

Original Article

Comprehensive identification of *FGFR1-4* alterations in 5 557 Chinese patients with solid tumors by next-generation sequencing

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Abstract: Deregulation of fibroblast growth factor receptor (FGFR) network is common in cancer due to activating mutations, gene amplifications and chromosomal translocations. Currently, various FGFR inhibitors are being developed. In order to optimize their clinical applications, understanding the frequencies and types of *FGFR* alterations in multiple cancer types appears to be extremely important. This study characterized *FGFR1-4* alterations in solid tumors by next-generation sequencing (NGS). Between Jun. 2019 and Aug. 2020, the sequencing data of 5 557 solid tumors of diverse types in the database of Simcere Diagnostics, Inc. (Nanjing, China) were retrospectively analyzed. A panel-based NGS assay was used to detect *FGFR1-4* alterations in tumor samples. 9.2% of cancer cases had *FGFR1-4* alterations, in which gene amplifications (51.5%) and mutations (40.7%) were frequent, whereas gene rearrangements were less common (10.0%). *FGFR1* was involved in 4.6% of 5 557 cases, *FGFR2* in 2.1%, *FGFR3* in 1.6%, and *FGFR4* in 1.4%. Of patients with *FGFR1-4* alterations, *TP53*, *MUC16*, *NSD3*, *MYC* and *LRP1B* genes were the top 5 mutant genes. *FGFR1-4* aberrations occurred in almost every type of solid tumors, with the most common tumor being endometrial carcinoma (22.2%), followed by sarcoma (17.3%), breast cancer (13.2%), gastric cancer (12.2%), and more. 0.6% of cancer cases harbored *FGFR1-4* fusions, with the most common fusion partner being *TACC3*. Two cases of GBM harboring *FGFR3-TACC3* fusions were responsive to anlotinib treatment. In conclusion, *FGFR1-4* alterations are prevalent in solid tumors of diverse types, with the majority being gene amplifications and mutations. *FGFR1-4* fusions only occur in a minority of cancer cases, and those with glioblastoma harboring *FGFR3-TACC3* fusions may benefit from anlotinib.

Keywords: Fibroblast growth factor receptors, next-generation sequencing, solid tumors, gene fusion, anlotinib

Introduction

Fibroblast growth factor receptors (FGFRs) pertain to a family of highly-conserved transmembrane tyrosine kinase receptors consisting of extracellular domain, transmembrane domain and intracellular tyrosine kinase domain, and are involved in various aspects of cancer biology, including cell proliferation, differentiation, migration, angiogenesis, and carcinogenesis [1]. There are four typical tyrosine kinase recep-

tors (FGFR1-4) and one without intracellular tyrosine kinase domain (FGFR5 or FGFR4) in humans [2]. Despite being encoded by different genes, FGFR1-4 share high homology in sequence identify ranging from 56% to 71% [3]. Fibroblast growth factors (FGFs) are the native ligands for FGFRs. When FGFs bind to their cognate receptor, the receptor will dimerize, subsequently resulting in phosphorylated intracellular domain of the receptor, intracellular signaling cascades and gene transcription [4].

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Table 1. Frequencies of FGFR1-4 alterations and relative distribution of alternation types in solid tumors (%)

Cancer types	N	All	FGFR1	FGFR2	FGFR3	FGFR4	Amplification	Mutation	Rearrangement
Endometrial cancer	36	22.2	8.3	11.1	8.3	2.8	0.0	22.2	2.8
Sarcoma	202	17.3	10.9	1.5	1.5	4.0	13.4	3.5	1.0
Breast cancer	106	13.2	8.5	2.8	0.9	0.9	8.5	3.3	0.0
Gastric cancer	254	12.2	3.5	7.9	0.8	0.4	9.1	2.8	3.1
Carcinoma of unknown primary	899	11.6	7.1	1.8	1.7	1.7	6.5	2.4	1.1
Colorectal cancer	437	11.0	6.2	1.8	3.9	0.7	5.0	6.4	0.5
Liver cancer	625	9.6	1.9	4.0	1.9	2.1	4.3	3.5	2.2
Biliary tract cancer	218	9.2	5.0	1.4	1.4	1.4	3.2	3.7	2.3
Esophageal cancer	98	9.2	6.1	2.0	1.0	0.0	6.1	3.1	0.0
Melanoma	105	8.6	2.9	1.0	3.8	1.0	2.9	4.8	1.0
Lung cancer	2262	6.9	3.8	1.0	1.1	1.4	3.1	3.8	0.3
Pancreatic cancer	152	6.6	5.3	0.0	1.3	0.0	5.3	1.3	0.0
Cervical cancer	67	6.0	1.5	1.5	3.0	0.0	1.5	4.5	0.0
Brain cancer	96	4.2	1.0	0.0	1.0	2.1	1.0	2.1	1.0

Constitutive activation of *FGFR* genes can be oncogenic due to point mutations, gene amplifications and chromosomal translocations [5-7]. FGFs, ligands of the receptors, could also strengthen the signaling in terms of oncogenesis and neoangiogenesis [8]. Specific *FGFR* alterations are identified in certain cancer types, such as *FGFR1* amplification in lung squamous cell carcinoma (LSQCC) and *FGFR3* mutations in bladder cancer, and some of them tend to be the driver abnormalities [9, 10]. There is evidence suggesting that variations of specific *FGFR* expression may be associated with the prognosis or sensitivity to cancer treatments [11, 12]. Donnem et al. found that co-expression of *FGFR-1*/platelet-derived growth factor-B (PDGF-B) and *FGF2*/vascular endothelial growth factor-3 (VEGF-3) was correlated with the poor survival of patients with non-small cell lung cancer (NSCLC) [13]. In contrast to those with wild-type *FGFR1*, the patients with *FGFR1* amplification had a worse progression-free survival (PFS) [14]. Additionally, the tumor *FGFR4* level was also found a significant predictor of response to lenvatinib in patients with advanced hepatocellular carcinoma (HCC) [15].

Considering that most *FGFR* alterations result in gain of function, it is rational to speculate that use of *FGFR* inhibitors to target these tumors would be beneficial [16]. Although non-selective *FGFR* inhibitors have been approved for cancer treatments, presence of various complications limits their use in clinic [17]. It seems very necessary to identify *FGFR* aberra-

tions that may predict the response to treatment in various cancer types and to verify their contributions to the onset of drug-resistant mechanisms. Next-generation sequencing (NGS) with the characteristics of high throughput, automation and low cost makes accurate and rapid identification of *FGFR* alterations practicable [18]. In this study, we characterized *FGFR1-4* alterations involving amplifications, mutations, and rearrangements in 5 557 Chinese patient samples across multiple solid tumors by NGS, aiming at providing a landscape for *FGFR* alterations in Chinese solid tumors and evidence for more effective use of pharmaceutical agents.

