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FGFR2 gene amplification status and its clinicopathologic significance in gastric carcinoma

위암에서 FGFR2 유전자 증폭의 임상병리학적 특성과 예후에 미치는 영향에 대한 연구

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FGFR2 gene amplification status and its clinicopathologic significance in gastric carcinoma

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Abstract

FGFR2 gene amplification status and its clinicopathologic significance in gastric carcinoma

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Introduction : Fibroblast growth factor receptor 2 (FGFR2) is a member of the FGFR receptor tyrosine kinase family, and FGFR2 gene amplification or missense mutation has been observed in various human cancers, including gastric carcinoma. Furthermore, recent studies have shown that anti-FGFR2 agents inhibit tumor progression in various human cancers, such as endometrial carcinoma and gastric carcinoma, which remains one of the most frequent causes of cancer-related death worldwide. We considered that knowledge of the status of FGFR2 gene amplification in gastric carcinoma may aid in targeted cancer therapy. **Methods**: In this study, FGFR2 amplification status was evaluated using fluorescence in situ hybridization (FISH) and quantitative real-time polymerase chain reaction (PCR) in 313 surgically resected gastric

carcinoma tissues. FGFR2 gene copy number alteration was also evaluated in 372 gastric carcinoma tissues by FISH method. In addition, potential associations between clinicopathological parameters and the presence of FGFR2 amplification or copy number gain was investigated and survival analysis was performed **Results**: In our study, the frequency of FGFR2 amplification was 4.0% and 4.5% in the 2 groups of patients. FGFR2 amplification was also found to be associated with a higher pathologic T stage (p=0.023), pathologic N stage (p=0.038) and distant metastasis (p=0.009). In addition, univariate analysis revealed that FGFR2 copy number gain as well as amplification was significantly associated with lower cancer-specific survival. **Conclusion**: We therefore found gastric carcinoma with FGFR2 amplification or FGFR2 copy number gain to be associated with advanced disease. We believe that the determination of FGFR2 gene status could allow the identification of a subset of cancers sensitive to targeted FGFR2 inhibitor - based therapies.

Key words: stomach neoplasm, fibroblast growth factor receptor type II, fluorescence in situ hybridization, gene amplification, survival analysis.

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Introduction

Although the incidence of gastric carcinoma has declined recently, gastric cancer is the fifth most common malignancy in the world and the third leading cause of cancer death worldwide(1). Furthermore, despite the progress made during the past decade on improvements in our understanding of gastric carcinoma, the prognosis of patients with advanced gastric carcinoma remains poor. In fact, the 5-year survival rate for stage IV gastric carcinoma stands at only 3%(2).

However, although genetic alterations, such as, gene amplification, microsatellite instability, and promoter methylation are known to occur during gastric carcinogenesis, the underlying molecular mechanisms that drive the development of gastric carcinoma are largely undetermined (3-5). Moreover, an understanding of the mechanism of gastric carcinogenesis is required for the development of new diagnostic, preventive, and therapeutic methods.

Protein tyrosine kinases are important regulators of intracellular signal transduction pathways and play a major role in the transductions of proliferative signals. Normally, their activities are tightly regulated, but they can become oncogenic when dysregulated by mutation or genetic alterations(6). Recently, tyrosine kinases have shown to be therapeutic targets

in various human cancers for example, KIT in gastrointestinal stromal tumors, Bcr-Abl in chronic myeloid leukemia, and EGFR in non-small cell lung cancer(7-9).

The fibroblast growth factor receptors (FGFRs) are members of the receptor tyrosine kinase family and encode transmembrane tyrosine kinase receptors involved in signaling by interacting with the fibroblast growth factors (FGFs). FGF proteins mediate various biological functions, which include the regulation of embryogenesis. Furthermore, during embryonic development, FGFs play a critical role in morphogenesis by regulating cell proliferation, differentiation, and migration. In adult organisms, FGFs participate in tissue repair, wound healing, and tumor angiogenesis, and FGF signaling has been shown to play an important role in tumorigenesis in prostate, skin, urothelial carcinoma, and hematologic malignancies(10, 11).

Dysregulation of the FGFR signaling pathway as a results of gene amplification, mutation, or receptor over expression is associated with cancer development and progression in several human cancers (12, 13).

Several SNPs of the FGFR2 gene have been associated with an increased risk of breast cancer in a genomewide study (14). Gene amplification of FGFR1 and FGFR2 is associated with

several human carcinomas (13), and FGFR1 amplification is reported in 22% of squamous cell lung cancers and 10% of ER + lung cancers (15, 16). Missense activating mutations of the FGFR2 gene occurs in various congenital skeletal abnormalities, such as Crouzon, Apert, and Pfeiffer syndromes (17, 18). Missense mutations of the FGFR2 gene occur in 10% of uterine endometrial carcinoma (19) and 10% of melanoma (20). FGFR3 mutations are found in 60% of urinary bladder cancers and FGFR4 mutations in 7.5% of rhabdomyosarcomas (21, 22). Accordingly, the FGFR pathway has attracted considerable interest as an oncogene and as a target for the development of therapeutic agents (23).

Recently, amplification of the FGFR gene has been studied in various human malignancies. FGFR2 gene amplification and overexpression has been reported in human breast cancers and gastric cancers (24, 25). FGFR1 amplification was reported in ER+ breast cancer and squamous cell lung carcinoma and FGFR2 amplification in triple negative breast and gastric carcinomas, however, FGFR1, 3, or 4 amplification was not observed in gastric carcinoma (26, 27).

The FGFR2 gene is located at chromosome 10q26 and encodes two isoforms (FGFR2b and FGFR2c), which function as FGF receptors. FGFR2 is activated by ligand binding, and this leads

to the autophosphorylation of the intracellular domains of receptors and receptor associated adaptors. The formation of these complexes results in the activation of several downstream pathways, including those of mitogen-activated protein kinase and phosphoinositide 3-kinase/Akt (11).

Despite these results, no large-scale study has been performed to evaluate the status of FGFR2 amplification in gastric carcinoma. In addition, clinicopathological characteristics and prognostic implication of FGFR2 gene amplification in gastric carcinoma have not been evaluated.

Therefore, the aim of this study was to evaluate FGFR2 gene amplification status in gastric cancer cell lines and a large number of gastric carcinoma tissues, using fluorescence in situ hybridization (FISH) and quantitative real-time polymerase chain reaction (PCR). We then investigated potential associations between FGFR2 amplification and a number of clinicopathological features, including survival. Additionally, we analyzed the FGFR2 gene copy number alteration status in gastric carcinoma tissue using FISH and evaluated the clinicopathological significance of FGFR2 gene copy number alteration in gastric carcinoma.

Materials and methods

1. Cell lines

Ten human gastric cancer cell lines (SNU-1, SNU-5, SNU-16, SNU-216, SNU-284, SNU-601, SNU-620, SNU-638, SNU-668, and SNU-719) were used in this study. All cell lines were obtained from the Korean Cell Line Bank (http://cellbank.snu.ac.kr, Seoul, Korea) and maintained in RPMI-1640 (JBI, Seoul, Korea). All culture media contained 10% fetal bovine serum (HyClone, Logan, UT), 100 U/mL penicillin, and 100 μg/mL streptomycin (Sigma Chemicals, St Louis, MO).

2. Reverse transcription-Polymerase chain reaction (PCR)

For RT-PCR, total RNA was isolated from 10 gastric cancer cell lines using TRIZOL reagent (GIBCO BRL, Grand Island, NY). To generate cDNA, total RNA (5 μg) was reverse-transcribed using MMLV-reverse transcriptase (Bioneer, Seoul, Korea) using random primers. PCR amplification was performed for 35 cycles at 95° C for 30 s, annealing at 50° C for 30 s, extension at 72° C for 1 min, and this was followed by a final

extension at 72° C for 10 min in a thermal cycler (Bioneer). To test cDNA integrity, the GAPDH gene was amplified in each sample. Primer sequences and annealing temperatures are provided in Table 1.

3. Western blot analysis

Cellular protein extracts from gastric cancer cell lines were prepared by dissociation using lysis buffer (iNtRON Biotechnology, Korea), protein levels were measured using BCA protein assay kits (Pierce Biotechnology Inc., Rockford, IL), and protein were separated on 8% SDS polyacrylamide gels using SDS-PAGE. The separated proteins were transferred onto reinforced PVDF membranes (Millipore Corporation, Bedford, Mass), and nonspecific sites on blots were blocked by incubation in TBS containing 0.1% Tween 20 and 5% non-fat dried milk for 1 h. Anti-FGFR2 (Abcam, USA, 1:2000) was used as the primary antibody. The membranes were incubated overnight at 4° C, washed with TBS, and incubated for 1 h at room temperature with anti-mouse HRP as the secondary antibody. After extensive washing, antigen-antibody complexes were visualized by ECL-staining (Amersham, Arlington, IL).

4. Gastric carcinoma patient samples

1) FGFR2 gene amplification in gastric carcinoma

Surgically resected gastric carcinoma specimens were obtained from 313 patients who underwent gastrectomy at Seoul National University Hospital between January 1, 2004 and December 31, 2004 (Cohort A).

