Multi-omics and cellular function analyses of human iPSC- and iMSC-derived extracellular vesicles in human articular chondrocytes

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INTRODUCTION: Osteoarthritis (OA) is a common degenerative joint disease marked by the terminal differentiation and apoptosis of articular chondrocytes. While mesenchymal stem cells (MSCs) and their cell-free product (such as secretome or extracellular vesicles) show promise for OA treatment, their clinical use is limited by low proliferation, aging effects, and challenges in large-scale exosome production. Induced pluripotent stem cells (iPSCs), with their high proliferative capacity, provide an alternative source for generating therapeutic exosomes. In our previous study, we demonstrated that iPSC-derived exosomes (iPSC-Exos) suppressed IL-1 and MMP13 expression and promoted chondrogenic markers in IL-1-stimulated human articular chondrocytes (hACs). Moreover, iPSC-derived MSCs may have more chondrogenic potential because of the multipotency for chondrogenesis. Therefore, this study further compares exosomes from iPSCs and iPSC-derived MSCs (iMSCs).

METHODS: The human iPSC line (IBMS-iPSC-01-02_feeder-free) will be generated and authenticated by the Taiwan Human Disease iPSC Service Consortium and the generation of hiMSCs derived from hiPSCs will be induced as Liu's paper. Normal human articular chondrocytes (HACs) (Clonetics™, USA) were cultured in Dulbecco's modified Eagle's medium. Surface antigens of hiPSCs and hiMSCs were analyzed using flow cytometry and fluorescence microscope. Ultra-high-speed centrifugation was employed to isolate extracellular vesicles (EVs) derived from hiPSCs and hiMSCs. The particle size and concentration of hiPSC-EV and hiMSC-EVs were determined using Nanoparticle Tracking Analysis (NTA). The proteomics and miRNA profile of hiPSC-EVs and hiMSC-EVs were analyzed by LC-Mass and miRNA NGS analysis. HACs were subjected to IL-1 treatment conditions, with or without hiPSC-EV or hiMSC-EV, to mimic osteoarthritis (OA) inflammation (n=4). Chondrogenic and inflammatory gene expression was assessed through Q-PCR, while alcian blue staining and β-Glucosidase staining were employed to evaluate glycosaminoglycan (GAG) synthesis and aging cells.

RESULTS: Human iPSCs expressed pluripotency markers SSEA-4 and Oct-4, confirming that the pluripotent state was maintained in the hiPSC line (Fig. 1A). Furthermore, characterization of hiPSC-derived MSCs revealed positive expression of surface markers CD90, CD29, CD73, and CD44, and negative expression of CD34 and CD45, indicating successful differentiation into hiMSCs (Fig 1B and C). Characterization of EVs from human iPSC and hiMSC were confirmed (data not showed). Proteomic and miRNA analyses revealed that hiPSC-EVs and hiMSC-EVs have distinct molecular compositions (Fig 2A and B). Specifically, the data showed that hiMSC-EVs were functionally enriched in proteins and miRNA related to cell junctions, cytoskeletal regulation, and focal adhesion, whereas hiPSC-EVs were enriched in proteins for maintaining pluripotency, stem cell populations, and ribosome function. Alcian blue staining showed that GAG synthesis was increased when treated iPSC-EVs and hiMSC-EVs under IL-1 treatments (Fig 3A). Moreover, hiPSC-EVs and hiMSC-EVs reduced aging cells by β-Glucosidase staining (Fig 3B). The inflammatory gene expression (IL-1 and MMP-13) increased with IL-1 treatment, but both hiPSC-EVs and hiMSC-EVs effectively reduced these inflammatory markers. Especially, IL-1 was more reduced after hiMSC-EVs treatment compared to hiPSC-EVs treatment (Fig 3C). While chondrogenic genes (COL-2) decreased under IL-1 conditions, post-treatment with either hiPSC-EVs or hiMSC-EVs restore their levels. Notably, the restorative effect of hiPSC-EVs was more potent than that of hiMSC-EVs.

