Incidence of FGFR2 Amplification and FGFR2 Fusion in Patients with Metastatic Cancer Using Clinical Sequencing

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Aberrations in the fibroblast growth factor receptor2 (FGFR2) gene, including genetic alterations and chromosomal rearrangements, lead to the development and progression of cancer with poor prognosis. However, the mechanisms underlying the FGFR2 signaling pathway to facilitate the development of FGFR2-targeted therapies have not been fully explored. Here, we examined the clinicopathological features of FGFR2 amplification and fusion in gastrointestinal tract/genitourinary tract cancers. FGFR2 amplification and fusion were identified in approximately 1.5% and 1.1% of all cancer types in 1,373 patients, respectively, with both FGFR2 amplification and fusion occurring together at a rate of approximately 0.6%. Of all cancer types screened, gastric cancer (GC) was the most common cancer type with FGFR2 amplification (87.5% of all FGFR2 amplification case) or fusion (46.7% of all cases). In addition, FGFR2 alteration had poorer overall survival (OS, 13.7 months vs. 50.2 months, P = 0.0001) and progression-free survival (PFS, 5.6 months vs. 11.4 months, P = 0.0005) than did those without FGFR2 alteration, respectively. Taken together, our data underscore to screen solid cancer patients for FGFR2 aberrations in oncology clinic.

1. Introduction

Fibroblast growth factor receptor 2 (FGFR2), which belongs to a family of highly conserved tyrosine kinase receptors (FGFR 1–4), has emerged as a critical oncogenic factor that controls cancer development and progression [1–4]. However, the underlying mechanisms of action of FGFR2 are not fully understood [2, 5]. Under normal conditions, the FGFR system contributes to the regulation of several developmental processes, including the induction of organogenesis and homeostasis [4, 6]. Dysfunction of the FGFR system results in uncontrolled cell proliferation, migration, and survival, leading to cancer [4, 6, 7]. Although FGFR2 aberrations, including gene amplification, fusion, mutation, and overexpression, have been detected in various types of cancer [3, 8–13], clinically available treatment options are limited.

Accumulating evidence indicates the significance of FGFR2 in the tumorigenesis and progression of gastric cancer (GC); genomic aberrations in FGFR2 have been linked to one of the most frequently occurring oncogenic aberrations [2, 14–17]. Although the incidence of FGFR2 aberrations is relatively low, it is associated with a poor prognosis in GC [18]. Alterations in FGFR genes, such as amplification,
mutation, and fusion, can lead to the aberrant activation of downstream components of FGFR-related pathways and induce mesenchymal transition as well as antia apoptotic responses in cancer cells [9, 19–21]. Based on recent studies involving selective pharmacological inhibition of FGFR2 in a model of FGFR2 amplification, this receptor is a promising therapeutic target in solid tumors, especially in GC [15, 17, 22].

Several clinical trials of FGFR-targeted therapy, including FGFR2, have been conducted in patients with GC [23–25]. In a recent phase 2 study, the addition of the anti-FGFR2 antibody to first-line chemotherapy demonstrated superior survival in FGFR2-overexpressed GC patient cohort (the FIGHT trial) when compared to chemotherapy alone [26].

We analyzed the clinicopathological features of FGFR2 aberrations, including amplification and fusion, in patients with solid tumors who received clinical next-generation sequencing (NGS) as part of the clinical practice. In this study, we aimed to demonstrate the incidence and prevalence of FGFR aberrations using a clinical NGS platform.

2. Results

2.1. Patient Characteristics. Between November 2019 and January 2021, 1,373 oncology patients diagnosed with metastatic solid tumors received a clinical sequencing panel at Samsung Medical Center. NGS was performed with TruSight Oncology 500 (TSO500) assay (Illumina, San Diego, CA, USA) as described previously [27–29]. The panel includes multiple variant types across 523 genes and enables quantitative assessment of MSI and TMB status. The patient cohort consisted of 1,373 patients with 24 different types of solid tumors. The most common cancer types were colorectal cancer (CRC, 452 cases, 32.9%), GC (327 cases, 23.8%), and sarcoma (139 cases, 10.1%) (Figure 1). In all, 28 patients (N = 28, 2.0%) had FGFR2 aberrations detected in their tumor by NGS panel. Of the 28 patient, 21 (1.5%) patients had FGFR2 amplification, and 15 (1.1%) patients had FGFR2 fusion alone. Of note, 8 (0.6%) patients had both FGFR2 amplification and FGFR2 fusion in their tumor specimen.

