Animal Biotechnology in Bioindustry : Why and How?

Seungkwon You

Division of Biotechnology and Genetic Engineering, College of Life & Environmental Science, Korea University

Normal cells proliferate generally a limited number doublings in culture and only rarely have they been shown to overcome cellular senescence and crisis stages, and immortalize spontaneously. I have established a number of non-virally and non-chemically immortalized embryo fibroblastic (EF) cell lines in continuous cell culture. These include the spontaneously immortalized cell line, DF-1 and several immortal EF cell lines derived from various embryonic tissues. I have previously demonstrated that all of the immortal EF cells established have rapid cell proliferation capacity compared to primary EF cells, presumably due to the deregulation of cell cycle regulators such as p53, E2F-1 and the numerous cyclins. DF-1 cells, in particular, were shown to proliferate more rapidly under normal culture conditions compared to other immortal EF cells, implicating other mechanisms may be important for regulating their growth. The possible mechanism(s) underlying the accelerated growth of DF-1 cells will be addressed in this study.

Briefly, the reactive oxygen species (ROS) are known as endogenous toxic oxidant damaging factors in a variety of cell types, and in response, the antioxidant genes have been implicated in cell proliferation, senescence, immortalization, and tumorigenesis. The expression of manganese superoxide dismutase mRNA was shown to increase in most of the immortal chicken embryo fibroblast (CEF) cells tested, while expression of catalase mRNA appeared to be dramatically decreased in all immortal CEF cells compared to their primary counterparts. The copper-zinc superoxide dismutase and glutathione peroxidase expressed relatively similar levels in both primary and immortal CEF cells. As primary and immortal DF-1 CEF cells were treated with 10-100 uM of hydrogen peroxide (concentrations known to be sub-lethal in human diploid fibroblasts),

immortal DF-1 CEF cells were shown to be more sensitive to hydrogen peroxide, and total cell numbers were dramatically reduced when compared with primary cell counterparts. This increased sensitivity to hydrogen peroxide in immortal DF-1 cells occurred without evident changes in either antioxidant gene expression, mitochondrial membrane potential, cell cycle distribution or chromatin condensation. However, the total number of dead cells without chromatin condensation was dramatically elevated in immortal DF-1 CEFs treated with hydrogen peroxide, indicating that the inhibition of immortal DF-1 cell growth by low concentrations of hydrogen peroxide may be due to increased necrotic cell death, but not apoptosis.

Furthermore, of the numerous genes analyzed, three mitochondrial-encoded genes (ATPase 8/6, 16S rRNA, and cytochrome b) were shown to express at higher levels in DF-1 cells compared to other primary and immortal CEF cells. The inhibition of mitochondrial translation by treatment of chloramphenicol markedly decreased ATP production and cell proliferation in DF-1 cells, while not effecting growth in either primary or other immortal CEF cells. This result suggests a correlation between rapid cell proliferation and the increased mitochondrial respiratory functions. We also determined that the increased transcripts of mitochondrial-encoded genes in DF-1 cells is due to the increased de novo transcript synthesis as shown by mitochondrial run-on assays, but not the results of either increased mitochondrial biogenesis or mitochondrial transcript half-lives. Taken together, the present studies suggest that transcriptional activation of mitochondrial-encoded genes and elevated respiratory function as well as the balanced antioxidant function might be important for cell proliferation in response to toxic oxidative damage by hydrogen peroxide and should be one of characteristics of rapidly dividing immortal cells.