Hypoxia-ADSC-Exo Rescues Inflammaging of Osteoarthritic Chondrocytes via NAD$^+$/SIRT Signaling Pathway

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INTRODUCTION: Osteoarthritis (OA) is the most common age-related degenerative joint disease. Inflammaging, linking inflammation and ageing, in senescent cells with the secretions of matrix-degrading proteins and proinflammatory cytokines. Nicotinamide adenine dinucleotide (NAD$^+$) and SIRT1 together regulate nuclear and mitochondrial functions and decline with many age-associated diseases. Restoring NAD$^+$ and combining sirtuin activation may be an effective anti-aging intervention. Our previous study has found that exosomes derived from hypoxia-cultured human adipose stem cells (H-ADSC-Exo) can alleviate articular chondrocyte inflammaging and osteoarthritis progression. This study aimed to investigate the anti-aging effects of H-ADSC-Exo on osteoarthritic chondrocytes and to explore the underlying molecular mechanisms. We hypothesized that H-ADSC-Exo might contain biochemical reactions, such as activated nicotinamide phosphoribosyltransferase (NAMPT), to increase NAD$^+$ biosynthesis and then ameliorate chondrocyte inflammaging.

METHODS: H-ADSC-Exo were derived from ADSCs cultured in 1 % O_2 and 10 % de-Exo-FBS for 48hrs. The cell senescence and biological effects of hypoxia-ADSC-Exo were tested on IL1-β-induced OA-like human articular chondrocytes (HACs) *in vitro*. **Human articular chondrocytes cultivation:** Primary human knee articular chondrocytes (HACs) were purchased from Clonetics™. **ADSCs cultivation:** ADSCs are purchased from StemPro® Human Adipose-Derived Stem Cells (Gibco®). **H-ADSCs-Exo isolation and characterization:** H-ADSCs-Exo were isolated by ultracentrifugation of CM derived from pre-cultured with ADSCs and characterization by Transmission electron microscopy (TEM) and Nanoparticle tracking analysis (NTA). **Cell senescence detection:** SA-β-gal cellular senescence assay kit was used. **DNA damage detection:** histone γ-H2AX Immunofluorescence stained and 8-OHdG content. **Superoxide quantification**: intra-cellular ROS detection by dihydroethidium (DHE) staining and mitochondria ROS detection by MitoSOX Red staining. **SOD activity and Catalase activity**: SOD Determination Kit and CheKine™ Micro Catalase (CAT) Activity Assay Kit. **NAD$^+$ and ATP level:** NAD$^+$/NADH was measured with the colorimetric NAD/NADH Quantitation Kit and the ATP Colorimetric Assay Kit. **Protein level analysis:** western blotting detection for NAMPT, SIRT1/6, SOD1/2, p16^{ink4a}.**Statistical analysis:** The data are expressed as the means ± SE from each experimental replicate. Statistical significance was evaluated by one-way analysis of variance (ANOVA), and multiple comparisons were performed using Scheffe's method. A $p<0.05$ was considered significant.

RESULTS SECTION: The results showed that the H-ADSC-Exo suppressed IL-1b induced cell senescence marker, SA-β-gal and p16^{ink4a}, and DNA damage marker, 8-OHdG, and histone H2AX phosphorylation (γH2AX) (Figure1). H-ADSC-Exo also promoted SOD1, SOD2, catalase activity, and mRNA/protein level to reduce intra-cellular and mitochondria ROS in IL-1b induced OA-like HACs (Figure 2). Furthermore, we detected the NAD$^+$ content in hypoxia-ADSC-Exo (data not shown), and treatment with it could increase NAD$^+$ and ATP content by promoting SIRT1 activity and protein level in OA-like HACs (Figure 3).

DISCUSSION: H-ADSC-Exo increased SOD1/2 and catalase activity to reduce ROS-induced DNA damage and HACs senescence via the NAD$^+$/SIRT signaling pathway.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings suggest that H-ADSC-Exo treatment may be an effective anti-aging therapeutic bioagent, providing hope to aging societies worldwide. The H-ADSC-Exo treatment may offer another strategy for future OA therapy.