P23 Identification of Oocyte-specific Diva-associated Proteins using Immunoprecipitation followed by Mass Spectrometry

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Objective: Previously, we have found that Diva is highly expressed in matured MII oocytes compared to immature GV oocytes in mouse (Yoon et al., 2005). We have characterized Diva as an ovary- and oocyte-specific transcript with negative correlation to the ovarian apoptotic cell death. In the present study, we identified the binding partner(s) of Diva by using immunoprecipitation and Mass Spectrometry (MS) analysis.

Materials and methods: We have used the overexpressed Flag-Diva system because finding of endogenous Diva-binding proteins in mouse ovaries was not successful with commercially available anti-Diva antibody. NIH/3T3 cells were transiently transfected with Flag-tagged mouse Diva construct for 24 hr, and immunoprecipitated with anti-Flag antibody. Untransfected NIH/3T3 cells were used as control. The immuno-isolated complexes were resolved by SDS-PAGE on a 12% gel followed by Coomassie Blue staining. For in-gel digestion, 15 bands of interest were excised manually and digested with trypsin. All mass spectra were acquired at a positive reflector mode by a 4700 Proteomics Analyzer (Applied Biosystems, Framingham, MA). Proteins were identified by searching the NCBI nonredundant database using MASCOT Peptide Mass Fingerprint software (Matrixscience, London).

Results: Diva-associated complexes were identified that formed in Flag-tagged mouse Diva-overexpressed NIH/3T3 cells via immunoprecipitation using anti-Flag-conjugated beads. Among the excised 15 bands, actin and actin-binding proteins such as tropomyosin, tropomodulin 3, α-actinin, and gelsolin were identified. Binding of Diva with actin and tropomyosin was confirmed by using immunoprecipitation followed by Western blotting. Both bindings were also detected endogenously in mouse ovaries indicating that Diva works with actin and tropomyosin.

Conclusions: This is the first report that immuno-isolated Diva-associated complexes are related to actin filament of the cytoskeletal system. Based on the results of the present study, the association of Diva with actin and tropomyosin, we suggest the role of oocyte-specific Diva in modulating the cytoskeletal systems during oocyte maturation. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ10-PG6-01GN13-0002).

P24 Down-regulation of PRDX2 Gene in Blastocysts from the Vitrified Mouse 2-cell Embryos

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Objectives: Expression and activity of peroxiredoxin 2 ($PRDX_2$), one of the antioxidant enzymes is regulated depending on the culture condition to regulate reactive oxygen species (ROS) toxicity and cell signaling. Frozen-thawed embryos are showed increased ROS production. However it is largely unknown the PRDXs mRNA expression level in the mouse blastocysts and the effects of vitrification on the PRDXs expression by the embryonic stages. In this study, we analyzed the PRDX2 expression in vitrified-thawed embryos after cultivation to blastocyst(98 hr time point after hCG injection) and analyzed embryonic development by the vitrified embryonic stages.

Materials and methods: 2-cell embryos were collected at 46 hr from oviduct of 6 weeks old ICR female mice primed with PMSG and hCG after 48 hr. Vitrification for 2-cell (post hCG48 hr), 8-cell (68 hr), and blastocyst (94hr) embryos was done as mentioned by Kuwayama and his colleague (2000) and modified straws. After thawing, each stage embryos were cultured additively up to 98hr time point post hCG injection. Expanded blastocyst were collected and then stained with Hoechst 33342 to count the cell number. Expression of PRDX2 mRNA was analyzed with quantitative RT-PCR.

Results: The developmental rate of the fresh 2-cell embryos was 88.6%. In contrast to that of the control, frozen-thawed embryonic developmental rates were significantly low; 55.3%, 56.9% and 80.7% (2-cell, 8-cell and blastocyst stage, respectively). The cell numbers of expanded blastocysts were decreased in 2-cell group (24.7 ± 3.2) and 8-cell group (36.0 ± 7.9) compared with the *in vitro* cultured control blastocyst (48.8 ± 6.3). Vitrified-thawing process did not affect to the blastocyst formation rate but it induced dramatic decrease of cell number of expanded blastocyst. The quantitative RT-PCR analysis showed a lower expression of PRDX in blastocysts obtained from 2-cell stage group, compared with fresh blastocysts or other cell stages groups.

Conclusions: PRDX2 is an important gene to regulate ROS during life cycle of cells. Indispensably exposure to osmotic, thermal or oxidative stresses during freezing and thawing of embryos result in stress to the cell and ROS production. In bovine, oxidative stress is evaluated around the major embryonic genome activation (Leyens et al., 2004). Previously we explored the decreased IL-6 expression after frozen-thawing at 2-cell stage. Put together with the present results, it is suggested that the decreased expression of PRDX2 is one of the cause for the poor development of vitrified 2-cell. Besides it may be the best way to get the best result in embryo cryopreservation to avoid freezing at the major embryonic genome activation stages, 2-cell stage in mouse.

Key words: Vitrification, PRDX2, Cell number, Developmental rate