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Poster Presentation

Imaging Action Potentials with Calcium Indicators Fura-4 in Motor Neuron Like Cells Derived Adipose Stem Cells

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Abstract

Cell replacement therapy has provided the basis for future clinical applications to treat central nervous system injury, a common result of car accidents. Induced functional motoneurons are an option for replacing the lost motoneurons. Adipose derived stem cells (ADSCs) are an appropriate source of cells for autologous cell therapy with the ability of neural differentiation. In this study, ADSCs were induced to neurospheres using B27 and bFGF as pre-inducer, and then neurospheres were induced to motoneuron-like cells (MNLCs) by Sonic hedgehog (SHH) and retinoic acid (RA) as inducers. ADSCs markers such as CD90, CD44, CD49d, CD106, CD31 and CD45 were measured by immunocytochemistry analysis and their multipotency were evaluated by incubation of the ADSCs with adipogenic, chondrogenic and osteogenic induction media. Then neurospheres derived ADSCs were evaluated by immunocytochemistry and RT-PCR assay. The expression of islet-1, oligo-2 and HLXB9 in induced MNLCs from neurospheres evaluated by RT-PCR and immunocytochemistry assay. To identify the functional MNLCs, a co-culture preparation of MNLCs and myocytes, calcium ion imaging and synaptic vesicle release was used. ADSCs treated with a mixture of pre-inducer (B27 and bFGF) and inducer factors (SHH and RA) adopted a morphology similar to motoneuron cells. Immunocytochemical staining and RT-PCR approved that the treated cells expressed the motoneuron markers islet-1; oligo-2 and HLXB9. The co-cultured with myocytes indicate the formation of neuromuscular connections between MNLCs and myocytes. After two week, MNLCs showed high HLXB9 expression, indicative of full differentiation. Also, the release rate of synaptic vesicles using FM1-43 in the induced MNLCs was 10 fold. Moreover a calcium imaging with fluo-4 results approved those functional excitatory synaptic connections can influence the activity of MNLCs. These results indicate ADSCs can be differentiated to a functional MNLCs phenotype and may be benefit for treatment of motoneuron diseases.

Keywords: Car Accident, Retinoic Acid, Sonic Hedgehog, Motoneuron, Fura-4.

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