# 学 位 論 文 の 要 旨

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学 位 論 文 名　Reconstitution of High-grade Serous Ovarian Carcinoma From Primary Fallopian Tube Secretory Epithelial Cells.

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## 論 文 内 容 の 要 旨

### INTRODUCTION

Epithelial ovarian cancer (EOC) is a heterogeneous group of neoplastic diseases that comprise the most lethal gynecologic malignancy worldwide. Several cellular origins have been reported for EOCs, including epithelial cells of the fallopian tube fimbriae, ovarian fimbriae, ovarian surface epithelium, inclusion cysts, peritoneal mesothelium, or endometriotic tissue. The hypothesis of fallopian tube fimbriae-derived high-grade serous ovarian carcinomas (HGSOCs), particularly with respect to fallopian tubal secretory epithelial cells (FTSECs), has been attracting increasing attention in recent years. Several genetic alterations are involved in HGSOC carcinogenesis, but the minimal requirement for tumor initiation remains unclear. In this study, our first challenge was to establish a method for immortalizing FTSECs while retaining their normal phenotype. Our second challenge was to identify the minimal number and specific combination of mutations in samples from patients with HGSOC that would be sufficient for malignant transformation of the immortalized FTSECs. We thus aimed to generate an in vitro stepwise model of carcinogenesis.

### MATERIALS AND METHODS

FTSECs were isolated from clinical samples and immortalised via cyclin D1, $CDK4^{R24C}$,

and *hTERT* overexpression. Various oncogenic mutations were mimicked by lentiviral transduction. For sequencing analysis, sections from the 34 tumor samples from patients with HGSOC were washed in cold PBS, minced to ~1-mm3 fragments, and digested with collagenase A (10 mg/mL) with mild agitation at 37 ℃ for 40 min. The samples were allowed to rest, and single tumor cells or small tumor cell clusters (<10 cells) were obtained in suspension after the large undigested tissue fragments settled at the bottom of the centrifuge tube. The tumor cells were washed with PBS, harvested with anti-Ep-CAM-coated magnetic beads, and then subjected to genomic DNA extraction. Samples with limited cell yields were expanded in an RPMI1640 medium with 10% FBS for 3 days, prior to genomic DNA isolation. All exons of TP53, PTEN, KRAS, and BRAF, as well as exons 9 and 20 of PIK3CA, were amplified by PCR using the primer sets mentioned in Supplementary Table 2. PCR products were purified using a PCR purification kit and then analyzed by direct sequencing.

The study protocol was approved by the Ethics Committee of Shimane University and written informed consent was obtained from all subjects.

All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University and they were handled according to our institutional guidelines.

## RESULTS AND DISCUSSION

Highly pure FTSECs were successfully isolated from clinical samples and immortalized by overexpressing cyclin D1, CDK4R24C, and hTERT, which enabled introduction of various genetic alterations. We found two distinct patterns of gene alterations essential for HGSOC carcinogenesis: p53/KRAS/AKT and p53/KRAS/c-Myc. Dominant-negative p53 expression alone or in combination with oncogenic KRAS (KRASV12), constitutively active AKT (CA-AKT), or c-Myc in immortalized cells failed to induce tumorigenic phenotypes; however, overexpression of either CA-AKT or c-Myc along with dominant-negative p53 and KRASV12 was sufficient to confer tumorigenic potential. Importantly, all transformed FTSECs formed tumors in nude mice, which were grossly, histologically, and immunohistochemically similar to human HGSOC. Sasaki et al. previously established a carcinogenetic model comprising OSE cells immortalized by p53 inactivation, oncogenic KRASV12, and c-Myc and Bcl-2 overexpression. However, tumor growth in mice revealed undifferentiated carcinomas devoid of PAX8 expression, which were not similar to human HGSOCs. These data therefore support our hypothesis that FTSECs are the origin of HGSOCs. Interestingly, mice harboring tumors with c-Myc amplifications displayed extensive metastases, consistent with the increased dissemination observed in their human counterparts. C-Myc is associated with cell proliferation

in vitro; therefore, this genetic abnormality may promote HGSOC progression.

## CONCLUSION

We identified two patterns of three genetic alterations (i.e., p53/KRASV12/c-Myc and p53/KRASV12/PI3K-AKT) that were essential for HGSOC development, and successfully established an in vitro step-wise model of carcinogenesis using immortalized FTSECs. This experimental model provides a foundation for future studies on early HGSOC development, and for identifying candidate targets for drug development and secreted biomarkers for early detection.