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General Summary

The main research topics of our department are the development of molecularly targeted agents for 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. Increased synthesis of indoleamine-2, 3-dioxygenase protein is associated with impaired survival in patients with serous, but not with other types, of ovarian cancer

We have previously reported that indoleamine-2,3-dioxygenase (IDO) is associated with paclitaxel resistance and that IDO serves as a marker of poor prognosis in ovarian serous adenocarcinomas. We further examined IDO expression of serous adenocarcinomas and of other types of ovarian cancer. After informed consent was obtained, expression of IDO protein was analyzed with immunohistochemical techniques for a total of 136 ovarian cancers, including 40 serous adenocarcinomas, 67 clear cell adenocarcinomas, 12 mucinous adenocarcinomas, and 17 endometrioid adenocarcinomas. Staining for IDO was positive in 57% of serous adenocarcinomas, 49% of clear cell adenocarcinomas, 25% of mucinous adenocarcinomas, and 76% of endometrioid adenocarcinomas. The Kaplan-Meier survival curve showed a clear relationship between staining score and overall survival for patients with advanced (stage III and IV) serous adenocarcinoma ($n=33$) who underwent optimal surgery and paclitaxel-carboplatin chemotherapy as a first-line regimen. There was no association between IDO staining score and overall survival in cases of clear cell adenocarcinoma. Eight of 11 cases (72.7%) of clear cell adenocarcinomas with endometriosis showed identical IDO staining patterns between clear cell adenocarcinomas and endometriosis. In 43 of 60 cases (71.6%) with lymph-node metastasis, the IDO staining patterns of the primary lesion and the metastatic site were identical. These results suggest that increased synthesis of IDO protein is associated with impaired survival only in serous ovarian cancer.

2. Study of CD147 expression as a novel biomarker in malignant gynecological tumors

Immunohistochemical studies have shown expression of CD147 (extracellular matrix

metalloprotease inducer, basigin) and matrix metalloproteinase (MMP) in several types of cancers and have suggested that they play important roles in tumor invasiveness and metastasis. We showed that CD147 is expressed in 97.3% of endometrial carcinomas and is correlated with various clinicopathological factors and with recurrence-free survival. The aim of our study was to further analyze CD147 expression in other malignant gynecological tumors with links to clinicopathological factors, including patient outcomes. In addition, we would like to clarify the functional significance of CD147 in cancer progression and examine the possibility of using CD147 as a novel biomarker or as a therapeutic target.

3. Pattern recognition in serum to diagnose ovarian cancer in a Japanese population: Preliminary results

This small proof-of-principle study demonstrates that there are profiles in the serum of Japanese patients with ovarian cancer that can be used to classify the presence of cancer. The information is similar to that in the sera of patients with ovarian cancer in the United States because N-dimensional clusters built on United States sera spectra were used to create cluster maps predictive of the Japanese samples. The model performed better than any existing single biomarker assay, although truly useful models await a much larger sample size and the use of independent validation sample sets to demonstrate the robustness of the models. These results encourage us to start a large scale, multisite collection of serum from Japanese patients with ovarian cancer to develop a serum profile assay for ovarian cancer.

4. MicroRNA expression profiles for cancers, including lung, breast, stomach, prostate, and colon, were examined to investigate the involvement of microRNA in carcinogenesis.

We found that microRNAs were differentially expressed in normal tissues and cancers and could contribute to cancer development and progression. The microRNA molecular profiles were also correlated with patient survival. These results indicate that microRNA expression profiles are diagnostic and prognostic markers of cancers.

5. Mesenchymal-to-epithelial transition during 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 the precursor lesion of ovarian carcinoma. On the basis of this hypothesis, we aimed to characterize 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. Immunohistochemical studies were performed with 10 normal ovaries containing 92 inclusion cysts and 4 normal 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, CA125, MOC31, TAG-72, Ber-EP4, and E-cadherin. We found that the 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% (10/10) with vimentin. In tubal epithelium the positive staining

rates for epithelial markers were 100% (4 of 4) with HBME-1, 100% (4 of 4) with vimentin, 100% (4 of 4) with EMA, 75% (3 of 4) with TAG-72, and 100% (4 of 4) with Ber-EP4. Inclusion cysts showed positive staining for both markers with an incidence of 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 cysts gain epithelial characteristics and lose mesencyhmal characteristics. These findings support the observation of a mesenchymal-to-epithelial transition during inclusion cyst formation from the ovarian surface epithelium.

