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Evaluation of fibroblast growth factor receptor 2 expression, heterogeneity and clinical significance in gastric cancer

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Evaluation of fibroblast growth factor receptor 2 expression, heterogeneity and clinical significance in gastric cancer

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ABSTRACT

Introduction: *Fibroblast growth factor receptor 2* (*FGFR2*) gene amplification promotes overexpression of FGFR2 protein, a target for therapeutics now in clinical development for gastric cancer (GC) patients. This study aimed to evaluate the protein and mRNA expressions of fibroblast growth factor receptor 2 (FGFR2) by immunohistochemistry (IHC) and mRNA *in situ* hybridization (ISH), respectively, and to assess the heterogeneity of FGFR2 expression in gastric cancer.

Methods: The study cohorts included patients who had gastric cancer and have undergone surgical resection from January 1, 2004, to December 31, 2005. Cases from 2 cohorts of patients with gastric cancer from the year 2005 (n = 362, training set) and 2004 (n = 413, validation set) were selected for the construction of tissue microarrays. One hundred thirty-five matched metastatic lymph nodes (LNs) were also selected from training set. They were used to assess the IHC and mRNA ISH status. DNA fluorescence *in situ* hybridization (FISH) for *FGFR2* was performed in training set. For 188 patients, *FGFR2* status by

DNA FISH was available.

Results: All *FGFR2*-amplified cases (n = 5) showed FGFR2 protein and mRNA overexpressions ($P < 0.001$) from training set. Kaplan-Meier survival analysis revealed that FGFR2 protein and mRNA overexpressions were significantly associated with poor overall survival from training set ($P < 0.001$ and $P = 0.012$, respectively), whereas no significant association with survival was observed in validation set. FGFR2 protein overexpression was found to be a negative prognostic indicator on multivariate analysis when the 2 cohorts were combined ($P = 0.043$). Intratumoral heterogeneity was defined as different results between tissue microarray cores. In FGFR2-positive primary gastric cancers, heterogeneous FGFR2 protein and mRNA overexpressions were observed in 5 of 9 (55.5%) and 18 of 21 (85.7%) cases, respectively. Discordant FGFR2 protein and mRNA expressions were observed in 5 of 9 (55.5%) and 4 of 14 (28.6%) pairings of primary tumor and regional lymph node metastases, respectively.

Conclusions: IHC using FGFR2 antibody would be suitable for evaluating FGFR2 overexpression. Intratumoral heterogeneity

and discrepant FGFR2 results in primary and regional metastatic lymph node are common in gastric cancer.

Keywords: Receptor, Fibroblast Growth Factor, Type 2; Stomach Neoplasms; Immunohistochemistry; In Situ Hybridization, mRNA; In Situ Hybridization, Fluorescence, DNA; Heterogeneous expression

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LIST OF ABBREVIATIONS

AGC: advanced gastric cancer

ASCO/CAP: American Joint Committee on Cancer/College of American Pathologists

CI: confidence interval

EGC: early gastric cancer

FFPE: formalin-fixed, paraffin-embedded

FGFR2: fibroblast growth factor receptor 2

FISH: fluorescence *in situ* hybridization

GC: gastric cancer

HER2: human epidermal growth factor receptor 2

IHC: immunohistochemistry

MD: moderately differentiated

mRNA ISH: messenger ribonucleic acid (mRNA) *in situ* hybridization

NA: not available

NPV: negative predictive value

PD: poorly differentiated

PPV: positive predictive value

qRT-PCR: quantitative real-time reverse transcription polymerase chain reaction

RSEM: RNA-Seq by Expectation-Maximization

SD: standard deviation

SRC: signet ring cell

TCGA: The Cancer Genome Atlas

UICC/AJCC: Union for International Cancer Control/American Joint Committee on Cancer

WD: well differentiated

INTRODUCTION

Gastric cancer is the fourth most commonly diagnosed cancer and the third most frequent cause of cancer deaths for male, worldwide (1). Its early detection and complete excision result in a 5-year survival rate of over 90% for patients with early stage disease in both the Western countries and Japan (2). However, the outcome of advanced gastric cancer is still bleak (3). Targeted agents including trastuzumab have been investigated for patients with advanced stage, and have been successful in treating metastatic gastric cancer (4). *Fibroblast growth factor receptor 2* (*FGFR2*) is one of four *FGFR* family members that encode transmembrane receptor tyrosine kinases (5). Alternative splicing of the *FGFR2* transcript gives rise to the IIIb isoform (FGFR2b) that is preferentially expressed in epithelial cells and determines ligand specificity (6, 7). A previous study showed that *FGFR2* amplified gastric cancer cell line OCUM2 and *FGFR2*-amplified patient gastric cancer tissue expressed FGFR2b isoform dominantly (8).

FGFR2 gene amplification in gastric cancer was first identified in the human gastric cancer cell line KATO-III (9), and can activate the *FGFR2* signaling pathway and thus promote the proliferation and survival of gastric cancer cells (10). FGFR2 amplification has been reported to occur in 2-9% of gastric cancers, and is associated with shorter survival (11-17). Recent studies on FGFR inhibitors in gastric cancer cell lines have given promising results (10, 18-21), suggesting that FGFR2 is a potential molecular target for the treatment of a subset of gastric cancers.

In previous studies, DNA fluorescence *in situ* hybridization (FISH) and quantitative real-time PCR (qRT-PCR) methods were used to evaluate FGFR2 status (11, 12). DNA FISH is regarded as the gold standard for the detection of oncogene amplification (13, 22), but it is expensive and time-consuming to perform. qRT-PCR does not allow *in situ* evaluation, and the presence of adjacent stromal cells and/or inflammatory cells make it difficult to obtain pure tumor samples. Immunohistochemistry (IHC) and mRNA *in situ* hybridization (ISH) are *in situ* methods using light microscopy

to assess protein expression and mRNA expression, respectively. Both are faster and more economical than DNA FISH and they make it easier to evaluate large areas of the tumor, which makes them appealing techniques for screening of *FGFR2* gene amplification or of FGFR2 protein overexpression. However, mRNA ISH and IHC are currently not standard for assessing FGFR2 status.

Intratumoral heterogeneity is frequently observed in gastric cancer, and clonal and phenotypic divergences of histologically mixed carcinomas have been reported (23). *Human epidermal growth factor receptor 2* (*HER2*) was shown to be expressed heterogeneously and at a higher level in gastric cancer than in breast cancer (24). Heterogeneity of gene amplification status within a tumor or between primary and paired metastatic lesions is considered to be an important potential cause of targeted therapy failure (24–26). Heterogeneous expression of oncogene has been well studied for *HER2.* The most commonly employed method involves randomly sampling multiple cores from tumors to constitute tissue microarray, and applying IHC staining (26).

Heterogeneous *FGFR2* amplification has recently been described in gastric cancer, but it has not yet been comprehensively studied (13, 16, 17). Su *et al.* (13) reported intratumoral heterogeneity of *FGFR2* gene amplification in 7 of 29 (24%) gastric cancer cases, and Ooi *et al.* (17) reported that 5 of 6 *FGFR2* gene-amplified gastric cancer cases showed focal (30-70%) amplification, and that only 1 case showed homogeneous staining. These studies did not focus on heterogeneous expression of FGFR2, and there has been no study targeting discordance between FGFR2 status of primary tumor and metastatic lymph nodes (LNs). Additional studies are needed to evaluate the prevalence of heterogeneous FGFR2 expression in gastric cancer.

The purpose of the present study is to validate FGFR2 IHC and mRNA ISH in FGFR2 assays and to assess the intratumoral heterogeneity and the discordance of FGFR2 between primary and metastatic gastric cancers. Other aims of this study are evaluation of the frequency and clinical implication of FGFR2 overexpression in gastric cancer by means of DNA, mRNA and protein.

MATERIALS AND METHODS

1. Patients and Specimens

362 cases of primary gastric carcinoma patients who underwent surgical resection in 2005 (training set) and another 413 cases in 2004 (validation set) at the Department of Pathology, Seoul National University Hospital were examined. Age, gender, WHO and Lauren's classification, pathological TNM (pTNM) stage (according to the 7th UICC/AJCC manual) (27), lymph node metastasis, and distant metastasis were evaluated by reviewing medical charts, pathological records, and glass slides. The median follow-up period was 58.7 months for training set (range, 0-80 months), and 49 months (range, 0-60 months) for validation set. The follow-up loss cases were regarded as censored data for the analysis of survival rates. No patient received preoperative chemotherapy or radiotherapy before surgery. Patients with stage II, III, or IV disease received postoperative chemotherapy using a 5-fluorouracil-based regimen (alone, plus mitomycin C, or plus cisplatin). The retrospective study protocol was reviewed and approved by the

Institutional Review Board of Seoul National University Hospital under the condition of anonymization (IRB No. H-1309-087-522).

