Prognostic significance of the co-overexpression of fibroblast growth factor receptors 1, 2 and 4 in gastric cancer

HIDEAKI MURASE[1], MIKITO INOKUCHI[1], YOKO TAKAGI[2], KEIJI KATO[1], KAZUYUKI KOJIMA[3] and KENICHI SUGIHARA[1]

Departments of [1]Surgical Oncology, [2]Translational Oncology and [3]Minimally Invasive Surgery, Tokyo Medical and Dental University, Tokyo 113-8519, Japan

Received February 2, 2014; Accepted April 30, 2014

DOI: 10.3892/mco.2014.293

Abstract. The overexpression of fibroblast growth factor receptor (FGFR) 2 is an established prognostic factor and treatment target in gastric cancer. However, the roles of other FGFRs have not been fully elucidated. In this study, we investigated the correlations of the expression of FGFR1-4 with clinicopathological characteristics and outcomes in gastric cancer. Tumor samples were obtained from 222 patients with gastric adenocarcinoma who underwent gastrectomy between 2003 and 2007. The expression of each FGFR was measured in the tumors by immunohistochemical analysis. The overexpression of FGFR1, FGFR2 or FGFR4 was found to be significantly associated with tumor progression, including depth of invasion, lymph node metastasis, pathological stage and distant metastasis or recurrent disease. Patients exhibiting overexpression of FGFR1, FGFR2 or FGFR4 had a significantly poorer disease-specific survival (DSS; P<0.001, P=0.008 and P<0.001, respectively). Moreover, the co-overexpression of all three FGFRs was significantly associated with a poorer DSS compared to the expression of none or only one of the FGFRs (P<0.001 and P=0.001, respectively) and it was found to be an independent prognostic factor (HR=1.71, 95% CI: 1.02-2.85, P=0.041). In conclusion, high expression of FGFR1, FGFR2 or FGFR4 was associated with tumor progression and poor survival in patients with gastric cancer. Similar to FGFR2, FGFR1 and FGFR4 may be considered as prognostic factors and treatment targets in gastric cancer.

Introduction

Gastric cancer is the second leading cause of cancer-related mortality worldwide, accounting for ~1 in 10 of all deaths from cancer (1). The outcome of gastric cancer is generally poor, with a 5-year relative survival of <30% in most countries (2). Although radical surgery remains the cornerstone of treatment for gastric cancer, surgery alone appears to have reached its limits in terms of local control and survival. The achievement of locoregional control remains difficult in the presence of advanced disease (3). The majority of patients with advanced gastric cancer receive palliative chemotherapy, which is associated with a median survival of 11-12 months (4). In addition to standard cytotoxic regimens, targeted therapies, using small molecules or antibodies designed to disrupt the activity of specific oncogenic signaling pathways, have recently emerged as a promising treatment strategy. A number of receptor tyrosine kinases (RTKs) have been associated with tumor progression and patient outcomes in various types of cancer. RTK inhibitors, such as human epidermal growth factor receptor (HER), have been evaluated and some have been used to treat gastrointestinal cancers. In a recent ToGA trial (5), trastuzumab, a monoclonal antibody against the p185HER2 protein, improved the overall survival of patients with HER2-positive tumors when combined with chemotherapy. However, only 7-17% of gastric cancer patients have HER2-positive tumors and are considered as suitable candidates for anti-HER2 therapy (6,7). Further investigations are required to increase the number of patients with gastric cancer for whom targeted treatments may be a viable clinical option.

The fibroblast growth factor receptor (FGFR) family (FGFR1-4) belongs to the receptor tyrosine kinase superfamily. FGFRs regulate fundamental developmental pathways by interacting with fibroblast growth factors (FGFs) and thereby control a wide range of events, extending from mesoderm patterning in the early embryo to the development of multiple organ systems (8,9). FGF signaling participates in several biological functions in the adult organism, including regulation of angiogenesis and wound repair. FGFRs are expressed on a number of different cell types and regulate key cell activities, such as proliferation, survival, migration and differentiation, which renders FGF signaling susceptible to subversion by cancer cells (10).

FGFR2 amplifications have been reported in 10% of gastric cancers, the majority of which are of the diffuse type (11). FGFR2 amplification may correlate with poor outcomes in patients with diffuse-type gastric cancer (12). Moreover, the

Correspondence to: Dr Mikito Inokuchi, Department of Surgical Oncology, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
E-mail: m-inokuchi.srg2@tmd.ac.jp

Key words: fibroblast growth factor receptor, gastric cancer, immunohistochemical analysis

presence of FGFR2 gene amplification in gastric cancer is associated with sensitivity to inhibition of FGFR signaling by tyrosine kinase inhibitors and monoclonal antibodies in preclinical models (13,14). Thus, FGFR2 has attracted considerable attention as a novel therapeutic candidate for the development of targeted anticancer agents (15).

In contrast to FGFR2, the roles of FGFR1, FGFR3 and FGFR4 have not been fully elucidated. Overexpression of these FGFRs in gastric cancer was reported by a few small studies (16-19). In this study, we aimed to investigate the correlations of FGFR1-4 immunohistochemical expression with clinicopathological characteristics and outcomes in gastric cancer.

