Levels of Double Strand-Breaks Repair Proteins change in mitochondria in response to Oxidative Stress

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INTRODUCTION: DNA double-strand breaks (DSBs) are hazardous DNA lesions that can lead to cell death or genomic instability. They can be generated by ionizing radiation (IR), which generates DSBs directly and indirectly via production of reactive oxygen species (ROS). In the nucleus, cells utilize two distinct pathways to repair DSBs, homologous recombination (HRR) and non-homologous end joining repair (NHEJ), but little is known about how these are repaired in the mtDNA. As the the HRR protein Rad51 was found to localize to mitochondria after exposure to ROS and IR, we investigate whether other DSB repair proteins follow a similar pattern.

MATERIAL AND METHODS: DSBs were induced in HEK293T cells by two methods: 100 µM of hydrogen peroxide (H$_2$O$_2$) for 30 minutes, followed by 0, 6 and 24 hours recovery, or 5 Gy of IR followed by 1, 3, 6 and 24 hours recovery. Mitochondria were isolated at the indicated times using differential centrifugation followed by Percoll gradient. The levels of Ku70/86 or Rad52 were determined by quantitative Western Blotting; band intensities were analyzed using ImageJ software and normalized by levels of VDAC1 or COX4 proteins.

RESULTS AND DISCUSSION: Two isoforms of Rad52 are found in mitochondria; after both treatments, the 43 kDa, but not the 50 kDa isoform, showed significant relocalization to mitochondria. On the other hand, Ku70 levels decreased in mitochondria at initial times after exposure (0 and 1 hour) but returned to basal levels afterwards. The levels of Ku86 subunit (found in mitochondria as a 45 kDa isoform) are being analyzed.

CONCLUSIONS: The mitochondrial relocalization of Rad52 after the exposure to the damaging agents suggests a direct role for this protein in the repair of DSB in mtDNA. Functional assays are being performed to corroborate this hypothesis.

Key words: DSBs repair, mitochondria.
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