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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. Profiling of actionable gene alterations in ovarian cancer by targeted deep sequencing

To construct a profile of therapeutically actionable gene alterations in the major histological types of ovarian cancer. Seventy-two Japanese patients with surgically resected ovarian cancers were selected from an original cohort consisting of 267 patients who had not received pre-treatment before surgery. Somatic mutations and copy number alterations at 740 hot spots in 46 cancer-related genes were detected by deep sequencing genomic DNA using a next generation sequencer. *TP53*, *PIK3CA*, and *KRAS* were the three genes with the highest frequency of mutations and were altered in 28 (38.9%), 18 (25.0%), and 10 (13.9%) ovarian cancer patients, respectively. Actionable mutations and/or copy number aberrations in nine other genes, *PIK3CA*, *KRAS*, *PTEN*, *ERBB2*, *RB1*, *CDKN2A*, *AKT1*, *CTNNB1*, and *NRAS*, were detected in 35 (48.6%) patients with ovarian cancer. These mutations tended to occur in a mutually exclusive manner. Non-serous histological type tumors showed frequent actionable gene alterations (32/47; 68.1%). The profile indicates that in the non-serous ovarian cancers found in this Japanese population there are frequent gene aberrations that link to therapy using molecular targeting drugs.

2. Promising Therapeutic Target of IL-6/IL-6R Signaling Pathway in Ovarian Clear Cell Carcinoma

Cytokine expression in a tumor microenvironment can impact both host defense against the tumor and tumor cell survival. We previously reported that ovarian clear cell carcinoma (CCC) showed a dominant Th-2 cytokine expression pattern driven largely by *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 strategy of treatment for CCC.

3. MicroRNA-21 is a Candidate Driver Gene for 17q23-1 25 Amplification in Ovarian Clear Cell Carcinoma

Ovarian clear cell carcinoma (CCC) has unique clinical characteristics and behaviors that differ from other histological types of epithelial ovarian carcinoma (EOC), including a frequent association with endometriosis and a highly chemoresistant nature, resulting in poor prognosis. However, factors underlying its malignant behavior are still poorly understood. The aim of this study was to investigate the role of *miR-21* in 17q23-25 amplification associated with CCC oncogenesis. We identified 17q23-25 copy number aberrations among 28 primary CCC tumors by using a comparative genomic hybridization method. Next, we measured expression levels of the candidate target genes, *miR-21* and *PPM1D*, for 17q23-25 amplification by real-time RT-PCR analysis and compared those data with copy number status and clinicopathological features. In addition, immunohistochemical analysis of PTEN (a potential target of *miR-21*) was performed using the same primary CCC cases. We investigated the biological significance of *miR-21* overexpression in CCC using a loss-of-function antisense approach. 17q23-25 amplification with both *miR-21* overexpression and PTEN protein loss was detected in 4/28 CCC cases (14.2 %). The patients with 17q23-25 amplification had significantly shorter progression-free and overall survival than those without 17q23-25 amplification (log-rank test: $p = 0.0496$; $p = 0.0469$, respectively). A significant correlation was observed between *miR-21* overexpression and endometriosis. Both *PTEN* mRNA and PTEN protein expression were increased by *miR-21* knockdown in CCC cells. We also confirmed that *miR-21* directly bound to the 3′-untranslated region of *PTEN* mRNA using a dual-luciferase reporter assay. *MiR-21* is a possible driver gene other than *PPM1D* for 17q23-25 amplification in CCC. Aberrant expression of *miR-21* by chromosomal amplification might play an important role in CCC carcinogenesis through the regulation of the *PTEN* tumor suppressor gene.

