Association of leukemia and mitochondrial diseases—A review

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ABSTRACT

Mitochondria play an important role in various metabolic pathways like oxidative phosphorylation free radical generation and apoptosis. Defects in mitochondrial function are responsible for a number of heterogenous clinical presentations along with development and progression of cancer. Decrease in cellular energy (ATP) production because of impaired oxidative phosphorylation is the most important cause for these underlying disorders. The present review article aims to provide current understanding of mitochondrial genetics and biology and relates the mt-DNA alterations in leukemia patients.

Keywords: Leukemia, mitochondrial DNA, oxidative phosphorylation

Introduction

Mitochondria are complex cellular organelles regulating various metabolic processes including urea cycle, gluconeogenesis, fatty acid oxidation, Krebs cycle, oxidative phosphorylation (OXPHOS) in the electron transport chain (ETC), and many others.[1]

Mitochondria are a unique organelle containing mitochondrial DNA (mt-DNA). The human mitochondrial genome is 16,569 base pair (bp) in length, with double-stranded circular DNA molecules containing 37 genes. The role of mitochondria in various human malignancies including leukemia has been long proposed and explored with varying outcomes. This genome also includes a noncoding displacement region (D-loop) that consists of 1122 bp (16,024–577 bp) of mitochondrial DNA. It acts as a promoter region for both the heavy and light strands of the mt-DNA and contains essential transcription and replication elements. The D-loop is a hot spot region for mt-DNA variations. Genetic variability in the D-loop region has been suggested to affect the function of the respiration chain, leading to high reactive oxygen species (ROS) levels and instability in the mt-DNA. Thus, it is not surprising that mitochondrial dysfunction has been linked to human degenerative diseases and cancers, including leukemia.[2]

Mitochondrial diseases refer to the group of genetic disorders that are mainly characterized by dysfunctional mitochondria and primarily affects the major cellular energy pathways like the ETC and therefore the production of adenosine triphosphate (ATP).[3]

However, with recent advances in molecular and genetic testing, mitochondrial dysfunctions are being recognized to be even more complex and thus categorized into 2 groups as a primary mitochondrial disease (PMD) and secondary mitochondrial dysfunction (SMD).[4]

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PMDs are associated with the germline mutations occurring in mitochondrial DNA (mt-DNA) and/or nuclear DNA (nuDNA) genes encoding ETC proteins. PMD occurs not only because of defective genes encoding ETC proteins, but also because of germline mutations in other nuDNA genes that affect OXPHOS function by impacting the production of the complex machinery needed for the ETC to perform optimally. In contrast, SMD can accompany many pathologic and the environmental factors and do not involve the genes encoding ETC proteins and accompanies many hereditary nonmitochondrial diseases.[5,6]

Mitochondrial DNA Heteroplasmy and Genetics

The mitochondrial genome is a compact, double-stranded, circular, 16,569 base pairs long molecule, consisting of 37 genes, including 13 polypeptides, 22 transfer RNA (tRNA), and 2 ribosomal RNA (rRNA) genes.[7]

The majority of proteins required for mitochondrial function are nuclear-encoded whereas the mt-DNA differs from nuDNA in various aspects (a) presence of multiple copies in a single cell thus both wild-type and mutant mt-DNA molecules can coexist, a condition known as heteroplasmy;[8,9] (b) maternal mode of inheritance; and (c) the absence of introns.[10]

The mutation rate of mt-DNA is much higher than nuDNA because of very few noncoding sequences and most importantly, its presence in the vicinity of high ROS rich environment (the OXPHOS system) in the inner mitochondrial membrane. The pathophysiology of mitochondrial diseases is complex and involves genetic mutations in mitochondrial DNA (mt-DNA) and nuDNA. This complex genetics means that mitochondrial diseases can have any pattern of inheritance, including autosomal and Xlinked inheritance for nuDNA mutations and maternal inheritance for mt-DNA mutations.[11] Most of the pathogenic mutations responsible for the expression of the disease phenotype are present in the heteroplasmic condition.[12]

There are a few exceptions like Leber hereditary optic neuropathy, where mutations are mostly present in the homoplasmic condition in both symptomatic and asymptomatic individuals. For causing any biochemical defect in the cell, and for the phenotypic expression of diseases, the percentage of mutant mt-DNA must exceed the tissue-specific critical threshold level. The difference in the level of heteroplasmy determines the clinical variability of diseases in different patients and in individuals within the same family. The mitochondrial diseases primarily affect the pathway of ATP generation that is OXPHOS but various cycles running inside the mitochondria like Krebs cycle and folate cycle, lack of enzyme intermediates, and accumulation of toxic substances might also have an impact on these diseases.

