Targeting Fibroblast Growth Factor Receptor (FGFR) with BGJ398 in a Gastric Cancer Model

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Abstract. *Aim: To assess the efficacy of targeting fibroblast growth factor receptor (FGFR) with the pan-FGFR inhibitor BGJ398 in a gastric cancer (GC) model. Materials and Methods: Expression of FGFRs was determined in GC cell lines (KKLS, MKN-45, TMK-1). Impact of the FGFR inhibitor BGJ398 on growth, motility, signaling, expression of transcription factors and secretion of vascular endothelial growth factor-A (VEGFA) was determined in vitro. Results were validated in subcutaneous tumor models. Results: In vitro, FGFR inhibition was most effective in KKLS cells (high FGFR1, FGFR2IIIc, no FGFR2IIIb expression) with inhibition of growth, motility, signaling, c-MYC expression and VEGFA secretion. BGJ398 showed some activity in MKN-45 cells (intermediate FGFR1, high FGFR2IIIb, low FGFR2IIIc expression), while TMK-1 cells (low FGFR1, no FGFR2IIIb and FGFR2IIIc expression) did not respond. Results were confirmed in vivo with strongest efficacy on growth in KKLS tumors and only minor impairment of TMK-1 lesions. Conclusion: Efficacy of FGFR inhibition is dependent on FGFR1 and FGFR2IIIc expression in GC models.*

Gastric cancer (GC) is the fifth most common solid malignancy and the second most frequent cause of cancer-related death worldwide (1). Overall, 5-year survival from GC is approximately 20-30%, with slightly better results in Japan due to the lack of screening programs in other countries (2). So far, surgical resection accompanied by neoadjuvant and adjuvant treatment is the standard-of-care for localized GC. In advanced GC, systemic chemotherapy provides only minor survival benefit with median survival time between 10 and 13 months (3). Therefore, novel therapeutic opportunities based on the increasing knowledge of molecular properties and potential pharmacological targets in GC are urgently needed to improve the prognosis of patients suffering from this disease.

The fibroblast growth factor/fibroblast growth factor receptor (FGF/FGFR) system consists of 18 ligands (FGFs) and four receptors (FGFR1-4) (4, 5). Upon ligand binding FGFRs activate several signaling cascades, particularly phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) and mitogen-activated protein kinases (MAPK)/extracellular-signal-regulated kinase (ERK) (6). In turn, this leads to regulation of diverse cellular functions which play a pivotal role not only in physiological homeostasis but also in carcinogenesis, *e.g.* proliferation, motility, angiogenesis, anti-apoptosis and drug resistance (5, 7). Regarding GC, Deng *et al.* found genomic alterations in *FGFR2* in 9% of tumors (8). Moreover, immunohistochemical assessment in 950 patients with GC showed overexpression of FGFR2 in 31.1% of the cases (9). Finally, overexpression of FGFR1, FGFR2 and FGFR4 was recently associated with tumor progression and poor prognosis in 222 patients with GC (10). Therefore, targeting FGFRs is an interesting approach for treatment of GC.

During the past decade, several FGFR inhibitors have been evaluated in preclinical and clinical studies up to phase III trials (5, 11). Nonetheless, most of the FGFR inhibitors in clinical trials such as Dovitinib (TKI258) or AZD4547 are not specific for FGFR, but also target vascular endothelial growth factor receptor (VEGFR) (AZD4547), platelet-derived growth factor receptor (PDGFR), Fms-like tyrosine kinase 3 (FLT3), v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT) and colony stimulating factor 1 (CSF1) (Dovitinib) (NCT01576380 and NCT01719549). BGJ398 is a potent selective and orally available pan-FGFR kinase inhibitor (12). BGJ398 has been used in several pre-clinical models including cholangiocarcinoma (13), endometrial

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cancer (14), head and neck cancer (15), hepatocellular carcinoma (HCC) (16) and colorectal cancer (17). Based on results from these experiments, BGJ398 has entered clinical phase I and II studies either alone or in combination with other targeted agents for the treatment of cancer including advanced solid malignancies, melanoma, cholangiocarcinoma and gastrointestinal stromal tumors (NCT01004224, NCT01820364, NCT02150967, NCT02257541).

