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The importance of GABAergic neurons in the spinal cord injury; in vitro generation of GABAergic neuron like cells

Shahram Darabi^{1, 2}, Taki Tiraihi^{1, 2}, Alireza Delshad³, Majid Sadeghizadeh⁴, Taher Taher², Hadi Kazem².

Objective: Pain is a major problem in the spinal cord injury. One of the approaches to treat spinal cord injury is γ -aminobutyric acid (GABA)ergic neuron cell therapy. Bone marrow stromal stem cells (BMSCs) represent an appropriate source of multipotent stem cells, which are ideal for various cell-based therapies. BMSCs can be differentiated into neuronal-like cells under appropriate conditions. This study examined the effectiveness of co-stimulation, in the induction of BMSCs to differentiate into GABAergic neuron-like cells (GNLCs).

Material and methods: BMSCs isolated from the femur and tibia bones of adult rats were cultured in Dulbecco's modified Eagle medium (DMEM)/F12 medium supplemented with 10% fetal bovine serum (FBS), preinduced with β -mercaptoethanol (β ME), and induced by using retinoic acid (RA) and creatine (Cr). Neurofilament 200 KDa (NF-H), neurofilament 160 KDa (NF-M), nestin, fibronectin (Fn), GABA, GAD65/67, and vesicular GABA transporter (VGAT) immunostaining were used to evaluate the transdifferentiation of BMSCs into GNLCs. The percentage of these immunoreactive cells was used to evaluate the effectiveness of the pre-induction and the induction assays. The gene expression levels for Fn, Oct-4, GABA, glutamic acid decarboxylase (GAD), and VGAT in BMSCs and GNLCs were examined using reverse transcription-polymerase chain reaction (RT-PCR) to monitor BMSC transdifferentiation into neuronal cells.

Results: The results of this study showed a 2-fold increase in GABAergic differentiation after costimulation with RA and Cr in the induction stage as compared with Cr alone, resulting in a 71.6% yield for GABAergic neurons. RT-PCR showed no expression of Oct-4 and Fn after the induction stage.

Conclusion: The results of this study showed that the application of β ME, RA, and Cr induced the transdifferentiation of BMSCs into GABAergic neurons.

Keywords: Bone marrow stromal cells (BMSC), GABAergic-like neurons, Retinoic acid, Cell therapy

1. Dept. of Anatomical Sciences, School of Medical Sciences, Tarbiat Modares University, P.O. Box 14155-4838, Tehran, Iran;

2Shefa neuroscience research center, Khatam-Al-Anbia Hospital, Tehran, Iran

3Department of Anatomy, Shahed University, Tehran, Iran

4Department of Genetics, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran