proportion (40%) of patients with second-line chemotherapy than that previously observed in any other trials. We think that patients with severe peritoneal metastasis who progressed during the first-line treatment were in extremely poor general condition, so most of the patients might have missed the opportunity to receive second-line chemotherapy.

In the present study none of the patients with severe peritoneal metastasis received an oral fluoropyrimidine- or cisplatin-containing regimen. We believe that these patients could not receive oral fluoropyrimidine plus cisplatin combination therapy for the following reasons: the instability of oral agent administration and absorption, the high risk of complications, and their inability to receive the adequate hydration that is required for renal protection from cisplatin. We need to develop novel chemotherapeutic regimens with non-oral agents, with no need for hydration, and with high feasibility for gastric cancer patients with severe peritoneal metastasis.

Although 5-FU is one of the most commonly used drugs in patients with gastrointestinal malignancies, systemic 5-FU chemotherapy has a limited response rate [9, 10, 14]. Several new cytotoxic agents, such as oxaliplatin and taxanes, have been proven to confer a survival benefit and to show promise as standard anticancer agents for patients with gastric cancer [6, 15, 16]. In Japan, oxaliplatin cannot be used for gastric cancer because it is not yet approved. On the other hand, paclitaxel is recognized as an effective agent for peritoneal metastasis because of its high molecular weight and bulky molecular structure, delaying its clearance from the peritoneal cavity [17, 18]. Currently, there are expectations for the use of paclitaxel as an agent for gastric cancer with peritoneal metastasis, and a randomized Phase II study (JCOG 0407) comparing the best available 5-FU (when the prior chemotherapy included bolus 5-FU, 5-FU ci was administered; for other cases, MTX/5-FU was administered) with wPTX in 5-FU-refractory gastric cancer patients with peritoneal metastasis has completed accrual and awaits final analysis. When paclitaxel was administered alone, it showed a 20–23% objective response rate in patients with advanced gastric cancer [19, 20]. In combination chemotherapy, paclitaxel has a synergistic effect in combination with 5-FU and there are no overlapping toxicities. We believe that a regimen with a high response rate against ascites may improve clinical symptoms in the early stage during the course of treatment in gastric cancer patients with severe peritoneal metastasis. Whether high therapeutic efficacy improves the prognosis has not yet been fully defined, and it is necessary to verify which is better: sequential treatment with a 5-FU-based regimen followed by paclitaxel or combined chemotherapy with 5-FU plus paclitaxel. We are conducting a clinical trial of 5-FU/*l*-LV plus wPTX (FLTAX) for gastric cancer patients with severe peritoneal metastasis, and a feasibility study is currently ongoing as a preliminary step.

In conclusion, 5-FU-based chemotherapy had marginal activity with tolerable toxicity in advanced gastric cancer patients with severe peritoneal metastasis. To achieve a better prognosis, we must investigate new feasible regimens with non-oral agents and no need for hydration for use in this study population.

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Conflict of interest We have no conflicts of interest to declare.

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ORIGINAL ARTICLE

Efficacy and safety of capecitabine plus cisplatin in Japanese patients with advanced or metastatic gastric cancer: subset analyses of the AVAGAST study and the ToGA study

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Abstract
Background Capecitabine plus cisplatin (XP) is recognized as one of the global standard first-line chemotherapy regimens for patients with metastatic gastric cancer (mGC). Recent multinational phase III trials in mGC have been conducted with XP as the control arm, although no data on XP in Japanese patients with mGC have been published to date. The AVAGAST (XP ± bevacizumab in mGC) and ToGA (XP ± trastuzumab in human epidermal growth factor receptor 2 [HER2]-positive mGC) studies were the first two global studies including Japanese mGC patients. The aim of this analysis was to investigate the efficacy and safety of XP in Japanese mGC patients, using AVAGAST and ToGA subgroup data.
Methods Efficacy and safety analyses were carried out in Japanese patients with mGC receiving XP alone, based on results from the AVAGAST and ToGA studies. There were differences in the target populations between the two

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studies; for example, the ToGA study limited patients to those with HER2-positive tumors; therefore, efficacy was evaluated separately.

Results Ninety-four Japanese patients in the AVAGAST study and 50 in the ToGA study received XP alone. Median overall and progression-free survivals were 14.2 and 5.7 months, respectively, in the AVAGAST study, and 17.7 and 5.6 months, respectively, in the ToGA study. Overall response rates were 49.2 % in the AVAGAST and 58.5 % in the ToGA study. Adverse events were generally mild; the most common grade 3/4 events were neutropenia, anemia, anorexia, and nausea.

Conclusions XP is effective and well tolerated in Japanese patients with mGC, and could be one of the standard regimens for the first-line treatment in this cohort.

Keywords Capecitabine · Cisplatin · Gastric cancer · Japanese patients · Subset analysis

Introduction

Gastric cancer remains one of the most common forms of cancer worldwide, with an incidence of approximately 870,000 new cases per year and 650,000 deaths per year [1, 2], accounting for about 9.9 % of new cancers [3]. In Japan, the incidence of gastric cancer is around 110,000 new cases each year, with around 50,000 deaths reported in 2009 [4]. Chemotherapy is the most effective treatment for patients with unresectable advanced and metastatic gastric cancer (mGC). The combination of a fluoropyrimidine (5-fluorouracil or an oral fluoropyrimidine) plus cisplatin has been one of the most commonly used regimens due to its activity and well-established toxicity profile.

Capecitabine is an oral fluoropyrimidine that undergoes a three-step enzymatic activation process, the last step of which occurs selectively within the tumor tissue itself. The comparable efficacy of regimens substituting capecitabine for infused 5-flurouracil has been directly studied in two phase III trials: the REAL-2 study and the ML17032 study. A meta-analysis of these two trials concluded that, compared with 5-flurouracil combinations, capecitabine combinations were associated with higher response rates (odds ratio 1.38, 95 % confidence interval [CI] 1.10–1.73) and better overall survival (hazard ratio for death 0.87, 95 % CI 0.77–0.98) [5]. The combination of capecitabine and cisplatin (XP) is therefore recognized worldwide as one of the standard first-line chemotherapy regimens for patients with mGC.

More recent phase III studies, including the AVAGAST, ToGA, and EXPAND studies, have focused on the benefit of adding a molecular targeting agent to the XP regimen (bevacizumab, trastuzumab, and cetuximab in each of these studies, respectively). The AVAGAST and ToGA studies (conducted in human epidermal growth factor receptor 2 [HER2]-positive mGC patients) were the first two global studies including Japanese patients with mGC. Japanese patients comprised 94 of 387 patients receiving XP alone in the AVAGAST study, and 50 of 290 patients receiving XP alone in the ToGA study.

In the Japanese *Gastric cancer treatment guideline (3rd edition)* [6], S-1 (a compound preparation containing the oral fluoropyrimidine tegafur) in combination with cisplatin (SP) is recommended as a standard first-line chemotherapy regimen in Japan. However, there has been no efficacy or safety information on the XP regimen for the Japanese population.

The aim of this analysis was to investigate the efficacy and safety of XP in Japanese patients with mGC using subgroup data from the AVAGAST study and the ToGA study. There were differences in the target populations in the two studies; for example, the ToGA study was limited to patients with tumors showing overexpression of HER2. Because it was not known whether the efficacy of chemotherapy differed between HER2-positive and -negative tumors, we evaluated efficacy in the ToGA and AVAGAST studies separately. For adverse events, we combined the data from the ToGA and AVAGAST studies.

Patients and methods

Patients

The main eligibility criteria common to both the studies above were as follows: metastatic or inoperable locally

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advanced adenocarcinoma of the stomach or gastro-esophageal junction; measurable (according to response evaluation criteria in solid tumors [RECIST]) or evaluable disease; adequate organ function; Eastern Cooperative Oncology Group (ECOG) performance status 0–2; written informed consent; creatinine clearance ≥60 mL/min; no previous adjuvant chemotherapy within 6 months; no previous chemotherapy for metastatic or locally advanced gastric cancer; and no history of other malignancies.

The ToGA study limited patients to those with HER2-positive tumors (centrally assessed, immunohistochemistry [IHC] 3+ and/or fluorescent in situ hybridization [FISH] +) [7], no congestive heart failure, and baseline left ventricular ejection fraction (LVEF) ≥50 %. In the AVAGAST study, the exclusion criteria included uncontrolled hypertension or clinically significant (i.e., active) cardiovascular disease.

Treatment

The AVAGAST study was a randomized double-blind placebo-controlled study, and the ToGA study was an open-label randomized controlled study. Patients were randomly assigned in a 1:1 ratio to receive monoclonal antibody therapy (bevacizumab in AVAGAST; trastuzumab in ToGA) plus chemotherapy (capecitabine or fluorouracil plus cisplatin), or chemotherapy alone.

The chemotherapy regimen for Japanese patients was exclusively XP in both studies. Cisplatin 80 mg/m^2 was given by intravenous infusion on day 1 of each 3-week cycle. Capecitabine 1,000 mg/m^2 was given orally twice a day on days 1–14 of the cycle followed by a 7-day rest period. XP doses were modified according to the hematological toxicities on the first day of a planned course (Table 1). If the absolute neutrophil count (ANC) was $<1 \times 10^9$/L and the platelet count was $<100 \times 10^9$/L, treatment was delayed; and then if hematological parameters did not recover within 3 weeks after the delay, treatment was discontinued. Cisplatin doses were also modified to account for renal function, as shown in Table 2.

Table 1 Dose modification for hematological toxicity on the first day of a planned course

ANC and platelet count	Capecitabine/cisplatin dose modification
$\geq 1.5 \times 10^9$ and $\geq 100 \times 10^9$/L	100 % of original dose of capecitabine and cisplatin given without a delay
$\geq 1 - < 1.5 \times 10^9$ and $\geq 100 \times 10^9$/L	75 % of original dose of capecitabine and cisplatin given without a delay
$< 1 \times 10^9$ and/or $< 100 \times 10^9$/L	Delay until recovery to ANC $\geq 1 \times 10^9$/L and platelets $\geq 100 \times 10^9$/L. Then, if ANC $\geq 1 - < 1.5 \times 10^9$/L, re-commence at 75 % of original dose of capecitabine and cisplatin. Thereafter in post-dose reduction treatment courses, if ANC $\geq 1.5 \times 10^9$/L, then re-commence at 100 % of original dose of capecitabine and cisplatin

ANC absolute neutrophil count

Assessment of response and toxicity

In both studies the primary endpoint was overall survival. Secondary endpoints included progression-free survival, time to progression, overall tumor response rate, clinical benefit rate (defined as patients without progressive disease in AVAGAST; and patients with best overall response of confirmed complete response, partial response, or stable disease in ToGA), duration of response, and safety. These endpoints were based on regular assessments of disease response and progression using RECIST criteria. In AVAGAST, tumor assessments were performed every 6 weeks for the first year after randomization and thereafter every 12 weeks until disease progression. In ToGA, tumor assessments were performed every 6 weeks until disease progression.

Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 3.0.

Statistical analysis

The analyses of overall survival and progression-free survival were based on the survival analysis; median time was estimated using the Kaplan–Meier method, the 95 % confidence interval (CI) for the median was calculated by the Brookmeyer-Crowley method. Overall tumor response rate was defined as the proportion of

Table 2 Dose modification of cisplatin in renal impairment

Creatinine clearance (mL/min)	AVAGAST	ToGA
≥60	100 % of dose	100 % of dose
51–59	Reduce dose by 25 %	Same dose of cisplatin in mg/m^2 as the value of the Ccr in mL/min
41–50	Reduce dose by 50 %	
≤40	Stop cisplatin permanently	Stop cisplatin permanently

Creatinine clearance (Ccr) was determined mainly by the Cockcroft and Gault calculation. If Ccr was evaluated by the direct method then dose modification was based on this

occurrence of either a confirmed complete or a partial best overall response.

Dose intensity was defined as the actual dose administered divided by the planned dose. In the AVAGAST study the planned dose of cisplatin was the entire dose of all six cycles, and that of capecitabine was the whole dose of an actual administered cycle. In the ToGA study the planned dose was the whole dose of an actual administered cycle.

Results

Patient characteristics

In the AVAGAST study between September 2007 and December 2008, a total of 774 patients were enrolled at 93 centers in 17 countries. There were 188 Japanese patients enrolled at 14 centers, with 94 patients in the XP group. In the ToGA study between September 2005 and December 2008, a total of 594 patients were enrolled at 122 centers in 24 countries; 102 patients were enrolled at 16 centers in Japan. One patient was excluded from the analysis as they received no treatment, resulting in a total of 101 patients being the subjects for the analysis, with 50 patients in the XP group.

Table 3 shows the demographics and baseline characteristics of the cohorts. Median follow-up times were 12.0 months (0.1–23.9 months) in the AVAGAST study and 17.1 months (1–49 months) in the ToGA study.

Table 3 Baseline patient characteristics

	AVAGAST[a] ($N = 94$)	ToGA[b] ($N = 50$)
Sex (%)		
Male	63 (67.0)	40 (80.0)
Female	31 (33.0)	10 (20.0)
Median age		
Years (range)	61.0 (36–78)	63.5 (45–81)
Extent of disease (%)		
Locally advanced	1 (1.1)	1 (2.0)
Metastatic	93 (98.9)	49 (98.0)
Primary tumor site (%)		
Stomach	88 (93.6)	44 (88.0)
Gastro-esophageal junction	6 (6.4)	6 (12.0)
Measurability of disease (%)		
Measurable	65 (69.1)	41 (82.0)
Non-measurable	29 (30.9)	9 (18.0)
ECOG performance status (%)		
0–1	94 (100.0)	50 (100.0)
Number of metastatic sites at baseline (%)		
0	1 (1.1)	–
1	34 (36.2)	–
≥2	59 (62.8)	–
1–2	–	32 (64.0)
>2	–	18 (36.0)
Type of gastric cancer[c] (%)		
Intestinal type	22 (23.4)	42 (84.0)
Diffuse type	65 (69.1)	4 (8.0)
Mixed type	7 (7.4)	4 (8.0)
Visceral metastasis (%)		
Liver metastasis	23 (24.5)	–
Liver or lung metastasis	–	33 (66.0)
History of treatment for gastric cancer (%)		
Prior gastrectomy	31 (33.0)	13 (26.0)
Prior chemotherapy	8 (8.5)	0

[a] The target population of the AVAGAST study was metastatic or inoperable locally advanced adenocarcinoma of the stomach or gastro-esophageal junction

[b] The target population of the ToGA study was human epidermal growth factor receptor 2 (HER2)-positive metastatic or inoperable locally advanced adenocarcinoma of the stomach or gastro-esophageal junction

[c] The type of gastric cancer is as described in the Lauren classification

Efficacy

In the AVAGAST study, the median overall survival was 14.2 months (95 % CI, 10.9–18.8 months; Fig. 1a) and median progression-free survival was 5.7 months (95 % CI, 5.3–7.0 months; Fig. 1b). Patients with measurable disease comprised 65 of the 94 patients. The overall tumor response rate was 49.2 % (32/65 patients) and the clinical benefit rate was 67.7 % (44/65 patients). Median time to progression was 5.6 months (95 % CI, 5.1–7.2 months) and median response duration was 6.9 months (95 % CI, 4.2–9.5 months; Table 4).

In the ToGA study, the median overall survival was 17.7 months (95 % CI, 12.0–24.0 months; Fig. 1c) and the median progression-free survival was 5.6 months (95 % CI, 5.0–7.0 months; Fig. 1d). Patients with measurable disease comprised 41 of the 50 patients. The overall tumor response rate was 58.5 % (24/41 patients), and the clinical benefit rate was 85.4 % (35/41 patients). Median time to progression was 5.6 months (95 % CI, 5.0–7.0 months) and median response duration was 4.3 months (95 % CI, 4.0–7.0 months; Table 4).

Safety

In the AVAGAST study, capecitabine exposure was as follows: the median number of treatment cycles was 7; the median total dose received was 243,900 mg; and the median dose intensity was 80 %. Cisplatin exposure was as

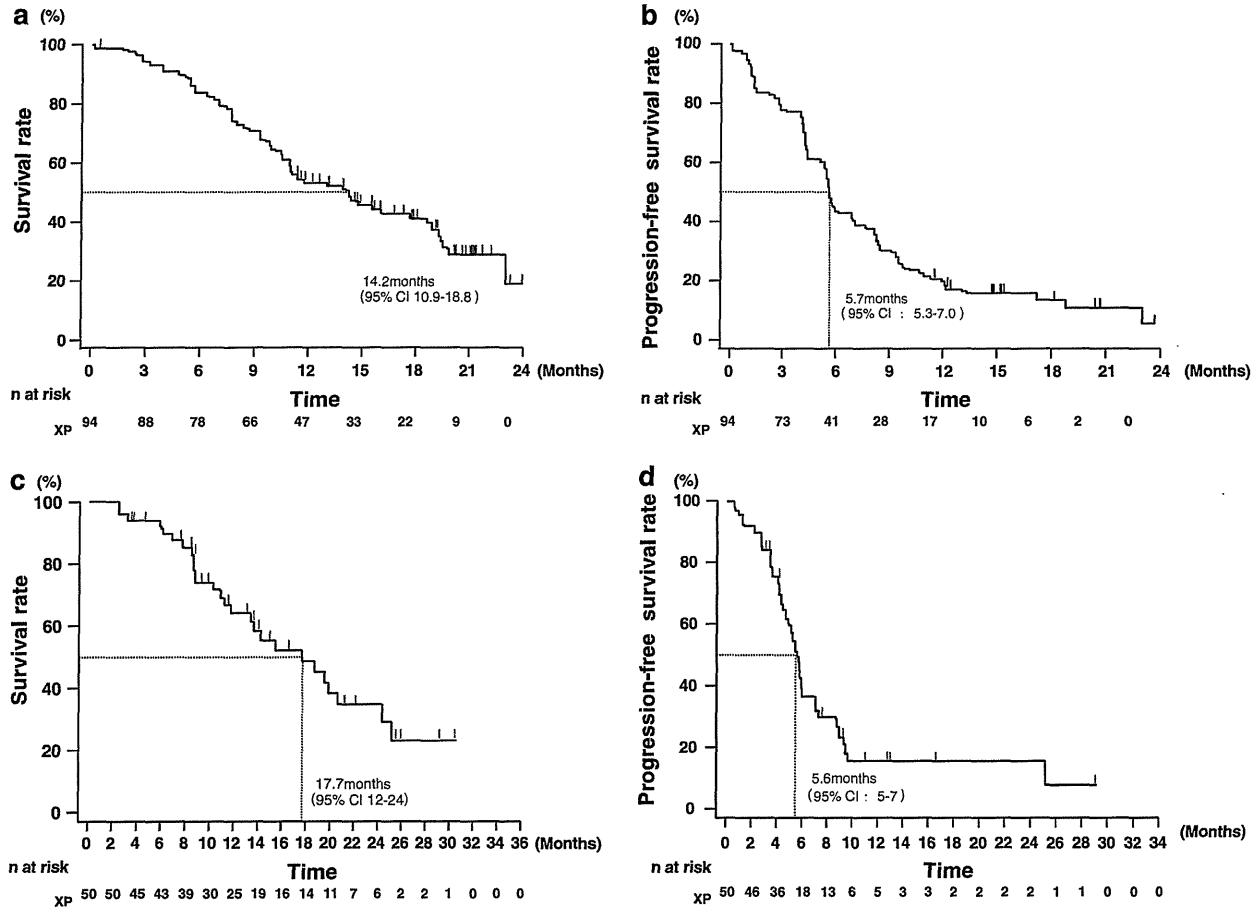

Fig. 1 **a** AVAGAST: overall survival. Median overall survival was 14.2 months (95 % confidence interval [*CI*], 10.9–18.8 months). **b** AVAGAST: progression-free survival. Median progression-free survival was 5.7 months (95 % CI, 5.3–7.0 months). **c** ToGA: overall survival. Median overall survival was 17.7 months (95 % CI, 12–24 months). **d** ToGA: progression-free survival. Median progression-free survival was 5.6 months (95 % CI, 5–7 months)

follows: the median number of treatment cycles was 6; the median total dose received was 539 mg; and the median dose intensity was 71 %.

In the ToGA study, capecitabine exposure was as follows: the median number of treatment cycles was 6; the median total dose received was 210,000 mg; and the median dose intensity was 84 %. Cisplatin exposure was as follows: the median number of treatment cycles was 6; the median total dose received was 569 mg; and the median dose intensity was 83.3 %.

Grade 3 or 4 adverse events occurred in 107 (74 %) patients and those events occurring at a frequency of greater than 10 % were as follows: neutropenia 45 %, anorexia 26 %, nausea 17 %, and anemia 13 %. Grade 3 or 4 diarrhea (4 %) and hand-foot syndrome (2 %) occurred at much lower frequencies compared with the incidences of each event in all grades. A full summary of the adverse events is shown in Table 5. Treatment was terminated due to adverse events for 12 (13 %) patients in the AVAGAST study and 4 (8 %) patients in the ToGA study. The type and severity of adverse events were consistent with previous reports of these drugs. There were no treatment-related deaths. In the AVAGAST study, the median time to initial onset of the first episode of neutropenia was 1.4 months (the start of cycle 3); for renal impairment it was 21 days (the start of cycle 2); and for nausea or vomiting it was 3 days (after the administration of cisplatin in cycle 1).