The aim of our study was to assess the effects of targeting FGFR with BGJ398 on GC cell lines. Our results suggest that treatment with BGJ398 impairs tumor growth *in vitro* and *in vivo* by effects on oncogenic signaling and angiogenesis, in particular when tumor cells express FGFR1 and FGFR2IIIc.

Materials and Methods

Cell lines, culture conditions and reagents. The human GC cell line TMK-1 was obtained from Dr. Eiichi Tahara (University of Hiroshima, Hiroshima, Japan), KKLS cells from Yutaka Takahashi (Cancer Research Institute, Kanazawa University, Kanazawa, Japan), MKN-45 cells from Professor Derek Zieker (University of Tuebingen, Tuebingen, Germany) and Hep3B cells from the American Type Culture Collection. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Lonza Group, Basel, Switzerland) supplemented with 10% fetal calf serum (FCS; Sigma-Aldrich, St. Louis, MO, USA) and maintained at 37˚C in a humidified atmosphere with 5% CO_2. The FGFR inhibitor BGJ398 (Novartis Oncology, Basel, Switzerland) was dissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich; *in vitro*) or water (*in vivo*).

Real-time PCR analysis. Expression levels of FGFR1-4 were assessed by real-time PCR from cultured cells incubated with and without BGJ398 (250 nM for 24 h). Total RNA was isolated and processed by spin column-based nucleic acid purification (NucleoSpin® RNA II; Macherey-Nagel, Düren, Germany). From each sample, a 1 μg aliquot was reversely transcribed into cDNA (Transcriptor First-Strand cDNA Synthesis Kit; Roche, Mannheim, Germany). Selected PCR primer pairs are described elsewhere (16). RT-PCR was performed using the LightCycler system and LightCycler® 480 SYBR Green I Master kit (Roche, Mannheim, Germany). 18S served as a housekeeping gene. Results were normalized to those for FGFR expression in Hep3B since this cell line expresses all isoforms.

Determination of cell growth by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) bromide assays. GC cells (10^3 cells) were seeded into 96-well plates. After 24 h, the cells were exposed to increasing concentrations of BGJ398 (0, 10, 100, 250, 500, 1,000, 2,500 nM) in 10% FCS-DMEM and under serum-starved conditions (1% FCS-DMEM) after a further 24, 48 and 72 h, MTT assays were used to determine cell growth as shown elsewhere (16).

Cell motility. Migration assays were conducted using modified Boyden chambers as described elsewhere (16). In brief, 5×10^4 cells were resuspended in 1% FCS-DMEM and seeded into 8-μm filter pore inserts (BD, Heidelberg, Germany). As chemoattractant, serum-rich medium (10% FCS) with and without BGJ398 (250 nM) was used. After 24 h, migrated cells were fixed, stained (Diff Quik, Medion Diagnostics, Miami, FL, USA) and counted in four random fields.

Western blotting. Western blotting was performed as described previously (18). Briefly, tumor cells were incubated with different concentrations of BGJ398 (0, 100, 250, 500 nM) and stimulated with 100 μM of the hypoxia mimic desferroxamine (DFX; Sigma-Aldrich) for 24 h. Whole cell lysates were then prepared and protein samples (30 μg) were subjected to a denaturating 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Sequentially, the membranes were probed with primary antibodies against the signaling intermediates $pAKT^{Ser473}$, AKT, $pERK^{Tyr202/204}$, ERK, c-MYC (Cell Signaling, Beverly, MA, USA); hypoxia-inducible factor-1α (HIF1α) and hypoxia-inducible factor-2 α (HIF2α) (Novus Biological, Littleton, CO, USA), and β-actin (Santa Cruz Biotechnologies, Dallas, TX, USA). Antibodies were detected by enhanced chemiluminescence (luminol and p-coumaric acid; Sigma-Aldrich).

Enzyme-linked immunosorbent assay (ELISA) for VEGFA. To determine changes in VEGFA secretion upon FGFR inhibition with BGJ398, an ELISA kit was used according to the manufacturer's instruction (BioSource, Nivelles, Belgium). GC cells were plated at 40-50% density, incubated with and without BGJ398 (250 nM) and stimulated by DFX (100 μM) for 24 h before collection of culture supernatants.

