

Synergistic antitumor effects of FGFR2 inhibitor with 5-fluorouracil on scirrhous gastric carcinoma

Masakazu Yashiro^{1,2}, Osamu Shinto¹, Kazunori Nakamura¹, Masashige Tendo¹, Tasuku Matsuoka¹, Taro Matsuzaki¹, Ryoji Kaizaki¹, Atsushi Miwa³ and Kosei Hirakawa¹

¹ Department of Surgical Oncology, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-Machi, Abeno-Ku, Osaka, Japan

² Oncology Institute of Geriatrics and Medical Science, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-Machi, Abeno-Ku, Osaka, Japan

³ Drug Discovery Research Laboratories, Kyowa Hakko Kirin Co., Ltd., Mishima, Shizuoka, Japan

Scirrhous gastric carcinoma (SGC) carries the highest mortality because of a frequent metastasis to lymph node (LN). S1, a 5-fluorouracil (5-FU) analog, is clinically available for gastric cancer at an advanced stage. Fibroblast growth factor receptor 2 (FGFR2) is required for the proliferation of SGC. The objective of this study is to clarify the benefit of a combination of S1 and kinase inhibitors including FGFR2 inhibitor Ki23057 in gastric cancer. OCUM-2MLN and KATO-III were derived from SGC. MKN-7 and MKN-74 were derived from non-SGC. MTT assay was used to examine the growth-inhibitory activity of 5 small-synthetic molecules including Ki23057, Sunitinib, Glivec, Lapatinib or SU11274, in cells cultured with 5-FU. Combination effects of 5-FU with Ki23057 on proliferation, apoptosis and mRNA expression were examined. S1 and/or Ki23057 were administered to murine models of SGC created by the orthotopic inoculation of OCUM-2MLN cells. Ki23057 at 100 nM significantly ($p < 0.01$) inhibited the proliferation and decreased the phosphorylation of FGFR2 in SGC cells, but not in non-SGC. Ki23057 showed synergistic antitumor effects for SGC cells in combination with 5-FU using CalcuSyn analysis, but Sunitinib, Glivec, Lapatinib and SU11274 did not. The combination of Ki23057 and 5-FU decreased *DPD* expression and increased apoptosis rates and *p21* expression level of SGC cells. The combined administration of S1 and Ki23057 significantly ($p < 0.05$) decreased orthotopic tumors as well as LN metastasis more effectively than S1 alone. These findings suggested that the combined treatment with 5-FU and Ki23057 produced synergistic antitumor effects and is therapeutically promising for SGC treatment.

Key words: gastric cancer, lymph node metastasis, fibroblast growth factor receptor-2 inhibitor, 5-fluorouracil, combination therapy

Abbreviations: SGC: scirrhous gastric carcinoma; LN: lymph node; FGFR2: fibroblast growth factor receptor 2; 5-FU: 5-fluorouracil; DPD: dihydropyrimidine dehydrogenase; PDGF-R: platelet-derived growth factor receptor; VEGF-R3: vascular endothelial cell growth factor receptor-3; EGFR: epidermal growth factor; DMEM: Dulbecco's modified Eagle medium; RTK: receptor tyrosine kinase; MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide; PI: propidium iodide; PDGF-R: platelet-derived growth factor receptor

Additional supporting information may be found in the online version of this article

Grant sponsor: Grants-in Aid for Scientific Research, Ministry of Education, Science, Sports, Culture and Technology of Japan; **Grant numbers:** 18591475, 20591073, 18390369; **Grant sponsors:** Grant-in Aid for the Japanese Society of Gastroenterology for Scientific Research, Grant-in Aid for Kobayashi Foundation for Innovative Cancer Chemotherapy, Grant-in Aid for the Sagawa Foundation for Cancer Research, Grant-in Aid for the Osaka Medical Research Foundation for Incurable Diseases

DOI: 10.1002/ijc.24763

History: Received 7 Feb 2009; Accepted 3 Jul 2009; Online 20 Jul 2009

Correspondence to: Masakazu Yashiro, Department of Surgical Oncology, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-Ku, Osaka 545-8585, Japan. Tel.:

+81-6-6645-3838, Fax: +81-6-6646-6450,

E-mail: m9312510@med.osaka-cu.ac.jp

The characteristic clinical features of scirrhous gastric carcinoma (SGC), a diffusely infiltrating type of gastric carcinomas also known as linitis plastica-type gastric carcinoma, include rapidly progressive growth and a high frequency of metastasis to the lymph node (LN).¹⁻³ SGC carries a worse prognosis among other types of gastric carcinomas, with 5-year survival rates in the range of 10–15%.^{4,5} Chemotherapy is frequently recommended treatment for LN metastasis of gastric cancer in Japan.⁶⁻⁹ A novel chemotherapeutic agent S1, a 5-fluorouracil (5-FU) analog, has recently become first-line chemotherapy for gastric cancer patients with LN metastasis.⁹ Even so, however, the median survival duration in patients with advanced SGC continues to be less than a year.^{6,7}

Fibroblast growth factor receptor 2 (FGFR2) overexpression has been reported in about 40% of advanced stomach cancer cases and is especially frequent in SGC.^{10,11} FGFR2 is a member of the FGFR receptor tyrosine kinase (RTK) family, which consists of 4 receptors and 23 ligands.¹² The signal through FGFR2 and FGF7 is important for the growth of SGC with an amplification of the activated *FGFR2* gene (*K-samII*).^{1,10,11,13} Ki23057, a newly developed FGFR2 inhibitor, competes with ATP for the binding site in the kinase and therefore blocks the autophosphorylation of FGFR2 as previously reported.^{14,15} Recent work, using FGFR2 inhibitors, has provided evidence that in cell lines expressing FGFR2, the kinase can be required for cancer cell proliferation.¹⁴⁻¹⁶