6. Characterization of mitochondria in cisplatin-resistant human ovarian carcinoma cells

Examination of mitochondrial transmembrane potentials revealed greater depolarization of platinum-resistant cells than of platinum-sensitive cells. Treatment of these cells with cisplatin or hydrogen peroxide (H_2O_2) induces complete destruction of mitochondrial DNA damage in sensitive cells, whereas only partial destruction of DNA was observed in resistant cells, suggesting mitochondria *per se* are resistant to cisplatin. Continuous oxygen consumption was significantly greater in sensitive cells than in resistant cells, and the amount of oxygen consumption was decreased 30% in resistant cells, suggesting mitochondrial respiratory malfunction in C13 cells. The resistance of mitochondrial DNA to cisplatin might be a main characteristic of the lower-level apoptosis induced by cisplatin.

Fetomaternal medicine

1. Which types of antiphospholipid antibody affect miscarriage or pregnancy-induced hypertension?

The aim of this study was to determine which types of antiphospholipid antibody affect miscarriage or pregnancy-induced hypertension. The results suggest that different types of of antiphospholipid antibody affect the pregnancy-induced hypertension group and miscarriage group, respectively.

2. Introduction of experimental intrauterine growth restriction in naive mice with purified IgG B_2-glycoprotein I-dependent anticardiolipin antibody

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 reported to be observed in some complications with pregnancy i.e., pregnancy-induced hypertension, intrauterine growth restriction (IUGR), 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 established an experimental model for APS using Fcγ receptor knock-out mice.

3. Interferon-γ-mediated IDO expression in peripheral monocytes is up-regulated by prolactin

IDO plays essential roles in successful pregnancy and is induced by type 1 cytokines. The receptors of type 1 cytokines share a structure and a signal transduction pathway

with prolactin. Because physiological levels of prolactin increased markedly during pregnancy, we examined the possible cooperation between prolactin and IDO expression. Therefore, to investigate IDO expression in CD14 cells, we obtained mononuclear cells from 12 healthy volunteers and cultured the cells for 24 to 48 hours in the presence or absence of stimuli (interferon [IFN]-γ: 5,100 IU/ml; prolactin: 10, 100, or 500 ng/ml). IDO expression was up-regulated only when prolactin were applied at physiological concentrations observed during pregnancy in combination with 5 IU/ml of IFN-γ; in contrast, prolactin or 5 IU/ml of IFN-γ alone had no effect. These findings suggest prolactin plays important roles in pregnancy maintenance, thereby up-regulating IDO induction. These findings also suggest that the physiological concentration of IFN-γ is important for pregnancy.

4. Does maternal exercise encourage the progression of uterine contractions and lead to premature delivery?

A rat model of excessive exercise during pregnancy was used to examine uterine smooth-muscle contractility and the effects on the uterine muscle of excessive exercise during pregnancy. Contraction of the uterine muscle was not induced by exercise on the 18th day of pregnancy. Even if excessive exercise caused a decrease in fetal-placenta blood flow, because fetal weight was smaller in exercised rats, contraction of the uterine muscle was not induced. Exercise did not cause premature delivery.

5. *IGFBP1* and *follistatin-like* 3 genes are significantly up-regulated in expression profiles of the IUGR placenta.

