Therapeutic Potential of IFN-γ–Preconditioned MSC-derived Extracellular Vesicles in Osteoarthritis

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INTRODUCTION:

Mesenchymal stromal cell (MSC)-derived extracellular vesicles (EVs) have emerged as promising cell-free therapeutic agents in regenerative medicine due to their ability to modulate immune responses through encapsulated bioactive molecules such as miRNAs.[1] In osteoarthritis (OA), persistent low-grade inflammation, particularly driven by pro-inflammatory M1 macrophages, plays a crucial role in cartilage degradation and disease progression.[2] We have previously demonstrated that MSC-derived EVs suppress OA progression in both in vitro model, assessing their effects on chondrocytes, and in vivo mouse OA model.[3] However, OA is a multifactorial disease involving complex interactions among various cell types, including macrophages, chondrocytes, and synoviocytes.[4] Therefore, understanding how EVs modulate these interactions is critical for optimizing therapeutic strategies. Recent evidence suggests that the therapeutic potential of EVs can be enhanced by preconditioning human adipose-derived mesenchymal stromal cells (ASCs) with inflammatory stimuli such as interferon-gamma (IFN-γ), but the optimal conditions and underlying mechanisms remain unclear.[5] This study aimed to investigate the therapeutic potential of IFN-γ–preconditioned ASC-derived EVs in osteoarthritis.

METHODS:

Human ASCs were selected as the source of small extracellular vesicles (sEVs; a subclass of EVs <200 nm in size) in this study, as they have been widely used in the clinical treatment of OA.[6] ASCs were obtained via liposuction aspirate from the abdominal subcutaneous adipose tissue of donor patients (N = 3), isolated using collagenase digestion, and cultured in DMEM/F12 supplemented with NeoSERA and recombinant human bFGF. Cultures were maintained at 37°C in 5% CO_2 with medium changes twice weekly. Passage 2–4 cells were used for all experiments. To confirm successful ASC isolation as MSCs, the cells were evaluated for their tri-lineage differentiation potential and surface marker expression, in accordance with ISCT guidelines.[7]ASCs were stimulated with IFN-γ at varying concentrations (1, 10, or 100 ng/mL) for 48 hours. Cell proliferation was assessed using a WST-8 assay, and cell viability was evaluated by measuring lactate dehydrogenase (LDH) release. Surface marker expression of ASCs (such as PD-L1, HLA-DR), and expression of immunomodulatory genes in ASCs (IDO1, CCL2, IL6, TGFB1) were assessed via flow cytometry and RT-qPCR, respectively. sEVs were isolated from conditioned media using tangential flow filtration (TFF) and further analyzed by ExoScreen assay for CD9/CD63 positivity.[8] Protein levels of IDO1 within EVs were also quantified. Soluble cytokine contamination was assessed to confirm effective washout during EVs isolation. sEVs collected from IFN-γ–stimulated ASCs were referred to as γ-sEVs, while those derived from unstimulated ASCs were termed naive sEVs (N-sEVs). They were added to cultured macrophages, and the proportion of inflammatory macrophages was evaluated.

RESULTS:

During culturing on plastic dishes, ASCs maintained their fibroblast-like spindle morphology. Their ability to differentiate into adipocytes, osteoblasts, and chondroblasts was confirmed. Flow cytometry analysis showed >95% positive expression for CD73, CD90, and CD105, and <2% expression for a negative marker cocktail (CD11b, CD19, CD31, CD45, HLA-DR), consistent with the ISCT criteria. The viability of ASCs remained above 95% across all IFN-γ stimulation conditions, while their proliferation capacity showed a mild, dose-dependent decline (Figure 1). A dose-dependent increase in PD-L1, CCL2, and IDO1 gene expression in ASCs was observed. The concentration of CD9-positive EVs decreased slightly with IFN-γ stimulation, while that of CD63-positive EVs increased in a concentration-dependent manner. IDO1 protein levels were significantly higher in γ-sEVs than in N-sEVs (Figure 3). Soluble cytokines were effectively removed during ultrafiltration, yielding highly purified preparations of sEVs and confirming that the observed effects were attributable to sEVs. These purified sEVs could potentially meet the quality requirements for clinical-grade applications. Functionally, γ-sEVs treatment reduced the proportion of M1 macrophages in vitro compared to the N-sEVs group, although statistical significance was not reached. The trend suggested a shift toward an anti-inflammatory phenotype.

DISCUSSION:

Our findings suggest that IFN-γ preconditioning modulates the immunoregulatory properties of MSC-EVs by enhancing IDO1 content and reducing macrophage inflammatory activation. These effects may contribute to improved therapeutic efficacy in inflammatory conditions such as OA.

SIGNIFICANCE:

This study demonstrates the potential of IFN-γ preconditioning to optimize the immunomodulatory function of MSC-EVs in OA therapy. Further investigations incorporating expanded in vitro and in vivo models, including gene expression profiling in target cell types such as chondrocytes and macrophages, may help elucidate the mechanisms of action of EV therapy. This approach could facilitate the identification of specific miRNA cargo involved in immunomodulation in OA.

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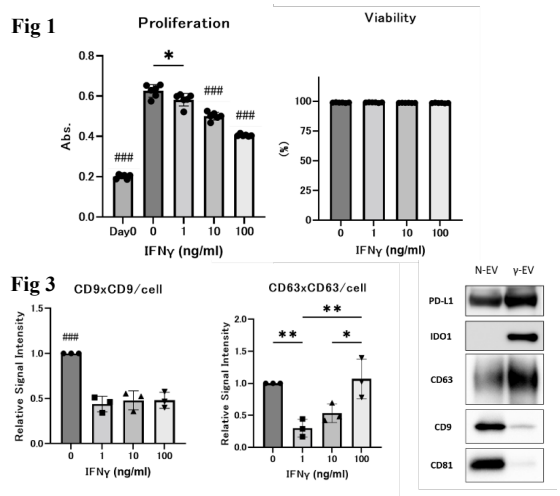
IMAGES:



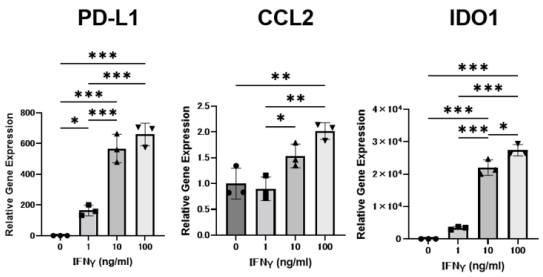


Figure 1. IFN-γ stimulation suppressed ASC proliferation in a dose-dependent manner, while cell viability remained above 95%.
Figure 2. IFN-γ stimulation upregulated the expression of inflammation-related genes such as PD-L1, CCL2, and IDO1 in a dose-dependent manner.
Figure 3. IFN-γ stimulation modulated the secretion levels of sEVs, surface markers such as CD9 and CD63, and cargo contents including IDO1 protein.