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Abstract of thesis

Cancer is one of the leading causes of death globally; about 1 in 6 deaths is due to it. The economic impact of cancer is significant and increasing. There are many types of cancer treatment, including surgery, radiation, chemotherapy, immunotherapy, hormone therapy, stem cell transplant and molecular-targeted therapy/precision medicine. Molecular-targeted therapies are based on the identification of oncogenic gene alterations and their specific inhibitors. They have demonstrated dramatic antitumor effect with reduced side effects and improved patient survival compared to conventional therapies. Some examples of the molecular-targeted therapies include Herceptin for epidermal growth factor receptor 2 amplification and overexpression in breast cancer patients, XALKORI for echinoderm microtubule-associated protein-like 4–anaplastic lymphoma kinase fusion oncogene-positive non–small-cell lung cancer, and Tarceva for EGFR-mutated non–small cell lung cancer.

The signaling pathway activated by FGFRs and their cognate ligands, i.e., fibroblast growth factors (FGF), plays an important role in the course of development from early embryogenesis to the formation of various organs. Aberrant activation of FGF/FGFR signaling promotes cellular proliferation, migration/invasion and angiogenesis in a variety of human cancers in several ways: amplification, fusion or mutations of FGFR family members. Oncogenic genetic alternations are identified in FGFR1, 2, 3 and 4 in human cancers. For example, FGFR1 amplifications are identified in breast, lung, gastric and bladder cancers. FGFR2 amplification, mutations and fusions are identified in breast, liver, uterine, lung, and gastric cancers. FGFR3 mutations and fusions are identified bladder and lung cancers. On the other

hand, oncogenic genetic alternation of FGFR4 is infrequently identified in cancers. FGFR4-N535K/D and V550E/L, were identified as oncogenic mutation in rhabdomyosarcoma (RMS), although other oncogenic alternations are unknown. The author investigated aberrant FGF19-FGFR4 signals and therapeutic efficacy of FGFR inhibitor on cancers.

In chapter I, the author investigated the pharmacological profile of novel FGFR inhibitor, ASP5878, and explored its potential therapeutic efficacy on FGF19-expressing Hepatocellular carcinoma (HCC), an aggressive cancer with poor prognosis. FGF19 is a physiologic ligand for FGFR4, which is expressed in and secreted from the small intestine and gallbladder and controls the catabolism of cholesterol in the liver through FGFR4 and coreceptor, bKlotho. Furthermore, in recent years, it has been found that FGF19 is involved in several types of cancers including HCC. FGF19 gene amplification has been reported to be involved in 14% of HCC patients, and FGF19 is overexpressed in approximately 50% of HCC patients. FGF19 expression correlates with poorer prognosis, recurrence, and tumor progression in HCC patients. FGF19-expressing transgenic mice spontaneously develop HCC, and the knockout of FGFR4 shows that it is required for tumorigenesis in this FGF19-mediated HCC model. HCC is an aggressive cancer with poor prognosis and the third most common cause of cancer related deaths worldwide. Surgical resection is the most successful treatment for early-stage HCC; however, 70% of patients have recurrence after 5 years. Sorafenib, a broad-spectrum kinase inhibitor, has been approved as a molecular targeted drug for surgically unresectable HCC and has been shown to improve the duration of survival to 10.7 months in comparison with 7.9 months in patients receiving placebo in a phase 3 trial. However, more effective therapeutic approaches are urgently required for unresectable HCC. To develop small molecule inhibitors for FGFR4, purified kinase protein that exhibited mutant kinase activity in *in vitro, in vivo* assays, and anti-tumor effect evaluation system (*in vitro, in vivo*) were established. Screening of chemical compounds was executed using these evaluation systems (including chemical library by HTS and in silico modeling and docking study), and finally, some promising candidate compounds including ASP5878 that is characterized extensively in this thesis, were identified. ASP5878 is a novel inhibitor of FGFR 1, 2, 3, and 4. It inhibits FGFR4 kinase activity with an IC50 of 3.5 nmol/L. ASP5878 potently suppressed the growth of the FGF19–expressing HCC cell lines Hep3B2.1-7, HuH-7, and JHH-7. In the Hep3B2.1-7 cell line, ASP5878 inhibited the phosphorylation of FGFR4 and its downstream signaling molecules as well as induced apoptosis. Oral administration of ASP5878 at 3 mg/kg induced sustained tumor regression in a subcutaneous xenograft mouse model using Hep3B2.1-7. In HuH-7, an orthotopic xenograft mouse model, ASP5878 induced complete tumor regression and dramatically extended the survival of the mice. These results suggest that ASP5878 is a potentially effective therapeutic agent for HCC patients with tumors expressing FGF19.