Patients and methods

Patients. Our study group comprised 222 patients with primary gastric adenocarcinoma who underwent surgical resection between January, 2003 and December, 2007 in the Department of Esophagogastric Surgery, Tokyo Medical and Dental University. Each tumor was classified according to the tumor-node-metastasis staging system recommended by the International Union Against Cancer. Of the 222 patients, 168 were men and 54 were women. The mean age of the patients was 64.6 years (range, 21-92 years). All the patients were evaluated for recurrent disease by tumor marker analysis or diagnostic imaging (computed tomography, ultrasonography, magnetic resonance imaging and endoscopy) every 3-6 months. Patients with distant metastasis or recurrent disease received chemotherapy with S-1 alone or combined chemotherapy. A total of 20 patients (9%) received adjuvant chemotherapy with S-1 following radical resection. All the patients were followed up until July, 2012. The median follow-up was 60 months (range, 3-111 months). A total of 77 patients (35%) succumbed to gastric cancer, 66 (30%) had recurrent disease and 11 (5%) died from other causes.

Immunostaining of the FGFR family. Immunohistochemical analysis was performed with the use of secondary antibodies conjugated to a peroxidase-labeled polymer [Histofine Simple Stain MAX PO (Multi); Nichirei Co., Tokyo, Japan]. Polyclonal rabbit antibodies against FGFR1, FGFR2, FGFR3 and FGFR4 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All the available hematoxylin and eosin-stained slides of the surgical specimens were reviewed. For each case, representative formalin-fixed, paraffin-embedded tissue blocks were selected for immunohistochemical studies and sliced into 4-μm sections. Following deparaffinization and rehydration, antigen retrieval was performed at 98˚C for 30 min, using a pH 6.0, 10 mmol/l sodium citrate buffer (Mitsubishi Chemical Medience Corporation, Tokyo, Japan) in a microwave processor (MI-77; Azumaya, Tokyo, Japan). Endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol. Subsequently, non-specific binding was blocked by treating the slides with 10% normal goat serum for 10 min at room temperature. The slides were incubated with the primary antibodies, including anti-FGFR1 (dilution, 1:100), anti-FGFR2 (dilution, 1:300), anti-FGFR3 (dilution, 1:500) and anti-FGFR4 (dilution, 1:100) in 1% bovine serum albumin/phosphate-buffered saline overnight at 4˚C. The sections were then incubated with Simple Stain Max PO (Multi) for 30 min at room temperature. The chromogen substrate was 3,3'-diaminobenzidine tetrahydrochloride solution (Histofine Simple Stain DAB solution; Nichirei Co.). Subsequently, the sections were counterstained with Mayer's hematoxylin (Wako, Tokyo, Japan). Negative controls were treated similarly, except for the antibodies being replaced by normal rabbit IgG (Santa Cruz Biotechnology, Inc.).

Interpretation of immunostaining results. The staining intensity was scored into four grades as follows: 0, no staining; 1, weakly positive; 2, moderately positive; and 3, strongly positive. The staining extent (positive frequency) was also scored into four grades according to the percentage of stained tumor cells as follows: 0, complete absence of staining; 1, ≤20%; 2, >20 to ≤50%; and 3, >50% stained cells. Composite scores were derived by addition of the intensity score and the staining extent score. For the statistical analysis, composite scores of ≥4 were defined as high expression and scores of <4 as low expression. Two investigators (Hideaki Murase and Yoko Takagi), who were blinded to the patients' outcomes separately counted the stained cancer cells. Any disagreements between the two investigators were resolved by reassessment and consensus.

Statistical analysis. The statistical analysis was performed using IBM SPSS Statistics 20 software (IBM, Inc., Armonk, NY, USA). The χ^2 test was used to investigate the possible associations between the expression of each FGFR receptor and clinicopathological variables. The χ^2 test was also used to assess the correlations between FGFR expressions. The Mann-Whitney U test was used to analyze the associations between FGFR expression and patient age. Kaplan-Meier curves were plotted to assess the effects of FGFR expression on disease-specific survival (DSS) and different DSS curves were compared using the log-rank test. Multivariate proportional Cox models were used to assess the prognostic significance of FGFR and of factors associated with DSS. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Immunohistochemical analysis of the FGFR family. Expression of FGFR1, FGFR2, FGFR3 and FGFR4 was mainly observed in the cytoplasm of cancer cells (Fig. 1) and fibroblasts in cancer tissue. Weak expression was observed in certain regions of the normal epithelium in proximity to the cancer cells. Among the 222 tumors investigated, the number of tumors exhibiting high FGFR expression were 66 (30%) for FGFR1, 114 (51%) for FGFR2, 142 (64%) for FGFR3 and 175 (79%) for FGFR4. High expression of FGFR1, FGFR2, FGFR3 or FGFR4 was significantly correlated with high expression of each of the other three proteins (Table I).

Association with clinicopathological variables. The clinicopathological variables are summarized in Table II. A high expression of FGFR1, FGFR2 and FGFR4 was significantly associated with the depth of tumor invasion (T3-T4 vs. T1-T2:

Table I. Correlations among the expressions of FGFR1, FGFR2, FGFR3 and FGFR4.

Expression level	FGFR2			FGFR3			FGFR4		
	Low	High	P-value	Low	High	P-value	Low	High	P-value
FGFR1									
Low	94	62	<0.001	64	92	0.022	43	113	<0.001
High	14	52		16	50		4	62	
FGFR2									
Low				49	59	0.005	39	69	<0.001
High				31	83		8	106	
FGFR3									
Low							26	54	0.003
High							21	121	

FGFR, fibroblast growth factor receptor.

