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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; perinatology, including fetal therapy; and the development of assisted reproductive techniques. These topics were investigated both experimentally and clinically.

Research Activities

Gynecology

1. Application of artificial intelligence for preoperative diagnostic and prognostic prediction, on the basis of blood biomarkers, in cases of epithelial ovarian cancer

A total of 334 patients with epithelial ovarian cancer (EOC) and 101 patients with benign ovarian tumors were randomly assigned to "training" and "test" cohorts. Seven supervised machine learning classifiers were used to derive diagnostic and prognostic information from 32 variables commonly available from pretreatment peripheral blood tests and age.

Ensemble methods combining weak decision trees showed the best performance in EOC prediction. For segregating EOC from benign ovarian tumors with Random Forest, the values for the highest accuracy was 92.4% and the area under the curve was 0.968. Unsupervised clustering analysis identified subgroups among patients with early-stage EOC and significantly worse survival. Machine learning systems can provide critical diagnostic and prognostic prediction for patients with EOC, and the use of predictive algorithms might facilitate personalized treatment options through pretreatment stratification of patients.

2. Identification of novel gene related to ovarian clear cell carcinoma proliferation with genome-scale CRISPR-Cas9 screens

By using genome-scale CRISPR-Cas9 screens against 4 ovarian clear cell carcinoma (OCCC) cell lines, we identified 1 candidate gene postulated to be involved in the growth of OCCC with mutations in both AT-rich interactive domain 1A (*ARID1A*) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*). While the viability of cell lines with these mutations was reduced upon small interfering RNA-mediated knockdown of the candidate gene, this knockdown did not cause any significant changes in gene expression. This result leads us to postulate that the candidate gene

induces apoptosis in a manner not yet characterized in the literature. To that end, we are now focusing on changes in cell metabolites and protein function upon knockdown of the candidate gene.

3. MicroRNA-34a as a new therapeutic target for ovarian cancer

MicroRNA-34a, which shows tumor-suppressive effects on several types of cancer, has been reported to be downregulated in ovarian high-grade serous carcinoma. In our present study, we aim to clarify the biology of microRNA-34a expression in high-grade serous carcinoma and the therapeutic viability of the microRNA on ovarian cancer.

4. Profiling of clinicopathological factors and actionable mutations with cervical cancer in the Japanese population

To elucidate the frequency of actionable genomic alterations in cervical cancers from Japanese patients, we performed the targeted sequencing for hotspot mutations in 50 cancer-related genes. Genomic DNAs extracted from formalin-fixed and paraffin-embedded tumor tissues were subjected to the analysis. Copy number alterations were detected with the TaqMan real-time quantitative polymerase chain reaction (PCR) assay in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA)*, erb-b2 receptor tyrosine kinase 2 (*ERBB2*), phosphatase and tensin homologue (*PTEN*), and serine/threonine kinase 11 (*STK11*). Human papillomavirus infection was confirmed with genetic testing and the in-situ hybridization method. The oncogenic or pathogenic variants registered in the OncoKB and ClinVar databases were defined as the actionable mutations in this study. We are processing the correlation analysis between genomic alteration, clinicopathological factors, and prognosis.

5. Invariant natural killer T cells are a unique subset that share properties of both T cells and natural killer cells

Invariant natural killer T cells (iNKT cells) express a semi-invariant T cell receptor that can interact with a glycolipid presenting molecule called CD1d. Upon activation with lipid antigen, iNKT cells can exert direct anticancer activity, behaving like CD8 T cells, and indirect anticancer activity through production of many cytokines. Our laboratory developed a new glycolipid named 7DW8-5, which showed higher affinity to CD1d and a stronger effect as an malaria vaccine adjuvant than does alpha-galactosylceramide. Therefore, we are investigating the antitumor effects of 7DW8-5 as iNKT cell-targeting immune therapy.

6. Development of a new diagnostic tool for OCCC using a cell-free circulating DNA detection method

We attempted to detect mutations by means of droplet digital PCR (ddPCR) in the genes *PIK3CA* and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) in cell-free (cf) DNA from patients who have OCCC. We were able to specifically detect *PIK3CA* mutation H1047R and *KRAS* point mutation G12D in cfDNA from patients with OCCC and monitor their response to therapy. Our results suggest that detection via ddPCR of mutations in cfDNA would be useful for diagnosing OCCC and predicting its recurrence.