Clinicopathological parameters such as age, sex, histological type, pathological stage, and the presence of lymphatic invasion, lymph node metastasis, and distant metastasis were evaluated by reviewing pathological records and medical charts. The mean age of the patients was 59 years, and 89.6% of patients had undergone a curative resection (R0 according to the guidelines of the International Union Against Cancer). Clinical outcomes were determined from surgery until death or December 31, 2009, which resulted in a mean follow-up period of 49 months (range 1-64 months). Cases lost to follow-up and deaths attributed to causes other than gastric carcinoma were censored during the survival analysis.

2) FGFR2 gene copy number alteration in gastric carcinoma

To evaluate FGFR2 copy number status in gastric carcinoma, we used different gastric carcinoma samples from that used in the FGFR2 gene amplification study. Surgically resected gastric carcinoma specimens were obtained from 372 patients who underwent gastrectomy at the Seoul National University

Hospital between January 1, 1996 and December 31, 1996 (Cohort B). The clinicopathological parameters such as age, sex, histological type, pathological stage, and the presence of lymphatic invasion, lymph node metastasis and distant metastasis were evaluated by reviewing pathological records and medical charts. The mean age of the patients was 56.89 years, and 92.04% of the patients had undergone curative resection (R0 according to the International Union Against Cancer guideline). The clinical outcome was determined from the date of surgery until death or December 31, 2003, which resulted in follow-up periods from 2 to 96 months (mean, 58.88 months). The cases lost to follow-up and deaths caused by reasons other than gastric carcinoma were censored during survival analysis.

5. Tissue array methods

Core tissue biopsy specimens (diameter 2 mm) were obtained from individual paraffin-embedded gastric carcinoma tissues (donor block) and arranged in new recipient paraffin blocks (tissue array blocks) using a trephine apparatus (Superbiochips Laboratories, Seoul, Republic of Korea). Non-neoplastic gastric mucosa specimens were included in each of the tissue array blocks. Each tissue array block contained up to 60 cores.

6. FGFR2 FISH

1) FGFR2 gene amplification

Dual color FISH assays were performed using the locus-specific identifier (LSI) FGFR2 spectrum orange/chromosome enumeration probe (CEP) 10 spectrum green probe (Macrogen, Seoul, Korea). Briefly, slides of 10 gastric carcinoma cell lines and 2 μm-sectioned deparaffinized and dehydrated tissue array slides were incubated in the pretreatment solution of Paraffin Pretreatment kits (Vysis) at 80° C for 30 min. After washing, the slides were treated with protease in protease buffer (from the same kit) at 37° C for 30 min and rinsed with wash buffer at room temperature for 5 min. The DNA probe set was applied onto areas selected based on the presence of tumor foci, and hybridization areas were covered with glass cover slips and sealed with rubber cement. Slides were incubated at 77° C for 6 min to denature chromosomal and probe DNA and then placed in a humidified chamber at 37° C for 16 h to enable hybridization to occur. Slides were then counterstained with 4'6-diamidine-2'-phenylindole dihydrochloride in antifade solution and examined under a fluorescence microscope (Olympus, Tokyo, japan) equipped with a triple band pass filter set (Vysis). After counting at least 40 tumor cell nuclei per

case, FGFR2 gene amplification was determined based on the presence of tight signals of FGFR2 (red) clusters or a ratio of signals of the FGFR2 (red)/CEP-10 (green) greater than or equal to 2.0.

2) FGFR2 gene copy number score

We analyzed FGFR2 gene copy number alteration in gastric carcinoma tissue. Gene amplification and polysomy were defined as per the criteria suggested by Hirsch et al (28).

Fifty nuclei were evaluated, and FGFR2 gene copy number and FGFR2/CEP10 ratio were calculated. The ratio was calculated by dividing the total FGFR2 gene signals by the total number of CEP10 signals. If the ratio was ≥2, FGFR2 gene amplification was observed, and the FISH result was scored with a value of 6. If the ratio was <2, and ≥10% of the tumor cells had 15 or more red signals, the FISH result was scored with a value of 6 and regarded as gene amplification. If the ratio was <2, and ≥40% tumor cells have 4 or more red signals, the FISH result was defined as high polysomy and was scored with a value of 5. If the ratio is <2 and 10%-39% tumor cells had 4 or more red signals, the FISH result was defined as low polysomy and was scored as value of 4. If the ratio was <2 and <10% of tumor cells had 4 or more red signals and ≥40% of tumor cells had 3 red signals, the FISH result was defined as

high trisomy and was scored with a value of 3. If the ratio was <2 and <10% tumor cells had 4 or more red signals and 10%–39% tumor cells had 3 red signals, the FISH result was defined as low trisomy and was scored with a value of 2. If the ratio was <2 and <10% tumor cells had 4 or more red signals and <10% tumor cells had 3 red signals, with most of the tumor cells having 2 or less red signals, the FISH result was defined as disomy and was scored with a value of 1.

7. Laser capture microdissection

This procedure was used to extract DNA from membrane-mounted slides. Briefly, 4 μm paraffin-embedded tissue sections were deparaffinized with xylene. Sections were rehydrated using graded ethanol series, stained with H&E, rinsed in tap water, and finally immersed in 100% ethanol. After drying, the areas of interest were identified. Tumor cells were selectively microdissected using a laser microdissection device (ION LMD, Jung Woo International, Co., Seoul, Korea). For DNA extraction, the dissected cells were collected into 50 μl of DNA extraction buffer, which was composed of 0.5% Tween 20, proteinase K (20 mg/ml), and 50% chelex-100 (Sigma Chemicals).

8. Quantitative real-time PCR

The relative copy number of the FGFR2 gene was determined by quantitative real-time PCR using a PRISM 7500 sequence detection kit (Applied Biosystems, Foster City, CA) and using the TaqMan method (fluorogenic MGB probes). Primers and probes are listed in table 1. PCR was performed using a PCR premix (TaKaRa Bio, Otsu, Shiga, Japan), which contained 500 nmol/L of each primer, 400 nmol/L of each probe, and 100 μL of each appropriately diluted genomic DNA sample in a 20 μL final reaction mixture. After 10 s of incubation at 95° C, gene amplification was performed over 40 cycles (denaturation for 5 s at 95° C and hybridization of probes and primers for 1 min at 60° C). PCRs were performed in duplicate. The standard curve method was used to calculate FGFR2 gene copy numbers in tumor DNA samples relative to the GAPDH gene.

9. Statistical analysis

The chi-square test, Kruskal-Wallis test or Fisher's exact (two-sided) test was used to compare FGFR2 gene status. Survival curves were drawn using the Kaplan Meier product-limit method and the significance of differences between survival curves was determined using the log-rank test.

Multivariate analysis was performed using Cox proportional hazards regression modeling. All statistical tests were two-sided, and statistical significance was accepted for p values of <0.05. The analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Inc., Chicago, IL).

Results

1. FGFR2 gene amplification in gastric cancer cell lines

To investigate whether FGFR2 gene amplification is involved in gastric carcinogenesis, we analyzed FGFR2 gene copy numbers in 10 gastric cancer cell lines using FISH. As shown in Figure 1A, high-level amplification of the FGFR2 gene was found in the SNU-16 cell line. We also performed FISH on gastric cancer cell line KATO, which is known to bear FGFR2 amplification to validate our FISH results(29). The KATO cell line showed FGFR2 gene amplification in our FISH study. In addition, RT-PCR was used to determine FGFR2 mRNA levels in the 10 gastric cell lines, and only SNU-16 showed strong FGFR2 mRNA expression (Figure 1B). We then investigated the FGFR2 protein levels in the 10 cell lines by western blotting and found that SNU-16 also expressed the FGFR2 protein (Figure 1C). These results confirmed that the SNU-16 gastric cancer cell line exhibits FGFR2 gene amplification and that it overexpresses FGFR2 at the mRNA and protein levels.

2. FGFR2 gene amplification in gastric carcinoma tissues

To investigate FGFR2 gene amplification status in gastric carcinoma tissues, FISH was performed on the 313 gastric carcinoma tissues. Ratios of the copy numbers of the FGFR2/CEP-10 were determined and found to range from 0.90 to 10.74 (Figure 2). Of the 313 cases, 14 cases (4.5%) showed FGFR2 amplification (Figure 3).

3. Associations between FGFR2 gene amplification and clinicopathologic findings

To explore the clinicopathological associations of FGFR2 gene amplification, we compared the clinical characteristics in gastric carcinoma patients with or without FGFR2 gene amplification. Table 2 summarizes the clinicopathological differences observed between these two patient groups. In particular, FGFR2 amplification was more frequently found in female patients (p=0.045) and in carcinomas which tended to invade deeper into the stomach wall (p=0.015). Furthermore, all FGFR2 gene-amplified cases were of advanced gastric carcinoma, and FGFR2 gene amplification was significantly associated with a higher pT stage (p=0.023). FGFR2 gene amplification was also significantly associated with the presence of venous (p=0.039) and lymphatic invasions (p=0.012). in total, 13 of the 14 gastric carcinoma cases

(92.9%) with FGFR2 gene amplification had lymphatic invasion, and FGFR2 gene amplification was associated with lymph node metastasis (p=0.038) and distant metastasis (p=0.009). Moreover, 5 of the 14 had distant metastasis at the time of surgery. Finally, FGFR2 gene amplification was significantly related to pathological tumor-node-metastasis (pTNM) stage (p=0.032). On the other hand, no significant association was found between FGFR2 amplification and histological type according to the Lauren (p=0.952) or WHO classifications (p=0.452).