Materials and methods

Between Jun. 2019 and Aug. 2020, the sequencing data of 5 557 solid tumors of diverse types (specific cancer types provided by submitting physicians and their corresponding number of cases are described in **Table 1**) in the database of Simcere Diagnostics, Co. Ltd. (Nanjing, China) for genomic profiling were retrospectively analyzed. All the patients included in the study were informed consent with respect to genetic testing and research. A panel-based NGS assay was used to detect *FGFR1-4* alterations in formalin-fixed, paraffin-embedded (FFPE) tumor samples. First, DNA was extracted from undyed FFPE sections with the proportion of tumor cells more than 20% and whole-blood samples and then purification and library preparation were performed. Second,

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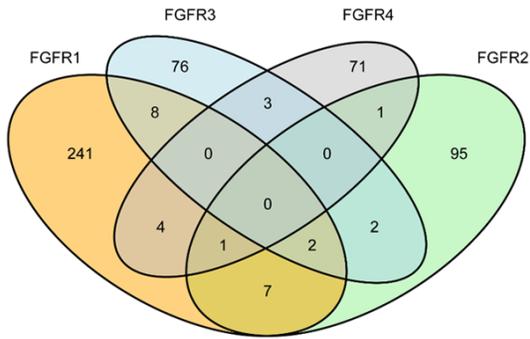


Figure 1. Number of cases with *FGFR1-4* aberrations in 5 557 solid tumors.

a probe with 2.29 Mbp in size was used for hybrid capture and enrichment in gene-specific regions where various aberrations of 539 cancer-related genes including single nucleotide variants, copy number variations, small insertions, deletions and gene arrangements were covered. At last, double-ended sequencing on the Illumina NovaSeq 6000 platform was conducted after the captured library was mixed, degenerated and diluted.

Results

Frequency of FGFR1-4 alterations in solid tumors

Of the 5 557 tumor samples sequenced, *FGFR1-4* alterations occurred in 511 cases, with an overall frequency of 9.2%. It should be noted that 28 cases had more than one alterations (**Figure 1**). *FGFR1* was influenced in 4.6% of 5 557 cases, *FGFR2* in 2.1%, *FGFR3* in 1.6%, and *FGFR4* in 1.4%. In contrast to *FGFR2-4* aberrations, *FGFR1* alterations were more common (**Figure 2A**). A great number of *FGFR1-4* alterations were gene amplifications (51.5%) and gene mutations (40.7%), with gene rearrangements being less common (10.0%; **Figure 2B**). The gene amplification was more prevalent in *FGFR1* (71.9%), whereas gene mutations were more frequent in *FGFR2-4* (50.0%, 68.1%, and 61.3%, respectively; **Figure 2C**). Among 511 patients with *FGFR1-4* aberrations, the top five mutant genes included *TP53* (66%), *MUC16* (27%), *NSD3* (27%), *MYC* (25%) and *LRP1B* (22%), among which amplification was more common in *NSD3* and *MYC* genes (**Figure 2C**). Most concurrently-mutated genes with *FGFR1-4* aberrations were found in cell cycle pathways, while those in RAS/MAPK and Wnt/ β -catenin

pathways were uncommon. Compared with mutations and fusions, *FGFR1-4* amplifications were relatively frequent in such pathways as PI3K-ALK, DNA repair, growth factor receptors and transcriptome (**Figure 2D**; **Table 2**). The specific forms of *FGFR1-4* variations are respectively shown in **Figure 3A-D**.

In **Table 1**, it could be observed that the frequencies of *FGFR1-4* alterations were different in solid tumors of diverse types, ranging from 4.2% to 22.2%. Endometrial carcinoma had the highest frequency of aberrations (22.2%), followed by sarcoma (17.3%), breast cancer (13.2%), gastric cancer (12.2%), carcinoma of unknown primary (11.6%), colorectal cancer (11.0%), liver cancer (9.6%), biliary tract cancer (9.2%), esophageal cancer (9.2%), melanoma (8.6%), lung cancer (6.9%), pancreatic cancer (6.6%), cervical cancer (6.0%) and brain cancer (4.2%). The proportions of *FGFR1-4* alterations in various cancer types are shown in **Figure 4A-D**.

Endometrial carcinoma

There were 36 cases of endometrial carcinoma in the dataset, with the overall frequency of 22.2% in *FGFR1-4* aberrations. A great number of aberrations were gene mutations, with gene rearrangements being less common and without gene amplifications (**Figure 5A**). *FGFR2* mutations were relatively common, including S252W (2 cases), N549K (1 case), D794Y (1 case) and E806K (1 case), and all of these were gain-of-function mutations.

Sarcoma

Totally 202 cases of sarcoma were involved in the dataset, and 35 cases of them harbored *FGFR1-4* variations. The gene amplifications were the most common alterations, especially *FGFR1* amplification (9.9%). *FGFR1*, *FGFR3* and *FGFR4* mutations varied from 0.5%-2.0% in sarcoma (**Figure 5B**).

Breast cancer

In the dataset, there were 106 breast cancer cases in total, mainly including invasive lobular carcinoma, invasive ductal carcinoma and invasive metaplastic carcinoma. About 13.2% of breast cancer cases harbored *FGFR1-4* alterations, with *FGFR1* amplification being the most frequent (6.6%), while *FGFR2* amplification and