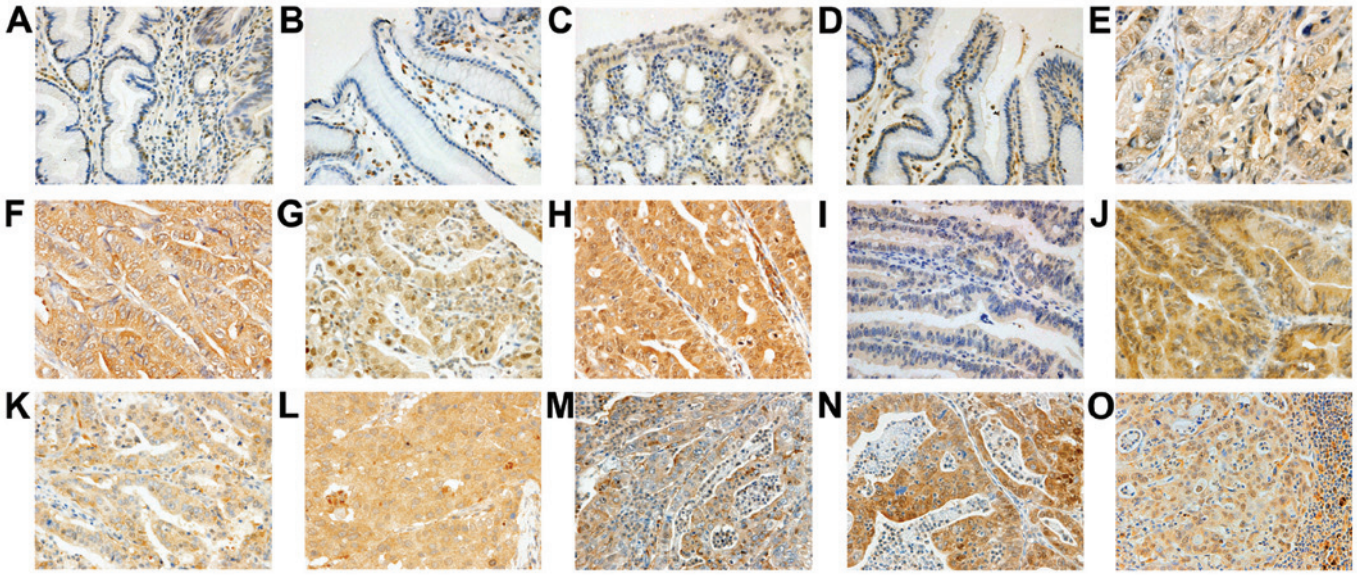

Figure 1. Immunostaining for fibroblast growth factor receptor (FGFR)1, FGFR2, FGFR3 and FGFR4. Representative primary gastric carcinomas exhibiting positive immunostaining for (A) FGFR1, (B) FGFR2, (C) FGFR3 and (D) FGFR4. Representative primary gastric carcinomas exhibiting immunostaining for FGFR1 with intensity scores of (E) 1 and (F) 3; immunostaining for FGFR2 with intensity scores of (G) 1 and (H) 3; immunostaining for FGFR3 with intensity scores of (I) 1 and (J) 2; and immunostaining for FGFR4 with intensity scores of (K) 1 and (L) 3. Representative metastatic lymph nodes exhibiting immunostaining for (M) FGFR1, (N) FGFR2 and (O) FGFR4. Magnification, x400.

P<0.001, P=0.011 and P<0.001, respectively), lymph node metastasis (P=0.002, P=0.011 and P<0.001, respectively), and tumor stage (III-IV vs. I-II: P=0.001, P=0.012 and P<0.001, respectively). Distant metastasis or recurrence was found in a significantly higher proportion of patients with high expression of FGFR1, FGFR2 and FGFR4 compared to those with low expression of these proteins (P<0.001, P=0.004 and P<0.001, respectively). As the high expression of FGFR1, FGFR2 and FGFR4 was significantly associated with lymph node metastasis, we immunohistochemically evaluated the expression of these proteins in lymph node metastases from 88 patients and compared it to their expression in the primary tumor. A high expression of FGFR1, FGFR2 and FGFR4 was found in 61 (69%), 44 (50%), and 67 (76%) patients, respectively. However, only FGFR4 exhibited a significant association between its expression in the primary tumor and that in metastatic lymph nodes (P=0.017) (Table III).

Association with DSS. High expression of FGFR1, FGFR2 and FGFR4 in the primary tumor was significantly associated with poorer DSS on the univariate analysis (P<0.001, P=0.008 and P<0.001, respectively) (Fig. 2). The 5-year DSS in patients with high expression of FGFR1, FGFR2 and FGFR4 was 57, 63 and 66%, respectively, compared to 77, 79 and 91%, respectively, in patients with low expression of these proteins (Table IV). On the multivariate analysis, the depth of tumor invasion and lymph node involvement were independent prognostic factors [hazard ratio (HR)=6.80, 95% confidence interval (CI): 2.63-15.6, P<0.001; and HR=4.48, 95% CI: 1.90-10.5, P=0.001, respectively], unlike

Table II. Correlations of the expressions of FGFR1, FGFR2, FGFR3 and FGFR4 with clinicopathological factors.

