P-21 Amniotic Fluid Cells Enhance the Differentiation of Umbilical Cord Blood CD34+ Cells into Dendritic Cells

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Background & Objectives: To develop a method to enhance the differentiation of CD34+ cells into dendritic cells by coculture with amniotic fluid cells.

Method: Amniotic fluid cells (AFC) were obtained from the amniotic fluid at 20 weeks of pregnancy. After extended subcultures, fibroblast-like cells were isolated and used for the coculture. CD34+ cells were isolated from the cord blood at full-term delivery using miniMACS separation system. They were cultivated alone or co-cultivated with AFC in RPMI-1640 containing 10% FBS, 50 ng/ml GM-CSF, 20 ng/ml IL-4, 25 ng/ml SCF and 20 ng/ml TNF-α. After culture for 2 weeks, cells were analyzed by FACS and Mixed Lymphocyte Reaction (MLR).

Results: After 4 passages, homogeneous population of fibroblast-like cells was obtained from the amniotic cells. RT-PCR analyses showed the expression of SCF, BMP-4, nestin, ADAM-12, FGF-5, vimentin, AFP and CK18 genes. When CD34+ cells were induced to differentiate into dendritic cells, total number of cells grown in the presence of AFC was greater by more than 2.6 fold compared to that in the absence of AFC. A similar percentage of differentiation, as analyzed for CD80, CD86, CD83, CD40 and HLA-DR markers, was shown in both cells cultivated alone and cocultured with AFC. Results of MLR analyses also showed a similar ratio of thymidine labeling, indicating little difference of dendritic cell differentiation between two culture methods.

Conclusions: AFC could enhance the differentiation of CD34+ cells into dendritic cells by significantly increasing the proliferation of CD34+ cells.

P-22 HIDE, a Testis Specific Deubiquitinating Enzyme, Interacts with HSP90

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Background & Objectives: Ubiquitination and deubiquitination are one of important protein modifica-

tion 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, HIDE (Hsp90 interacting deubiquitinating enzyme), which interacts with heat shock protein 90 (Hsp90). We characterized the catalytic activity, the interaction with Hsp90, the tissue distribution, and the localization of HIDE. Interestingly, HIDE proteins were detected only in the testis, and the expression is limited in the Sertoli cells and meiotic germ cells.

Method: To search for the conserved domains of HIDE, 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 HIDE and Hsp90 was confirmed by co-immunoprecipitation. Localization of HIDE and Hsp90 within cell lines was studied by immunofluorescence microscopy, and distribution of HIDE within the testis was studied by immunohistochemistry.

Results: HIDE 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 HIDE was merged with that of Hsp90 in the cytoplasm. The catalytic domain of HIDE contains a zinc finger domain, and deubiquitinates the monoubiquitin moiety of ubiquitin-β-galactosidase, but does not reduce the global pool of polyubiquitin chains. HIDE transcripts were highly expressed in the testis, muscle and heart. However, HIDE proteins were detected only in the testis. The immunostaining analysis revealed that HIDE is highly expressed in meiotic spermatocytes and Sertoli cells, but absent in Leydig cells, spermatogonia and sperms. We suggest that HIDE is highly expressed in meiotic germ cells but not in pre-meiotic germ cells and differentiated sperms, and may function during spermatogenesis.

Conclusions: HIDE transcript was highly expressed in the testis, heart, and muscle, but HIDE proteins were detected only in the testis. HIDE interacted with Hsp90, and HIDE was co-localized with Hsp90 within the cytoplasm. HIDE is highly expressed in the meiotic germ cells in the testis.

P-23 생식주기과 착상기를 전후의 생쥐 자궁에서 Junctional Adhesion Molecule-1 (JAM-1)의 발현 및 Steroid에 의한 발현의 조절

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Background & Objectives: 상피조직의 apical side에 형성되는 밀착결합 (Tight Junction, TJ)은 혈액-조직 사이의 확산장벽을 형성하여 조직 특이적 특수 환경 조성에 중요한 역할을 한다. 밀착결합은 occludin, claudins 등 integral membrane protein과 ZO-1, JAM 등의 plaque protein으로 구성되며 세포질 골격 및 다양한 신호전달 분자와 복합체를 형성한다. 따라서 다양한 조직에서 세포 내외부의 신호에 반응하여 그 구조와 기능이 역동적으로 조절된다. 자궁내막은 생식주기와 착상을 위한 준비과정 동안 주로 난소 스테로이드의 영향 하에 구조 및 기능적 분화를 진행한다. 자궁내막에 존재하는 상피와 혈