Mitochondrial DNA: The Alternative Molecular Marker for Cancer Detection and Treatment

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ABSTRACT
Mitochondrion being an organelle plays a major role in many metabolic and bio synthetic pathways. Cancer is strongly associated with changes in cellular metabolism, with a characteristic metabolic shift toward aerobic glycolysis in transformed cells. The recent resurgence of interest in the study of mitochondria has come into the light due to recognition of the fact that the genetic or metabolic alterations in this organelle are causative or contributing factors in a variety of human diseases including cancer. A variety of mitochondrial dna mutations are caused by oxidative damage via ROS that are generated either endogenously during oxidative phosphorylation or by exogenous sources and other mutagens. The ability of adaptation of mitochondrial function has been recognized as crucial to the changes that occur in cancer cells. Due to the presence of this unique property of abundance and homoplasmic nature of mitochondria it makes mt DNA an attractive molecular marker for cancer. These alterations in mitochondrial structure and function might prove clinically useful either as markers for the early detection of cancer or as unique molecular sites against which novel and selective chemotherapeutic agents might be targeted. This review suggests that mitochondrion is one such target.

Key words: Mitochondrial DNA, Mutation, Molecular Marker, Cancer detection, Saliva.

INTRODUCTION
Mitochondria, although an organelle, are known as cellular power plants and their main function is to manufacture ATP which is utilized as source of energy in the body. Mitochondria are considered as gatekeepers of life and death of most cells in the body as they help to regulate signalling, metabolism, and energy production needed for cellular function. Apart from nucleus, mitochondria are the only sole component present in the cell to possess DNA. Although the mitochondrial (mt) proteins have their origin in cell nucleus but these proteins are imported into mitochondria. These proteins are responsible for energy production in mt DNA and any damage to the proteins can cripple the ability of mitochondria to produce energy.

For decades it has been assumed that mitochondria are inherited solely through the maternal line. MtDNA is typically passed on only from mother during sexual reproduction, meaning that the mitochondria are clones. This means that there is little change in mtDNA from generation to generation unlike nuclear DNA which changes by 50% each generation. Due to the presence of this unique property of abundance and homoplasmic nature of mitochondria it makes mt DNA an attractive molecular marker for cancer. These alterations in mitochondrial structure and function might prove clinically useful either as markers for the early detection of cancer or as unique molecular sites against which novel and selective chemotherapeutic agents might be targeted. This review suggests that mitochondrion is one such target.

their mitochondria from their father. These findings came into the light while they were trying to discover why one of their patients suffered extreme fatigue during exercise. They found that the patient had an extremely normal heart and lungs and his muscles appeared healthy. But they discovered that his muscles absorbed very little oxygen so they examined the genetic sequence of his mitochondrion and found two mutations in his mitochondrial DNA – one of which was responsible for his extreme fatigue. On further investigation of the mutations and sequencing of the DNA of his mother, father and uncle, they found that the sequence matched those to his father and uncle. The muscles biopsies showed that about 90% of their mitochondria came from his father but the mitochondrial in his blood, hair roots and fibroblasts came entirely from his mother. The two mutations appear to have arisen spontaneously during or shortly after conception. Inheritance of parental mitochondrial DNA is probably very rare. Evolutionary biologists often date the divergence of species by the differences in genetic sequences in mt DNA. Even if paternal DNA is inherited very rarely it could invalidate many of their findings. It will also have implications for scientists investigating inherited metabolic diseases.

Mitochondrial DNA (mtDNA) mutation in cancer

Mitochondria are one of the most important organelles that play a major role in many metabolic and biosynthetic pathways. The ability of adaptation of mitochondrial function has been recognized as crucial to the changes that occur in cancer cells. Heteroplasmy has been displayed by disease-causing mtDNA mutations, and the percentages of mutated mtDNA determine the severity of mitochondrial dysfunction.\(^7\)\(^8\) Cancer is strongly associated with changes in cellular metabolism, with a characteristic metabolic shift toward aerobic glycolysis in transformed cells.\(^7\)\(^8\) mtDNA is prone to mutations due to its proximity to ROS generation and lack of DNA repair system. The frequency of mtDNA mutations in cancer cells is 10-fold higher than nuclear DNA mutations, and many alterations to the mtDNA that can be detected in tumour cells potentially alter mitochondrial function. Apart from that mtDNA alterations can be detected in the premalignant stage itself.\(^4\) Mitochondrial DNA is particularly susceptible to damage by reactive oxygen species (ROS), such as superoxide radical (O\(_2^-\)), hydrogen peroxide (H\(_2\)O\(_2\)) and hydroxyl radical (OH\(^-\)), owing to the lack of the protective histone backbone and complex DNA repair mechanisms associated with nuclear DNA.\(^5\) It is well documented that a variety of mitochondrial mutations are caused by oxidative damage via ROS that are generated either endogenously during oxidative phosphorylation or by exogenous sources and other mutagens.\(^8\)\(^10\)