		FGFR1			FGFR2			FGFR3			FGFR4		
Clinicopathological factors	n	Low (156)	High (66)	P-value	Low (108)	High (114)	P-value	Low (80)	High (142)	P-value	Low (47)	High (175)	P-value
Age (years)				0.36			0.036			0.38			0.006
<70	142	103	39		77	65		48	94		38	104	
≥70	80	53	27		31	49		32	48		9	71	
Gender				1.00			1.00			1.00			0.70
Female	54	38	16		26	28		19	35		10	44	
Male	168	118	50		82	86		61	107		37	131	
Main location				0.20			0.029			0.39			0.84
Middle or lower	177	128	49		93	84		61	116		37	140	
Upper	45	28	17		15	30		19	26		10	35	
WHO pathological type				0.47			0.023			0.002			0.74
Differentiated	106	77	29		43	63		27	79		21	85	
Undifferentiated	116	79	37		65	51		53	63		26	90	
Depth of invasion				<0.001			0.011			0.58			<0.001
T1/2	118	96	22		67	51		45	73		41	77	
T3/4	104	60	44		41	63		35	69		6	98	
Lymphatic invasion				0.001			0.020			0.45			<0.001
Negative	69	59	10		42	27		22	47		26	43	
Positive	153	97	56		66	87		58	95		21	132	
Venous invasion				0.019			0.004			0.66			<0.001
Negative	73	59	14		46	27		28	45		30	43	
Positive	149	97	52		62	87		52	97		17	132	
LN metastasis				0.002			0.011			0.41			<0.001
Negative (N0)	114	91	23		65	49		38	76		36	78	
Positive (N1/2/3)	108	65	43		43	65		42	66		11	97	
Stage				0.001			0.012			0.47			<0.001
I/II	141	110	31		78	63		48	93		42	99	
III/IV	81	46	35		30	51		32	49		5	76	
Distant metastasis or recurrence				<0.001			0.004			0.29			<0.001
Negative	152	119	33		84	68		51	101		43	109	
Positive	70	37	33		24	46		29	41		4	66	

FGFR, fibroblast growth factor receptor; WHO, World Health Organization; LN, lymph node.

FGFR1, FGFR2 and FGFR4 (HR=1.20, 95% CI: 0.73-2.00, P=0.47; HR=1.32, 95% CI: 0.77-2.24, P=0.31; and HR=0.96, 95% CI: 0.32-2.90, P=0.95, respectively).

Co-overexpression of FGFR1, FGFR2 and FGFR4. The co-overexpression of FGFR1, FGFR2 and FGFR4 in the primary tumors was found to be significantly associated with a poorer DSS compared to the expression of none or only one of these proteins (P<0.001 and P=0.001). The 5-year DSS was 55, 65, 78 and 92% in patients exhibiting high expression of all three, two, one and none of these FGFRs, respectively (Fig. 3). Although tumor stage was the most significant prognostic factor (HR=22.3, 95% CI: 10.1-49.6, P<0.001), the co-overexpression of these three FGFRs was also identified as an independent prognostic factor (HR=1.71, 95% CI: 1.02-2.85, P=0.041) (Table V).

Discussion

Our results suggested that high expression of FGFR1, FGFR2 or FGFR4 may be crucial in tumor progression, metastasis and outcomes in gastric cancer patients. Moreover, the co-overexpression of FGFR1, FGFR2 and FGFR4 was found to be an independent prognostic factor in gastric cancer.

FGFR-dependent signaling occurs through two main pathways: via the intracellular receptor substrates FGFR substrate 2 (FRS2) and phospholipase Cg (PLCg), ultimately upregulating the Ras-dependent mitogen-activated protein