The transmission of disease-causing mt-DNA from heteroplasmic mother to the offspring shows a high degree of genetic and phenotypic variability between siblings and is well explained by a phenomenon known as "mitochondrial genetic bottleneck." The comparison of the heteroplasmic level in offspring with those of oocytes at different stages of development has revealed that the bottleneck occurs in the early stages of oogenesis. During this process, there is a significant reduction in the number of mt-DNA molecules (the bottleneck). Following fertilization, heteroplasmic mt-DNA mutation present in the oocyte segregates to either of the 2 daughter cells. This is a random process, which generates variability in the transmitted mutation load to the offspring thus making it possible for an unaffected heteroplasmic female individual to have children, who are either unaffected or mildly to severely affected.[13]

The majority of pathogenetic mitochondrial DNA (mt-DNA) mutations are heteroplasmic, with a mixture of mutated and wild-type mt-DNA inside an individual cell. High levels of heteroplasmy refer to cells with high levels of mutant mt-DNA and low levels of wild-type mt-DNA, whereas low levels of heteroplasmy refer to cells with low levels of mutant mt-DNA but high levels of wild-type mt-DNA. Studies in single cells from patients with mitochondrial diseases have shown that the level of mutated and wild-type mt-DNA is very important for determining the cellular phenotype. For example, cells become respiratory deficient if they contain high levels of mutated mt-DNA and low levels of wild-type mt-DNA (i.e. high levels of heteroplasmy). The threshold at which this deficiency occurs depends on the precise mutation and the cell type. Typically, high percentage levels of mutated mt-DNA (>50%) are required to result in cellular defects, but some mt-DNA mutations only generate a deficiency if present at very high levels (typically mttRNA mutations) and others (such as single, large-scale mt-DNA deletions) produce a deficiency when there is ~60% deleted mt-DNA.[14]

Mitochondrial physiology and ROS

The main physiological function of mitochondria is the production of ATP by OXPHOS and the essential metabolites to accomplish the bioenergetics and biosynthetic demands of normal and cancer cells. Three major and important aspects of OXPHOS involved in mitochondrial pathogenesis are (i) energy production, (ii) ROS production, and (iii) apoptosis. Carbon fuels are utilized by mitochondria to produce ATP. The sources of carbon pools are pyruvate generated from glycolysis, amino acids like glutamine, and fatty acids. The Krebs cycle in the mitochondrial matrix uses these carbon fuels to generate the reducing equivalents nicotinamide adenine dinucleotide hydrogen (NADH) and flavin adenine dinucleotide hydrogen ($FADH_2$), which subsequently pass their electrons to the ETC. The transfer of electrons is coupled to the efflux of hydrogen ions from the matrix to the intermembrane space by mitochondrial complexes I, III, and IV. The 2 main components generated by the proton-motive force are the membrane potential that occurs from the net movement of positive charges across the inner mitochondrial membrane and the pH gradient. Most of the energy largely supplied by the membrane potential is reserved in the gradient. Complex V uses this proton motive force to generate ATP from adenosine diphosphate and P_i.

Thus, the mitochondrial membrane potential is crucial to maintain the physiological function of the ETC in order to produce ATP. A significant loss of mitochondrial membrane potential renders cells depleted of energy with subsequent death. Besides, the generation of NADH and FADH$_2$, the Krebs cycle also produces intermediates that can fuel into multiple biosynthetic ways to synthesize glucose, amino acids, lipids, heme, and nucleotides. Hence, mitochondria serve as a center for both catabolic and anabolic metabolism. ROS is a byproduct of the mitochondrial ETC. Free radicals are mainly generated in the inner mitochondrial membrane during the process of OXPHOS. The important role of ROS has been implicated in the regulation of growth and survival of cancer and structural damages to cells along with lipids, membranes, and DNA.[15]

Leukemia and Mitochondrial Diseases

Inadequate production of ATP or energy is considered as the mainstay for most of the mitochondrial pathologies, which result in multisystemic disorders. Till date, >300 mutations have been reported that are known to cause a spectrum of mitochondrial diseases.

Mutations in genes encoding mitochondrial proteins have been linked to cancer. For example, defects in succinate dehydrogenase (SDH), also known as complex II of the ETC, may lead to increased oxygen production and increased sensitivity to oxidative stress.

