IS-AC-4-6  The differential expression of microRNA between human first trimester villous and extravillous trophoblast cells: PCR array-based approach

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[Objective] Villous trophoblast (VT) differentiates into extravillous trophoblast (EVT) : this requires gene expression changes between VT and EVT, meaning that expression of microRNA (miRNA) naturally changes between the two. We demonstrated placental expression of placenta-specific miRNA clusters. We examined whether expression of miRNAs, especially placenta-specific miRNAs, change between VT and EVT. [Methods] We obtained informed consent and the approval of Ethics Committee. PCR-array based analysis was employed to examine the expressions of 756 miRNAs between VT and EVT (minced chorionic villi) and EVT (isolated from explanted chorionic villi) (10-11 weeks at legal abortion). [Results] Of 756 miRNAs, 160 were detected, of which 31 and 5 were originated from chromosome 19 cluster (C19MC) and C14MC miRNAs, respectively. Of 160, 29 (29/160: 18%) showed statistically different expressions between the two, with all 29 down-regulated in EVT than VT, with 9 belonging to C19MC and 4 to C14MC miRNAs. The miR-100 and -145 expressions were markedly reduced: EVT expressed them less than 1/10 than VT. [Conclusion] Expression of 29 miRNAs including placenta-specific miRNAs changed between VT vs. EVT, with all reduced in EVT. While differentiating from VT into EVT, trophoblast cells lost some miRNA expression (especially placenta-specific miRNAs), thereby regulating corresponding gene expression.

IS-AC-5-1  Human induced pluripotent stem cells (iPS cell) differentiate to hCG-producing trophoblast (For elucidation of the various obstetrical and trophoblastic diseases)

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[Objective] The characteristics of the induced pluripotent stem cells (iPS cells) are useful for a disease study and treatment. However, the model construction about the placental development of the human is difficult, so we haven’t understood about it yet. This study tried differentiation to hCG-producing trophoblast, using these iPS cells to elucidate placentation. [Methods] The iPS cells on feeder were subcultured to the xenoid-free environment, and we added BMP4 for about 10 days. We evaluate morphologic change and hormone level of culture media supernatant. After collecting cells, we extracted RNA and made cDNA to measure the gene expression of the trophoblast markers in RT-PCR. immunohistochemistry was underwent about CDX2 and HCG. [Results] After about 5days incubation, a morphological change and the expression of trophoblast markers in RT-PCR was confirmed, and the hormone level of HCG and progesterone in supernatant were rose. Furthermore the CDX2 and HCG in the immunohistochemistry were positive. [Conclusion] Now we try to transfect a vector of the PPARγ gene to the iPS cells and differentiate to hCG-producing trophoblast. Then it will be transplanted to mouse to investigate a function in vivo and to construct placenta model. The differentiation of the trophoblast should greatly contribute to elucidation and treatment of the various obstetrical and trophoblastic diseases.

IS-AC-5-2  Exacerbation of endometriosis by dysfunction of regulatory T cells in mouse and human

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[Objective] Endometriosis is a chronic inflammatory disorder that is associated with the altered immune response. Regulatory T cells (Treg) play a key role in maintaining immune homeostasis. Here we demonstrate the role of Treg in endometriosis. [Methods] A mouse model of endometriosis was made by transplanting donor mouse uterine fragments into the abdominal cavity of recipient mice. Foxp3-ires-DTR/GFP [DFG] C57BL/6 mice were used as Treg cell-depleted model. Endometrioma, endometrium, peritoneal fluid, and peripheral blood were obtained from women with and without endometriosis. Three Treg cell fractions (resting Treg, activated Treg, non-Treg) in human CD4+ cells were examined by FACS. This study was approved by the IRB and informed consent was obtained from each patient. [Results] In DFG mice, the number (P<0.05) and weight (P<0.01) of endometriotic lesions, and the serum level of IL-6 (P<0.01) were significantly increased compared to those in control mice. In women with endometriosis, the proportion of activated Treg in endometrioma (2.4±1.7%, P<0.01) and endometrium (2.0±1.1%, P<0.01), but not in peritoneal fluid or peripheral blood, was significantly decreased compared with that in women without endometriosis (5.2±4.5%). [Conclusion] An enhanced inflammatory response caused by reduced activated Treg cells may be involved in the progression of endometriosis.