4. Quantitative real-time PCR of FGFR2 in FGFR2-amplified gastric carcinoma tissues

FGFR2 DNA levels in gastric carcinoma tissues were assessed by quantitative real-time PCR, which was performed on all 14 FISH determined FGFR2-amplified gastric carcinoma tissues and on 14 FGFR2 non-amplified tissues. The calculated FGFR2/GAPDH ratios were higher in FISH-positive cases than in negative cases . When a FGFR2/GAPDH ratio of ≥2.0 was taken as positive for FGFR2 gene amplification, 92.6% (26 of 28) of quantitative real-time PCR results matched FISH results. Furthermore, 13 of 14 FGFR2-amplified gastric carcinoma tissues as determined by FISH

were confirmed to be amplified by quantitative real-time PCR. One FGFR2 FISH-negative gastric carcinoma showed a high FGFR2 DNA level by quantitative real-time PCR, whereas the others had a low FGFR2 DNA level by quantitative real-time PCR. A FGFR2/GAPDH gene ratio cutoff of 2.0 yielded a sensitivity of 92.9% and a specificity of 92.9% using FISH results as the standard (Figure 4).

5. FGFR2 amplification affecting the overall survival rate of gastric carcinoma patients

First, we evaluated the overall survival rate of the 313 patients (Cohort A)with the available follow-up data. The mean duration of follow-up was 40.0 months after surgery. During follow-up, 94 of the 313 patients (30.1%) died. To determine whether FISH-determined FGFR2 gene amplification affected survival, we prepared Kaplan-Meier survival curves and analyzed them statistically. In total, 8 of the 14 patients with FGFR2 amplification died of cancer. The median survival for patients with FGFR2 gene amplification was 34.9 months. In comparison, the median survival of patients without FGFR2 amplification was 50.8 months. The overall survival rate of patients with FGFR2 gene amplification, as determined by the log-rank test, was significantly lower than that of patients without FGFR2

gene amplification (p=0.012) (Figure 5).

FGFR2 amplification was not found in early gastric carcinoma cases therefore, we performed survival analysis excluding early gastric carcinoma cases. The survival rate of FGFR2 amplified gastric carcinoma was lower than that of non-amplified gastric carcinoma patients, but this was not statistically significant (p=0.182) (Figure 6A). The results of the evaluation of the survival rate of gastric carcinoma patients in pathological tumor stage 3 or 4 are shown in figure 6B. The survival rate of FGFR2-amplified gastric carcinoma patients was lower than that of FGFR2 non-amplified patients, and this was statistically insignificant (p=0.318)

However, no significant difference was found based on multivariate analysis between patients with a FGFR2-amplified gastric carcinoma and those with a FGFR2 non-amplified gastric carcinoma. pTNM stage was the strongest predictive factor (p<0.001)(Table 3).

6. FGFR2 copy number alteration in gastric carcinoma tissue

The results were obtained from a total of 372 gastric carcinoma cases from patients who underwent gastrectomy in SNUH in 1996 (Cohort B). FGFR2 amplification frequency was 4.0% (15

of 372 cases) and did not differ significantly from the previous FGFR2 gene amplification study of gastric carcinoma patients (Cohort A). FGFR2 polysomy was observed in 10.8% patients of in this group. In summary, FGFR2 gene copy number gain including polysomy and amplification was found in 14.8% (55 cases) of 372 gastric carcinoma tissues (Fugure 7, Table 4).

7. FGFR2 copy number gain of gastric carcinoma and its clinicopathological features

To assess the significance of FGFR2 gene copy number, the clinicopathological parameters were compared with the FGFR2 score. We regarded FGFR2 FISH scores 4, 5, and 6 as FGFR2 gene copy number gain. In this study, the frequency of FGFR2 gene copy number gain was 14.8% (55 of 372 cases). The FGFR2 copy number gain, which includes score 4, 5, and 6, was more common in male patients (p=0.030) and more common in younger age (less than 65 years) (p=0.045) (Table 5). There was no association of FGFR2 copy number gain with histological subtype according to Lauren's classification (p=0.457) and WHO classification (p=0.757), pathological tumor stage (p=0.248), metastasis stage (p=0.115) or tumor location (p=0.215). Although gastric carcinomas with FGFR2 copy number gain showed a higher pN stage, this was statistically

not significant (p=0.106).

In this cohort, the FGFR2 amplification frequency was 4.0% (15 of 372 cases). Table 6 shows the clinicopathological differences between FGFR2-amplified and non-amplified gastric carcinomas from the Cohort B. FGFR2-amplified gastric carcinomas were associated with duffuse-type gastric carcinoma of Lauren classification (p=0.044) and poorly differentiated type according to the WHO classification (p=0.013). Lymph node metastasis (p=0.035) was also associated with FGFR2 amplification in gastric carcinoma. There were no associations of age, sex, pathologic T stage, or pathologic M stage between the cases of FGFR2-amplified and non-amplified gastric carcinomas.

8. FGFR2 copy number gain and patient survival

Results of the univariate analysis revealed that the FGFR2 copy number score was a significant survival parameter (p=0.021) (Figure 8A), and FGFR2 copy number gain (score 4, 5, and 6) was also associated with a low survival rate (p=0.024) (Figure 8B). However, in multivariate analysis, the high FGFR2 copy number gain was not a predictive factor for survival (Table 7). TNM stage and residual tumor after surgery are the significant predictive factors in multivariate analysis. Using Kaplan-Meir

method by log-rank test, FGFR2 amplified gastric carcinoma showed poor survival rate (p=0.003) (Figure 8C). It is a marginally significant survival predictor (p=0.112) in multivariate analysis (Table 8). In high pathological T stages, including pT3 and pT4, FGFR2 gene copy number gain significantly affected patients' survival regardless of pathological T stage (p=0.029) (Figure 9A). The FGFR2 gene amplification was also associated with poor prognosis in high pathological T stages, but was not statistically significant (p=0.079) (Figure 9B).

Table 1. Primers, probes and annealing temperatures used for RT-PCR and quantitative PCR analysis

Method	Gene	Probe	Sequence	Annealing Temp.
RT-PCR	FGFR2	Forward	AGCCAGAAATGTTTTGGTAA	50℃
		Reverse	TCAGCAGCTTAAAAAGTTCC	
	GAPDH	Forward	CCCCGGTTTCTATAAATTGAGC	60℃
		Reverse	CACCTGGCGACGCAAAAG	
Q-PCR	FGFR2	Probe	FAM-TCGTCTAGCCTTTTCTTTT-MGBNFQ	
		Forward	CCCCCTCCACAATCATTCCT	
		Reverse	ACCGGCGGCCTAGAAAAC	
	GAPDH	Probe	VIC-AAAAGAGCTAGGAAGGACAGGCAACTTGGC-TAMRA	
		Forward	CTCCCCACACACATGCACTTAC	
		Reverse	CCTAGTCCCAGGGCTTTGATT'	

Table 2. Comparison of FGFR2 amplified and FGFR2 non-amplified gastric carcinomas according to the clinicopathological features (Cohort A).

Parameter	FGFR2 amp(-) n=299	FGFR2 amp (+) n=14	*p* value
Sex (%)			**0.045**
Male	222 (74.2%)	7 (50.0%)	
Female	77 (25.8%)	7 (50.0%)	
Age			0.393
0-65	199(66.6%)	10 (71.4%)	
66-	100(33.4%)	4 (28.6%)	
Lauren classification			0.952
Intestinal	133 (44.5%)	7 (50.0%)	
Diffuse	109 (36.5%)	5 (35.7%)	
Mixed	54 (18.1%)	2 (14.3%)	
Undetermined	3 (1.0%)	0 (0.0%)	
WHO classification			0.452
Papillary	1 (0.3%)	0 (0.0%)	
WD	25 (8.4%)	0 (0.0%)	
MD	109 (36.5%)	5 (35.7%)	
PD	106 (35.5%)	4 (28.6%)	
Mucinous	9 (3.0%)	2 (14.3%)	
SRC	45 (15.1%)	3 (21.4%)	
UD	3 (1.0%)	0 (0.0%)	
Others	1 (0.3%)	0 (0.0%)	
Blood vessel invasion			**0.039**
Absent	254 (84.9%)	9 (64.3%)	
Present	45 (15.1%)	5 (35.7%)	
Lymphatic invasion			**0.012**
Absent	122 (40.8%)	1 (7.1%)	
Present	177 (59.2%)	13 (92.9%)	

Tumor invasion			0.015
EGC	90 (30.1%)	0 (0.0%)	
AGC	209 (69.9%)	14 (100.0%)	
T class			0.023
T1	90 (30.1%)	0 (0%)	
T2	50 (16.7%)	4 (28.6%)	
T3	103 (34.4%)	4 (28.69%)	
T4a	48 (16.1%)	6 (42.9%)	
T4b	8 (2.7%)	0 (0%)	
N class			0.038
pN0	131 (43.8%)	2 (14.3%)	
pN1	44 (14.7%)	1 (7.1%)	
pN2	38 (12.7%)	3 (21.4%)	
pN3a	46 (15.4%)	6 (42.9%)	
pN3b	40 (13.4%)	2 (14.3%)	
Distant metastasis			0.009
Absent	264 (88.3%)	9 (64.3%)	
Present	35 (11.7%)	5 (35.7%)	
TNM stage			0.032
I	106 (35.5%)	2 (14.3%)	
II	77 (25.8%)	2 (14.3%)	
III	81 (27.1%)	5 (35.7%)	
IV	35 (11.7%)	5 (35.7%)	
Location			0.366
Low	158 (52.8%)	4 (28.6%)	
Middle	74 (24.7%)	5 (35.7%)	
Upper	54(18.1%)	4 (28.6%)	
Whole	13 (4.4%)	1 (7.1%)	

W/D; well differentiated tubular adenocarcinoma, M/D; moderately differentiated tubular adenocarcinoma. P/D; poorly differentiated tubular adenocarcinoma, SRC; signet ring cell carcinoma. U/D; undifferentiated carcinoma.