2. Cell lines and cell culture

Eighteen human gastric cancer cell lines (SNU-1, -5, -16, -216, -484, -520, -601, -620, -638, -668 ,-719, AGS, KATO-III, NCI-H87, MKN-1, -28, -45, -74) were used in this study. All cell lines were obtained from the Korean Cell Line Bank (http://cellbank.snu.ac.kr, Seoul, Korea). Cells were maintained in RPMI-1640 (JBI, Seoul, Korea). All culture media contained 10% fetal bovine serum (FBS, HyClone, Logan, UT), 100 U/mL of penicillin, and 100 μg/mL of streptomycin (Sigma Chemicals, St Louis, MO). When the number of cells reached about 5 million, the cells were washed with phosphate-buffered saline (PBS), and then harvested. After that, 3 ml of trypsin-EDTA (1×) was added, and after a 3-minute break, and added 10 ml of RPMI-1640 with 10% FBS. And it was transferred to a centrifuge tube and centrifuged at 1000 rpm for 5 min, after which it was fixated by 10% formalin. In order to

harvest the cells in suspension, it was taken and centrifuged at 1000 rpm for 5 min. Then, it was washed by PBS and fixated. After the samples were centrifuged at 1000 rpm for 5 min, they were mixed with 60°C 6% agarose and the final concentration of agarose was 3%. Once they were solidified, they were bathed in 10% formalin, and then paraffin-embedded.

3. Tissue Array Method

Array blocks obtained from patients with gastric cancer were prepared as previously described (Superbiochips Laboratories, Seoul, Korea) (28). Briefly, representative tissues cores (2 mm in diameter) were taken from individual paraffin-embedded gastric cancer samples and arranged in new tissue array blocks using a trephine apparatus. Non-neoplastic gastric mucosa specimens were included in each of the array blocks, which contained up to 60 cores. To evaluate intratumoral heterogeneity, tissue arrays were constructed by sampling 3 cores from each primary tumor in multiple paraffin blocks (training set only). Two cores were obtained from submucosal or deeper invasive sites and one core from the mucosal site. In

cases with lymph node metastasis, tissue arrays were constructed separately. One core for each case was obtained from the paraffin block containing the largest metastatic tumor. Adequate case was defined as a tumor occupying more than 10% of the core area.

4. DNA fluorescence *in situ* Hybridization

Dual-color Zytolight kits (Zytovision, Bremerhaven, Germany) were used for DNA FISH. Briefly, sections were deparaffinized and dehydrated, and then incubated in 20% sodium bisulphate/×2 standard saline citrate (×2 SSC) at 43°C for 20 min. This was followed by treatment with proteinase K at 37°C for 25 min. Denaturation, hybridization, and post-hybridization washing were carried out according to the manufacturer's instructions. Slides were counterstained with 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI) and examined under a fluorescence microscope (Olympus, Tokyo, Japan). After counting at least 50 tumor cell nuclei per core, *FGFR2* amplification was defined as a ratio of *FGFR2* signal (green) to chromosome 10 centromere signal (red) of ≥2.0.

5. mRNA *in situ* Hybridization

ISH detection of *FGFR2* mRNA was performed manually with the RNAscope 2.0 FFPE Assay Kit (Advanced Cell Diagnostics, Hayward, CA, USA) according to the manufacturer's instructions (https://acdbio.com/technical-support/user-manuals), as previously described (29). Briefly, unstained slides were deparaffinized and endogenous peroxidases were blocked first. Endogenous proteases were also digested. After hybridizing target probe and washing the slides, signal amplification and washing were repeated six times. 3,3'-diaminobenzidine (DAB) mixture was used for colorimetric reaction. Positive staining was indicated by brown punctate dots present in the tumor cells. *FGFR2* mRNA expression was scored according to the RNAscope 2.0 FFPE Assay Kit instructions (30, 31): no staining or less than 1 dot/cell under a 40× objective lens (score 0), 1-3 dots/cell under a 20-40× objective lens (score 1), 4-10 dots/cell and no or very few clusters of dots under a 20-40× objective lens (score 2), >10 dots/cell and <10% of positive cells have dot clusters under a

20× objective lens (score 3), and >10 dots/cell and >10% of positive cells have dot clusters under a 20× objective (score 4). Tissue microarrays with a score of 3 or 4 were considered to be showing *FGFR2* mRNA overexpression. Each tissue array block was stained for *ubiquitin C* (*UBC*) mRNA, which serves as the positive control, and only those with a score of 4 were used in the analysis.

6. Immunohistochemistry

Immunohistochemical staining was performed using an automated immunostainer (Bond-Max, Leica Microsystems, Wetzlar, Germany) according to the manufacturer's instructions. Anti-FGFR2b monoclonal antibody (FPR2-D; Five Prime Therapeutics, Inc., South San Francisco, CA, USA; 1:20), which is specific for the FGFR2-IIIb splice variant of human FGFR2, was used as a primary antibody (8, 32). Another commercial anti-FGFR2 monoclonal antibody (ab58201; Abcam, Cambridge, UK; 1:800) was tested for a comparison of FGFR2 immunoreactivity. Cancer cells showing membranous staining, regardless of the presence of cytoplasmic staining, were

considered to be positive. Immunostaining of FGFR2b was evaluated according to 2013 ASCO/CAP recommendations for HER2 testing in breast cancer (33): score 3, circumferential membrane staining that is complete, intense, and within more than 10% of the malignant cells; score 2, circumferential membrane staining that is incomplete and/or weak/moderate and within > 10% of tumor cells, or complete and circumferential membrane staining that is intense and within ≤ 10% of tumor cells; score 1, incomplete membrane staining that is faint/barely perceptible and within > 10% of tumor cells; score 0, no staining is observed, or membrane staining that is incomplete and is faint/barely perceptible and within ≤ 10% of tumor cells. Scores of 2+ or 3+ were considered positive and scores of 0 or 1+ were considered negative (2 grade system).

7. Data set of the Cancer Genome Atlas

Publicly available data for stomach adenocarcinoma patients were obtained from The Cancer Genome Atlas Project (TCGA) (34). Clinicopathological parameters were acquired from the 'Master Patient Table'. Level 4 RNA Expression data made by

IlluminaGA RNASeq and IlluminaHiseq RNASeq (Illumina Sequencing Technology, San Diego, CA, USA) were used for mRNA expression. mRNA expression and methylation statuses were taken from 'Associated Data Files' (https://tcga-data.nci.nih.gov/docs/publications/stad_2014/). These data were frozen on February 2, 2014. Such data has been confirmed though comparison with data downloaded from cBioPortal (http://www.cbioportal.org) (35, 36).

8. Statistical Analysis

Survival curves were plotted using the Kaplan-Meier product-limit method, and the significance of differences between survival curves was determined using the log-rank test. Multivariate survival analysis was performed using the Cox proportional hazards model. The χ^2 test or Fisher's exact test (2-sided) was used to determine the associations between parameters. Student's t-test was used in the analysis of age factor. Results were considered significant when P values were < 0.05 (two-sided). Statistical analyses were conducted using the SPSS 21.0 statistical software package (IBM Corp., Armonk,

NY, USA), MedCalc free statistical calculators (https://www.medcalc.org/calc/, MedCalc Software, Ostend, Belgium) for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and R (version 3.1.1, The R Foundation for Statistical Computing; http://www.R-project.org) with RStudio (version 0.97.248, RStudio Inc., http://rstudio.org/) for Freeman-Halton test (extended Fisher's exact test).

RESULTS

FGFR2 expression status in gastric cancer cell lines

FGFR2 IHC and mRNA ISH were performed on cell block obtained from 18 human gastric cancer cell lines (SNU-1, -5, -16, -216, -484, -520, -601, -620, -638, -668 ,-719, AGS, KATO-III, NCI-H87, MKN-1, -28, -45, -74). KATO-III and SNU-16 cell lines showed FGFR2 protein and mRNA overexpressions, whereas 16 other cell lines did not show the expression in IHC and in mRNA ISH (Figure 1). *FGFR2* gene amplification in gastric cancer was first identified in the human gastric cancer cell line KATO-III (9). Kim *et al.* (20) reported *FGFR2* gene expression in 38 gastric cancer cell lines by qRT-PCR, and found KATO-III and SNU-16 have *FGFR2* amplification. Jung *et al.* (11) also studied *FGFR2* gene amplification in ten human gastric cancer cell lines by DNA FISH (SNU-1, SNU-5, SNU-16, SNU-216, SNU-284, SNU-601, SNU-620, SNU-638, SNU-668, and SNU-719), and found *FGFR2* gene amplification in SNU-16. The reports from

some previous studies regarding FGFR2 amplification in gastric cancer cell lines are in Table 1.