4. Evaluation of maintenance docetaxel after paclitaxel and carboplatin combination chemotherapy in ovarian cancer

To test the concept of taxane sequencing, this feasibility trial evaluated maintenance docetaxel after paclitaxel and carboplatin combination chemotherapy in patients with stage Ic-IV ovarian cancer. All patients received debulking surgery followed by paclitaxel and carboplatin chemotherapy. Maintenance docetaxel started at an initial dose of 70 mg/m^2 every 4 weeks for 6 cycles and was extended to 10 cycles when effective. Stage subsets in 20 eligible patients were as follows: Ic, 1 patient (5 %), II, 1 patient (5 %), III, 13 patients (65 %), and IV, 5 patients (25 %). Neutropenia was common (40 % with grade 3 or 4) and was most frequent during first or second cycle although the disabling peripheral neuropathy was not observed. Twelve patients completed protocol therapy (6≦cycles), while 8 patients failed to complete 6-course chemotherapy, because of progressive disease (5 patients) or grade 4 toxicities (3 patients). Median overall survival from the start of maintenance chemotherapy was 39 months with median follow up period of 40 months and 1-year survival rate was 100 %. Those data were translated into the following conclusion; no less than 6 cycles of single-agent docetaxel maintenance chemotherapy is feasible and generally tolerable to women with advanced ovarian cancer who attained a clinically defined response to initial paclitaxel and carboplatin

based chemotherapy.

5. Feasibility Study of the Laparoscopic Approach for Borderline Ovarian Tumors

Compared to ovarian cancers, borderline ovarian tumors (BOTs) primarily present at an early stage in younger patients and have an excellent overall prognosis. Clinical management of BOTs during reproductive age has been modified from radical surgery to fertility-sparing surgery. However, the accurate diagnosis of BOTs prior to surgery is currently difficult. The aim of this study was to evaluate the feasibility of the laparoscopic approach for BOTs in terms of clinical outcome, including pre and intra-operative diagnosis. From January 2005 through December 2012, we retrospectively reviewed the clinical and surgical parameters of patients undergoing surgery for epithelial BOTs at our institution. A total of 119 BOTs were analyzed. For initial surgery, 111 (93%) underwent a laparotomy, and 8 (7%) underwent laparoscopic surgery, All the cases that underwent laparoscopic surgery were selected under a preoperative diagnosis of adenoma. Among 119 BOTs, 70 (64%) had solid areas and 50 (82%) had contrast enhancements in the tumor that was revealed by magnetic resonance imaging. The accuracy of intraoperative frozen section diagnosis was 84%. The incidence of tumor rupture during surgery was significantly higher in the laparoscopic surgery group compared to laparotomy group ($P = 0.0007$); however, there was no significant difference in the recurrence rate between stage Ia and Ic (b) patients. Pre- and intra-operative characterization of ovarian tumors using enhanced imaging studies and frozen section is clinically important. Although further studies are needed, with appropriate patient selection, laparoscopic surgery might be an acceptable intervention for young women with BOTs.

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 aß2GPI 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 second 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. Immunohistochemical localization of bilirubin oxidation in human placenta

Pregnancy is a state of oxidative stress, and in certain pathologic pregnancies the stress has been recognized to be heightened. However, the response in human placenta has not been fully clarified. Bilirubin is an intrinsic antioxidant, produced from heme through biliverdin which is catalyzed by heme oxigenase 1 (HO-1) and generates oxidative metabolites called biopyrrins as a result of the reaction with reactive oxygen species. Then in this study, to elucidate whether the oxidative stress affects placental physiology and function, this heme catalytic pathway and oxidative response in human placenta were morphologically investigated. Placental tissues from 10 patients with pre-eclampsia and 7 patients with uncomplicated preterm deliveries were examined immunohistochemically with monoclonal antibodies against bilirubin (24G7) and HO-1 (EP1391Y). Immunoreactivity with EP139Y was demonstrated around decidual spiral arteries, especially those with atherosis, in areas of infarction and villous stroma accompanied by perivillous fibrin deposits, and in syncytial knots. Immunoreactivity for 24G7 was identified around areas of infarction and decidual vasculopathy. Staining for bilirubin and HO-1 was more diffuse and more intense in cases of pre-eclampsia than in uncomplicated cases. This is the first report to demonstrate the localization of HO-1 and biopyrrins immunohistochemically in the human placenta.

Reproductive endocrinology

In 2013 we researched infertility counseling, endoscopic surgery, fertility preservation undergoing suffer from gonadotoxic agents, and women's sport medicine.

We assessed how the information is provided for infertility treatment. The desire for counseling is low among persons who do not have information about the treatment.Infertility counseling about the "aging of the egg" and the "end of treatment" had increased. We have also examined the relationship between body-mass index and endometriosis for the early detection of this condition. We have examined fertility preservation with respect to the growing need for it in our hospital. The need for oral conceptive pills by female athletes to treat dysmenorrhea was considered. Numerous cases of recurrent pregnancy loss in clinical trials were also followed.

Publications

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