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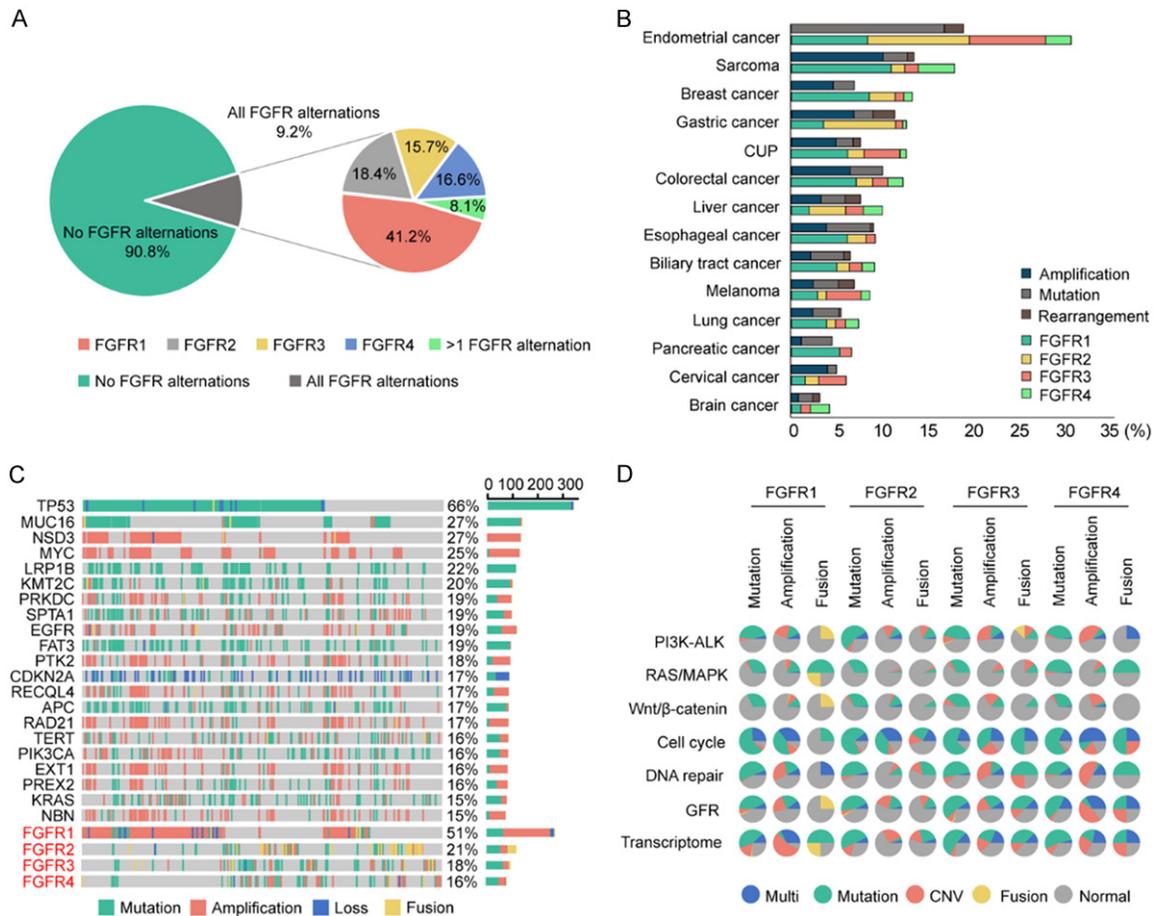


Figure 2. Frequency and distribution of *FGFR1-4* variations in solid tumors. **A:** Frequency of *FGFR1-4* alterations in all cases (left); relative proportion of *FGFR1-4* alterations in all cases harboring *FGFR1-4* aberrations (right). It should be noted that 28 cases had more than one alterations. **B:** Distribution of *FGFR1-4* variations in all cancer types. The horizontal axis represents the percentage of tumors with aberrations. For each cancer type, the frequency of *FGFR1-4* variations is described as the percentage of all cancer cases analyzed, and the distribution and type of variations are normalized to 100%. **C:** Landscape of genomic aberrations of *FGFR1-4* in solid tumors. **D:** The pie chart shows the frequency and types of concurrently mutant genes with *FGFR1-4* mutations, amplifications and fusions in seven major pathways.

Table 2. List of common genes in different pathways

Pathways	Genes
PI3K-AKT	AKT3, TSC2, PTEN, mTOR, PIK3CA, RPTOR, TSC1, TCS2, RICOTR, PI3K3R2, PI3K3CB, INPP4B, STK11, PPP2R1A, PIK3R1, AKT2, RHEB, PDK1, AKT1
RAS/MAPK	KRAS, MAP2K1, NF1, NF2
Wnt/ β -catenin	AXIN1, AXIN2, RNF43, WNT7B, WNT108, AMER1, CHD4, TCF7L2, WNT10A
Cell cycle	TP53, TERT, CTNNB1, ATM, APC, CDK4, CCND1, CCND3, CCND2, CDKN2A, RB1, CCNE1, CDK6, CDK8, CCKN2A/B, CDKN1B CDKN2B
DNA repair	ATR, ATM, BACA1, BRCA2, MLH1, MSH2, MSH6, FANCA, BRIP1, BAP1, CHEK2, ERCC2, FANCA/D2/E/L, MUTYH, NBN, PMS2, RAD50/51B, WRN
Transcriptome	IGF1R, RET, ERBB2, EGFR, KIT, PDGFRA, KDR, ERBB4, FLT4, PDGFRB, MET, FLT3, ERBB3
Growth factor receptors	MYC, SOX2, KDM6A, SMARCA4, RUNX1, MYCN, EZH2, NKX2, KMT2D, SMARCB1, DNMT3A, MYCL1, SOX10, ARID2, ASXL1, DNMT3A, EP300, KMT2C, KMT2D, NSD3, PBRM1, SMARCB1, SETD2, BAP1, IDH1, IDH2

FGFR1-4 mutations less common (0.9%-1.9%; **Figure 5C**). *PIK3CA* aberrations were extraordinarily prevalent in breast cancer [19]. In our

dataset, 2 out of 5 cases with *FGFR* mutations (40.0%) had *PIK3CA* mutations, including *PIK3CA-H1047R*, *PIK3CA-H1048R*, and