Subcutaneous GC models. Experiments were approved by the Institutional Animal Care and Use Committee of the University of Regensburg and the regional authorities (54-2532.1-1/13). In addition, experiments were conducted according to "Guidelines for the Welfare of Animals in Experimental Neoplasia" published by The United Kingdom Coordinating Committee on Cancer Research (19). Cancer cells (1×10^6) were subcutaneously injected into the right flank of 6- to 8-week-old athymic nude mice (Crl:NU(NCr)-*Foxn1*^{nu}, n=6-8 animals per group; Charles River, Sulzfeld, Germany), as described elsewhere (20). Mice were randomized and assigned to control or treatment groups. The BGJ398 application (10 mg/kg/d from Monday to Friday *via* oral gavage) was initiated when tumors reached a size of approximately 80 mm^3. Tumor diameters were measured and volumes calculated ($width^2 \times length \times 0.5$). The experiment was terminated after 22, 25 and 30 days for with TMK-1, KKLS and MKN-45 tumors, respectively. Tumors were excised, weighed and processed for further experiments.

Immunohistochemical analysis of tumor vascularization. To determine CD31-positive vessel area, cryosections of tumor tissue were fixed in cold acetone and chloroform, washed with phosphate-buffered saline and exposed to primary antibody against CD31 (1:50; Pharmingen, Heidelberg, Germany); a secondary antibody AlexaFluor 488 (1:200; Live Technologies, Carlsbad, CA, USA) was then applied as described elsewhere (16).

Statistical analysis. Statistical analyses were performed using SigmaStat (Version 3.0; Systat Software, San Jose, CA, USA) . Results of *in vivo* experiments were analyzed for significant outliers using Grubb's test (www.graphpad.com). The two-sided Student's *t*-test was applied for analysis of *in vitro* data. Results for migration assays and PCR are shown relative to those of controls. Expression of FGFRs in all GC cell lines was normalized to that of Hep3B since this tumor cell line expresses all FGFRs. All results were confirmed in independent experiments and are expressed as the mean±SEM.

