PB-05

Identification of Genes Involved in Primordial-primary Follicle Transition by Suppression Subtractive Hybridization

Chang-Eun Park, Se-Jin Yoon, Eun-Hyun Jeon, Young-Hoon Kim,
Sook-Hwan Lee, Kyung-Ah Lee
Infertility Medical Center, CHA General Hospital, College of Medicine,
Pochon CHA University

Recruitment of primordial follicles(PMF) is crucial for female fertility. however, factors and mechanisms that regulate this process is poorly understood. The present study was conducted to obtain an inclusive view of the gene expression and to identify novel factors and their pathways of regulating PMF arrest and/or growth initiation.

Ovaries from one-day neonatal(consists of oocyte and PMF) and five-day old(consists of PMF and primary follicles, PRIF) mice were collected, either total RNA or mRNA was isolated, and suppression subtractive hybridization(SSH) was used to isolate and clone genes that differentially expressed in day 1 and day 5 ovaries. Confirmation that some of these genes are differentially expressed in PMF and/or in PRIF was accomplished by using laser captured microdissection(LCM), RT-PCR, in situ hybridization(ISH) and/or immunohistochemistry(IHC).

In toto, 357 clones were sequenced and analyzed by BLAST and RIKEN program. Sequences of 330 clones significantly matched database entries while 27 clones were novel. Forty-two and 47 different genes were identified as differentially expressed in day 1 and day 5 ovaries, respectively, while 7 genes were expressed in both stages of ovaries. Day 5-subtracted library included several genes known as markers for growing follicles, such as ZP2, MATER, and fetuin. Among the genes with assigned functions, 23.8% was associated with cell cycle/apoptosis regulation, 7.1% with cellular structure, 11.9% with metabolism, 26.2% with signal transduction, and 31.0% with gene/protein expression in day 1; while 10.6%, 17.0%, 23.5%, 25.5%, and 23.4% in day 5, respectively. Genes such as GDF-8, Lats2, Septin2, and Wee1 were the highly expressed genes in PMF, while HSP84, Laminin2, MATER, MTi7, PTP, and Wrn were highly expressed genes in PRIF.

We have successfully discovered list of genes expressed in day 1 and day 5 ovaries and confirmed that some of them are differentially expressed in PMF and/or PRIF. Gene expression profile from the present study would provide insight for the future study on the mechanism(s) involved in primordial-primary follicular transition. *This work was Supported by Korean Health 21 RND Project, Ministry of Health and Welfare, Korea (01-PJ10-PG6-01GN13-0002).*

Key words) ***Primordial follicle, Primary follcile, Suppression subtractive hybridization, Laser captured microdissection***