**Mitochondrial Dysfunction Induces MMP-13 Expression via Production of ROS in Primary Human Chondrocytes**

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**Introduction:** Osteoarthritis (OA) is the most common chronic joint disease and is characterized by joint articular cartilage degradation. Chondrocytes are the only cell type in cartilage and are responsible for its regulation and repair. An upregulation of catabolic processes, such as Matrix Metallopeptidase 13 (MMP-13) secretion, degrades cartilage collagen fibers resulting in cartilage degeneration. Mitochondrial dysfunction has been associated with reactive oxygen species (ROS), however, its effect on OA is unclear. Our objective was to determine the effect of mitochondrial dysfunction on MMP-13 expression. We hypothesized that mitochondrial dysfunction induces MMP-13 expression through ROS production in primary human OA chondrocytes.

**Methods:** Primary human OA chondrocytes and cartilage explants were prepared from unaffected areas of knee OA cartilage (n=3) and treated with Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) (30 µM) to mimic mitochondrial dysfunction. Mitochondrial membrane depolarization and ROS levels were determined by staining with JC-1 and MitoSOX Red dyes respectively, followed by flow cytometry. MMP-13 expression was detected via Western blotting, ELISA, and immunohistochemistry.

**Results:** CCCP treatment of primary human OA chondrocytes induced mitochondrial dysfunction as determined by mitochondrial membrane potential loss. Induction of mitochondrial dysfunction via CCCP significantly upregulated mitochondrial ROS levels. Induction of oxidative stress by CCCP or H2O2 in chondrocytes induced MMP-13 expression. Upregulation of MMP-13 expression was dependent on time and dose of CCCP treatment. To rule out the possibility of chondrocyte dedifferentiation, we prepared human OA cartilage explants and treated with CCCP. Interestingly, in human OA cartilage explants, CCCP treatment upregulated MMP-13 expression. To confirm that mitochondrial dysfunction upregulated MMP-13 expression through ROS production, we treated primary human OA chondrocytes with MitoTempo antioxidant, followed by CCCP. Interestingly, mitochondrial ROS inhibition suppressed CCCP induced MMP-13 expression.

**Conclusion:** Our data demonstrates that mitochondrial dysfunction upregulated MMP-13 expression through the production of mitochondrial ROS in primary human OA chondrocytes.