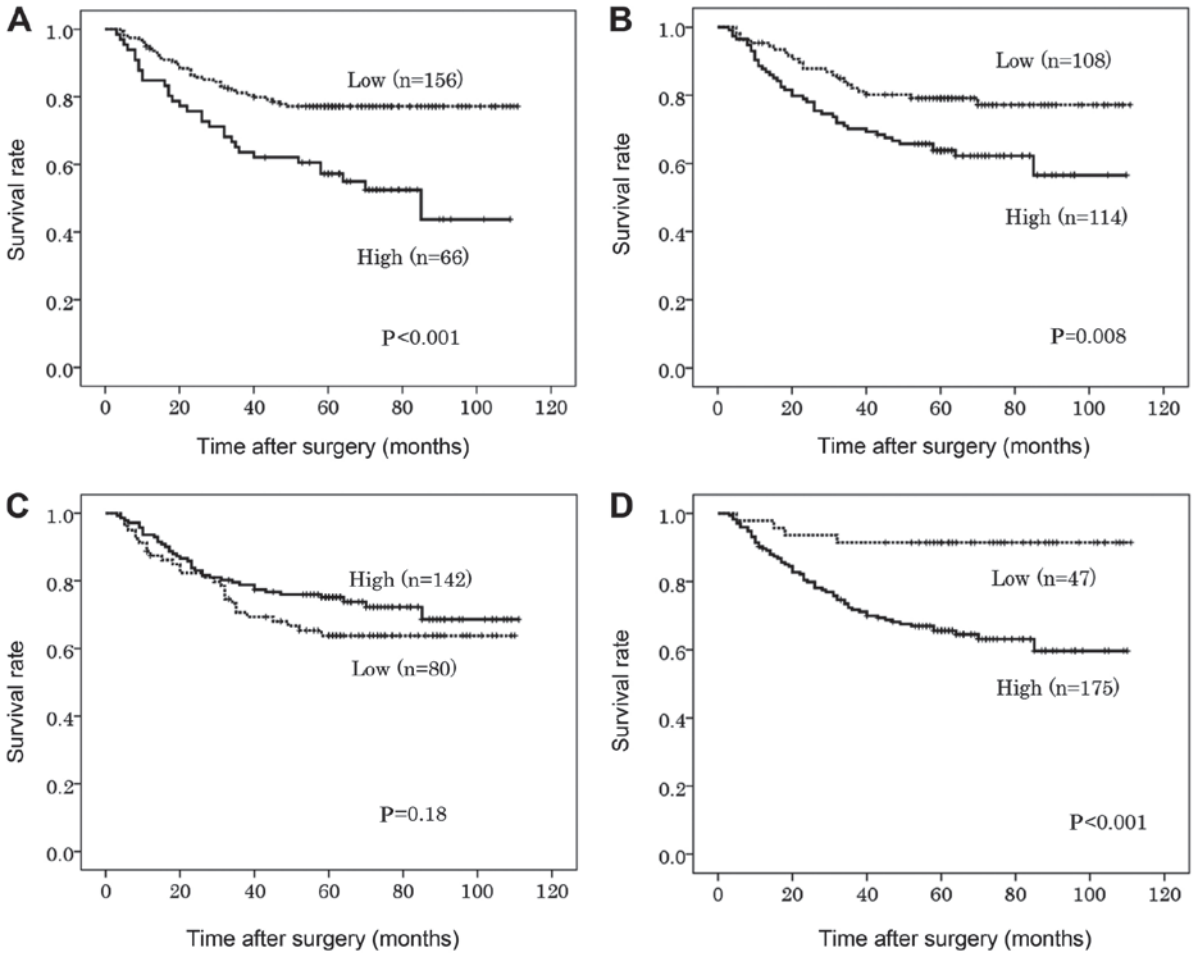

Figure 2. Survival of all patients. Kaplan-Meier curves for the disease-specific survival of patients with expression of (A) fibroblast growth factor receptor (FGFR)1, (B) FGFR2, (C) FGFR3 and (D) FGFR4 in the study group as a whole.

Table III. Correlations of FGFR1, FGFR2, FGFR3 and FGFR4 expression between primary tumors and metastatic lymph nodes.

Expression level	Metastatic lymph nodes		
	Low	High	P-value
Primary tumor			
FGFR1			
Low	17	38	0.95
High	10	23	
FGFR2			
Low	21	14	0.13
High	23	30	
FGFR4			
Low	5	3	0.017
High	16	64	

FGFR, fibroblast growth factor receptor.

kinase, and the Ras-independent phosphoinositide 3-kinase-Akt signaling pathways (15). Other pathways may also be activated by FGFRs, including STAT-dependent signaling (20). Although all four FGFRs generally signal through a similar network of pathways, a number of qualitative and quantitative differences have been identified and FGFR-specific differences in signaling pathways associated with genetic alterations of each FGFR have been confirmed in different types of cancer (21-24).

FGFR1 amplification was previously identified in breast (25), ovarian (26), bladder (27) and lung cancer (28). In gastric cancer, a previous study reported the presence of FGFR1 amplifications in 12 (50%) of the 24 cases and FGFR1 protein was overexpressed in 37 (61%) of the 61 specimens on immunohistochemical analysis using a monoclonal antibody that differed from the one used in the present study. However, that study reported no significant correlation between FGFR1 expression and clinicopathological characteristics (17). To the best of our knowledge, the present study is the first to demonstrate that the high expression of FGFR1 is associated with poor survival in gastric cancer. The overexpression of FGFR1 was also found to be correlated with liver metastasis in colorectal cancer (29), whereas the amplification and overexpression of FGFR1 may contribute to poor outcomes in luminal-type breast cancer by driving anchorage-independent proliferation and resistance to endocrine therapy (25). The co-overexpression of FGF1 and FGFR1 has also been associated with poor outcomes in esophageal squamous cell carcinoma (30); however, we did not assess FGF expression in this study.

Table IV. Prognostic factors in univariate and multivariate Cox proportional-hazards regression models for DSS in the study group as a whole.

Prognostic factors	Univariate (log-rank)		Multivariate		
	5-year DSS (%)	P-value	HR	95% CI	P-value
Age (years)					
<70	72				
≥70	69	0.37			
Gender					
Female	72				
Male	71	0.81			
Main location					
Middle or lower	74				
Upper	62	0.15			
WHO pathological type					
Differentiated	80		1		
Undifferentiated	62	0.004	1.42	0.84-2.40	0.18
Depth of invasion					
T1/2	97		1		
T3/4	54	<0.001	6.80	2.63-15.6	<0.001
LN metastasis					
Negative	95		1		
Positive	46	<0.001	4.48	1.90-10.5	0.001
FGFR1					
Low	77		1		
High	57	<0.001	1.20	0.73-2.00	0.47
FGFR2					
Low	79		1		
High	64	0.008	1.32	0.77-2.24	0.31
FGFR3					
Low	64				
High	75	0.18			
FGFR4					
Low	91		1		
High	66	<0.001	0.96	0.32-2.90	0.95

FGFR, fibroblast growth factor receptor; DSS, disease-specific survival; LN, lymph node; HR, hazard ratio; CI, confidence interval.

The FGFR4 Gly388Arg polymorphism has attracted considerable attention since the discovery of this germline polymorphism by Bange *et al* (31). In the human FGFR4 gene, a single-nucleotide polymorphism (SNP) from G to A at codon 388 at exon 9 changes the amino acid sequence of FGFR4 from glycine to arginine (Gly388 to Arg388). FGFR4 Gly388Arg was found to be associated with poor outcomes in breast (32), ovarian (33), lung (34) and gastric cancer (18). FGFR4 amplification was also found in pancreatic (35), renal cell (36) and gastric cancer (19). Ye *et al* (19) reported that the high expression of FGFR4 is associated with lymph node metastasis and a trend toward worse survival. Our findings are consistent with the findings of that study.

FGFR2 amplification was previously reported in gastric (37) and breast cancer (38), whereas FGFR2 missense mutations have been identified in gastric (39), lung (40), ovarian (41) and endometrial cancer (42), as well as in melanoma (43). FGFR2 genetic amplification or mutation leads to abnormal activation of the FGFR2 signaling pathway and contributes to carcinogenesis and tumor progression in gastric cancer. Overexpression of FGFR2 protein was detected on immunohistochemical staining in 20 of 38 diffuse-type gastric cancers, but in none of 11 intestinal-type lesions (44). FGFR2 amplifications were found in 10% of gastric cancers, the majority of which were of the undifferentiated type (11). Furthermore, FGFR2 amplification may correlate with poor outcomes in undifferentiated gastric cancer (12). In the present study, a high expression of FGFR2 was observed in undifferentiated as well as in differentiated-type gastric cancer. Although high expression of FGFR2 was not identified as an independent prognostic factor, it was significantly associated

Table V. Prognostic factors in multivariate Cox proportional-hazards regression models for disease-specific survival of patients with co-overexpression of FGFR1, FGFR2 and FGFR4.

