D109 Ceramide-Induced Apoptotic Cell Death in Mouse Granulosa Cells

Jong-Hoon Kim*, GyoungMi Kim, Sang Soo Kim and Yong-Dal Yoon
Department of Biology, College of Natural Sciences, Hanyang University

In mammalian ovary, the majority(>99%) of ovarian follicles undergo atresia. Recent studies have shown that this phenomenon is mediated via granulosa cell apoptosis. Ceramide, a product of sphingomyelin hydrolysis, has been proposed as a novel lipid second messenger with specific roles in mediating antiproliferative responses including apoptosis and cell cycle arrest. In this present study, we have examined the effect of ceramide on apoptotic cell death of granulosa cells *in vitro*. The granulosa cells were harvested by squeezing the antral follicles from the immature mice(3-4weeks) and cultured in MEM medium with 10% fetal bovine serum. The cells were treated with various concentrations of ceramide(0 to 50μM)or dihydroceramide and cultured for 24h. Cell death was determined by MTT cell viability assay and immunohistochemical(IHC) staining for proliferating cell nuclear antigen(PCNA). Apoptosis was evaluated by fluorescent staining, *in situ* 3'-end labeling(TUNEL), DNA fragmentation assay, and flowcytometry. Ceramide treatment induced apoptotic cell death in granulosa cells in time- and concentration-dependent manners(12.5μM), but dihydroceramide, closely related lipid analog of ceramide, had no effect. The PCNA stain was decreased clearly in dose-dependent way. The flowcytometric analysis showed the significant increases of A_0 cells by ceramide treatments. These results provide evidence for ceramide as lipid second messenger of apoptosis and suggest that ceramide may interact specifically with an intracellular in mouse granulosa cell.

D110 Regulation of Mouse Follicular Bad and Bax Gene Expressions by Steroids

Han Seung Kang, Wae Lee Kim, Chang Sook Shin
and Sung Goo Kang
Department of Biology, Inje University

The purpose of this study was to investigate the effects of steroid hormones on the expression of bad and bax genes. 20 day-old mice ovaries were obtained at 0, 24, 48, 72 hours time intervals after the injection of PMSG. The expressions of both genes in ovary were decreased at 48 hours after PMSG injections. Also the preovulatory follicles cultured for 24 hours in plain culture media, the expression of bad and bax genes was increased. We have found that treatment with testosterone increased the expression of bad and bax genes of preovulatory follicles in a dose-dependent manner. In contrast, each of progesterone and estogen suppressed expression of bad and bax genes.

These data suggest that gonadal steroid hormones may play an important roles in the regulation of mouse follicular differentiation via the expressions of bad and bax genes. (HRC - 96)