P-56 Expression of Membrane-Type Matrix Metalloproteinase 1 (MT1-MMP) and 2 (MT2-MMP) in Human Granulosa Cells of Preovulatory Follicles

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During its growth and periovulatory period, a mammalian follicle undergoes extensive remodeling. While FSH and LH are the main hormonal inducers of the events, many factors are known to be involved in the process of remodeling. Membrane-type matrix metalloproteinases (MT-MMP) are the enzymes which are located in cell membrane and proteolytically digest many extracellular matrix components and cleave other secretory pro-MMPs to make them active. Due o these activities, MT-MMPs are known to play important roles in the mechanisms of differentiation, tissue remodeling and cancerous invasion of various tissues. In this study, expressions of MT1- and MT2--MMP are examined with regards to their possible role in the late follicular growth in human. By using RT-PCR technique, granulosa cells (GC) obtained from either large follicles (>18 mm) or small follicles (<18 mm) were shown to contain mRNAs of both MT1- and MT 2-MMP. However, mRNA expressions of MT3- and MT4-MMP were barely seen regardless of the size of follicles. When the protein expression patterns of both MT1- and MT2-MMP were examined using their specific antibodies, both proteins were detected after western blotting. Interestingly, the intensities of both protein bands obtained from the GC of small follicles appeared to be much stronger than those from the GC of larger follicles. Moreover the band intensity of MT2-MMP was stronger than that of MT1-MMP. From these observations, it is suggested that MT1- and MT2-MMPs possibly involved in tissue remodeling during late follicular growth in human.

P-57 Expression of Membrane-Type Matrix Metalloproteinase 1 (MT1-MMP) and 2 (MT2-MMP) in Mouse Cycling and Preimplantation Uteri

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To see if MT-MMPs might be involved in tissue remodeling of uterus during estrous cycle and preimplantation period in mouse, expression patterns of both MT1-MMP and MT2-MMP were investigated using RT-PCR and western blotting techniques. Expression of both mRNAs of MT1- and MT2-MMP were observed throughout the estrous cycles, namely proestrus, estrus, metestrus and diestrus. The expression of MT1-MMP protein having molecular weight of 55 kDa was also observed throughout the estrous cycle in oviductal tissue homogenates of each estrous stage that

were blotted against anti-human MT1-MMP antibody following SDS-PAGE. And the band intensity of each stage tissue was not the same from each other. Preimplantation uteri examined at day 1, 2, 3, and 4 of pregnancy also revealed distinct mRNA expressions of both MT1- and MT2-MMP and there was no difference among the band intensities. However, as pregnancy progresses, the amount of MT1-MMP protein expression appears to increase such that uterine tissue homogenate of day 4 pregnancy gave the most intense band while that of day 1 was the weakest. Anti-human MT2-MMP antibody failed to detect any mouse protein antigen on the blot. From these observations, it is suggested that MT1-MMP and possibly MT2-MMP might play an important role in remodeling process of mouse uterine tissue during preimplantation period.

P-58 Expression of Membrane-Type Matrix Metalloproteinase 1 (MT1-MMP) and 2 (MT2-MMP) in Mouse Oocyte, Ovary and Oviduct

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The possible roles of membrane-type matrix metalloproteinase 1 (MT1-MMP) and 2 (MT2-MMP) in the meiotic maturation of oocytes and in the remodeling process of ovarian and oviductal tissues were investigated using RT-PCR and western blot techniques. mRNA of MT1-MMP were found to be expressed in mouse GV oocytes only and there was no discernible RT-PCR product in PB oocytes whether they were grown *in vivo* or *in vitro*. However, RT-PCR product of mRNA of MT2-MMP was observed in both GV and PB oocytes but the band intensity of GV oocytes was much stronger than that of PB oocytes. When mouse ovarian tissues obtained at prepubertal, 48 hr post PMSG, 10 hr post hCG, 15 hr post hCG or from pregnant mice at 48 hr post hCG were examined, the amount of RT-PCR products of both MT1- and MT2-MMP appeared to be the same regardless whether the ovaries were stimulated by hormone or not. Protein expression of MT1-MMP was also observed in all of the above ovarian tissues whereas MT2-MMP protein was not detected at all in the same samples. Interestingly the band intensity of MT1-MMP protein was observed to be stronger in the ovaries just prior to ovulation than before hCG stimulation and after ovulation. However, it was the highest in the ovaries of pregnant mice. Both RT-PCR products and proteins of MT1- and MT2-MMP in oviductal tissues did not reveal any significant difference among the samples of periovulatory periods. However, the oviducts of pregnant mice gave an intense signal when blotted against anti-MT1-MMP antibody although the intensity of its RT-PCR product was not stronger than that of any other tissues samples. Anti-human MT2-MMP antibody failed to detect MT2-MMP protein in the mouse tissues although gold-labeled antibody and silver-enhance method was used. Based upon these observations, it is suggested that MT1-MMP might play a role in the follicular rupture prior to ovulation and possibly involved in the remodeling of oviductal tissues at the time of pregnancy.