2.2. FGFR2 Amplification Was Predominantly Detected in Gastric Cancer. Of the 21 patients with FGFR2 amplification, 18 (85.7%) had GC, followed by sarcoma (N = 2, 9.5%) and CRC (N = 1, 4.8%). For GC patients, the incidence of FGFR2 amplification, FGFR2 fusion, and the cooccurrence of FGFR2 amplification and fusion were 18 (5.5%), 7 (2.1%), and 7 (2.1%) patients, respectively. The median age of all patients was 48 years (range, 20–60 years) (Figures 2(a) and 2(d)). There were no cases of FGFR2 deletion in our cohort. Of note, patients with FGFR2 amplification had MSS and TMB low (less than 10 mutations/mB), indicating that FGFR2 amplification predominantly occurs in the MSS subtype. In the FGFR2-amplification cohort (N = 28), the copy number ranged from 4.3 to 274 (Figure 2(b)). Six patients (28.5%) had over 50 copies of FGFR2 in their tumor specimens. We performed immunohistochemistry against the 22C3 antibody to examine whether the FGFR2 amplification was correlated with the level of PD-L1 expression. The combined positive score (CPS) for PD-L1, defined as the number of PD-L1+ cells, including tumor cells and immune cells (macrophages and lymphocytes), relative to the total number of tumor cells, is an important biomarker of cancer progression, with a CPS ≥ 1 considered a positive PD-L1 tumor. Of the 28 patients with FGFR2 aberration, 9 patients had PD-L1 data available. Of 9 patients, 5 patients had PD-L1 negative tumor while 4 patients had PD-L1+ tumor (Figures 2(c) and 2(d)).

2.3. The Incidence of FGFR2 Gene Fusion with Various Partners in GC. FGFR2 fusions are the result of gene rearrangements and have been observed in different types of cancer with different incidence per cancer type [30–36]. Approximately 1.1% of the patients (15/1,373) had FGFR2 fusions. The most common cancer types where FGFR2 fusion was detected were GC (N = 7, 46.7%), followed by common bile duct (CBD) cancer (N = 5, 33.3%), HCC (N = 1, 6.7%), pancreatic cancer (N = 1, 6.7%), and sarcoma (N = 1, 6.7%) (Figures 3(a) and 3(b)). The FGFR2 fusion partners included TACC2, BICC1, BTBD16, WAC, HFM1, HOOK1, INPP5F, C10orf90, and WDR11 (Figure 3(b), top panel). FGFR2 was combined with the TACC2 gene in 4 of 15 patients with FGFR2 fusion (26.7%). In addition, BICC1-FGFR2 gene fusion was detected in 3 CBD and pancreatic cancer patients (3/15 cases, 20%), and BTBD16-FGFR2 gene fusions were detected in 1 GC and 1 sarcoma patient (2/15 cases, 13.3%) (Figure 3(c)). Other FGFR2 fusions were partnered with WAC, HFM1, HOOK1, INPP5F, C10orf90, and WDR11 (Figure 3(c)). Similar to those with FGFR2 amplification, all patients with FGFR2 fusions had MSS and TMB-low status by NGS (Figure 3(b), bottom panel). Interestingly, there were 7 out of 9 patients with FGFR2 fusion and positive PD-L1 CPS. Interestingly, all 5 CBD cancer patients with FGFR2 fusion had PD-L1 positive tumor (Figure 3(b), bottom panel).

2.4. Cooccurrence of FGFR2 Amplification and Fusion. Lastly, we surveyed the incidence of cancer patients with concurrent FGFR2 amplification and FGFR2 fusion in their tumor specimen. There were 8 patients with concurrent FGFR2 amplification and FGFR2 fusion (Figures 4(a), 4(b), and 4(d), top panel). Notably, approximately 87.5% of the cases with concurrent FGFR2 amplification and FGFR2 fusion occurred in GC patients (Figures 4(c) and 4(d)). Of the 28 patients with FGFR2 aberration, 15 patients had additional tissue specimens available for FGFR2 IHC. Of the 13 patients tested for FGFR2 IHC, 8 (61.5%) patients had FGFR2 overexpression by IHC (2+ in 4 patients and 3+ in 5 patients) (Figure 4(d)). Interestingly, three GC patients with concurrent FGFR2 amplification and FGFR2 fusion (3/7 cases, 42.8%) had FGFR2 protein overexpression in their tumor (Figure 4(d)). Representative image of FGFR2 protein overexpression by IHC is provided in Figure 4(e).

