Poster Presentations

PP-11

Investigation of Non-Classical MHC Class I Molecules in Mesenchymal Stem Cells Derived from Wharton's Jelly

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Objectives: Mesenchymal stem cells (MSCs) can be derived from various sources, including Wharton's jelly (WJ). WJ-derived MSCs stand out with their advantages of easy isolation, expandability, and notable immunomodulatory and anti-inflammatory effects. Recent studies have demonstrated that MSCs derived from WJ express high levels of non-classical MHC-class I molecule, human leukocyte antigens (HLA)-G. This study investigates the effects of interferon-gamma (IFN-γ) stimulation on HLA-G expression in WJ-derived MSCs after treatment with the demethylating agent 5-AZA. MSCs are multipotent somatic stem cells with the capacity to differentiate into various cell types (1,2). Among the mechanisms underlying the immunosuppressive properties of MSCs, the HLA-G molecule has been shown to play an important role (3). HLA-G gene expression significantly decreases in serial passages of MSC cultures (4). DNA methylation is one of the key epigenetic mechanisms controlling gene expression (5). Demethylation in the promoter region of a gene is associated with transcriptional activation (6). Based on this information, adding DNA demethylating agents to cell cultures may help maintain stable HLA-G expression across all passages for therapeutic applications.

The expression of MHC class I genes can also be induced by various cytokines. IFN-γ, a proinflammatory cytokine, is the most potent inducer of *MHC class I* gene expression (7). This study aims to elucidate the effects of HLA-G promoter region methylation and IFN-γ stimulation on *HLA-G* gene expression in MSCs derived from WJ.

Materials and Methods: WJ was isolated from umbilical cord tissue and cultured in an appropriate incubator. Cell characterization was performed using a flow cytometer. Following MSC characterization, 10 µM 5-AZA was applied to passage 1 (P1) cells for 72 hours. After this treatment, one group was stimulated with 10 ng/mL IFN-γ for 3 days after removing 5-AZA, while the other group was stimulated with IFN-γ without removing 5-AZA. Soluble HLA-G levels in cell culture supernatants from all groups were analyzed by ELISA, and the results were subjected to statistical analysis.

Results: HLA-G levels in P1 samples treated with 10 µM 5-AZA for 72 hours were significantly higher compared to control (p=0.007) (Figure 1). After removing 5-AZA and stimulating with IFN-γ, HLA-G levels increased but were not statistically significant (p=0.172) (Figure 2A). In contrast, when 5-AZA was not removed, HLA-G levels were significantly higher compared to the control group (p=0.026) (Figure 2B). HLA-G levels in the P1 group.

Discussion: One of the primary mediators in the immunomodulatory effects of MSCs is the HLA-G molecule (8). Increased HLA-G expression is correlated with the immunosuppressive effects of MSCs (9). This study demonstrates that demethylating agents like 5-AZA and cytokines like IFN-γ can enhance HLA-G expression. The combination of continuous 5-AZA presence with IFN-γ stimulation is more effective than removing 5-AZA before stimulation. Future research will contribute to a deeper understanding of these mechanisms.

Keywords: HLA-G, mesenchymal stem cell, Wharton's jelly

References

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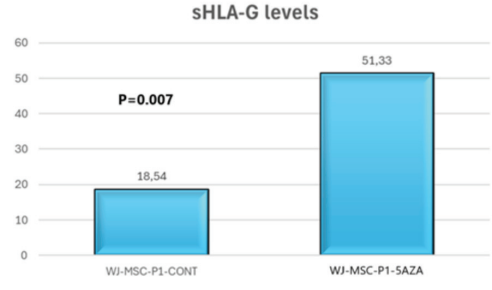

Figure 1. Intra-group comparisons of HLA.
HLA: Human leukocyte antigens

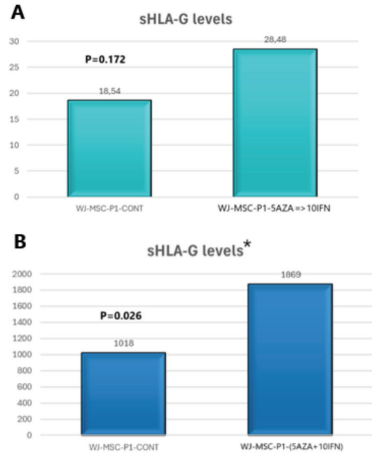

Figure 2. Intra-group comparisons of HLA-G levels in the P1 group.
**: A different ELISA kit was used for measurement, HLA: Human leukocyte antigens*