SC. Translational genomics and recent advances in oral squamous cell carcinoma. Semin. Cancer Biol,2020:61:71–83. doi: 10.1016/j.semcancer.2019.09.011.
3. Afrasiabi M, Seydi E, Rahimi S, Tahmasebi G, Jahanbani J, Pourahmad J. The selective toxicity of

superparamagnetic iron oxide nanoparticles (SPIONs) on oral squamous cell carcinoma (OSCC) by targeting their mitochondria. J Biochem Mol Toxicol,2021:35(6):1-8. doi: 10.1002/jbt.22769. Epub 2021: 11. PMID: 33704875.
4. Bai J, Wu L, Wang X, Wang Y, Shang Z, Jiang E, *et al*. Roles of Mitochondria in Oral Squamous Cell Carcinoma Therapy: Friend or Foe? Cancers (Basel),2022 22:14(23):5723. doi: 10.3390/cancers14235723. PMID: 36497206: PMCID: PMC9738284.
5. Jiang M, Li B. STAT3 and Its Targeting Inhibitors in Oral Squamous Cell Carcinoma. Cells,2022:11(19):3131. doi: 10.3390/cells11193131. PMID: 36231093: PMCID: PMC9563058.
6. Prior SL, Griffiths AP, Lewis PD. A study of mitochondrial DNA D-loop mutations and p53 status in nonmelanoma skin cancer. Br J Dermatol,2009:161(5):1067-71. doi: 10.1111/j.1365-2133.2009.09304. x. Epub 2009 11. PMID: 19624548.
7. Zhu W, Qin W, Bradley P, Wessel A, Puckett CL, Sauter ER. Mitochondrial DNA mutations in breast cancer tissue and in matched nipple aspirate fluid. Carcinogenesis,2005:26(1):145-52. doi: 10.1093/carcin/bgh282. Epub 2004: 16. PMID: 15375011.
8. Yokoe H, Nomura H, Yamano Y, Fushimi K, Sakamoto Y, Ogawara K, *et al*. Characterization of intracellular superoxide dismutase alterations in premalignant and malignant lesions of the oral cavity: correlation with lymph node metastasis. J Cancer Res Clin Oncol,2009:135(11):1625-33. doi: 10.1007/s00432-009-0610-8. Epub 2009: 12. PMID: 19521720.
9. Challen C, Brown H, Cai C, Betts G, Paterson I, Sloan P, *et al*. Mitochondrial DNA mutations in head and neck cancer are infrequent and lack prognostic utility. Br J Cancer,2011:104(8):1319-24. doi: 10.1038/bjc.2011.96. Epub 2011: 22. PMID: 21427725: PMCID: PMC3078603.
10. Dasgupta S, Koch R, Westra WH, Califano JA, Ha PK, Sidransky D, *et al*. Mitochondrial DNA mutation in normal: gins and tumors of recurrent head and neck squamous cell carcinoma patients. Cancer Prev Res (Phila),2010:3(9):1205-11. doi: 10.1158/1940-6207.C:-10-0018. Epub 2010: 26. PMID: 20660573: PMCID: PMC3040952.
11. Carvalho AC, Kowalski LP, Campos AH, Soares FA, Carvalho AL, Vettore AL. Clinical significance of molecular alterations in histologically negative surgical: gins of head and neckcancer patients. OralOncol.2012:48(3):2408.doi:10.1016/j.oraloncology.2011.10.018. Epub 2011 21. PMID: 22104250.
12. Liu SA, Jiang RS, Wang WY, Lin JC. Somatic mutations in the D-loop of mitochondrial DNA in head and neck squamous cell carcinoma. Head Neck,2015:37(6):878-83. doi: 10.1002/hed.23680. Epub 2014: 27. PMID: 24976238.
13. Allegra E, Garozzo A, Lombardo N, De Clemente M, Carey TE. Mutations and polymorphisms in mitochondrial DNA in head and neck cancer cell lines. Acta Otorhinolaryngol Ital,2006:26(4):185-90. PMID: 18236634: PMCID: PMC2639997.
14. Lièvre A, Blons H, Houllier AM, Laccourreye O, Brasnu D, Beaune P, *et al*. Clinicopathological significance of mitochondrial D-Loop mutations in head and neck carcinoma. Br J Cancer,2006: 13:94(5):692-7. doi: 10.1038/sj.bjc.6602993. PMID: 16495928: PMCID: PMC2361200.
15. Lièvre A, Chapusot C, Bouvier AM, Zinzindohoué F, Piard F, Roignot P, *et al*. Clinical value of mitochondrial mutations in colorectal cancer. J Clin Oncol,2005 20:23(15):3517-25. doi: 10.1200/JCO.2005.07.044. PMID: 15908662.
16. Quintela-Fandino M, Hitt R, Medina PP, Ga:ra S, Manso L, Cortes-Funes H, *et al*. DNA-repair gene polymorphisms predict favorable clinical outcome among patients with advanced squamous cell carcinoma of the head and neck treated with cisplatin-based induction chemotherapy. J Clin Oncol,2006:24(26):4333-9. doi: 10.1200/JCO.2006.05.8768. Epub 2006: 8. PMID: 16896002.
17. Dasgupta S, Koch R, Westra WH, Califano JA, Ha PK, Sidransky D, *et al*. Mitochondrial DNA mutation in normal: gins and tumors of recurrent head and neck squamous cell carcinoma patients. Cancer Prev Res (Phila),2010:3(9):1205-11. doi: 10.1158/1940-6207. C:-10-0018. Epub 2010: 26. PMID: 20660573: PMCID: PMC3040952.
18. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet,2005:39:359–407. doi: 10.1146/annurev.genet.39.110304.095751