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Formation of Mitochondrial Genome Concatemers as an Alternative Mechanism Promoting Oncogenic Transformation of Lymphoid Cells

Felipe Bedoya, Ph.D. and Peter G. Medveczky, M.D.

Department of Molecular Medicine, School of Basic Biomedical Sciences College of Medicine, University of South Florida, Tampa, FL.

Summary

We have previously shown that AIDS-associated lymphomas and lymphoma cell lines contain mitochondrial genome concatemers not present in normal T-lymphocytes. Since cellular homeostasis and energy production rely heavily on mitochondrial DNA (mtDNA) stability, mutations in the mtDNA have long been linked to the development of various types of cancers. In most of the cases, however, neoplastically transformed cells harbor non-mutated mtDNA. Herein, we propose an alternative mechanism that shows how the formation of mitochondrial genome concatemers may promote oncogenic transformation of normal lymphoid progenitor cells when no mtDNA mutations or chromosomal aberrations are present. We detected high reactive oxygen species (ROS) levels in the lymphoma samples tested despite no identification of putative mutations in the coding mtDNA. We propose that the formation of atypical mtDNA configurations (i.e. dimers and concatemers) interferes with normal mitochondrial function. Unstable mitochondria lead to abnormal assembly and dysfunction of the oxidative phosphorylation (OXPHOS) complexes, eventually leading to oxidative stress from elevated production of intracellular ROS. ROS have been reported to activate transcription factors associated with cellular proliferation and apoptosis inhibition. Therefore, we hypothesize that formation of mitochondrial genome concatemers can augment endogenous ROS levels capable of promoting oncogenic transformation of normal lymphoid progenitor cells.

Introduction

Mitochondria are subcellular organelles central to energy production harnessed mainly through the OXPHOS system (1). They are assembled from the coordinate expression of nuclear DNA and mtDNA genes (2). In humans, the mtDNA encodes 37 genes essential for OXPHOS (3). It has been long known that mitochondrial function and OXPHOS are dysregulated in many types of cancers, and that mtDNA-derived transcripts are upregulated (4-7). However, the mechanism by which defects in OXPHOS and increased mitochondrial gene expression occur in cancer cells remains poorly understood.

Data from published and ongoing research in our laboratory show that AIDS-associated lymphoma samples and transformed cells of lymphoid origin contain mitochondrial genome

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*Address correspondence to: Peter G. Medveczky, M.D., Department of Molecular Medicine, College of Medicine, University of South Florida, 12901 Bruce B. Downs Blvd. MDC 7, Tampa, FL 33612, USA. Fax: 813-396-9188; pmedvecz@health.usf.edu.

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concatemers that are not present in normal lymphocytes (8). Formation of such atypical mtDNA structures may be a direct mechanism to promote neoplastic transformation of lymphoid progenitors that has not been addressed before. There is increasing evidence that mtDNA defects are associated with various types of cancers (9,10). Mutations in the mtDNA lead to deficient OXPHOS resulting in high intracellular levels of ROS (11,12). Increased ROS production has been proposed to be an important factor in tumorigenesis in association with inactivation of tumor suppressor factors such as *p16ink4a* and p53 (13). Further, ROS act as secondary messengers that can interact with cell division regulatory factors such as AP-1, NF- κ B and APE/ref-1 (14,15). However, so far, no compelling mechanism has been put forward to elucidate how elevated ROS levels may occur in transformed cells (e.g. in leukemia or lymphoma), when no mutations in the mtDNA or in the nuclear OXPHOS-coding genes are identified. We propose an alternative model that addresses this issue.

Hypothesis

We hypothesize that the formation of atypical configurations of the mitochondrial genome (dimers or concatemers) promotes oncogenic transformation of a partially differentiated population of lymphoid cells. We propose that during lymphomagenesis, defects in factors associated with maintenance or processing of the mtDNA (e.g. DNAPol- γ or mtDNA binding factors) in lymphoid progenitor cells lead to the formation of mitochondrial genome concatemers affecting normal mitochondrial gene expression, which results in improper assembly of the OXPHOS system. Defects in OXPHOS are known to increase the production of mitochondrion-associated ROS (16,17). Elevated ROS have been reported to stimulate factors associated with cellular proliferation and apoptosis inhibition (14,15), that ultimately result in a rapid and sustained expansion of a lymphoid precursor population which correlates with the pathophysiological phenotype observed in individuals with lymphoma (Figure 1).

Discussion

Mitochondrial dysfunction has long been associated with cancer (18-20). Because mtDNA is essential for OXPHOS assembly and function, mtDNA mutations have been reported in different types of cancer by several groups (11,21,22). These mutations cause oncogenic transformation, metastasis and aging by presumably increasing the production of intracellular ROS, characteristic of deficient OXPHOS (23). Our data show that all of the tumor-suspension samples (obtained from patients with AIDS-associated lymphoma) and lymphoma cell lines examined contained mitochondrial genome concatemers (8). Formation of mtDNA concatemers may represent an alternative mechanism that explains the elevated production of intracellular ROS observed in cancerous cells for which no mtDNA mutations are identified.

Herein, we propose that defects in factors associated with mtDNA maintenance are associated with the formation of mitochondrial genome concatemers, which can interfere with normal mtDNA transcription, affecting mtDNA gene expression and OXPHOS assembly. Higher ROS levels derived from defective OXPHOS machinery can promote the selection and expansion of a sub-population of lymphoid progenitor cells resulting ultimately in the development of lymphoma.

To test our hypothesis that mitochondrial genome concatemers promote cellular transformation of lymphoid cells, transmitochondrial cybrids will be made containing either dimeric, concatemeric or wild-type mtDNA, and similar nuclear backgrounds as in normal lymphocytes (24). Flow cytometry assays using dihydroethidium (DHE) and Annexin-V staining will be performed to determine endogenous ROS production and cellular apoptosis, respectively. Colony formation on methyl cellulose culture medium will be used to determine number of

colonies of transmitochondrial cybrids. The number of colonies in culture media indicates the enrichment of cybrid populations within a specific configuration of mtDNA.

We have also been successful in the production of human T-lymphocytic cell lines by immortalization with Herpesvirus *saimiri* (25,26). These cell lines will be used to determine whether mitochondrial genome concatemers are formed as a consequence of cellular transformation. The presence of mtDNA concatemers will be indicative that cellular transformation triggers the formation of this type of mtDNA suprastructures and not vice versa. If this is the case, we will perform an increasing time-point analysis of mtDNA configuration after viral infection to determine how cellular transformation, mtDNA concatemer formation and ROS production correlate in *H.saimiri*-immortalized lymphocytes.

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figure 1. Schematic diagram of proposed mechanism of induction of oncogenic transformation by formation and maintenance of mitochondrial genome concatemers

Cells containing mitochondrial genome concatemers show increased mtDNA levels and higher ROS production (8). Neoplastically transformed cells upregulate mtDNA gene expression (24), generally resulting in a defective (uncoupled) OXPHOS system (11,23), which produces higher ROS levels. ROS can inhibit tumor suppressor factors (p16ink4a, p53) and induce transcription factor activation (AP-1, NF-B, APE), which results in cellular proliferation (27, 28).