Table 3. Multivariate Cox proportional hazard models for predictors of overall survival in 313 gastric carcinomas (Cohort A).

Parameters	Odds ratio (95% CI)	*p* value
FGFR2 amplification		0.422
Positive versus negative	1.357(0.644-2.860)	
pTNM stage		<0.001
II versus I	5.817 (1.822-18.570)	
III versus I	30.153 (10.572-86.004)	
IV versus I	44.190 (14.859-131.421)	
Radical surgery		0.003
R1 versus R0	1.913 (0.458-7.982)	
R2 versus R0	2.781 (1.529-5.058)	

CI, confidence interval.

Table 4. FGFR2 gene copy number score in 372 gastric carcinoma tissues according to FISH results

FGFR2 FISH Score	Number of cases	FGFR2 copy number gain	Number of cases
1 (disomy)	238 (64.0%)	Absent	317
2 (low trisomy)	67 (18.0%)		(85.2%)
3 (high trisomy)	12 (3.2%)		
4 (low polysomy)	36 (9.7%)	Present	55
5 (high polysomy)	4 (1.1%)		(14.8%)
6 (amplification)	15 (4.0%)		
Total		372	

Table 5. Comparison of gastric carcinomas with FGFR2 copy number gain and absent of FGFR2 gene copy number gain according to clinicopathological features (Cohort B)

	FGFR2 gene copy number gain			*p* value
	Absent	Present		
	Score 1,2,3	Score 4,5,6	Total	
Total	317	55	372	
Sex				**0.030**
Male	213 (67.2%)	45 (81.8%)	258 (69.4%)	
Female	104 (32.8%)	10 (18.2%)	114 (30.6%)	
Age				**0.045**
0–65	247 (78.2%)	36 (65.5%)	283 (76.1%)	
66–	70 (21.8%)	19 (34.5%)	89 (23.9%)	
Lauren classification				0.425
Intestinal	152 (47.9%)	25 (45.5%)	177 (47.6%)	
Diffuse	155 (48.7%)	26 (47.3%)	181 (48.7%)	
Mixed	10 (3.2%)	4 (7.3%)	14 (3.8%)	
WHO classification				0.757
W/D	14 (4.4%)	3 (5.5%)	17 (4.6%)	
M/D	147 (46.4%)	25 (45.5%)	172 (46.2%)	
P/D	102 (32.2%)	16 (29.1%)	118 (31.7%)	
Mucinous	28 (8.8%)	5 (9.1%)	33 (8.9%)	
SRC	26 (8.2%)	6 (10.9%)	32 (8.6%)	
Tumor invasion				0.387
EGC	93 (29.3%)	13 (23.6%)	106 (28.5%)	
AGC	224 (70.7%)	42 (76.4%)	266 (71.5%)	

T class				0.248
T1	67(21.1%)	6 (10.9%)	73 (19.8%)	
T2	26 (8.2%)	7 (12.7%)	33 (8.9%)	
T3	118 (37.2%)	23 (41.8%)	141 (37.9%)	
T4a	98 (30.9%)	19 (34.5%)	117 (31.5%)	
T4b	8 (2.5%)	0 (0.0%)	8 (2.2%)	
N class				0.106
N0	116 (36.6%)	14 (25.5%)	130 (34.9%)	
N1	49 (15.5%)	13 (23.6 %)	62 (16.7%)	
N2	63 (19.9%)	6 (10.9%)	69 (18.5%))	
N3a	51 (16.1%)	11 (20.0%)	62 (16.7%)	
N3b	38 (12.0%)	11 (20.0%)	49 (13.2%)	
Distant metastasis				0.115
Absent	300 (94.6%)	49 (89.1%)	349 (93.8%)	
Present	17 (5.4%)	6 (10.9%)	23 (6.2%)	
Lymphatic invasion				0.257
Absent	204 (64.4%)	31 (56.4%)	235 (63.2%)	
Present	113 (35.6%)	24 (43.6%)	137(36.8%)	
Blood vessel invasion				0.716
Absent	298 (94.0%)	51 (92.7%)	349 (93.8%)	
Present	19 (6.0%)	4 (7.3%)	23 (6.2%)	
Location				0.215
Low	191 (60.3%)	40 (74.1%)	231 (62.3%)	
Middle	59 (18.6%)	4 (7.4%)	63 (17.0%)	
Upper	38 (12.0%)	6 (11.1%)	44 (11.9%)	
whole	29 (9.1%)	4 (7.4%)	33 (8.9%)	

Table 6. Comparison of FGFR2 amplified and FGFR2 non-amplified gastric carcinomas according to clinicopathological features (Cohort B).

	FGFR2			*p* value
	Non-amplification Score 1~5	Amplification score 6	Total	
Total	357	15	372	
Sex				0.169
Male	250 (70.0%)	8 (53.3%)	258 (69.4%)	
Female	107 (30.0%)	7 (46.7%)	114 (30.6%)	
Age				0.383
0-65	273 (76.5%)	10 (66.7%)	283 (76.1%)	
66-	84 (23.5%)	5 (33.3%)	89 (23.9%)	
Lauren classification				**0.044**
Intestinal	174 (48.7%)	3 (20.0%)	177 (47.6%)	
Diffuse	169 (47.3%)	12 (80.0%)	181 (48.7%)	
Mixed	14 (3.9%)	0 (0.0%)	14 (3.8%)	
WHO classification				**0.013**
W/D	17 (4.8%)	0 (0.0%)	17 (4.6%)	
M/D	170 (47.6%)	2 (13.3%)	172 (46.2%)	
P/D	110 (30.8%)	8 (53.3%)	118 (31.7%)	
Mucinous	30 (8.4%)	3 (20.0%)	33 (8.9%)	
SRC	30 (8.4%)	2 (13.3%)	32 (8.6%)	
Tumor invasion				0.149
EGC	104(29.1%)	2(13.3%)	106(28.5%)	
AGC	253 (70.9%)	13(86.7%)	266(71.5%)	

T class				0.406
T1	71 (19.9%)	2 (13.3%)	73 (19.6%)	
T2	33 (9.2%)	0 (0.0%)	33 (8.9%)	
T3	133 (37.3%)	8 (53.3%)	141(37.9%)	
T4a	112 (31.4%)	5 (33.3%)	117(31.5%)	
T4b	8 (2.2%)	0 (0.0%)	8 (2.2%)	
N class				0.035
N0	129 (36.1%)	1 (6.7%)	130(34.9%)	
N1	57 (16.0%)	5 (33.3%)	62 (16.7%)	
N2	66 (18.5%)	3 (20.0%)	69(18.5%))	
N3a	62 (17.4%)	0 (0.0%)	62 (16.7%)	
N3b	43 (12.0%)	6 (40.0%)	49 (13.2%)	
Distant metastasis				0.235
Absent	336 (94.1%)	13 (86.7%)	349(93.8%)	
Present	21 (5.9%)	2 (13.3%)	23 (6.2%)	
Lymphatic invasion				0.058
No	229 (64.1%)	6 (40.0%)	235 (63.2%)	
Yes	128 (35.9%)	9 (60.0%)	137(36.8%))	
Blood vessel invasion				0.235
No	336 (94.1%)	13 (86.7%)	349 (93.8%)	
Yes	21 (5.9%)	2 (13.3%)	23 (6.2%)	
Location				0.225
Lower	219 (61.3%)	12 (85.7%)	231 (62.3%)	
Middle	63 (17.6%)	0 (0.0%)	63 (17.0%)	
Upper	43 (12.0%)	1 (7.1%)	44 (11.9%)	
Whole	32 (9.0%)	1 (7.1 %)	33 (8.9%)	

Table 7. Multivariate analysis of predictive factors including FGFR2 copy number gain for survival in Cohort B (Cox proportional hazards model)

Parameters	Odds ratio (95% CI)	*p* value
FGFR2 copy number gain		0.491
Positive versus negative	1.156(0.766−1.745)	
pTNM stage		<0.001
II versus I	5.149 (1.792−14.800)	
III versus I	20.492 (5.286−79.435)	
IV versus I	22.394 (8.237−60.884)	
Radical surgery		0.001
R1 versus R0	2.750 (1.219−6.206)	
R2 versus R0	3.959 (1.542−10.167)	

CI, confidence interval.

Table 8. Multivariate analysis of predictive factors including FGFR2 amplification for survival in Cohort B (Cox proportional hazards model)

Parameters	Odds ratio (95% CI)	*p* value
FGFR2 amplification		0.112
Positive versus negative	1.658(0.889−3.091)	
pTNM stage		<0.001
II versus I	5.195 (1.808−14.928)	
III versus I	20.964 (5.456−80.551)	
IV versus I	22.181 (8.158−60.308)	
Radical surgery		0.001
R1 versus R0	2.787 (1.233−6.296)	
R2 versus R0	4.023 (1.616−10.020)	

CI, confidence interval.