Discussion

In our analysis of the efficacy of XP, comparing the results from the XP group of Japanese subjects in the AVAGAST study with those of the overall chemotherapy group [8] showed that median progression-free survival times (5.7 vs. 5.3 months) were equivalent, but the median survival time (14.2 vs. 10.1 months) was longer for Japanese subjects. A similar comparison of the results from the XP group of

Table 4 Analysis of efficacy

Endpoints	AVAGAST[a] ($N = 94$)	ToGA[b] ($N = 50$)
Median overall survival (months) (95 % CI)	14.2 (10.9–18.8)	17.7 (12–24)
Median progression-free survival (months) (95 % CI)	5.7 (5.3–7.0)	5.6 (5–7)
Median time to progression (months) (95 % CI)	5.6 (5.1–7.2)	5.6 (5–7)
Response rate[c] (%)	49.2 (32/65)	58.5 (24/41)
Clinical benefit rate[c] (%)	67.7 (44/65)	85.4 (35/41)
Median response duration (months) (95 % CI)	6.9 (4.2–9.5)	4.3 (4–7)

95 % CI 95 % confidence interval

[a] The target population of the AVAGAST study was metastatic or inoperable locally advanced adenocarcinoma of the stomach or gastro-esophageal junction

[b] The target population of the ToGA study was HER2-positive metastatic or inoperable locally advanced adenocarcinoma of the stomach or gastro-esophageal junction (GEJ)

[c] Measurable disease

Japanese subjects in the ToGA study with those of the overall chemotherapy group [7] also showed median progression-free survival times (5.6 vs. 5.5 months) to be equivalent, with median survival times of 17.7 vs. 11.1 months; again, appreciably longer in Japanese subjects. In Japanese patients, the second-line regimen, after the failure of the first-line treatment, was usually irinotecan- or taxane-based [9, 10], which might have led to the favorable results in two Japanese studies.

Safety analysis demonstrated similar tolerability in the XP group of Japanese subjects to that of the overall chemotherapy group. In the AVAGAST study, this was shown by the median dose intensity of capecitabine (80.0 vs. 87.0 %), median dose intensity of cisplatin (71.0 vs. 71.0 %), and the incidence of grade 3 or higher adverse events (76 vs. 77 %), and in the ToGA study by the median dose intensity of capecitabine (84.0 vs. 86.7 %), the median dose intensity of cisplatin (83.3 vs. 91.1 %), and the incidence of grade 3 or higher adverse events (72 vs. 68 %). These results provide ample evidence of the efficacy and tolerability of XP in Japanese patients.

Table 5 Summary of adverse events

	AVAGAST[a] ($N = 94$)		ToGA[b] ($N = 50$)		Total ($N = 144$)	
	All grades *N* (%)	Grade 3 *N* (%)	All grades *N* (%)	Grade 3 *N* (%)	All grades *N* (%)	Grade 3 *N* (%)
Total	94 (100)	71 (76)	50 (100)	36 (72)	144 (100)	107 (74)
Hematological toxicities						
Neutropenia	63 (67)	45 (48)	34 (68)	20 (40)	97 (67)	65 (45)
Thrombocytopenia	19 (20)	2 (2)	8 (16)	3 (6)	27 (19)	5 (3)
Anemia	16 (17)	10 (11)	11 (22)	8 (16)	27 (19)	18 (13)
Febrile neutropenia	5 (5)	5 (5)	3 (6)	3 (6)	8 (6)	8 (6)
Non-hematological toxicities						
Nausea	84 (89)	18 (19)	44 (88)	7 (14)	128 (89)	25 (17)
Vomiting	60 (64)	6 (6)	28 (56)	2 (4)	88 (61)	8 (6)
Diarrhea	51 (54)	4 (4)	24 (48)	2 (4)	75 (52)	6 (4)
Stomatitis	34 (36)	1 (1)	16 (32)	1 (2)	50 (35)	2 (1)
Abdominal pain	12 (13)	1 (1)	3 (6)	–	15 (10)	1 (<1)
Hand-foot syndrome	54 (57)	2 (2)	23 (46)	1 (2)	77 (53)	3 (2)
Rash	19 (20)	–	5 (10)	–	24 (17)	–
Anorexia	83 (88)	27 (29)	46 (92)	10 (20)	129 (90)	37 (26)
Fatigue	69 (73)	5 (5)	26 (52)	4 (8)	95 (66)	9 (6)
Peripheral neuropathy	28 (30)	2 (2)	10 (20)	–	38 (26)	2 (1)
Renal impairment	17 (18)	3 (3)	27 (54)	–	44 (31)	3 (2)
Increased lacrimation	2 (2)	–	1 (2)	–	3 (2)	–

[a] The target population of the AVAGAST study was metastatic or inoperable locally advanced adenocarcinoma of the stomach or gastro-esophageal junction

[b] The target population of the ToGA study was HER2-positive metastatic or inoperable locally advanced adenocarcinoma of the stomach or gastro-esophageal junction

Comparing the Japanese XP groups in the AVAGAST and ToGA studies, the incidence of intestinal-type gastric cancer in the ToGA study (which limited patients to HER2-positive tumors only) was higher than that in the AVAGAST study (84.0 vs. 23.4 %). The incidence of non-measurable disease in the AVAGAST study was higher than that in the ToGA study (30.9 vs. 18.0 %) because patients with peritoneal disease, which could be diagnosed by laparoscopy or laparotomy, were allowed to enter the AVAGAST study, as they were generally regarded as having evaluable disease. In the analysis of efficacy, median progression-free survival was equivalent (AVAGAST, 5.7 months vs. ToGA, 5.6 months). However, median survival time was longer in the ToGA study (AVAGAST, 14.2 months vs. ToGA, 17.7 months) although the reason for this is unknown. The profiles and frequencies of adverse events were similar in both studies. The follow-up period of the AVAGAST study was shorter than that of the ToGA study and further follow up might be necessary.

The dose modification methods used for cisplatin in patients who demonstrated decreased creatinine clearance were different in the AVAGAST and ToGA studies (Table 2). However, given that there were no major differences between the AVAGAST study and the ToGA study in terms of efficacy, safety, or the percentage of patients with cisplatin dose reduction up to cycle 6 in the Japanese XP group, and considering the fact that the dose modification method for cisplatin in the AVAGAST study is simpler, the dose modification method for cisplatin in the AVAGAST study is recommended to be adopted as the standard approach for XP in Japan.

It is also important to note that the durations of treatment with capecitabine were different in the AVAGAST and ToGA studies. In the AVAGAST study capecitabine could be continued until disease progression, but in the ToGA study it could be continued initially up to cycle 6. After July 2007, owing to a protocol amendment in the second half of the study, capecitabine could subsequently be continued until disease progression. Although maintenance treatment with capecitabine has been proven effective in colorectal cancer, it has not been studied in mGC. Debate continues on the effectiveness of maintenance therapy in gastric cancer and further investigations are required before this becomes common practice.

Based on the results of the JCOG 9912 study [11] and SPIRITS study [12], the Japanese *Gastric cancer treatment guideline (3rd edition)* [6] recommends the combination of S-1 plus cisplatin (SP) as the standard first-line chemotherapy for unresectable advanced or mGC. A comparison of the SP cohort in the SPIRITS study [12] and the Japanese XP cohort in the AVAGAST study showed their main clinical characteristics to be similar. In the analysis of efficacy, the median survival time was 13.0 months for SP and 14.2 months for XP. The 1-year survival rate was 54.1 % for SP and 53.7 % for XP, demonstrating that the results for the Japanese XP group were not inferior to those for SP. Grade 3/4 adverse events (incidence of $\geq$10 %) were neutropenia, anorexia, anemia, leucopenia, and nausea for SP, and the events and frequencies were similar in the Japanese XP group. There were also no major differences in the frequencies of grade 3/4 diarrhea or hand-foot syndrome.

Considering that the global standard for patients with mGC is combination therapy with a fluoropyrimidine plus a platinum compound, it is possible that XP could be ranked alongside SP as one of the standard regimens in the first-line treatment of unresectable advanced or mGC in Japan. However, capecitabine and S-1 are of different drug designs and it has been reported that predictive factors for the efficacy of these two agents are different [13, 14]. It is anticipated that further research will establish the appropriate use of each fluoropyrimidine, using biomarkers and other measures.

By adjusting the dose according to the criteria for dose modification and discontinuation (Tables 1, 2), the XP regimen permits the continuation of treatment when adverse reactions occur. It will be important to know which adverse reactions may occur, and when. In the Japanese XP cohort of the AVAGAST study the main adverse events that led to interruption, dose modification, or discontinuation of capecitabine or cisplatin included neutropenia, renal impairment, and nausea or vomiting. In the AVAGAST study, the median time to the initial onset of the first episode of neutropenia was 1.4 months (the start of cycle 3); for renal impairment it was 21 days (the start of cycle 2); and for nausea or vomiting it was 3 days (after the administration of cisplatin in cycle 1).

For Japanese patients, no phase I trial combining XP in a 3-week cycle has been conducted. In the AVAGAST study, dose modification of capecitabine due to adverse events was reported in 83 patients (88.3 %), but discontinuation due to adverse events occurred in only 6 patients (6 %). The starting dose of cisplatin was 80 mg/m^2, but, as illustrated in the analysis in Fig. 2, about 50 % of the patients required a dose reduction in the second cycle. It is considered that there might be a more feasible dosing schedule for Japanese patients. Dose reduction of cisplatin due to adverse events (including laboratory abnormalities) was reported in 75 patients (79.8 %), but discontinuation due to adverse events was necessary in just 7 patients (7 %). Treatment was continued by adjusting the dose in such patients. Given the above observations, it will be important to cautiously monitor the adverse events that are the main causes of dose modification, i.e., neutropenia, renal impairment, and nausea or vomiting, and appropriately adjust the dose up to the

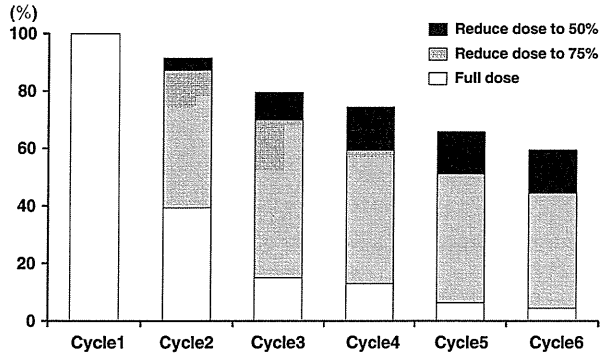

Fig. 2 AVAGAST: situation of cisplatin dose reduction by treatment cycle. The dose of cisplatin was reduced from 80 to 60 mg/m^2 at cycle 2 in about 50 % of the patients. Cisplatin therapy was continued even at cycle 6

time at which cisplatin is given in combination, and in particular at least until the third cycle after starting XP treatment for gastric cancer.

