

Evaluating the effects of NGF, RA and Shh growth factors on murine embryonic stem cell differentiation into neurons and oligocytes

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Abstract

Introduction: Embryonic stem (ES) cells are pluripotent cells that can differentiate into all types of specific adult cells and are used for cell therapy in certain diseases. It seems that the inductive factors may be used to produce neurons and gliocytes out of ES cells for the treatment of some nervous system diseases in future. In this research, pluripotent and undifferentiated murine embryonic cells were cultured and effects of the growth factors NGF, RA and Shh were studied for the induction of neural and glial differentiations.

Materials & Methods: CCB ES cell line derived from mouse strain 129 were cultured on inactivated embryonic fibroblasts from C57/BL6 mice and embryonic bodies (EBs) were prepared and transplanted onto culture plates covered by fibronectin and growth factors NGF, Shh and RA with respective concentrations of 50 and 100 ng/ml, 300 and 500 ng/ml and 1 μM and in some groups bFGF with a concentration of 20 ng/ml were added to the specific culture medium for neural cell precursors to induce cell differentiation into neural cells. For studying differentiation into many types of neurons and oligodendrocyte, gene expression of specific neural genes such as nestin, Nkx2-2, Nurr1, S100 and Olig-2 were assessed by RT-PCR and immunocytochemistry assays were used to confirm the presence of MAP-2 protein.

Results: In this study, genetic evaluations showed that each of the previously mentioned growth factors activate some molecular mechanisms that have essential effects on differentiation into different kinds of nervous system cells by affecting the expression of marker genes. Cytochemistry of these cells by monoclonal MAP-2 antibody showed the resultant cells have dendrites that are specific for neural cells or neurons.

Conclusion: The findings indicated that pluripotent and undifferentiated CCB ES cells underwent differentiation into neural cells and oligodendrocytes under the effects of NGF, Shh and RA growth factors and this neural induction was confirmed by the presence of molecular and antigenic markers in these cells. Each of these factors was effective in the induction of differentiation of specialized neural cells and even different concentrations of the factors induced particular cell differentiations.

Key Words: Murine, Embryonic stem cells, Differentiation, Neuron, RA, NGF, Shh.

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