学 位 論 文 の 要 旨

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論 文 内 容 の 要 旨

INTRODUCTION

The p110α catalytic subunit of PI3K or phosphatidylinositol 3kinase (*PIK3CA*) is an oncogene, located on chromosome 3q26.3, which is mutated in several types of cancer. *PIK3CA* mutations increase PI3K activity, cell survival, motility, and cell cycle progression. Activated PI3K/AKT oncogenic signaling pathway regulates the expression of several downstream target genes that inhibit apoptosis and promote cell proliferation. Somatic mutations of *PIK3CA* have been shown playing an important role in the pathogenesis of ovarian clear cell carcinoma. Unlike the more common serous ovarian cancers, ovarian clear cell carcinoma is more frequently resistant to conventional platinum based chemotherapy, which worsens its prognosis. Therefore a need exists for the targeted therapies. We analyzed the relationship between *PIK3CA* mutation in ovarian clear cell carcinomas from Japanese patients and various clinicopathological variables. To clarify the role of PI3K/AKT activation in ovarian clear cell carcinomas harboring *PIK3CA* mutations, we inactivated the PI3K/AKT/mTOR pathway in ovarian carcinoma cells using potent inhibitors of PI3K or mTOR, LY294002 or temsirolimus, respectively, and a dual inhibitor of PI3K and mTOR, NVP-BEZ235.

MATERIALS AND METHODS

We used formalin-fixed, paraffin-embedded tissue samples of 71 ovarian cancers including 56 clear cell carcinomas and 15 high grade serous carcinomas. Acquisition of tissue specimens and clinical information was approved by the institutional review board. The paraffin tissue

blocks were organized into tissue microarrays, each made by removing 3-mm diameter cores of tumor from the block. For *in vitro* study we used both ovarian serous carcinoma and ovarian clear cell carcinoma cell lines. Genomic DNA was purified from all of the cell lines and paraffin-embedded tissues for PCR analysis and nucleotide sequencing of *PIK3CA, KRAS* and *BRAF.* Expression levels of the phosphorylated form of AKT (p-AKT) and mTOR (p-mTOR) were assessed by immunohistochemistry on tissue microarrays. For the clinicopathological and survival analysis, patients with no or weak expression were assigned to the low-expression group, and those with moderate or strong expression were assigned to the high-expression group. We performed western blot analysis to compare p-AKT or p-mTOR expression with *PIK3CA* mutation and MTT cell growth assay to examine the cell viability after treating with PI3K/mTOR inhibitors.

RESULTS AND DISCUSSION

Somatic mutations of *PIK3CA* were identified in 16 (28.6%) of 56 ovarian clear cell carcinoma samples. All of the *PIK3CA* mutations were missense and mapped to exon 9 (helical domain) and exon 20 (kinase domain). Somatic mutations of *KRAS* were identified in 5.4% of cases. In contrast, no mutation of *BRAF* was identified in the tested samples. The frequency of *PIK3CA* mutations in ovarian high grade serous carcinomas (0.0%: 0/15) was significantly lower than in clear cell carcinomas (28.6%: 16/56) ($P<0.05$).

There was no significant correlation between *PIK3CA* mutations and FIGO stage, CA125 levels, Ki-67 labeling index or the status of residual tumor. *PIK3CA* mutation was significantly correlated with younger age ($P=0.04$). *PIK3CA* mutation tended to be more frequent in tumors associated with endometriosis. However, the difference was not statistically significant ($P=0.17$). In addition, there were no significant relationship between *PIK3CA* mutations and p-AKT expression ($P=0.39$), p-mTOR expression ($P=0.07$), except the relationship between p-AKT expression and p-mTOR expression ($P=0.01$). These findings suggest other molecular mechanisms may be required for activating the PI3K–AKT pathway in ovarian clear cell carcinoma, or that p110α has functions distinct from PI3K–AKT regulation. In *PIK3CA* mutated tumors, the PI3K/AKT pathway is probably the principal pathway for carcinogenesis and progression, however, AKT is activated by several factors in addition to *PIK3CA* mutation (e.g., EGFR/HER-2). Therefore, it is plausible that *PIK3CA* mutation status and AKT activation may impact tumor behavior differently.

We examined the prognostic effect of *PIK3CA* mutations, p-AKT and p-mTOR expression. The activating *PIK3CA* mutation correlated with favorable overall survival in patients with ovarian clear cell carcinoma treated with platinum-based chemotherapy ($P=0.03$). Activating mutation in *PIK3CA* tended to correlate with longer progression-free survival in patients with ovarian clear cell carcinoma treated with platinum–based chemotherapy. However, the difference was not statistically significant ($P=0.10$). There was no significant relationship between p-AKT expression and overall/progression-free survival ($P=0.43$, $P=0.31$, respectively). There was a

significant relationship between p-mTOR expression and favorable progression-free survival but not overall survival (P=0.04, P=0.18, respectively).

PIK3CA mutation may be associated with a less aggressive phenotype. In this study, *PIK3CA* mutations were associated with a more favorable prognosis. The mutations also tended to be more frequent in tumors associated with endometriosis. It has been reported that ovarian clear cell carcinomas associated with endometriosis had a more favorable outcome in comparison to ovarian clear cell carcinomas without endometriosis. Taken together, these results suggest that tumors with *PIK3CA* mutations may represent a more indolent subset of ovarian clear cell carcinoma.

Although the biological roles of the PI3K/AKT/mTOR pathways in human cancer have been thoroughly investigated, it is not known whether these pathways mediate the effect of activating *PIK3CA* mutations on tumor progression of ovarian clear cell carcinoma. In this study, we analyzed the genotype-phenotype correlation of ovarian clear cell carcinoma cells using three different PI3K/mTOR inhibitors. Unexpectedly, mutational status was not correlated with growth inhibition by any of the three inhibitors. Treatment with the PI3K/mTOR inhibitors failed to inhibit proliferation (<50% of DMSO control) in four of the cell lines harboring *PIK3CA* mutations. In contrast, proliferation was inhibited in some cell lines with wild-type *PIK3CA*. Cell viability following treatment with the PI3K/mTOR inhibitors was not impacted in the three ovarian cancer cell lines harboring either *KRAS* or *BRAF* mutations. This is in contrast to a recent report demonstrating that *PIK3CA* and *KRAS* mutations predict the response to the mTOR inhibitor everolimus in colorectal and breast carcinomas. This discrepancy may be due to differences in organ specific oncogenic pathways. However, *PIK3CA* mutation warrants further investigation in the application of targeted PI3K/mTOR inhibitors in ovarian clear cell carcinoma.

CONCLUSION

Our observations suggest that the subgroup of ovarian clear cell carcinoma harboring *PIK3CA* mutation were associated with a more favorable prognosis, while they did not predict sensitivity of ovarian clear cell carcinoma cells to PI3K/mTOR inhibitors. There was no association of *PIK3CA* mutations with positive p-AKT and positive p-mTOR expression, suggesting that the PI3K/AKT/m-TOR pathway may be activated by other molecular mechanism. As our findings were based on a retrospective analysis of a relatively small number of patients, a prospective study is required to confirm the role of *PIK3CA* mutation on the prognosis of ovarian clear cell carcinoma.