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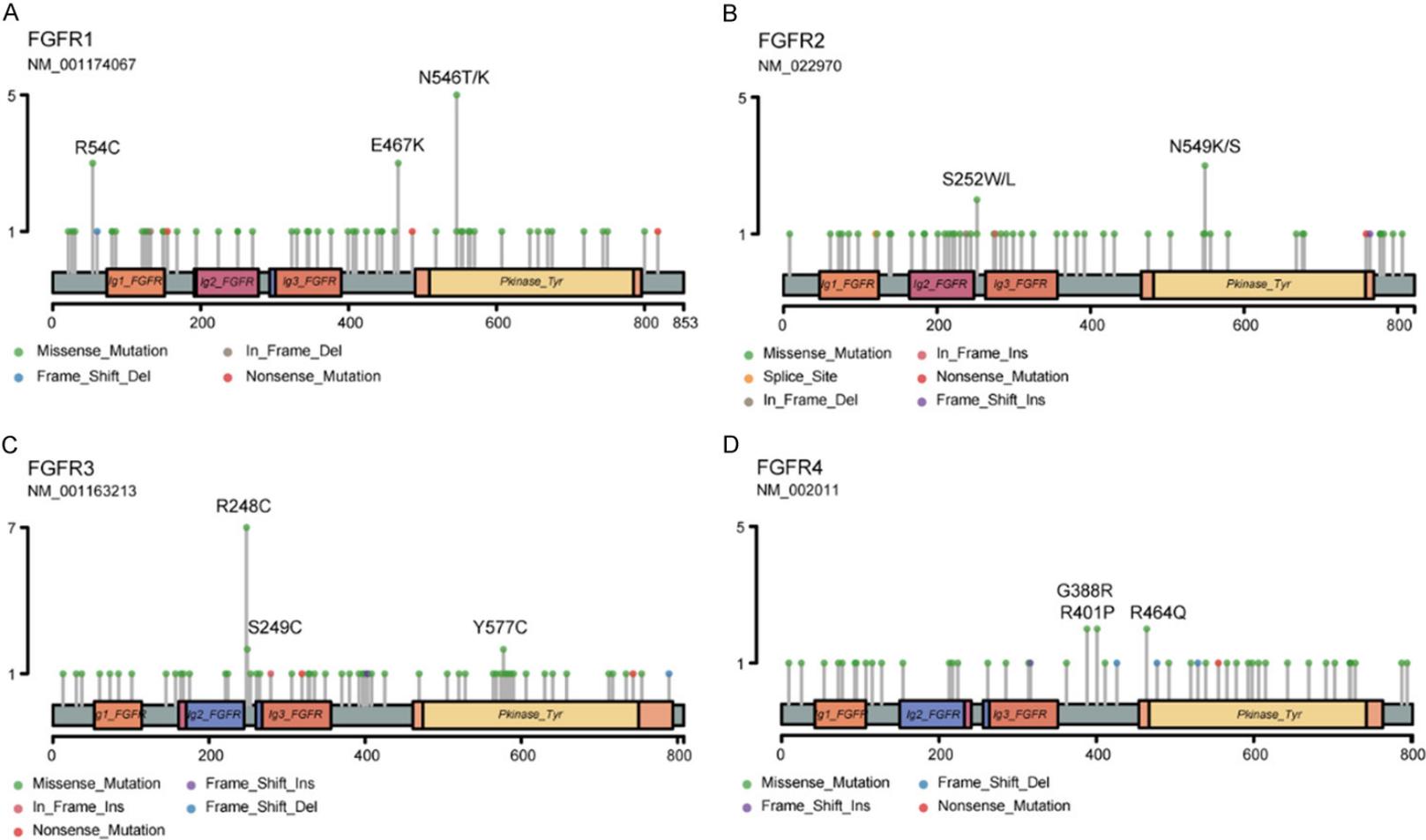


Figure 3. Specific types of FGFR1-4 variations. A: FGFR1; B: FGFR2; C: FGFR3; D: FGFR4.

