論　文　の　内　容　の　要　旨

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論文題目　　A Study on the Role of Ski in Regulating Ovarian Function
（卵巣機能調節における Ski の役割に関する研究）

The establishment of cell associations and interactions is an important component of tissue remodeling, tissue morphogenesis and development. The ovary, probably more than other organs, exemplifies such events. Changes in cell associations and segregation of cell populations are characteristics of follicular development, ovulation, formation of corpus luteum (CL) and the demise of CL. The granulosa cells of the ovarian follicle have a number of options available to them, namely, to remain quiescent, to proliferate, to differentiate, or to be eliminated by programmed cell death (apoptosis). The molecular mechanisms that regulate apoptosis during follicular atresia has been extensively studied, and recent studies have suggested that the destiny of the developing follicles (continual growth and eventual ovulation or atresia) is dependent on the fate of the granulosa cell (survival vs. apoptosis), which is determined by the coordinated actions, balance of opposing activities and interactions of cell survival and death factors. It is widely accepted that

gonadotropins either directly or indirectly regulate the expression of many proteins in the ovary, including growth factors, enzymes, and transcription factors that may impact multiple signaling cascades. Although the endocrinological regulatory mechanisms involved in follicular development and atresia have been characterized to a large extent, the precise temporal and molecular mechanisms underlying the regulation of these processes remain unknown.

C-Ski, a proto-oncogene, has been identified as the cellular homologue of *v-Ski* that was originally identified as the transforming gene of the avian Sloan-Kettering retroviruses, which transform chicken embryonic fibroblasts and lead to anchorage-independent growth. Ski protein is a nuclear transcriptional factor that does not bind DNA directly. Due to its unique binding properties with multiple factors, Ski could posses various roles in both the regulation of cellular proliferation and differentiation. Ovary is one of the tissues in which *c-Ski* expression has been identified but the role of this gene is unknown. Therefore, in the present thesis, the author aimed to locate Ski protein in rat ovaries to predict the possible involvement of Ski in follicular development, atresia and luteinization.

In Chapter I, the author examined the localization of Ski in the rat ovaries of the estrous cycles and having single generation of developing and atretic follicles in order to predict the possible involvement of Ski in follicular development and atresia. Ovaries obtained on the day of estrus were subjected to immunohistochemical analysis for Ski and proliferating cell nuclear antigen (PCNA) in combination with terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL). Ski was expressed in granulosa cells that were positive for TUNEL, but negative for PCNA in the follicles during the estrous cycle. Expression of Ski in TUNEL-positive granulosa cells, but not in PCNA-positive granulosa cells, was also verified in immature hypophysectomized rats having a single generation of developing and atretic follicles by treatment with 40 IU of equine chorionic gonadotropin (eCG). On day 2 after eCG administration, where preovulatory follicles with

PCNA-positive granulosa cells were evident, neither Ski- nor TUNEL-positive granulosa cells were observed. On day 4 after eCG administration, a numerous number of follicles with TUNEL-positive granulosa cells appeared, indicating that these follicles were undergoing to atresia. TUNEL-positive granulosa cells in these atretic follicles were mostly positive for Ski, confirming the above findings that Ski is profoundly expressed in the granulosa cells of atretic follicles, but not in growing follicles. Taken together, these results suggest that Ski plays a role in apoptosis of granulosa cells during follicular atresia.

In response to the luteinizing hormone (LH) surge, a preovulatory follicle embarks on a terminal differentiation pathway (luteinization) that transforms granulosa and theca cells of a preovulatory follicle into luteal cells to form CL. Luteinizing follicular cells then undergo specific morphological changes as well as physiological alterations in their transition to luteal cells. In Chapter I, the author demonstrated the presence of Ski in granulosa cells of atretic follicles, suggesting the role of Ski as a proapoptotic factor. The preliminary experiments demonstrated *c-Ski* mRNA is abundantly expressed in luteal cells of the CL, suggesting the possible involvement of Ski in regulating luteinization. Thus, in Chapter Ⅱ, the author examined Ski protein localization in the rat ovaries during ovulation and subsequent CL formation in order to predict the possible involvement of Ski in luteinization. Immunohistochemical analysis of Ski was performed on ovarian sections obtained from rats having single generation of CL. Follicular growth was induced in immature rats with eCG, then followed by 15 IU of human chorionic gonadotropin (hCG) to induce subsequent ovulation and luteinization. In this model, ovulatory rupture occurred 12 h after hCG injection. Ski was expressed in luteinizing granulosa cells at 6 and 12 h after hCG injection, and its expression was persisted after the formation of CL. No Ski-positive granulosa cells were observed in preovulatory follicles unless hCG was injected as was shown in Chapter I. Quantitative analysis revealed that the proportion of Ski-positive cells at 24 h after hCG injection was higher than that of

the previous time points (before 12 h), suggesting that the number of Ski-positive cells increased after ovulation. Taken together, the results indicate that Ski expression is induced in granulosa cells by the effect of hCG (LH) and suggest that Ski may also play a role during luteinization of granulosa cells as well as apoptosis of granulosa cells during follicular atresia as shown in Chapter I.

Then, the author examined whether hCG (LH) directly induces gene expression of *c-Ski* in granulosa cells. Unexpectedly, *c-Ski* mRNA was expressed in granulosa cells regardless of hCG (LH) treatment *in vivo* and *in vitro*, and its expression level was unaffected by hCG (LH) treatment. The observation that *c-Ski* mRNA is expressed in granulosa cells before hCG injection, while Ski protein is absent, raises the possibility that the amount of Ski protein is regulated at the translational (including degradation), but not transcriptional, level during luteinization of granulosa cells. Of the factors reported so far, the author picked up *Arkadia* as a possible candidate that regulates Ski protein level, since *Arkadia* is shown to be responsible for the degradation of Ski protein. Thus, the author examined the expression level of *Arkadia* mRNA during luteinization of granulosa cells by real-time PCR. Contrary to the author's expectation, the level of *Arkadia* mRNA expression was unchanged during luteinization of granulosa cells, suggesting that contribution of *Arkadia* in the regulation of Ski protein level during luteinization might be little if any.

The results obtained in the present thesis clearly demonstrated that Ski protein is expressed in granulosa cells during follicular atresia and luteinization, but not in those in proliferating phase, suggesting that Ski plays multiple roles in apoptosis and differentiation of granulosa cells. In addition, the presence of the novel regulatory mechanism of Ski protein in response to hCG (LH) at translational or degradation level was also suggested. Further studies to explore the precise role of Ski as well as its regulatory mechanism must bring a new insight to understand the follicular atresia and postovulatory luteinization.