学 位 論 文 の 要 旨

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学　位　論　文　名	Association Between KRAS and PIK3CA Mutations and Progesterone Resistance in Endometriotic Epithelial Cell Line
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論 文 内 容 の 要 旨

INTRODUCTION

Endometriosis causes dysmenorrhea, chronic pelvic pain, dyspareunia, painful defecation or infertility and affects quality of life of reproductive aged women. Mechanisms of endometriosis-related pains are thought to be caused by complex pathways as following: (1) Cyclic release of pain mediators and inflammatory mediators activate nociceptors resulting in dysmenorrhea and cyclic lower abdominal pain; (2) Increased sensory nerve fibers and decreased sympathetic nerve fibers, and an imbalance of proinflammatory and anti-inflammatory sympathetic neurotransmitters cause acyclic chronic pelvic pain (neurogenic inflammation); and (3) Cyclic and repeated pain increase the nociceptive fields and lead to spinal hyperalgesia resulting in painful defecation.

The major medical treatment for endometriosis-related pain is an administration of progestins such as dienogest (DNG) or medroxyprogesterone acetate (MPA), however, progestin resistance in terms of lesion growth and/or pain is sometimes observed. Although endometriosis is a benign disease, the cancer-associated mutations such as *KRAS* and *PIK3CA* genes are detected in the epithelial cells at a certain frequency. In a previous study, we established an immortalized endometriotic epithelial cell lines derived from the patients with endometriosis and

found that introduction of *KRAS* or *PIK3CA* mutations into them significantly promoted the cell proliferation, migration and invasion *in vitro*, suggesting the biological role of these mutations for aggressive behavior of the disease. Here, we examined whether *KRAS* or *PIK3CA* mutations observed in endometriotic epithelial cells are involved in progestin-resistance.

MATERIALS AND METHODS

We collected, purified and primary-cultured epithelial cells from a surgically removed ovarian endometrioma. Then we immortalized them by the transduction with c-DNA for *hTERT*, *cyclin D1*, and mutant *CDK4* ($CDK4^{R24C}$) via lentivirus-mediated gene transfer, and named it as HMOsisEC10. We further overexpressed cDNA for *progesterone receptor B* (PR-B) into this cell line. Additionally, we introduced *KRAS* ($KRAS^{V12}$) and *PIK3CA* ($PIK3CA^{E545K}$) mutant alleles by lentivirus vector infection into these cells, named as HMOsisEC10 KRAS-PRB and HMOsisEC10 PIK3CA-PRB, respectively.

Under the treatment of these cells with DNG or MPA at 15μM, we examined the ability of migration, invasion and proliferation via wound healing assay, Matrigel invasion assay and MTT assay. Furthermore, the expressions of pain- and angiogenesis-related factors were evaluated by real-time quantitative PCR (qPCR), such as Cox-2, interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), monocyte chemotactic protein-1 (MCP-1), and cytochrome P 19A1 (CYP19A1).

The study protocol was approved by the Research Ethics Committee of Shimane University.

RESULTS AND DISCUSSION

Immortalized HMOsisEC10 cell line lacked PR expression unlike original endometriotic epithelial cells, probably due to long-term culture during immortal process. PR has two isoforms, PR-A, and PR-B. PR-B is known to exhibit potent transcriptional activity to various target genes, whereas PR-A partly functions as a transcriptional repressor. Since progestin-responsiveness of endometriotic tissues is mediated by PR-B, rather than PR-A, we sought to transduce PR-B into HMOsisEC10 cells. Therefore, we overexpressed cDNA for PR-B into this cell line and confirmed that this manipulation successfully led to sufficient expression of PR-B by western blot analysis. We further introduced *KRAS* ($KRAS^{V12}$) and *PIK3CA* ($PIK3CA^{E545K}$) mutant alleles by lentivirus vector infection into these cells, named as

HMOsisEC10 KRAS-PRB and HMOsisEC10 PIK3CA-PRB, respectively.

These cells apparently showed progestin sensitivity in relation to cell growth, migration, invasion and various pain- and angiogenesis-related factors. However, in HMOsisEC10 KRAS-PRB cells, pain-related COX-2 and mPGES-1 expressions were not repressed by progestin treatment, showing progestin-resistance. Thus, some pain-related factors are not responsive to progestin in the presence of oncogenic *KRAS* mutations.

It is reported that progestin resistance of endometriosis may be partly due to the suppression of PR expression in the target tissues, although endometriotic tissues usually express PR. We hypothesized that the existence of oncogenic *KRAS* or *PIK3CA* mutation detected in endometriotic epithelial cells may play some roles in progestin resistance of endometriosis. The present study found that HMOsisEC10 KRAS-PRB or HMOsisEC10 PIK3CA-PRB responded to DNG or MPA, leading to the inhibition of cell growth, migration and invasion. However, interestingly, expressions of pain-related factors, COX-2 and mPGES-1 were not inhibited by progestin treatment in HMOsisEC10KRAS-PRB cells. Therefore, we speculate that *KRAS* mutation may be involved in progestin resistance to endometriosis-related pain.

CONCLUSION

Our results suggest that *KRAS* and *PIK3CA* mutation may not be associated with progestin resistance to aggressive behavior, but *KRAS* mutation may be involved in progestin resistance in terms of pain.