FGFR1-4 alterations in Chinese solid tumors by NGS

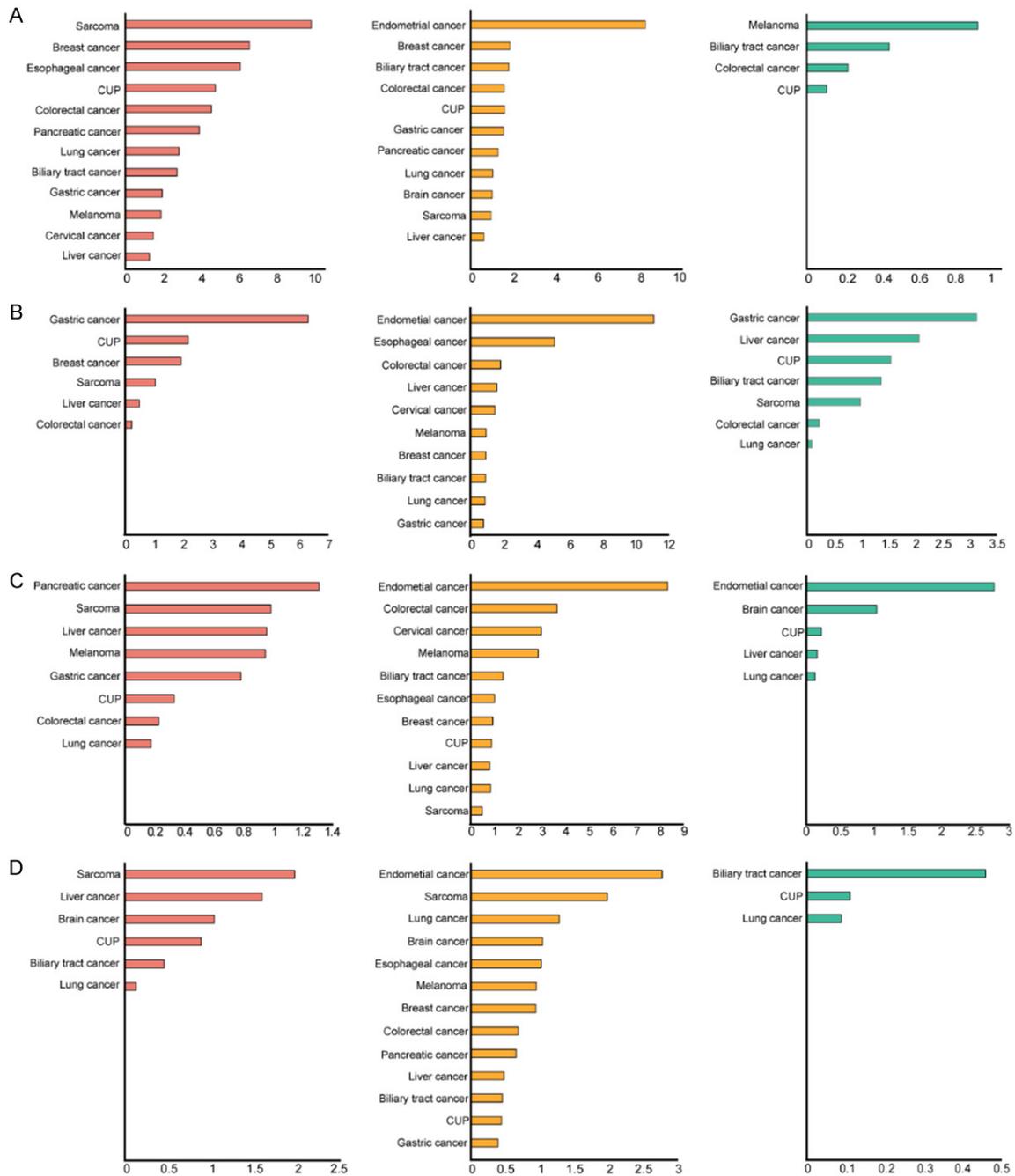


Figure 4. Proportions of *FGFR1-4* alterations in various cancer types. The horizontal axis represents the percentage of tumors with aberrations. A: *FGFR1*; B: *FGFR2*; C: *FGFR3*; D: *FGFR4*. Orange: gene amplification; Yellow: gene mutation; Green: gene rearrangement.

PIK3CA-C378Y. It should be noted that these 2 cases both suffered from *PIK3CA-H1047R*.

Lung cancer

A total of 2 262 lung cancer cases were involved in the dataset, including adenocarcinoma,

squamous cell carcinoma, adenosquamous carcinoma and small cell carcinoma. Approximately 6.9% of lung cancer cases analyzed harbored *FGFR1-4* alterations, the most frequent of which was *FGFR1* amplification (2.9%), while *FGFR1-4* mutations were less common (0.8%-1.3%), and *FGFR3-4* amplifications and

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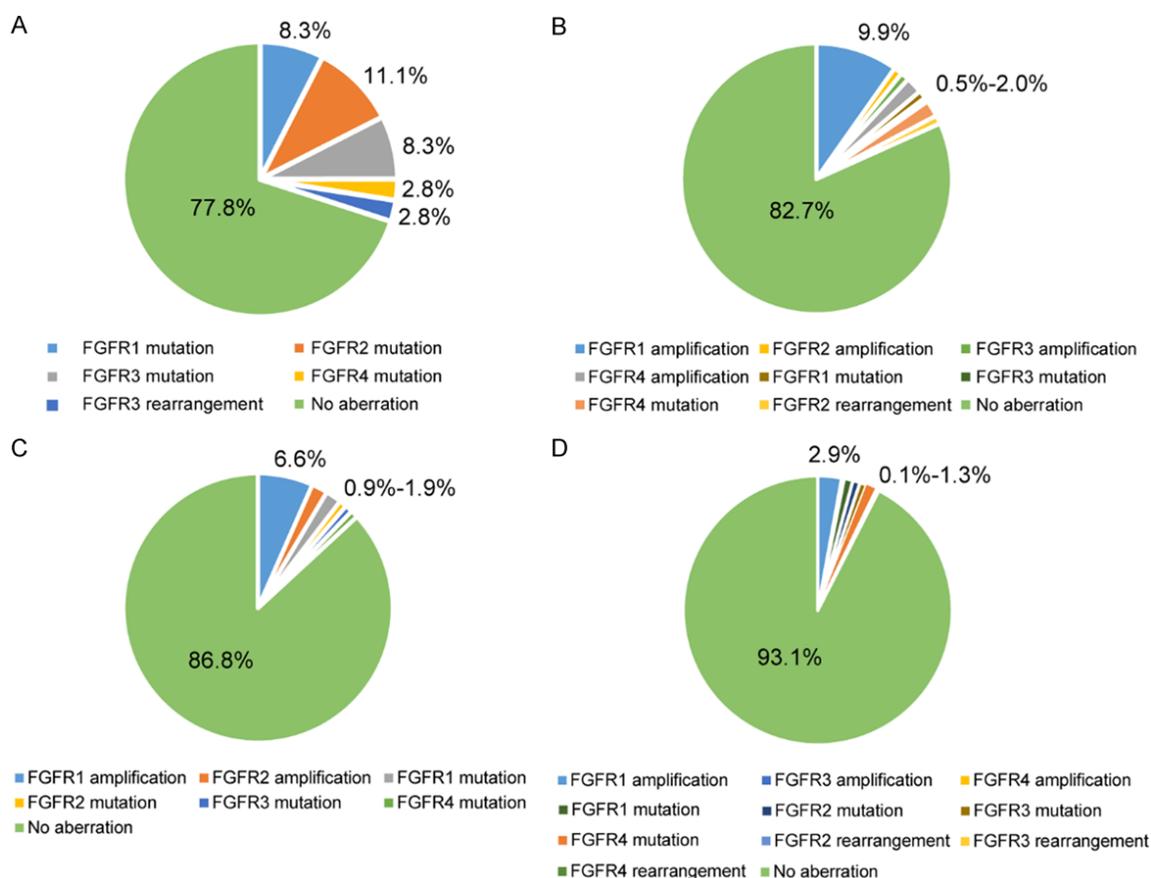


Figure 5. Frequency of *FGFR1-4* aberrations in specific cancer types. A: Endometrial cancer; B: Sarcoma; C: Breast cancer; D: Lung cancer.

FGFR2-4 rearrangements were rare (0.1%-0.2%) (**Figure 5D**). In our previous CHOICE study including 245 cases of NSCLC [20], the frequencies of *FGFR1*, *FGFR2*, *FGFR3* and *FGFR4* mutations were 2.45%, 0.41%, 2.04%, and 0.41%, respectively, further suggesting that the frequencies of *FGFR1-4* mutations were low in Chinese lung cancer patients.

Gene fusions

The genes fusing with *FGFR1-4* in our dataset are summarized in **Table 3** and **Figure 6**. It could be observed that 31 cases (0.6%, 31/5557) harbored fusions of *FGFR* genes with other genes, a majority of which were with *FGFR2* (24 cases). Transforming acidic coiled-coil containing protein 3 (*TACC3*) was the most common fusion partner (5 cases), followed by *TACC2* (2 cases) and *SH3GLB1* (2 cases). The other fusion partners, including *BICC1*, *CAT*, *TDRD1*, *CCDC158*, *CIT*, *TXLNB*, *NKD2*, *CUX2*,

HK1, *DOCK1*, *TACC1* and more, were found in single cases. Of 31 cases, gene fusions occurred in 10 cases of liver cancer, 8 of carcinoma of unknown primary, 5 of lung cancer, 2 of gastric cancer, 2 of sarcoma, 2 of biliary tract cancer, 1 of renal cancer and 1 of brain cancer.

