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FGF2: a novel druggable target for glioblastoma?

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Abstract

Introduction: Fibroblast growth factors (FGFs) constitute important mitogens in development, tissue homeostasis and cancer. FGF2 is well known to regulate self-renewal of multiple stem cell types, thus is widely used in stem cell culture paradigms and has been adopted for cultivating growth of cancer stem cells *ex vivo*. Recent work has shed light on the functions of FGF2 in brain tumors, particularly malignant glioma, demonstrating that FGF2 also increases self-renewal of glioblastoma cancer stem cells. This review highlights the potential of targeting FGF2 signaling for anti-cancer therapy.

Areas covered: Based on the current literature, we describe the expression of FGF ligands and the FGFR family of receptor tyrosine kinases in the normal brain and in glioblastoma. In addition, we discuss FGF/FGFR signaling, including the function of heparin/heparan sulfate proteoglycans in facilitating FGF signaling. We speculate on avenues for potential therapeutic targeting of the FGF2-FGF receptor signaling axis in glioblastoma and the associated challenges envisioned with these approaches.

Expert opinion: More precise targeting of FGF/FGFR signaling holds potential for improved anti-cancer therapeutics with less adverse effects. Future development of specific models and inhibitors could provide a ‘pharmacological toolbox’ for targeting diverse ligand/receptor combinations.

Article Highlights

- Functions of FGF2 in neural development and brain cancer
- Overview of FGFR structure and signaling cascades
- Discussion of known FGFR functions in glioblastoma
- Synopsis of current pharmacological tools to block FGFR signaling
- Summary of current clinical trials testing FGFR inhibitors in brain cancer
- New strategies for therapeutic targeting FGF2 in glioblastoma

1. Fibroblast growth factors

Fibroblast growth factors (FGFs) encompass a family of 22 separate known members (reviewed in [1]) that were originally studied as early as the 1960s [2] for their function in driving fibroblast growth, but they were formally purified and characterized nearly a decade later [3]. Despite being a large family of growth factors, FGFs signal via 4 FGF receptors (FGFRs) (reviewed in [4]) with diverse tissue and developmental stage expression. FGF/FGFR signaling has a multitude of roles in development including proliferation, self-renewal, epithelial to mesenchymal transition, and invasion. In addition, FGFRs serve as a cellular entry point for a variety of downstream signaling pathways (reviewed in [4]). Given its importance in development, FGF signaling has also been of great interest in a variety of malignant tumors, where it plays similar roles in proliferation, survival, self-renewal of cancer stem cell (CSC) populations, and invasion. In this review, we focus on the known functions of FGF/FGFRs in glioblastoma (GBM), the most common primary malignant brain tumor that is associated with a poor prognosis and therapeutic resistance.

2. FGF2 in neural development

Studies uncovering the importance of FGF signaling in the developing brain have led to great advancements in neural stem cell culture that have been adopted for use in studying GBM CSCs *in vitro*. Specifically, basic FGF (bFGF, FGF2) was shown to be essential for the proliferation of mouse neural stem and progenitor cells in a variety of initial cell culture studies using both neurosphere culture and immortalized neural stem cells [5,6]. The requirement for FGF2 was confirmed in human neural stem and progenitor cells in neurosphere cultures, with the added requirement for heparan sulfate proteoglycans to stabilize FGF2 [7,8]. These culture approaches were leveraged for the identification and expansion of CSCs from GBM patients, as described later. While additional studies in neural development and adult neural stem cells revealed an equally important role for epidermal growth factor (EGF), development studies showed FGF2 was essential in early neural development during neural stem cell expansion while EGF became more dominant later during neural progenitor cell expansion and early differentiation into neurons [9]. Studies in neural stem cells from a variety of development stages revealed diverse expression of FGFRs with high FGFR1 and FGFR4 expression in neuroepithelial cells [10] and expression of FGFR1 and FGFR3 in neural stem cells in the ventricular zone [11], which signal to drive symmetric self-renewing stem cell division. Mouse knockout studies further confirmed an important role for FGFRs1, 2, and 3 in mid-brain development in a redundant manner [12], and deletion of FGFRs1, 2, and 3 resulted in complete loss of the dorsal telencephalon [13], a neural stem cell enriched region in the developing brain.

