Department of Obstetrics and Gynecology

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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

Gynecologic oncology

1. Genetic analysis of FOXL2 in adult-type granulosa cell tumors

Genetically, 97% of aGCT express heterozygous c.402C>G FOXL2 mutation. We analyzed the clinical data of 56 aGCT patients to find a marker of recurrence, and also compared the FOXL2 status in 5 matched primary and recurrent samples to address the role of FOXL2 in potential mechanisms of recurrence. The genetic analysis showed all the samples expressed heterozygous c.402C>G FOXL2 mutation and the FOXL2 protein expression. This finding adds further credence to the concept that the c.402C>G FOXL2 mutation is oncogenic and integral to this disease.

2. Sensitivity to conventional chemotherapeutic drugs according to ARID1A deficiency of ovarian carcinoma cell

Mutations of ARID1A gene are frequently observed in OCCC and ovarian endometrioid carcinoma (ENOCa). The aim of this study is to reveal associations of ARID1A deficiency with sensitivity to conventional chemotherapy. We are investigating availability of information of ARID1A status in the stage of drug selection for OCCC and ENOCa by comparison of sensitivity for conventional drugs in cancer cells with or without ARID1A mutation.

3. Prognostic impact of interleukin-6 expression in stage I ovarian clear cell carcinoma

We investigated tumor biology and prognostic factors for stage I OCCC from a clinicopathological perspective, including the expression of ARID1A and IL-6. A retrospective cohort study of 192 patients with stage I OCCC treated at a single institution was performed. The multivariate analysis indicated that substage classification and IL-6 expression status were associated with poor OS ($p = 0.010$ and $p = 0.027$, respectively). IL-6 molecular stratification may be crucial in optimizing therapeutic strategies for early stage OCCC to improve survival.

4. CRISPR-Cas9 knockout screening in human ovarian clear cell carcinoma cell lines

Using the Toronto KnockOut (TKO) CRISPR Library Version 3, prepared from a library

of approximately 80,000 sgRNA sequences, with three or four designs relating to the human genome of approximately 20,000 genes, screening was carried out with OCCC cell lines, which have both ARID1A and PIK3CA mutations, and cell lines, which have no mutations characteristic of OCCC. Findings in this study is expected that identification of the ARID1A and PIK3CA mutations, their signal pathways, and related novel treatment targets, and also identification of factors that promote tumorigenicity without mediation by the ARID1A and PIK3CA mutations, will facilitate establishment of targeted therapies for OCCC.

5. Molecular profiling in malignant gynecologic tumors

Studies of molecular characteristics in cervical cancer and carcinosarcoma/sarcoma of the uterus have been limited, especially in Japanese population. We clarify those by targeted sequencing and analyze correlation between moleculer features and clinical factors.

6. MicroRNA as a therapeutic target for ovarian cancer

MicroRNA-34a, which shows tumor-suppressive effects on several types of cancer, is reported to be down-regulated in ovarian high-grade serous carcinoma (HGSC). In our present study, we aim to clarify the mechanisms of microRNA-34a expression in HGCS and the therapeutic availability of the microRNA on ovarian cancer.

7. Development of immunological treatment targeting NKT cell

iNKT cells are a unique subset of T cells that share properties of both T cells and natural killer cells. we are investigating effect of 7DW8-5 against tumor and other optimal iNKT cell targeting immune therapy against tumor.

8. Molecular characterization of chemo-sensitivity in OCCC

To identify predictive biomarker of chemo-sensitivity in OCCC, we performed immunohistochemistry and targeted sequencing. Although we suspected that chemo-response might be due to misclassification of a subset of OCCC as HGSOC, or be associated with the low proportion of OCCC with TP53 mutation, these were not associated with chemo-response. Rather, low level expression of HNF1B and Ki67 appear to be associated with a particularly favorable outcome.

Perinatology

1. Fetal therapy model of myelomeningocele with three-dimensional skin using amniotic fluid-derived iPS cells

We generated iPS cells from amniotic fluid. Our iPS cells differentiated into keratinocytes with high expression level of epithelial markers. Furthermore, these iPS-derived keratinocytes were successfully reconstructed into multilayered epidermis. Through transplantation of “Artificial Skin”, the defects of the myelomeningocele model in rat fetuses were successfully treated.

2. Amniotic fluid cell-derived Down syndrome induced iPS cells exhibited reversion to intact disomy 21

We aimed to correct chromosome 21 trisomy cells to disomy cells in vitro through generation of iPS cells. We have successfully obtained revertant cells with intact chromosome 21 diploids from the trisomy cells. Trisomy-rescued stem cells with the same/similar genetic background serve as good controls for Down syndrome cells when elucidating the pathology of Down syndrome by comparing the properties of their differentiated deriva-

tives.
3. To develop methods for extracting targeted genomic/epigenomic information from crudely mixed genomic/epigenomic information
We are developing a new method for analyzing fetal DNA using purified circulating fetal cells in maternal peripheral blood. And we analyze for recurrent abortions and undiagnosed perinatal diseases using genome-wide single-nucleotide polymorphism microarray, exome analysis and methylation analysis.
4. Prenatal determination of fetal RHD focused on the difference of haplotype
We identified three haplotypes covering more than 99% of the RHD negative in Japanese population, and developed a new method to accurately distinguish target genes by high resolution polymorphism analysis using next generation sequencers. As a result, it is possible to accurately identify the fetal genotype from cell free DNA and to perform prenatal diagnosis of RHD blood type adapted to the Japanese population.
5. Changes in oxytocin-sensitive cells in amygdala with labor experience
Central action of oxytocin in the brain has been received much attention in recent years. We focus on the amygdala involved in emotion and pain and are working on elucidating the change of oxytocin-sensitive cells in the amygdala for perinatal period.

Reproductive endocrinology
1. Improving reproductive technology make fertility preservation possible including for prepubertal females
A limitation of this approach is our lack of knowledge about egg quality in the pediatric population where a large fraction of individuals may be in the pubertal transition. To determine the effects of pubertal transition on egg quality, we examined how the yield and size of oocytes collected from antral follicles are affected by animal age during the pubertal transition and quantified the following meiotic parameters in oocytes isolated in each cohort. Using a mouse model, we found that the egg quality in the pre-pubertal cohort was decreased. These findings suggest that we can obtain matured oocytes including pre-pubertal cohort but that the egg quality in this cohort will be decreased, resulting in the high incidence of aneuploidy. These results provide important opportunities to understand the effects of puberty on egg quality as well as to improve the fertility of young female patients.

Publications

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