Chromosomal translocations that can result in fusion proteins play their oncogenic roles in cancer by over-expressing another normal gene or creating a chimeric gene where parts of two genes are fused. Take *FGFR3-TACC3* for example, the *TACC* domain mediating microtubule binding is fused with the entire *FGFR3* kinase domain. The MAPK pathway can be activated by the fusion genes transfected into the normal urothelial cells in humans, highlighting the presence of active signaling in fusion genes. Moreover, the cell lines with fusion genes were found extremely sensitive to FGFR-selective agents, suggesting that the presence of fusion

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Table 3. The genes fusing with FGFR1-4 in the dataset (31 cases)

Gene partner, chromosome location	Specific fusion (n)	Tumor types (n)
SH3GLB1, chromosome 1: 87191379	FGFR2-SH3GLB1 (2)	Liver cancer (2)
BICC1, chromosome 10: 60426432	FGFR2-BICC1 (1)	Liver cancer (1)
TACC2, chromosome 10: 123996320/123991849	FGFR2-TACC2 (2)	Renal cancer (1), gastric cancer (1)
TACC3, chromosome 4: 1743533/1737371/1739077/1740504/1739733	FGFR3-TACC3 (5)	CUP (3), lung cancer (1), brain cancer (1)
CAT, chromosome 11: 34463828	FGFR2-CAT (1)	Lung cancer (1)
TDRD1, chromosome 10: 115939394	FGFR2-TDRD1 (1)	Liver cancer (1)
CCDC158, chromosome 4: 77292242	FGFR2-CCDC158 (1)	CUP (1)
CIT, chromosome 12: 120186475	FGFR2-CIT (1)	Liver cancer (1)
TXLNB, chromosome 6: 139595138	FGFR2-TXLNB (1)	Liver cancer (1)
NKD2, chromosome 5: 1011532	FGFR2-NKD2 (1)	Sarcoma (1)
CUX2, chromosome 12: 111570060	FGFR2-CUX2 (1)	Sarcoma (1)
HK1, chromosome 10: 71051284	FGFR2-HK1 (1)	Liver cancer (1)
EIF4ENIF1, chromosome 22: 31884504	FGFR2-EIF4ENIF1 (1)	CUP (1)
DOCK1, chromosome 10: 129093385	FGFR2-DOCK1 (1)	Gastric cancer (1)
TACC1, chromosome 8: 38683244	FGFR2-TACC1 (1)	Biliary tract cancer (1)
TP63, chromosome 3: 189457592	FGFR2-TP63 (1)	Lung cancer (1)
PAH, chromosome 12: 103300452	FGFR2-PAH (1)	Liver cancer (1)
TRIM8, chromosome 10: 104408969	FGFR2-TRIM8 (1)	CUP (1)
ERC1, chromosome 12: 1241041	FGFR2-ERC1 (1)	Biliary tract cancer (1)
CASP7, chromosome 10: 115441363	FGFR2-CASP7 (1)	Liver cancer (1)
LGSN, chromosome 6: 64006372	FGFR2-LGSN (1)	Liver cancer (1)
ZNF346, chromosome 5: 176472292	ZNF346-FGFR4 (1)	Lung cancer (1)
UVSSA, chromosome 4: 1377083	UVSSA-FGFR3 (1)	Lung cancer (1)
LINC01580, chromosome 15: 94446896	LINC01580-FGFR2 (1)	CUP (1)
NRAP, chromosome 10: 115383150	FGFR2-NRAP (1)	CUP (1)

Notes: CUP, carcinoma of unknown primary.

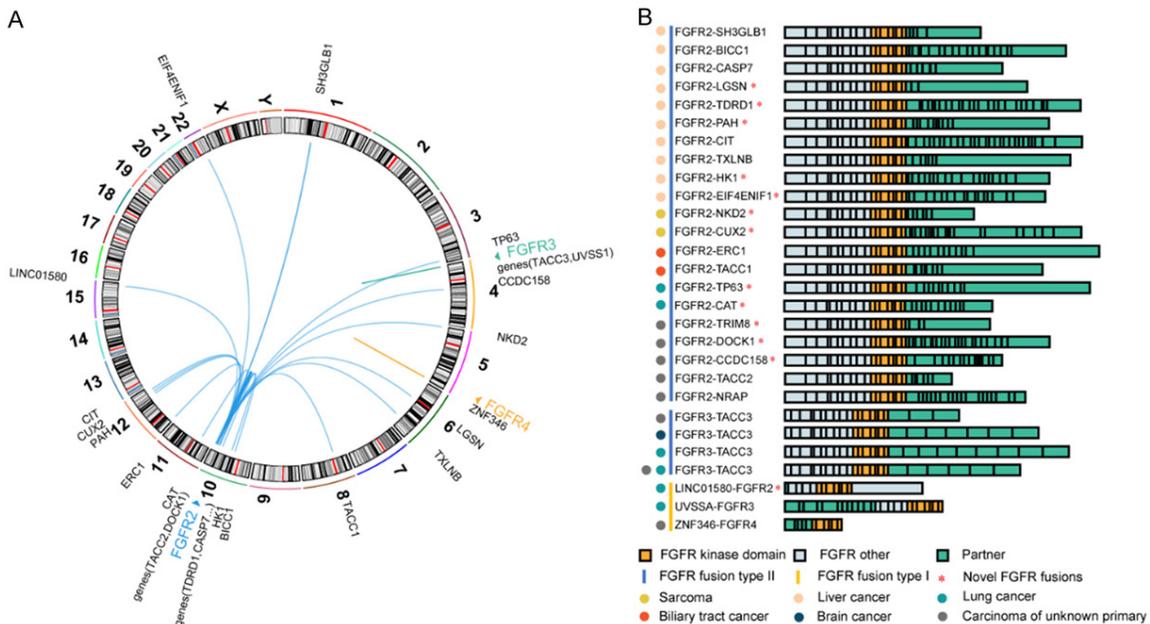


Figure 6. Distribution of the genes fusing with FGFR1-4 in all cancer cases. A: The Circos plot graphically depicts FGFR and their fusion partners, showing specific chromosomal locations of fusion partners. B: Schematic of all FGFR fusion proteins identified, at scale with exons represented by individual boxes. The upstream partner is colored grey, with FGFR kinase domain colored orange and other FGFR exons colored green.