While there is a clear expression pattern of FGFRs in neural stem cells and a key requirement for FGFRs in neural stem cell function and neural development, there is limited insight into the ligand/receptor spatial interaction *in vivo*. Inferences have been made as to the sources of FGF2 and localization pattern in the neural stem cell niche, and careful examination of the cytoarchitecture of these regions has revealed thin fractones of laminin-enriched extracellular matrix enriched in bound FGF2 [14–16]. These observations provide a paradigm for localized FGFR signaling and a cellular mechanism by which neural stem cells can directly access FGFs embedded in their surrounding microenvironment. These earlier studies of FGFRs in the developing mouse brain and requirement of FGF2 in human neural stem cell cultures have served as the foundation for investigation of this signaling axis in GBM CSCs as described in section 4.

3. FGFR domains and signaling cascade

FGFR1-4 are encoded by 4 different genes and together they represent a distinct class of receptor tyrosine kinases (RTKs). The structures of FGFR1-4 consist of an extracellular ligand binding domain, a transmembrane domain, and an intracellular domain that interacts with cytoplasmic molecules and transduces FGFR signaling (**Figure 1**) [17–19]. Various FGFs can activate each FGF receptor, and some FGFs can activate multiple receptors. For instance, FGF2 can activate FGFRs1-4. Distinct from other classes of RTKs, the extracellular domains of FGFRs contain three immunoglobulin-like (Ig) loops: Ig-I, Ig-II, Ig-III (reviewed in [20]), with the junction between the Ig-II and Ig-III subdomains specifying their affinity for FGF ligands [21–23]. Alternative splicing of this region in each of the FGFR1-3 genes results in the generation of nearly 50 FGFR isoforms that have different affinity for FGF2. This is specifically achieved by alternative splicing of the Ig-III domain, which results in Ig-IIIb and Ig-IIIc isoforms encoded by exons 8 and 9, respectively. Different affinities of the Ig-IIIb and Ig-IIIc isoforms for FGF2 affect receptor sensitivity to this ligand and enable fine-tuning of FGF2 signaling across different cell types in the same tissue depending on their FGFR profile [17,24]; FGF2 binds more efficiently to the FGFR2/3-IIIc isoforms than the FGFR2/3-IIIb isoforms [26]. The expression of Ig-IIIb and Ig-IIIc is tissue specific, as IIIb is more prevalent in epithelial cells and IIIc is more prevalent in mesenchymal tissues [27]. Further, alternatively spliced ! and ∀ isoforms depend on the presence or absence of the Ig-I domain (encoded by exon 3). The truncated ! isoforms lack auto-inhibitory functions and are oncogenic. Finally, ligand-receptor affinity and sensitivity is further influenced by co-receptors like heparin/heparan sulfate proteoglycans (HSPGs) and klotho family members [28], as previously described [17,29–31].

FGFRs are RTKs, thus their intracellular domains consist of a juxtamembrane domain, two tyrosine kinase domains, and a C-terminal tail. Receptor dimerization is necessary for signal transduction, resulting in physical proximity of the cytoplasmatic tyrosine kinase domains, which then reciprocally phosphorylate each other (termed "transphosphorylation"). For this to happen, the hydroxyl group of the tyrosine residues receives a #-phospho group from ATP, which triggers subsequent phosphorylation events on downstream targets [32,33]. These phosphorylation events create binding sites for adaptor proteins, which facilitate specific cellular responses through the activation of an extensive network of signaling pathways [34] (**Figure 1**). Nuclear magnetic resonance coupled with FRET experiments of different FGFs bound to their receptors indicated FGFRs can dimerize and are phosphorylated in the absence of ligand, but FGF1 and FGF2 binding led to conformational changes and additional phosphorylation events on the cytoplasmic tails of FGFR1 and FGFR3. Interestingly, FGFR2 phosphorylation did not increase upon FGF1/FGF2 ligand binding. In addition, FGF1 and FGF2 binding led to distinct structural conformations, with FGF2 binding causing decreased distance between the transmembrane domains of FGFR and increased cytoplasmic phosphorylation [35]. This finding may hint at the prognostic relevance of FGF2 in GBM described below. As part of the RTK family, FGFRs orchestrate the activation of multi-protein complexes associated with cell proliferation, survival, cytoskeletal regulation and receptor degradation [36]. FGFR-mediated cell survival is mainly promoted by the activation of PI3K/AKT and STATs signaling [37], while FGFR-resultant RAC/JNK/p38 and RAS/MEK/MAPK signaling are relevant for cell proliferation [23,24,37]. In addition, FGF2-induced activation of the MAPK cascade is regulated by the Sprouty proteins Spry3 and Spry4. Spry3 accelerates MAPK-induced proliferation and migration while this effect is counterbalanced by Srpy4 [38].

