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Abstract of thesis

Incidence of cancer has been increasing exponentially in last one decade. Defined by disorderly and uncontrolled growth of cells that severely impairs QOL of patients, cancer therapies face enormous challenges. Among the tumor types developed in various organs, lung cancer is one of the most frequent and fetal cancer and hence efficacious therapy for these patients is desired. Although several therapies including surgery, radiotherapy, and chemotherapy have been developed so far, systemic chemotherapy remains a major therapeutic option for lung cancer patients due to the difficulties in its early detection and diagnosis. Recent translational research has revealed that existence of key genomic changes for cancer development. These are called “oncogenic drivers” and have been identified for lung cancer. In this premises, the molecular target agents targeting these driver mutations have been in demand and are expected to provide better therapeutic outcome. Among them, drug discovery and development of kinase inhibitors against EGFR are remarkable due to their high mutation frequency in non-small cell lung cancer (NSCLC). EGFR-tyrosine kinase inhibitor (TKI) has been established as the first choice of treatment for NSCLC harboring EGFR mutations. On the other hand, clinical research has demonstrated the existence of EGFR mutations that exhibit resistance to treatment with EGFR-TKIs. Especially, the patients harboring EGFR with in-frame insertion mutation in exon 20 region has been shown to be poor responders to the existing EGFR-TKI therapy

compared to other common mutations. Preclinical studies have revealed that the EGFR-TKIs which have acquired FDA approval has a potency to inhibit kinase activity of not only exon 20 insertion mutant-EGFR but also wild type (WT) EGFR. Such non-selective response implied to maintain plasma concentrations of the compounds low in clinical settings. On the other hand, newer EGFR-TKI, which shows inhibitory activity against exon 20 mutant-EGFR while spared WT EGFR, is deemed useful in cancer therapy for these population with high unmet medical need.

The author has conducted compound screening with recombinant human enzymes and genetically engineered cell lines. As a result, TAS6417 was identified as an EGFR inhibitor with a unique scaffold. He has conducted extensive experiments on inhibitory activity, selectivity, and binding mode in biochemical assays. In mobility-shift assay, he confirmed inhibitory activity of TAS6417 against EGFR and its mutants, and demonstrated EGFR selectivity in a panel of over 250 kinases. By MS analysis and dissociation assay with recombinant enzyme of EGFR D770_N771insNPG (an exon 20 insertion mutation), he found that TAS6417 bound covalently to cysteine residue at 797, and showed irreversible or slow-dissociation kinetics. Furthermore, the author employed cell lines genetically engineered to express human WT EGFR and exon 20 insertion mutant-EGFRs, and undertook extensive analysis about cellular potency and mutation selectivity. He exposed the cells to TAS6417 that showed potent inhibitory activity against exon 20 insertion mutations with a superior mutation selectivity to the representative EGFR-TKIs such as Afatinib and Erlotinib. Based on the data, the author revealed that TAS6417 is a potent and selective kinase inhibitor targeting EGFR exon 20 insertion mutations.

As the next step, the author conducted *in vitro* and *in vivo* experiments with cancer cell lines to assess the potential of TAS6417 for anticancer agent. He used a variety of NSCLC cell lines with EGFR mutations including exon 20 insertion mutations and human primary keratinocytes of which cell growth is regulated by WT EGFR. In cell proliferation assay, he found that TAS6417 inhibited the growth of cancer cells harboring EGFR exon 20 insertion mutations. It was mediated by inhibition of phosphorylation of EGFR and its signal transduction including MAPK pathway and PI3K pathway, leading to caspase activation in cancer cells. While the inhibitory activity of TAS6417 in cancer cell lines was comparable or higher than that of Afatinib, its effect on normal keratinocytes was less than Afatinib. The author further evaluated *in vivo* activity in mice subcutaneously implanted with genetically engineered cells expressing the representative EGFR exon 20 insertion mutations such as D770_N771insSVD and H773_V774insNPH. In these models, Afatinib showed minimal or moderate suppression of tumor growth. In contrast, oral administration of TAS6417 exerted a significant inhibition of tumor growth associated with intratumoral target inhibition and plasma concentration profile over time. It was also seen to achieve a partial to complete remission of tumors in patient derived xenograft models, which is more predictable model than xenograft model using cultured cells. In addition, in vivo experiment in mouse orthotropic model revealed survival benefit of TAS6417 with a good tolerability. Taken together, the author demonstrated that TAS6417 was selectively toxic to cancer cells with EGFR exon 20 insertion mutation, but its effect to normal cells was moderate. Furthermore, this mutation selective characteristics led to a superior antitumor activity to Afatinib in subcutaneous or intrapulmonary implantation

model. On the basis of these data, the author proposed TAS6417 as a potential anticancer agent that warrants further investigation in clinical trial.

There are various EGFR mutations such as common mutations (exon 19 deletions and L858R mutation), and the third most common mutation is exon 20 insertion mutation, followed by G719X and L861Q mutations. The author investigated inhibition spectrum of TAS6417 in EGFR mutations. Combined with the results on pharmacodynamics analysis and cell proliferation assay, he found that TAS6417 had cellular potency and mutation selectivity against not only common mutations but also G719X and L861Q mutations, which differed from other EGFR-TKIs. Consistent with the *in vitro* property, TAS6417 showed an intensive antitumor activity in allograft models such as EGFR G719A and EGFR G719A+T790M. The author provided the experimental evidence that TAS6417 had a unique spectrum and selectivity against EGFR mutations.

Abstract of assessment result

【Review】

The applicant discovered TAS6417, a new EGFR-TKI, and investigated its pharmacological characteristics. Furthermore, the applicant demonstrated that TAS6417 served as irreversible or slow-dissociation kinetics against EGFR, and inhibited EGFR exon 20 insertion mutations while sparing WT EGFR, resulting in selective cytotoxicity to cancer cells. In addition to biological mode of action including EGFR signal inhibition and caspase activity induction, he proved anticancer activity of TAS6417, which was superior to that of afatinib, in several models such as patient-derived xenograft models and intrapulmonary implanted model, related to clinical prediction of cancer response and survival benefit, respectively. The applicant also found the unique and broad spectrum to EGFR mutations other than exon 20 insertion mutations by in vitro and in vivo experiments. The study passes the criteria of originality, well planning and accomplishment. The objective of research is clear and well understood. Experimental models used are well suited. Interpretation of the results is clear and reasonable. Data analyses have been done using appropriate tools. The results and discussion sections are well written and interpreted. The study contributes significantly in the field of innovation and disease mechanisms with special reference to understanding of cancer and its interventions.

【Result】

The final examination committee conducted a meeting as a final examination on 01/07/2019. 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】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Disease Mechanism.