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