P-6 In vitro Neuronal Cell Differentiation Derived from Human Carcinoma Stem Cells

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Objectives: Human teratocarcinoma stem Ntera2 cells are able to generate post-mitotic neurons and for this reason these cells can provide an important tool to study human neurogenesis in vitro. This study was to examine whether highly purified neuron populations can be obtained from the Ntera2 cells.

Methods: For neuron differentiation, 2×10^6 Ntera2 cells were plated on 0.05% PEI coated 100mm dish in advanced Dulbecco's modified Eagle's medium/F12 and treated with 10 μM all-trans retinoic acid (RA) for 4 weeks (phase 1). Following RA treatment, the neural precursor cells were re-plated onto poly orinithin plus lamimin (P-O/L) coated dish and neuron cells were selected in N2 medium including 0.001mM cytosine arabinoside, 0.01 mM fluorodeoxyuridine and 0.01 mM uridine for 4 weeks (phase 2). For the production of mature neuron cells, phase 2 cells were re-plated on P-O/L dish in N2 medium for 1-3 weeks (phase 3). Neuronal gene and protein expression during the neuron differentiation of Ntera2 cell were analyzed by RT-PCR and immunocytochemistry, respectively.

Results: After treatment of RA to the Ntera2 cells for 4 weeks, nestine expression of neural progenitor marker was strongly detected. In the next step, treatment with mitotic inhibitors for 4 weeks induced robustly increased expression of Pax2, Pax6, Tubuline, MAP2 and GFAP of neuron differentiating Ntera2 cells, while nestin expression was decreased. In addition, treatment of RA for 4 weeks and subsequent mitotic inhibitors for 4 weeks showed that strong positive expression of NCAM, MAP2, Tuj1 and GFAP protein and negative expression of nestine protein by immunocytochemistry. Also, robust A2B5 expression was detected in the additional culture of N2 medium for 2 weeks of phase 3.

Conclusion: This result indicates that RA and mitotic inhibitors treatment can induce efficiently higher numbers of neuron differentiation from Ntera2 cells. Thus, information of the expression pattern of different neuronal genes during Ntera2 commitment could be used to investigate alterations in molecular pathways involved in the human neuronal differentiation.