INVESTIGATION OF THE EFFECT OF PRP AS A NATURAL SUPPLEMENT ON DIFFERENTIATION OF PSCS INTO MSCS

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Introduction. Platelet-rich-plasma (PRP) of peripheral blood contains valuable natural growth factors, such as: PDGF, TGF, PDAF, VEGF, EGF and presents a suitable alternative to xenogeneic supplements for *ex vivo* propagation of mesenchymal stem cells (MSCs). Currently, for differentiation of MSCs from pluripotent stem cells (PSC) a fetal bovine serum (FBS) as a cell culture supplement is commonly used with FGF, PDGF, EGF. Replacement of the FBS with PRP to support the cell growth may reduce risks of transfer xenogeneic infection. Hence, differentiation using PRP presents a promising alternative for generation of pluripotent-derived MSCs (PD-MSCs). In this regard, the aim was to study the effect of PRP lysate as the natural supplement on differentiation of PSCs into MSCs.

Methods. Karyotypically normal and homogeneous PSCs were maintained in xeno-free and feeder-independent conditions. Pluripotent characteristics of PSCs were assessed with alkaline phosphatase staining, immunofluorescence and RT-PCR analysis. Isolation of PRP from healthy donor was performed using PRP kit (Neogenesis, Korea). PRP lysate preparation was conducted using freeze-thawing cycles and ultracentrifugation. For MSCs differentiation, PSCs were cultured in E8 xeno-free medium (Gibco, USA) supplemented with PRP lysate for period of 11 days. For the control, the same cells were cultured in complete E8 xeno-free medium. After differentiation, the obtained PD-MSCs were characterized using morphological and immunofluorescence analysis.

Results. Our results demonstrated that PRP lysate is able to induce the differentiation of PSCs to MSCs. The first morphological changes of PSCs were appeared on the 8-th day. In these cells the lack of expression of the transcription factor Oct-4 was identified on the 11-th day. The data of immunofluorescence analysis showed the expression of MSC markers, including membrane glycoprotein - CD105, which was detected on the 11-th day of differentiation. Moreover, it was revealed that generated PD-MSCs had spindle-shape cell morphology, which is characteristic of the normal MSCs, and high proliferation potential.

Conclusion. Our preliminary data shows that the use of PRP lysate as the natural supplement of important growth factors have significant potential for reproducible, cost-effective generation of biocompatible and immunomodulatory PD-MSCs for clinical cell therapy.