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論文概要
(Summary of the Thesis/Dissertation)

Doctoral Program in Life Science Innovation
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1. Title of the Thesis/Dissertation :
Inhibitor of HER2 Kinase – Its Potential for Cancer Therapies

2. Summary (800 – 1,000 words in English)

HER2 is a member of ERBB family kinases, and activated through the formation of homo-, or hetero dimers with HER2 or other ERBB family kinases. Amplified or overexpressed *HER2* gene is an activation mechanism of HER2 kinase, and a well-established therapeutic target in breast and gastric cancer. In addition, HER2 somatic mutations were reported as an alternative HER2 activation mechanism in breast cancer. Those *HER2* gene abnormalities are recognized as an oncogenic driver gene, and inhibition of HER2 is expected to lead the antitumor effect. To date, these HER2 gene abnormalities were reported in various cancers including bladder, biliary tract, lung, and colorectal cancer. There are many cancer patients caused by *HER2* gene abnormalities. Although HER2 targeting antibodies, including trastuzumab, pertsuzumab, and a HER2 directing antibody-drug conjugate (ADC), T-DM1, are approved for use in patients with HER2-overexpressing breast cancers, there is still a need for effective therapies in patients who are refractory to HER2-targeting antibodies or ADC. Lapatinib, a small molecule, non-covalent, reversible HER2/EGFR (epidermal growth factor receptor) dual inhibitor, is available in treatment for HER2 overexpressed breast cancer. However, the clinical result is not satisfactory, due to the insufficient potency. Afatinib and neratinib are small molecule, non-selective, covalent-binding, pan-ERBB inhibitors. The issue of these covalent pan-ERBB inhibitors is a severe diarrhea due to the EGFR inhibition. Therefore, HER2 selective, potent inhibitor is desired to improve the clinical benefit and QOL of cancer patients. To provide a novel therapeutic option for cancer patients with HER2 activated tumors, the original screening scheme was set up. Leveraging the compound library in TAIHO and the established screening flow, primary hit compounds were identified. After derivatization of the hit compounds with lead compound optimization approach, a candidate inhibitor has successfully been discovered. To develop the inhibitor in human trials as an antitumor medicine, the author evaluated the antitumor profile of the identified compound in various preclinical models *in vitro* and *in vivo* whether the compound can go into the next preclinical evaluation stage and the subsequent a clinical trial stage. Furthermore, the author investigated the antitumor mechanism of the inhibitor.

Firstly, to characterize the antitumor profile and mode of action of the identified small molecule inhibitor, the author investigated the binding mode, pharmacodynamics of the compound in cancer cells and tumors in mouse xenograft models. Mass spectrometry (MS) analysis and a dilution assay were conducted to evaluate the irreversible covalent binding of TAS0728 with HER2 kinase. Unlike lapatinib, TAS0728 covalently bound to HER2 and inhibited the kinase activity irreversibly. To evaluate the effect of TAS0728 and lapatinib on HER2 signaling, the phosphorylation level of HER2 and the downstream pathway molecules in HER2 positive breast cancer SKBR3 cells were examined after 3 or 48 hours treatment by Western blot analysis. In SKBR3 cells, although lapatinib initially inhibited phosphorylation of HER2 and the downstream pathway after 3 hours incubation, reactivation of those molecules was observed after 48 hours of continuous exposure.

In contrast, TAS0728 exhibited a sustained and robust inhibition of the HER2 signaling pathway. Notably, in the TAS0728 treated group, apoptosis induction associated with BIM (an apoptosis inducer) stabilization was observed. Pharmacokinetics and pharmacodynamics analysis in mice revealed that sustained target inhibition was observed after elimination of TAS0728 in the plasma of mice. Consistently, TAS0728 induced tumor shrinkage associated with enhanced apoptosis marker induction in mouse xenograft models bearing NCI-N87 (HER2 activated gastric cancer). Furthermore, TAS0728 showed the superior survival benefit compare to lapatinib in an NCI-N87 peritoneal dissemination model. No evident toxicity was observed in the mice during the treatment with TAS0728.

Secondly, the author aimed to address the risk of side-effect of the compound. In an enzyme kinase panel of 386 kinases, TAS0728 at 0.1 μmol/L showed an inhibition rate of greater than 80% (≥ 80% inhibition of activity) for three of the 386 kinases tested. TAS0728 showed a potent inhibitory activity for wild type HER2, while little activity against the majority of other kinases including EGFR. TAS0728 did not bind to the 68 targets of non-kinases. Cellular pharmacodynamic evaluation in HER2 amplified SKBR3 cells and EGFR amplified A431 cells revealed that TAS0728's selectivity between HER2 and EGFR was higher than afatinib. In growth inhibition assays with various cell lines, TAS0728 exhibited antiproliferative activity only against HER2 activated cells across various cell lines. *In vivo*, although TAS0728 and afatinib induced tumor shrinkage in HER2 amplified xenograft models, the therapeutic window of TAS0728 was higher than that of afatinib.

Lastly, the author investigated whether TAS0728 has antitumor activity for tumor models resistant to established anti-HER2 antibody or ADC therapy. NCI-N87 mouse xenograft model or a PDX (Patient Derived Xenograft) mouse model were used. In the NCI-N87 xenograft model, although T-DM1 was no longer effective once the tumor started to regrowth during T-DM1 treatment, switching to TAS0728 was effective and induced the tumor regression again. Furthermore, TAS0728's antitumor effect was evaluated in a PDX model derived from a HER2 over-expressed breast cancer patient who showed resistance to trastuzumab/pertuzumab-based therapy, T-DM1 and other chemotherapies. In the PDX model resistant to established anti-HER2 antibody therapy, TAS0728 exhibited the potent antitumor effect.

The author has revealed that TAS0728 is an orally available, potent antitumor agent for HER2 activated tumors. This compound showed the irreversible inhibition of HER2 kinase activity and exhibits the potent antitumor activity through the sustained inhibition of HER2 kinase activity and the subsequent apoptosis induction in cancer cells. The high specificity for HER2 of the compound allows tumor selective cytotoxicity for HER2 activated tumors and the large therapeutic window in mice models. TAS0728 was also effective in tumor models resistant to established HER2 targeting antibodies or ADC, implicating that TAS0728 has a distinct different mode of action compared to HER2 targeting antibodies or ADC. Therefore, TAS0728 is a promising therapeutic option for HER2 activated cancer and would be expected to have an improved therapeutic window compared to current HER2 inhibitors. The assessment of TAS0728 in human is ongoing in the United States and European Union countries.