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. Integrated Copy Number and Expression Analysis of Chemoresistant Ovarian Carcinomas

Women with serous ovarian cancer are often intrinsically refractory to platinum-taxol—based treatment or become resistant on relapse. Because accurately predicting the response to chemotherapy remains possible, we sought to identify somatic DNA copy number variation (CNV) associated with primary resistance in advanced-stage disease. Genome-wide frequency and the level of CNV in 118 ovarian tumors were measured with single nucleotide polymorphism microarrays. A well-defined subset of 85 advanced-stage serous tumors was then used to relate CNV to primary resistance to treatment. The discovery-based approach was complemented by quantitative polymerase chain reaction analysis of copy number of 12 candidate genes previously reported to be associated with clinical outcome in ovarian cancer. Likely CNV targets and tumor molecular subtypes were further characterized by gene expression profiling. Amplification of 19q12, containing cyclin E (CCNE1) and 20q11.22-q13.12, mapping immediately adjacent to the steroid receptor co-activator NCOA3, was significantly associated with a poor response to primary treatment. From previously reported associations of copy number with outcome, only the amplification status of CCNE1 was validated as a marker for primary chemoresistance. Chemoresistant tumors with high CCNE1 copy number and protein expression were predictably associated with increased cellular proliferation, as were a subset of treatment-responsive patients, suggesting a cell-cycle—independent role for CCNE1 in modulating chemoresponse. Patients with poor clinical outcomes and without CCNE1 amplification over-expressed genes involved in extracellular matrix deposition. Our findings identify 2 distinct mechanisms of primary treatment failure in serous ovarian cancer, involving CCNE1 amplification and enhan-

ced extracellular matrix deposition.

2. Mesenchymal-to-epithelial transition during the inclusion cyst formation from human ovarian surface epithelium

Most surface epithelial-stromal tumors of the ovary are thought to arise from epithelial inclusion cysts. Thus, these cysts are precursor lesions of ovarian carcinoma. On the basis of this hypothesis, we aimed to characterize the human ovarian surface epithelium in which the mesenchymal-to-epithelial transition occurs in the process of inclusion cyst formation. We used specimens from 9 patients with endometrial cancer who underwent hysterectomy and bilateral salpingo-oophorectomy. Immuohistochemical studies were performed of 10 normal ovaries containing 92 inclusion cysts and 4 normal fallopian tubes to examine the expression of antigen markers, including calretinin, podoplanin, D2-40, thrombomodulin, human bone marrow endothelial (HBME)-1, vimentin, epithelial membrane antigen (EMA), WT1, carbohydrate antigen (CA) 125, MOC31, tumor-associated glycoprotein (TAG) 72, Ber-EP4, and E-cadherin. We found that positive staining rates for mesothelial markers in normal ovarian surface epithelium were 100% (10 of 10) with calretinin, 80% (8 of 10) with podoplanin, 80% (8 of 10) with D2-40, 70% (7 of 10) with thrombomodulin, 100% (10 of 10) with HBME-1, and 100% (10 of 10) with vimentin; that positive staining rates for epithelial markers in tubal epithelium were 100% (4 of 4) with HBME-1, 100% (4 of 4) with vimentin, 100% (4 of 4) with EMA, and 75% (3 of 4) with TAG-72, and 100% (4 of 4) with Ber-EP4; and that positive staining rates for both markers in inclusion cysts were 51.1% (47 of 92) with HBME-1, 44.6% (41 of 92) with vimentin, 65.2% (60 of 92) with TAG-72, and 88.0% (81 of 92) with Ber-EP4. Ovarian surface epithelium has both mesencyhmal and epithelial characteristics. In contrast, inclusion cyst gains more epithelial characteristics with the loss of mesencyhmal characteristics. These findings support a mesenchymal-to-epithelial transition during inclusion cyst formation from ovarian surface epithelium.

3. MicroRNA (miRNA) expression profiles for cancers, including those of the lung, breast, stomach, prostate, and colon, were examined to investigate the miRNA involvement in carcinogenesis. We are now investigating the roles of miRNA in the resistance of human ovarian cancer cells to paclitaxel. Our findings may have significant implications for therapeutic strategies aiming to overcome cancer cell chemoresistance.

4. A randomized trial of retroperitoneal closure versus opening has been registered to prevent lymphedema after lymphedenectomy for patients with uterine cervical cancer or endometrial cancer. A total of 150 cases will be registered; 64 cases had been registered by March 2009.

5. This small proof-of-principle study has demonstrated that there are profiles in the serum of Japanese ovarian cancer patients that can be used to classify the presence of cancer. The information is similar to that in ovarian cancer sera from the United States because N-dimensional clusters built on United States sera spectra created cluster maps predictive of the Japanese samples. The model performed better than 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.

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. Recently, APS has also been observed with some complications of pregnancy, e.g., pregnancy-induced hypertension, intrauterine growth restriction, and late fetal death. However, little is known about how APS is involved in these complications. The Fc receptor for IgG (Fcγ receptor) is implicated in some autoimmune diseases. To investigate the pathological significance of the Fcγ receptor in APS and complications of pregnancy, we have attempted to establish an experimental model for APS using Fcγ receptor knock-out mice.

We examined the presence of antiphospholipid antibodies in patients who had obstetrical complications, and investigated placental pathology.

2. Many patients with recurrent pregnancy loss become infertile or have repeated spontaneous abortions after infertility therapy. These transitional conditions have not been researched so far. We have investigated the different possible causes and clinical manifestations of these conditions from the perspective of reproductive failure.

3. Establishment of an immortalized human extravillous trophoblast cell line by retroviral infection of E6/E7/human telomerase reverse transcriptase

Investigation into the function of human trophoblasts has been restricted by a lack of suitable cell models. We aimed to obtain long-lived human normal trophoblast cell lines that would serve as ideal *in vitro* cell models. Primary human trophoblast cells were derived from the placenta of a woman who had undergone 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 (hTERT). Characterization of the cell line was performed. Immunocytochemical staining for human chorionic gonadotrophin chain β, cytokeratin 7, human leukocyte antigen G, and CD9 indicated an extravillous trophoblastic phenotype. Transwell insert invasion assay showed the invasiveness of this cell line, and gelatin zymography showed secretion of matrix metalloproteinases 2 and 9. Karyotype analysis showed almost normal chromosomal number with small deviations ranging from 46 to 48, and a nude mouse assay showed no tumorigenecity. This newly immortalized cell line, HChEpC1b, will provide a useful model for the study of extravillous trophoblast function.

4. Dynamics of biopyrrins in pregnant women and newborns

Biopyrrins are bilirubin oxidative metabolites that can be measured in pregnant women to monitor psychological stress. Urinary biopyrrin levels were significantly elevated by delivery from 4.22 ± 0.47 to 7.33 ± 0.68 (U/g•Cre). Induction of delivery increased postpartum biopyrrin levels by 89% (10.74 ± 1.37 vs 5.67 ± 0.80 (Ug•Cre). These data suggest that pregnancy and delivery increase psychological stress and that delivery induction increases stress even more.

Reproductive endocrinology

1. Most of the mechanisms for achieving pregnancy have been clarified owing to advances in assisted reproductive technology. Nevertheless, the mechanism of implanta-

tion remains unclear.

CD147 is expressed at high levels on cell surfaces of various tumors and stimulates matrix metalloproteinases. We hypothesized that CD147 may play 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 levels of CD147 and matrix metalloproteinase 2 mRNA in human endometrium were significantly decreased during the secretory phase in controlled ovarian hyperstimulation cycles.

Publications

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