Use of mtDNA mutation as biomarkers in cancer

Many cancer-related mitochondrial defects have been recognized at the organelle level, including abnormal activity of aerobic respiratory chain subunits, decreased oxidation of NADH-linked substrates and altered expression of mtDNA and mutations of mtDNA.\(^11\) Mutations in mtDNA have been observed in a number of cancers including breast, colon and rectum, stomach, prostate, bladder, head and neck, and lung. As mtDNA does not contain any intron sequences, mutations that do occur would lie in coding or regulatory regions and are potentially biologically significant. The types of mutations observed in mtDNA ranges from point mutations, to deletions, and duplications. Most tumours contain homoplasmic mutant mtDNA because of the clonal nature of cancers.\(^12\) Due to the presence of this unique property of abundance and homoplasmic nature of mitochondria it makes mtDNA an attractive molecular marker of cancer.\(^11\) Mutant mtDNA in tumour cells is reported to be 220 times as abundant as a mutated nuclear marker.

A recent study have shown that mutated mtDNA is readily detectable in urine, blood and saliva samples from patients with bladder, head and neck and lung cancers. Thus mtDNA mutation might prove to be an extremely useful biomarker for the detection of many cancers. Ongoing research on DNA repair genes involved in maintaining the genetic integrity of the mitochondrial genome combined with further analysis of the nature of mtDNA mutations will greatly aid progress in this area.\(^11\)\(^12\) Also, screening tools such as MitoChip oligonucleotide arrays make it possible to screen for many factors at the same time.\(^12\)

Mitochondria as a target for detection and treatment of cancer

The recent resurgence of interest in the study of mitochondria has come into the light due to recognition of the fact that the genetic or metabolic alterations in this organelle are causative or contributing factors in a variety of human diseases including cancer. There are differences between the mitochondria of normal cells and cancer cells at the genetic, molecular and biochemical levels.\(^13\)\(^14\) these alterations in mitochondrial structure and function might prove clinically useful either as markers for the early detection of cancer or as unique molecular sites against which novel and selective chemotherapeutic agents might be targeted.

For the basis of developing new treatments it is important to understand the basic biology of mitochondrial disease. Several strategies have been employed to try and correct the underlying genetic defect. Understanding of mitochondrial biochemistry and genetics has important implications for investigation of suspected mitochondrial disease.\(^14\) at present the management of mitochondrial disease is largely supportive and aimed at identifying, preventing and treating complications wherever possible. Pharmacological treatments have been used with varying degrees of success. The aim is to reduce the proportion of mutated mtDNA to sub threshold levels. Making a specific genetic diagnosis is helpful in various ways. It allows a comparison of that individual with other patients providing some guide to prognosis and highlighting complications that may evolve over time. Retrospective studies suggest that measuring the percentage level of mutated mtDNA in the mother will provide some guidance. This could be achieved by adding more wild type mtDNA or by removing mutated mtDNA.\(^15\)\(^16\)

Addition of wild type of mtDNA is done by process called as gene shifting which involves moving of wild type mitochondrial genomes from one compartment to another. For example the precursor satellite cells present in skeletal muscle proliferate and fuse with the juxtapositionary mature skeletal fibres when the cell is subjected to stress and exercise.\(^16\) In some patients with mtDNA myopathy, the percentage of mutated mtDNA in satellite cells is lower than the level in affected skeletal muscle. Induction of satellite cell proliferation is done by injecting a toxin into muscle (such as bupivacaine) or by exercising the muscle. These techniques have been shown to deliver wild type mtDNA from the satellite cell compartments into mature muscle fibres and reduce the proportion of mutated mtDNA within affected tissues and to correct the biochemical defect. Although it showed that exercise also improves the strength and stamina of patients with mtDNA myopathy, but it may also increase the amount of mutated mtDNA in the muscle leading to short term improvements that may be detrimental in longer terms.\(^16\)\(^17\)

Strategies that have been employed to remove mutated mtDNA is by developing synthetic molecules that bind to mutated mtDNA molecules and prevent them from replicating but allowing wild type mtDNA replication to continue unimpeded. Although this approach worked in vitro and it appears that the anti-genomic molecules can be delivered into mitochondria but it has not been possible to influence the level of heteroplasmy in living cells. An alternative approach is to use drugs that select against mutated mtDNA in dividing cells allowing wild type mtDNA levels to increase.\(^16\)\(^17\) Looking at the drawbacks the best strategy is to remove all mutated mtDNA at early stage in development by nuclear transfer. By removing the nucleus from an affected zygote with mtDNA mutation and inserting it into a healthy enucleated donor with normal mtDNA it should be possible to form healthy offspring that do not harbour the mtDNA defect thereby preventing the disease in that individual and also preventing further transmission of the disease.\(^16\)