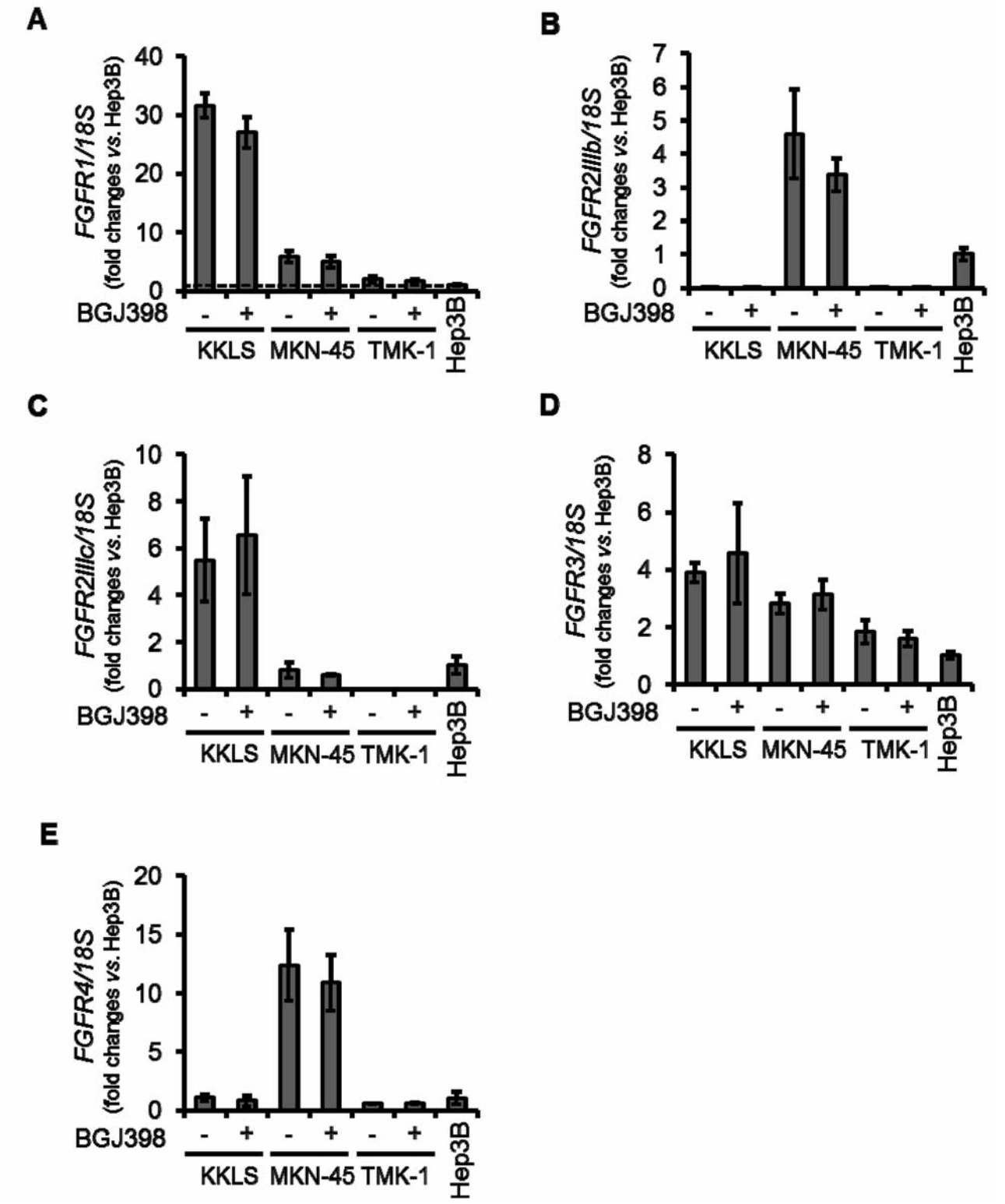

Figure 1. *Expression of fibroblast growth factor receptors (FGFRs) in gastric cancer cell lines in vitro. A: FGFR1 was expressed in all three GC cell lines KKLS, MKN-45 and TMK-1. B: FGFR2IIIb was only detected in MKN-45 but not in KKLS and TMK-1 cells. C: FGFR2IIIc was expressed in KKLS and to lesser amount in MKN-45 cells. In TMK1 cells, this FGFR isoform was not expressed. D: FGFR3 was found in all three cell lines at similar amounts. E: FGFR4 was expressed in KKLS, MKN-45 and TMK-1 cells. FGFR blockade by BGJ398 did not affect the receptor expression in any of the three cell lines. Results are normalized to those for Hep3B (set at 1; dotted line). Data are the mean±SEM of n=3.*