4. FGFRs in GBM

FGFRs have different expression levels and functions in GBM (**Table 1**). Recently, FGFR1 has been identified as a crucial target for inhibition of FGFR signaling in GBM as multiple studies have shown a positive association between glioma progression and FGF2-FGFR1 expression and function. For instance, FGFR1 was shown to be a key regulator of glioma growth [39] and invasion [40]. Moreover, FGFR1 was shown to induce GBM cell radioresistance through PLC-#, which is also involved in the regulation of hypoxia and apoptosis [41,42]. In addition, the role of FGFR1 as a GBM CSC regulator has been recently discovered [43,44]. FGFR1 is specifically expressed in GBM CSCs and translates FGF2 stimuli to regulate crucial stem-cell associated transcription factors including ZEB1, SOX2 and OLIG2. Importantly, FGFR1 is the only FGFR reported to promote tumorigenicity *in vivo* [43].

These results outline a new way of isolating GBM CSCs and offer a potential novel therapeutic target to treat GBM. In addition, elevated Spry3 expression and reduced or absent Spry4 expression have been detected in GBM, consistent with increased activation of FGF2-mediated MAPK signaling [38].

The functional relevance of FGFR2 and FGFR3 signaling in GBM is not yet completely understood. These receptors have been associated with differentiated cancer cells, and are expressed in patient-derived human GBM lines [43], but may not be relevant for CSC function. In agreement with this notion, reduced FGFR2 expression including both its IIIb and IIIc isoforms has been linked with higher tumor grade and poorer survival in glioma patients [45]. While FGFR1 knockdown reduced patient-derived GBM CSC sphere forming capacity, knockdown of FGFR2 and FGFR3 in GBM CSCs did not affect sphere formation [43]. However, Ma *et al.* demonstrated that FGFR2 drives phosphorylation of Y240 on PTEN in human GBM samples, identifying a potential mechanism of FGFR2-mediated radioresistance in GBM [46]. As for FGFR3, an oncogenic FGFR3 form fused to TACC3 (Transforming acidic coiled-coil-containing protein 3) has been identified [47]. This mutation occurs in 3% of gliomas and induces ligand-independent activation of the FGFR pathway and chromosomal instability [47]. Glioma cells carrying the FGFR3-TACC fusion overcome the detrimental effects of these chromosomal alteration due to their growth advantage [48].

5. Targeting FGF2 signaling in GBM

FGF2 is a known oncogenic factor in GBM [49] as it contributes to glioma growth [50], vascularization [51,52] and GBM CSC self-renewal [53]. Therefore, the FGF2/FGFRs system is potentially an important target for therapeutic approaches to inhibit progression and spread of this disease. The relevance of therapeutic targeting of FGF2 (**Figure 1,** number 2) is highlighted by experimental studies using FGF2-specific anti-sense oligonucleotides or antibodies to block glioma cell proliferation [39,54] and angiogenesis [55,56], which increased survival in animal models of glioma. While these studies provide proof-of-concept, pharmacological targeting of FGF2 *in vivo* has not yet been attempted. The development of inhibitors that interfere with the binding of FGF2 to its cell surface receptors [57] enables a more direct approach for pharmacological blocking of FGF2/FGFR signaling (**Figure 1,** number 1). Indeed, Foglieni *et al.* have identified new small-molecule inhibitors of FGF2 aiming to mimic the function of thrombospondin, a protein that endogenously blocks angiogenesis through FGF2 interaction and sequestration [57,58]. Similar to thrombospondin, these small molecules block FGF2 activity by directly interfering with the FGF2/HSPG binding and by allosterically affecting FGF2/FGFR interaction. The authors described that these

inhibitors reduced endothelial cell proliferation and vessel sprouting from aortic rings embedded in Matrigel in the presence of FGF2. Ronca *et al.*, have also identified a small molecule (NSC12) with anti-angiogenic characteristics that functions as a multi-FGF trap by blocking the FGF/FGFR interaction. NSC12 showed anti-cancer efficiency *in vivo* [59]. Recently, we have used these compounds [57] in primary GBM patient-derived cells showing a reduction in sphere/colony formation capacity *in vitro* [43]. This work highlights the importance of targeting FGF2 to inhibit CSC maintenance and provides proof-of-concept that blocking FGF2 may be therapeutically beneficial. Further work is needed to establish whether these FGF2 blocking agents are able to cross the blood-brain barrier, and whether they show efficacy *in vivo*.