Role of tumour suppressor gene SDH mutation in tumour formation

UK researchers based at the University of Glasgow’s Beaton institute for cancer research have found out how the excessive buildup of simple metabolic molecules in mitochondria can trigger a sequence of events that lead to tumour growth. The discovery increases the understanding of the molecular basis of the several types of cancer which is crucial for the development of new ways to prevent, diagnose, and treat the disease. The defect in the genes that code for the mitochondria’s energy generating machinery is tumour suppressor which can lead to cancer. But still now it is unclear as to how mutations in these genes resulted in the disease.\(^18\) Emphasis has been given to the role of tumour suppressor gene called SDH which encodes for a molecule called succinate dehydrogenase.
Mitochondrial DNA (mtDNA) mutations within ND2 gene of histological normal parotid salivary gland tissue of smokers has been demonstrated as molecular biomarkers for smoking induced mt DNA damage by Lewis et al. According to them mtDNA mutations are present in oncocyes within Warthin's tumour. In addition, Warthin's tumours contain morphologically abnormal and respiratory deficient mitochondria. Certain mtDNA mutations within the ND2 gene were elevated in smokers and were a potential biomarker for smoking induced mtDNA damage prior to histological changes. The discovery of A:T to G:C and G:C to A:T transition mutations at nt 4767 and 4853 can be considered indicative of oxidative damage to the mitochondrial genome. Oral squamous cell carcinoma cells are strongly related to cigarette smoking and therefore PCR and direct sequencing was used to establish whether mtDNA mutations were also present in oral SCC which could be used as additional biomarkers for smoking associated DNA damage.

Apart from mutations in the ND2 gene, the mitochondrial D-loop was also analysed. Three mutation hotspot were observed in D-loop at nt 146, 152, 186, two of which (nt 146, 152) have also been implicated in oesophageal SCC, another smoking related cancer. The mutation hotspot observed in nt 186 has not been previously been reported in other tumours. Furthermore it is shown that the mutations previously reported within the ND2 gene in normal parotid tissue of smokers were not evident in these samples, but that a mutation hotspot occurs at nucleotide 4917 in oral SCC. It is concluded that the mtDNA mutations hotspot found in this study in particular nt186 are potential biomarkers for oral SCC.

D-loop mutations should be considered as a cancer biomarker that may be useful for the early detection of HNSCC in individuals at risk of this cancer. The presence of these mutations in saliva and serum of tobacco and alcohol consumers’ should be investigated in further studies in order to evaluate their relevance in the screening of these cancers in association with other tumour specific molecular alterations.

MtDNA as a marker in histopathologic grading in premalignant and malignant head and neck lesions

Mitochondria are highly susceptible to oxidative damage and mitochondrial function decreases with oxidative damage, overall mitochondrial DNA content increases to compensate for general mitochondrial dysfunction. Head and neck squamous cell carcinomas arise through premalignant intermediates and may be merely morphologic manifestations of accumulated genetic alterations. In keeping with this molecular tumour progression model, studies have shown that mtDNA increases according to histopathology grade, a phenomenon that may be a feedback mechanism that compensates for a generalised decline in respiratory chain function. Therefore, high mtDNA content may be another marker of genetic alteration, a measure of relative DNA injury, and a surrogate measure of histopathology grade.

Role of mitochondrial dysfunction in cancer therapy

Mitochondrial dysfunction also has important implications in cancer therapy. This has been demonstrated by measuring the cell survival of a cervical tumour cell lines (with parental mitochondrial function i.e. Rho+) and its derivative isogenic cell line that completely lacked mtDNA (with dysfunctional mitochondria i.e. Rho) after the exposure to a variety of anticancer agents. It was found that mitochondrial dysfunction leads to increased cell survival after exposure to cancer therapeutic agents such as Adriamycin and porphyrin catalysed photo toxicity. By contrast no measurable difference was found in the cell survival of Rho+ and Rho0 cells to high doses of ionizing radiation. These results underscore the importance of mitochondrial genome in development of therapeutic drugs.

CONCLUSION

The involvement and important role of mitochondria in cancer transformation is underlined by several mechanisms, including the mutagenesis of mtDNA. Each aspect of the association between mitochondria and cancer can be exploited for its usefulness in anticancer therapy. Traditional chemotherapies aimed at DNA replication in actively dividing cells have achieved only limited success in the treatment of cancer largely because of their lack of specificity for cells of tumorigenic origin. It is important therefore to search for novel cellular targets that are sufficiently different between normal cells and cancer cells so as to provide a basis for selective cytoxicity. As this review suggests the mitochondrion is one such target.

REFERENCES


