

Glutathione is the Major Defensive Mechanism against Oxidative Stress in Human Embryonic Stem Cell

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Embryonic stem (ES) cells, derived from preimplantation embryo, are able to differentiate into various types of cells consisting the whole body, or pluripotency. In contrast, terminally differentiated cells do not usually alter their nature but frequently die or transform if they are exposed to inappropriate external stimulations. 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, human ES cells (MB03) and terminally differentiated cells (HeLa), we examined the ability of these two types of cells in confronting a severe oxidative insult, that is H₂O₂. Approximately 1×10^4 cells were plated in 96 well plate and serum starved for overnight. The conditioned cells were exposed to a various concentration of H₂O₂ for 24 hrs and loaded with neutral red (50 μ g/ml) for 4 hrs, washed with PBS for 2 min three times, and entrapped dye was dissolved out using acetic ethanol. Cytotoxicity was determined by reading the amount of dye in the medium using microplate reader equipped with 575 nm filter. Relative amount of the dye entrapped within MB03 or HeLa were not significantly different when cells were exposed up to 0.4 mM H₂O₂. However, this sharply decreased down to 0.12% in HeLa cells when the cells were exposed to 0.8 mM H₂O₂, while it was approximately 54% in MB03 suggesting that this concentration of H₂O₂ is the defensive threshold for HeLa cells. The resistance to oxidative stimulation reversed, however, when cells were co-treated with BSO (L-buthionine- [S,R] -sulfoximine) which chelates intracellular GSH. This result suggests that cellular GSH is the major defensive mechanism of human ES cells. Induction of enzymes involved in GSH metabolism and type of cell death is currently being studied.

Key words) *Oxidative stress, Defensive mechanism, GSH (Glutathione)*