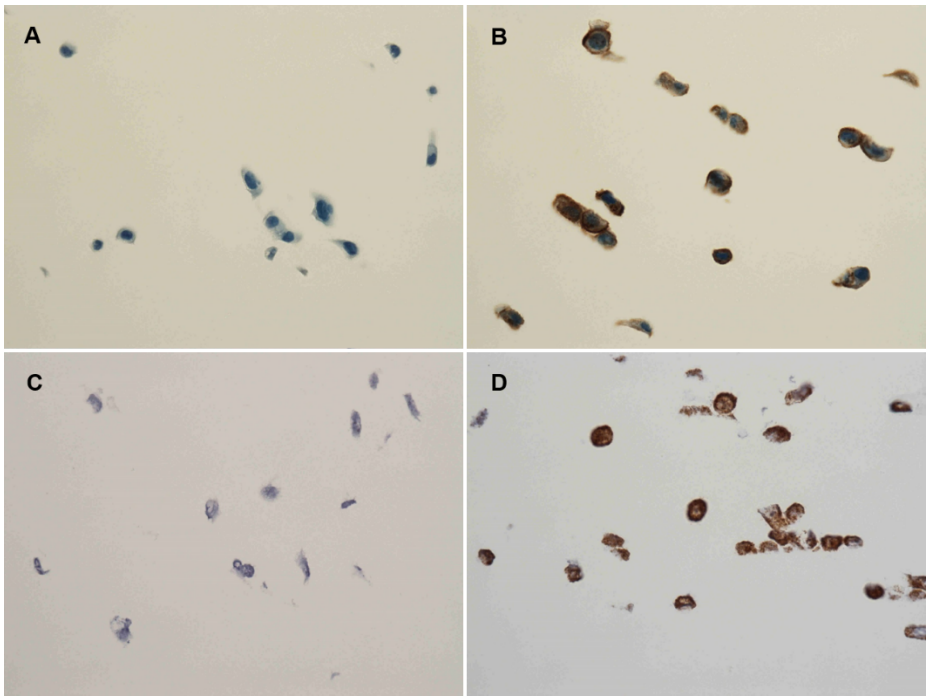


Figure 1. Representative images of immunohistochemical and mRNA *in situ* hybridization showing FGFR2 expression in human gastric cancer cell block using cell lines (x400). FGFR2 protein expression was not observed in SNU-601 (A). FGFR2 protein overexpression was observed in KATO-III (B). *FGFR2* mRNA expression was not identified in SNU-601 (C). *FGFR2* mRNA overexpression was observed in KATO-III (D).

Table 1. Summary of the previous studies reported the results of FGFR2 amplification in gastric cancer cell lines.

Year	Methods	Cell lines	References
1990	cDNA cloning	KATO-III	Hattori, *et al.* (37)
2012	qRT-PCR	KATO-III, SNU-16, HSC-39, HSC-43	Matsumoto, *et al.* (12)
2012	DNA FISH	SNU-16	Jung, *et al.* (11)
2014	qRT-PCR, DNA FISH	KATO-III, SNU-16, OCUM-2M, HSC-39	Kim, *et al.* (20)
Present study	IHC, mRNA ISH on cell block	KATO-III, SNU-16	

Validation of FGFR2 IHC and *FGFR2* mRNA ISH by comparison with dual-color DNA FISH

DNA FISH for FGFR2 was performed for 188 primary gastric cancer cases (Figure 2). IHC and mRNA ISH were performed on the same array block (A2; submucosa or deeper tissue). When slides were analyzed for FGFR2 IHC staining, FGFR2 positivity was represented by brown membranous and less intense cytoplasmic staining. Positive staining for FGFR2 mRNA ISH was indicated by brown punctate dots present in the nucleus and cytoplasm of tumor cells. *FGFR2* gene amplification was found in 5 (2.7%) of primary gastric cancers. All *FGFR2* gene-amplified cases showed FGFR2 protein overexpression and a *FGFR2* mRNA ISH score of 4 ($P < 0.001$). *FGFR2* gene amplification was not identified in FGFR2 IHC or *FGFR2* mRNA ISH negative cancers (Table 2). Taking FISH for *FGFR2* DNA as the gold standard, sensitivity of FGFR2 IHC was 100%, specificity was 100%, PPV was 100%, and NPV was also 100%. Sensitivity of *FGFR2* mRNA ISH was 100%, specificity was 98.4%, PPV was 62.5%, and NPV was 100%.

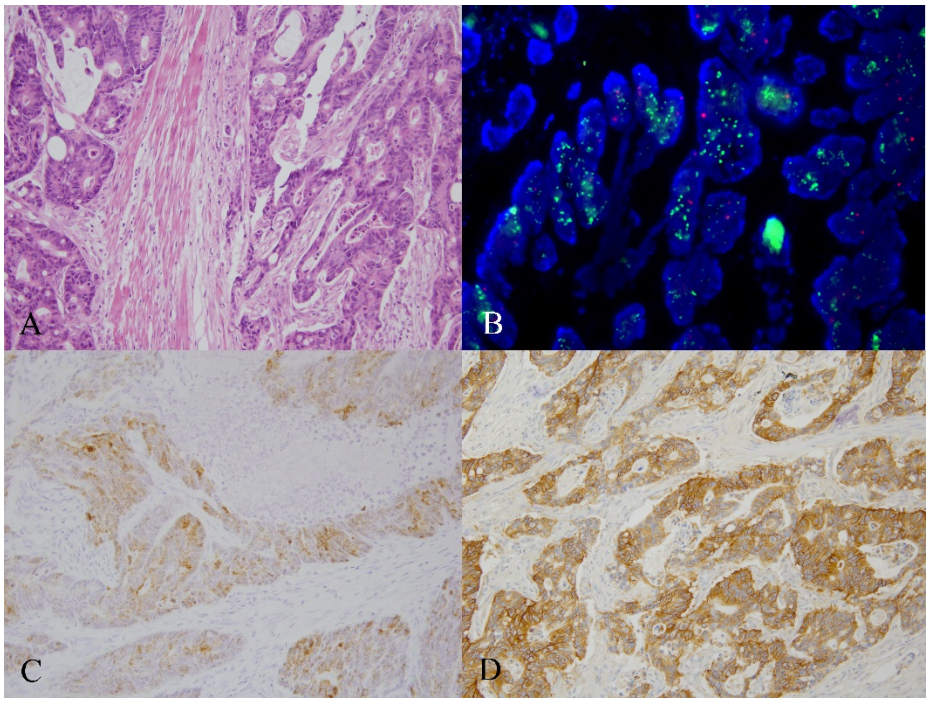


Figure 2. Photomicrographs of FGFR2 protein and mRNA expressions in primary gastric cancer. (A) Histologic features of *FGFR2* amplified gastric cancer. Hematoxylin and eosin stain. ×200. (B) *FGFR2* gene amplification evaluated by fluorescence *in situ* hybridization using dual-color (green, FGFR2 signal; red, chromosome 10 centromere signal) break-apart probes. ×1000. (C) *FGFR2* mRNA expression evaluated by mRNA *in situ* hybridization. ×200. (D) FGFR2 protein expression evaluated by immunohistochemistry. ×200.

Table 2. Comparison of FGFR2 IHC (using FPR2-D), mRNA ISH and dual-color DNA FISH findings (a part of training set).

	DNA FISH		*P* value
	Non-amplified	Amplified	
IHC (n = 188)			< 0.001*
Negative	183	0	
Positive	0	5	
mRNA ISH (n = 188)			< 0.001*
Negative	180	0	
Positive (Score 3 or 4)	3	5	

Fisher's exact test was used in each analysis.

* Statistically significant

Comparison of IHC results between FPR2-D and commercial FGFR2 antibody

Commercial monoclonal anti-FGFR2 antibody (ab58201), commonly used in precedent studies (38, 39), was used for IHC. Table 3 shows the comparison data with *FGFR2* DNA FISH and IHC using ab58201. FGFR2 IHC positivity using ab58201 had no correlation with *FGFR2* gene amplification, as evaluated by DNA FISH (P = 0.616). Taking *FGFR2* DNA FISH as the gold standard, sensitivity of FGFR2 IHC using ab58201 was found to be 25.0%, and specificity was found to be 78.95 %. PPV was 2.4%, and NPV was 98.0%. From 356 cases of training set, FGFR2 protein overexpression was identified in 2.2% (8 cases) by FPR2-D and the FGFR2 positive rate by ab58201 was 23.8% (85 cases) (Table 4). This difference may have been caused by the low sensitivity of the antibody previous used in studies for FGFR2 protein expression. Predictive functions between FGFR2b-specific IHC antibody, mRNA ISH, and commercial antibody are summarized in Figure 3.

