Department of Obstetrics and Gynecology

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General Summary

The main research topics of our department are the development of molecularly targeted agents for gynecologic tumors, including ovarian cancer, clarification of the mechanisms of successful pregnancy, and the development of assisted reproductive techniques. These topics were investigated both experimentally and clinically.

Research Activities

Gynecologic oncology

1. CD147 (extracellular matrix metalloprotease inducer, basigin) plays important roles in tumor invasiveness and metastasis by activation of matrix metalloproteinases (MMPs). We showed that CD147 was expressed in 97.5% of endometrial carcinomas and in 83.1% of cervical squamous cell carcinomas. Especially in endometrial carcinomas, CD147 expression is correlated with various clinicopathological factors (stage, grade, lymph node metastasis, lymphatic vessel infiltration, distant metastasis, presence of ascites) and with recurrence-free survival. The aims of our study were to clarify the functional significance of CD147 in cancer progression and to examine the possibility of using CD147 as a novel biomarker or as a therapeutic target.

2. Genomic identification of significant targets in cancer analysis in ovarian carcinomas We analyzed somatic DNA copy number variation in various histological types of 78 ovarian carcinomas using genomic identification of significant targets in cancer analysis. Regions that were significantly (p-value <0.01) more common in serous tumors, after corrections for multiple hypothesis testing (indicating nonrandom distribution across subtypes), included (in order of significance): 8p23.3, 6q24.3, 11p15.5, 8p21.2, 16q22.1, 22q13.31, 4q22.1, 5q22.2, 7p22.3, and 14q24.2. Although no regions of copy number variation were significantly associated with any histological subtype other than serous tumors, an overrepresentation of amplification of 20q13.2 (ZNF217), 17q12 (CCL4), and 8q13.2-q21.11 (NCOA2) was observed in clear-cell tumors, and amplification of 1q21.3 (CTMP) and 1q42.13 (RAB4A) was observed in endometrioid tumors.

3. Japanese ovarian cancer patients that can classify the presence of cancer. The information is similar to that of sera of patients with ovarian cancer in the United States because N-dimensional clusters built on United States sera spectra created cluster maps predictive of the Japanese samples. The performance of the model was better than that

of any of the existing single-biomarker assays, although truly useful models await a much larger sample size and the use of independent validation sample sets to demonstrate their robustness. These results encourage us to start a large-scale, multisite collection of sera from Japanese patients with ovarian cancer to develop a Japanese ovarian cancer serum profile assay.

4. MicroRNA (miRNA) expression profiles for cancers, including those of the lung, breast, stomach, prostate, and colon, were examined to investigate the involvement of miRNA in carcinogenesis. We are now investigating the roles of miRNA in the resistance of human ovarian cancer cells to paclitaxel. This research has significant implications for therapeutic strategies for overcoming cancer cell chemoresistance.

5. Mitochondrial ultrastructure-associated chemotherapy response in ovarian cancer A mitochondrial scoring system was developed in association with platinum sensitivity in ovarian cancer and was applied to clinical samples. Twenty-four cases were included in this study. Excellent correlation to chemosensitivity was noted for 2 factors: electron density and distribution pattern. The total score of these 2 factors in 9 sensitive cases was 1.44 and was 3.58 in 19 resistant cases ($P<0.001$). Receptor operative characteristics analysis revealed that the total cut-off score was 3 point. Scores with this system were strongly correlated with response, and this result suggests that this system would be of great value as a biomarker for chemosensitivity.

6. The prognostic factors in patients with advanced epithelial ovarian cancer have been identified by many investigators. We performed a study to construct a simple and powerful prognostic index (PI) of epithelial ovarian cancer (PIEPOC). The four prognostic factors that remained independently significant in the analysis were age, performance status, cell type, and size of residual disease. On the basis of the regression function, patients were classified into three risk groups, i.e., low risk (PI: 0–2), intermediate risk (PI: 3), and high risk (PI: 4–6). The PIEPOC was equally predictive in a validation sample ($n=230$), identifying 3 groups (5-year survival rate: 67% in the low-risk group, 43% in intermediate-risk group, 17% in the high-risk group).