The present research is the first analysis focusing on the efficacy and safety of XP in Japanese patients with advanced gastric cancer. Based on the results of this study, XP is considered to be acceptable as a standard control arm in Japanese patients. Furthermore, by modifying the doses of XP to manage adverse reactions, XP could be one of the standard regimens for the first-line treatment of unresectable advanced or recurrent gastric cancer in Japan.

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SRPX2 Is a Novel Chondroitin Sulfate Proteoglycan That Is Overexpressed in Gastrointestinal Cancer

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Abstract

SRPX2 (Sushi repeat-containing protein, X-linked 2) has recently emerged as a multifunctional protein that is involved in seizure disorders, angiogenesis and cellular adhesion. Here, we analyzed this protein biochemically. SRPX2 protein was secreted with a highly posttranslational modification. Chondroitinase ABC treatment completely decreased the molecular mass of purified SRPX2 protein to its predicted size, whereas heparitinase, keratanase and hyaluroinidase did not. Secreted SRPX2 protein was also detected using an anti-chondroitin sulfate antibody. These results indicate that SRPX2 is a novel chondroitin sulfate proteoglycan (CSPG). Furthermore, a binding assay revealed that hepatocyte growth factor dose-dependently binds to SRPX2 protein, and a ligand-glycosaminoglycans interaction was speculated to be likely in proteoglycans. Regarding its molecular architecture, SRPX2 has sushi repeat modules similar to four other CSPGs/lecticans; however, the molecular architecture of SRPX2 seems to be quite different from that of the lecticans. Taken together, we found that SRPX2 is a novel CSPG that is overexpressed in gastrointestinal cancer cells. Our findings provide key glycobiological insight into SRPX2 in cancer cells and demonstrate that SRPX2 is a new member of the cancer-related proteoglycan family.

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Introduction

Sushi repeat protein X-linked 2 (SRPX2) was first identified as a gene up-regulated in pro-B leukemia cells and was described as sushi-repeat protein up-regulated in leukemia (SPRUL, [1]). Several years later, SRPX2 was found to be responsible for rolandic seizures associated with oral and speech dyspraxia and mental retardation [2]. The disease-causing mutation (N327S) and a second mutation (Y72S) of SRPX2 were identified, and these mutations resulted in the gain-of-N-glycosylated form of the mutant protein [2]. Although the molecular and biological functions of SRPX2 have been unknown for a long time, a recent study clearly demonstrated that SRPX2 binds to urokinase plasminogen activator receptor (uPAR) in a ligand/receptor interaction and that SRPX2 mutations led to an increase in the SRPX2/uPAR binding affinity [3]. In the vascular endothelial cells, Srpx2 regulates endothelial cell migration and tube formation, and the interaction of SRPX2 and uPAR is also involved in the early phases of endothelial remodeling during angiogenesis [4].

Recently, we demonstrated that SRPX2 is overexpressed in gastric cancer tissue and that expression was associated with a poor clinical outcome [5]. SRPX2 enhances cellular migration and adhesion in gastric cancer cells and, interestingly, the conditioned-medium obtained from SRPX2-producing cells increased the cellular migration activity and cellular adhesion [5]. We further examined SRPX2, focusing on a biochemical analysis in this study.

Materials and Methods

Cell culture

HEK293 was maintained in DMEM medium and SNU-16 and MKN7 were maintained in RPMI1640 medium supplemented with 10% FBS. HUVEC (human umbilical vein endothelial cells) was maintained in Humedia-EG2 (KURABO, Tokyo, Japan) medium with 1% FBS under the addition of EGF and FGF-2. The cells were maintained in a 5% CO_2-humidified atmosphere at 37°C. These cell lines were obtained from the Japanese Collection of Research Bioresources Collection (Sennan-shi, Osaka).

Western blotting analysis

The western blotting analysis has been previously described [6]. In belief, cell pellets were lysed in RIPA buffer (Tris-HCl: 50 mM, pH 7.4; NP-40: 1%; Na-deoxycholate: 0.25%; NaCl: 150 mM; EDTA: 1 mM; phenylmethyl-sulfonyl fluoride: 1 mM; aprotinin,

leupeptin, pepstatin: 1 mg/ml each; Na3VO4: 1 mM; NaF: 1 mM). Cell extracts were electrophoresed on 7.5% (w/v) polyacrylamide gels and transferred to a polyvinylidene di-fluoride membrane (Nihon Millipore, Tokyo, Japan). The membrane was incubated in Tris-buffered saline containing 0.5% Tween 20 with 3% BSA and then reacted with the primary antibodies and the HRP-conjugated secondary antibody for 90 min each. Visualization was achieved with an enhanced chemiluminescent detection reagent (Amersham Biosciences, Buckinghamshire, UK). The following antibodies were used: anti-HA high affinity (Roche Applied Science, Mannheim, Germany), anti-SRPX2 [5] and anti-chondroitin sulfate (CS-56; Seikagaku Kogyo, Tokyo, Japan).

Detection of endogenous SRPX2 protein

The culture medium was dialyzed against 50 mM of ammonium bicarbonate and lyophilized. The residue was dissolved in 50 mM of Tris-HCl (pH 7.4) and centrifuged at 20,000 rpm for 30 min. The supernatant was filtered through a 0.22-μm filter. The filtrate was subjected to fast protein liquid chromatography (FPLC; GE Healthcare UK Ltd. Buckinghamshire, England) separation on HiTrap Q HP columns (5 mL; GE Healthcare). The columns were equilibrated with 50 mM of Tris-HCl (pH 7.4). The samples were then injected onto the columns, which were washed with the same buffer and eluted at a flow rate of 4 mL/min using a linear gradient consisting of 0–2 M NaCl in 50 mM Tris-HCl (pH 7.4) over 45 min. The SRPX2 protein-containing fractions were then performed using gel-filtration chromatography (Superdex200 column, 16 mm×60 mm; GE Healthcare).

Expression constructs and purification of SRPX2-HA/His protein

The method for producing the expression constructs was previously described [5]. Empty and SRPX2-HA/His vectors were then transfected into HEK293 cells using FuGENE6 transfection reagent (Roche Diagnostics, Basel, Switzerland), and the cells were then selected with hygromycin. The stable transfectant HEK293 cells were designated as HEK293-Mock and HEK293-SRPX2-HA/His. The conditioned medium of the HEK293-Mock and HEK293-SRPX2-HA/His cells was subjected to FPLC loading at 3 mL/min on a 5-mL HisTrap HP column (GE Healthcare). The bound protein was washed with 15 mL of wash buffer (WB: 50 mM Na_2HPO_4, 10 mM Tris-HCl, 20 mM imidazole [pH 8.0] and 600 mM NaCl,) and eluted in elution buffer (EB: WB+230 mM imidazole). The SRPX2-HA/His protein-containing fractions were applied to an FPLC Superdex200 column (16 mm×60 mm; GE Healthcare) equilibrated with 0.15 M of ammonium bicarbonate. Elution was carried out using the same buffer at a flow rate of 1 mL/min. The SRPX2-HA/His-containing fractions were verified using western blotting and lyophilized.

Digestion of SRPX2 by specific GAG-degrading enzymes

Purified SRPX2-HA/His protein was digested with several specific enzymes including chondroitinase ABC and chondroitinase AC II (0.1 units in 40 mM Tris-HCl, 40 mM sodium acetate [pH 8.0] at 37°C for 2 h), chondroitinase B (0.02 units in 20 mM Tris-HCl, 0.25 μM calcium acetate [pH 7.5] at 37°C for 2 h), heparinase I and heparinase II (0.05 units in 5 mM calcium acetate, 50 mM sodium acetate [pH 7.0] 37°C for h), keratanase (0.1 units in 7.5 μM Tris-HCl [pH 7.4] at 37°C for 2 h), and hyaluroinidase (0.02 M acetate buffer, 0.15 M NaCl [pH 6.0] at 60°C for 2 h). Enzymes were purchased from Seikagaku Kogyo. The samples were then analyzed using western blotting.

Binding Assays

An IAsys resonant mirror biosensor (Affinity Sensors, Cambridge, UK) with a carboxymethyl dextran-sensing cuvette was used to determine the kinetic constants of hepatocyte growth factor (HGF) binding to immobilized SRPX2-HA/His. SRPX2-HA/His was dissolved in 10 mM sodium formate (pH 4.0) and immobilized on the carboxymethyl dextran surface of the cuvette, according to the manufacturer's instructions. Binding experiments were performed in PBS. Changes in the resonant angle were monitored at 1-s intervals for approximately 600 s. Experiments were performed at 25°C with a stirrer speed of 80 rpm. The binding parameters were calculated from the association and dissociation phases of the binding reactions using the non-linear curve fitting FastFit (Affinity Sensors). Bovine serum albumin (BSA) was used as a control.

Microarray data

The clinical samples of the paired colorectal cancers (CRCs), microarray procedure and analysis method have been previously described [7]. This study was approved by the institutional review board, and written informed consent was obtained from all the patients. All microarray data has been deposited to Center for Information Biology gene Expression database (CIBEX, http://cibex.nig.ac.jp/index.jsp) as accession number #CBX205. All data is MIAME compliant and that the raw data has been deposited in a MIAME compliant database (CIBEX), as detailed on the MGED Society website http://www.mged.org/Workgroups/MIAME/miame.html.

Patients and samples

The 30 CRC and 10 paired non-cancerous colonic mucosa samples were analyzed using real-time RT-PCR. The RNA extraction method and the quality check protocol have been previously described [7]. This study was approved by the institutional review board of the National Cancer Center Hospital, and written informed consent was obtained from all the patients.

Real-time reverse transcription PCR and western blot analysis

The methods used in this section have been previously described [5].

Results

Overexpression of SRPX2 in CRC tissues

We evaluated the mRNA expression of *SRPX2* in clinical samples of CRCs in addition to its homologue *SRPX* (*SRPX1*) using microarray data. *SRPX2* expression was markedly up-regulated (20.5 fold, $p = 0.00014$) in cancer tissues, compared with paired noncancerous mucosa samples, whereas the putative tumor suppressor gene *SRPX* was down-regulated (0.7 fold, $p = 0.029$) in cancer (Fig. 1). The result indicates that *SRPX2* is overexpressed in CRC during carcinogenesis and tumor progression, unlike *SRPX*. Real-time RT-PCR for the 30 CRC and 10 paired non-cancerous colonic mucosa samples confirmed that *SRPX2* mRNA was markedly overexpressed in the CRC samples but was only expressed at a very low level in non-cancerous colonic mucosa (Figure 1B).