Table 3. Comparison of FGFR2 IHC (using ab58201) and DNA FISH (a part of training set, n = 194).

	DNA FISH		*P* value
	Non-amplified	Amplified	
IHC (n = 194)			0.616
Negative	150	3	
Positive	40	1	

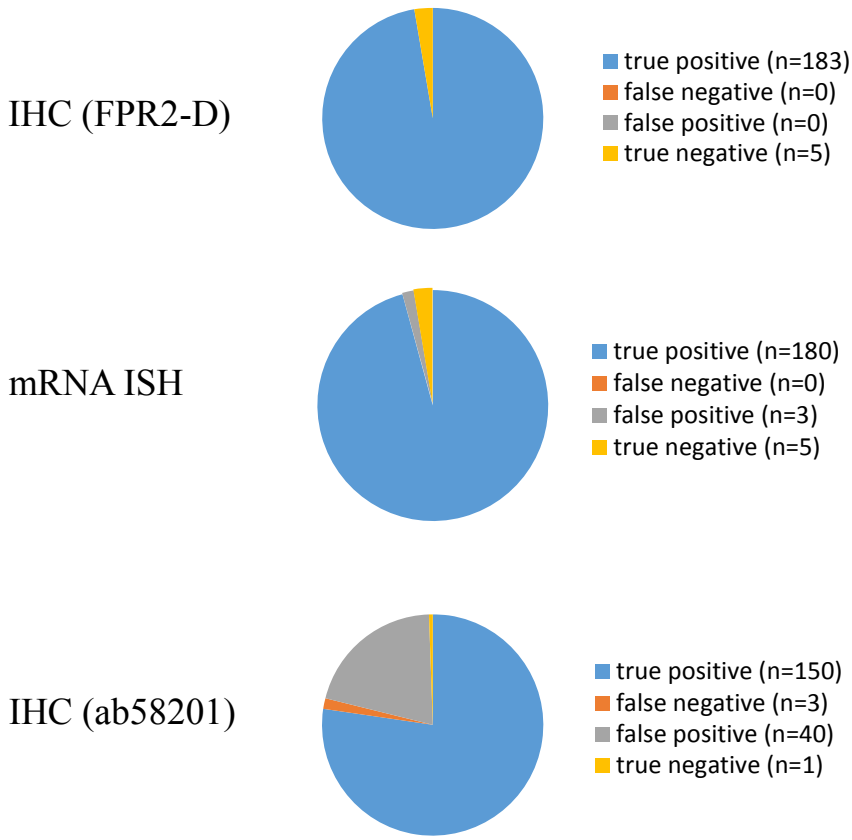
Fisher's exact test was used in analysis.

Table 4. Comparison of two FGFR2 antibodies (FPR2-D and ab58201) for IHC (n = 356).

	FPR2-D		*P* value
	Negative	Positive	
ab58201			1.000
Negative	265	6	
Positive	83	2	

Fisher's exact test was used in analysis.

Figure 3. Comparison of predictive functions between FGFR2b-specific IHC antibody (FPR2-D), mRNA ISH, and commercial antibody (ab58201).



FGFR2 expression status by IHC and mRNA ISH and clinicopathological characteristics (training set)

Among 362 primary gastric cancers of training set, FGFR2 protein overexpression was identified in 9 (2.5%) by IHC, and mRNA overexpression was identified in 21 (5.8%) by mRNA ISH. There was a strong association between FGFR2 protein and mRNA expressions ($P < 0.001$, Table 5).

The clinicopathological features of FGFR2 positive and negative tumors are summarized in Table 6. FGFR2 protein overexpression was associated with perineural invasion ($P = 0.015$). However, FGFR2 protein and mRNA expression status showed no significant differences with respect to patient age, gender, histologic subtype, lymphatic invasion, venous invasion, lymph node and distant metastasis ($P > 0.05$).

In univariate survival analysis, FGFR2 protein and mRNA overexpression were significantly associated with a poor outcome ($P < 0.001$ and $P = 0.012$, respectively; Figure 4). When the analysis was restricted to the advanced disease (stage III–IV gastric cancer), FGFR2 protein and *FGFR2* mRNA overexpression was also significantly associated with poor

survival (P = 0.019 and P = 0.014, respectively). Multivariate Cox hazard models revealed that *FGFR2* mRNA overexpression was an independent biomarker of poor survival (P = 0.016; Table 7), after adjustment for tumor invasion, lymph node and distant metastasis, which were all significant prognostic factors in the univariate analyses.

Table 5. Relationship between results of FGFR2 IHC and mRNA ISH (training set).

	FGFR2 mRNA ISH			*P* value
	Negative	Positive	Total	
FGFR2 IHC				<0.001*
Negative	340 (93.9)	13 (3.6)	353 (97.5)	
Positive	1 (0.3)	8 (2.2)	9 (2.5)	
Total	341 (94.2)	21 (5.8)	362 (100)	

*Statistically significant

Values are numbers (%)

Table 6. Clinicopathological characteristics related to FGFR2 protein and mRNA expression status (training set).

	FGFR2 IHC (n = 362)			*FGFR2* mRNA ISH (n = 362)		
	Positive	Negative	*P*	Positive	Negative	*P*
Age (mean ± SD, yr)	55 ± 17	58 ± 12	0.585	58 ± 15	58 ± 12	0.945
Gender			0.501			0.459
Male	7 (77.8)	227 (64.3)		12 (57.1)	222 (65.1)	
Female	2 (22.2)	126 (35.7)		9 (42.9)	119 (34.9)	
WHO classification			0.529			0.339
WD	0	14 (4.0)		0	14 (4.1)	
MD	1 (11.1)	115 (32.6)		4 (19.0)	112 (32.8)	
PD	7 (77.8)	162 (45.9)		14 (66.7)	155 (45.5)	
SRC	1 (11.1)	38 (10.8)		3 (14.3)	36 (10.6)	
Others	0	24 (6.6)		0	24 (6.6)	
Lauren classification			0.756			0.230
Intestinal	3 (33.3)	172 (48.7)		7 (33.3)	168 (49.3)	
Diffuse	5 (55.5)	136 (38.5)		10 (47.6)	131 (38.4)	
Mixed	1 (11.1)	41 (11.6)		3 (14.3)	39 (11.4)	
Indeterminate	0	4 (1.1)		1 (4.8)	3 (0.9)	
Tumor invasion			1.000			1.000
EGC	0	22 (6.2)		1 (4.8)	21 (6.2)	
AGC	9 (100.0)	331 (93.8)		20 (95.2)	320 (93.8)	
Lymphatic invasion			0.055			0.712
Not identified	0	122 (34.6)		6 (30.0)	116 (34.0)	

Present	8 (100.0)	231 (65.4)		14 (70.0)	225 (66.0)	
Venous invasion			0.149			0.113
Not identified	6 (66.7)	300 (85.0)		15 (71.4)	291 (85.3)	
Present	3 (33.3)	53 (15.0)		6 (28.6)	50 (14.7)	
Perineural invasion			0.015*			0.174
Not identified	0	139 (39.4)		5 (23.8)	134 (39.3)	
Present	9 (100.0)	214 (60.6)		16 (76.2)	207 (60.7)	
pTNM stage			0.059			0.690
I	0	67 (19.0)		3 (14.3)	64 (18.8)	
II	1 (11.1)	124 (35.1)		7 (33.3)	118 (34.6)	
III	6 (66.7)	136 (38.5)		8 (38.1)	134 (39.3)	
IV	2 (22.2)	26 (7.4)		3 (14.3)	25 (7.3)	
Lymph node metastasis			0.062			0.435
Absent	0	114 (32.3)		5 (23.8)	109 (32.0)	
Present	9 (100.0)	239 (67.7)		16 (76.2)	232 (68.0)	
Distant metastasis			0.148			0.215
Absent	7 (77.8)	327 (92.6)		18 (85.7)	316 (92.7)	
Present	2 (22.2)	26 (7.4)		3 (14.3)	25 (7.3)	
Total	9 (2.5)	353 (97.5)		21 (5.8)	341 (94.2)	

* Statistically significant

Chi-square or Fisher's exact test was used in the analysis of each factor except age (Student's t-test).

Figure 4. Kaplan-Meier survival plots for the 362 gastric cancer patients (training set) according to (A) FGFR2 protein overexpression and (B) and mRNA overexpression.