We next examined the correlation between PD-L1 expression and FGFR2 genomic alterations (FGFR2+ or FGFR2−) in GC patient cohort (N = 327) (Figure 4). There were fewer FGFR2+ GC patients with PD-L1 expression...
A number of studies have reported that FGFR2 amplification is associated with GC development and progression, although the proportion of GC cases exhibiting FGFR2 amplification is relatively low (up to ∼5%) [2, 14–17]. Furthermore, high-level FGFR2 amplification has been suggested to be associated with poor prognosis in several types of cancer, including GC [15, 16], CRC [14, 23], and breast cancer [5]. However, the clinical significance of FGFR2 gene aberrations remains controversial, and further investigations to characterize FGFR2 genetic alterations are needed. In this study, we showed that the rate of FGFR2 gene amplification was higher in GC than in other types of cancer, consistent with previous studies [17, 22, 25] indicating an incidence of FGFR2 amplification in GC of approximately 1.3%.

We also confirmed that the occurrence of FGFR2 amplification was correlated with potential biomarkers, including MSI status and PD-L1 expression. FGFR2 has been reported to promote PD-L1 expression in a xenograft mouse model and induce PD-L1 expression via the JAK/STAT3 signaling pathway, causing apoptosis of T lymphocytes in CRC [37]. Consistent with a previous report [32], a strong correlation between FGFR2 amplification and PD-L1 expression was observed in CBD cancer. However, we found that FGFR2 amplification was unlikely to affect PD-L1 expression levels in gastric cancer, with a broad range of PD-L1 expression levels detected regardless of FGFR2 copy number. Nevertheless, the results of correlation analysis for FGFR2 gene amplification and PD-L1 should be interpreted with caution because samples comprising >57.1% of the total sample set were excluded (i.e., samples for which data were not available). Therefore,
Further studies are needed to elucidate the relationship between FGFR2 amplification and PD-L1 expression. Chromosomal rearrangement of the FGFR2 gene is the most common type of FGFR gene fusion, with its frequency exceeding those of FGFR1 and FGFR3 [33]. It has been reported that FGFR2 fusions occur with diverse fusion partners, especially in cholangiocarcinoma, and these genetic aberrations of FGFR2 induce cancer cell proliferation and tumorigenesis [32–35]. Consistent with the literature [33, 35, 36], the results of the present study showed fusion of the FGFR2 gene with nine different partners, namely, TACC2, BICC1, BTBD16, WAC, HFM1, HOOK1, INPP5F, C10orf90, and WDR11, in five different types of cancer. Among these fusions, FGFR2-TACC2 and FGFR2-BICC1 were observed in gastric and CBD cancers. Interestingly, FGFR2 gene fusions are often correlated with high levels of FGFR2 amplification.

**Figure 2**: Clinicopathological landscape of patients with different cancer types exhibiting FGFR2 amplification. (a) Venn diagram showing the percentage distribution of tumor types with FGFR2 amplification (21 cases): GC (85.7%), sarcoma (9.5%), and CRC (4.8%). (b) FGFR2 copy number. (c) Analysis of the correlation between PD-L1 level and FGFR2 copy number. (d) Patient profiles, including the distribution of FGFR2 copy number of all patients, tumor type and FGFR2 amplification level (top), age and sex (middle), and MSI, TMB, and PD-L1 status (bottom). MSI: microsatellite instability; PD-L1: programmed cell death-ligand 1; TMB: tumor mutational burden.
PD-L1 expression in CBD cancer. In contrast, there was no correlation between FGFR2 gene fusion and PD-L1 expression in gastric cancer. However, all cases of GC exhibited positive PD-L1 expression regardless of whether FGFR2 genetic alterations were present (CPS, 1–10), suggesting that anti-PD-L1 therapy may be beneficial in the treatment of gastric cancer. Although there was a relatively weak correlation between FGFR2 genetic alteration and the biomarker PD-L1, the blockade of FGFR2 aberrations in human cancers is considered to be a promising approach for targeted therapy.

Based on our results and others [37, 38], FGFR2 IHC using FGFR2 antibody may not be a useful diagnostic utility to identify cancer patients with FGFR2 aberration in their tumor specimen. In the FIGHT trial, FGFR2b antibody was used as a screening method. However, FGFR2 antibody for the FIGHT trial was developed using their FGFR2b specific antibody [18] for patient screening.

It may be possible to treat advanced GC by regulating the FGFR signaling pathway, which may serve as a prognostic molecular target. Several reports have supported the efficacy of monoclonal antibodies targeting FGFR2, such as PRO-007 [11], AZD4547 [17, 22], futibatinib [39], and bemarituzumab [26], in reducing GC progression. In addition, AZD4547 was shown to exert antitumor activity in GC lines with FGFR2 amplification [22].