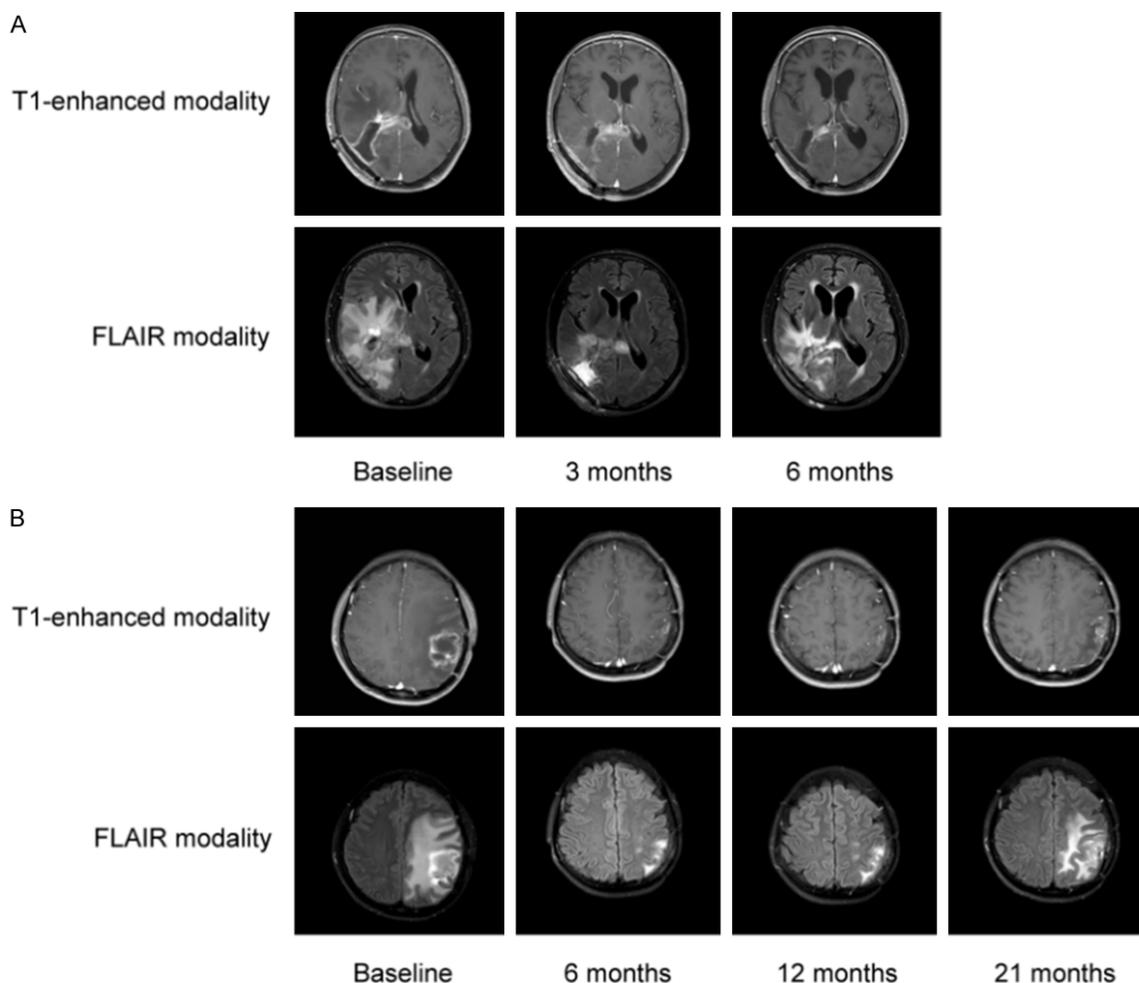


Figure 7. MRI images of two cases with *FGFR3-TACC3* fusions at different time points after anlotinib use. A: A 65-year-old women diagnosed as glioblastoma multiforme in the right splenium of corpus callosum; B: A 46-year-old women with glioma in the left thalamus and parietal lobe.

genes may be a potential therapeutic target in cancer cells [21]. Studies have shown that *FGFR-TACC* fusions may be conducive to identifying a subset of patients with glioblastoma multiforme (GBM) who would get benefits from targeted *FGFR* kinase inhibition [22, 23]. Here, we shared the treatment experience of two cases of GBM with *FGFR3-TACC3* fusions who both achieved partial responses (PR) after use of anlotinib.

Case presentation: The first patient with *FGFR3-TACC3* fusion was a 65-year-old women diagnosed as GBM in the right splenium of corpus callosum. She underwent GBM resection on Feb. 13, 2020, recovered well and left hospital after radiotherapy concurrently with temozolomide chemotherapy. Later, she successively

visited our hospital and 5 cycles of temozolomide chemotherapy were given. On May 4, 2020, she began to use anlotinib (12 mg) every other day, and achieved PR after use for 3 months. Until now, the patient is alive, and still on the treatment of anlotinib. Magnetic resonance imaging (MRI) images of this patient at different time points after use of anlotinib are shown in **Figure 7A**.

The second patient with *FGFR3-TACC3* fusion and *FGFR3* amplification was a 46-year-old woman with GBM in the left thalamus and parietal lobe. She visited the hospital on October 1, 2017, with the chief complaint of 3 sudden onsets of epilepsy, and underwent GBM resection on October 10, 2017. The patient first came to our hospital on November 29, 2017

and underwent intensity modulated radiation therapy concurrently with temozolomide chemotherapy. Later, she was successively treated with temozolomide, nedaplatin and bevacizumab, but residual recurrence occurred on June 5, 2018, then 1 cycle of temozolomide chemotherapy was administrated. On July 4, 2018, the patient received 1 cycle of temozolomide chemotherapy again, and then used anlotinib (12 mg, every other day) for 21 months and maintenance chemotherapy of temozolomide to date. She got PR after using anlotinib for 2 months. This patient is still alive now. Her MRI images at different time points after use of anlotinib are shown in **Figure 7B**.

