ISOLATON AND ENRICHMENT OF RAT BONE MARROW MESENCHYMAL STEM CELLS

S. Baidosova[1,2] , V. Kumasheva[1,2] , T. Kerimbayev [3,] V. Aleynikov [3], N. Aldiyarova [3], Vyacheslav Ogay[1,2]

[1] *Stem Cell Laboratory, National Center for Biotechnology (Astana, Kazakhstan)*
[2] *National Laboratory Astana, Nazarbayev University (Astana , Kazakhstan)*
[3] *National Center for Neurosurgery (Astana, Kazakhstan)*
s.baidossova@gmail.com

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Introduction: Mesenchymal stem cells (MSCs) are a promising tool for cell therapy, because of their specific characteristics, which mimic partially those of embryonic stem cells, but with some advantages in terms of availability, expandability, transplantability and ethical implications. These cells can be isolated from the bone marrow and enriched with two mesenchymal surface markers CD54 and CD90 by immunomagnetic separation. In this regard, the purpose of this study was to isolate and enrich rat MSCs from bone marrow.

Methods: Bone marrow-derived cells were isolated from Wistar mature rats. Isolated cells were adhered to plastic surface and expanded during in vitro culture. CD54 and CD90 monoclonal antibodies were used to enrich MSCs by immunomagnetic separation. Enriched rat CD54+CD90+ bone marrow MSCs were characterized by immunocytochemistry, CFU-assay and multilineage differentiation assay.

Results: Bone marrow-derived cells were successfully isolated from femurs and tibias of mature rat. After 48 hours, the cells with spindle-shaped morphology had attached to the tissue culture flask and gradually grow as colonies. When culture reached approximately 80% confluency, the cells were detached and prepared for immunomagnetic separation. Our results showed that approximately 15% CD54+CD90+ MSCs can be isolated from total amount of heterogeneous bone marrow-derived cells. The study on functional activity showed that separated CD54+CD90+ MSCs posses high clonogenic growth capacity and can effective differentiate into adipocytes, chondrocytes and osteoblasts under appropriate experimental conditions. In addition, immunocytochemical analysis revealed that CD54+CD90+ MSCs highly expressed CD73 and CD105, but they do not express the hematopoietic marker CD45.

Conclusion: Thus, our results confirmed that immunomagnetic separation based on CD54 and CD90 is a suitable method to enrich the subpopulation of CD54+CD90+ MSCs from rat bone marrow.