学 位 論 文 の 要 旨

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学 位 論 文 名　Establishment of a Novel In Vitro Model of Endometriosis With Oncogenic *KRAS* and *PIK3CA* Mutations for Understanding the Underlying Biology and Molecular Pathogenesis

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論 文 内 容 の 要 旨

INTRODUCTION

Endometriosis is a common gynecological condition that causes pelvic pain and infertility. Despite having normal histological features, several cells bear cancer-associated somatic mutations that result in local tissue invasion but rarely metastasize. Several cancer-associated genes, such as *KRAS* and *PIK3CA*, are frequently mutated in the endometriotic epithelium. However, the functional behavior and molecular pathogenesis of this disorder remain unclear. In this study, we developed an immortalized endometriotic epithelial cell line with mutations in *KRAS* and *PIK3CA*, which are genes associated with aggressive behaviors, such as increased cell migration, invasion, and proliferation. In addition, we performed microarray analyses of immortalized endometriotic epithelial cell lines (*KRAS* and *PIK3CA* mutant) to identify migration and invasion-related gene signatures.

MATERIALS AND METHODS

Human endometriotic tissue samples were obtained from ovarian endometriotic cysts from a patient who underwent a laparoscopic ovarian cystectomy. Immortalized ovarian endometriotic epithelial cells were successfully generated through transduction with *hTERT*, *cyclin D1*, and

mutant *CDK4* (*CDK4*^{R24C}: an inhibitor-resistant form of *CDK4*) via lentivirus-mediated gene transfer using the Gateway system. We also established two new cell lines through transfection of mutant *KRAS* and mutant *PIK3CA*. The established cell lines were named as HMOsisEC10 (wild type), HMOsisEC10 *KRAS* (mutant), and HMOsisEC10 *PIK3CA* (mutant) which were used in our study. Next, we have conducted an In Vitro migration, invasion and proliferation assay by using these immortalized cell lines to analyze the cellular functional behavior. To evaluate the transformation phenotype, an anchorage-independent assay and nude mice xenograft assay were performed. We next performed the whole-exome sequencing to identify whether the immortal HMOsisEC10 cell line had acquired any tumor-specific somatic mutations. In order to recognize the genes that were regulated in endometriosis pathway, a global microarray analysis was undertaken. The study protocol was approved by the Research Ethics Committee of Shimane University. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

RESULTS AND DISCUSSION

The experimental cell line HMOsisEC10 was developed from ovarian endometriotic epithelial cells without any evidence of cancer. Subsequently, we established two new cell lines through transfection of *KRAS* and *PIK3CA*. Western blot using pan-cytokeratin showed that the epithelial morphology was highly detectable in the HMOsisEC10 immortal cell line. Western blot assays also showed that the RAS/ERK signaling pathway was strongly activated in the HMOsisEC10 *KRAS* mutant cells, whereas the PI3K/AKT signaling pathway was activated in the HMOsisEC10 *PIK3CA* mutant cells. To confirm the presence of any type of somatic mutation, we subjected the developed cell line to whole-exome sequencing analysis, which revealed no somatic or germline mutations including single-nucleotide variations, insertions, or deletions. The results of In Vitro assays showed that the immortalized *PIK3CA* and *KRAS* mutant cell lines exhibit a higher degree of cell proliferation, invasion, and migration indicating these mutations played causative roles in the aggressive behavior of endometriosis. However, these changes were not sufficient to induce the development of cancer from endometriosis by using anchorage dependent assay and nude mice xenograft assay. Microarray analysis identified a cluster of gene signatures; among them, two significantly upregulated cancer-related genes *LOX* and *PTX3* were associated with cell proliferation, invasion, and migration capabilities. Furthermore, siRNA knockdown of the two genes markedly reduced the metastatic ability of the cells. These results suggest that endometriosis with *KRAS* or *PIK3CA* mutations can significantly enhance cell migration, invasion, and proliferation by upregulating *LOX* and *PTX3*.

CONCLUSION

In summary, by using the immortalized endometriotic cell line, we have established two new mutant cell lines, HMOsisEC10 *KRAS* and HMOsisEC10 *PIK3CA*, which significantly enhance cell migration, invasion, and proliferation by upregulating *LOX* and *PTX3*. We propose that *LOX* and *PTX3* silencing using small molecules could be an alternative therapeutic regimen for severe endometriosis.