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A modified alkaline comet assay to measure base excision repair in mitochondria

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Base excision repair (BER) is the major repair pathway for oxidative DNA damage removal, taking place in nuclei and mitochondria. Both in nuclear and mitochondrial DNA (mtDNA), the BER process is initiated by DNA glycosylases, such as 8-oxoguanine DNA glycosylase 1 (OGG1), that recognize and incise N-glycosidic bonds between the damaged base and deoxyribose. MtDNA is exposed to reactive oxygen species more than nuclear DNA due to its proximity to the electron transport chain. Functional BER, keeping mtDNA intact, is necessary for the proper cell energetic metabolism and for preventing mtDNA mutations leading to various aging-related diseases. The comet-based in vitro DNA repair assay is generally used to monitor BER activity in whole cell lysates. However, comet assay protocols for assessing BER activity only in mitochondria were until now missing. Our modified comet-based BER assay does not use a whole cell lysate as in the conventional protocol but a mitochondrial protein extract enabling assessment of the BER activity exclusively in mitochondria.

BER activity was assessed using crude mitochondria pellets isolated, using Percoll density gradient ultracentrifugation, from homogenized liver tissues of Zucker fatty and spontaneously hypertensive (ZSF1) rats; including obese and lean hypertensive rats investigated at ages 8-9, 22-23, and 34-35 weeks old (n=8-10/group). BER activity was compared to healthy control Wistar rats (n=8); 22-23 weeks old. The purity of isolated mitochondria was checked and confirmed on a Western blot. Pilot data showed that BER activity in mitochondria from obese (n=3) is about 1.3-fold lower than lean (n=4) ZSF1 rats (P=0.03; BER activity = 25.2% ± 4.8 versus 34.4% ± 2.9), while average mitochondrial BER activity of Wistar control rats was similar to the lean ZSF1 rats (33.0% ± 8.0). Analysis of all other comet assay images is ongoing and a more complete data set will be presented at the EEMGS meeting.

Currently, we are verifying our approach on mitochondrial proteins and whole cell lysates from wild-type and Ogg1-deficient (Ogg1-/-) mouse embryonic fibroblasts (MEFs). The study is funded by the Programme EXCELES (LX22NPO5102).

Keywords:
Base excision repair; alkaline comet assay; mitochondria; oxidative mtDNA damage.