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学位の種類	博士（医学）
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学位授与の要件	学位規則第４条第１項該当
審査研究科	人間総合科学研究科
学位論文題目	Analysis of *PIK3CA* mutation in stage IIB to IVA cervical cancers treated by concurrent chemoradiotherapy with weekly cisplatin(シスプラチン毎週投与併用同時化学放射線治療が行われた IIB～IVA 期子宮頸癌患者における *PIK3CA* 遺伝子変異の解析)
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論文の内容の要旨

Abstract of Thesis

[Purpose] The standard treatment for locally advanced cervical cancer is cisplatin-based concurrent chemoradiotherapy (CCRT). On the other hand, *PIK3CA* gene is closely related with cancer including cervical cancer. Mutations of the *PIK3CA* gene are associated with poor prognosis in patients with different solid tumors of different organs, but they can lead to a favorable response to PI3-kinase/AKT inhibitors. Moreover, the PI3-kinase/AKT/mTOR is the most commonly activated pathway in human cancer, present in 30-50% of all tumors. *PIK3CA* mutations and modification of p110α are reported to be the most frequent driver mutations in cancer. Although the activated PI3-kinase/AKT pathway is known to be involved in both cisplatin-resistance and radioresistance, to date only a few studies have reported significant associations between *PIK3CA* gene mutational status and outcome by CCRT in the disease. The aim of this study was to clarify the prognostic significance of *PIK3CA* mutational status in cervical cancers treated by CCRT.

[Materials and Methods] The author analyzed *PIK3CA* mutation in 59 patients diagnosed with stages IIB to IVA cervical carcinomas primarily treated by CCRT with weekly cisplatin at our institution. She used formalin-fixed paraffin-embedded biopsy specimens before treatment. Fifty-seven out of 59 patients (97%) were locally advanced cancers with stages IIIA to IVA. She prepared 8 to 10 slides of 5 μm FFPE tissues, and scratched the tissues from the slides into an autoclaved tube using sterile surgical blades. She used the blackPREP DNA Kit (GenoStaff, Tokyo, Japan) for DNA extraction according to the manufacturer's instructions. DNA quality and concentration were

examined by Nanodrop. The isolated DNAs were submitted to Eurofins Genomics (Tokyo, Japan) for direct sequencing. The author used BioLign software (version 4.0.6.2, http://en.bio-soft.net/) to look for the presence of mutations.

Clinicopathological data and patient survival were compared according to *PIK3CA* mutational status. She further performed cell viability assay examining the effect of a PI3-kinase inhibitor copanlisib (BAY80-6946) in combination with cisplatin on cell proliferation of CaSki cells harboring *PIK3CA* mutation. Cisplatin was purchased from Wako (Osaka, Japan). Copanlisib was purchased from LKT Laboratories (St. Paul, MN, USA). Cells in the log growth phase were seeded on 96-well plates at 2,000 cells per well. Twenty-four hours later, cells were treated with increasing doses of cisplatin (0.01-100 μM), copanlisib (1-10,000 nM), or cisplatin plus copanlisib. Cellular proliferation was monitored after 72 hours of treatment using a WST-1 assay as follows. WST-1 (Dojindo, Kumamoto, Japan) and 1-methoxy PMS (Dojindo) were added to the wells and incubated for 4 hours, and then the absorbance at 450 nm was measured and normalized relative to the absorbance of cell cultures treated with DMSO alone. The mean and SD were obtained from independent 3 experiments.

[Results] *PIK3CA* mutation was found in 7 out of 59 patients (12%). Five (71%) of the mutations were mapped on the helical domain, and one (14%) on the kinase domain of the p110α protein. No significant clinical differences were observed according to *PIK3CA* mutational status. Patients with wild-type *PIK3CA* showed significantly improved cancer-specific survival as compared with mutated patients (p=0.044). Subsequent survival analyses revealed that *PIK3CA* mutation was a significant prognostic factor for poor overall survival (multivariate adjusted HR, 3.88; 95% CI, 1.28-11.78; p=0.017) and cancer-specific survival (multivariate adjusted HR, 3.60; 95% CI, 1.19-10.95; p=0.024). Next, she performed cell viability assay to examine the effect of a PI3-kinase inhibitor copanlisib (BAY80-6946) in combination with cisplatin on cell proliferation of CaSki cells harboring heterozygous *PIK3CA* mutation. By treatment with copanlisib in combination with cisplatin, IC_{50} decreased from 7.32 μM to 6.04 μM. From the numerical value of the calculated Combination Index, this effect was found to be synergistic rather than additive effect.

[Discussion and Conclusion] The mutational analysis of this study found *PIK3CA* mutations in 12% of the patients, which is relatively lower than the results previously reported (13-36%), possibly due to difference in quality of samples for DNA extraction, as she used archival FFPE biopsy specimens from patients with mostly locally advanced cancers before treatment. Her subsequent survival analyses revealed that patients with mutant *PIK3CA* had significantly worse cancer-specific survival than those with wild type, and that *PIK3CA* mutation was a significant prognostic factor for poor overall and cancer-specific survival. These findings suggest that molecular inhibitors targeting the PI3-kinase/AKT pathway may improve the outcome by CCRT in cervical cancers harboring *PIK3CA* mutation, providing significant implications for novel treatment strategy based on precision medicine in the disease.

審査の結果の要旨

Abstract of assessment result

（**General Comments**）

This study is very challenging and unique, since the author used the real biopsied and surgically resected materials fixed by formalin to study the relationship between *PIK3CA* mutational status and patients' outcome after CCRT. The

conclusion is very clear but this study contains several limitations. First, the total sample size is small, which may have also impacted on the small number of *PIK3CA* mutations found. Secondly, only one cell line, *PIK3CA*-mutated CaSki, was used in the cell viability assay. However, the results and conclusion are almost acceptable and further additional experiments are recommended.

（**Assessment**）

The final examination committee conducted a meeting as a final examination on October 3rd, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（**Conclusion**）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.