**Figure 3:** Clinicopathological landscape of patients exhibiting FGFR2 fusion among all types of cancer. (a) Venn diagram showing the percentage distribution of tumor types with FGFR2 fusion (15 cases): GC (46.7%), CBD cancer (33.3%), sarcoma (6.7%), pancreatic cancer (6.7%), and HCC (6.7%). (b) Patient profiles, including tumor type and FGFR2 fusion partner genes (top), age and sex (middle), and MSI, TMB, and PD-L1 status (bottom). (c) FGFR2 fusions with nine different partner genes were detected: TACC2, BIVV1, BTBD16, WAC, HFM1, HOOK1, INPP5F, C10orf90, and WDF11.
Figure 4: Genomic landscape of patients exhibiting cooccurrence of FGFR2 amplification and fusion. (a) Venn diagram showing the percentage distribution of patients with FGFR2 genomic alterations, including amplification (red), fusion (sky blue), and cooccurrence of amplification and fusion (purple) ($N = 28$). (b, c) Numbers of patients exhibiting FGFR2 amplification, fusion, and cooccurrence of amplification and fusion and the percentage of cases with each tumor type: green, gastric cancer; magenta, sarcoma. (d) Comprehensive clinical characteristics of and the structural alterations present in patients, including tumor type, presence of amplification, presence of fusion, and FGFR2 IHC (top), age and sex (middle), and MSI, TMB, and PD-L1 status (bottom). (e) Representative immunohistochemistry results of FGFR2 showing strong cytoplasmic staining in the tumor cells. Scale bar: 100 $\mu$m and 80 $\mu$m. (f) Analysis of correlations between the PD-L1 expression level and FGFR2 aberrations in GC ($N = 327$, FGFR2+ : black circles and FGFR2− : gray circles). (g) Alluvial diagram representing the flow from tumor types to FGFR2 alteration and disease response to chemotherapy. (h) Overall survival (OS, top) and progression-free survival (PFS, bottom) curves calculated using the Kaplan–Meier method for groups classified according to FGFR2 alteration. CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.
Phase 1 and 2 clinical studies conducted in GC evaluating the efficacy of various inhibitors targeting FGFR have been reported [23–25]. In particular, pemigatinib, a small molecular inhibitor of FGFR, has shown efficacy and safety in patients with metastatic solid tumors with FGFR alteration in phase 2 and 3 clinical trials (FIGHT-201,202,302) [40–42] and has received accelerated approval in the USA for the treatment of metastatic CCA harboring FGFR2 gene rearrangements or fusions. This clinical evidence led to the design of another phase 2 trial in refractory, metastatic GC patients (the FiGhTeR trial) [43]. Bemarituzumab, a first-in-class humanized IgG2 monoclonal antibody plus first-line chemotherapy (oxaliplatin, 5-fluorouracil, leucovorin, and FOLFOX), demonstrated improved overall survival in patients with FGFR2+ GC [44].

4. Conclusion

In conclusion, we observed FGFR2 aberrations, that is, gene amplification and fusion, within a cohort of Korean patients at our precision oncology clinic. NGS screening identified 21 patients as positive for FGFR2 amplification (1.5%) and 15 patients as positive for FGFR2 fusion (1.1%), with eight patients testing positive for both FGFR2 amplification and fusion (0.6%). High levels of PD-L1 expression are more closely related to FGFR2 fusion in CBD cancer. Besides, we found that patients with FGFR2 alteration had poorer OS and PFS than patients without FGFR2 alteration. Although further prospective studies in larger cohorts are required to determine the relationships between FGFR2 aberrations and several potent biomarkers, including PD-L1 levels, there is accumulating evidence for the efficacy of therapeutic strategies involving the regulation of the FGFR signaling pathway, supporting the potential development of FGFR2-targeted therapy for precision medicine.

5. Materials and Methods

5.1. Patient Enrollment. The collection of specimens and associated clinical data used in this study was approved by the Institutional Review Board of Samsung Medical Center (IRB# 2021-09-052). All patients who participated in this study provided written informed consent prior to enrollment and specimen collection. This study was performed in accordance with the principles of the Helsinki Declaration and the Korean Good Clinical Practice guidelines.

