Mitochondrial transfer of adipose-derived stem cells recovers mitochondrial and cartilage functions of senescent chondrocytes

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ABSTRACT INTRODUCTION:
Autologous chondrocyte implantation (ACI) represents the gold standard treatment for articular cartilage defects. However, clinical outcomes of ACI are not optimal for the fibrocartilage formed in articular cartilage defect. The process of in vitro monolayer culture expansion has been shown to induce senescence in chondrocytes. Studies indicate that fibrocartilage formation in articular cartilage defect is due to chondrocyte senescence. Chondrocytes senescence is closely associated with both mitochondrial dysfunction and cartilage dysfunction. Mitochondrial transfer emerges as a potential strategy for restoring mitochondrial function. Adipose-derived stem cells (ADSCs) are known to transfer mitochondria to recipient cells for tissue regeneration. In this study, we investigate the mitochondrial transfer from ADSCs to senescent chondrocytes on mitochondrial and cartilage functions of senescent chondrocytes.

METHODS:
For induction of senescence, the chondrocytes were in vitro monolayer culture expansion for at least 5 passages, and then the cells were treated with ADSCs mitochondrial transfer. The chondrocytes were divided into three groups: 1. Young group: chondrocytes without in vitro monolayer culture expansion, 2. Senescence group: chondrocytes were in vitro monolayer culture expansion for at least 5 passages to induce senescence, 3. Senescence + Mito group: senescent chondrocytes were treated with ADSC mitochondrial transfer. Senescence of chondrocytes were identified through detecting the expression of P16 and P21. ADSC mitochondria were isolated using an isolation kit, and then transferred to senescent chondrocytes by co-culture for a 3-day period. The mitochondrial function including oxygen consumption rate (OCR), and energy analysis parameters (basal respiration, ATP production, maximal respiration, spare respiratory capacity, coupling efficiency, proton leak, non-mitochondrial oxygen consumption and spare respiratory capacity as a %) of young and senescent chondrocytes were assessed using the Seahorse XF analyzer. Mitochondrial function was also evaluated using JC-10. The cartilage functions (osteocalcin, SOX9, BMP2, type collagen II, RUNX2, ALP, type I collagen, and type X collagen) of chondrocytes were analyzed by Real-time PCR.

RESULTS:
The chondrocytes in senescence group show positive for P16 and P21, while only P21 was found in young group (Figure 1). The mitochondrial function analysis shows chondrocytes in senescence group were significantly increased in OCR, and basal respiration, ATP production, max respiration and Spare respiratory when compared with young group (Figure 1). After ADSC mitochondrial transfer, the OCR was increased in senescence + Mito group than in senescence group indicating ADSC mitochondria affect senescence chondrocytes. The JC-10 results show the mitochondrial membrane potential were recovered after ADSC mitochondrial transfer (Figure 2). Compared with senescence group, the mRNA level of osteocalcin, SOX9, BMP2 and type II collagen were recovered, while RUNX2, ALP, type I collagen and type X collagen were suppressed in senescence + Mito group indicating cartilage functions is recovered after ADSC mitochondrial transfer (Figure 3).

DISCUSSION:
Based on these results, we found that mitochondrial transfer from ADSC into senescent chondrocytes recover mitochondrial function and cartilage function. This may be used for improving chondrocyte function in ACI.

SIGNIFICANCE/CLINICAL RELEVANCE:
Mitochondrial transfer of ADSCs recovers mitochondrial and cartilage functions of senescent chondrocytes.

Fig.1. Analysis of senescence markers and cellular energy metabolism in young and senescence chondrocytes. A. The senescence biomarkers of P16 and P21expressions. B: The measurements of OCR. C: The measurement of basal respiration, ATP production, maximal respiration, spare respiratory capacity, coupling efficiency, proton leak, non-mitochondrial oxygen consumption and spare respiratory capacity as a %.

Fig.2. The effect of ADSC mitochondrial transfer on mitochondrial function of chondrocytes. A. The chondrocytes staining with DAPI (blue) and cell tracker (Green). B. The chondrocytes transferred with ADSC mitochondria. DAPI (blue), cell tracker (Green) and ADSC mitochondria (red). B: The OCR measurement of chondrocytes in senescence and senescence + Mito. C: The mitochondrial membrane potential of chondrocytes in young, senescence and senescence + Mito.

Fig.3. Analysis of young, senescence and senescence + Mito chondrocytes of cartilage function markers (osteocalcin, SOX9, BMP2, type collagen II, RUNX2, ALP, type I collagen, and type X collagen) by real-time PCR.