Identification of Double Strand Break Repair Proteins in Mammalian Mitochondria and Their Role in Genome Integrity

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INTRODUCTION: DNA is constantly exposed to damaging agents from both endogenous and exogenous sources. DNA double-strand breaks (DSBs) are highly toxic lesions that can drive genetic instability and cell death. All known organisms use two major pathways for repairing DSBs: homologous recombination (HR) and nonhomologous DNA end joining (NHEJ). These pathways have been well characterized in the nucleus, but there is only genetic evidence that they exist in mitochondria.

MATERIAL AND METHODS: Mitochondria were isolated from human embryonic kidney cells (HEK293T) by differential centrifugation followed by Percoll gradient. Mitochondrial localization of DSBR proteins was assessed by Western blot. Expression of proteins of interest was diminished via transfection of specific short hairpin RNA (shRNA) and checked by Western blot. mtDNA copy number and deletions was measured by Real Time Quantitative PCR (qPCR).

DISCUSSION AND RESULTS: We tested mitochondrial localization of essential proteins of the HR (Rad51, Rad52) and NHEJ (Ku70/86, DNA-PKCs and ATM) pathways. We found similar isoforms of Ku70, Rad51, ATM and DNA-PKCs are found in nucleus and mitochondria. We detected two isoforms of Rad52 in mitochondrial extracts, while four isoforms were detected in nuclear extracts. A smaller isoform of Ku80 was detected in mitochondrial extracts (45KDa), distinct from the nuclear isoform (80KDa) in the N-Terminus region. Immunoprecipitation experiments confirmed distinct Ku heterodimers in nucleus and mitochondria, 70/86 and 70/40 respectively. Cells with decreased expression of Rad52 and Ku70 were generated. Rad52 knockdown cells showed decreased mtDNA copy number and increased deletions in the mitochondrial genome.

CONCLUSION: All DSBR proteins investigated here were found in mammalian mitochondrial extracts, suggesting that these pathways operate in this compartment. Decreased Rad52 expression resulted in loss of mtDNA copy number, indicating that his protein is necessary to mitochondria genome maintenance. Using in vitro approaches, we are now investigating how Rad52 participates in mtDNA repair.

Keys Word: mitochondria, DNA repair

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