B-4 Identification and Characterization of Centrosomal Protein Recognized by Newly Developed Monoclonal Antibodies in Mouse Oocyte & Preimplentation Embryos

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Introduction and Aims: In most animal cells, centrosomes are instrumental for organization of the spindle poles, and involved in many other cytoplasmic activities including cell cycle-related kinetic activity. The oocytes from mouse do not contain centrosomes, yet they form bipolar spindles during meiosis. And contribution of sperm about embryo centrosomes is not clear at present. In this study we developed and characterized monocronal antibodies recognizing centrosomes in both mouse oocytes and somitic cells.

Materials and Methods: Indirect immunocytochemistry was used to examine the antigen distributions and attractions during cell cycle in both meiotic and mitotic divisions.

Results: Discrete spots were recognized in the oocytes undergoing maturation as evidenced by two monocloned antibodies, 1D and 2D.

In preimplantation embryos, the antigens were found abundantly in blastomeres throughout the cytoplasm. Interestingly the polarized distribution of the antigens were found in the opposite side of the trophectodermal cells to the inner cell mass during blastocyst stage. The staining intensity suggests that the protein(s) recognized by the monocronal antibodies are present at very low level in the oocyte, whereas are synthesized rapidly during embryonic development.

Conclusion: The antibody should provide a useful tool for definitely ooplasmic factors involved in centrosomal organization in the mouse.

B-5 Expression of GnRH-II in Human Endometrium During the Normal Menstrual Cycle and First Trimester

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Objective: We aimed to demonstrate the expression of GnRH-II mRNA and peptide in cycling human endometrium and first trimester decidua.

Methods: Neststed RT-PCR was performed to identify GnRH-II mRNA in the endometrium (n=6) and the sequencing analysis was carried out. For immunohistochemistry, 23 endometrial samples (proliferative: n=4, early secretory: n=8, mid-secretory: n=6, late secretory: n=5) and decidual samples obtained during 5~10 wk of gestation (n=7) were examined. The expression of GnRH-II peptide in each phase of the

menstrual cycle was assessed semi-quantitatively by cumulative histologic score (HSCORE) and mean HSCORE was analyzed by ANOVA.

Results: All of the endometrial samples expressed two spliced variants of GnRH-II mRNA and the short variant had a 21-bp deletion in GnRH-associated peptide (GAP). Immunoreactive GnRH-II was localized in both stromal and glandular epithelial cells during the entire menstrual phase. In glandular epithelial cells, the mean (SEM) HSCORE of early and mid-secretory phase (3.5±0.1, 3.3±0.3, respectively) were significantly higher ($p<0.05$) than those of proliferative and late secretory phase (2.1±0.4, 2.6±0.3, respectively). In stromal cells, the mean HSCORE of early and mid-secretory phase (3.0±0.2 and 3.1±0.3, respectively) were significantly higher ($p<0.05$) than those of proliferative and later secretory phase (1.6±0.1 and 2.1±0.1, respectively). During the first trimester, decidualized stromal cells and glandular epithelial cells showed strong intensity of irGnRH-II.

Conclusion: Our study demonstrated that the second isoform of GnRH (GnRH-II) was expressed in cycling human endometrium and first trimester decidua. We suggest that a local expression of endometrial GnRH-II peptide, noted during the early and mid-secretory phase, may play an important role in human embryonic development and implantation. Moreover, maintenance of GnRH-II peptide expression in first trimester decidua may be involved in early pregnancy.

B-6 Laser Captured Microdissection (LCM)을 이용한 유전자 발현에 대한 연구: 난자의 RNA 추출 및 증폭을 위한 최소 한도의 확립

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목 적: 분자생물학적 기술의 발전은 조직으로부터 개별적인 세포단위의 유전적, 기능적인 변화를 찾는 수준에까지 진행되었다. LCM의 기법을 접목하면 여러 가지 세포가 혼합되어 있는 복잡한 조직 또는 매우 적은 양의 시료로부터 특정세포만을 선택적으로 얻어 특이적 유전자 발현을 연구할 수 있는 강점이 있다. 본 연구의 목적은 LCM을 이용하여 mRNA 수준에서 유전자 발현을 분석하기 위해 RT-PCR 실험을 할 때 필요한 최소한의 세포의 수를 결정하고, 이때 mRNA 추출 및 증폭을 위한 여러 가지 실험조건을 확립하는 것이었다.

재료 및 방법: 난자를 이용함으로써 세포의 숫자를 정확하게 조절할 수 있었으며, house-keeeping 유전자인 glyceraldehyde-3-phosphate-dehydrogenase (GAPDH)의 유전자 발현을 연구함으로써 다른 세포를 연구할 때 본 연구 결과가 유용하게 사용될 수 있도록 했다. 3주된 ICR 생쥐에 PMSG를 주사 후, 48시간에 난소를 적출하여 OCT compound로 포매한 후 cryostat을 이용해 7 μm로 절편하였다. 제작된 슬라이드를 70% EtOH에 고정한 후, Hematoxylin-Eosin으로 염색하고 마지막으로 xylene으로 5분 동안 탈수한 후 실온에서 20분 동안 말린 후 LCM으로 난자를 procure (포획) 하였다. 이때 laser beam은 선택하고자 하는 세포의 크기에 따라 7.5 μm ~ 30 μm diameter까지 spot size를 조절하였고, power는 조직의 상태에 따라 20~40 mW 범위에서 사용하였다. 포획한 세포가 붙어 있는 transfer film cap은 GITC (guani-