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General Summary

The main research topics of the Department of Obstetrics and Gynecology are oncology, perinatology, reproductive endocrinology, and women's health. Researchers for each field commit to basic and clinical research to overcome clinically or biologically relevant issues with the latest technology and experimental methods.

Research Activities

Gynecologic Oncology

1. Identifying common targetable gene alteration among different tumor locations in advanced ovarian clear cell carcinoma

Ovarian clear cell carcinoma (OCCC) is a tumor that is resistant to conventional chemotherapy and demands novel molecularly targeted therapy. To identify common targetable mutations we perform gene sequencing of specimens of primary tumors from different locations and a metastasized tumor from 1 patient. This research may clarify the true target of OCCC.

2. Exploring novel target gene related to ovarian cancer carcinogenesis

Employing genome-scale clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 screens against 4 OCCC cell lines, we identified 1 candidate gene postulated to be involved in the growth of OCCC. Although the viability of cell lines with these mutations was reduced upon small interfering RNA- or short hairpin RNA-mediated knockdown of the candidate gene, this knockdown did not cause any significant changes in gene expression. On the basis of this finding, we postulate that the candidate gene induces cell death in a way not characterized in the literature. Therefore, we are focusing on changes in cell metabolites and protein function upon knockdown of the candidate gene.

3. Therapeutic preferability of gemcitabine for AT-rich interactive domain-containing protein 1A-deficient OCCC

A unique genomic feature of OCCC is frequent deficiency of the AT-rich interactive domain 1A gene (*ARID1A*). The present study was performed to investigate standard chemotherapeutic options suitable for *ARID1A*-deficient OCCC. Drugs with selective toxic-

ity to *ARID1A*-deficient OCCC cells were identified among 6 cytotoxic drugs used in standard chemotherapy for OCCC. Both *ARID1A*-knockout and *ARID1A*-deficient OCCC cells had selective sensitivity to gemcitabine. Growth of OCCC xenografts with *ARID1A* deficiency was inhibited by the administration of gemcitabine. Furthermore, of 7 patients of a retrospective cohort who had OCCC and received single-agent therapy with gemcitabine, 3 patients with *ARID1A*-deficient OCCC had significantly longer progression-free survival after gemcitabine treatment than did 4 patients with *ARID1A*-proficient OCCC. Patients with *ARID1A*-deficient OCCC might benefit from gemcitabine treatment.

4. Profiling of genomic alterations and clinicopathological factors with cervical cancer in the Japanese population

A potentially curative resection procedure was performed at the National Cancer Center Hospital for 154 patients with cervical cancer. Genomic DNA samples were analyzed with targeted sequencing, copy number assays, and human papillomavirus genotyping. Specific genomic alteration profiles were observed in patients with cervical cancer. These profiles were correlated with certain histological types and human papillomavirus genotypes. We detected actionable genomic alterations in 54 (35%) patients with cervical cancer. Furthermore, alterations of the serine/threonine kinase 11 gene (*STK11*) caused a poorer prognosis in patients with cervical cancer and in The Cancer Genome Atlas dataset. We have proposed the prognostic value of *STK11* genomic alterations.

5. The role of human epidermal growth factor receptor 3 expression in the resistance of ovarian cancer to chemotherapy

Human epidermal growth factor receptor (HER) 3, a member of the HER family, which also includes epidermal growth factor receptor and HER2, is expressed in approximately 50% of patients with ovarian cancer and has been reported to be a negative prognostic factor. A preclinical study showed that HER3 expression and chemoresistance are corelated. Therefore, we are investigating the relationship of HER3 expression and chemotherapy by means of clinical tumor specimens. This study might provide important information for overcoming the chemoresistance of ovarian cancer and, hopefully, for making HER3 an appropriate treatment target. This study is in collaboration with National Cancer Center Hospital East and Daiichi-Sankyo.

6. Gene function analysis of VUS (variant undertermined significance)

Genetic panel tests have become a basis of cancer precision medicine. However, few patients have received molecularly targeted therapy matched to known mutations; therefore, more mutations related to the efficacy of existing anticancer drugs must be identified. We are focusing on mutations in the extracellular domain of REarranged during Transition (RET) kinase as a model of mutants whose significance needs to be clarified. In some mutants, a gain-of-function property, such as transforming ability and growth factor-independent growth, was observed in tumor cell lines. The findings were confirmed by the result that RET tyrosine kinase inhibitors inhibit cell growth. Thus, the therapeutic significance of many druggable mutations might not have been investigated because of their rarity and unclustered features. The significance and promise of uncharacterized mutations in molecularly targeted therapy will be investigated by employing RET as a model.

