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. Cyclin D1 is a prognostic factor for advanced serous ovarian cancer

We have previously shown with high-resolution oligonucleotide copy number analysis that cyclin E (*CCNE1*) amplification is strongly associated with the resistance to treatment of serous ovarian cancer. We focused on 66 cases of advanced serous epithelial ovarian cancer (EOC) and investigated the associations between the expression of G1-S phase regulatory proteins and clinicopathological variables. Immunohistochemical analyses of cyclin D1, pRb, p16, p53, $p27^{Kip1}$, $p21^{Waf1/Cip1}$, and cyclin E were performed on formalin-fixed tissue sections collected from primary surgical specimens. The correlations between the expression of these proteins and the clinicopathological variables, including progression-free survival (PFS), overall survival (OS), and chemosensitivity, were examined. Univariate analysis showed that overexpression of cyclin D1 was positively correlated with reduced PFS ($p=0.00062$) and OS ($p=0.00037$). Reduced expression of $p27^{Kip1}$ tended to be associated with shorter OS ($p=0.064$). Multivariate analysis showed that overexpression of cyclin D1 ($p=0.0019$), reduced expression of $p27^{Kip1}$ ($p=0.042$), and residual tumor volume ($p=0.0092$) were independent predictors of OS. Overexpression of cyclin D1 ($p=0.011$) and residual tumor volume ($p=0.006$) were significantly associated with first-line chemosensitivity. In advanced serous EOC, overexpression of cyclin D1 contributes greatly to poor prognosis owing, perhaps in part, to chemoresistance. Cyclin D1 is a possible target for overcoming the refractory nature of advanced serous EOC.

2. Cytokine gene expression signature in ovarian cancer

Host defenses against tumors are controlled by several immunological mediators, including cytokines, that play important roles in the host-tumor immune-system conflict. Alterations of cytokine expression and an imbalance in the helper T types 1 and 2 cell

cytokine response have previously been shown in ovarian cancer. In this study, we sought to clarify whether the cytokine gene expression profile affects the development or progression or both of ovarian cancer.

3. Copy number analysis identifies novel interactions between genomic loci in ovarian cancer

Ovarian cancer is a heterogeneous disease with complex genomic alterations, and, consequently, studies to date have had difficulty determining the most relevant copy number alterations (CNAs). We obtained genome-wide CNA data from 4 different single nucleotide polymorphism array platforms for a final data set of 398 ovarian tumors, mostly of the serous histological subtype. Frequent CNAs targeted many thousands of genes; however, high-level amplicons and homozygous deletions enabled filtering of this list to the most relevant. The large data set enabled refinement of minimal regions and the identification of rare amplicons, such as those at 1p34 and 20q11. We performed a novel co-occurrence analysis to assess the cooperation and exclusivity of CNAs and analyzed their relationships to patient outcome. Positive associations were identified between gains on 19 and 20q, gain of 20q, and loss of X and between several regions of loss, particularly 17q. We found weak correlations of genomic loci, such as 19q12, with clinical outcome. We also assessed genomic instability measures and found a correlation of the number of higher amplitude gains with poorer OS. By assembling the largest collection of ovarian copy number data to date we have been able to identify the most frequent aberrations and their interactions.

4. IL‐6-STAT3-HIF signaling and therapeutic response to the angiogenesis inhibitor sunitinib in ovarian clear cell cancer.

Ovarian clear cell adenocarcinoma (OCCA) is an uncommon histotype that is generally refractory to platinum-based chemotherapy. Here, we analyzed the most comprehensive gene expression and copy number data sets compiled to date to identify potential therapeutic targets of OCCA.

Gene expression and DNA copy number analysis were performed with primary human OCCA tumor specimens, and findings were confirmed with immunohistochemical studies of tissue microarrays. Circulating levels of interleukin (IL) 6 were measured in the serum of patients with OCCA or high-grade serous cancers and were related to PFS and OS. Two patients were treated with sunitinib, and their therapeutic responses were measured clinically and with positron emission tomography.