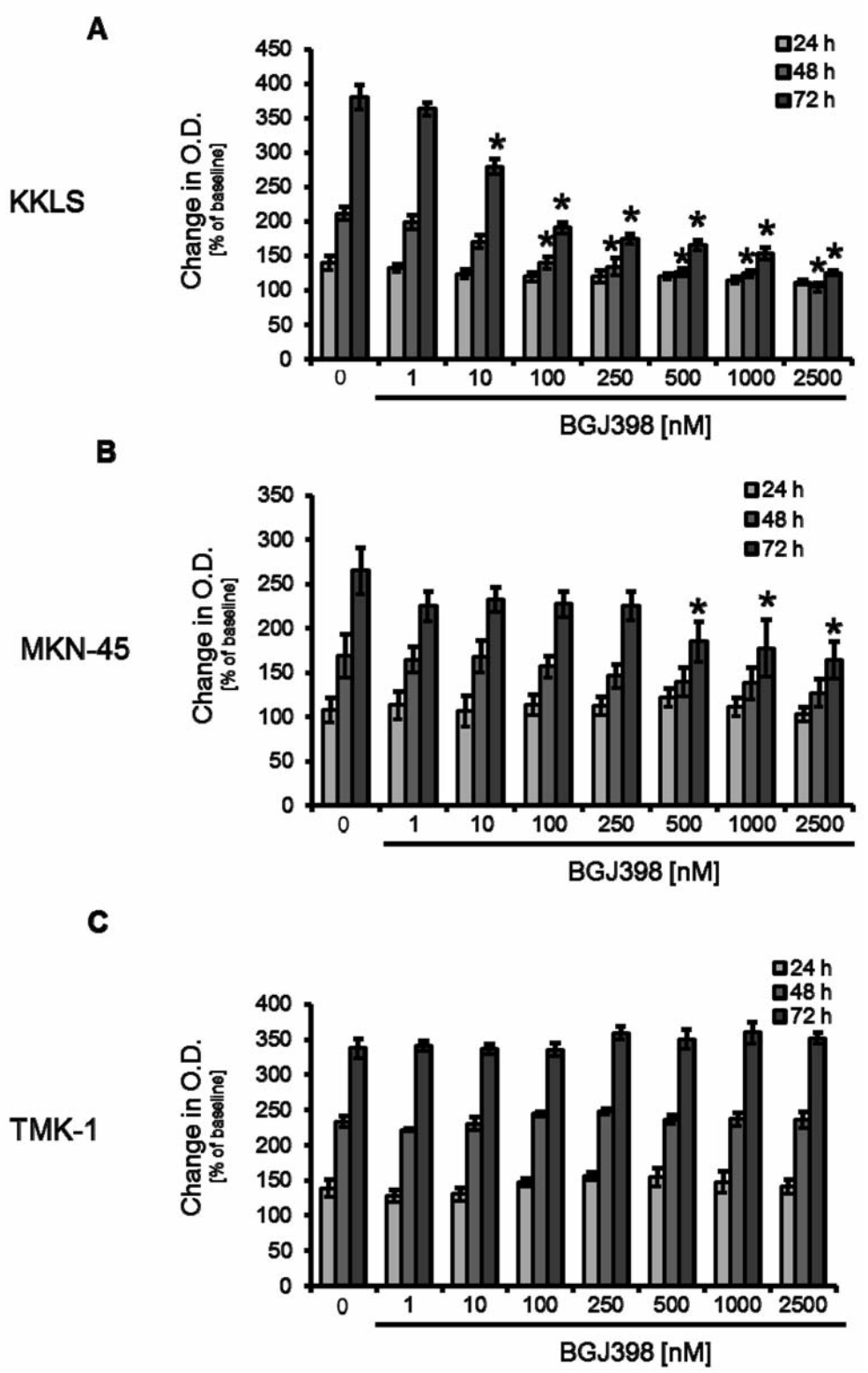

Figure 2. *MTT assays of gastric cancer (GC) cell lines treated with fibroblast growth factor receptor (FGFR) inhibitor BGJ398. A: FGFR inhibitor BGJ398 significantly impaired growth of KKLS GC cells, from 100 nM after 48 h and 10 nM after 72 h (*p<0.05). B: Less substantial inhibitory effects were detected in MKN-45 cells after 72 h, from 500 nM (*p<0.05). C: In TMK-1 cells, FGFR blockade did not impact tumor cell growth. Data are the mean±SEM of n=3.*