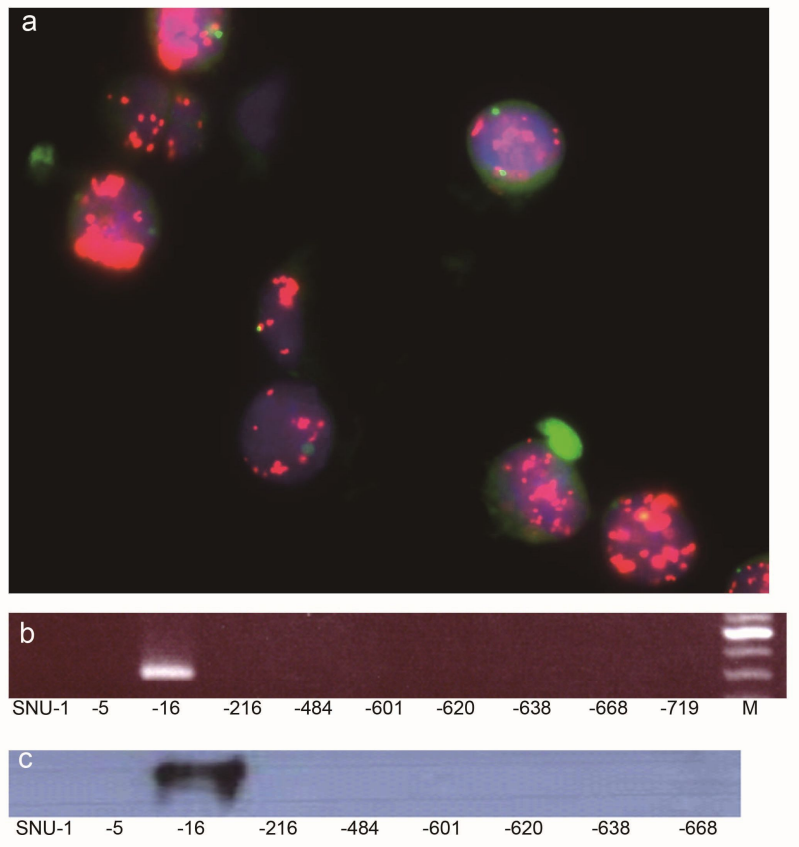


Figure 1. FGFR2 gene status in gastric carcinoma cell lines.

A) FISH validation shows high-level amplification of target probe (red signal) to chromosome enumeration probe (CEP) 10 (green signal) in SNU-16 (ratio of green to red signal: 9.0). B) RT-PCR demonstrates strong RNA expression in SNU-16 (281bp). C) FGFR2 protein (120kD) is strongly expressed in SNU-16 cell lines in western blot analysis.

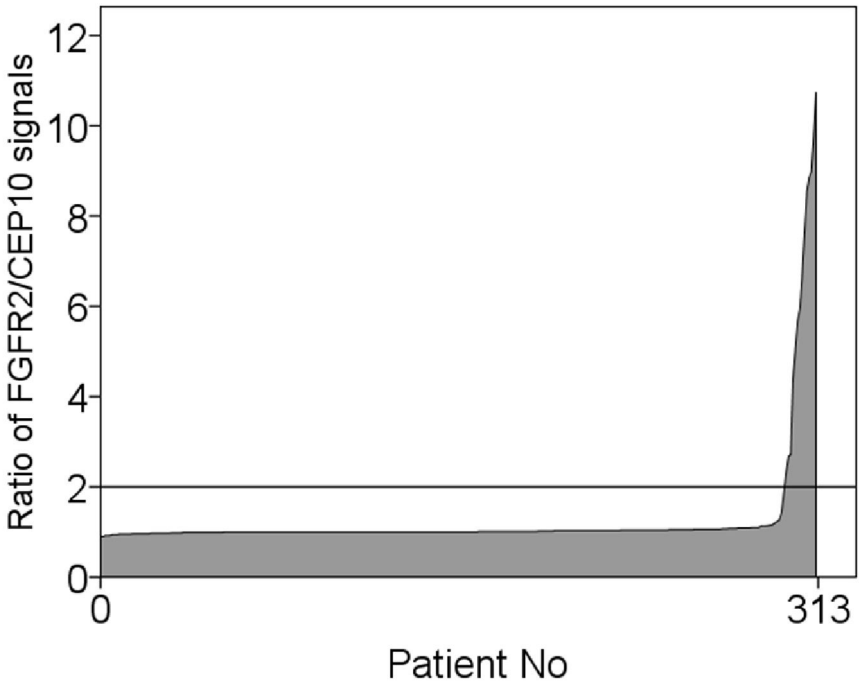


Figure 2. Ratios of signals of the FGFR2 (red)/CEP10 (green) distribution. FGFR2 gene amplification was defined by a ratio of signal of FGFR2/CEP 10 was ≥2.0. Fourteen of 313 cases were categorized as FGFR2 gene amplified gastric carcinoma.

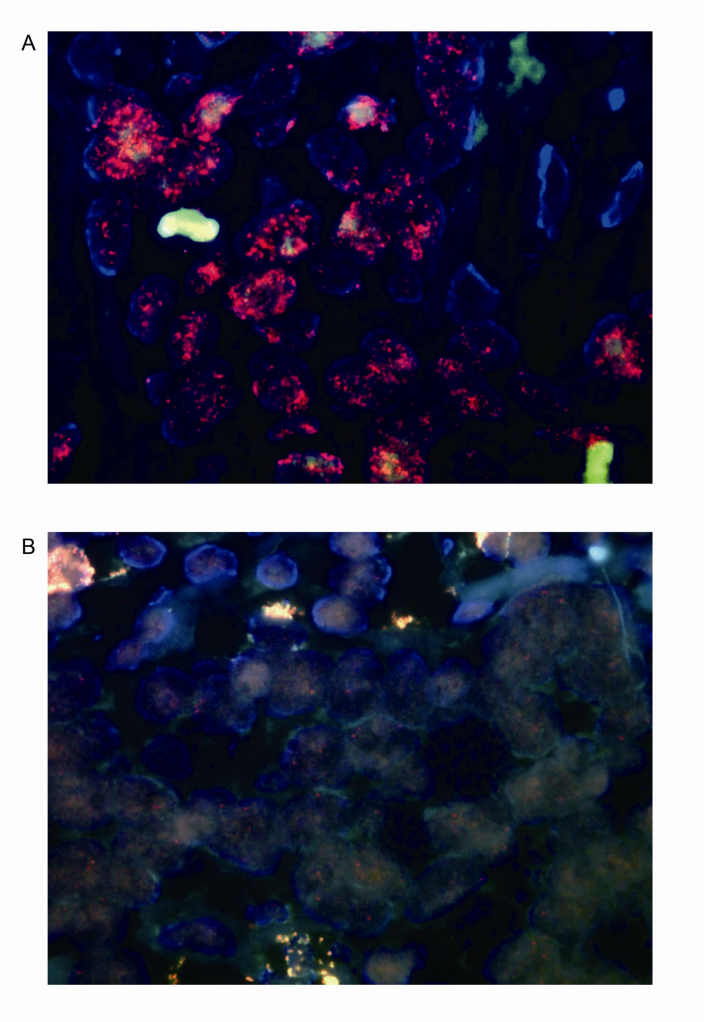


Figure 3. Fluorescence in situ hybridization analyses of FGFR2 gene status in gastric carcinoma tissues. A) FISH analysis of gastric carcinoma with FGFR2 gene amplification. The ratio of red signals of FGFR2/ green signals of CEP10 was more than 2. B) FISH analysis of gastric carcinoma without FGFR2 gene amplification. The ration of red signals of FGFR2/green signals of CEP10 was 1.0. (Original magnification X1000)

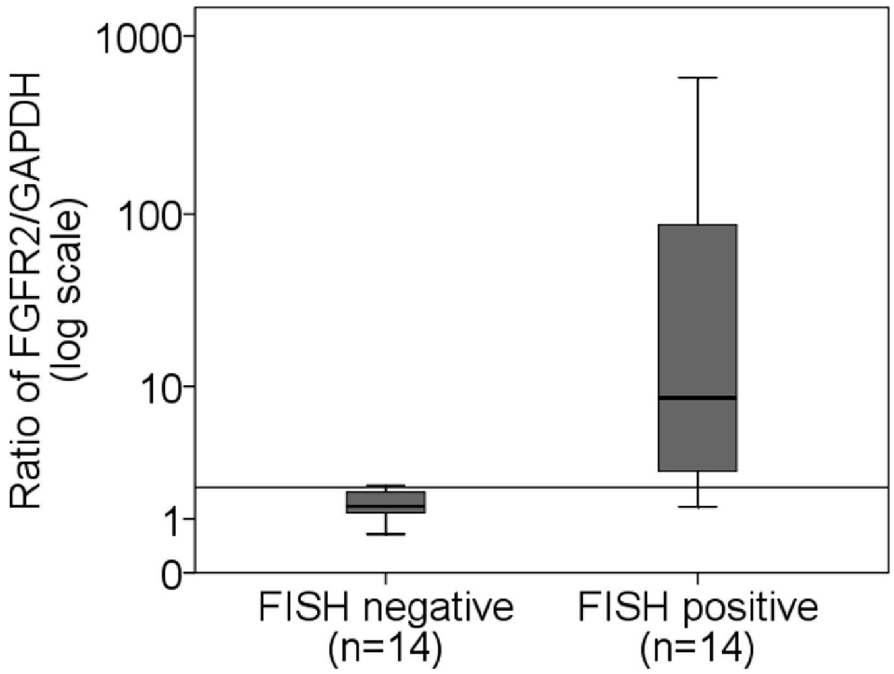


Figure 4. Scatter plot of FGFR2/GAPDH ratios determined by quantitative real-time PCR for patients with positive and negative FISH results. The horizontal line indicates the optimal cutoff for the FGFR2/GAPDH ratio, 2.0.