7. Exploring novel genetic factors of adult granulosa cell tumor related to tumorigenesis and treatment
Adult type granulosa cell tumor (aGCT) is a rare ovarian tumor whose treatment is based on insufficient evidence or on the treatment of other ovarian cancers. The aGCT is characterized by late recurrence, but the treatment of aGCT other than complete resection has not been established. A recent study has shown that more than 90% of aGCTs harbor forkhead transcription factor L2 C402G mutations and telomerase reverse transcriptase promoter C228T mutations are associated with a poorer prognosis, but the validation in the Japanese cohort is not sufficient. The aim of this study is to detect the novel mechanism of tumorigenesis and to establish the effective treatment of this tumor through the complete genomic analysis of clinical samples.
8. MicroRNA as a 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 study, we provided the rationale for microRNA-34a replacement being a promising therapeutic strategy for this ovarian carcinoma.
9. Antiangiogenic therapy for OCCC with high interleukin 6 expression
Interleukin (IL) 6 is reported to be a potential treatment biomarker for antiangiogenic drugs against ovarian cancer. Despite OCCC having high IL-6 expression, whether antiangiogenic therapy is suitable remains unclear. We found that IL-6 is related to the effect of an antiangiogenic agent *in vivo* and that both vascular endothelial growth factor, which is the main target of the antiangiogenic agent, and other factors, such as angiopoietin, play roles between IL-6 and antiangiogenic therapy for OCCC.

Perinatology
1. Protective effect of ferroptotic cell-derived blebs
Ferroptosis is a nonapoptotic, iron-dependent form of programmed cell death caused by depleting glutathione and inactivating the phospholipid peroxidase glutathione peroxidase 4. Morphological characteristics of ferroptosis include cell rounding followed by plasma membrane rupture at the end stage of the cell death process. Plasma membrane changes include the formation of blebs, which are usually created via the local detachment of the cortex from the membrane with a spherical protrusion followed by plasma membrane rupture. Blebs reportedly have dynamic features connected to dramatic cellular reorganization with roles in the cytokinesis, cell spreading, virus uptake, apoptosis, and locomotion of tumors and embryonic cells. However, little has been reported on the functional analysis of blebs in ferroptosis. BeWo cells form blebs during the process of ferroptosis. We examined the functional role of blebs. We collected conditioned medium containing blebs from ferroptotic BeWo cells, treated the recipient cells with the conditioned medium, and examined cell viability with a lactate dehydrogenase releasing assay. We found that cells treated with the conditioned medium showed greater viability than did control cells, indicating that ferroptic cells might secret factors that have protective effects against cell death into blebs.
2. Genomics and epigenetics research in the 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
3. Amplicon sequencing-based noninvasive fetal genotyping for *RHD*-positive D antigen-negative alleles
Cell-free DNA-based fetal Rh blood group D antigen gene (*RHD*) genotyping might eliminate the necessary of routine anti-D immunoglobulin administration to RhD-negative pregnant women to avoid infant hemolytic disease from maternal anti-fetal Rh antigen alloantibodies. However, current *RHD* deletion detection methods do not address the higher *RHD*-positive D antigen-negative allele rates in non-white populations. We developed an amplicon-sequencing method to estimate the paternally inherited fetal *RHD* allele from 4 major *RHD* alleles in the Japanese population: D antigen-positive (*RHD**01, 92.9%) and D antigen-negative (*RHD**01N.01, 6.6%; *RHD**01EL.01, 0.3%; *RHD**01N.04, 0.1%) alleles, using cell-free DNA from the blood plasma of pregnant women (Takahashi K, et al: Clinical Chemistry 65:10 1307-1316, 2019). This method allows targeted anti-D immunoglobulin to be administered in East Asian countries and increases the accuracy of fetal *RHD* genotyping implemented nationally in several European countries. We have started a prospective study.
4. Development of cell therapy for a mouse model of hypophosphatasia
The aim of this research is to develop a new approach of treatment for hypophosphatasia. We established a mouse model of hypophosphatasia and are developing an alkaline phosphatase overexpressing cell line. This study is supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science in 2019.

Publications

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