6. Targeting FGFRs

As described in the previous section, FGF2 signaling can be inhibited in experimental models by blocking ligand-receptor binding. In addition to interference with ligand/receptor interactions, FGF2 signaling can be targeted at the level of its cognate receptors. Although this approach is not specific to FGF2 signaling over other FGF family members, the differential expression of FGFRs on GBM CSCs (i.e. high FGFR2, mid FGFR1&3, and absent FGFR4) [43] may enable targeted approaches for GBM therapy. Current pharmaceutical development is most advanced for small molecule RTK inhibitors (**Figure 1,** number 3), which are in clinical trials for several malignancies, including GBM (**Table 2**). A number of different small molecule inhibitors of FGFR tyrosine kinases have been developed (e.g. PD173074, BGJ398, AZD4547), which show good selectivity of FGFRs over other RTKs, and in some instances selectivity for FGFR1-3 over FGFR4 (reviewed in [60]). In preclinical studies, Ma *et al.*, showed that AZD4547 increased the anti-tumor effects of irradiation, decreasing tumor growth and prolonging survival in mice orthotopically transplanted with patient derived GBM CSCs [46]. In addition, in a glioma xenograft model where FGFR3-TACC3–transformed astrocytes were orthotopically transplanted, treatment with PD173074 inhibited tumor growth, and treatment with AZD4547 prolonged survival of glioma-bearing mice [47]. Recently, Holzhauser *et al.,* showed that the combination of AZD4547 with a PI3K inhibitor (BEZ235) is more efficient for the treatment of pediatric medulloblastoma than the single administration of AZD4547 or BEZ235 [61]. Currently there are no clinical-grade inhibitors with selectivity for individual FGFRs or their isoforms [31]. Given the possibility that FGFR1-3 may have divergent functions in GBM and considering the prevalent expression of these receptors on other cell types in the brain, development of inhibitors with selectivity for individual FGFRs is desirable. Indeed, Jang *et al.* have recently used a FGFR1 specific inhibitor (SSR128129E) *in vitro* and *in vivo* xenograft models showing a sensitization of chemo-refractory cancers such as breast and

cervical cancers [62,63]. It will be interesting to evaluate the efficacy of this and future FGFR1-specific inhibitors to cross the blood-brain barrier and for targeting of the FGF2-FGFR1 axis in GBM CSCs *in vivo*.

In our recent study, we found that FGF2 activates FGFR1 on the CSC cell surface, inducing expression of stem cell-associated transcription factors through ERK1/2 signaling [43], which is one of the main RAS signal-regulated kinase pathways hyperactivated in GBM [64,65]. Therefore, FGF2-FGFR1 signaling may also be targeted indirectly by using ERK1/2 inhibitors capable of crossing the blood-brain barrier [66]. Nonetheless, patient response to treatments targeting single molecules of the MAPK family in GBM has been minimal. One of the known off-target effects related to this therapy resistance is the upregulation of RTKs [67]. In addition, ERK1/2 inhibition can promote the activation of other signaling effectors such as STAT3 and AKT in GBM [67,68] which highlights the need of combinatorial therapies to treat GBM.

The recent identification of a new targetable axis in GBM where FGF2 is released into the tumor microenvironment by ADAMDEC1, a protease of the ADAM family of zinc proteases [43], presents another avenue for therapeutic intervention (**Figure 1,** number 4). As GBM CSCs secrete ADAMDEC1 exclusively, blocking ADAMDEC1 activity could be exploited to specifically prevent access of GBM CSCs to FGF2. It will be interesting to see development of small-molecule inhibitors against this protease and to test their efficacy in experimental models of GBM, aggressive pediatric brain tumors [69] and pediatric medulloblastoma [61], which may all benefit from novel therapies targeting FGFR signaling. Initial studies indicate the identification of such compounds may be possible using classical biochemical screening approaches [70].