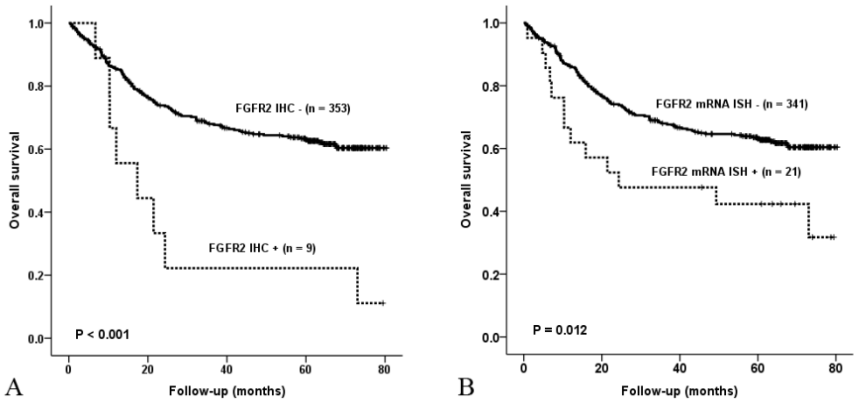


Table 7. Multivariate Cox proportional hazard models for predictors of overall survival (training set).

Variables	Hazard ratio	*P* value
FGFR2 IHC		
IHC expression (positive vs. negative)	1.869 (0.905–3.860)	0.091
Tumor invasion (EGC vs. AGC)	3.133 (1.151–8.534)	0.025*
Lymph node metastasis (no vs. yes)	4.245 (2.462–7.319)	< 0.001*
Distant metastasis (no vs. yes)	4.592 (2.918–7.227)	< 0.001*
FGFR2 mRNA ISH		
mRNA ISH expression (positive vs. negative)	2.028 (1.141–3.603)	0.016*
Tumor invasion (EGC vs. AGC)	3.219 (1.183–8.763)	0.022*
Lymph node metastasis (no vs. yes)	4.303 (2.499–7.409)	< 0.001*
Distant metastasis (no vs. yes)	4.832 (3.074–7.595)	< 0.001*

95% confidence intervals are indicated in parentheses

*Statistically significant

FGFR2 expression status by IHC and mRNA ISH and clinicopathological characteristics (validation set)

Among 413 primary gastric cancers of validation set, FGFR2 protein overexpression was identified in 10 (2.4%), and mRNA overexpression was identified in 15 (3.6%). There was a strong association between FGFR2 protein and mRNA expression ($P < 0.001$, Table 8).

The clinicopathological features of FGFR2 protein and mRNA positive and negative tumors are summarized in Table 9. *FGFR2* mRNA overexpression was associated with moderately differentiated histology ($P = 0.029$), higher frequency of advanced gastric cancer (AGC) ($P = 0.015$), and lymphatic invasion ($P = 0.033$). FGFR2 protein and mRNA expression status showed no significant differences with respect to patient age, gender, histologic subtype, lymphatic invasion, venous invasion, lymph node and distant metastasis.

On univariate survival analysis, FGFR2 protein and mRNA overexpression was not significantly associated with a poor outcome ($P = 0.153$ and $P = 0.802$, respectively; Figure 5).

Table 8. Relationship between results of FGFR2 IHC and mRNA ISH (validation set).

	FGFR2 mRNA ISH			*P* value
	Negative	Positive	Total	
FGFR2 IHC				<0.001*
Negative	395 (99.2)	8 (53.3)	403 (97.6)	
Positive	3 (0.8)	7 (46.7)	10 (2.4)	
Total	398 (96.4)	15 (3.6)	413 (100)	

*Statistically significant

Values are numbers (%)

Table 9. Clinicopathological characteristics related to FGFR2 protein and mRNA expression status (validation set).

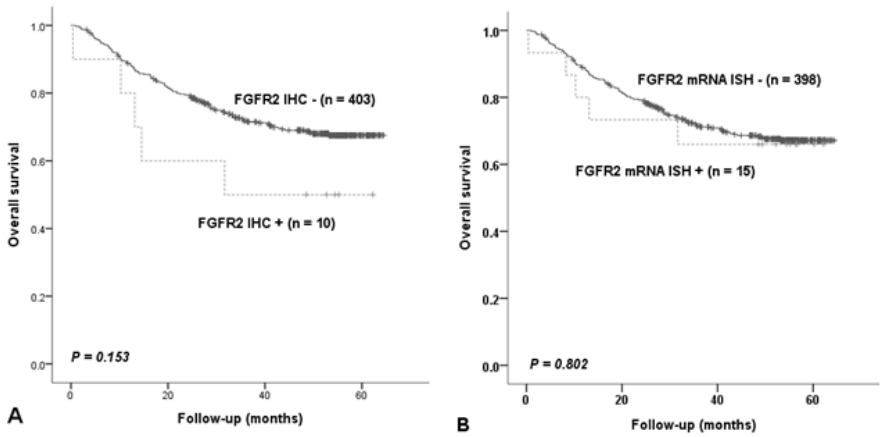
	FGFR2 IHC (n = 413)			*FGFR2* mRNA ISH (n = 413)		
	Positive	Negative	*P*	Positive	Negative	*P*
Age (mean ± SD, yr)	63 ± 9	58 ± 13	0.152	64 ± 11	57 ± 13	0.053
Gender			0.732			0.573
Male	8 (80.0)	289 (71.7)		12 (80.0)	285 (71.6)	
Female	2 (20.0)	114(28.3)		3 (20.0)	113 (28.4)	
WHO classification			0.082			0.029
WD	0	29 (7.2)		0	29 (7.3)	
MD	1 (10.0)	139 (34.5)		3 (20.0)	137 (34.4)	
PD	4 (40.0)	143 (35.5)		7 (46.7)	140 (35.2)	
SRC	3 (30.0)	74 (18.4)		2 (13.3)	75 (18.8)	
Others	2 (20.0)	18 (4.4)		3 (20.0)	17 (4.3)	
Lauren classification			0.524			0.979
Intestinal	5 (50.0)	166 (41.2)		6 (40.0)	165 (41.5)	
Diffuse	5 (50.0)	163 (40.4)		6 (40.0)	162 (40.7)	
Mixed	0	71 (17.6)		3 (20.0)	68 (17.1)	
Indeterminate	0	3 (0.7)		0	3 (0.8)	
Tumor invasion			0.068			0.015
EGC	0	114 (28.3)		0	114 (28.6)	
AGC	10 (100)	289 (71.7)		15(100)	284 (71.4)	
Lymphatic invasion			0.328			0.033
Not identified	2 (20.0)	164 (40.7)		2 (13.3)	164 (41.2)	

Present	8 (80.0)	239 (59.3)		13 (86.7)	234 (58.8)	
Venous invasion			0.088			0.163
Not identified	6 (60.0)	332 (82.4)		10 (66.7)	328 (82.4)	
Present	4 (40.0)	71 (17.6)		5 (33.3)	70 (17.6)	
Perineural invasion			0.543			1.000
Not identified	4 (40.0)	203 (50.4)		8 (53.3)	199 (50.0)	
Present	6 (60.0)	200 (49.6)		7 (46.7)	199 (50.0)	
pTNM stage			0.124			0.176
I	0	138 (34.7)		2 (13.3)	136 (34.6)	
II	4 (40.0)	85 (21.4)		6 (40.0)	83 (21.1)	
III	4 (40.0)	128 (32.2)		6 (40.0)	126 (32.1)	
IV	2 (20.0)	47 (11.8)		1 (6.7)	48 (12.2)	
Lymph node metastasis			0.054			0.115
Absent	1 (10.0)	166 (41.2)		3 (20.0)	164 (41.2)	
Present	9 (90.0)	237 (58.8)		12 (80.0)	234 (58.8)	
Distant metastasis			0.337			1.000
Absent	8 (80.0)	356 (88.3)		14 (93.3)	350 (87.9)	
Present	2 (20.0)	47 (11.7)		1 (6.7)	48 (12.1)	
Total	10 (2.4)	403 (97.6)		15 (3.6)	398 (96.4)	

* Statistically significant

Chi-square or Fisher's exact test was used in the analysis of each factor except age (Student's t-test).

Figure 5. Kaplan-Meier survival plots for 413 gastric cancer patients (validation set) according to (A) FGFR2 protein and (B) mRNA overexpression.



FGFR2 expression status by IHC and mRNA ISH and clinicopathological characteristics (total population)

When training set and validation set are combined, positivity of IHC was 2.5% (19 out of 775) and that of mRNA ISH was 4.7% (36 out of 775). In univariate survival analysis, FGFR2 protein overexpression was significantly associated with a poor outcome ($P < 0.001$; Figure 6A). Within the scope of advanced disease (stage III–IV gastric cancer), FGFR2 protein overexpression was also significantly associated with poor survival ($P = 0.021$; Figure 6B). Multivariate Cox hazard models revealed that FGFR2 protein overexpression was an independent biomarker of poor survival ($P = 0.043$; Table 10) after adjustment for tumor invasion, Lymph node metastasis and distant metastasis. Survival difference was observed when only stage III–IV groups were analyzed ($P = 0.039$, Table 11).

