P53 Functional beatings after chemical-induced differentiations of myoblast and cardioblast

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Objectives: The final differentiation of myo- and cardioblast should be specified by the appearance of the spontaneous beating cells. Although many studies reported the differentiation of the progenitor cells, few reports were found to demonstrate the functional differentiation such as cardiac beating using C2C12 and H9C2 cells.

Materials and Methods: To differentiate the myo- & cardioblast to muscle and cardiac-like cells, respectively, several drugs were tested to obtain efficient functional beating. The cells were differentiated in low serum medium with and without dimethulsulfoxide (DMSO), retinoic acid (RA), dibutyryl cyclic adenosine monophosphate (dbcAMP) or lithium chloride (LiCl). After differentiation, the cells analyzed by immunocytochemistry and RT-PCR using various biomarkers including α-cardiac actin, atrial natriuretic peptide, α-myosin heavy chain, GATA4, cardiac troponin I, cardiac troponin C and α-sarcomeric actin, and the appearance of beating activity.

Results: Sub-confluent cells were induced to differentiate into muscle-like cells at 4 days after culture in differentiation medium. Cells had a muscle-like morphology except for DMSO. In both dbcAMP and LiCl, the cells showed more advanced morphology of differentiation than those in other chemicals. When these cells were stained with the markers, no muscle-like morphology was also found in DMSO-treated cells. After 12 days of differentiation, beating cells were observed in RA-, dbcAMP- and LiCl-treated cells. The cells in LiCl had more beating cells than those in other chemicals. RT-PCR analysis also showed a similar biomarker expressions.

Conclusions: These results suggest that LiCl may be helpful for differentiations of myoblast and cardioblast into uniform functional units.

Key words: C2C12, H9C2, cardiac differentiation, RT-PCR, beating

P54 Expressions of a Mouse Neurotrimin Promoter-driven GFP/LacZ Dual Reporter in Differentiating Neural Cell Lineages

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Objectives: Neurotrimin (Ntm) is a member of the family of neural cell adhesion molecules. Its expression pattern suggests that Ntm promotes axonal fasciculation, guides nerve fibers to specific targets and stabilizes synapses with synaptogenesis. We previously reported mouse Ntm cDNA (AF282980) and established its expression patterns in the hippocampus and the cerebellum of adult brain. To determine cellular specificity and regulation of Ntm in developing synaptogenesis, we analyzed newly cloned promoter of Ntm in mouse primary brain cells or mouse N2a neuroblastoma cells.

Materials and Methods: To begin with, we cloned the 5'-upstream region (promoter) of 2.5kb inserted into a vector containing a GFP/LacZ dual reporter, designated pNtmGL. And we analyzed expression of pNtmGL in mouse primary brain cells or mouse N2a neuroblastoma cells with neural stem cell and neuronal cell lineage markers, Nestin, Tuj1, MAP2b and GFAP.

Results: The pNtmGL was used to demonstrate its promoter activity and cell-specific expression in both mouse primary brain cells and N2a cells *in vitro*. The results showed that simultaneous expressions of GFP/LacZ are found with early neural cell marker, Tuj1 in pNtmGL-transfected primary brain cells. When the transfected N2a cells were analyzed by immunocytochemistry and RT-PCR with neural cell lineage markers including nestin, Tuj1 and MAP2b during differentiation, GFP-LacZ and either nestin or Tuj1 were concurrently expressed in neurally-differentiating N2a cells. Through chemical agent treatment study, we found that 2.5kb 5' upstream sequence of Ntm promoter containing cAMP response element is activated by dibutyryl cyclic AMP and 17β-estradiol.

Conclusions: Taken these together, the cell-specific promoter activity and expression of NtmGL suggest that the expression of the dual reporter gene should allow us to establish an endogenous Ntm expression patterns not only in neural differentiation but also during the developing nervous system. These results also suggest that the Ntm promoter can be used as a crucial marker for neural stem cells and neurons.

Key words: Ntm promoter, GFP/LacZ (GL) dual reporter, pNtmGL