7. Conclusion

The expression and demonstrated function of FGF signaling (specifically FGF2/FGFR1) in GBM and specifically in GBM CSCs warrants excitement and justifies efforts to develop strategies to inhibit these molecules for therapeutic use. Attenuating GBM CSC function via specific blocking of FGFR1 is a promising and alternative approach to the targeting of soluble factors. However, as the kinase domains of FGFR1-4 exhibit a high degree of homology with each other, there may be issues with specificity. In addition, given the importance of FGF/FGFR pathways in normal neural development as discussed in section 2, it will be prudent to utilize established non-neoplastic (stem) cell culture paradigms for assessing the therapeutic window of FGF/FGFR inhibitors. In light of these complications, identifiying alternative targeting mechanisms for FGF signaling in GBM should be a priority, and we

believe efforts should be focused on identifying unique mechanisms used by cancer cells to upregulate this pathway in GBM that are not present in normal neural cells. For example, our observation of ADAMDEC1 being secreted and leading to increased FGF2 release from GBM CSCs could be exploited for therapeutic development with potentially fewer off-target effects compared to targeting of individual receptors. Molecular genetic observations in GBM have also provided an opportunity for development of GBM-specific FGF signaling approaches, namely those involving the FGFR3-TACC fusion. For targeting of this fusion event to be exploited, a genetic mouse model of GBM driven by this fusion could be a platform for the assessment of potentially clinical relevant inhibitors. FGFR targeted therapies are currently being investigated in early phase clinical trials. If significant toxicities are observed due to a small therapeutic window, more advanced strategies could be tested. For instance, advancements in chimeric-antigen receptor immunotherapies may eventually pave the way for bispecific inhibition of FGFR1 and another putative cancer stem cell marker to increase specificity over normal neural stem and progenitor cells. As we continue to study this pathway in GBM, including its role in resistance to standard of care therapies, we hope that additional targeting opportunities will unfold.

8. Expert opinion

Pharmacological targeting of the FGF2/FGFR axis is an exciting avenue for GBM therapy. High-resolution structural information of FGFRs including their ligand-binding sites and co-receptors exists and is prerequisite for development of new compounds that interfere with this signaling axis. For instance, structural changes in the ternary receptor complex induced by the binding of specific FGF ligands could potentially be exploited for the design of inhibitors that not only target a particular receptor, but a combination of ligand and receptor. Currently, existing FGFR inhibitors target the tyrosine kinase domain, which offers the advantage of disrupting an essential step in transducing downstream signaling and thereby provides highly reliable inhibition. Conversely, these compounds typically do not discriminate very well between different FGFRs, partly because the tyrosine kinase domain is highly homologous across the four FGFRs. More precise targeting of specific receptor and ligands combinations could reduce off-target effects and/or other adverse effects (e.g. 30-50% of patients treated with AZD4547 showed hyperphosphataemia, whereas monoclonal antibodies against FGFR1 resulted in unexpected weight loss in preclinical animal studies).

More recent preclinical studies have identified compounds that block ligand/receptor interactions for FGF2. These could provide useful lead structures for further development of precision inhibitors, eventually generating a ‘pharmacological toolbox’ for specific blockade of a number of ligand/receptor combinations. Ideally, this would enable highly selective

interference with FGFR signaling pathways relevant for cancer progression while preserving essential functions of these receptors in non-neoplastic tissues. This could potentially limit adverse effects, while simultaneously increasing anti-cancer efficacy. Such a 'toolbox' would be useful beyond GBM, enabling targeting FGFR signaling in other cancers as well where different FGF ligands crucially activate this axis.

Several issues need to be overcome to achieve this goal:

(i) Additional high resolution structural data needs to be generated for different permutations of FGFRs, FGF ligands and co-receptors. Currently, the RCSB protein data bank contains structural data for four FGFs bound to FGFR1, three ligands bound to FGFR2, and FGF1 bound to FGFR3. Better understanding of the structural relationship between different FGFRs and their ligands will be essential to dissect the biology of these receptors as well as for precise pharmacological targeting.

(ii) Additional small-molecule inhibitors should be developed that block FGF/FGFR interactions based on currently known compounds. For instance, inhibitors of FGF2 binding to FGFRs are based on FGF2-binding capacity of Thrombospondin-1 or Pentraxin-3 [57–59]. Development of structural derivatives of these tool compounds may enable further optimization of their inhibitory activity and/or other pharmacological parameters. Similar strategies may also be employed to develop inhibitors for other FGF ligands.

(iii) Assays should be developed to screen novel inhibitors for their efficacy against cancer cells and for their safety in normal tissues. Blocking specific FGFR/ligand interactions will likely improve targeting of GBM over non-neoplastic cells, but this needs to be validated in appropriate settings.

