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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. Vitamin D receptor polymorphisms and the prognosis of patients with epithelial ovarian cancer

Recently, the vitamin D receptor (VDR) polymorphism FokI was shown to be associated with increased susceptibility to ovarian cancer. We examined whether VDR FokI polymorphisms affect the prognosis of patients with epithelial ovarian cancer. The VDR polymorphisms from FokI in 101 patients with epithelial ovarian cancer were genotyped by sequencing. Overall survival was compared between FokI single nucleotide polymorphisms by means of Kaplan-Meier survival analysis, log-rank tests, and the Cox proportional hazard model adjusted for International Federation of Gynecology and Obstetrics stages, postoperative chemotherapy, histologic type, and the presence of residual tumor. Hazard ratios, adjusted hazard ratios, and 95% confidence intervals were determined. The FokI C/C genotype was associated with a better prognosis than was the C/T or T/T genotype (log-rank test: $P=0.008$; adjusted hazard ratio: 0.16; 95% confidence interval: 0.05 to 0.57; $P=0.004$). Thirty months after surgery, 90% of patients with the FokI C/C genotype were still alive; in contrast, 66% of patients with the C/T or T/T genotype were alive. When the cancer stage was restricted to II to IV, 84% of patients with the FokI C/C genotype were still alive: in contrast, only 50% of patients with the C/T or T/T genotype were alive. These results suggest that the VDR polymorphisms from the FokI genotype are associated with the improved prognosis of patients with epithelial ovarian cancer.

2. 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 upon relapse. Because the response to chemother-

apy cannot be accurately predicted, we sought to identify somatic DNA copy number variation (CNV) associated with primary resistance in advanced-stage disease. The 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 with 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. On the basis of previously reported copy number associations 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 outcomes but without *CCNE1* amplification overexpressed genes involved in extracellular matrix deposition. Our findings have identified 2 distinct mechanisms of primary treatment failure in serous ovarian cancer, involving *CCNE1* amplification and enhanced extracellular matrix deposition.

3. Mesenchymal-to-epithelial transition during the formation of inclusion cysts 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 the precursor lesions of ovarian carcinoma. On the basis of this hypothesis, we aimed to characterize human ovarian surface epithelium in which mesenchymal-to-epithelial transition occurs in the process of inclusion cyst formation. We examined specimens from 9 patients with endometrial cancer who underwent hysterectomy and bilateral salpingo-oophorectomy. Immuohistochemical studies were performed of 10 healthy ovaries containing 92 inclusion cysts and 4 healthy fallopian tubes to examine the expression of antigen markers, including calretinin, podoplanin, D2–40, thrombomodulin, human bone marrow endothelial 1 (HBME-1), vimentin, epithelial membrane antigen (EMA), Wilms tumor 1 (WT1), CA125, MOC31, TAG-72, Ber-EP4, and E-cadherin. We found that the staining rates for mesothelial markers in healthy ovarian surface epithelium were 100% (10 of 10) for calretinin, 80% (8 of 10) for podoplanin, 80% (8 of 10) for D2-40, 70% (7 of 10) for thrombomodulin, 100% (10 of 10) for HBME-1, and 100% (10/10) for vimentin. Staining rates for epithelial markers in tubal epithelium were 100% (4 of 4) for HBME-1, 100% (4 of 4) for vimentin, 100% (4 of 4) for EMA, 75% (3 of 4) for TAG-72, and 100% (4 of 4) for Ber-EP4. Staining rates for markers of both types in inclusion cysts were 51.1% (47 of 92) for HBME-1, 44.6% (41 of 92) for vimentin, 65.2% (60 of 92) for TAG-72, and 88. 0% (81 of 92) for Ber-EP4. Ovarian surface epithelium has characteristics of both mesencyhme and epithelium. In contrast, inclusion cysts gain epithelial characteristics and lose mesencyhmal characteristics. These findings support the notion that a

mesenchymal-to-epithelial transition occurs during the formation of inclusion cysts from ovarian surface epithelium.

4. 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 T helper (Th) 1/Th2 cytokine response have been shown in ovarian cancer. In this study, we sought to clarify whether the cytokine gene expression profile affects the development and progression of ovarian cancer.

5. A randomized study of retroperitoneal closure

A randomized study of retroperitoneal closure versu sopening after lymphadenectomy for both uterine cervical cancer and endometrial cancer has been completed because 200 cases were registered.

A clinical study of robotic surgery using the da Vinci Surgical System has been started.

Fetomaternal Medicine

1. Investigation of the effects of antiphospholipid antibodies on obstetric complications

Antiphospholipid syndrome (APS) is a clinical entity manifested by arterial and venous thromboses and recurrent miscarriages which is caused by antiphospholipid antibodies. Recently, APS has 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 APS is involved in these complications. The Fc receptor for IgG (Fcγ receptor) is implicated in several autoimmune diseases. To investigate the pathological significance of the Fcγ receptor in APS and the complications of pregnancy, we created an experimental model for APS using Fcγ receptor knock-out mice.

Furthermore, we examined the presence of antiphospolipid antibodies in patients who had these obstetrical complications, concerning no risk patients.

2. The effect of antiphospolipid antibodies in unexplained infertility

Patient who experience recurrent pregnancy loss often become infertile or tend to have repeated spontaneous abortions after treatment for infertility. Those transitional conditions have not been noticed so far. We investigated the differences in possible causes and clinical status among these conditions from the perspective of reproductive failure.

Reproductive endocrinology

CD147 expression in implantation-stage endometrium

Many aspects of how pregnancy is achieved have been clarified with the development of assisted reproductive technologies, but the implantation period remains unclear.

CD147 is expressed at high levels on the surfaces of various tumor cells, stimulates matrix metalloproteinases (MMPs), and plays an important role in successful implantation. A unique mechanism for focal MMP expression may exist in endometrium during the human implantation period, but this remains unknown.

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 hyperstimula-

tion cycles.
Levels of CD147 and MMP2 messenger RNA in human endometrium were significantly decreased in the secretory phase of controlled ovarian hyperstimulation cycles.

Publications

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