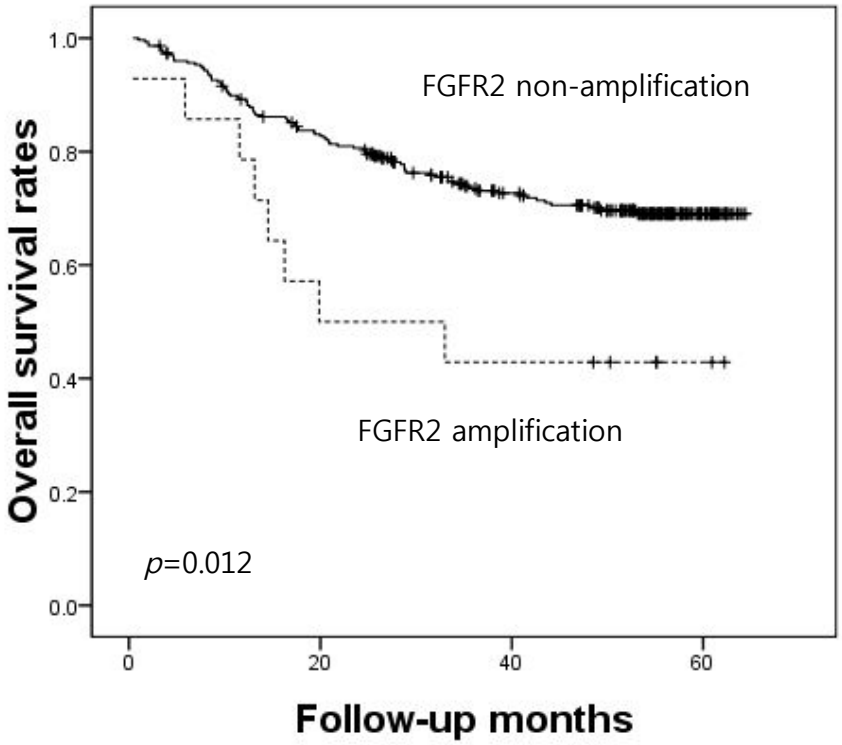
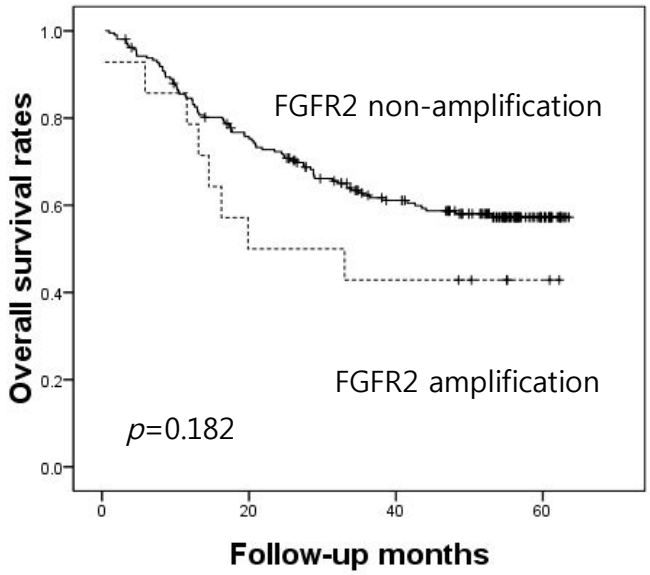


Figure 5. The Survival curves of Kaplan-Meier method by log-rank test in 313 gastric carcinomas (Cohort A). Survival curve shows that FGFR2 gene amplified cancer has an unfavorable prognosis compared with non-amplified cancer (p=0.012). Solid line, FGFR2 non-amplified cases; broken line, FGFR2 amplified cases.

A


Overall survival rates
1.0
0.8
0.6
0.4
0.2
0.0
FGFR2 non-amplification
FGFR2 amplification
p=0.182
0
20
40
60
Follow-up months

B

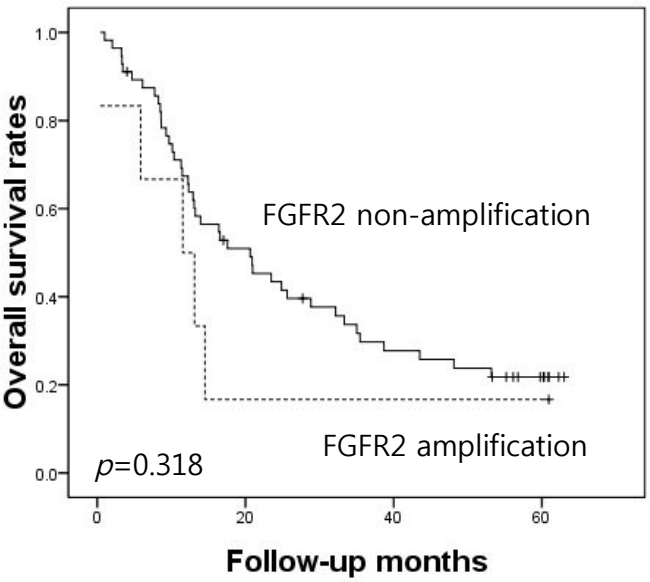
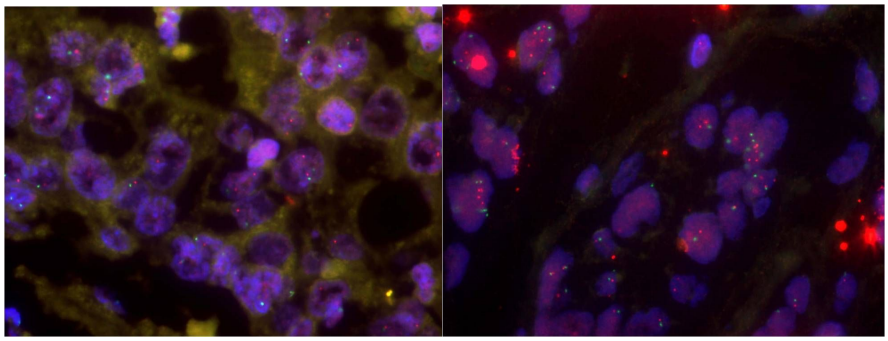

Overall survival rates
1.0
0.8
0.6
0.4
0.2
0.0
FGFR2 non-amplification
FGFR2 amplification
p=0.318
0
20
40
60
Follow-up months

Figure 6. The survival curves of Kaplan-Meier method by log-rank test in advanced disease (Cohort A).

A). The Survival curves of Kaplan-Meier method by log-rank test in advanced gastric carcinoma. Survival curve shows that FGFR2 gene amplified cancer has an unfavorable prognosis compared with non-amplified cancer, but there was no statistically significance (p=0.182).

B). Kaplan-Meier survival analysis of FGFR2 amplification at pathologic T stage 3 or 4. Survival curve shows that FGFR2 gene amplified cancer has an unfavorable prognosis compared with non-amplified cancer, but there was no statistically significance (p=0.318). Solid line, FGFR2 non-amplified cases; broken line, FGFR2 amplified cases.

A B



C D

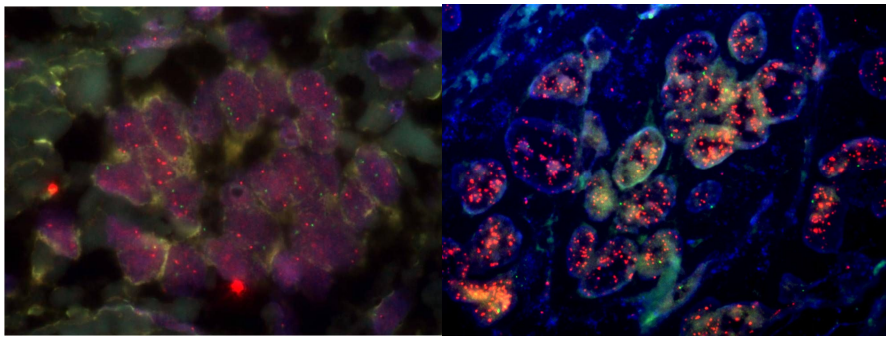
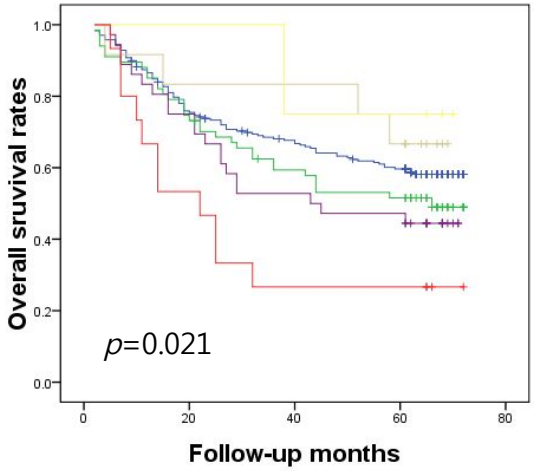


