10:00~11:40 SESSION (I) 좌 장 : 조동제·이상호

Microarray-based Identification of Genes Involved in Early Follicular Development

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When ovarian follicles are formed, they enter a resting, primordial stage that persists for a period of time that varies from follicle to follicle.[1] Most of them remain in a resting stage until they either degenerates or some signals activate them to enter the growth phase during which the follicles undergo a dramatic course of cellular proliferation and differentiation. Initiation of primordial follicle growth is essential for female reproduction. However, the mechanisms that initiate follicular growth of these resting primordial follicles are completely unknown.

Mammalian follicle growth is a complex process regulated by various extraovarian and intraovarian factors.[2] Compared to the many studies on growth and maturation of antral follicles, relatively few have been conducted on the growth of primordial follicles, and factor(s) that control the onset of primordial follicle development is poorly understood.[3,4] To date, the research on the primordial-primary follicle transition has mostly involved functional analysis of the known genes while culturing the neonatal ovaries in vitro. The primordial to primary follicle transition was promoted by adding the following factors to the culture media: kit ligand (KL; 3); nerve growth factor (NGF; 4); growth differentiation factor-9 (GDF-9; 5); basic fibroblast growth factor (bFGF; 6); leukemia inhibitory factor (LIF; 7); insulin[8]; or bone morphogenetic protein-7 (BMP-7; 9). In contrast to these stimulating factors, anti-mullerian hormone (AMH) inhibits primordial follicular growth.[10]

If we could reveal list of genes expressed during early folliculogenesis, particularly at the primordial-primary follicle transition period, it would give insight to study the regulating mechanism of this specific process. Therefore, our objective was to identify differentially expressed the genes exclusively in primordial or primary follicles that may play important roles in the arrest of primordial follicles and/or initiation of their growth into primary follicles. We employed the several genome-wide, high throughput methods of to identify differentially expressed genes between primordial follicles and primary follicles. The suppression subtractive hybridization (SSH) method has been a powerful approach to isolate and identify differential genes in a variety of experimental settings.[11~13] Difficulty in identifying genes responsible for follicle development often arises when a gene is expressed at low levels. We did a highly accurate and sensitive PCR that can be used to amplify the minute amounts of material available in oocytes or embryos using the annealing control primer (ACP; Seegene, Seoul, Korea). This primer anneals specifically to the template and allows only genuine product to be amplified, thus eliminating false-positive results. Using this a new innovative technology, ACP-PCR, we identified genes that were differentially expressed in different systems.[14,15] We found several genes with oocyte-selective or granulosa-

selective expression among the list of genes obtained by ACP-PCR.

Recently, many studies reported listed of genes that are up- and down-regulated at in the between two groups using microarray analysis, and we succeeded in profiling gene expression in the uterus using amplified RNA for the cDNA microarray.[16] By using the same system again, we successfully identified valuable list of genes differentially expressed between primordial, primary, and secondary follicles. We are analyzing their ovarian mRNA and protein expression after we categorized genes by their functions, such as cell cycle, growth and its maintenance, apoptosis, extracellular matrix, cytoskeleton, and signal transduction. We found several genes with oocyte-selective or granulosa-selective expression.

In summary, we obtained list of genes expressed in the mouse ovaries at early stage of follicular development by using SSH and ACP-PCR. We also obtained a list of differentially expressed genes between primordial, primary, and secondary follicles by using DNA Chip analysis. The lists per se and the characterization and/or functional analysis of these genes would provide the insight for the future study on mechanisms involved in primordial-primary follicle transition and theca layer formation in the ovary.

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