Discussion

In this study, overall 9.2% of patients with solid tumors were found to harbor *FGFR1-4* alterations, with the majority being gene amplifications (mostly *FGFR1* amplification) and mutations. *FGFR1* was influenced in 4.6% of 5 557 cases, *FGFR2* in 2.1%, *FGFR3* in 1.6%, and *FGFR4* in 1.4%. The frequencies of *TP53*, *MUC16*, *NSD3*, *MYC* and *LRP1B* mutations were relatively high in patients with *FGFR1-4* aberrations, and most concurrently-mutated genes were identified in the cell cycle pathway. In solid tumors, the frequencies of *FGFR1-4* aberrations varied from 4.2% to 22.2%. In addition, FGFR fusions were also identified in 0.6% of cancer cases, among which liver cancer showed the highest fusion frequency. The subsequent case studies suggested that GBM patients with *FGFR3-TACC3* fusions may derive benefits from anlotinib.

Up to 22.2% of *FGFR1-4* aberrations occurred in cases with endometrial cancer in our dataset, mostly being activating mutations in *FGFR2*, such as *S252W* and *N549K*. *FGFR2-S252W* mutation can increase the affinity to FGF ligands, especially FGF9, which may be extremely crucial for endometrial cancer due to its extensive presence in endometrial stroma [24]. Interestingly, in endometrial cancer, it appears to be mutually exclusive in *FGFR2* and *KRAS* mutations, indicating redundancy with respect to activation of MAPK pathway [25].

There were 13.2% of breast cancer cases with *FGFR1-4* alterations in our dataset, with the majority being *FGFR1* amplification (6.6%)

which had been identified to be a significant prognostic predictor for the patients with breast cancer and were associated with endocrine therapy resistance [26-28]. Additionally, the prognosis of patients with *FGFR1* and human epidermal growth factor receptor 1 or 2 (*HER1/2*) co-amplification may be worse than those with *FGFR1* or *HER1/2* amplification alone or no amplification [29]. These findings all highlighted the importance of evaluating *FGFR1* status. Lucitanib, an inhibitor of VEGF1-3, *FGFR1-3*, and platelet-derived growth factor receptor (PDGFR) α/β , exerts a certain effect on *FGFR1*-amplified breast cancer, but its anti-tumor activity is modest in HR+/HER2-metastatic breast cancer, with conspicuous hypertension-related toxicity [30]. Therefore, it still needs more studies to explore the potential role of FGFR inhibition in reversing endocrine therapy resistance.

Although *FGFR* variations did not seem to segregate well with histological changes, it was observed that some certain cancers harbored more frequent alterations. In our study, *FGFR1* amplification was not found in endometrial cancer, but more commonly in sarcoma, breast cancer and lung cancer. Of 2 262 lung cancer cases sequenced, 6.9% of them was detected to have *FGFR1-4* aberrations, especially *FGFR1* amplification. Previous studies also showed the high frequency of *FGFR1* amplification in LSQCC [31, 32], but it was not analyzed in details in our study due to insufficient cases with LSQCC. Currently, except for erdafitinib approved by Food and Drug Administration, a selective pan-FGFR tyrosine kinase inhibitor, other selective FGFR inhibitors for various cancer types are under way, such as AZD4547, Ly2874455, CH5183284, NVP-BGJ398, and more [3].

In our study, it was also observed *FGFR* gene fusions (especially *FGFR2* and *FGFR3*) in a small number of the cases, with the most common fusion partner being *TACC3*, which might be attributed to the fact that *FGFR3* was close to *TACC3* on chromosome 4p16 [33]. The emergence of the *TACC* coiled-coil domain can enhance *FGFR3* activity by phosphorylating more miscellaneously constituted tyrosine kinase residues in *FGFR3* protein and activating MAPK kinase [34]. *FGFR3-TACC3* fusions can result in loss of an miR-99a binding site

within the 3'-untranslated region of FGFR3, leading to the release of FGFR3 signaling from miR-99a-dependent inhibition and reinforcement of tumor progression in contrast to wild-type *FGFR3* [35]. Emerging evidence suggests that the constitutional kinase activity of *FGFR3-TACC3* fusion protein can be suppressed by FGFR inhibitors [22, 36, 37]. Anlotinib is an orally administrated, small-molecule receptor tyrosine kinase inhibitor that can target VEGFR, FGFR, PDGFR and c-kit, with inhibitory effects on tumor cell proliferation and tumor angiogenesis. It can suppress the activation of FGFRs by inhibiting the phosphorylation of FGFR1 with the inhibition rate of 45.0% (p-FGFR1/FGFR1) at 1 μ M, and an IC_{50} value of 25 nM in AN3Ca cells overexpressing a FGFR2 mutant protein has been reported [38]. In our study, two GBM patients harboring *FGFR3-TACC3* fusions both achieved PR after using anlotinib, suggesting that *FGFR3-TACC3* fusions might be a potential biomarker for administration of anlotinib to treat GBM, which was confirmed by our previous study [39]. Moreover, there are also evidences showing that GBM patients harboring *FGFR3-TACC3* fusions may benefit from targeted FGFR kinase inhibition [40, 41].

A major strength of this study was that *FGFR* aberrations were comprehensively analyzed in a large group of Chinese patients with diverse cancer types, which may provide a better landscape for *FGFR* alterations in solid tumors. Additionally, other *FGFR* fusion partners (*SH3GLB1*, *BICC1*, *CAT*, *TDRD1*, *CCDC158*, *CIT*, *TXLNB*, *NKD2*, *CUX2*, *HK1*, and *DOCK1*) were also identified except for TACC, particularly the most common fusion partner TACC3. However, some limitations should also be interpreted cautiously. First, the number of patients with each cancer type was based on the number of tumor samples presented by physicians for genomic profiling, which may lead to the bias in sample size. Second, we did not evaluate whether some novel alterations of *FGFRs* had functional effects in *in vitro* or *in vivo* models. Further clinical relevance of these alterations needs investigation.

Conclusions

FGFR1-4 aberrations are common in solid tumors of diverse types, approximately account-

ing for 4.2%-22.2%. Of *FGFR1-4* variations, gene amplifications and gene mutations are more frequent, especially *FGFR1* amplifications. The frequencies of *TP53*, *MUC16*, *NSD3*, *MYC* and *LRP1B* mutations are relatively high in patients with *FGFR1-4* aberrations. *FGFR* fusions (mostly *FGFR2* and *FGFR3*) occur in 0.6% of cancer cases, and its relevant case studies suggest that GBM patients with *FGFR3-TACC3* fusions may derive benefits from anlotinib.

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Two cases of GBM with *FGFR3-TACC3* fusions voluntarily participated in the study and signed the informed consent form. The study was in accordance with regulations of the Declaration of Helsinki.

Disclosure of conflict of interest

None.

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