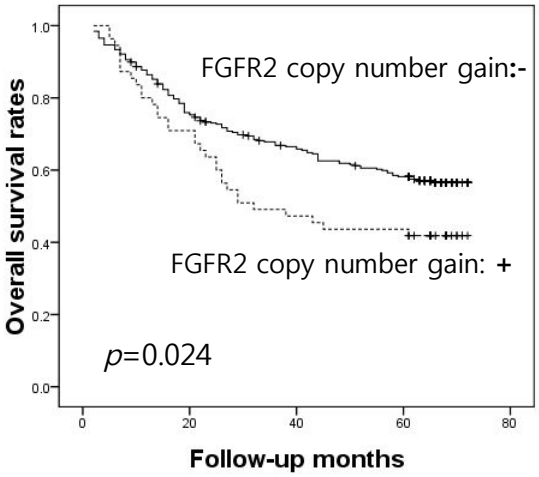
Figure 7. Fluorescence in situ hybridization analysis of FGFR2 gene status in 372 gastric carcinoma tissues (Cohort B).

A) FGFR2 gene disomy. B) FGFR2 gene low polysomy. C) FGFR2 gene high polysomy. D) FGFR2 amplification. (Original magnification X1000)

A


Overall sruvival rates
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Follow-up months
p=0.021

B


Overall survival rates
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Follow-up months
FGFR2 copy number gain:-
FGFR2 copy number gain: +
p=0.024

C

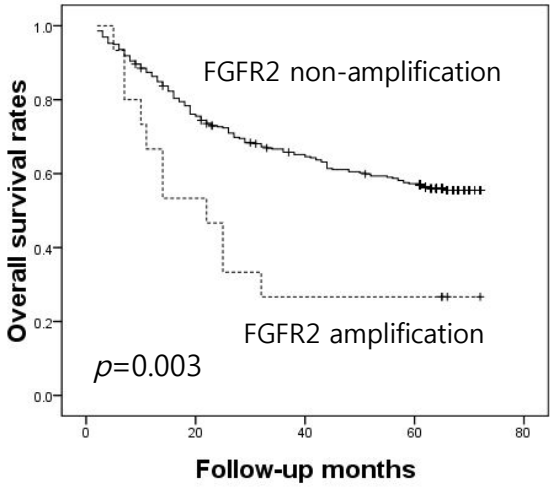

Overall survival rates
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Follow-up months
FGFR2 non-amplification
FGFR2 amplification
p=0.003

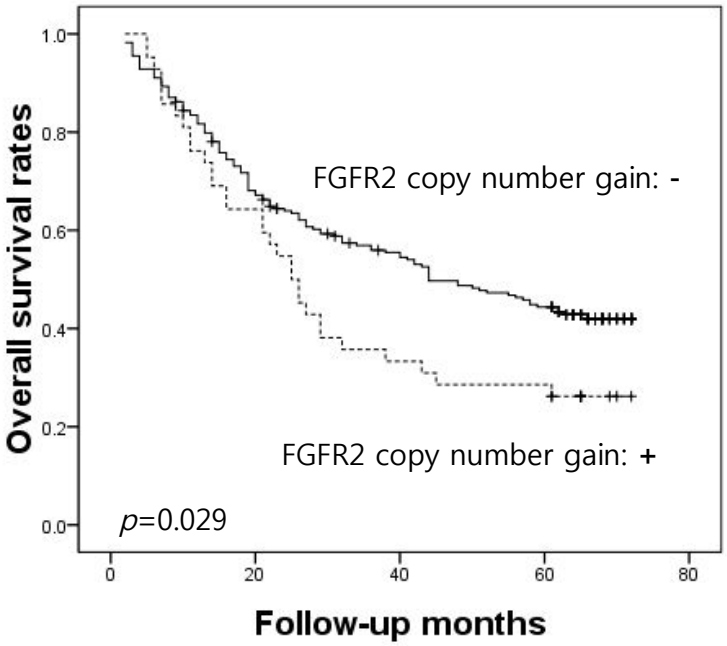
Figure 8. The Survival curves of Kaplan-Meier method by log-rank test in 372 gastric carcinomas (Cohort B)

A) Survival rate differences were found according to FGFR2 gene copy number score (p=0.021). Blue line, disomy; green line, low trisomy; olive green line, high trisomy; purple line, low polysomy; yellow line, high polysomy; red line, amplification

B) Survival curve shows that gastric carcinoma patient with FGFR2 gene copy number gain has an unfavorable prognosis compared with absent of FGFR2 copy number gain (p=0.024).

C) Survival curve shows that FGFR2 amplified gastric carcinoma has an unfavorable prognosis compared with non-amplified cancer (p=0.003). Solid line, FGFR2 non-amplified cases; broken line, FGFR2 amplified cases.

A


Overall survival rates
1.0
0.8
0.6
0.4
0.2
0.0
FGFR2 copy number gain: -
FGFR2 copy number gain: +
p=0.029
0
20
40
60
80
Follow-up months

B

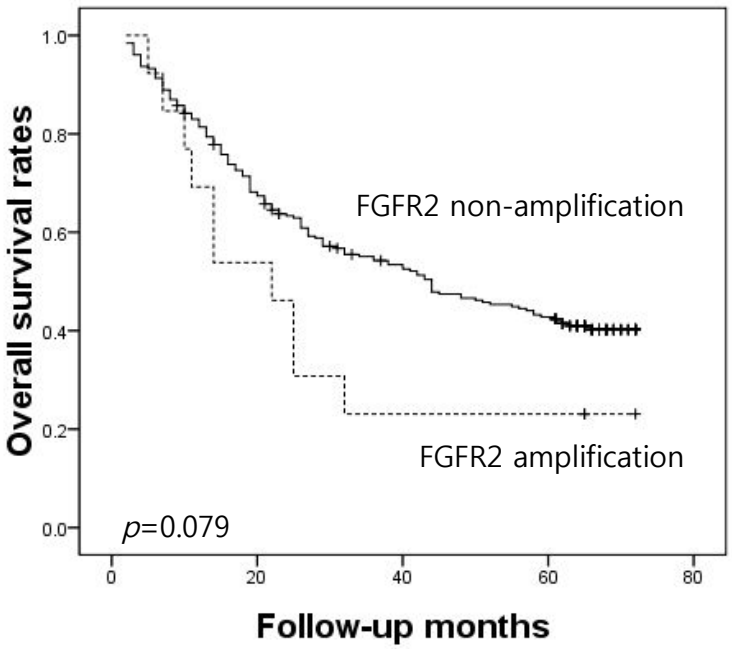

Overall survival rates
1.0
0.8
0.6
0.4
0.2
0.0
FGFR2 non-amplification
FGFR2 amplification
p=0.079
0
20
40
60
80
Follow-up months

Figure 9. Kaplan-Meier survival analysis with gastric carcinomas of pathologic T stage 3 or 4 (Cohort B)

A). Survival curve shows that cases with FGFR2 copy number gain has an unfavorable prognosis compared with absent of FGFR2 copy number gain (p=0.029).

B). Survival curve shows that FGFR2 gene amplified cancer has an unfavorable prognosis compared with non-amplified cancer, but there was no statistical significance (p=0.079). Solid line, FGFR2 non-amplified cases; broken line, FGFR2 amplified cases.

Discussion

Recently, the FGFR gene has become an attractive therapeutic target, and several small molecule inhibitors against FGFRs are currently undergoing clinical trials. Endometrial cancer cell lines with activating FGFR2 mutations have been reported to be selectively sensitive to the pan-FGFR inhibitor PD173074 in an *in vitro* study, and Ki23057, a broad-range tyrosine kinase inhibitor, has been reported to inhibit FGFR1, FGFR2 and VEGF2 tyrosine kinases. Ki23057 has also been reported to inhibit the proliferation of gastric cancer cells exhibiting FGFR2 gene amplification (30). The FGFR inhibitor, AZD4547 effectively inhibited the phospholylation of FGFR2 and its downstream signaling molecules and apoptosis in an FGFR2-amplified gastric cancer cell line and significant dose dependent tumor growth inhibition in FGFR2 amplified xenograft (31). In a preclinical study of gastric carcinoma, FGFR2 amplification was associated with increased tumor cell proliferation and tumor cell survival. FGFR2-amplified gastric carcinoma cells also showed sensitivity to drugs targeting the FGFR pathway, such as the FGFR selective small molecule inhibitors AZD4547 and BGJ398 and anti FGFR2 antibodies (32-34).

In gastric carcinoma, there have been several reports of FGFR2 amplification or overexpression. However, reported

incidences of FGFR2 overexpression vary widely. In a series of gastric carcinoma patients, the results of immunohistochemical staining and in situ hybridization revealed that 20 of 38 undifferentiated-type gastric carcinomas overexpressed the FGFR2 protein, but none of the 11 well-differentiated subtypes overexpressed the FGFR2 protein. These result suggests that the overall incidence of FGFR2 overexpression in gastric carcinoma is as high as 40% (35). Other studies reported FGFR2 gene amplification in approximately 3%-12.5% of gastric carcinomas and that FGFR2 gene amplification is associated with a poorly differentiated subtype (35-37). In addition, each study employed a different method to assess FGFR2 gene status, including RT-PCR, SNP arrays, IHC, or FISH.

