P19 Effects of letrozole on proliferation and apoptosis in cultured leiomyoma cells treated with prostaglandin E2

Myoung-Seok Han, M.D., Moon-Seok Cha, M.D., Ji-Young Kim,* M.Sc., Jong-Min Kim,* Ph.D.

Department of Obstetrics and Gynecology, Dong-A University, College of Medicine, Busan, Korea, Department of Anatomy and Cell Biology, Dong-A University, College of Medicine, Busan, Korea

Objectives: This study aimed to elucidate the direct effect of letrozole on proliferation and apoptosis of cultured leiomyoma cells treated with prostaglandin E2 (PG E2)

Materials and Methods: Three leiomyoma tissues were obtained from three patients and these leiomyoma cells were cultured *in-vitro*. Percentages of antiproliferative cells were evaluated by the MTT assay and the apoptosis was assessed with sub-G1 cell counts by flowcytometry and Western blot analysis.

Results: Treatment with 100 uM letrozole to the cultured leiomyoma cells for 48 hours showed 52.10 ± 8.52% of cell survival, assayed by MTT, but did not induce the apoptosis. Combined treatment with 100 uM letrozole plus 10 uM PG E2 for 48 hours resulted in a significantly lower percentage of survival: 25.94 ± 4.46% and significant percentages of apoptosis were induced: 31.55 ± 4.43%. This apoptotic cell rate was compared with that of letrozole only treated cell group: 5.91 ± 1.08%, which were assessed through sub-G1 cell analysis by the flowcytometry. The increased expression of cleaved caspase-3 was found in letrozole plus PG E_2 treated cell group and the increased expression of aromatase was found in PG E_2 treated cell group on the basis of Western blot analysis.

Conclusions: The present results demonstrated that letrozole inhibits the growth and induces apoptosis of leiomyoma cells through blocking the aromatase up-regulated by PG E_2 treatment. These findings support the further investigation of aromatase inhibitor as a medical treatment option in leiomyoma.

Key words: leiomyoma, aromatase inhibitor, apoptosis, prostaglandin, MTT

P20 Guided Differentiation of Human Embryonic Stem Cells into Hepatocytes: From Endoderm Specification to Hepatic Maturation

Woo DH, Kim SE, Kim BK, Gil JE and Kim JH

Division of Biotechnology, College of Life Sciences & Biotechnology, Korea University, Seoul, Korea

Objectives: Human embryonic stem (hES) cells are an attractive system for the study of human development and may be a source for the regenerative medicine including liver diseases. Although previous studies have demonstrated the potential of hES cells to differentiate into a wide spectrum of cell types, few reports have focused on the differentiation of hES cells into hepatic lineage. Here, we directly determine if the early specification of endoderm from hES cells and if the subsequent hepatic differentiation can be achieved from this source.

Materials and Methods: To activate Wnt/β-catenin signaling pathway embryoid bodies (EBs) were culture with or without lithium chloride (LiCl) during EB formation (day2~day4). To induce hepatic differentiation, the LiCl-treated EBs were plated onto laminin-coated plate and further differentiated in the presence or absence of 20 ng/ml hepatocytes growth factor (HGF) or 10 ng/ml oncostatin M (OSM). RT-PCR and immunostaining were performed to analyze the expression patterns of genes and proteins, involved in the germ layer formation and hepatic differentiation. We also confirmed the functional activities of hES cell-derived hepatocytes by γ-glutamyl transpeptidase (GGT) staining, indocyanine green (ICG) uptake test, and periodic acid schiff (PAS) staining as well as biochemical analysis for urea secretion and glucose production.

Results: Exposure to LiCl at an appropriate concentration induced nuclear translocation of β-catenin in hES cell-derived EBs and coincidentally up-regulated expressions of endoderm-specific genes (Foxa2, Sox17, and GATA4) and early hepatic genes (Hex, Hnf4, and Prox1). Those endodermal EBs were efficiently differentiated into functional hepatocytes by HGF. hES cell-derived hepatocytes highly expressed liver-specific genes such as albumin, α-fetoprotein, α1-antitrypsin, transthyretin, and thrombin-antithrombin as well as liver-specific protein such as albumin, α-fetoprotein, GATA4, cytokeratin 8, and cytokeratin 18. The hES cell-derived cells also showed the physiological and biochemical activities that are similar to those of hepatocytes in the body.

Conclusions: Our results reveal that the definitive endoderm can be derived from hES cells in defined culture conditions by activating β-catenin signaling, and show that HGF could further differentiate the endoderm into functional hepatocytes. These findings are expected to facilitate the use of hES cells as a useful tool in the regenerative medicine and drug discovery.

Key words: Human Embryonic Stem Cells, Endoderm, Hepatocyte, HGF, LiCl