The clinicopathological features of IUGR remain unclear, and no effective therapy has been established for IUGR. To our knowledge, this is the first study to use microarray analysis to identify differentially expressed genes in the IUGR placenta. The expression profiles of 9121 genes were examined with cDNA microarray analysis using mRNA from an appropriate for gestational age (AGA) placenta and an IUGR placenta from discordant dichorionic twins. Up-regulation of the *IGFBP1* and *follistatin*-like 3 genes was detected in the IUGR placenta, with a balanced differential degree of 20.7 ± 1.3 and 13.1 ± 2.1, respectively, whereas the balanced differential degrees of other genes were 2.6 or less. The expression of the *IGFBP1* and *follistatin*-like 3 genes in 4 single IUGR and 4 AGA placentas was also examined with the reverse transcriptase polymerase chain reaction. Consistent with our data in discordant chorionic twin placentas, 3 of 4 IUGR placentas showed up-regulation of the *IGFBP1* gene, and all 4 IUGR placentas showed up-regulation of the *follistatin*-like 3 gene when compared with AGA placentas. Our results suggest that *IGFBP1* and *follistatin*-like 3 are highly up-regulated in the placenta in IUGR. *IGFBP1* and *follistatin*-like 3 are known critical regulators of fetal growth and differentiation. Pathways associated with these genes might be promising targets in molecularly-targeted therapy for IUGR

Reproductive endocrinology

We reported outcomes of treatment with laparoscopic ovarian drilling in cases of polycystic ovary syndrome, which causes follicular growth disorder, ovulation disorder. The effects of mental pressure during treatments were also examined.

Publications

Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce MM, Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006; **9:** 189–98.

Motegi M, Takakura S, Takano H, Tanaka T, Ochiai K. Adjuvant chemotherapy in a pregnant patient with endodermal sinus tumor of the ovary: a case report. *Obstet Gynecol* 2007; **109:** 537–40.

Okamoto A, Endo H, Kalionis B, Shinya M, Saito M, Nikaido T, Tanaka T. *IGFBP1* and *Follistatin*-like 3 genes are significantly up-regulated in expression profiles of the IUGR placenta. *Placenta* 2006; **27:** 317–21.

Ueda K, Yamada K, Urashima M, Ishibashi Y, Shirai M, Nikaido T, Takahashi H, OkamotoA, Saito M, Yasuda M, Ohkawa K, Tanaka T. Association of extracellular matrix metalloproteinase inducer in endometrial carcinoma with patient outcomes and clinicopathologenesis using monoclonal antibody 12C3. *Oncol Rep* 2007; **17:** 731–5.

Takao M, Okamoto A, Nikaido T, Urashima M, Takakura S, Saito M, Saito M, Okamoto S, Takikawa O, Sasaki H, Yasuda M, Ochiai K, Tanaka T. Increased synthesis of indoleamine-2,3-dioxygenase protein is positively associated with impaired survival in patients with serous-type, but not with other types of, ovarian cancer. *Oncol Rep* 2007; **17:** 1333–9.

Hirama M, Isonishi S, Yasuda M, Ishikawa H. Characterization of mitochondria in cisplatin resistant human ovarian carcinoma cells. *Oncol Rep* 2006; **16:** 997–1002.

Niimi S, Kiyokawa T, Takakura S, Ochiai K, Tanaka T. Recurrent small cell carcinoma of the ovary treated with docetaxel: a case report. *Int J Gynecol Cancer* 2006; **16:** 1944–66.

Takakura S, Saito M, Ueda K, Motegi M, Takao M, Yamada K, Okamoto A, Niimi S, Sasaki H, Tanaka T, Ochiai K. Irinotecan hydrochloride (CPT-11) and cisplatin as first-line chemotherapy for ovarian clear cell adenocarcinoma. *Int Surg* in press

Isonishi S, Hirama M, Saitou M, Yasuda M, Tanaka T. Primary chemotherapy-associated effect of second-line chemotherapy on survival of patients with advanced ovarian cancer. *Int J Clin Oncol* 2006; **11:** 236–42.

Reviews and Books

Ochiai K. Ransougan no subete. Tokyo: Medical Review; 2006.

Okamoto A, et al, editors. Guideline for endometrial cancer. Tokyo: Kaneharashuppan; 2006.

Yasuda M. Yokuwakaru Ransougan no subete (in Japanese). Osaka: Nagaishoten; 2007.