The FGFR3-TACC fusion is considered a driving genetic event in GBM pathogenesis due to its abilty to lead to tumorigenicity in primary *Ink4A;Arf*$^{-/-}$ astrocytes and anchorage independent growth of fibroblasts. Likewise, stereotactic transduction of mouse hippocampus with FGFR3-TACC lentivirus in combination with p53 knockdown leads to invasive malignant gliomas in mice. Given these findings and homogenous expression of FGFR3-TACC fusions detected in primary GBM tumors, patients with gliomas carrying this fusion may represent the group most likely to benefit from FGFR-targeted therapies [48]. As discussed earlier, preclinical studies demonstated that AZD4547 prolongs survival of FGFR3-TACC^{+} glioma bearing mice. AZD4547 has been tested in a Phase II clinical trial of recurrent malignant

gliomas expressing the FGFR-TACC fusion (NCT02824133) but has yet to demonstrate efficacy in the human setting. Therefore, specific, brain penetrant inhibitors for this fusion should continue to be developed and tested in mouse models of FGFR-TACC driven GBM.

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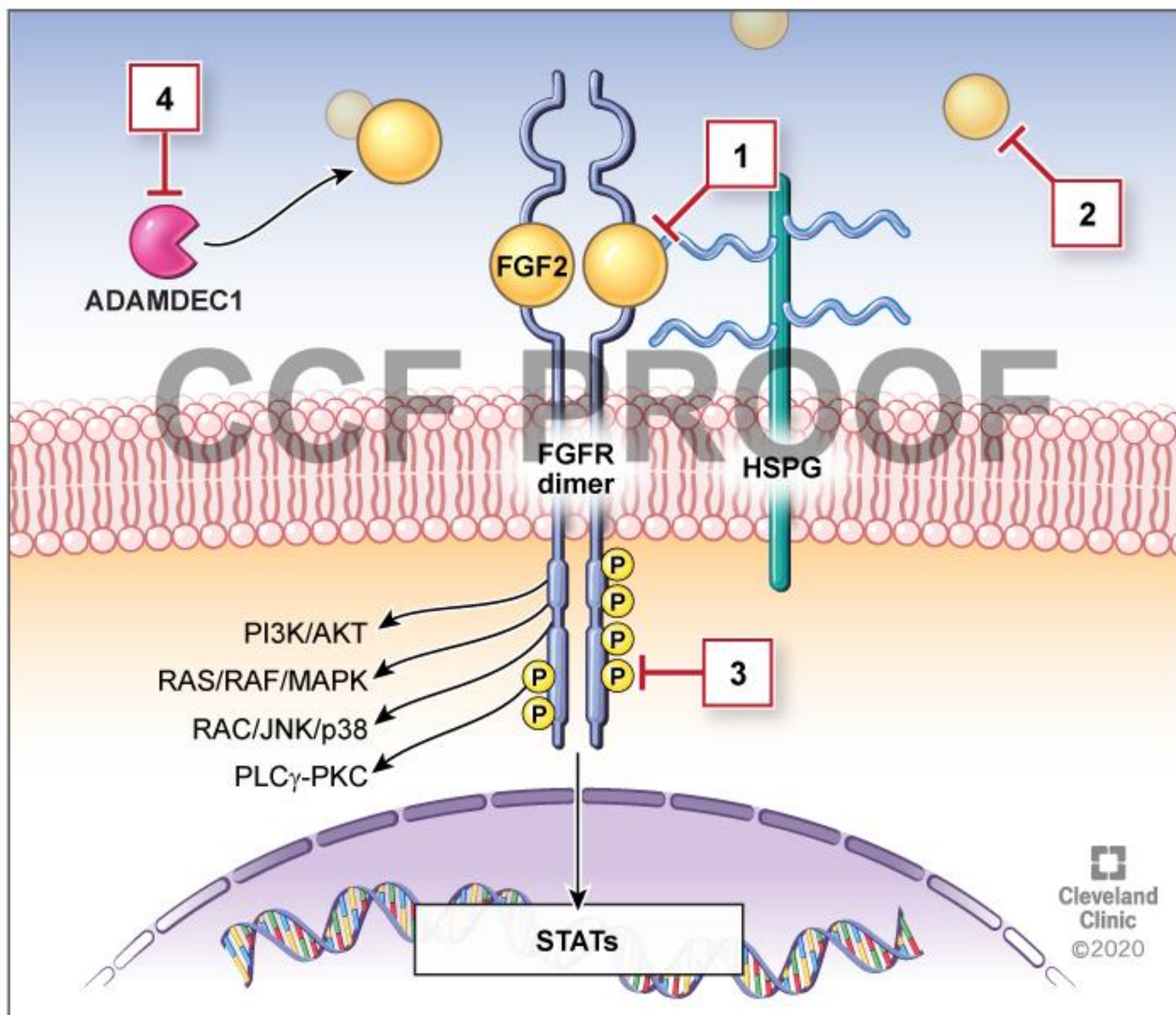
Figures and Tables

