

Exploring the effects of human iPSC extracellular vesicles on enhancing the chondrogenic function in human articular chondrocytes

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INTRODUCTION: Osteoarthritis (OA) stands as a globally significant orthopedic and joint ailment. Mesenchymal stem cells (MSCs) have found application across various orthopedic conditions, including OA. Nevertheless, the clinical utilization of MSCs faces several drawbacks. Recent research has unveiled a pivotal role in tissue repair played by extracellular vesicles (EVs) that secreted by MSCs. However, a major challenge in Exos deployment lies in their scarcity, owing to the constrained proliferative capacity of MSCs. This limitation could potentially be mitigated by utilizing induced pluripotent stem cells (iPSCs), known for their robust proliferation. The current study seeks to probe the potential of iPSC-derived exosomes (iPSC-EVs) in enhancing chondrogenic function and countering inflammatory responses. Our hypothesis posits that iPSC-EVs hold the capability to enhance chondrogenic functions, thereby holding promise as a viable avenue for osteoarthritis treatment.

METHODS: Human iPSC-derived extracellular vesicles (hiPSC-EVs) were isolated through ultra-high-speed centrifugation, and the size and concentration of these exosomes were determined using Nanoparticle Tracking Analysis (NTA). Western blotting was employed to identify positive markers (ALIX, TSG101, CD63, CD81) and the negative marker (Calreticulin) for exosomes. The human articular chondrocytes (HACs) were treated with/without hiPSC-EVs under IL-1 treatment to mimic the inflammatory conditions of osteoarthritis (OA). The chondrogenic and inflammatory genes were evaluated by Q-PCR, and the GAG synthesis were also tested by alcain blue staining and GAG synthesis kit.

RESULTS: Our findings revealed that the concentration of hiPSC-EVs measured $2.75 \pm 0.88 \times 10^{10}$ particles/ml, accompanied by a particle size of 150.33 ± 17.00 nm. Positive markers ALIX, TSG101, CD63, and CD81 were expressed within hiPSC-Exos, whereas the negative marker Calreticulin was absent (Fig. 1). These observations confirmed the characterization of hiPSC-Exos. Alcian blue staining demonstrated the augmentation of glycosaminoglycan (GAG) synthesis by human iPSC-EVs (Fig. 2). When subjected to IL-1 treatment, the expression of inflammatory genes IL-1, MMP-13, and TNF- α increased, but this upregulation was attenuated by iPSC-EVs. Moreover, hiPSC-EVs treatment under IL-1 conditions led to heightened expression of chondrogenic genes COL-2 and aggrecan compared with IL-1 alone treatment. These results collectively underscore the favorable chondrogenic effects of iPSC-EVs and their potential to reduce the inflammatory response in HACs.

DISCUSSION: In our study, it was demonstrated that hiPSCs could be excellent candidates for obtaining EVs-derived products due to their high proliferative ability. Additionally, hiPSC-EVs were observed to mitigate the inflammatory response following IL-1 treatments and enhance the chondrogenic function in HACs. These findings indicate that hiPSC-EVs exert beneficial effects in reducing the damage induced by IL-1 in HACs.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): These results show that hiPSC-EVs have the potential to treat OA and maintain chondrocyte functions. It is expected that their application in OA treatment will be further explored in the future.

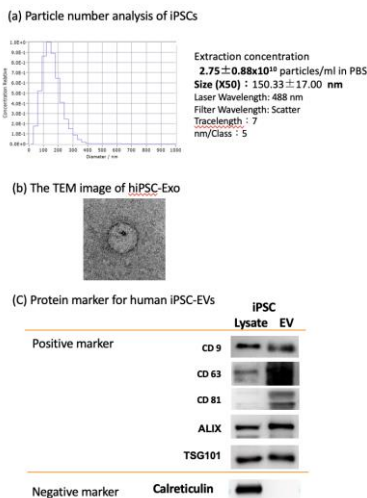


Fig 1. Characterization of EVs derived from human iPSCs. (a) Particle size distribution and concentration measured. The data showed that the average size of EVs is 118.7 nm. The concentration of iPSC EVs is 4×10^8 particles/ml iPSC medium. (b) The positive protein marker, ALIX, TSG101, CD63 and CD9 have detected in hiPSC-EVs.

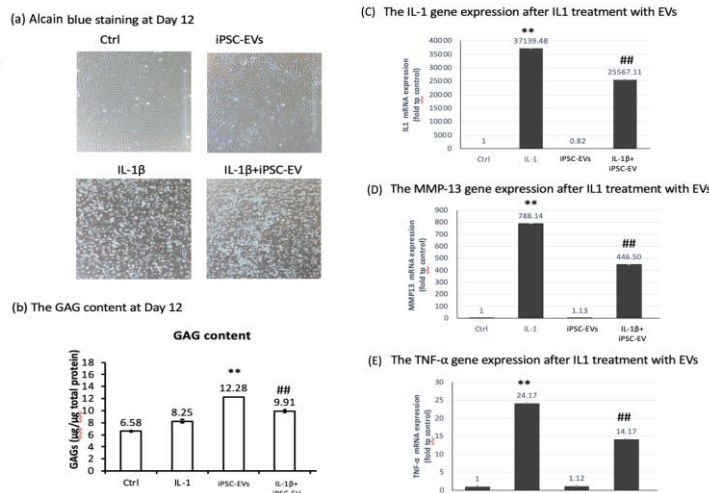


Fig. 2. The cell functions of human articular chondrocytes (HACs) after IL-1 and iPSC-EV treatments. (A) The cells showed that less Alcain blue positive staining after IL-1 treatment, and hiPSC-EV reversed the phenomenon. (B) The quantification of GAG content. (C) The IL-1 gene expression increased after IL-1 treatment in HACs, and hiPSC-EVs reversed the effect. (D) The MMP-13 gene expression increased after IL-1 treatment in HACs, and hiPSC-EVs reversed the effect. (E) The TNF- α gene expression increased after IL-1 treatment in HACs, and hiPSC-EVs reversed the effect. (**, compared with control group; ##, compared with IL-1 group)

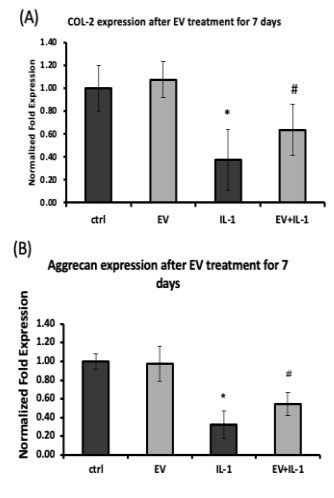


Fig. 3. The gene expression of COL-2 and aggrecan in human articular chondrocytes (HACs) after IL-1 and iPSC-EV treatments. (a) The COL-2 gene expression decreased after IL-1 treatment in HACs, and hiPSC-EVs reversed the effect that increased the COL-2 gene expression. (b) The aggrecan gene expression was also decreased after IL-1 treatment in HACs, and hiPSC-EV reversed the effect. (*, compared with control group; #, compared with IL-1 group)