Figure 6. Kaplan-Meier survival plots according to FGFR2 protein overexpression in total population. (A) Total overall survival. (B) Overall survival in stage III-IV group.

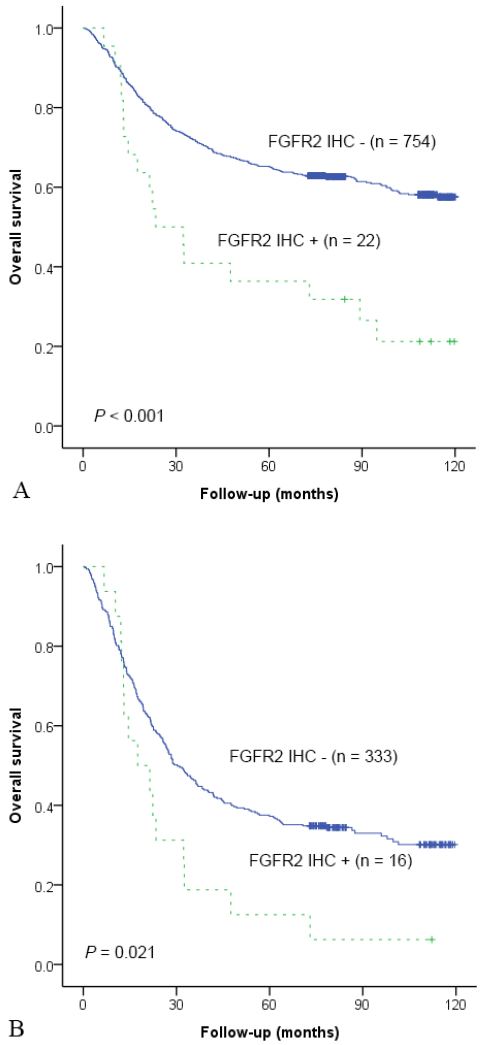


Table 10. Multivariate Cox proportional hazard models for predictors of overall survival (total population).

Variables	Hazard ratio	*P* value
IHC expression (positive vs. negative)	1.662 (1.017-2.719)	0.043*
Tumor invasion (EGC vs. AGC)	2.375 (1.482-3.805)	<0.001*
Lymph node metastasis (no vs. yes)	3.652 (2.609-5.114)	<0.001*
Distant metastasis (no vs. yes)	5.245 (3.975-6.922)	<0.001*

95% confidence intervals are indicated in parentheses

*Statistically significant

Table 11. Multivariate Cox proportional hazard models for predictors of overall survival (stage III-IV).

Variables	Hazard ratio	*P* value
IHC expression (positive vs. negative)	1.680 (1.026-2.751)	0.039*
Tumor invasion (EGC vs. AGC)	1.607 (0.957-2.699)	0.073
Lymph node metastasis (no vs. yes)	2.002 (1.332-3.008)	0.001*
Distant metastasis (no vs. yes)	3.651 (2.746-4.852)	<0.001*

95% confidence intervals are indicated in parentheses

*Statistically significant

FGFR2 mRNA expression in TCGA cohort

To validate our findings further, data from The Cancer Genome Atlas (TCGA) stomach adenocarcinoma cohort were analyzed (34). Among 265 gastric cancer patients, mean *FGFR2* mRNA expression was 13.2 (range, 0.05 to 310.0; RNASeq V2 RSEM). The cut-off was chosen as the mean plus 2 times of standard deviation, which was 70.2 (Figure 7); Accordingly, *FGFR2* mRNA overexpression was 5 out of 265 gastric cancer patients (1.9%), and was not significantly associated with any clinicopathological parameters (Table 12). During the follow-up period, 48 (18.1%) of 265 patients were passed away. Using Kaplan-Meier estimates, the median survival for patients with high *FGFR2* expression was 293.0 days, while the median survival of the patients with low *FGFR2* expression was 1393.7 days. While it could be observed that poor survival was related to high FGFR2 expression, log-rank test for survival analysis failed to show a significant survival difference (P = 0.060, Figure 8).

Table 12. Clinicopathological characteristics related to *FGFR2* mRNA expression status from TCGA.

	FGFR2 mRNA (n = 265)		*P* value
	High expression (n = 5)	Low expression (n = 260)	
Total	5 (1.9)	260 (98.1)	
Age (mean ± SD, yr)	71 ± 8	66 ± 11	0.317
Gender			0.376
Male	2 (40.0)	161 (61.9)	
Female	3 (60.0)	99 (38.1)	
WHO classification			0.814
Papillary/Tubular	2 (40.0)	141 (54.2)	
Poorly cohesive carcinoma	2 (40.0)	63 (24.2)	
Others	1 (20.0)	56 (21.5)	
Lauren classification			0.792
Intestinal	3 (60.0)	170 (65.4)	
Diffuse	2 (40.0)	63 (24.2)	
Mixed/NA	0	27 (10.4)	
Tumor invasion			0.199
EGC	1 (20.0)	10 (4.0)	
AGC	4 (80.0)	241 (96.0)	
pTNM stage			0.612
I	1 (20.0)	31 (12.7)	
II	1 (20.0)	104 (42.4)	
III	3 (60.0)	92 (37.6)	
IV	0	18 (7.3)	

Chi-square test and Fisher's exact test were used in each analysis except for the analysis of age factor (Student t-test)

Figure 7. Distribution of *FGFR2* mRNA expression on TCGA (n = 265).

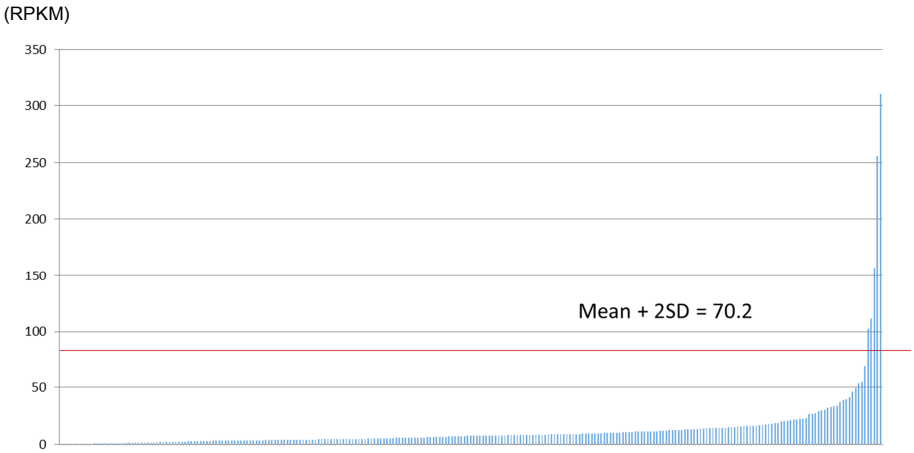
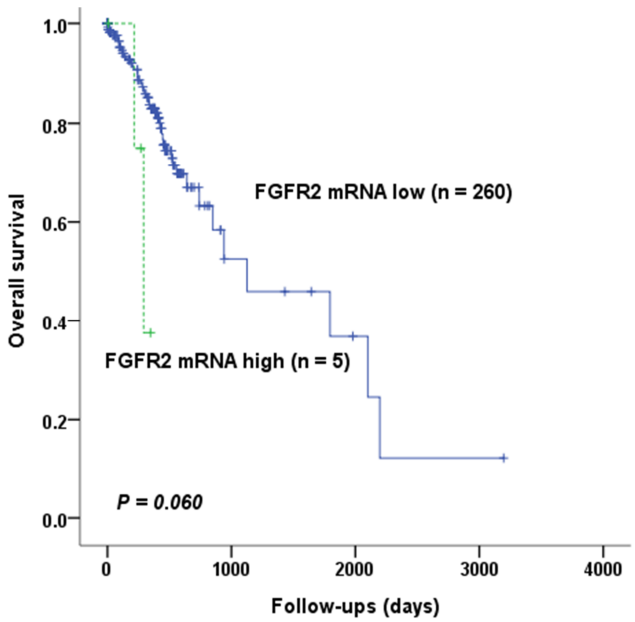


Figure 8. Kaplan-Meier curves showing the effect of *FGFR2* mRNA expression on patient survival (TCGA).



Intratumoral heterogeneity of FGFR2 in primary gastric tumors

FGFR2 protein and mRNA expression were examined in thee different areas of each primary cancer in the training set. Two cores of tissues were obtained from submucosa or deeper area, and another core was obtained from mucosa (Figure 9). Intratumoral heterogeneity is defined as a case where the staining status of a tissue core is different from that of another core from the same tumor, as studied for in HER2 expression in gastric cancer (26). Such has been was observed in 5 of 9 FGFR2 IHC-positive cases (55.5%) and 18 of 21 mRNA ISH-positive cases (85.7%) (Table 13). All FGFR2 IHC positive cases exhibited mRNA ISH positivity. A representative paraffin tumor block ("full section") was tested for each FGFR2 positive case. Full-section staining was performed for all 9 cases by IHC and 19 of 21 cases by mRNA ISH. In the former, the average proportion of positively stained area was 45.9% (81.2% in concordant cases and 17.6% in discordant cases) (Figure 10A). The average proportion of positively stained area in the other 19 tumors was 21.4% (63.3% in concordant cases and 14.8% in discordant cases) (Figure 10B). There was no

residual tumor tissue to obtain full sections in 2 of the 21 mRNA ISH positive cases. A representative image of intratumoral heterogeneity is shown in Figure 11.