Prognostic factors	Multivariate		
	HR	95% CI	P-value
WHO pathological type			
Differentiated	1		
Undifferentiated	1.29	0.77-2.19	0.33
Stage			
Stage I/II	1		
Stage III/IV	22.3	10.1-49.6	<0.001
Co-overexpression of FGFR1, 2 and 4			
Others	1		
All high	1.71	1.02-2.85	0.041

FGFR, fibroblast growth factor receptor; WHO, World Health Organisation; HR, hazard ratio; CI, confidence interval.

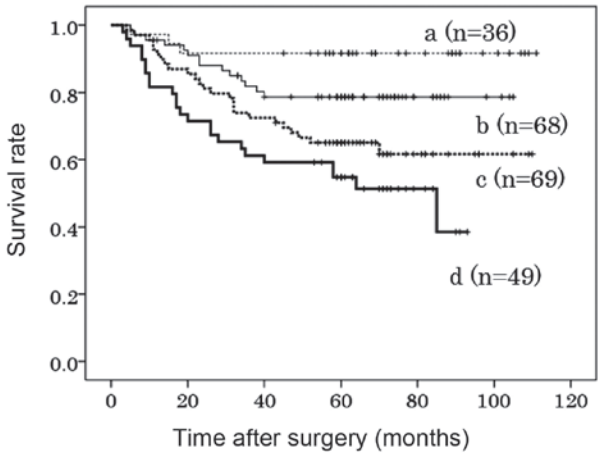

Figure 3. Survival of patients with co-overexpression of fibroblast growth factor receptor (FGFR)1, FGFR2 and FGFR4. Kaplan-Meier curves for the disease-specific survival of patients with co-overexpression of FGFR1, FGFR2 and FGFR4. a, none highly expressed; b, one highly expressed; c, two highly expressed; d, all highly expressed.

with poorer survival. Our findings are consistent with the results of previous studies.

FGFR3 amplification has been rarely reported in cancer (45,46). FGFR3 mutations have been identified in several types of cancer, including cervical cancer (47), multiple myeloma, prostate cancer (48) and spermatocytic seminomas (49). Bladder cancer exhibits the most clearly established association with FGFR3 mutations, which are strongly associated with low-grade non-invasive disease (50). Overexpression of FGFR3 was reportedly associated with low-stage bladder cancers on immunohistochemical analysis (51). However, overexpression of FGFR3 has also been associated with poor differentiation and high nuclear grade in hepatocellular carcinoma (52). The overexpression of FGFR3 in invasive breast cancer was not significantly associated with specific clinicopathological characteristics, although it was suggested to be a candidate marker for a poor prognosis (53). In this study, the expression of FGFR3 was not significantly associated with clinicopathological findings or survival. Shin *et al* (16) investigated the expression of the FGFRs in gastric cancer tissues and cell lines on northern blot analysis, ribonuclease protection assay and immunohistochemical analysis and reported that the mRNAs of FGFR1, FGFR2 and FGFR4 were upregulated in cancer tissues, whereas FGFR3 mRNA was not. These FGFR mRNAs were coexpressed in various combinations of two or three in the same tissue. The immunohistochemical analysis confirmed specific staining of multiple FGFRs, excluding FGFR3, in cancer specimens. In the present study, a high expression of FGFR3 was detected in addition to that of the other three FGFRs. The discrepancies among studies may be attributed to the differences in disease stage or the techniques used for immunohistochemical analysis.

We also evaluated the expression of FGFR1, FGFR2 and FGFR4 in metastatic lymph nodes. To the best of our knowledge, FGFRs in metastatic sites of gastric cancer had not been previously investigated. The expression of FGFR1 and FGFR2 differed between the primary tumors and lymph node metastases and were significantly correlated with the expression of only FGFR4. The differences in FGFR1 or FGFR2 expression among different tumor sites may represent a challenge regarding chemotherapy against these molecular targets.

FGFR-targeted therapeutics using small-molecule compounds that inhibit binding of FGF to FGFR is an active topic in the field of clinical oncology (54). Ki23057, a FGFR2 inhibitor, was reported to enhance the chemosensitivity of drug-resistant gastric cancer cells (55). Inhibition of FGFR2 signaling by AZD4547, a selective inhibitor of FGFR1, FGFR2 and FGFR3, was shown to significantly inhibit tumor growth in a dose-dependent manner in FGFR2-amplified xenografts (56). AZD4547 is currently being compared to paclitaxel as second-line treatment for patients with gastric cancer whose tumors exhibit FGFR2 gene amplification (NCT01457846, SHINE). Monoclonal antibodies that selectively recognize FGF or FGFR represent additional options for FGFR-targeted cancer therapy. Anti-FGFR2 monoclonal antibodies inhibit the *in vivo* growth of SNU-16 and OCUM-2M gastric cancer cells with FGFR2 gene amplification (13). Our results suggested that a selective inhibitor of FGFR1, FGFR2 and FGFR4 or the combined use of anti-FGFR1, -FGFR2 and -FGFR4 monoclonal antibodies may represent an effective FGFR-targeted therapy for gastric cancer.