We found specific overexpression of the IL-6-STAT3-HIF (IL-6-signal transducer and activator of transcription 3-hypoxia-induced factor) pathway in OCCA tumors compared with that in high-grade serous cancers. Expression of parathyroid hormone-like hormone and high circulating levels of IL-6 in patients with OCCA may explain the frequent occurrence of hypercalcemia of malignancy and thromboembolic events in OCCA. We describe the amplification of several receptor tyrosine kinases, most notably MET, and suggest other potential therapeutic targets. We report sustained clinical and functional imaging responses in 2 patients with chemotherapy-resistant OCCA who were treated with sunitinib, thus showing significant parallels with renal clear-cell cancer.

Our findings highlight important therapeutic targets in OCCA, suggest that more extensive clinical trials with sunitinib in OCCA are warranted, and provide significant impetus

to the growing realization that OCCA is molecularly and clinically distinct from other forms of ovarian cancer.

5. Differential mitochondrial scoring associated with chemotherapeutic responses in patients with ovarian cancer

Ultrathin sections of surgical specimens from patients with ovarian cancer were examined with electron microscopy. Clinical response was compared with mitochondrial scores composed of 7 independent mitochondrial morphological features. The total mitochondrial score was 5.13 in responsive cases and 11.41 in resistant cases. Receiver operating characteristic analysis revealed that the resistant total cut-off score was ≥10 points. After a median follow-up period of 20 months, 11 patients have relapsed. The PFS curves showed a difference in favor of the low-scoring patients over high-scoring patients.

6. Clinical and prognostic value of the presence of irregular giant nuclear cells in pT1 ovarian clear cell carcinomas

We aimed to identify irregular giant nuclear cells (IGNCs) with a simple method in clinical practice and to evaluate their prognostic value in pT1 ovarian clear cell carcinomas (OCCCs). Eighty-seven patients with pT1 OCCCs who underwent initial surgery were retrospectively assessed. Survival rates were significantly lower in IGNC-positive patients than in IGNC-negative patients (adjusted hazard ratio=14; 95% confidence interval= 2.7–768). Prognostic differences were not identified for other factors. IGNC identification on 28 available touch imprint cytology smears predicted IGNC identification on paraffin-embedded tissue sections (sensitivity=50.0%, specificity=100%, P=0.007). The presence of IGNCs has clinical and prognostic value for pT1 OCCCs.

Fetomaternal Medicine

1. Does the presence of antiphospholipid antibodies affect the etiology of unexplained infertility as it does that of recurrent spontaneous abortion?

Thrombophilias, such as antiphospholipid antibody (APLA) syndrome, are known to play a role in recurrent spontaneous abortion; however, their effect on unexplained infertility remains controversial and poorly understood. The aim of the present study was to investigate the prevalence of several thrombophilic markers in patients with unexplained infertility so that better use can be made of infertility treatments. In women with unexplained infertility, APLAs of any type tended to have a higher prevalence than in healthy pregnant women; therefore, APLAs might affect reproductive function. We suggest that women with unexplained infertility should undergo screening examinations for APLAs. We intend to investigate more cases.

2. The effect of APLAs on fetal and placental growth

APLAs have been proposed as a possible cause of fetal growth restriction (FGR) related to placental dysfunction. Of the numerous APLAs, however, which are involved is unclear. We revealed the APLA profile implicated in FGR to establish the therapeutic principles for pregnancy in patients with APLAs. In addition, we examined the effects of low-dose aspirin and heparin on the incidence of FGR.

Reproductive endocrinology

1. Study of ending *in vitro* fertilization treatment for infertile women 40 years or older.

Dealing with infertile women 40 years or older who cannot conceive despite *in vitro* fertilization (IVF) treatment can be difficult. The ending of infertility treatment is rarely discussed, although counseling is recognized as being important for infertile patients. We studied the results of IVF treatment in infertile women 40 years or older. We also used a questionnaire survey to examine the ending of IVF treatment for such women.

Publications

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