Secreted SRPX2 protein is suspected to be modified posttranslationally

The predicted molecular mass of SRPX2 protein was 53 kDa; however, western blotting revealed that the molecular mass of the secreted SRPX2 protein was highly increased, with smeared bands at an apparent molecular mass of 100–150 kDa in SNU-16 and

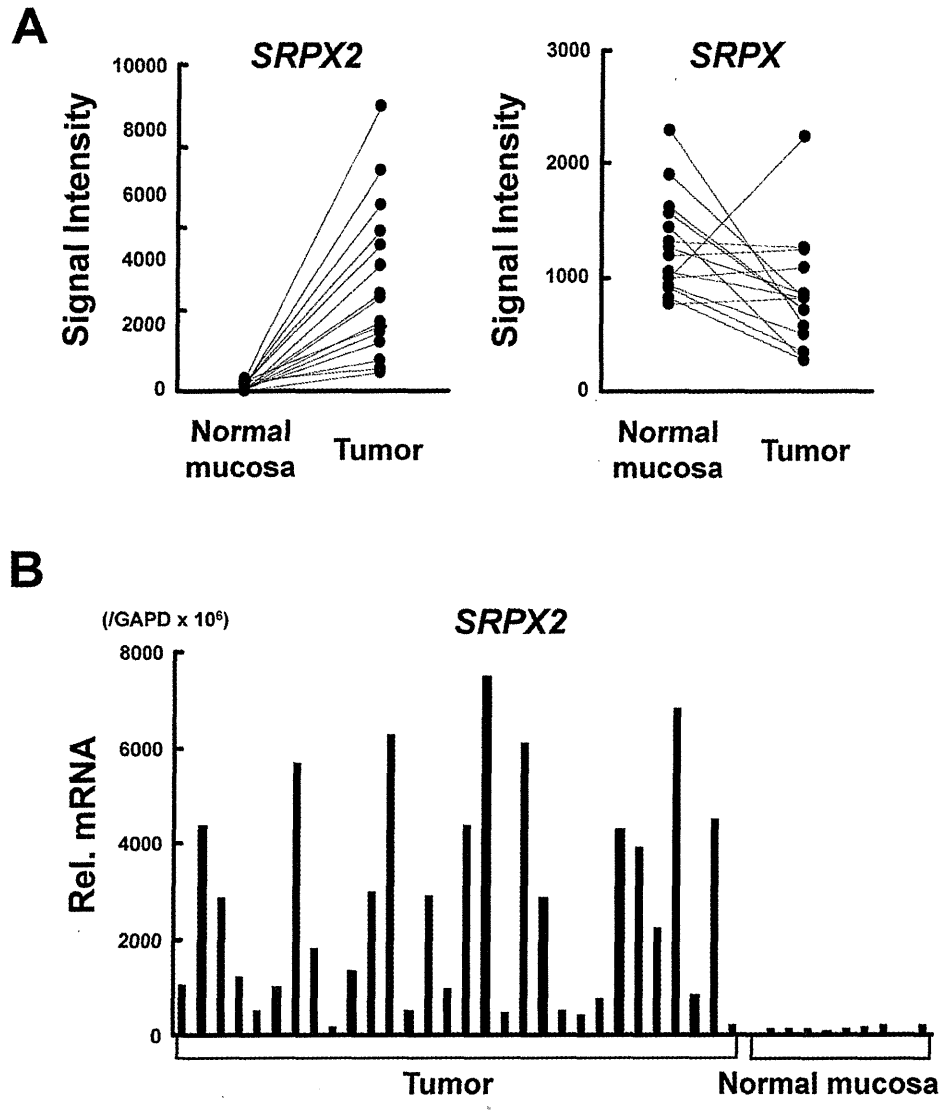

Figure 1. SRPX2 is overexpressed in colorectal cancer (CRC). (A) The mRNA expression of *SRPX2* and its homologue *SRPX* in 15 CRC and paired normal colonic mucosa specimens. The values indicate the normalized signal intensity obtained from the microarray data. (B) mRNA expression levels of *SRPX2* determined using real-time RT-PCR. CRC: colorectal cancer, Rel mRNA: normalized mRNA expression levels (*SRPX2/GAPD*×10^6).
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MKN7 cell lines (Fig. 2A). Next, we evaluated the exogenously expressed SRPX2 protein derived from HEK293-Mock and HEK293-SRPX2-HA/His cells. The molecular mass of intracellular SRPX2 protein was similar to the predicted size, while the molecular mass of the secreted-SRPX2 protein was highly increased (100–150 kDa). Smeared bands were also detected using both anti-HA and anti-SRPX2 antibodies (Fig. 2B). The non-smeared bands at 120 kDa in cell lysate are endogenous SRPX2. These results suggested that secreted SRPX2 protein may undergo posttranslational modifications.

SRPX2 is a novel chondroitin sulfate proteoglycan

Based on the appearance of the smeared bands at a highly increased molecular mass, we hypothesized that SRPX2 is a proteoglycan with glycosaminoglycan (GAG) chains. Accordingly, we treated purified-SRPX2 protein obtained from the cultured medium of HEK293-Mock (empty control) or HEK293-SRPX2-HA/His cells with chondroitinase ABC, heparitinase 1, heparitinase 2, keratanase, chondroitinase AcII, chondroitinase B, and hyaluroinidase. Western blotting revealed that the molecular mass of the secreted SRPX2 protein was clearly decreased by chondroitinase ABC digestion, but not by heparitinase or keratanase or hyaluroinidase (Fig. 3A, 3B). Further chondroitinase treatment showed that chondroitinase ABC and chondroitinase AcII completely digested GAGs on SRPX2, but that chondroitinase B partially digested these chains (Fig. 3B). A small digested SRPX2 protein was also detected using anti-SRPX2 antibody (Fig. 3C, 3D). These results indicate that SRPX2 contains chondroitin sulfate GAG chains and is a novel chondroitin sulfate proteoglycan (CSPG). In addition, the partial digestion by chondroitinase B suggests that a dermatan sulfate component may be included in the chondroitin sulfate GAG chains. Next, we

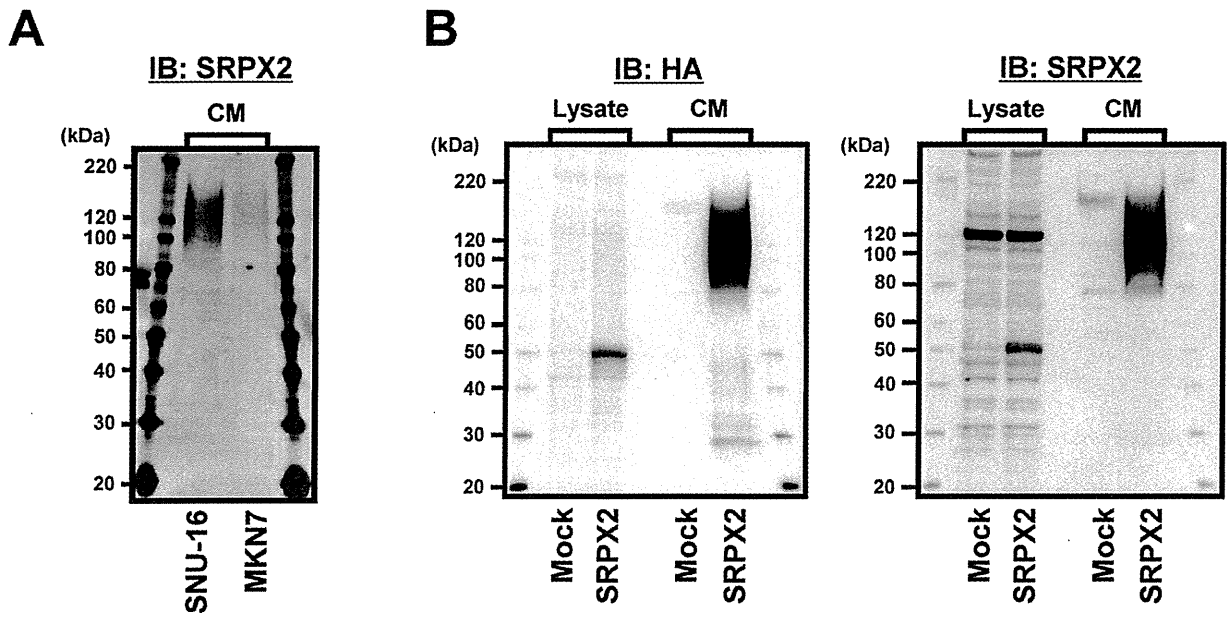

Figure 2. Secreted SRPX2 protein is suspected to be modified posttranslationally. (A) Secreted form of endogenous SRPX2 protein obtained from culture medium (CM) in SNU-16 and MKN7 cells. CM was subjected to ion exchange chromatography and used for western blotting analysis using anti-SRPX2 antibody. (B) Western blotting for exogenous SRPX2 protein obtained from cell lysate and CM using anti-SRPX2 and anti-HA antibody. Stable transfectant HEK293 cells, introducing the full-length cDNA fragment encoding human SRPX2 with HA and the His-tag vector or empty vector, were used for analysis. The non-smeared bands at 120 kDa in cell lysate are endogenous SRPX2. Mock: HEK293-Mock cells, SRPX2: HEK293-SRPX2-HA/His cells. IB: immunoblotting, Lysate: cell lysate, CM: culture medium.
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confirmed the results of enzymatic digestion against endogenous SRPX2 from HUVEC using western blotting with anti-SRPX2 antibody and a similar result was obtained (Fig. 4A). Anti-chondroitin sulfate antibody (CS-56) also detected the chondroitin sulfate GAG on SRPX2 (Fig. 4B). The non-smeared bands at 120 kDa in cell lysate are endogenous SRPX2.

HGF binds to SRPX2

It is well known that several ligands including HGF, heparin-binding EGF-like growth factor, fibroblast growth factor 2 and vascular endothelial growth factor are capable of binding to the GAG chain and that such interactions are considered to be a unique characteristic of GAGs and proteoglycans [8]. According to a report on CSPG endocan and HGF binding [9], we examined the interaction between HGF and GAGs using an IAsys resonant mirror biosensor. HGF dose-dependently bound to the GAGs of SRPX2, while control BSA did not (Fig. 5A). The K_d value of this interaction, calculated from the ratio of K_{diss}/K_{ass}, was 5.6 nM; these data were similar to those for previously reported data on HGF and endocan [9]. Next, we examined the biological function of SRPX2 on HGF. HGF increased the proliferation of HUVECs, and the addition of purified SRPX2 protein into the medium significantly increased HGF-induced proliferation (Figure 5B). These results suggest that the interaction of HGF with SRPX2 has a positive effect on angiogenesis.