In conclusion, high expression of FGFR1, FGFR2 or FGFR4 may be associated with tumor progression and poor survival in patients with gastric cancer. Similar to FGFR2, FGFR1 and FGFR4 may represent future prognostic factors and treatment targets in gastric cancer.

Acknowledgements

K.M., M.I. and K.S. were responsible for drafting the manuscript. K.M., K.K. and Y.T. contributed to the immunohistochemical analysis. M.I. and K.K. contributed to the analysis and interpretation of data.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
2. Brenner H, Rothenbacher D and Arndt V: Epidemiology of stomach cancer. Methods Mol Biol 472: 467-477, 2009.
3. Hartgrink HH, Jansen EP, van Grieken NC and van de Velde CJ: Gastric cancer. Lancet 374: 477-490, 2009.
4. Cunningham D, Starling N, Rao S, *et al*: Capecitabine and oxaliplatin for advanced esophagogastric cancer. N Engl J Med 358: 36-46, 2008.
5. Bang YJ, Van Cutsem E, Feyereislova A, *et al*: Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 376: 687-697, 2010.
6. Gravalos C and Jimeno A: HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. Ann Oncol 19: 1523-1529, 2008.
7. Hofmann M, Stoss O, Shi D, *et al*: Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathology 52: 797-805, 2008.
8. Kimelman D and Kirschner M: Synergistic induction of mesoderm by FGF and TGF-beta and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. Cell 51: 869-877, 1987.
9. De Moerlooze L, Spencer-Dene B, Revest JM, Hajihosseini M, Rosewell I and Dickson C: An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal-epithelial signalling during mouse organogenesis. Development 127: 483-492, 2000.
10. Turner N and Grose R: Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer 10: 116-129, 2010.
11. Kunii K, Davis L, Gorenstein J, *et al*: FGFR2-amplified gastric cancer cell lines require FGFR2 and Erbb3 signaling for growth and survival. Cancer Res 68: 2340-2348, 2008.
12. Toyokawa T, Yashiro M and Hirakawa K: Co-expression of keratinocyte growth factor and K-sam is an independent prognostic factor in gastric carcinoma. Oncol Rep 21: 875-880, 2009.
13. Zhao WM, Wang L, Park H, *et al*: Monoclonal antibodies to fibroblast growth factor receptor 2 effectively inhibit growth of gastric tumor xenografts. Clin Cancer Res 16: 5750-5758, 2010.
14. Zhao G, Li WY, Chen D, *et al*: A novel, selective inhibitor of fibroblast growth factor receptors that shows a potent broad spectrum of antitumor activity in several tumor xenograft models. Mol Cancer Ther 10: 2200-2210, 2011.
15. Brooks AN, Kilgour E and Smith PD: Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. Clin Cancer Res 18: 1855-1862, 2012.
16. Shin EY, Lee BH, Yang JH, *et al*: Up-regulation and co-expression of fibroblast growth factor receptors in human gastric cancer. J Cancer Res Clin Oncol 126: 519-528, 2000.
17. Oki M, Yamamoto H, Taniguchi H, Adachi Y, Imai K and Shinomura Y: Overexpression of the receptor tyrosine kinase EphA4 in human gastric cancers. World J Gastroenterol 14: 5650-5656, 2008.
18. Ye Y, Shi Y, Zhou Y, *et al*: The fibroblast growth factor receptor-4 Arg388 allele is associated with gastric cancer progression. Ann Surg Oncol 17: 3354-3361, 2010.
19. Ye YW, Zhou Y, Yuan L, *et al*: Fibroblast growth factor receptor 4 regulates proliferation and antiapoptosis during gastric cancer progression. Cancer 117: 5304-5313, 2011.
20. Eswarakumar VP, Lax I and Schlessinger J: Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 16: 139-149, 2005.
21. Vainikka S, Joukov V, Wennström S, Bergman M, Pelicci PG and Alitalo K: Signal transduction by fibroblast growth factor receptor-4 (FGFR-4). Comparison with FGFR-1. J Biol Chem 269: 18320-18326, 1994.
22. Wang JK, Gao G and Goldfarb M: Fibroblast growth factor receptors have different signaling and mitogenic potentials. Mol Cell Biol 14: 181-188, 1994.
23. Shaoul E, Reich-Slotky R, Berman B and Ron D: Fibroblast growth factor receptors display both common and distinct signaling pathways. Oncogene 10: 1553-1561, 1995.
24. Xian W, Schwertfeger KL and Rosen JM: Distinct roles of fibroblast growth factor receptor 1 and 2 in regulating cell survival and epithelial mesenchymal transition. Mol Endocrinol 21: 987-1000, 2007.
25. Turner N, Pearson A, Sharpe R, *et al*: FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. Cancer Res 70: 2085-2094, 2010.
26. Theillet C, Adelaide J, Louason G, *et al*: FGFRI and PLAT genes and DNA amplification at 8p12 in breast and ovarian cancers. Genes Chromosomes Cancer 7: 219-226, 1993.
27. Simon R, Richter J, Wagner U, *et al*: High-throughput tissue microarray analysis of 3p25 (RAF1) and 8p12 (FGFR1) copy number alterations in urinary bladder cancer. Cancer Res 61: 4514-4519, 2001.
28. Weiss J, Sos ML, Seidel D, *et al*: Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2: 62ra93, 2010.
29. Sato T, Oshima T, Yoshihara K, *et al*: Overexpression of the fibroblast growth factor receptor-1 gene correlates with liver metastasis in colorectal cancer. Oncol Rep 21: 211-216, 2009.
30. Sugiura K, Ozawa S, Kitagawa Y, Ueda M and Kitajima M: Co-expression of aFGF and FGFR-1 is predictive of a poor prognosis in patients with esophageal squamous cell carcinoma. Oncol Rep 17: 557-564, 2007.
31. Bange J, Prechtl D, Cheburkin Y, *et al*: Cancer progression and tumor cell motility are associated with the FGFR4 Arg(388) allele. Cancer Res 62: 840-847, 2002.
32. Thussbas C, Nahrig J, Streit S, *et al*: FGFR4 Arg388 allele is associated with resistance to adjuvant therapy in primary breast cancer. J Clin Oncol 24: 3747-3755, 2006.
33. Marmé F, Hielscher T, Hug S, *et al*: Fibroblast growth factor receptor 4 gene (FGFR4) 388Arg allele predicts prolonged survival and platinum sensitivity in advanced ovarian cancer. Int J Cancer 131: E586-E591, 2012.
34. Frullanti E, Berking C, Harbeck N, *et al*: Meta and pooled analyses of FGFR4 Gly388Arg polymorphism as a cancer prognostic factor. Eur J Cancer Prev 20: 340-347, 2011.
35. Leung HY, Gullick WJ and Lemoine NR: Expression and functional activity of fibroblast growth factors and their receptors in human pancreatic cancer. Int J Cancer 59: 667-675, 1994.
36. Takahashi A, Sasaki H, Kim SJ, *et al*: Identification of receptor genes in renal cell carcinoma associated with angiogenesis by differential hybridization technique. Biochem Biophys Res Commun 257: 855-859, 1999.
37. Hattori Y, Odagiri H, Nakatani H, *et al*: K-sam, an amplified gene in stomach cancer, is a member of the heparin-binding growth factor receptor genes. Proc Natl Acad Sci USA 87: 5983-5987, 1990.
38. Heiskanen M, Kononen J, Bärlund M, *et al*: CGH, cDNA and tissue microarray analyses implicate FGFR2 amplification in a small subset of breast tumors. Anal Cell Pathol 22: 229-234, 2001.
39. Jang JH, Shin KH and Park JG: Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers. Cancer Res 61: 3541-3543, 2001.
40. Davies H, Hunter C, Smith R, *et al*: Somatic mutations of the protein kinase gene family in human lung cancer. Cancer Res 65: 7591-7595, 2005.
41. Byron SA, Gartside MG, Wellens CL, *et al*: FGFR2 mutations are rare across histologic subtypes of ovarian cancer. Gynecol Oncol 117: 125-129, 2010.
42. Pollock PM, Gartside MG, Dejeza LC, *et al*: Frequent activating FGFR2 mutations in endometrial carcinomas parallel germline mutations associated with craniosynostosis and skeletal dysplasia syndromes. Oncogene 26: 7158-7162, 2007.
43. Gartside MG, Chen H, Ibrahimi OA, *et al*: Loss-of-function fibroblast growth factor receptor-2 mutations in melanoma. Mol Cancer Res 7: 41-54, 2009.
44. Hattori Y, Itoh H, Uchino S, *et al*: Immunohistochemical detection of K-sam protein in stomach cancer. Clin Cancer Res 2: 1373-1381, 1996.
45. Nord H, Segersten U, Sandgren J, *et al*: Focal amplifications are associated with high grade and recurrences in stage Ta bladder carcinoma. Int J Cancer 126: 1390-1402, 2010.
46. Khnykin D, Troen G, Berner JM and Delabie A: The expression of fibroblast growth factors and their receptors in Hodgkin's lymphoma. J Pathol 208: 431-438, 2006.
47. Rosty C, Aubriot MH, Cappellen D, *et al*: Clinical and biological characteristics of cervical neoplasias with FGFR3 mutation. Mol Cancer 4: 15, 2005.
48. Hernández S, de Muga S, Agell L, *et al*: FGFR3 mutations in prostate cancer: association with low-grade tumors. Modern Pathol 22: 848-856, 2009.

