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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. Identification of a novel long noncoding RNA involved in the tumorigenicity of ovarian clear cell carcinoma

Ovarian clear cell carcinoma (CCC) has a poor prognosis because of its chemoresistance. Therefore, identifying novel therapeutic targets is important. In this study, we have identified ASBEL, a novel antisense-noncoding (nc) RNA of the ANA/BTG3 gene, that is required for the tumorigenicity of CCC. Knockdown of ASBEL induces apoptosis in the CCC cell line JHOC5 and reduces the tumorigenicity of CCC in xenograft experiments. When ASBEL was knocked down, protein level of ANA was increased whereas ANA messenger (m) RNA didn't change. The ANA mRNA is retailed in the nucleus by forming duplexes with ASBEL. When ASBEL was knocked down, ANA mRNA translocates to the cytoplasm and ANA protein is translated. Through this mechanism, ASBEL suppresses the functions of tumor suppressor gene ANA. Thus, ASBEL is a promising target for CCC diagnosis and therapy.

2. A pilot study of CD147 protein expression in epithelial ovarian cancer using monoclonal antibody 12C3

CD147 is a membrane glycoprotein that is expressed in various cancer cells and is involved in tumor invasion and metastasis by inducing stromal fibroblastic cells to produce matrix metalloproteinases. This study was performed to evaluate the relationship between CD147 expression and various clinicopathologic variables, including histological grade and survival, in a small sample of human ovarian cancers. Paraffin-embedded surgical tissue samples from 25 patients with ovarian serous and endometrioid adenocarcinomas were stained with an anti-CD147 antibody for immunohistochemical analysis. The CD147 protein was expressed in 84.0% (21 of 25 cases) of cancerous lesions but not in normal lesions. The CD147 expression by ovarian cancer cells was negatively

correlated with overall survival but was not correlated with histological grade. These results suggest that measurement of CD147 expression will enhance our understanding of the pathophysiology of epithelial ovarian cancer.

3. Promising therapeutic target of the interleukin 6 signaling pathway in ovarian CCC

Cytokine expression in a tumor microenvironment can affect both host defense against the tumor and tumor-cell survival. We have previously reported that ovarian CCC shows a dominant helper T type 2 cytokine expression pattern driven largely by interleukin (IL)-6 expression. The unique cytokine expression pattern found in CCC may be involved in the pathogenesis of this subtype. Modulation of IL-6 expression or its related signaling pathway may be a promising treatment strategy for CCC.

4. Feasibility study of paclitaxel plus carboplatin in patients with endometrial cancer: a Japan Kanto Tumor Board study

The optimal chemotherapy regimen for patients with endometrial cancer has not been established. We assessed the feasibility of postoperative chemotherapy with paclitaxel plus carboplatin for patients with endometrial cancer. Patients with newly diagnosed endometrial cancer received paclitaxel (180 mg/m^2) and carboplatin (area under the curve, 6 mg/mL/minute) every 3 weeks. Treatment was continued until disease progression or the completion of 6 cycles. Toxicities were evaluated every cycle according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. Sixty patients were registered from December 2005 through November 2006. Forty-four of 60 (73.3%) patients completed all 6 planned cycles. Grades 3 and 4 hematologic toxicities were: leukopenia (61.7%), neutropenia (95.0%), anemia (21.7%), and thrombocytopenia (5.0%). Neutropenia caused 6 patients to discontinue treatment. Grade 3 nonhematologic toxicities were: nausea (3.3%), vomiting (1.7%), neuropathy (5.0%), myalgia (6.7%), and constipation (1.7%). No grade 4 nonhematologic toxicity occurred. We believe postoperative chemotherapy with paclitaxel plus carboplatin is feasible for patients with endometrial cancer.

5. Development of a second-generation photodynamic therapy for cervical cancer to reduce photosensitivity and shorten the length of stay

Uterine cervix conization has become the standard uterus-preserving treatment for early-stage cervical cancer. However, because conization increases the risks of subsequent premature birth, low birth weight, and cesarean delivery (as reported in *The Lancet*, 2006), the 2011 guidelines of the Japan Society of Gynecologic Oncology for the treatment cervical cancer call for patients to be fully informed of these risks before undergoing conization. On the other hand, photophrin photodynamic therapy (PDT) for cervical cancer is associated with a high complete-response rate (97%) and low obstetrical risk, but PDT is not recommended as the standard treatment for cervical cancer because of the significant photosensitivity induced by photophrin and the long hospital stay, which can be 3 weeks. Therefore, to develop a second-generation PDT for cervical cancer with reduced photosensitivity and shorter hospital stays, we tested, in collaboration with Professor Kunio Awazu of the Osaka University Department of Engineering, a semiconductor laser (PD laser) connected to an existing probe for uterine cervix irradiation. First, we developed a fibre channel adapter to connect an irradiation probe for lung cancer to an existing probe for uterine cervix irradiation. We then connected the main apparatus of

the PD laser to a direct irradiation probe for lung cancer, to which we connected to the uterine cervix irradiation probe in tandem through the fibre channel adapter. We performed laser irradiation under different experimental conditions. Next year, we are planning to perform a phase I clinical trial of second-generation PDT with the photosensitizer talaporfin sodium (Laserphyrin, Meiji Seika Pharma Co. Ltd., Tokyo) to produce less photosensitivity.

