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# Inter-genomic cross talk between mitochondria and the nucleus plays an important role in tumorigenesis

Keshav K. Singh[a,*], Mariola Kulawiec[a], Ivan Still[a], Mohamed M. Desouki[a], Joseph Geradts[b], Sei-Ichi Matsui[a]

[a]*Department of Cancer Genetics, Cell and Virus Building, Room 247, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA*
[b]*Department of Pathology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA*

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**Abstract**

Mitochondrial dysfunction is a hallmark of cancer cells. Consistent with this phenotype mutations in mitochondrial genome have been reported in all cancers examined to date. However, it is not clear whether mitochondrial genomic status in human cells affects nuclear genome stability and whether proteins involved in inter-genomic cross talk are involved in tumorigenesis. Using cell culture model and cybrid cell technology, we provide evidence that mitochondrial genetic status impacts nuclear genome stability in human cells. In particular our studies demonstrate 1) that depletion of mitochondrial genome ($rho^0$) leads to chromosomal instability (CIN) reported to be present in variety of human tumors and 2) $rho^0$ cells show transformed phenotype. Our study also demonstrates that mitochondrial genetic status plays a key role in regulation of a multifunctional protein APE1 (also known as Ref1 or HAP1) involved in transcription and DNA repair in the nucleus and the mitochondria. Interestingly we found that altered expression of APE1 in $rho^0$ cells and tumorigenic phenotype can be reversed by exogenous transfer of wild type mitochondria in $rho^0$ cells. Furthermore, we demonstrate that APE1 expression is altered in variety of primary tumors. Taken together, these studies suggest that inter-genomic cross talk between mitochondria and the nucleus plays an important role in tumorigenesis and that APE1 mediates this process.
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*Keywords:* Mitochondria; Inter-genomic; Nucleus; Chromosomal instability; Tumorigenesis; APE1

## 1. Introduction

Mitochondria perform multiple essential cellular functions (Modica-Napolitano and Singh, 2002, 2004). Although the mitochondrial and nuclear genomes are physically distinct, there is a high degree of functional interdependence between the two genomes. Of the 82 structural subunits that make up the oxidative phosphorylation system in the mitochondria, the mitochondrial genome encodes 13 subunits (see below) and the rest of the subunits are encoded by the nuclear genome. Correct mitochondrial functions depend on an orchestrated cross talk between the nuclear and mitochondrial genomes.

The mitochondrial genome is a small 16.6 unit molecule that encodes 13 subunits of the respiratory chain complexes, 22 tRNAs and 2 ribosomal RNAs. Mammalian cells typically contain $10^3$–$10^4$ copies of mitochondrial DNA (mtDNA). Unlike nuclear DNA, mammalian mtDNA contains no introns, has no protective histones and is exposed to deleterious reactive oxygen species generated by oxidative phosphorylation. In addition, replication of mtDNA might be error prone. The accumulation of mutations in mtDNA is approximately tenfold greater than that in nuclear DNA (Grossman and Shoubridge, 1996, Johns, 1995, Penta et al., 2001). Mutations in mtDNA have been reported in mito-

*Abbreviations:* mtDNA, Mitochondrial DNA; CIN, Chromosomal instability; SKY, Spectral karyotyping; IHC, Immunohistochemistry; APE1, Apurinic/apyrimidinic endonuclease 1; TARP, Tissue arrays research program.

* Corresponding author. Tel.: +1 716 845 8017; fax: +1 716 845 1047.
*E-mail address:* keshav.singh@roswellpark.org (K.K. Singh).

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chondrial diseases and in a variety of cancers, including ovarian, thyroid, salivary, kidney, liver, lung, colon, gastric, brain, bladder, head and neck, and breast cancers, and leukemia (reviewed in Penta et al., 2001 and in Modica-Napolitano and Singh, 2002, 2004). Deletions, point mutations, insertions and duplications have been detected throughout the genome, and certain mutations in mtDNA are associated with specific cancers. For example, a 40 bp insertion localized in the COX I gene appears to be specific for renal cell oncocytomas (Welter et al., 1989), and a deletion mutation resulting in the loss of mtDNA within NADH dehydrogenase subunit III is a phenotype associated with renal cell carcinoma (Selvanayagam and Rajaraman, 1996). The D-loop region appears particularly susceptible to DNA mutations. Both hepatocellular carcinoma (Nomoto et al., 2002) and breast cancer (Parrella et al., 2001) are associated with certain deletion/insertion mutations in the C-tract, a hotspot and a potential replication start site within the D-loop of the mitochondrial genome. These studies suggest that mutations in mtDNA is a common feature of cancer cells. Unfortunately, to date it is not clear whether mutations in the mitochondrial genome affect nuclear genome stability and whether inter-genomic cross talk is involved in tumorigenesis.