SRPX2 has unique molecular architectures compared with other sushi repeat module-containing CSPG

Data from publicly available databases (http://smart.embl-heidelberg.de/) and a previous report [10] showed that SRPX2 has three sushi repeat modules (also known as complement control protein modules or short consensus repeats) and one hyaline domain (Fig. 6). Interestingly, four CSPG (agrrecan, versican, neurocan and brevican; also known as lecticans) are present among the sushi repeat module-containing family, and their common molecular architectures consist of one immunoglobulin-like domain, 2~4 LINK domains, one EGF-like domain, one C-type lectin, and one sushi repeat module (Fig. 6). The presence of a sushi repeat module and classification as a CSPG are the same for SRPX2 and lecticans, but the other molecular architectures of SRPX2 are quite different.

Taken together, these findings indicate that SRPX2 is a novel CSPG that is overexpressed in gastrointestinal cancer cells.

Discussion

The extensive use and structural diversity of sushi repeat modules presumably reflects the versatility of a structural scaffold that has been adapted by evolution to suit many purposes, both architectural and functional, such as the mediation of specific protein-protein and protein-carbohydrate interactions [10–12]. Meanwhile, SRPX2 has one hyaline domain, which appears to be involved in cellular adhesion. Hyaline domains have been identified in several eukaryotic proteins and are often associated with sushi repeat modules or arranged in multiple copies [13]. These characteristics of the molecular architectures of SRPX2, based on knowledge of protein-protein interactions, may contribute to ligand/receptor interactions between SRPX2 and uPAR, with implications for disorders of the language cortex, cognition, and angiogenesis [3,4].

We have demonstrated that SRPX2 is a novel CSPG, suggesting that SRPX2 may have additional as yet unknown biological functions as a proteoglycan, including interactions with

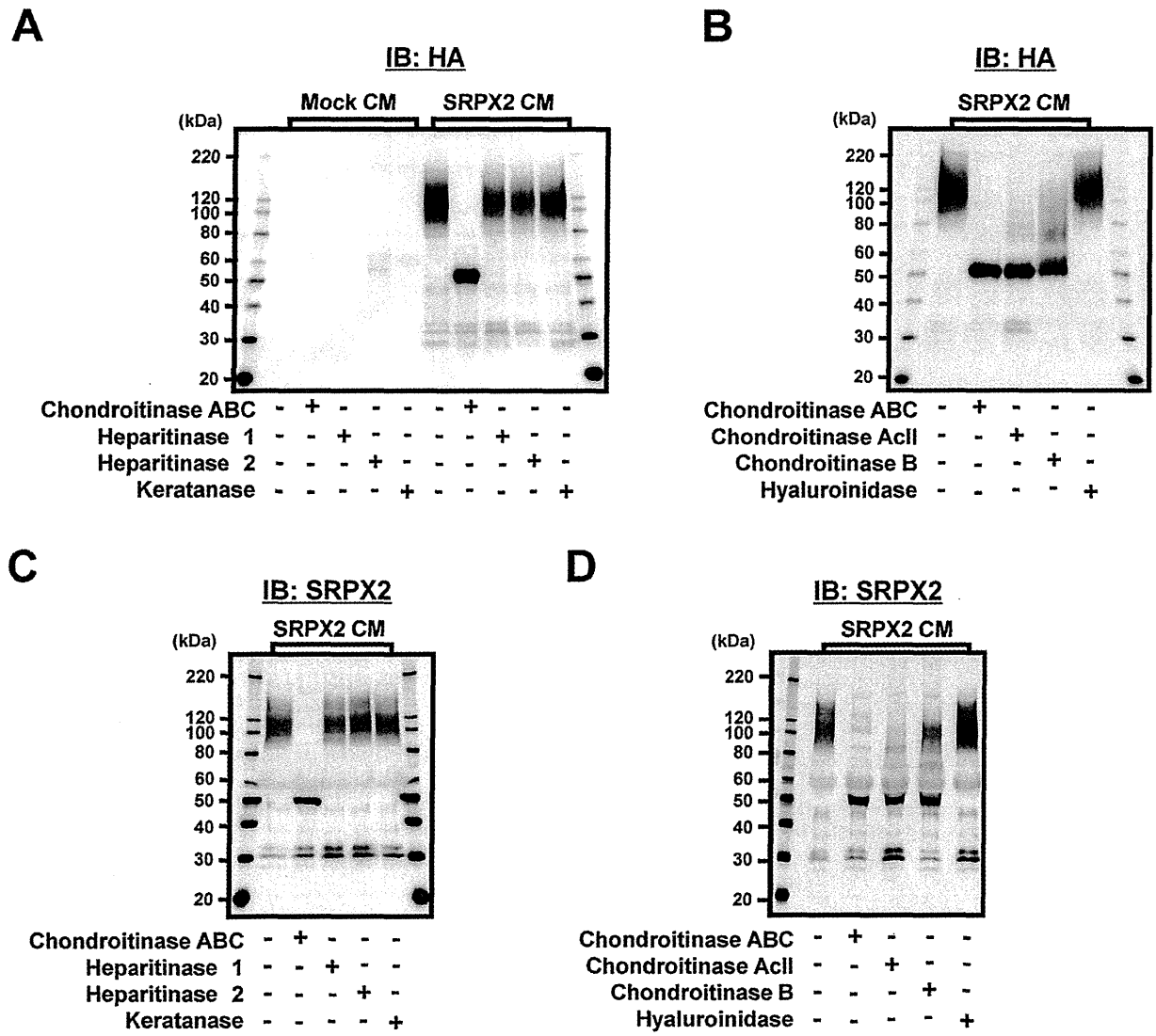

Figure 3. Effects of chondroitinases on SRPX2. (A, B) Purified SRPX2 protein obtained from cultured medium of HEK293-Mock or HEK293-SRPX2-HA/His cells were digested with chondroitinase ABC, heparitinase 1, heparitinase 2, keratanase, chondroitinase AcII, chondroitinase B and hyaluroinidase. The effect of digestion of the glycosaminoglycan chains was detected using western blotting using anti-HA (A, B) and anti-SRPX2 (C, D) antibody. IB: immunoblotting, CM: culture medium. Mock: HEK293-Mock cells, SRPX2: HEK293-SRPX2-HA/His cells.
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various extracellular signaling molecules such as growth factors, morphogens, enzymes and chemokines and/or may act at the cell-extracellular-matrix interface to modulate cell signaling. The conditioned-medium of SRPX2-producing cells markedly enhanced cellular adhesion in various cancer cell lines [5]; this result can be explained by the biological function of SRPX2 as a proteoglycan. In addition, although we have only demonstrated that HGF can bind to SRPX2, our results suggest that other known GAG-interacting ligands may be capable of binding to the GAG chain of SRPX2. Therefore, the function of ligand-SRPX2 binding may widely affect the activities of signaling pathway critical to cancer cells, including cellular proliferation, apoptosis, migration and survival [14]. In addition, SRPX2 was found to be secreted and may act as an extracellular matrix protein similar to other proteoglycans; indeed coating the culture dish with SRPX2 protein markedly enhanced cellular adhesion [5], supporting this idea.

Vascular endothelial cells HUVEC markedly express SRPX2 to the same extent as high-expressing cancer cell lines [5]. A recent report demonstrated that Srpx2 is a novel mediator of angiogenesis and a key molecule involved in the invasive migration of angiogenic endothelium through its role as a ligand for vascular uPAR [4]. Our findings also support the involvement of SRPX2 in angiogenesis from another aspect of proteoglycans. Since endocan is well-known as a vascular endothelial cells-specific CSPG [8], SRPX2 may be categorized as a vascular-related CSPG similar to endocan.

In conclusion, we found that SRPX2 is a novel chondroitin sulfate proteoglycan that is overexpressed in gastrointestinal

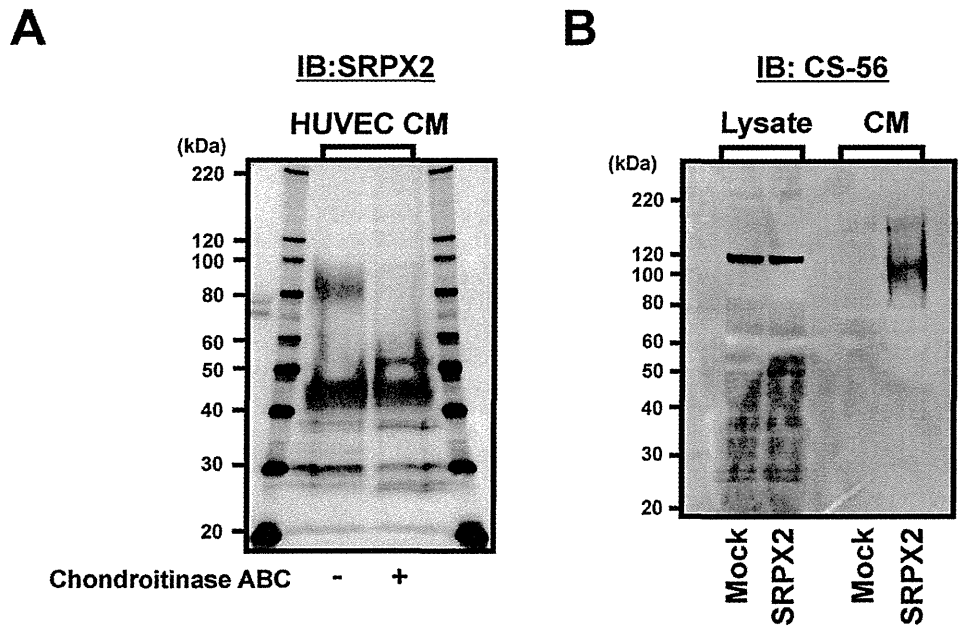

Figure 4. Detection of chondroitin sulfate glycosaminoglycan and binding of HGF to SRPX2. (A) Chondroitinase ABC digestion for endogenous SRPX2 protein derived from HUVEC (human umbilical vein endothelial cells). The SRPX2 protein was detected using anti-SRPX2 antibody. (B) Western blotting for SRPX2 protein using anti-chondroitin sulfate antibody (CS-56). The non-smeared bands at 120 kDa in cell lysate are endogenous SRPX2. IB: immunoblotting, Lysate: cell lysate, CM: culture medium. Mock: HEK293-Mock cells, SRPX2: HEK293-SRPX2-HA/His cells.
doi:10.1371/journal.pone.0027922.g004

