P-32 Alteration of Gene Expression Pattern during the Course of Differentiation of hES Cells into Neural Cells

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Objective: Studying molecular mechanisms that regulate cellular differentiation is very important to define differentiation stages of hES cells. Using a microarray-based expression analysis, we have examined differential gene expression profiles during hES (MB03) cell differentiation into neural cells.

Materials and Methods: For microarray, RNA was isolated from each stages of differentiation in vitro; i) undifferentiated hES cells, ii) embryoid bodies (EBs) forming stage, iii) differentiating stage, iv) differentiated cell stage. Growth conditions in each stages were as follows; EBs (for 4 days) were produced from hES cells in suspension culture, differentiating stage cells were in N2 medium supplemented with 20 ng/ml of b-FGF for 7 days on gelatin coated dish, and then finally differentiated cells were grown in N2 neural cell differentiation medium without b-FGF for 2 weeks. To examine gene expression profile during the course of hES cell differentiation, mRNAs from four stages were labeled and competitively hybridized to cDNA chip containing 3,000 mesenchymal cell derived gene sequences.

Results: Analysis of cDNA microarray identified 17 up-regulated genes and 4 down-regulated genes from stage i to ii, 6 up-regulated genes and 8 down-regulated genes from stage ii to iii, while 2 up-regulated genes and 2 down-regulated genes from stage iii to iv. These changes were reproducible and represent changes in the expression of development and differentiation enhancing factor, translation initiation factor, and cell surface antigens.

Conclusion: These identifications provide clues in understanding important molecular information for further functional studies of hES cell differentiation, although it was not sufficient to evaluate the large-scale changes in gene expression during neural cell differentiation.

P-33 *In vitro* Differentiation of P-mES and mES Cells into Functional Neurons

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Objective: This study was to investigate the probabilities of parthenogenetic mouse embryonic stem (P-mES02) cells and *in vitro* fertilization mES (mES01) cells differentiating into tyrosine hydroxylase expressing neuronal cells *in vitro* by several different factors.

Materials and Methods: P-mES or mES cells were cultured in ES media without mLIF 4 days until ES cell aggregations (embryoid bodies, EBs) were formed. To induce the neural cell differentiation *in vitro*, the