Leukemias are classified into 4 main categories, based on the type of white blood cell affected (lymphoid vs. myeloid) and characteristics of the disease (acute vs. chronic):

Based on the characteristics of disease classified as:
(a) Acute leukemia
(b) Chronic leukemia.

Based on types of white cell counts affected classified as:
A) Myelogenous leukemia
B) Lymphocytic leukemia.

Acute leukemias

Acute leukemia develops from early cells, called "blasts." Blasts are young cells that divide frequently. In acute leukemia cells, these cells do not stop dividing as their normal counterparts do.

Chronic leukemias

In chronic leukemia, the leukemia cells come from mature, abnormal cells. The cells thrive for too long and accumulate. The cells grow slowly.

Myelogenous leukemia

Myelogenous leukemia develops from myeloid cells. The disease can either be chronic or acute, referred to as chronic myelogenous leukemia (CML), or acute myelogenous leukemia (AML).

Lymphocytic leukemia

Lymphocytic leukemia develops from cells called lymphoblasts or lymphocytes in the blood marrow. The disease can be acute or chronic, referred to as chronic lymphocytic leukemia or acute lymphocytic leukemia (ALL).[16]

No direct cause-effect relationship has been established between mitochondrial mutation and hematological malignancy. Nonetheless, the progression of hematological malignancy is characterized by mt-DNA mutations with a low heteroplasmic mutation load in early stages of myelodysplastic disease syndrome (MDS); however, advanced stages of MDS are accompanied by a dramatic increase in the mt-DNA mutations, reaching homoplasmy with AML.

AML is a devastating and heterogenous hematological malignancy characterized by the uncontrolled proliferation of undifferentiated myeloid progenitor cells known as blasts. The altered metabolic state of AML cells and the role of the mitochondria has been shown to contribute to its pathogenesis. Mutations in the mitochondrial enzyme isocitrate dehydrogenase-2 have been identified as oncogenic drivers of AML. They convert alpha-ketoglutarate into the R-enantiomer of 2-hydroxyglutarate, which is associated with DNA hypermethylation, epigenetic changes, reduced ATP synthase activity, and overall reduced mitochondrial energy metabolism.[17]

Alterations in mt-DNA in hematologic malignancies were first discovered by Clayton and Vinograd[18] in 1967 through electron microscopy to examine the structure of mt-DNA in leukemic leukocytes using cesium chloride-ethidium density centrifugation. It was concluded that alternate mt-DNA structures exist that comprised circular dimers, catenated dimers, and catenated trimers. Normal cells also showed the presence of these alternate mt-DNA structures but an unusually high percentage of them exist in leukemic cells as compared with normal controls.[18]

In a follow-up study, the leukocytes of 14 patients with acute and chronic granulocytic leukemias were examined and it was concluded that circular dimers were found in all 14 patients, but not in leukocytes from 3 healthy donors. Later following chemotherapeutic treatment the percentage of circular dimers decreased in some leukemia patients, suggesting that the severity of leukemia may be related to the presence of circular dimers.[19]

The decrease of circular mt-DNA dimers after chemotherapy also suggests a possibility that the leukemia cells containing this mt-DNA abnormality may be more vulnerable to elimination by therapeutic agents. Analysis of mt-DNA from AML cells revealed that the origins of abnormal mt-DNA structures could be traced back to the bone marrow.

A separate study in leukemia cells from patients with ALL identified mt-DNA point mutations in 11 of 30 patients.[20]

Mutations of mt-DNA have also been identified in patients with MDS. Gattermann *et al.* described mutations in cytochrome *b*, cytochrome *c* oxidases I and II, and ATPase 8 in MDS cells.[21,22]

The more recent study further confirmed the increased mutations in the mitochondrially-encoded *COX-I* and *COX-II* genes.[23]

Apoptosis and Cell Survival

Apoptosis refers to the biological process by which the cells die in a well-controlled or programmed manner and because of significant research in mitochondrial biology indicates that the mitochondria play an important role in apoptosis.[24] Various proapoptotic proteins encoded by the nuDNA like cytochrome c, apoptosis-inducing factor (AIF), endonuclease G, and Smac/Direct IAP-Binding protein with Low PI (DIABLO) reside within the mitochondria and once these protein factors are released from mitochondria, they trigger a series of biochemical events leading to activation of apoptotic signaling cascades.[25]

Because apoptosis plays a critical role in cancer development and in the cellular response to anticancer agents, the significance of mt-DNA mutations in cancer is obviously an important area of the current investigation.

