Exosomal miR125b-5p Derived from Adipose-Derived Mesenchymal Stem Cells Alleviates Cell Senescence by Suppressing CDKN2A(P16^{ink4a}) in Human Articular Chondrocytes

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INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease and affects elderly individuals worldwide. Our previous study found that exosomes derived from hypoxia-cultured human adipose stem cells (H-ADSC-Exo) can slow the progression of osteoarthritis. However, the exact molecular mechanism remains fully elucidated, and extensive research has found that miRNA may play a role in regulating gene expression, yielding beneficial results through these miRNAs. In this study, we evaluate the miRNA profile of H-ADSC-Exo and compare the mRNA profile of HACs treated with or without H-ADSC-Exo to look for miRNA-mRNA connected genes. We found that one of the miRNAs (miR125b-5p) exhibited high expression, and its target gene (p16^{ink4a}) was also predicted to be regulated by miR-125b-5p. Our aim is to investigate whether the H-ADSC-Exo-miR125b-5p has an anti-aging effect.

METHODS: H-ADSC-Exo were derived from ADSCs cultured in 1 % O_2 and 10 % de-Exo-FBS for 48 hours. **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 characterized by Transmission electron microscopy (TEM) and Nanoparticle tracking analysis (NTA). **QRT-PCR analysis**: CDKN2A, CDKN1A, p53, TNFa, IL1b, IL6, CXCL8, MMP13, ADAMT5, COX2, CEBPB, b-actin mRNA expression and miR125b-5p miRNA expression. **Protein level analysis:** western blotting detection for CDKN2A (p16^{ink4a}), and β-actin as a reference gene. **Cell viability** by CCK8 analysis and **cell proliferation** by BrdU assay. **Cell senescence detection:** The SA-β-gal cellular senescence assay kit was used. **Cell function analysis**: cell migration and GAG deposition by Alcian blue stain and DMMB assay. **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: The results showed that the miR-125b-5p suppressed CDKN2A (p16^{ink4a}) mRNA and protein expression (Figure 1A and B). We also found that miR125b-5p expression decreased markedly with increasing cell passage number, whereas p16^{INK4a} protein levels showed a corresponding increase (Figure 1C and D). We transfected miR-125b-5p mimic in HACs and found that it promotes cell viability and proliferation (Figure 2A and B). We also found that overexpression of miR-125b-5p can suppress the expression of inflammation-related genes, including TNFα, IL-1β, IL-6, CXCL8, MMP13, ADAMT5, COX2, and CEBPB, but not the p53 gene (Figure 2C and D). To demonstrate that H-ADSC-Exo plays a major role in cell senescence and migration, and that these functions are regulated by miR125b-5p, we treated HACs with H-ADSC-Exo, a miR125b-5p mimic, or H-ADSC-Exo in combination with a miR125b-5p inhibitor. The results showed that treating HACs with H-ADSC-Exo and miR-125b-5p mimic suppressed CDKN2A mRNA expression, but had the opposite effect when H-ADSC-Exo was combined with the miR-125b-5p inhibitor (Figure 2E and F). Cell senescence also yields similar results, where H-ADSC-Exo and miR-125b-5p mimic can suppress SA-β-gal stain but reverse the effect when combined with a miR-125b-5p inhibitor (Figure 3B). HACs' function, eg, GAG deposition and migration, was promoted when HACs were treated with H-ADSC-Exo and miR-125b-5p mimic, and inhibited by H-ADSC-Exo combined with a miR-125b-5p inhibitor (Figure 3A, B, and D).

SIGNIFICANCE/CLINICAL RELEVANCE: miR-125b-5p can reduce human articular chondrocyte (HAC) senescence and inflammatory factor expression, while promoting glycosaminoglycan (GAG) deposition, migration, and proliferation by suppressing CDKN2A (p16^{ink4a}). Human ADSC-derived exosomes (H-ADSC-Exo) are enriched in miR-125b-5p and therefore exert effective anti-inflammaging effects, enhance cell viability, and may represent a promising therapeutic bioagent for future osteoarthritis (OA) treatment.