Figure 1. FGFR signaling pathway and its targetable axis. FGFR possesses three domains: an extracellular domain, a single transmembrane domain and an intracellular domain. The FGF-FGFR complex is stabilized by heparin/HS chains of the HSPG. After ligand-receptor binding, FGFRs dimerize and activate multiple signal transduction pathways through phosphorylation of their tyrosine kinase domain. These pathways (STATs, PI3K/AKT, RAS/RAC/JNKs/p38, RAS/RAF/MAPK/ERK, PLCγ/PKC) induce the expression of specific target genes related to cell proliferation, survival and self-renewal. In CSCs, ADAMDEC1 releases FGF2 into the tumor microenvironment, which mediates the activation of stem cell associated transcription factors contributing to GBM CSC self-renewal and maintenance. Boxes **1-4** indicate possible targetable axes to pharmacologically inhibit the FGFR signaling pathway.



Receptor	Expression levels in GBM	Function
FGFR1	High mRNA [71] and protein levels [72].	Regulates proliferation [23,53], migration [40] radioresistance [41,42], GBM CSC maintenance *in vitro* and tumorigenicity *in vivo* [43].
FGFR2	Low mRNA [73] and protein levels [72]	Possible tumor suppressor role that needs to be further assessed [73]. Potential regulator of radioresistance [46].
FGFR3	High FGFR3-TACC copy number in GBM patients with this mutation [74,75]	FGFR3-TACC fusion's oncogenic function leads to increased tumor proliferation *in vitro* and tumorigenicity *in vivo* [74]
FGFR4	Different results depending on article [39,43]	No differences in patient survival between FGFR4 high versus low expressing GBMs [43]

Table 1. Expression and function of FGFR1-4 in GBM. Protein and mRNA expression levels differ significantly among FGFRs in GBM. FGFR1 and FGFR3-TACC are highly expressed, FGFR2 is reported to be lowly expressed, while inconclusive results are identified for FGFR4. The function of each FGFR describes the relevance of these receptors in GBM.

Compound	Selectivity	C s (Glioma or GBM)	Dates and Locations	Phase of trial / Clinical
Anlotinib (AL3818)	Broad (VEGFR, Kit, PDGFR, FGFR)	NCT04004975	2019 – ongoing, Shangdong, CN	Phase II / not available
Nintedanib (BIBF-1120)	RTK (VEGFR, PDGFR, FGFR) and NRTK (Lck, Lyn, Src)	NCT01666600	2012-2017, Heidelberg, DE	Phase II / not available
		NCT01251484	2010-2012, Copenhagen, DK	Phase II / not available
		NCT01380782	2011-2014, Boston/Cleveland/ Virginia, USA	Phase II / nintedanib is not active against recurrent high-grade glioma [76]
Erdafitinib (JNJ-42756493)	FGFR1-4	NCT03210714	2017 – ongoing, multicentric	Phase II / not available
Ponatinib (AP24534)	FGFR1-4	NCT02478164	2015-2018, Boston, USA	Phase II / lack of efficacy
TAS-120	FGFR1-4	NCT02052778	2014 – ongoing, multicentric	Phase II / not available
AZD4547	FGFR1-3 (IGF1R, KDR)	NCT02824133	2016 – 2019, Paris, FR	Phase II / not available
BGJ398	FGFR1-3	NCT01975701	2003 – 2019, multicentric	Phase II / limited efficacy; 65% patients needed dose reductions or interruptions*
		NCT02150967	2014 – ongoing, multicentric	Phase II / not available

Table 2: Clinical trials involving FGFR inhibitors in glioma. Listed are small-molecule inhibitors either in currently ongoing or recently completed clinical trials. Included clinical trials are either exclusive for glioma or include brain cancers as part of a larger cohort of cancer patients. * indication was no longer pursued after outlicensing of BGJ398.