Gene copy number evaluation in tumor cells using FISH is the widely accepted gold-standard method. In this study, we evaluated FGFR2 gene status in gastric cancer cell lines and gastric carcinoma tissues using FISH. Additionally, we performed quantitative real-time PCR and compared the results with FISH results. We also examined the relationships between FGFR2 gene copy number alteration and clinicopathological features and evaluated the prognostic implications of FGFR2 gene copy number gain and amplification in gastric carcinoma

patients.

Frequency of FISH-determined FGFR2 amplification was 4.5% in Cohort A. FGFR2 gene amplification was more frequent in female patients and was found to be associated with blood vessel and lymphatic invasion. FGFR2 amplification was also significantly associated with lymph node metastasis and distant metastasis, and all 14 cases of FGFR2-amplified gastric carcinoma patients were advanced gastric carcinoma.

In the Cohort B, the FGFR2 amplification rate was 4.0%. The frequency of FGFR2 gene copy number gain, including polysomy and amplification, was 14.8%. FGFR2 amplification was associated with histological type and pathologic N stage and lymphatic invasion. Previously, it has been reported that FGFR2 gene overexpression is frequently found in diffuse-type gastric carcinoma and that FGFR2 gene amplification is found in gastric carcinoma of the mucinous type and in poorly differentiated gastric carcinoma (35, 37). In the present study, five FGFR2 amplified cases (35.7%) were of the diffuse type and two (14.3%) were of the mixed type of Lauren's histological classification in Cohort A. No significant association was found between histological subtype and FGFR2 gene amplification in the Cohort A. However, in the Cohort B, WHO classification and Lauren histological type were statistically

significantly associated with FGFR2 amplification.

These results of Cohort B differ from the results of the Cohort A. This may be a consequence of the fact that FGFR2 amplification is a rare event in gastric carcinoma and that this causes selection bias in studies on the association between FGFR2 gene status and pathological characteristics.

In clinical practice, the intratumoral heterogeneity of HER2 amplification is a common observation, and the frequency of intratumoral HER2 heterogeneity in gastric carcinoma has been reported previously (38, 39). Additionally, there was also been a report of FGFR2 amplification heterogeneity (40).

In the present study, we evaluated FGFR2 status using the TMA method. Therefore, these conflict with the results from two gastric carcinoma patients group, which could be due to tumor heterogeneity or sampling bias. These studies indicate the need for larger tissue samples and more detailed guidelines for the assessment of FGFR2 gene status.

In a recent study of FGFR2 amplification, there was a report that FGFR2 gene amplification was found in metastatic lesions but not in corresponding primary lesions in gastric carcinoma(37). Our study also showed that FGFR2 amplification is associated with distant metastasis (p=0.009) in the Cohort A. These results suggest that FGFR2 gene amplification

contributes to the development of metastasis.

In survival analysis, FGFR2 gene amplification has prognostic implications in gastric carcinoma patients. FGFR2 gene copy number gain was also significantly associated with lower survival rate in gastric carcinoma patients. This finding suggests that FGFR2 gene copy number gain can be a predictor of clinical outcome.

These results agree with those of other previous studies, which were conducted on 20 advanced gastric carcinoma tissues, in which FGFR mRNA and bFGF double positive gastric carcinomas were found to more frequently exhibit serosal invasion and to be associated with poor survival (41). In 136 gastric carcinoma tissues, FGFR2 expression was found to be positively associated with poor prognosis (42).

In summary, although FGFR2 amplification was not found to be a strong independent prognostic factor based on multivariate analysis, our results revealed that FGFR2 gene amplification and gene copy number gain are meaningful prognostic factors in gastric carcinoma patients.

In the present study, we also performed HER-2 and c-MET FISH analysis, but co-amplification of FGFR2 with HER2/c-MET was not found. In another previous study, two gastric carcinoma cases showed FGFR2 and HER2 co-amplification,

however, FGFR2 and HER2 amplification were found in different tumor cells (40). These findings reflect the multiple genetic and epigenetic abnormalities exhibited by cancer cells, but despite this complexity, cancer cell survival and growth can often be impaired by the inactivation of a single oncogene (43).

In the present study, we used FISH, which is considered the gold standard for the detection of gene amplification. However, FISH is expensive and time consuming when a large series of samples must be analyzed; and thus, we also assessed FGFR2 gene amplification using quantitative real-time PCR and then compared PCR to FISH results. Quantitative real-time PCR results were found to be correlated with FISH results (p<0.001), with high sensitivity (92.9%) and specificity (92.9%). In fact, only 2 of the 28 gastric carcinoma cases examined showed discrepant results. In particular, one case reported positive by FISH was proved a false negative by the quantitative real-time PCR method, with a low FGFR2/GAPDH ratio, which may have been a sampling error due to heavy normal cell contamination or DNA degradation. Therefore, although FISH remains the standard for determining gene amplification status, quantitative real-time PCR may provide an alternative means for determining the presence of FGFR2 gene amplification. More experience with DNA extraction is needed

before judgments can be made on the value of quantitative real-time PCR.

In summary, we examined FGFR2 amplification in the largest number of gastric carcinoma tissues, and the rate of FGFR2 gene amplification in all samples was found to be 4.0%-4.5%, and FGFR2 amplification was found to be strongly associated with tumor invasion depth, lymph node metastasis, and distant metastasis, which indicates that the FGFR2 gene plays an important role in gastric carcinoma progression and metastasis. Currently, clinical trials are being undertaken on selective FGFR2 inhibitors in gastric carcinoma patients. We hope that our findings aid patient selection and the interpretation of clinical trial results.

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국문초록

서론: FGFR2 는 발생과정 중에 배아의 성장 및 분화를 조절하는 인자이다. 이전의 여러 연구에서 FGFR2 의 과 발현, 증폭 및 변이는 인간의 다양한 암 종에서 관찰되었다. 또한 최근 여러 암에서 표적치료제의 개발이 진행되고 있으며 위암에서는 항 FGFR2 약제가 암의 진행을 억제한다고 보고된바 있다. 아직도 위암은 아시아에서는 높은 사망률을 보이는 암종이다. 이 연구에서는 위암조직에서 FGFR2 유전자증폭의 빈도를 조사하고 임상병리학적 특성 및 예후에 미치는 영향을 알아보았다.

방법: 수술적 방법으로 암 절제술을 받은 313 명의 위암환자에서 채취한 조직으로 FGFR2 형광 제자리 부합 법을 시행하여 FGFR2 유전자의 증폭을 조사하였다. 추가로 정량적 중합효소 연쇄반응 실험을 시행하여 두 가지 방법으로 얻은 결과를 비교하였다. 다음으로 또 다른 372 명의 환자군 에서 얻은 조직으로 FGFR2 유전자증폭 및 복사 개수 이득을 조사하였다. 다음으로 FGFR2 유전자의 증폭 및 복사 개수 이득을 보이는 위암의 임상병리학적 특성을 분석하고 생존분석을 시행하여 예후에 미치는 영향을 연구하였다.

연구결과: 첫 번째 실험 군에서 FGFR2 유전자의 증폭은 313 예의 위암환자 중에 14예에서 (4.5%) 관찰되었으며 더 깊은 침윤 (p=0.023), 더 빈번한 림프절전이 (p=0.038) 및 원격전이 (p=0.009)와 관련성을 보였다. 생존분석결과 FGFR2 유전자의 증폭은 위암환자의 낮은 생존율과 관련이 있었다. 두 번째 실험

군에서 FGFR2 유전자의 복제 개수 획득은 372예의 위암조직에서 14.8%인 55예 에서 관찰되었으며, 생존분석에서 낮은 생존율과 관련성이 있었다. FGFR2 유전자증폭은 372예 중 15예 (4.0%)에서 관찰되었으며 위암의 조직학적 분류 및 림프절전이와 관련성을 보였다. 생존분석에서 FGFR2 유전자증폭은 첫 번째 환자군과 마찬가지로 낮은 생존율과 관련성을 보였다. 추가로 시행한 정량적 중합효소 연쇄반응 실험결과 FGFR2 유전자증폭의 진단에 92.9%의 민감도 및 특이도를 보였다.

결론: 위암에서의 FGFR2 유전자증폭은 진행성 위암에서 더 빈번히 관찰되었으며 위암의 침윤 및 림프절전이 원격전이와 관련성이 있었다. 최근까지도 진행성 위암은 높은 사망률을 보이고 있으며 이러한 환자군 에서 예후 향상을 위한 표적치료제 개발이 진행 중에 있다. 특히 최근 위암에서 항 FGFR2 약제가 개발 중이며 이 연구결과는 항 FGFR2 약제를 이용한 치료에 민감한 진행성 위암 환자군 선별 및 결과해석에 도움을 줄 수 있을 것으로 생각된다. 궁극적으로는 이 연구가 진행성 위암환자의 예후향상에 기여할 것으로 기대한다.

주요어: 위암, 예후, FGFR2, 생존분석, 암유전자, 증폭, 형광 제자리 부합법

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