Mouse Mitochondria Do Not Contain 3-methyladenine DNA Glycosylase

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DNA repair systems protect genome integrity from damages generated by exogenous and endogenous agents. Due to its localization, mitochondrial DNA accumulates more damage than nuclear DNA. The base excision repair (BER) pathway is the main repair mechanism for single strand breaks and DNA base modifications, and is the predominant repair system in mitochondria. In mammals, 3-methyladenine DNA glycosylase (AAG) initiates BER of alkylated bases, playing an important role in protecting against the genotoxic effects of alkylating agents, including several clinically relevant chemotherapeutic agents. Mitochondrial localization of AAG was recently described in human cells. It was also reported that mice overexpressing AAG are hypersensitive to alkylating agents while knockout animals and cells are resistant, indicating that alkylation sensitivity can paradoxically be a direct result of AAG-initiated repair on alkylated substrates, influencing cellular homeostasis. Thus, we evaluated the presence of AAG in mouse mitochondria. *In silico* prediction of murine AAG (mAAG) targeting to mitochondria and their mitochondrial targeting sequences (MTSs), by the MitoProt II and iPSORT, indicated that mAAG did not display a canonical MTS, and have a low probability for mitochondrial localization (score = 0.6634). Western blotting analysis, using anti-AAG monoclonal antibody, did not detect AAG in C2C12 cells or mouse brain mitochondrial extracts, while it was clearly detected in nuclear extracts from both cells and tissue. The mitochondrial extracts were shown to be free of nuclear contamination using anti-VDAC1 as mitochondrial marker and anti-PCNA or histone H3 antibodies as nuclear markers. Also, mAAG could not be co-localized with mitochondria in C2C12 by immunofluorescence using anti-AAG monoclonal antibody and MitoTracker Orange CMTMRos as mitochondria marker. Together, these data indicate that mAAG does not localize in mitochondria of unstressed cells. Further analysis by fluorescence-based incision assay will investigate whether an incision activity toward alkylated bases is detected in murine mitochondria.

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