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

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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.