7. Serum indoleamine 2,3-dioxygenase expression was positively correlated with impaired survival in patients with serous-type ovarian cancer.

We have previously reported that the indoleamine 2,3-dioxygenase (IDO) screened with the GeneChip is positively correlated with paclitaxel resistance and with impaired survival in patients with serous-type ovarian cancer. We established an enzyme-linked immunosorbent assay with an anti-IDO antibody for serum and measured serum IDO titers in 26 types of ovarian cancer. We compared the expression pattern in surgical specimens and the corresponding serum IDO titer and found a positive correlation in the serous type. These results suggest that the serum IDO level is a biomarker for serous-type ovarian cancer.

Fetomaternal medicine

1. Antiphospholipid syndrome (APS) is a clinical entity manifested by arterial and venous thromboses and recurrent miscarriages, and is caused by antiphospholipid antibodies. APS has also been observed in some complications of pregnancy, *e.g.,* pregnancy-induced hypertension, intrauterine growth restriction, and late fetal death.

However, little is known about how antiphospholipid antibodies (APLs) is involved in these complications. To be identified mechanisms of APLs in obstetrical complications by investigating the pathological significance, we established an experimental model for APS using wild type, Fc-knock-out mice and C_3-knockout mice.

2. IDO plays essential roles in successful pregnancy and is induced by type 1 cytokines. The receptors of type 1 cytokines share a structure and a signal transduction pathway with prolactin. Because physiological levels of prolactin increase markedly during pregnancy, we examined a possible connection between prolactin and IDO expression. To investigate IDO expression in CD14 cells, we obtained mononuclear cells from 12 healthy persons and cultured the cells for 24 to 48 hours in the presence or absence of stimuli (interferon [IFN]-γ: 5,100 IU/ml; prolactin: 10, 100, or 500 ng/ml). IDO expression was up-regulated only when prolactin at physiological concentrations during pregnancy were present with 5 IU/ml of IFN-γ, whereas only prolactin and 5 IU/ml of IFN-γ were without effect. These findings suggest prolactin plays important roles in the maintenance of pregnancy, thereby up-regulating IDO, and support the view that the physiological concentration of IFN-γ is important for pregnancy.

3. Establishment of an immortalized human extravillous trophoblast cell line by infection with retroviral expression vectors

Studies of the function of human trophoblasts have been limited by a lack of suitable cell models. We aimed to obtain human normal trophoblast cell lines with long lifespans and consequently provide an ideal *in vitro* cell model. Primary human trophoblast cells were derived from the placenta of a woman undergoing elective abortion during the 7th week of gestation. The cells were immortalized by infection with retroviral expression vectors containing type 16 human papillomavirus E6 and E7 in combination with human telomerase reverse transcriptase. The characteristics of the cell line were analyzed. Immunocytochemical studies revealed staining for human chorionic gonadotrophin chain β, cytokeratin 7, HLA-G, and CD9 and an extravillous trophoblastic phenotype. Transwell insert invasion assay showed the invasiveness of this cell line, and gelatin zymography detected the secretion of MMP-2 and MMP-9. Karyotype analysis showed nearly normal chromosomal numbers with small deviations ranging from 46 to 48, and nude mouse assay showed no tumorigenecity. This newly immortalized cell line, HChEpC1b, will provide a useful model for the study of extravillous trophoblast function.

Reproductive endocrinology

1. Most of the mechanisms for achieving pregnancy have been clarified due to the development of assisted reproductive technololgies. However, the mechanism of implantation remains unclear.

CD147 is expressed at high levels on the surfaces of various tumor cells and stimulates MMPs. We hypothesized that CD147 plays an important role in implantation. The aim of this study was to determine the expression and hormonal regulation of the CD147 gene during the human implantation period in controlled ovarian hyperstimulation cycles. We found that CD147 and MMP-2 mRNAs in human endometrium during the

secretory phases were significantly decreased in controlled ovarian hyperstimulation cycles.

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