Method: Northern blot analysis of ovaries obtained from PMSG/hCG-treated immature animals revealed the rapid and transient induction of Nurr1 and Nor1 transcripts by hCG, reaching a maximum at 1 h, and decline to control levels at 3 h.

Results: The major cell types expressing NGFI-B subfamily, such as NGFI-B, Nurr1 and Nor1, were granulosa cells of preovulatory follicles and theca/interstitial cells. However, unlike the expression of NGFI-B in theca cells, Nurr1 and Nor1 are not present in theca cells in the ovaries obtained 2 days after PMSG treatment. In preovulatory follicles cultured in serum-free medium, Nurr1 and Nor1 transcripts were transiently induced by LH, reaching a peak within 1 h.

Conclusions: These results demonstrate the cell type-specific expression and gonadotropin induction of Nurr1 and Nor1 in granulosa cells of preovulatory follicles, similar to that of NGFI-B. We suggest that Nurr1, Nor1 and NGFI-B share many roles during ovulatory process.

O-18(기초) Ubiquitin Specific Protease USP-t and its Putative Substrates in Mouse Testis

Eung-Ji Lee, Minu Seong, Myung-Sun Kim, Yong-Soo Kim, Min-Young Lee, Kwang-Hyun Baek

Graduate School of Life Science and Biotechnology, Pochon CHA University

Background & Objectives: Ubiquitination and deubiquitination are critical protein modification systems in cellular metabolism and homeostasis. Several deubiquitinating enzymes are known to function as important regulators in signal transduction. In this experiment, we analyzed characteristics of USP-t (Ubiquitin-Specific Protease-Testis specific) and candidate binding proteins.

Method: We searched several conserved domains of USP-t and identified expression pattern at RNA and protein level using northern blot and western blot analysis. To know whether USP-t has deubiquitinating activity or not, we performed Ub-β-gal assay and using Nedd8 construct, we identified deneddylating activity of USP-t. And then, using immunostaining analysis, we identified expression pattern of USP-t within the testis. For finding binding proteins of USP-t, we performed Co-IP. And from the result, we found several candidate binding proteins of USP-t including HSP90. The binding between USP-t and HSP90 were conformed by IP/western blot analysis and Co-localization analysis.

Results: USP-t has two CS domains, a putative Hsp90 binding domain, and the cellular localization of USP-t was merged with that of Hsp90 in the cytoplasm. The catalytic domain of USP-t contains a zinc finger domain, and Ub-β-galactosidase assay revealed that it deubiquitinates the monoubiquitin moiety of ubiquitin-β-galactosidase, but does not reduce the global pool of polyubiquitin chains. Transcripts of USP-t were highly expressed in the testis, heart, and muscle, but USP-t proteins were detected only in the testis. The immunostaining analysis revealed that USP-t is highly expressed in the meiotic germ cells in the testis. And using co-immunoprecipitation assay we identified several putative substrates including cadherin, leucine rich repeat neuronal protein, zinc finger protein, coronin and elongin.

Conclusions: A deubiquitinating enzyme USP-t is highly expressed in the testis at RNA and protein levels and it has both deubiquitinating activity and deneddylating activity. USP-t interacts with Hsp90 in vivo and is co-localized with Hsp90 in the cytoplasm. Also, USP-t is highly expressed in the meiotic germ cells within the testis and we identified several binding proteins interacting with USP-t.

O-19(기초) Evaluation of DNA Extraction Methods to Assess Amplification Rate from Single Cells for Preimplantation Genetic Diagnosis

Jung Ah Yoon[1], Sung Ah Kim[1], Moon Joo Kang[1], Sun Kyung Oh[1], Tae Eun Kim[2], Jong Kwan Jun[1,2], Seung-Yup Ku[1,2], Seok Hyun Kim[1,2], Young Min Choi[1,2], Jung Gu Kim[1,2], Shin Yong Moon[1,2]

[1]Institute of Reproductive Medicine and Population, Medical Research Center,
[2]Department of Obstetrics and Gynecology, College of Medicine,
Seoul National University

Background & Objectives: In preimplantation genetic diagnosis (PGD), a rapid and accurate assay has been required. We have therefore evaluated methods of preparation of DNA from single cells for amplification and preimplantation genetic diagnosis.

Method: We designed Dystrophin gene exon 51 and sex determination gene ZFX/ZFY multiplex primer pairs that can work well together from single lymphocyte in one reaction tube. Amplification efficiencies were compared between DNA extraction by (A) lysed in distilled water at 96℃ for 15 mins followed by 10℃ 15 mins; (B) lysis in distilled water with freeze-thaw liquid nitrogen, then boiling; and (C) alkaline lysis buffer (ALB; 200 mmol/l KOH, 50 mmol/l dithiothreitol), heated to 65℃; (D) Proteinase K/SDS (17 μM Sodium Dodecyl Sulfate, 125 μg/ml Proteinase K), heated to 96℃ for 15 mins followed by 10℃ 15 mins.

Results: The efficiency of the DNA amplification from single lymphocytes was 94.1% following method A; 90.1% with B; 99.0% with C; and 100% with D. Results of amplification rate of dystrophin gene exon 51 was 96.0%, 88.0%, 100% and 100%, and amplification rate of sex determination gene ZFX/ZFY was 92.3%, 92.3%, 98.0% and 100%, respectively.

Conclusions: The Proteinase K/SDS lysis method was most efficient for extracting DNA from a single cell and should be particularly useful for preimplantation genetic diagnosis.