DISCUSSION: Our study demonstrated that proteomic and NGS results showed iMSC-Exos were enriched in proteins and miRNAs linked to regeneration, immune modulation, and signaling (e.g., PI3K-Akt, ECM interactions), including miR-125b-5p and miR-29a-3p. In contrast, iPSC-Exos contained miRNAs like miR-302b-3p associated with pluripotency. Both extracellular vesicle types reduced inflammation and restored COL-2 in IL-1-treated hACs, with iMSC-Exos showing greater therapeutic efficacy on anti-inflammaging. These results underscore the positive impact of both hiPSC-EVs and hiMSC-EVs in mitigating IL-1-induced damage in HACs.

SIGNIFICANCE/CLINICAL RELEVANCE: These results showed that hiPSC-EVs and hiMSC-EVs have potential to treat OA and maintain the chondrocyte functions. It is expected to explore the application in OA treatment in vivo in the future.

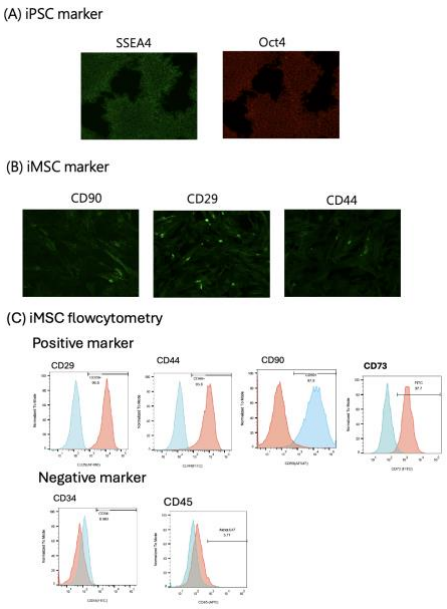

Fig 1. **Characterization of human iPSC and hiMSC**. (A) hiPSC markers (B)hiMSC markers (C) hiMSC markers by flowcytometry.

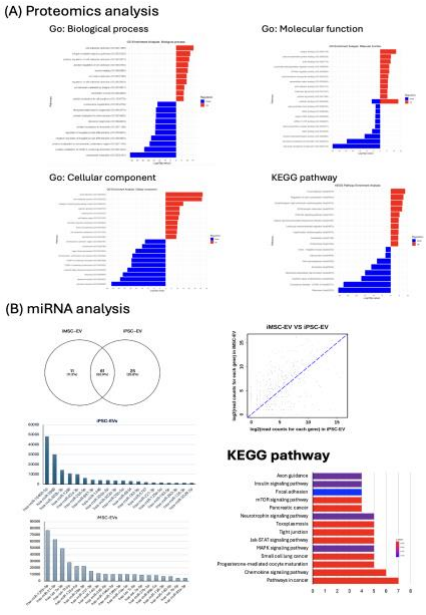

Fig 2. **Proteomics and miRNA analysis between hiPSC and hiMSC** (A) Proteomics (B)miRNAs.

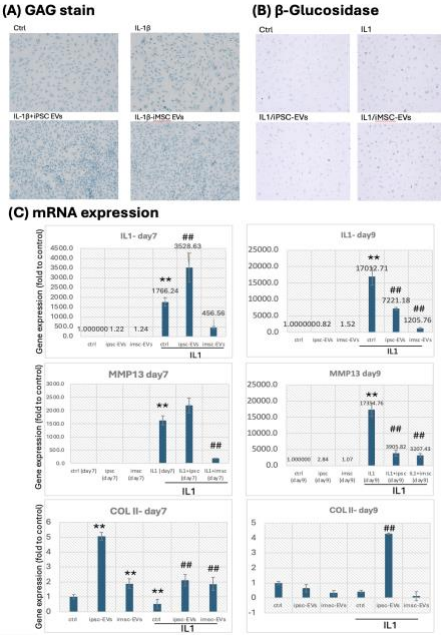

Fig 3. **The cellular function in hACs** (A) GAG staining (B) β-Glucosidase staining (C) mRNA expression.