By IHC, five (55.5%) cases were not stained in invasion front (Con4, Dis2, Dis3, Dis4, Dis5 in Figure 8). One (11.1%) case showed heterogeneous staining in invasion front (Dis1), and 3 cases showed FGFR2 positivity in invasion front (Con1, Con2, Con3).

Figure 9. Strategy for evaluation of FGFR2 heterogeneous expression in primary gastric cancer. A2 and A7: cancer tissue from submucosa or deeper area, B2: cancer tissue from mucosa.

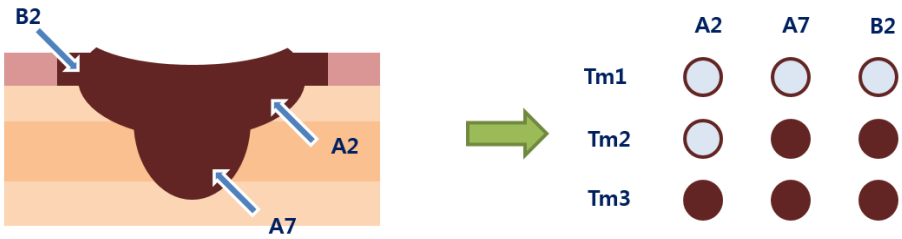


Table 13. Summary of FGFR2 IHC and mRNA ISH results in three different areas of primary gastric cancers.

	IHC	mRNA ISH
Negative	353 (97.5)	341 (94.2)
Positive	9 (2.5)	21 (5.8)
Homogeneous	4 (44.4)	3 (14.3)
Heterogeneous	5 (55.5)	18 (85.7)
Total	362	362

Figure 10. Areas of FGFR2 overexpression in a full section of a primary tumor evaluated semi-quantitatively using (A) immunohistochemistry and (B) mRNA *in situ* hybridization. Abbreviations: Con, homogeneous (concordant) overexpression of FGFR2 (i.e. FGFR2 overexpression in 3 different tissue array cores of each case; shaded); Dis, heterogeneous (discordant) overexpression of FGFR2 (i.e. different FGFR2 expression intensities were observed between the tissue array cores in each case; not shaded). Upper line: average area of homogeneous overexpression in the tissue array study. Lower line: average area of heterogeneous overexpression in the tissue array study.

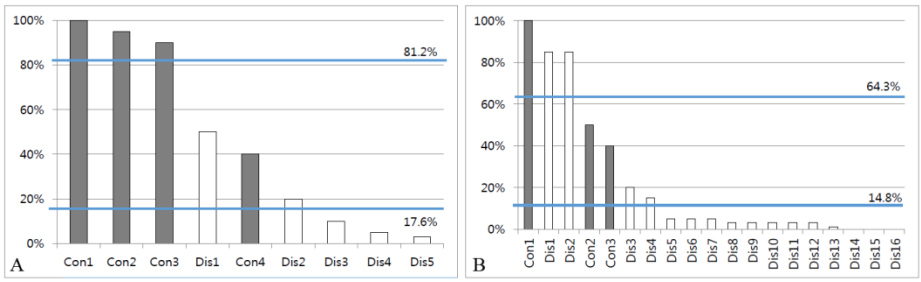
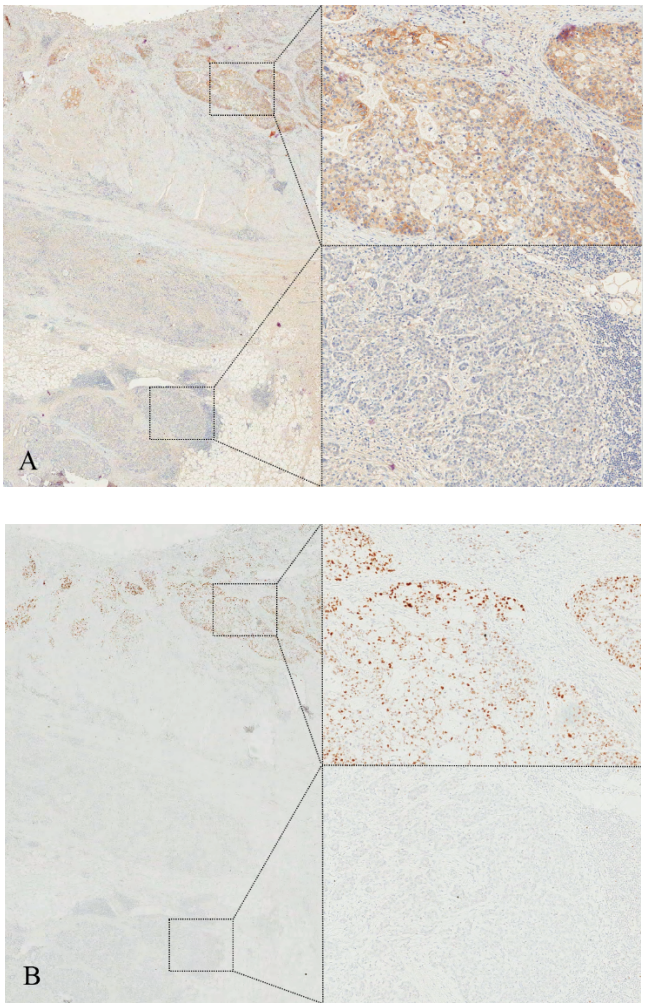


Figure 11. Representative full section image of heterogeneous FGFR2 expression. FGFR2 protein expression was evaluated by immunohistochemistry (A) and *FGFR2* mRNA expression was assessed by mRNA *in situ* hybridization (B).



Discordance of FGFR2 expression between primary gastric tumor and synchronous metastatic lymph nodes

One hundred thirty five pairs of primary tumors and synchronous metastatic regional lymph nodes were evaluated for the discordant expression of FGFR2 (Table 14). Using IHC, 9 cases among 135 cases (6.7%) showed FGFR2 overexpression, and discordance between FGRF2 expressions in primary tumor and metastatic lymph node was noted in 4 out of 9 cases (44.4%). Of these four cases, three showed negative conversion (i.e., FGFR2 protein showed overexpression in primary tumor, but not in lymph node). The one remaining case was negative in primary tumor but showed overexpression in the metastatic lymph node (i.e., positive conversion). In full section staining for the three negative conversion cases, FGFR2 expression was observed in 10%, 5%, and 3% of the primary tumor area, respectively. FGFR2 expression was not observed in the primary tumor by full section staining in single positive conversion case. In this case, dual-color DNA FISH revealed *FGFR2* gene amplification in the metastatic lymph nodes.

Using mRNA ISH, 14 cases among 135 cases (10.4%) showed *FGFR2* overexpression, and discordance between *FGRF2* expressions in primary tumor and metastatic lymph node was noted in 10 out of 14 cases (71.4%).

Table 14. Comparison of FGFR2 protein and mRNA expressions in primary gastric cancers and synchronous metastatic lymph nodes.

	IHC	mRNA ISH
Negative	126 (93.3)	121 (89.6)
Positive in primary GC or metastaic LN	9 (6.7)	14 (10.4)
Concordant cases	5 (55.5)	4 (28.6)
Discordant cases	4 (44.4)	10 (71.4)
Total	135	135

DISCUSSION

This study showed FGFR2 expressions in gastric cancer by using DNA FISH, mRNA ISH, and IHC simultaneously, a particularly powerful approach as all of these assays were performed *in situ*. The proportion of positive tumors was 2.7%, 5.8%, and 2.5% by DNA FISH, mRNA ISH, and IHC, respectively (training set), and 3.6% and 2.4% by mRNA ISH and IHC, respectively (validation set).

Targeted drugs are currently being developed for patients with gastric cancer exhibiting *FGFR2* gene amplification (10, 18–21, 40). A phase II *FGFR2*–targeted trial has been completed (not yet published) (41), and another study is in progress (42). DNA FISH has been widely accepted as the gold standard for assessing gene amplification in tumors (13, 43). However, it has a number of limitations, especially in the evaluation of large resected samples. Pathologists frequently use IHC as a practical screening tool for genetic alterations and selection of patients for clinical trials. HER2 protein

overexpression in breast cancer (44) and gastric cancer (45) and anaplastic lymphoma kinase (ALK) protein expression in lung cancer (43) are examples of therapeutically significant proteins that can be evaluated by IHC.

