# 学 位 論 文 の 要 旨

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| 学 位 論 文 名 | Clinicopathological and Biological Analysis of *PIK3CA* Mutation in Ovarian Clear Cell Carcinoma |
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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.

論文審査及び最終試験又は学力の確認の結果の要旨

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論文審査の結果の要旨

わが国では卵巣癌全体に明細胞腺癌（Ovarian clear cell carcinoma: OCCC）が占める割合が約25%と諸外国に比較して高い。OCCC症例は病期 I, II期で診断される割合が多く、他の組織型に比較して発症年齢が低い。一方、OCCCは抗癌剤に対する感受性が低く、同じ進行期では他の組織型に比較して予後不良とされる。ホスファチジルイノシトール 3-キナーゼ (PI3K)-Akt経路は下流のmTORにより細胞増殖や生存シグナルを制御し、さまざまな癌腫で異常が報告されている。申請者らは、新たな治療戦略の探索のため、OCCCにおけるPI3K, catalytic alpha polypeptide (*PIK3CA*)の遺伝子変異解析を行い、臨床病理学的、生物学意義を検討した。インフォームドコンセント及び、倫理委員会での承認の得られたOCCC検体を用い、*PIK3CA*、*KRAS*、*BRAF*の遺伝子突然変異をPCR-direct sequenceを用いて検討し、臨床病理学的因子、患者予後との関連について評価した。PI3Kの下流タンパク質であるAKT2、mTORの免疫染色を行い、*PIK3CA*の遺伝子変異との関連を検討した。OCCC細胞株を用い、*PIK3CA*の遺伝子変異とPI3K阻害剤であるLY294002、mTOR阻害剤であるTemsirolimus、両者のdual-inhibitorであるNVP-BEZ235に対する感受性との相関を検討した。OCCCの28.6%に*PIK3CA*遺伝子変異を認めた。*KRAS*の遺伝子変異は5.4%、*BRAF*は0%であった。*PIK3CA*遺伝子変異と下流タンパク質であるp-AKT、p-mTOR発現には相関はなかった。*PIK3CA*遺伝子変異は若年者に有意に多かった。*PIK3CA*遺伝子変異のあるOCCC患者は有意に全生存期間が長かった。OCCC細胞株において*PIK3CA*の遺伝子変異とLY294002、Temsirolimus、NVP-BEZ235の感受性との有意な相関は見られなかった。また、*KRAS*、*BRAF*の遺伝子変異も同様の結果であった。OCCCにおいてPI3K/AKT/mTOR経路の活性化は*PIK3CA*の遺伝子変異以外に重要な要素がある可能性が示された。また、OCCCにおいて*PIK3CA*の遺伝子変異は予後良好の予測マーカーとなる可能性があるが、mTOR阻害剤の効果予測のバイオマーカーとはならない可能性が示された。
以上より、OCCCにおけるPI3K/AKT/mTOR経路の役割と阻害薬の抗腫瘍効果が明らかとなり、本研究は今後の臨床試験に対する有用な情報を提供する意義のあるものと判断される。

最終試験又は学力の確認の結果の要旨

申請者は、卵巣明細胞腺癌において PI3K-AKT-mTOR カスケードについて検討した。本研究により、*PIK3CA* の遺伝子変異は、mTOR 阻害剤の効果予測のバイオマーカーとならない可能性が示された。公開審査における質疑応答も的確で背景、関連する分野の知識も充分であり、学位授与に値すると判断した。（主査：礒部 威）

申請者は、アジア人に多い卵巣明細胞腺癌では、他の癌腫と異なり *PIK3CA* 遺伝子変異が p-AKT, p-mTOR 発現と関連がなく，患者の良好な予後と関連があること、ヒト卵巣癌細胞株においてこの変異が、PI3K 経路の阻害因子に対する感受性を上げないことを明らかにした。治療薬開発に関わる重要な結果であり、関連分野の知識も豊富であり学位授与に値すると判断した。（副査：丸山 理留敬）

申請者は、欧米に比して本邦で頻度が高い卵巣明細胞腺癌において、PIK3CAの遺伝子変異をターゲットとしてその臨床病理学的および生物学的解析を行い、PIK3CA変異型の本態を解明した。最終試験でも質疑応答に明確に回答され、博士の学位にふさわしいと判断した。（副査：竹下 治男）

（備考）要旨は、それぞれ４００字程度とする。