7. Impact of mismatch repair protein and PD-L1 expression for therapeutic stratification in Japanese ovarian clear cell carcinoma

The study cohort comprised 113 patients with OCCC treated at a single institution. Protein expression levels in ARID1A, MLH1, PMS2, MSH2, MSH6, and PD-L1 were evalu-

ated by immunohistochemistry (IHC). MMR proteins disappeared in two cases (1.8%), specifically those who had synchronous double cancer with endometrial carcinoma and a family history of Lynch syndrome-related tumor. PD-L1 expression was diffused in 17 cases (15.0%). The diffused PD-L1 expression was significantly associated with ARID1A expression loss ($p = 0.003$), but no other correlations existed between PD-L1 expression and clinical parameters including prognosis. Although only few Japanese OCCC cases showed MMR deficiency as evaluated by IHC, immune checkpoint signals were activated even in MMR-intact OCCC, possibly through ARID1A interaction.

Perinatology

1. Amplicon-sequencing-based noninvasive fetal genotyping for *RHD*-positive D antigen-negative alleles

Fetal *RHD* genotyping via cfDNA might prevent unnecessary antibody application. We developed an amplicon-sequencing method that can estimate the type of paternally inherited fetal *RHD* allele from 4 major *RHD* alleles in the Japanese population. This method correctly determines the RhD blood type of the fetus even when RhD-negative pregnant women possess an *RHD*-positive-D antigen-negative allele. Because of its high accuracy and reasonable cost, this method is considered the first reliable noninvasive fetal *RHD* genotyping method for Japanese and other East Asian populations.

2. Retinoid acid-induced placental vascular hypoplasia in rats

Retinoic acid, a vitamin A derivative, has been suggested to be associated with preeclampsia. Therefore, we compared the expression of retinoic acid receptors and responders in the placentas of pregnant women with preeclampsia or with a normal pregnancy. Retinoid receptor expression was observed in the placenta of patients with pregnant hypertensive nephropathy as compared to that of patients with a normal pregnancy.

3. Development of novel cell therapy for fetal hypophosphatasia

The aim of our research is to develop a novel fetal therapy for hypophosphatasia. We established a mouse model of hypophosphatasia and are developing an alkaline phosphatase-expressing cell line. This study will be supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science in 2019.

4. Genomics and epigenetics research in perinatal region

The following studies were performed to develop methods for extracting targeted genomic/epigenomic information from crudely mixed genomic/epigenomic information.

a. Single-cell DNA sequencing of fetal cells in maternal peripheral blood for noninvasive prenatal diagnosis

b. The possibility of using placenta-specific interindividual differences in genome-wide DNA methylation profiles to assess intrauterine environments

c. Investigation via whole-genome single nucleotide polymorphism arrays of novel candidate genetic factors causing recurrent abortions in Japanese women

d. Genetic/epigenetic analyses for undiagnosed and rare perinatal diseases

5. Autonomous trisomic rescue of Down syndrome cells

In our study, we continuously cultivated induced pluripotent stem cells (iPSCs) with chromosome 21 trisomy and unexpectedly obtained revertant cells with normal chromosome 21 diploids from the trisomic cells. Repeated experiments revealed that this trisomy res-

cue was not due to mosaicism of chromosome 21 diploid cells and had occurred at an extremely high frequency. We herewith report the spontaneous correction from chromosome 21 trisomy to disomy without genetic manipulation, chemical treatment, or exposure to irradiation. The revertant diploid cells will possibly serve as a reference for drug screening and a raw material of regenerative medicinal products for cell-based therapy.

Reproductive endocrinology

1. The effect on fertility of molecularly targeted chemotherapies

We are studying the effects on ovaries of novel anticancer drugs, such as molecularly targeted drugs. Molecularly targeted drugs target specific molecules to suppress the increase of cancer and are said to have few side effects. However, their effect on ovaries is unknown. We are assessing the effects on ovaries of several molecularly targeted drugs.

Publications

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