It has been unknown whether previously reported FGFR2 IHC data reflected the *FGFR2* gene amplification rate. Six previous studies reported that FGFR2 protein overexpression evaluated by IHC occurred in 21-57% of gastric cancers (9, 38, 46-49), but they did not evaluate the relationship between IHC and DNA FISH results. Popular commercial mouse monoclonal anti-FGFR2 antibody (ab58201) did not function properly in our study (Figure 10). FGFR2 positive rate was 22.6% by ab58201. This difference is thought to be the low sensitivity of ab58201. Stomach cancer cell lines expressed b isoform of FGFR2 (8), and Deshpande *et al.* (32) showed an expression pattern of FGFR2IIIb-specific antibody (FPR2-D) in gastric cancer.

The incidence of *FGFR2* gene amplification is reported to be 2-9% in gastric cancer (11-17, 50). Our IHC and mRNA ISH data concur with previously reported results using DNA

FISH. These two methods can therefore potentially be used as primary evaluation tools for screening *FGFR2*–amplified gastric cancer.

This study also emphasized that FGFR2 protein and mRNA overexpression is highly heterogeneous in gastric cancer. Some authors mentioned such heterogeneity of *FGFR2* gene amplification (13, 17). Discordance appears to be caused by intratumoral heterogeneity since the majority of discordant cases showed negative conversion in IHC. Similar phenomena were reported from some studies about heterogeneous expressions of HER2 in gastric carcinoma (24–26). It has been suggested that heterogeneous HER2 expression could be a cause of Herceptin® (trastuzumab) resistance (51, 52). Similarly, heterogeneity might make FGFR2 less attractive as a drug target.

It might be inadequate to evaluate FGFR2 expression in small biopsy samples from inoperable cases. Das *et al.* (16) reported that one gastric cancer case showed heterogeneous FGFR2 gene amplification in the blocks despite none being found in the biopsy specimen. In the heterogeneous cases, 17.6%

of total area was positive by IHC and 14.8% was positive by mRNA ISH in full sections (Figure 10). Given that cases with at least 10% or higher HER2 positive gastric cancer are eligible for targeted treatment (4, 45), just a small portion of FGFR2 positive cells in a biopsy might reflect a significant number of clones with *FGFR2* gene amplification in the whole tumor. Multiple biopsies or a deep biopsy would be good alternatives in these situations.

Tumors carrying FGFR2 protein and mRNA overexpressions were not substantially associated with other clinicopathological parameters. It is not surprising that the findings are also not consistent with the published multicenter studies (11, 13).

This study has a number of limitations. First, this study used tissue microarray to sample the tumor specimens, which may not fully represent and could underestimate the true number of FGFR2 overexpression cases. In addition, our study was conducted with an Asian population, and the rate of FGFR2 overexpression cases may differ by ethnicity. Considering the low overall rate of FGFR2 overexpression and its heterogeneity,

it seems advisable to perform screening with IHC or mRNA ISH rather than DNA FISH for surgically resected specimens. It is suggested that both the primary tumor and lymph node metastases should be analyzed using FGFR2 IHC or mRNA ISH to select patients correctly and thus fully examine the efficacy of FGFR2-targeted therapy in gastric cancer. Conducting a DNA FISH study for evaluating prognosis is not very cost-effective, and IHC can be a reasonable and more economical alternative. To establish a scoring guideline like the one used for HER2 IHC (44), further research on FGFR2 IHC and/or *FGFR2* mRNA ISH is needed.

In summary, only a small subset of gastric cancers shows FGFR2 overexpression, and the overexpression is heterogeneous in most of the cases. Both FGFR2b-specific IHC and *FGFR2* mRNA ISH results correspond to FGFR2 gene amplification in gastric cancer. These methods therefore offer a sensitive and practical approach to supplement for DNA FISH. Both could be used for selecting patients in further studies of FGFR2-targeted therapy in gastric cancer as potential companion diagnosis.

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국문 초록

배경: 위암은 발생률 및 사망률의 관점에서 볼 때, 한국인에서 가장 대표적인 악성종양이지만 진행암 환자에게 사용할 수 있는 표적치료제는 매우 한정되어 있다. 위암에서 섬유모세포수용인자 수용체 2 (fibroblast growth factor receptor 2, *FGFR2*) 유전자 변이가 있으며, FGFR2 억제제가 이러한 위암의 성장을 억제시킨다는 보고에 근거하여 FGFR2 억제제를 이용한 임상시험이 진행되고 있으므로, FGFR2 억제제에 감수성이 있는 위암을 쉽게 선별하는 방법이 필요한 실정이다. *FGFR2* 유전자 변이의 대부분이 DNA 증폭이므로, *FGFR2* DNA 형광제자리부합법 (fluorescence *in situ* hybridization, FISH)을 이용하여 위암을 선별하는 방법이 사용되고 있으나, 진단기준이 정립되어 있지 않을 뿐 아니라, 비용과 시간이 많이 소요되는 단점이 있다. 본 연구는 FGFR2 단백을 측정하는 면역조직화학염색법 (immunohistochemistry, IHC) 및 mRNA의 발현 정도를 관찰하는 mRNA 제자리부합법 (mRNA *in situ* hybrization, mRNA ISH)의 결과가 FGFR2 변이 위암을 진단하는 데 유용한지를 검증하고자 하였으며, FGFR2 과발현이 임상예후적 인자로 작용하는지를 알아보고, FGFR2 발현의 불균질성을 원발성위암과 전이 림프절에서 알아보고자 하였다.

방법: 서울대학교병원에서 시행된 위암에 대한 수술적 절제검체를 대상으로 하였다. 2005년에 얻어진 검체 362례와 짝지어진 전이성 림프절조직 135례, 그리고 2004년에 얻어진 검체 413례를 바탕으로 tissue microarray (TMA)를 제작하였고, 이를 대상으로 FGFR2 IHC와 mRNA ISH를 사용하여 단백질 및 mRNA 발현을 평가하였다. 188례에 대해서는 *FGFR2* DNA FISH를 함께 시행하였다.

결과: *FGFR2* 유전자의 증폭을 보이는 위암환자의 조직은 모두 FGFR2 단백질 및 mRNA의 과발현이 관찰되었고, (5/188, $P<0.001$), IHC 결과와 mRNA ISH 결과 간에 일치율이 매우 높았다. 2005년 환자군을 바탕으로 생존에 대한 단변량분석시 FGFR2 단백질 및 mRNA 과발현은 불량한 예후와 유의한 관련성을 보였고 ($P<0.001$ 및 $P=0.012$), 다변량분석에서 *FGFR2* mRNA 과발현은 종양의 침윤, 국소 림프절 전이여부 및 원격 전이여부와 무관하게 나쁜 예후를 예측하는 인자로 나타났다 ($P=0.016$). 2004년 환자군에서는 FGFR2 발현과 생존률이 유의한 관련성을 보이지 않았으나, 두 군을 합하여 다변량분석을 시행하였을 때에도 FGFR2 단백질 과발현은 불량한 예후를 보였다 ($P=0.043$). FGFR2 발현의 균질성을 평가하기 위해 원발성 위암의 서로 다른 세 부위에 대해 FGFR2 IHC 및 mRNA ISH를 시행하니,

IHC에서 양성을 나타냈던 9례 중 5례 (55.5%), mRNA ISH 방법에서 양성을 나타냈던 21례 중 18례 (85.7%)에서 불균질한 발현이 관찰되었다. 원발성 위암과 전이 림프절 사이의 발현의 불일치는 IHC에서는 9례 중 5례 (55.5%), mRNA ISH에서는 14례 중 4례 (28.6%)로서, 발현의 불균질성이 상당히 높음을 확인하였다.

결론: 본 연구는 FGFR2 IHC 및 mRNA ISH 두 가지 방법이 *FGFR2* 유전자 변이를 선별하는 방법으로 유용하다는 점과 FGFR2의 과발현이 위암 환자의 불량한 예후와 관련이 있음을 확인하였다. 추가로 FGFR2 과발현이 부위에 따라 불균질하게 나타남을 관찰하였는 바, 표적유전자 발현의 불균질성은 표적치료 실패의 원인 중 하나로 지목되고 있으며, 작은 생검조직만으로 FGFR2의 발현을 측정하는 방법이 제한적이라는 주장의 근거가 된다. 이상의 연구 결과는 FGFR2 억제제를 이용한 위암 환자의 임상연구에 도움이 될 것으로 기대된다.

주요어: 위암; 섬유모세포성장인자 수용체 2; 면역조직화학염색법; mRNA 제자리부합법; DNA 형광제자리부합법; 불균질 발현

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