Hypoxia-ADSC-Exo Rescues Inflamming of Osteoarthritic Chondrocytes via NAD+/SIRT Signaling Pathway

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INTRODUCTION: Osteoarthritis (OA) is the most common age-related degenerative joint disease. Inflamming, linking inflammation and ageing, in senescent cells with the secretion of matrix-degrading proteins and proinflammatory cytokines. Nicotinamide adenine dinucleotide (NAD+) and SIRT1/6 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 inflamming 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 inflamming.

METHODS: H-ADSC-Exo were derived from ADSCs cultured in 1% O2 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, p16ink4a. 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 p16ink4a, and DNA damage marker, 8-OHdG, and histone H2AX phosphorylation (γH2AX) (Figure 1). 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.