P-47 Human Embryonic Stem Cells Experience a Typical Apoptotic Process upon Oxidative Stress

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Background & Objectives: Embryonic stem (ES) cells, derived from preimplantation oocytes, are able to differentiate into various types of cells consisting the whole body, or pluripotency. In addition to the plasticity, ES cells are expected to be different from terminally differentiated cells in very many ways, such as patterns of gene expressions, ability and response of the cells in confronting environmental stimulations, metabolism, and growth rate. As a model system to differentiate these two types of cells, ES cells (MB03) and terminally differentiated cells (HeLa).

Method: we examined the ability of these two types of cells in confronting a severe oxidative insult, that is H_2O_2. Ratio of dying cells as determined by the relative amount of dye neutral red entrapped within the cells after the exposures.

Results: Cell death rates were not significantly different when either MB03 or HeLa were exposed up to 0.4 mM H_2O_2. However, relative amount of dye entrapped within the cells sharply decreased down to 0.12% in HeLa cells when the cells were exposed to 0.8 mM H_2O_2, while it was approximately 54% in MB03. Pretreatment of cells with BSO (GSH chelator) and measurement of GSH content results suggest that cellular GSH is the major defensive mechanism of human ES cells. Induction of apoptosis in ES cell was confirmed by DNA laddering, induction of Bax, and chromatin condensation.

Conclusions: In summary, ES cells 1) are extremely resistant to oxidative stress, 2) utilize GSH as a major defensive mechanism. and 3) experience apoptosis upon exposure to oxidative stress.

P-48 Genetically Modified Human Embryonic Stem Cells Expressing Nurr1 and Their Differentiation into Tyrosine Hydroxylase Positive Cells in vitro

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Background & Objectives: As an effort to direct differentiation of human embryonic stem (hES, MB03) cells to dopamine-producing neuronal cells, Nurr1 was transfected using conventional transfection protocol into MB03 and examined the expression of tyrosine hydroylase (TH) after differentiation induced by retinoic acid (RA) and ascorbic acid (AA).

Method: Experimentally, cells were transfected with linearized Nurr1 cDNA in pcDNA3.1(+)-hyg overnight followed by selection in medium containing hygromycin-B (150 μg/ml). Expression of Nurr1

mRNA was confirmed by RT-PCR and protein by immunocytochemistry in the drug resistant clones. In order to study the effect of Nurr1 protein on the differentiation pattern of ES cells, one of the positive clones (MBNr24) was allowed to form embryoid body (EB) for 2 days and were induced to differentiate for another 4 days using RA (1 μM) and AA (50 mM) (2-/4+ protocol) followed by selection in N2 medium for 10 or 20 days.

Results: After 10 days in N2 medium, cells immunoreactive to anti-GFAP, anti-TH, or anti-NF200 antibodies were 38.8%, 11%, and 20.5%, respectively. After 20 days in N2 medium, cells expressing GFAP, TH, or NF200 were 28%, 15% and 44.8%, respectively but approximately 9% of MB03 expressed TH protein when the cells were induced to differentiate using a similar prorocol.

Conclusions: These results suggest that ectopic expression of Nurr1 enhances generation of TH+ cells as well as neuronal cells when hES cells were differentiated by 2-/4+ protocol.

P-49 Improvement of Motor Behavior of Parkinson's Disease Animal Model by Nurr1-transfected Human Embryonic Stem Cells

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Background & Objectives: The purpose of this study is to evaluate an efficacy of in vitro differentiated human embryonic stem (hES, MB03) cells expressing Nurr1 in relief of symptomatic motor behavior of Parkinson's disease (PD) animal models.

Method: MB03 was genetically modified to express Nurr1 protein and was induced to differentiate according to 2-/4+ protocol using retinoic acid and ascorbic acid. The differentiation-induced cells were selected for 10 to 20 days thereafter in N2 medium. Upon selection, cells expressing GFAP, TH, or NF200 were 38.8%, 11%, and 20.5%, respectively. In order to examine therapeutic effects of the differentiated cells in PD animal model, rats were unilaterally lesioned by administration of 6-hydroxydopamine HCl (6-OHDA) into medial forebrain region (MFB, AP -4.4 mm, ML 1.2 mm, DV 7.8 mm with incision bar set at -2.4 mm), as a reference to bregma and the surface of the skull. Confirmation of successful lesion by apomorphine-induced rotational behavior, differentiated cells were transplanted into the striatum (AP 1.0, ML 3.5, DV -5.0; AP 0.6, ML 2.5, DV -4.5).

Results: Improvements of asymmetric motor behavior by the transplanta- tion were examined every two weeks after the surgery. In two weeks, numbers of rotation by the experimental rats were -14.8+33.9% ($p<0.05$) of the number before transplantation, however, the ratio increased slightly to 13.6+56.3% in six weeks. In contrast, the ratio of sham-grafted animals ranged from 112.3+8.5% to 139.2+28.9% during the examination.

Conclusions: This result suggests that MBNr24 cells differentiated in vitro survived at least for 6 weeks when grafted into brains of PD animal model, and that symptomatic motor behavior was improved.