5.2. Tumor DNA Extraction. Genomic DNA was acquired from formalin-fixed paraffin-embedded (FFPE) tissue sections (generally measuring 6–10 nm) and then purified using a commercial kit (Qiagen AllPrep DNA/RNA FFPE kit; Qiagen, Venlo, the Netherlands). The concentration of the purified DNA was determined using a Qubit DSDNA HS Assay kit (Thermo Fisher Scientific, Waltham, MA). Aliquots of 40 ng of DNA from each sample were used for DNA library preparation. The DNA integrity number (DIN) was obtained to determine the size of the DNA fragments, and the DNA quality was determined using the Genomic DNA ScreenTape assay (Agilent Technologies, Santa Clara, CA) on an Agilent 2200 TapeStation system (Agilent Technologies, Santa Clara, CA, USA).

5.3. Library Preparation, Sequencing, and Data Analysis. DNA libraries of all samples were prepared using a hybrid capture-based TruSight Oncology 500 (TSO 500) DNA/RNA Next Seq kit (Illumina, San Diego, CA, USA) in accordance with the manufacturer’s protocol. Unique molecular identifiers (UMIs) to determine unique coverage at each position and increase accuracy during sequencing were used in the TSO 500, which allowed the detection of variants and variant allele frequency (VAF) while simultaneously suppressing errors, providing high specificity.

Sequence data were examined to identify clinically relevant classes of genomic alterations, including copy number variation (CNV) and rearrangements/fusions. An average CNV ≥ 4 was considered amplification and < 1 was considered a loss. Only gain was measured in the TSO 500 CNV files, and RNA translocation supporting reads > 4–12 is considered a translocation, subject to the quality of the sample. Filtered data exported from the TSO 500 pipeline [28] were annotated using the Ensembl Variant Effect Predictor (VEP) Annotation Engine [28] with information from databases, such as gnomAD genome and exome, 1000 genomes, dbSNP, COSMIC, RefSeq, ClinVar, and Ensembl. Genomic changes were categorized according to the 4-tier system proposed by the American Society of Clinical Oncology and the College of American Pathologists [29]. The TMB and MSI were recorded from the TSO 500 pipeline. In brief, the TMB detection is done by analyzing the following parameters: filtering any variant with an observed allele count ≥ 10 in any of the gnomAD exome, genome, and 1000 genomes databases, including variants in the coding region (RefSeq Cds) and variant frequency ≥ 5% of the total coding region with coverage > 50x. SNVs and indels: nonsynonymous and synonymous variant MSI status was determined using the calculated data from microsatellite sites relative to a set of normal baseline samples. The MSI score was determined by the percentage of unstable MSI sites to the total number of assessed MSI sites.

5.4. Immunohistochemistry (IHC). GC FGFR2 IHC was performed using Benchmark XT (Ventana, Tucson, AZ, USA). Fixed tissues were embedded in paraffin blocks and sectioned at 3 μm thickness. Each section was deparaffinized in xylene, and antigen retrieval was performed. Samples were incubated with anti-FGFR2 using a Dako Autostainer Link 48 (Agilent Technologies, Santa Clara, CA, USA). All IHC samples were scanned using a ScanScope Aperio AT Turbo slide scanner (Leica Microsystems, Melbourne, Australia).

5.5. Statistical Analysis. Overall survival (OS) period was defined as the time from initiation to chemotherapy until the date of death. Progression-free survival (PFS) was defined as the time from initiation to chemotherapy to the date of disease progression or all-cause mortality. Kaplan–Meier estimates were used in the analysis of all time to event variables, and the 95% confidence interval for the median time to event was computed. Survival analysis was
performed using R for windows (version 4.1.2, https://cran.r-project.org/bin/windows/base/), and RStudio desktop 1.4 was used for drawing graphics (RStudio Team, 250 Northern Ave, Boston, MA 02210, USA; https://www.rstudio.com/products/rstudio/download/). Correlations between PD-L1 expression and the presence or absence of FGFR2 genetic alterations were estimated using Pearson’s correlation analysis using Prism 5 (GraphPad Software, USA).

Data Availability
The data used to support the findings of this study are included within the article.

Conflicts of Interest
The authors declare that there is no conflict of interest.

Authors’ Contributions
Sujin Hyung and Boram Han contributed equally to this work.

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References
[23] A. Hollebecque, T. Doi, O. Saavedra et al., “A phase II study of futibatinib (TAS-120) in patients (pts) with advanced (adv) solid tumors harboring fibroblast growth factor receptor


[27] H. Kim, J. Y. Hong, J. Lee et al., “Clinical sequencing to assess tumor mutational burden as a useful biomarker to immunotherapy in various solid tumors,” *Therapeutic Advances in Medical Oncology*, vol. 13, p. 175883592199299, 2021.


