学 位 論 文 の 要 旨

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学　位　論　文　名　Establishment of a Novel In Vitro and In Vivo Model to Understand Molecular Carcinogenesis of Endometriosis-Related Ovarian Neoplasms

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論 文 内 容 の 要 旨

INTRODUCTION

Endometriosis, an estrogen-dependent condition affecting 7–15% of women of reproductive age, is associated with increased risk of epithelial ovarian cancers, particularly ovarian clear cell carcinoma (OCCC) and endometrioid carcinoma (OEC), known as endometriosis-related ovarian neoplasms (ERONs). Most ERONs are believed to arise from benign endometriotic cysts, especially in older patients or those with larger cysts. In Japan, the standardized incidence ratio of ovarian cancer in women with endometriotic cysts is 8.95%, with 39% OCCC and 35% OEC. ERONs follow the type I ovarian cancer pathway, with *ARID1A* mutations identified as key early events present in around 95% of OCCCs and 30% of OECs. Other recurrent mutations include *PIK3CA*, *PTEN*, *KRAS*, *PPP2R1A*, and *ZNF21*, suggesting a multistep molecular evolution. Although early-stage OCCC has a favorable prognosis, advanced cases show poor response to platinum-based chemotherapy. OEC generally has a better outcome, but recurrence and drug resistance remain challenges. Despite extensive genetic studies, the exact molecular mechanisms and combinations of alterations driving ERON development are not fully understood.

To investigate this, we established a stepwise In Vivo and In Vitro carcinogenesis model using immortalized endometriotic epithelial cells, introducing defined genetic alterations and evaluating tumorigenicity in immunocompromised mice.

MATERIALS AND METHODS

Benign endometrial tissue was obtained from a 53-year-old patient with an ovarian endometrioma at Shimane University Hospital. Primary cells were isolated and immortalized by introducing cyclin D1, CDK4R24C, and hTERT, and the resulting cell line was named HMOsisEC7. The epithelial origin of the cells was confirmed by immunocytochemistry (ICC) and western blotting. Immunocytochemistry (ICC) and western blot were carried out to confirm the epithelial origin. Population doubling assay and whole-exome profiling were carried out with the aim to determine the growth curve and germline mutation. HMOsisEC7 cells were then genetically modified to knock out or overexpress key cancer-related genes, including *ARID1A*, *KRAS*, *PIK3CA*, *AKT*, and *MYC*, resulting in the development of eight new cell lines. Western blotting was used to confirm the mutational status of each line. Functional assays, including cell proliferation, migration, invasion, and anchorage-independent growth, were conducted using various cell densities (10,000, 3,000, and 25,000 cells/well). Tumorigenic potential was evaluated through subcutaneous xenograft experiments in SCID and nude mice (2.5×10^7 cells/mL). Immunohistochemistry of xenograft tumors was performed using antibodies against pan-cytokeratin, PAX8, HNF-1B, ARID1A, and c-Myc.

Statistical analysis was conducted using Student's t-test (SPSS software), with a p-value < 0.05 considered statistically significant. The study protocols were approved by the Research Ethics Committee of Shimane University and all experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

RESULTS AND DISCUSSION

We established a novel immortalized endometriotic epithelial cell line, HMOsisEC7, derived from a patient with ovarian endometriosis. Cellular senescence was overcome through lentiviral transduction of $CDK4^{R24C}$, cyclin D1, and hTERT, resulting in stable immortalized cells that retained epithelial morphology and showed no tumorigenic potential in vivo. Analysis of The Cancer Genome Atlas (TCGA) revealed frequent mutations in *ARID1A*, *PIK3CA*, *KRAS*, AKT, and MYC in ERONs. Based on this, we hypothesized that *ARID1A* loss serves as a critical initiating event in ERON development. To investigate this, we used CRISPR-Cas9 to knock out *ARID1A* in HMOsisEC7 cells. We then introduced additional oncogenic alterations either individually or in combination, including mutant *KRAS*, mutant *PIK3CA*, constitutively active AKT, and overexpressed c-Myc. Functional characterization of the genetically modified cells revealed that triple-mutant combinations such as *ARID1A* KO + *KRAS* MT + c-Myc or *ARID1A* KO + AKT + c-Myc significantly enhanced cell proliferation, migration, invasion, and colony formation compared to parental or double-mutant cells. Importantly, only these triple-mutant cells formed tumors when injected into immunodeficient mice. Histological analysis of the

resulting tumors showed distinct phenotypes depending on the host immune background: tumors in SCID mice resembled ovarian clear cell carcinoma (OCCC), while those in nude mice resembled endometrioid ovarian carcinoma (OEC). This divergence suggests that not only the genetic alterations but also the tumor immune microenvironment (TIME), particularly the role of B cells may influence histological outcomes. These findings established a stepwise in vitro and in vivo model that mimics ERON progression from benign endometriotic epithelium to malignant transformation. Unlike high-grade serous ovarian carcinoma, which commonly harbors TP53 mutations, ERONs exhibit a distinct mutational spectrum involving *ARID1A*, *PIK3CA*, *KRAS*, AKT, and MYC. Our model supports this distinct pathogenesis, demonstrating that a combination of *ARID1A* loss, RAS/ERK or PI3K/AKT pathway activation, and c-Myc overexpression is sufficient to drive malignant transformation and tumorigenesis. Finally, the observation that identical genetic backgrounds produce different tumor histotypes in distinct immunodeficient models reinforces the influence of TIME, in line with recent studies suggesting that histological plasticity arises from an interplay between genetic/epigenetic alterations, cell state, and microenvironmental context. While our model successfully recapitulates key features of ERON pathogenesis and diverse histology due to maybe B cell, further investigations are essential to explore drug responsiveness and to characterize the TIME more precisely. These efforts will be critical to understand histological divergence and may inform the development of personalized therapeutic strategies for ERON patients.

CONCLUSION

We successfully created an in vitro and in vivo stepwise carcinogenesis model using immortalized endometriotic epithelial cells, in which introducing three specific genetic combinations (*ARID1A* KO/*KRAS* MT/overexpressed c-Myc or *ARID1A* KO/overexpressed AKT/overexpressed c-Myc) enabled the cells to form apparent tumors in immunocompromised mice, confirming that they are essential drivers for the development of ERONs. Of particular interest is the histological diversity depending upon the mouse species inoculated with the same genetic mutational cell line; OCCC phenotypes in SCID mice and OEC phenotypes in nude mice, suggesting the possible involvement of TIME (especially B-cell signaling) on histological outcomes. This model has the potential to facilitate future research on ERON development and the advancement of targeted therapies.