Our studies conducted in yeast *Saccharomyces cerevisiae* model system suggest that mutations in the mitochondrial genome cause nuclear genomic instability (Rasmussen et al., 2003). We also identified that nuclear genome stability was mediated by Rev1p dependent error prone repair pathway (Rasmussen et al., 2003). Using a human cell culture model our recent study provided evidence that mitochondrial genomic dysfunction leads to impaired oxidative DNA repair in the nucleus (Delsite et al., 2003). Our study also revealed that mitochondrial dysfunction leads to elevated expression of MnSOD which causes resistance to apoptosis (Park et al. 2004). In the present paper, we analyzed the importance of the mitochondrial genome in chromosomal instability (CIN) and its role in tumorigenesis. This study reveal that depletion of mitochondrial genome ($rho^0$) leads to 1) chromosomal instability 2) altered expression of APE1, a DNA repair gene and 3) observed instability in nuclear genome plays a critical role in tumorigenesis. Furthermore, our study for the first time suggest that the tumorigenesis phenotype and the altered expression of APE1 can be reversed by transfer of exogenous wild type mitochondrial genome into $rho^0$ cells. These studies suggest that inter-genomic cross talk between mitochondria and the nucleus plays an important role in tumorigenesis.

## 2. Method

### 2.1. Cell cultures

Human osteosarcoma cell line 143B and 143B $rho^0$ and derived cybrid cell lines were a kind gift of G. Manfredi (Columbia University, New York City). The cybrid cells were isolated by transfer of platelets from a normal volunteer into human mtDNA depleted ($rho^0$) 143B cells (Gajewski et al., 2003). Cell lines were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (Sigma, St. Louis, MO), 100 units/ ml penicillin and 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA) and 50 μg/ml uridine (Sigma, St. Louis, MO). All cell lines were maintained in a humidified 5% $CO_2$ atmosphere at 37 °C (Singh et al., 1999).

### 2.2. Spectral karyotyping analysis (SKY)

After mitotic arrest for 2 h with Colcemid, cells were harvested and treated with hypotonic solution according to the standard protocol. Chromosome slides were prepared using air-drying methods. After sequential digestion with RNAse and pepsin according to the procedure recommended by Applied Spectral Imaging, Inc. (ASI: Carlsbad, CA), the chromosomes were denatured in 70% formamide and hybridized with human SKY paint probes tagged with various nucleotide analogues (i.e., a mixture of individual chromosome DNAs prepared by flow-sorting and PCR amplification). The multiple fluorescence color images of chromosomes generated by Rhodamine, Texas-Red, Cy5, FITC and Cy5.5 were captured using a Nikon microscope equipped with a Spectral cube and Interferometer module and analyzed using SKY View software (version 1.62). Chromosome number and chromosomal rearrangements or alterations including simple balanced translocation or unbalanced (or non-reciprocal) translocation, deletion and duplication, were analyzed to determine the lineage of individual knock-out cell lines, compared to the original wild type counterpart (Matsui et al., 2002, 2003).

### 2.3. Assay for changes in tumorigenic phenotype

In vitro assays for changes in tumorigenic phenotype was assayed as previously described in Lauffart et al. (2003). For soft agar assays, cells were seeded at 5000 cells per well in 0.35% agar in 12 well plates. Colonies 50 μm or greater were scored as positive after 2 weeks of growth. Invasion assay was performed using the Matrigel Invasion Chamber assay (Becton Dickinson, Bedford, MA, USA) according to manufacturer's instructions. Cells ($5 \times 10^4$) in culture medium lacking serum were introduced to the upper side of the invasion chamber. For the purpose of this experiment, culture medium containing 10% serum was used as the chemoattractant. After 24 h at 37 °C, the membrane was removed and invading cells on the lower surface fixed and stained. Cells were visualized by microscopy and counted. For all assays, each data point was performed in triplicate and differences between cell lines analyzed using one way ANOVA followed by Dunnett's Multiple Comparison Post Test (Graphpad Prism Version 3.0, Graphpad Prism Software Inc.).

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