Effect of Genotoxic Stress on mt-DNA and Hematological Malignancy

Genotoxic stress' has been proposed to be an early event in cell transformation, but the mechanisms for this have remained unknown. Genotoxic stress leads to ROS-catalyzed oxidation producing rogue nucleotides in DNA. However, it has been shown that accumulation of inosine triphosphate (ITP)/deoxy-inosine triphosphate (dITP) can also lead to DNA instability. Reduced ITPase activity produces accumulation of the rogue nucleotides ITP and dITP and xanthosine triphosphate, and these rogue nucleotides may potentially increase the mutational load in mitochondria, slowly leading towards loss of cellular integrity, with either of 2 outcomes: cell death (by apoptosis) or malignancy.

Chronic genotoxic stress particularly may affect mt-DNA of bone marrow cells, producing late-onset, and precancerous cell transformation that may progress to cellular constitutional imbalance and hematologic malignancy. Alterations in mt-DNA have been identified and associated with solid tumors in bladder, breast, colon, head and neck, kidney, liver, lung and stomach, and in the hematologic malignancies leukemia and lymphoma.[26]

RAC Proteins and Leukemic Stem Cells

Leukemic stem cells reside within bone marrow niches that maintain their relatively quiescent state and convey resistance to conventional treatment. RAC GTPases act as crucial mediators that convert inactive guanosine diphosphate-bound state into an active state in which they are guanosine triphosphate-bound.

RACs are activated by various signaling events such as activation of tyrosine kinase receptors, G protein-coupled receptors, and cell-to-cell contacts. These further lead to various cytoskeleton rearrangements. The RAC family consists of RAC1, RAC2, and RAC3, which show tissue-specific expression distribution. RAC1 and RAC2 are both expressed in the hematopoietic system and play an important role in hematopoiesis.

RAC activity has also been implicated in the disease initiation and maintenance in various murine leukemia models, including BCR-ABL and MLL-AF9-driven transformation. In accordance with that, inhibition of RAC activity has been proven a successful strategy to target primary human AML and CML cells *in vitro* and *in vivo*. Despite the growing understanding of their role in murine models, our insight into molecular mechanisms by which RAC proteins contribute to the development of human leukemia remains limited.[27]

The mitochondrial integrity and mitochondrial membrane potential in BCR-ABL-transformed human hematopoietic stem/progenitor cells (HSPCs) depend on RAC2. RAC2 interacts with a set of mitochondrial proteins including SAM50 and Metaxin1 and downregulates SAM50 thus impairing the proliferation and replicating capacity of BCR-ABL-expressing cells, again associated with a decreased mitochondrial membrane potential.

RAC2 is necessary for the proper mitochondrial function in BCR-ABL-expressing HSPCs and that inhibiting RAC2 could aid in the targeting of those primitive leukemic cells.[27]

Implications for clinical practice

The primary care physician is the first contact of a patient for the consultation of illness. Early diagnosis and a multidisciplinary approach are key components of managing the various types of leukemias. Increased awareness and research in this field have facilitated the identification of risk factors and causation pathways. Certain therapies have shown a promise that needs evaluation in prospective clinical trials. Mitochondrial metabolism is a key factor for cancer therapy. Leukemia is a disease that has many mitochondrial metabolic dependencies. However, the nature of metabolic networks that enable abhorrent cell proliferation, cell-cell communication, evasion of apoptosis, and drug resistance remains poorly understood. The therapeutic compounds used against the target mitochondrial DNA further limits the cancer growth. In order to best target the leukemic cells, treatment strategy must incorporate the use of mitochondrial targeting agents that may enable a chance at a cure for currently incurable cancer.[28] A new class of anticancer drug named mitocans has been developed that targets mitochondria at distinct crucial points to promote their dysfunction and subsequent cell death.[29,30]

Conclusions

Chronic oxidative stress in cancer cells and mutations in mt-DNA are responsible for the various malignancies including different

types of leukemia. Mutations in mt-DNA and alterations in respiratory activities lead to abnormal expression of mt-DNA-encoded proteins that are responsible for solid tumors and hematological malignancies. Future molecular and genetic studies should be carried out to relate mt-DNA mutations in cancer development that contribute to the prevention and treatment of leukemia.

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Conflicts of interest

There are no conflicts of interest.

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