49. Goriely A, Hansen RMS, Taylor IB, *et al*: Activating mutations in FGFR3 and HRAS reveal a shared genetic origin for congenital disorders and testicular tumors. Nat Genet 41: 1247-1252, 2009.
50. van Oers JM, Wild PJ, Burger M, *et al*: FGFR3 mutations and a normal CK20 staining pattern define low-grade noninvasive urothelial bladder tumours. Eur Urol 52: 760-768, 2007.
51. Mhawrech-Fauceglia P, Cheney RT, Fischer G, Beek A and Herrmann FR: FGFR3 and p53 protein expressions in patients with pTa and pT1 urothelial bladder cancer. Eur J Surg Oncol 32: 231-237, 2006.
52. Qiu WH, Zhou BS, Chu PGG, *et al*: Over-expression of fibroblast growth factor receptor 3 in human hepatocellular carcinoma. World J Gastroenterol 11: 5266-5272, 2005.
53. Kuroso K, Imai Y, Kobayashi M, *et al*: Immunohistochemical detection of fibroblast growth factor receptor 3 in human breast cancer: correlation with clinicopathological/molecular parameters and prognosis. Pathobiology 77: 231-240, 2010.
54. Katoh M and Nakagama H: FGF receptors: cancer biology and therapeutics. Med Res Rev 34: 280-300, 2013.
55. Qiu H, Yashiro M, Zhang X, Miwa A and Hirakawa K: A FGFR2 inhibitor, Ki23057, enhances the chemosensitivity of drug-resistant gastric cancer cells. Cancer Lett 307: 47-52, 2011.
56. Xie L, Su X, Zhang L, *et al*: FGFR2 gene amplification in gastric cancer predicts sensitivity to the selective FGFR inhibitor AZD4547. Clin Cancer Res 19: 2572-2583, 2013.