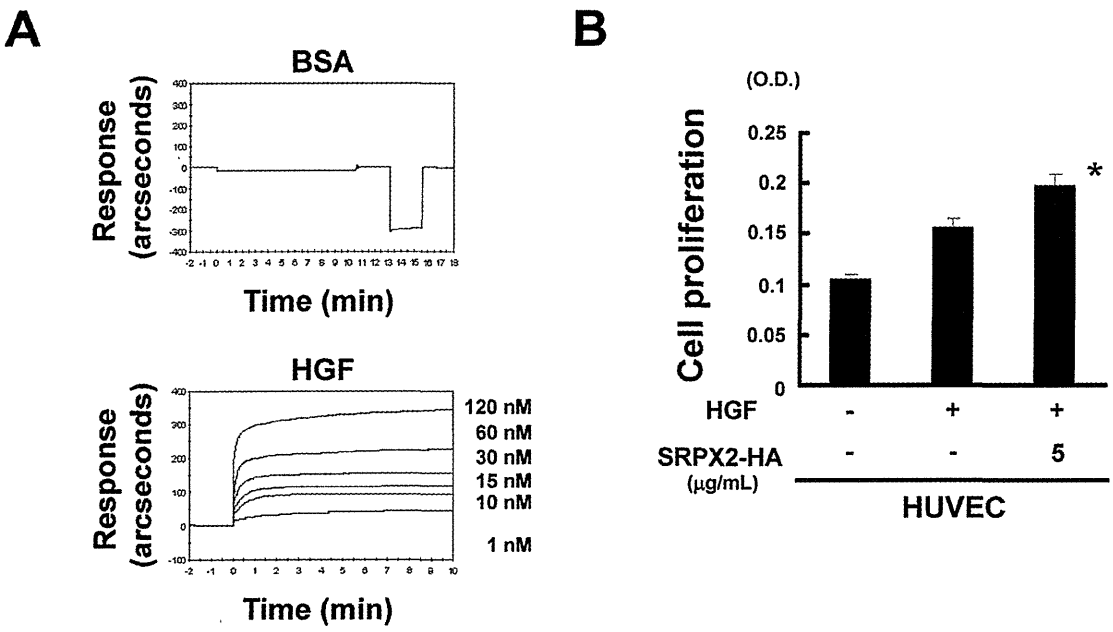

Figure 5. Binding of HGF to SRPX2 at the indicated concentrations. (A) IAsys resonant mirror biosensor was used for analysis. Bovine serum albumin (BSA) was used as a negative control. (B) Cell proliferation of HUVECs evaluated using an MTT assay. The HUVECs were stimulated with or without 10 ng/mL of HGF and 5 μg/mL of purified SRPX2 protein for 72 hours. *, SRPX2 (−) vs. (+), $p<0.05$.
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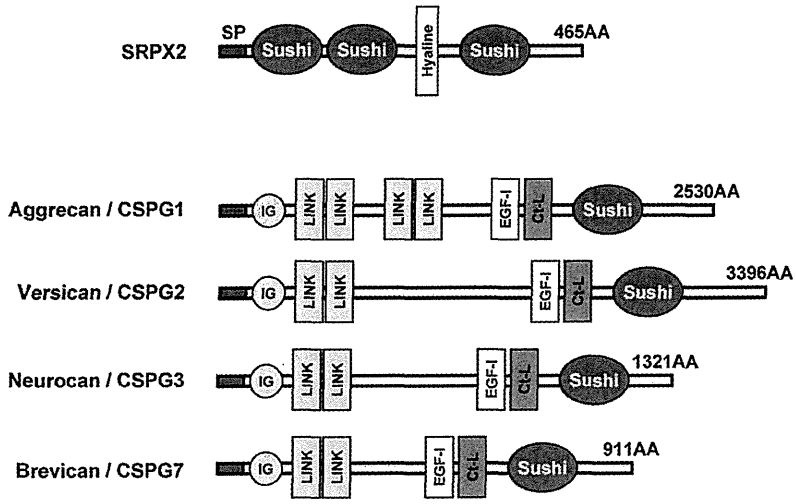

Figure 6. Molecular architectures of SRPX2. The data was obtained from the public database SMART (http://smart.embl-heidelberg.de/). SRPX2 has three sushi repeat modules and one hyaline domain. Four sushi repeat module-containing CSPG (agrrecan, versican, neurocan and brevican; also known as lecticans) are also shown. SP: signal peptides, AA: amino acids. Sushi: sushi repeat modules/CCP/short consensus repeats, Hyaline: hyaline domain, IG: immunoglobulin-like, LINK: hyaluronan-binding, EGF-I: EGF-like (Ca^{2+}-binding), Ct-L: C-type lectin.
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cancer. Our findings provide key glycobiological knowledge of this protein in cancer cells.

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Author Contributions

Conceived and designed the experiments: TA K. Nakagawa K. Nishio. Performed the experiments: KT DT KA KF KM H. Kaneda KK KY YF. Analyzed the data: TA H. Kimura KY IO. Contributed reagents/materials/analysis tools: YY. Wrote the paper: KT TA K. Nishio.

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The Need for a New Fluoropyrimidine in Advanced Gastric Cancer Treatment

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Abstract

Fluoropyrimidines have shown efficacy against a variety of cancers and have evolved into a range of different uses and formulations. These drugs have been tested extensively as monotherapies and as part of numerous different chemotherapy combinations. The efficacy and safety profile of bolus intravenous (IV) 5-fluorouracil (5-FU) has been improved by continuous IV administration. The availability of the first 5-FU oral form in Europe, capecitabine, has added a clear value in terms of convenience for patients, while forcing physicians and nurses to learn how to manage the toxicity profile of this compound. S-1 is a new oral formulation combining a 5-FU prodrug (tegafur) and two targeted modulators of its metabolism (gimeracil and oteracil) preserving the efficacy and improving the safety of the prodrug. S-1 has become the backbone treatment for advanced gastric cancer in Japan since its introduction in 1999. Extensive experience from clinical trials, post-marketing studies and patient registries of over 4,000 patients in Japan show that S-1 has improved measures of survival in advanced gastric cancer and has an acceptable safety profile. S-1 has recently been approved for advanced gastric cancer treatment in Europe. Since 5-FU metabolism differs between Asian and Caucasian populations, the introduction of S-1 in Caucasians has necessitated an entirely new clinical trial programme. Phase I trials indicated different dose levels were necessary in Westerners versus Asians (25 versus 40 mg/m²). In the FLAGS study of over 1,000 patients with advanced gastric cancer (the largest study ever conducted in this indication), S-1 plus cisplatin was demonstrated to be non-inferior in efficacy but superior in safety to 5-FU + cisplatin. Based on these results, S-1 was approved in Europe in March 2011 under the trade name Teysuno®. Further clinical trials are in progress to evaluate S-1 in advanced gastric cancer (AGC) and its use as part of triplet therapies is currently being investigated. This will further define the role of S-1 as a key part of advanced gastric cancer management in Western countries.

Keywords

Fluoropyrimidines, advanced gastric cancer, 5-fluorouracil (5-FU), S-1 (tegafur/gimeracil/oteracil), Caucasian

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The fluoropyrimidine drug 5-fluorouracil (5-FU) was originally patented in the US by Heidelberger and Duschinsky in 1957. Since that time, the use of the drug has been extensively developed and its analogues have played a continuing and pivotal role in cancer treatment. 5-FU was for a long time the only drug administered to gastric cancer patients but it subsequently evolved and underwent multiple manipulations, being administered with other compounds and in new schedules to increase its efficacy and safety.

In current clinical practice, fluoropyrimidines are likely to remain a central part of gastric cancer treatment for the foreseeable future; they are the backbone of most of the treatment regimens used in gastro-intestinal cancers. Efficacy and reduced toxicity are key requirements for oncologists and patients. New fluoropyrimidines in clinical development have considerable advantages over older drugs in terms of efficacy and safety. S-1 is a new formulation that consists of a 5-FU prodrug and two targeted modulators of its metabolism, preserving the efficacy and improving the safety of the prodrug.

Following the recent approval of S-1 for use in Western populations after more than 10 years of extensive experience in Japan, the history of the fluoropyrimidines in advanced gastric cancer will be considered, and evidence supporting the use of S-1 from early phases to more recent clinical studies will be reviewed.

Are all Fluoropyrimidines Equal?

The use of fluoropyrimidines in the treatment of advanced gastric cancer has undergone considerable development. In 1968, IV 5-FU was approved in Europe for the treatment of gastric carcinoma. In 2007, capecitabine became available for the first-line treatment of advanced gastric cancer in combination with a platinum salt. In 2011, S-1 was approved for first-line therapy of advanced gastric cancer in combination with cisplatin.

Table 1: Incidence of Hand-foot Syndrome on Comparative Studies of S-1

Study	Treatment Arms	Incidence of HFS (%) All Grades	Incidence of HFS (%) Grades 3–4
FLAGS (Ajani et al., 2010)[3]	S-1 + cisplatin (n=527)	5.4	0.19
	5-FU + cisplatin (n=526)	2.6	0.39
REAL-2 (Cunningham et al., 2008)[4]	epirubicin + cisplatin + capecitabine (n=250)	45.9	10.3
	epirubicin + oxaliplatin + capecitabine (n=244)	39.3	3.1
	epirubicin + cisplatin + 5-FU (n=263)	29.8	4.3
	epirubicin + oxaliplatin + 5-FU (n=245)	28.9	2.7
ML17032 (Ryu and Kang, 2009)[5]	capecitabine + cisplatin (n=160)	22	3.9
	5-FU + cisplatin (n=156)	4	0
SOX vs CAPOX (Kim et al., 2012)[6]	S-1 + oxaliplatin (n=65)	3.1	0
	capecitabine + oxaliplatin (n=64)	25	1.6

5-FU = 5-fluorouracil; FLAGS = First-Line advanced gastric cancer study; REAL2 = Randomized ECF for Advanced and Locally Advanced Esophagogastric Cancer study 2; S-1 = tegafur/gimeracil/oteracil combination (Teysuno™).

Four avenues have been exploited in order to improve the therapeutic index of 5-FU. The first avenue consisted of biochemical modulation with methotrexate, leucovorin and interferon.[1,2] The second avenue involved optimising the dosing schedule. For many years, it has been believed that continuous infusion of 5-FU channels its mechanism of action toward the DNA pathway – the basis of its anti-tumour activity – whereas a bolus dose diverts its activity toward the RNA pathway, which is believed to be the main cause of toxicity. Fifty-six years later, this is not an established fact, merely a supposition. The third avenue involved the development of oral agents for the convenience of clinicians and patients through the development of the 5-FU prodrugs. Finally, the fourth avenue focused on the metabolism of the agent. An increased understanding of each step of the 5-FU metabolism has allowed the selection of modulators targeting key enzymes involved in 5-FU degradation and activation.

