P1 Identification of coagulation factors involved in recurrent pregnancy loss

Kim MS[1], Kim YS[1], Lee EJ[1], Ajjappala BA[1], Lee SH[1], Choi BC[2], Cha KY[1], Baek KH[1]

[1]Cell and Gene Therapy Research Institute, Graduate School of Life Science and Biotechnology, Pochon CHA University, CHA General Hospital; [2]Department of Obstetrics and Gynecology, CL Women's Hospital

Objectives: Aberration of regulating processes can lead to a number of problems including RPL (recurrent pregnancy loss) during pregnancy. RPL, defined as the loss of three or more consecutive pregnancies prior to the 20th weeks of gestation, affects up to 5% of child-bearing population. This pregnancy loss is the most common complication of pregnancy, as ~10-15% of human conception terminates in a clinically detected spontaneous abortion. However, the specific proteins involved in this problem are not well defined. We investigated that coagulation factors (fibrinogen γ and antithrombin) play an important role for maintaining the normal pregnancy and these proteins are able to use as clinical biomarkers for RPL.

Materials and Methods: In follicular fluids from RPL patients and normal patients, we performed 2-DE and Coomassie blue and silver staining prior to the analysis of the differential protein expressions using image analysis software and mass spectrometry. For confirmation of protein expression, we carried out Western blot analysis using anti-fibrinogen γ and anti-antithrombin antibodies. In addition, semi-quantitative RT-PCR and real-time PCR were used to investigate expression of these genes in RNA levels.

Results: 2-DE analysis and protemics tools from follicular fluid from RPL and normal patients revealed that 5 proteins (complement component C3c chain E, fibrinogen γ, antithrombin, angiotensinogen, and hemopexin precursor) are down-regulated in RPL patients. We confirmed these expression patterns by Western blotting, RT-PCR, and real-time PCR. Interestingly, we showed that only fibrinogen γ, but not fibrinogen α and β type, were down-regulated by western blot analysis. In RNA levels, we showed that these genes were down-regulated by semi-quantitative RT-PCR and real-time PCR.

Conclusions: We have identified for the first time RPL-associated proteins in follicular fluids of RPL patients. For this confirmation, we used proteomic tools and confirmed by Western blotting, RT-PCR, and real-time PCR. In this study, we defined 5 proteins (complement component C3c chain E, fibrinogen γ, antithrombin, angiotensinogen, and hemopexin precursor) which are down-regulated in follicular fluid from RPL patients. These proteins may be helpful in understanding the molecular mechanism of the causes of RPL and will provide some insight into the prognosis of pregnancies with a high risk of RPL as biomarkers and management of those pregnancies.

Key words: recurrent pregnancy loss (RPL), 2-DE analysis, coagulation factors (fibrinogen γ and antithrombin), semi-quantitative RT-PCR, real-time PCR

P2 USP-t, a testis specific deubiquitinating enzyme, interacts with HSP90

Baek KH, Seong M, Kim MS, Lee EJ, Kim YS, Kim YK, Lee HJ

Cell and Gene Therapy Research Institute, Graduate School of Life Science and Biotechnology, Pochon CHA University, CHA General Hospital

Objectives: Ubiquitination and deubiquitination are one of important protein modification systems in cellular metabolism and homeostasis. Recently, several deubiquitinating enzymes are known to function as important regulators in signal transduction. In this way, we found a deubiquitinating enzyme, USP-t (Ubiquitin-Specific Protease-Testis specific), which interacts with heat shock protein 90 (Hsp90). We characterized the catalytic activity, the interaction with Hsp90, the tissue distribution, and the localization of USP-t. Interestingly, USP-t proteins were detected only in the testis, and the expression is limited in the Sertoli cells and meiotic germ cells. Specific binding proteins with USP-t are being analyzed.

Materials and Methods: To search for the conserved domains of USP-t, we scanned program using several databases. Transcript distribution was studied by Northern blot analysis, and protein distribution was studied by western blotting. Deubiquitinating activity was confirmed by the Ub-β-galactosidase assay. Interaction between USP-t and Hsp90 was confirmed by co-immunoprecipitation. Localization of USP-t and Hsp90 within cell lines was studied by immunofluorescence microscopy, and distribution of USP-t within the testis was studied by immunohistochemistry. To identify for putative substrate for USP-t, we performed immunoprecipitation and silver staining, and confirmed by mass spectrometry (MS) analysis.

Results: USP-t has two CS (a domain conserved in CHORD-containing protein and SGT1) domains, which were previously known as 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 deubiquitinates the monoubiquitin moiety of ubiquitin-β-galactosidase, but does not reduce the global pool of polyubiquitin chains. USP-t transcripts were highly expressed in the testis, muscle and heart. However, USP-t proteins were detected only in the testis. The immunostaining analysis revealed that USP-t is highly expressed in meiotic spermatocytes and Sertoli cells, but absent in Leydig cells, spermatogonia and sperms. We suggest that USP-t is highly expressed in meiotic germ cells but not in pre-meiotic germ cells and differentiated sperms, and may function during spermatogenesis. And we screened putative substrates for USP-t and isolated cadherin 4, leucine rich repeat neuronal 3, zinc finger protein 226, coronin, and elongin C.

Conclusions: Transcripts of USP-t were highly expressed in the testis, heart, and muscle, but USP-t proteins were detected only in the testis. USP-t interacted with Hsp90. USP-t was co-localized with Hsp90 within the cytoplasm. USP-t is highly expressed in the meiotic germ cells in the testis. USP-t binds with several putative substrates including cadherin 4, leucine rich repeat neuronal 3, zinc finger protein 226, coronin, and elongin C.

Key words: deubiquitinating enzyme, heat shock protein 90 (Hsp90), USP-t (Ubiquitin-Specific Protease-Testis specific), cadherin 4