学 位 論 文 の 要 旨

氏名　PUJA DEY

学 位 論 文 名　Development of Low-Grade Serous Ovarian Carcinoma From Benign Ovarian Serous Cystadenoma Cells.

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著 者 名　Puja Dey, Kentaro Nakayama, Sultana Razia, Masako Ishikawa, Tomoka Ishibashi, Hitomi Yamashita, Kosuke Kanno, Seiya Sato, Tohru Kiyono and Satoru Kyo.

論 文 内 容 の 要 旨

INTRODUCTION

Low grade serous ovarian carcinoma is constituting a relatively unusual distinctive type of tumor that tends to occur in younger patients, have apathetic progression and long-term survival where have an association with chemoresistance compare to other type of ovarian carcinoma. Although several genetic alterations are involved for the progression of low-grade serous ovarian carcinoma (LGSOC), but the specific combination of mutations required remains unclear. Several studies showed that in Western countries, *KRAS* (16–54%) or *BRAF* (2–33%) mutations are involved the carcinogenic pathway of LGSOC, indicating that KRAS/BRAF/ERK signaling pathway is thought to play an essential for developing LGSOCs. Beside this we reported that, serous adenofibroma or adenofibroma, atypical proliferative serous tumor (APST), and noninvasive micropapillary serous borderline tumor (MPSC) all the developmental stage were synchronous with a Japanese case of LGSOC and mutational analysis of *KRAS* or *BRAF* genes of different pathological regions revealed no oncogenic mutation. However, *KRAS* or *BRAF* mutations may not contribute to tumor progression in Japanese cases of LGSOCs. Subsequently, we observed a high frequency of *PIK3CA* mutations (60%) were present instead of *KRAS* mutation in Japanese cases of LGSOCs, indicating, activation of the PIK3CA/AKT signaling pathway may play a significant role in the carcinogenesis of Japanese LGSOCs. However, accumulating all the evidence of molecular alterations, the specific combination of genetic mutations required for the progression of LGSOC has not been identified still yet. The purpose

of this study to establish a stepwise model for low grade serous ovarian carcinoma from serous cystadenoma epithelial cells with the identification of genetic variation.

MATERIALS AND METHODS

Clinical sample of serous cystadenoma tissue was collected via laparoscopic bilateral salpingo-oophorectomy from a postmenopausal 53-year-old woman. After primary isolation cells were immortalized via cyclin D1, CDK4R24C, and hTERT gene transfection and named HOVs-cyst-1. Immunocytochemistry (ICC) and western blot was carried out to confirm the epithelial origin. Population doubling assay and whole-exome Profiling was carried out with the aim to determine growth curve and germline mutation. Furthermore, oncogenic mutations *KRAS* and *PIK3CA* were separately and concurrently introduced in immortalized HOV-cyst-1 cells, thoroughly created three new cell line, named HOVs-cyst-1*KRAS* mutant, HOVs-cyst-1*PIK3CA* mutant, and HOVs-cyst-1(*KRAS* + *PIK3CA*) mutant cell lines. For the conformation of mutational state western blot analysis was performed. Cell functions were subsequently analyzed via in-vitro assays such as invasion, migration and cell proliferation, anchorage-independent assay with density of 10000, 3000, 25000cells/well. Nude mouse xenograft experiments (2.5×10^7cells/mL) were carried out to confirm transform phenotype. Immunohistochemistry of mouse xenograft tumor was done with deparaffinized sections which were incubated overnight with primary antibody pan-cytokeratin, PAX8, vimentin, p53, ER, and PR then wash and samples were examined under a light microscope. Statistical analyses were carried out via Student's t-test using the SPSS software and p-value < 0.05 was considered statistically significant. All the study protocol was 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

Ovarian serous cystadenoma cells were successfully formed and immortalized but did not find any identical differences indicating that our immortalized serous cystadenoma epithelial cells were pure, non-transmitted and did not dedifferentiate with long time-based culture. We found the phenotypic expression of immortalized cystadenoma epithelial HOVs-cyst-1 cells which showed diffused pan-cytokeratin as well as PAX8 expression suggesting that immortalized HOVs-cyst-1 cells were of Müllerian and epithelial origin. Beside this HOVs-cyst-1 cells were positive for the expression of vimentin, indicating that some of these cells may have undergone epithelial to mesenchymal transition. Western blot analysis revealed that phosphor-MAPK was expressed in single mutant *KRAS* as well as bi-mutant (*KRAS+PIK3CA*) cells whereas, phosphor-AKT was expressed in single mutant *PIK3CA* as well as bi-mutant (*KRAS+PIK3CA*)

HOVs-cyst-1cells. Our results suggesting that *KRAS* mutant cells were characterized by the expression of the RAS/ERK signaling pathway proteins, whereas the *PIK3CA* mutant cells highly expressed members of the PI3K/AKT signaling pathway. Furthermore, the HOVs-cyst-1(*KRAS* + *PIK3CA*) mutant cells exhibited activation of both the RAS/ERK and PI3K/AKT signaling pathways. The biological and functional behavior of the mutant cells was carried out in-vitro assay and found that HOVs-cyst-1 cells that contain both *KRAS* and *PIK3CA* mutations showed higher migration, proliferation and invasion ability than wild-type HOVs-cyst-1 cells ($p < 0.01$). So, the results of our in vitro experiments indicated that the oncogenic potential was higher in double oncogenic mutant HOVs-cyst-1cells than in single oncogenic mutant HOVs-cyst-1 cells. The concurrence of *KRAS* and *PIK3CA* mutations in cells create hyperactivation of the RAS/ERK and PI3K/AKT pathways, as a result uncontrolled cell proliferation and metastasis. In addition, bi-mutant (*KRAS+PIK3CA*) exhibited tumorigenic potentiality, suggesting that simultaneous activation of the KRAS/ERK and PIK3CA/AKT signaling pathways may play an essential role in the development of LGSOCs carcinogenesis. Beside this the histological examination of tumors with double mutant HOVs-cyst-1cells showed a micropapillary pattern with small, uniform papillae with serous epithelial cells and diffuse pan-cytokeratin and PAX8 expression. The xenograft and histological results have indicated that Müllerian epithelial serous cystadenomas cells are a percussor of LGSOCs. This is the first report an in vitro carcinogenesis model for LGSOCs with two oncogenic mutational combination. Together all of these genetic findings of the present study based on the in vitro carcinogenic model of LGSOC was successful.

CONCLUSION

In summary, we found that both *KRAS* and *PIK3CA* mutations play an essential role in the development of LGSOCs by activation of the RAS/ERK/MAPK and PIK3CA/AKT signaling pathways and successfully developed a stepwise model for in vitro using immortalized serous cystadenoma cells. Our results suggest that carcinogenesis from benign tumors requires only two oncogenic mutations, that benign tumors are one step up in the three stages of carcinogenesis compared with normal epithelial cells. This stepwise in vitro carcinogenic model of LGSOCs will contribute to further research on the etiopathogenesis of LGSOCs.