Advances in understanding of the mechanism of action of 5-FU have led to the development of strategies that increase its anticancer activity. 5-FU alone is inactive and needs to be activated to the deoxy-nucleotide level, with the formation of 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP), by the action of thymidine phosphorylase (TP). FdUMP blocks thymidylate synthase (TS), the enzyme responsible for the last and crucial step of catalysis in DNA synthesis. 5-FU can also be activated via its conversion to 5-fluorouridine-5'-monophosphate (FUMP), which is incorporated into RNA as a nucleotide analogue that subsequently induces malfunction. Normally, 85 % of 5-FU is catabolised without being activated through its degradation to fluoro-beta-alanine by the enzyme dihydropyrimidine dehydrogenase (DPD). Without this degradation the level of active metabolites will increase substantially, causing intolerable toxicity.

S-1 is the international non-proprietary name (INN) of a fixed combination of three compounds that was selected to enhance 5-FU therapeutic effect while reducing toxicity. The first component is a 5-FU prodrug tegafur that has a sugar moiety attached to the fluorinated base and allows a reproducible high level absorption of 5-FU-based compound in the gastrointestinal (GI) tract. The second component is 5-chloro-2,4-dihydroxypyridine (CDHP or gimeracil), which inhibits dihydropyrimidine dehydrogenase (DPD). The third component is a potassium oxonate (OXO or oteracil) compound that inhibits orotate phosphoribosyl transferase (OPRT) at the start of the pathway of activation to FUMP. S-1 is marketed under different brand names around the world: TS-1® in Japan and Teysuno® in Europe.

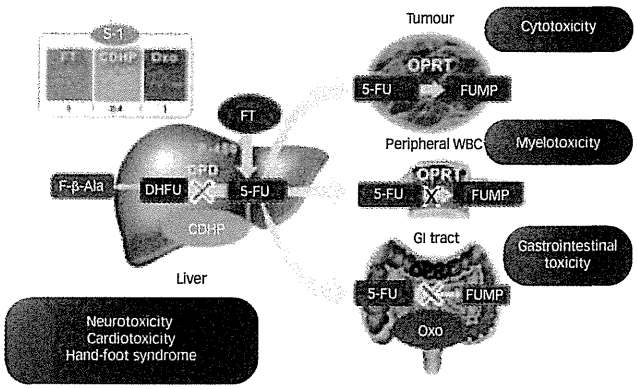
The formulation of S-1 has three possible beneficial consequences. Firstly, in blocking DPD, gimeracil allows a dramatic decrease in the quantity of 5-FU prodrug needed to achieve the active AUC of 5-FU compared with the dose of 1,000 mg/m² bid for capecitabine. In fact, the 25 mg/m² bid dose of tegafur provided by S-1 is 40 fold lower than that of capecitabine, resulting in a comparative dose of metabolised 5-FU of 2.5 %. Additionally, the direct action on DPD decreases the accumulation of fluoro-beta-alanine (Yamada et al., 2003) and the consequent occurrence of hand-foot syndrome (HFS) and other toxicities associated with this 5-FU catabolite. Secondly, oteracil blocks OPRT, leading to a decrease in activation of 5-FU into FUMP, especially in normal tissues, and, therefore, a decrease in gastrointestinal and haematological toxicities. Thirdly, 5-FU can be transformed in the tumour cells in its active form so that it is no longer broken down and is channelled towards FdUMP, triggering a more selective antineoplastic activity. *Figure 1* summarises the mechanism of action of this agent and demonstrates the way in which S-1 interferes and affects the metabolism of 5-FU in a targeted way.

In terms of reducing fluoro-beta-alanine levels by blocking the catabolism of 5-FU, all clinically available fluoropyrimidines can be distinguished into two classes: those that inhibit degradation, known as DPD inhibitory fluoropyrimidines (DIF), including tegafur-uracil, eniluracil and S-1, and those that do not contain a catabolism inhibitor such as IV 5-FU and capecitabine. Essentially, the DIF type of blocking agents substantially reduces fluoro-beta-alanine production and would be expected to reduce toxicity such as the incidence of HFS.

Patients receiving drugs of the DIF category have very low levels of HFS all grades, whereas patients receiving capecitabine show a high incidence of HFS, as do recipients of continuous infusion FU with bolus. When DIF compounds, including S-1, are employed, the incidence of grade 3-4 HFS is reduced to almost zero, as opposed to a 10 % level of severe toxicity with non-DIF compounds. In cancer treatments, theory and preclinical data rarely correspond exactly; but these findings are an example of a correspondence between a postulated mechanism and actual clinical data.

Animal studies involving treatment with a fluoropyrimidine without interference with the RNA-directed pathway, including the activation modulator, resulted in a serious degradation of the intestinal villi and crypts. When oteracil was added however, the anti-RNA effect was blocked and the intestinal villi were protected, as well as the crypts. In a study of 3,800 patients with gastric cancer who were receiving at least fluoropyrimidine plus oteracil treatment and listed in Japanese registry, the incidence of Grade 3–4 diarrhoea was only 2 %.[8] This

Figure 1: The Mechanism of Action of 5-fluorouracil showing the Inhibitory Action of S-1 (Teysuno®)



5-FU = 5-fluorouracil; CDPH = 5-chlorodihydro-pyrimidine; DHFU = 5', 6'- dihydro-5-fluorouracil; DPD = dihydropyrimidine dehydrogenase; FT = 1-(2-tetrahydrofuryl)-5-fluorouracil; FUMP = 5-fluorouridine-5'-monophosphate; OPRT = orotate phosphoribosyl transferase; Oxo = potassium oxonate; WBC = white blood cells. Source: data presented by Y Yamada.

value is consistent with clinical experience but has not been observed in randomised trials. More accurate reporting of the occurrence of diarrhoea in clinical trials conducted in Caucasians is needed to demonstrate the shorter duration and better response to treatment of these symptoms with S-1 than with other 5-FU preparations.

Pharmacokinetic analysis of 5-FU showed that to achieve similar drug exposure, expressed as area under the curve (AUC), in non-Asian patients as in Asian patients, the dose should be reduced. This finding indicates that the metabolism of S-1 is substantially different between Western and Asian populations. As a result of this observation, investigators repeated the entire series of clinical trials that had been carried out in Asian patient populations to adapt the drug to Western populations. Pharmacokinetic studies showed that compared with continuous infusion, the levels of fluoro-beta-alanine obtained with S-1 were approximately five times lower than those obtained with 5-FU. The levels for capecitabine, however, were approximately ten-fold higher.

Several studies have compared S-1 with capecitabine both indirectly and directly. In terms of efficacy, the indirect comparisons in randomised Phase III trials have provided strong evidence to support the use of S-1. The ML17032 study evaluating capecitabine plus cisplatin versus 5-FU plus cisplatin found non-inferiority of the capecitabine + cisplatin regimen.[5] The large (n=1,053) First-line advanced gastric cancer study (FLAGS) evaluating S-1 plus cisplatin versus 5-FU plus cisplatin showed non-inferiority of the S-1 plus cisplatin regimen.[3] From these results it may be concluded that in efficacy terms, capecitabine is roughly equal to IV 5-FU and that S-1 is equal to IV 5-FU. However, it cannot be concluded that S-1 is completely equal to capecitabine. In terms of safety, the FLAGS study found that S-1 had a significantly improved safety profile compared to IV 5-FU and cisplatin.[3]

To obtain meaningful safety comparisons between different cancer treatments, a comparable criterion must be defined. HFS is one of the most frequent adverse events necessitating a dose reduction or treatment interruption for capecitabine. The frequency of this parameter and the ability to monitor it in patients has made it a criterion of choice. Moreover, capecitabine-based regimens have shown an overall incidence of HFS of approximately 50 % and a severe incidence of approximately 10 %, whereas, in the FLAGS study, S-1 showed an incidence of less than 6 % with severe grade incidence of less than 1 % (see *Table 1*).

In addition to the randomised Phase III studies, a Phase II study conducted in South Korea (n=129) compared S-1 + oxaliplatin (SOX) with capecitabine + oxaliplatin (CAPOX) in advanced gastric cancer patients.[6] Both the SOX and CAPOX regimens were equally active and well tolerated. A 25 % incidence of all grades of HFS was observed for CAPOX versus only 3.1 % for SOX. In addition, a 1.6 % incidence of grade 3/4 HFS was seen in the CAPOX group versus 0 % in the SOX group. Grade 3/4 neuropathy, nausea, vomiting and asthenia were also less frequent with SOX.

The occurrence of a relevant adverse event, HFS, in indirect Phase III and direct Phase II comparisons of the different oral 5-FUs raises the following question for oncologists: "What will be your choice in daily practice for the treatment of your patients with advanced gastric cancer?"

S-1 (Teysuno®) – 10 Years of Savoir-faire in Asia

The mortality rate due to gastric cancer in Japan has decreased continuously since the 1960s. Gastric cancer, however, remains the second highest cause of cancer-related death, ranking second for males and third for females in Japan.[9] The five-year survival rates for gastric cancer between 1990 and 1994 at the National Cancer Center Hospital in Japan were not satisfactory at any disease stage, particularly for stage IV for which the rate is less than 10 %.[10]

In the 1960s, the fully active agent 5-FU became available. At the time, drugs were approved by the Japanese Pharmaceutical and Medical Devices Agency (PMDA) according to their response rates, and there was no requirement for Phase III survival benefits data. In the 1990s, irinotecan and taxanes were marketed, as well as S-1, and the survival time was prolonged from 7–8 months to one year. Subsequently, in the 21st century, a more specifically targeted drug, trastuzumab, has been developed for human epidermal growth factor receptor 2 (HER-2)-positive subgroup, constituting the only recent progress in biological therapies in the gastric cancer field.

Data of two late Phase II studies conducted in patients with advanced gastric cancer reported response rate for S-1 monotherapy of 49.0 and 44.2 %, respectively.[11,12] S-1 was very effective at the primary disease site for 39 and 28.9 %, respectively. The frequency of side effects was generally very low. The most commonly observed grade 3/4 toxicities were neutropenia and diarrhoea, with only a 2 % incidence rate. Moreover, the median OS were 250 and 207 days, whereas the one year OS rates were 37 and 36 %, respectively.

In the Japanese Nationwide Post-Marketing Survey of S-1, involving 3,808 Japanese patients, the toxicity profile of S-1 was shown to be similar to that reported in the late Phase II studies. Diarrhoea of grade ≥3 was only 2 %, but the incidence of neutropenia was 6 %. The toxicity of S-1, especially haematological toxicity, was related to creatinine clearance. This was not surprising given that CDHP, which is a DPD inhibitor, is excreted from the kidney. In patients with renal failure, the incidence of haematological toxicities was higher: 40 % (8/20) for a standard initial dose versus 23.5 % (4/17) for a reduced initial dose (for patients with creatinine clearance values <30 mL/min).[8] Therefore, when administering S-1, renal function must be checked and dose adjustments must be made according to age and gender.