In Chapter II, the author has identified novel oncogenic FGFR4 mutation in gastric cancer and investigated the function. Different from FGFR1, 2 and 3, limited number of oncogenic alternations have been identified in FGFR4. Oncogenic mutations of FGFR4 occur in RMS, in which FGFR4 is highly expressed. High FGFR4 expression correlates with tumor progression and survival of patients with gastric cancer. The author therefore hypothesized that FGFR4 plays an important role in gastric cancer and that mutations that activate the protein tyrosine kinase activity of FGFR4 promote an aggressive phenotype of gastric cancer. Gastric cancer is one of the leading causes of cancer death worldwide. Intensive investigations of anticancer treatments for gastric cancer during the past three decades have not significantly improved the poor prognosis of patients with unresectable advanced or recurrent gastric cancer, and improved therapies are required. The development of trastuzumab, a monoclonal antibody targeting ERBB2, is

a successful example of translational genetic profiling and precision medicine applied to gastric cancer Novel findings of genetic features help uncover molecular mechanisms and identify effective therapeutic targets for gastric cancer. The G636C-FGFR4 tyrosine kinase domain mutation was found in 1 of 83 primary human gastric tumors. The G636C mutation increased FGFR4 autophosphorylation, activation of FGFR4 downstream signaling molecules and enhanced anchorage-independent cell growth when expressed in NIH/3T3 cells. 3D-structural analysis and modelling of FGFR4 suggest that G636C destabilizes an auto-inhibitory conformation and stabilizes an active conformation, leading to increased kinase activation. Ba/F3 cell lines expressing the G636C-FGFR4 mutant were significantly more sensitive to ASP5878, a selective FGFR inhibitor, than the control. Oral administration of ASP5878 significantly inhibited the growth of tumors in mice engrafted with G636C-FGFR4/3T3 cells. Together, these results demonstrate that mutationally activated FGFR4 acts as an oncoprotein. These findings support the therapeutic targeting of FGFR4 in gastric cancer.

In conclusion, this study indicates that aberrant FGF19-FGFR4 signals is involved in HCC and gastric cancer, and that ASP5878 is a potentially effective therapeutic agent for cancer patients with tumors expressing FGF19 and G636C-FGFR4 mutations.

Abstract of assessment result

【Review】

In this dissertation, important findings in current investigation are; I) Identification of ASP5878 as a novel, potent and selective FGFR inhibitor, II) Demonstration of ASP5878's dramatic anti-tumor efficacy for FGF19-expressing HCC, III) Identification of novel FGFR4 oncogenic mutation, G636C, in gastric cancer and IV) Demonstration of ASP5878's dramatic anti-tumor efficacy for G636C-FGFR4 positive tumor. Taken together, these findings showed that ASP5878 may be a promising candidate for cancer patients who possess aberrant FGF19-FGFR4 signal such as FGF19-expression and G636C mutation.

This research will contribute to i) develop FGFR inhibitors for cancer patients with aberrant FGF19-FGFR4 signal and advancing our understanding of the disease mechanism and 2) lead to the development of new drugs that will dramatically improve the oncology society.

【Result】

The final examination committee conducted a meeting as a final examination on 02 June 2020. 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 Science.