6. Malignant ovarian tumor in pregnancy

The aim of this study was to investigate the effect on pregnancy of the histological findings of malignant ovarian tumors. This retrospective study involved 41 patients treated for ovarian malignancy during pregnancy from 1985 through 2010. The median age of patients was 30 years (age range, 20 to 41 years). Disease stage was stage I in 38 patients (92%) and stages II, III, and IV in 1 patient (2%) each. The ovarian malignancies were borderline malignancy in 23 cases (56%), epithelial ovarian cancer in 10 cases (24%), germ cell tumors in 7 cases (17%), and a sex cord stromal tumor in 1 case. All patients underwent primary surgery: cystectomy in 6 patients (14%), unilateral salpingo-oophorectomy in 32 patients (78%), and hysterectomy with bilateral salpingo-oophorectomy in 3 patients (7%). The outcomes of pregnancy were delivery of a live newborn in 29 cases (70%), which included 21 cases of borderline tumors (91%), 2 cases of ovarian cancer (20%), and 6 cases of nonepithelial tumor (75%); termination to perform the standard treatment for ovarian malignancy in 8 cases; and spontaneous abortion in 2 cases. In pregnant women, ovarian cancer was less frequent than in an age-matched, statistically corrected control cohort of nonpregnant women based on a Japanese annual report (10 of 33 [30%] vs. control [6%]; ovarian cancer/[ovarian cancer + borderline tumor], $P = 0.001$). Pregnant women with ovarian cancer preferred to prioritize treatment at the expense of their offspring, while those with borderline tumors or nonepithelial tumors were more likely to successfully deliver live newborns.

Perinatology

1. Multiple injections of anti-mouse β2-glycoprotein 1 antibody induce FcR gamma-dependent fetal growth restriction in mice

Antiphospholipid syndrome (APS) is characterized by the presence of circulating antiphospholipid antibodies (aPLs). It is also a leading cause of thromboembolic events, repeated miscarriages, and fetal loss and is a major risk factor for fetal growth restriction (FGR) and pre-eclampsia. Anti-β2 glycoprotein I (aβ2GPI) antibody is a human aPL that is considered a specific and important marker for APS. We developed a murine model of FGR by administering multiple injections of WBCAL-1, a well-characterized mouse aβ2GPI monoclonal antibody. Administration of WBCAL-1, but not of the control antibody of the same isotype and saline, into pregnant mice decreased the size of fetuses and placentas without affecting the number of delivered pups. Also, a significant increase in urinary albumin and electron microscopic changes, such as splitting layers of basal membranes in the placental labyrinth and rearrangement of pores in glomerular endothelial cells, were observed in WBCAL-1-treated mice. However, injection of WBCAL-1 did not induce any changes in blood pressure or in typical indicators of blood thromboembolic symptoms. Furthermore, our present findings suggest that proteinuria

is a symptom associated with APS-related FGR with placental and renal tissue injuries and that FcR-gamma is a molecular target for preventing aβ2GPI antibody-mediated obstetric pathologies.

2. Cytotrophoblast alterations in placentas from disorders associated with aPLs and dysregulated clotting factors

Both aPLs and clotting factor disorders have been recognized as causes of placental insufficiency and obstetrical complications. Approximately 30% of patients have aPLs (14.5%) or clotting factor disorders (12.7%). To investigate the effect of a combined aspirin and heparin therapy on next-pregnancy outcome, we compared pregnancy outcomes and placental pathologies in 2 successive pregnancies in the same patients with aPLs or clotting factor disorders. Therapies in the 2nd pregnancy were effective in terms of delivery week for all cases, and in terms of fetal weight, which increased significantly in patients with aPLs compared with those in patients with clotting factor disorders. The fibrin regions in the extravillous areas of the placenta increased significantly in patients with clotting factor disorders but not in patients with aPLs. Hence, there is a need to further assess the effects of aPLs and clotting factor disorders on placental development.

3. Pathological characteristics of placentas obtained from women with obstetrical complications caused by aPLs

We investigated the effect of aPLs on the biology of the villous trophoblast in placentas stained with antibodies against Ki-67 and cytokeratin 7 to selectively identify proliferating villous cytotrophoblasts. Images were randomly selected, and cytotrophoblasts were counted. Although cytotrophoblast proliferation was reduced in all placentas, the total number of cytotrophoblasts decreased only in placentas from aPL-positive women. Hence, we suggest that aPLs inhibit cytotrophoblast proliferation throughout pregnancy.

Reproductive endocrinology

In 2012 we researched infertility, recurrent pregnancy loss, and endoscopic surgery. We assessed infertility treatments and perinatal outcomes after endoscopic surgery for patients of reproductive age. We found that endoscopic surgery did not have severe adverse effects on treatments for infertility or perinatal outcomes in patients of reproductive age.

We investigated infertility counseling. Our “Infertility Class” was seen as providing information about ending treatment for infertility. We recognized that genetic information and emotional support in the termination of treatment were of high importance in counseling by physicians and that the way genetic information is provided by each expert, such as counselors, nurses, and medical geneticists, should be discussed.

We investigated the relationship of anti-Mullerian hormone (AMH) with the cumulative pregnancy rate and the abortion rate. AMH might be used as a marker for the cumulative pregnancy rate. The abortion rate might be lower among cases with higher levels of AMH.

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