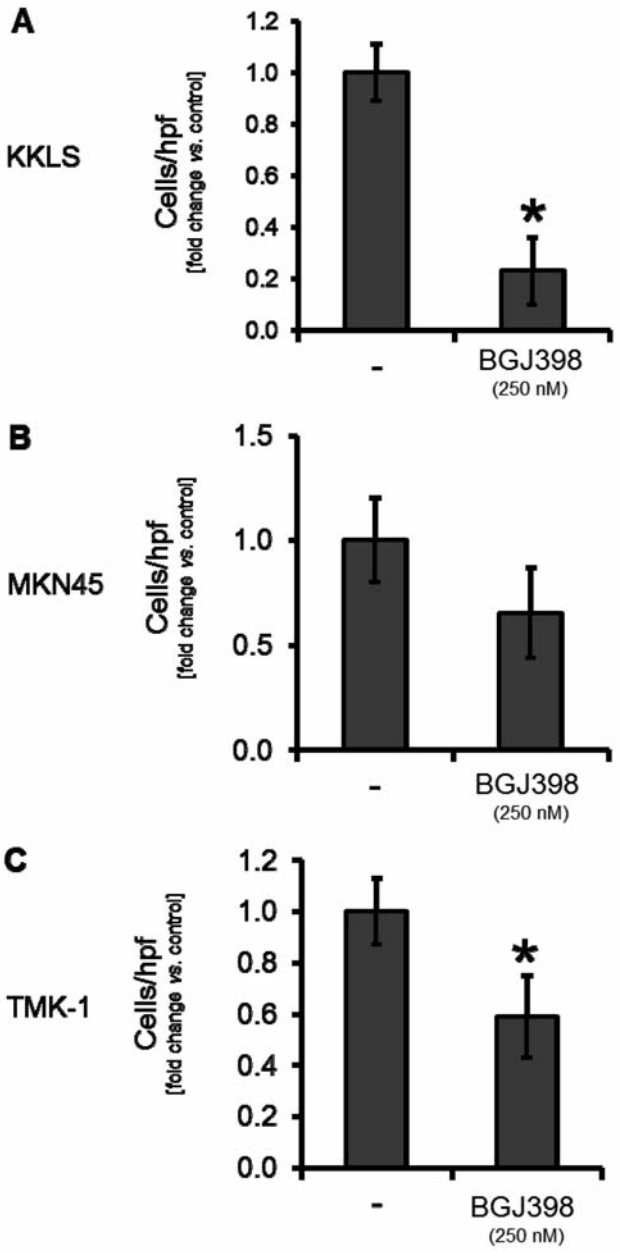

Figure 3. *Impact of targeting fibroblast growth factor receptor (FGFR) on motility of GC cell lines. A: FGFR blockade by BGJ398 significantly reduced migration of KKLS GC cells (*p<0.05). B: Treated MKN-45 cells also revealed a trend towards having a lower migration rate but this effect did not reach statistical significance. C: In TMK-1 cells, targeting FGFR significantly impaired cell motility (*p<0.05). Data are the mean±SEM of n=3.*

Results

Expression of FGFR1-4 in GC cell lines. Firstly, the expression of FGFRs was determined in GC cell lines KKLS, MKN-45 and TMK-1 to evaluate potential targets for BGJ398. The HCC cell line Hep3B served as a positive control since this cell line has been shown to express all FGFRs (16). The results showed that FGFR1, -3 and -4 can be detected in all three GC cell lines. In contrast, FGFR2IIIb was only found expressed in MKN-45 cells (very weakly in KKLS and TMK-1 cells), whereas FGFR2IIIc was found in KKLS and, to a lesser extent in MKN-45 but not in TMK-1 cells. Treatment with BGJ398 (250 nM, 24 h) did not have an impact on the receptor expression in any of the three cell lines (Figure 1A-E). In summary, our results show that FGFRs are expressed in GC cell lines, but differences regarding expression of FGFR1, FGFR2IIIb and FGFR2IIIc exist.

Different effects of targeting FGFR with BGJ398 on growth of GC cell lines in vitro. The growth-inhibitory capability of FGFR inhibition with BGJ398 was assessed by MTT assay. The results show a significant inhibition of growth of KKLS cells upon incubation with BGJ398 from 100 nM after 48 h and 10 nM after 72 h (Figure 2A). In contrast, growth inhibition of MKN-45 cells was only observed after 72 h incubation from 500 nM BGJ398 (Figure 2B). Finally, the TMK-1 GC cell line was not affected by FGFR blockade (Figure 2C). In conclusion, BGJ398 has significant growth-inhibitory effects on KKLS and little effect on MKN-45 cells; TMK-1 cells were essentially unaffected by FGFR blockade.

Inhibition of GC cell motility in vitro. Subsequently, we evaluated the functional consequences of FGFR blockade in the three GC cell lines. After 24 h, BGJ398 (250 nM) led to significant inhibition of tumor cell motility in KKLS and TMK-1 cells (Figure 3A and C). In MKN-45 a trend towards reduced migration was detected but this did not reach statistical significance (Figure 3B). Nonetheless, these findings show that FGFR inhibition can reduce cancer cell motility.

Impact of BGJ398 on signaling intermediates and expression of transcription factors in vitro. Next we investigated the consequences of FGFR inhibition by BGJ398 on ERK and AKT signaling. In KKLS cells, complete abrogation of ERK phosphorylation and dose-dependent reduction of AKT phosphorylation was detected (Figure 4A). In MKN-45 cells, BGJ398 led to dose-dependent inhibition of ERK phosphorylation but had no impact on AKT (Figure 4C). Treatment of TMK-1 cells with the FGFR inhibitor BGJ398 had no effect on either ERK or AKT (Figure 4E). Furthermore, modulation of transcription factors HIF1α, HIF2α and c-MYC by BGJ398 were analyzed under normoxic conditions and upon DFX-induced hypoxia. In all three cell lines, HIF1α was only detected upon hypoxia induction, while HIF2α was present under both conditions to a similar extent. Nonetheless, FGFR blockade did not affect either HIF1α or HIF2α expression *in vitro* (Figure 4B, D and F). Regarding c-MYC, a complete abrogation of its expression was found in KKLS cells (Figure 4B), whereas

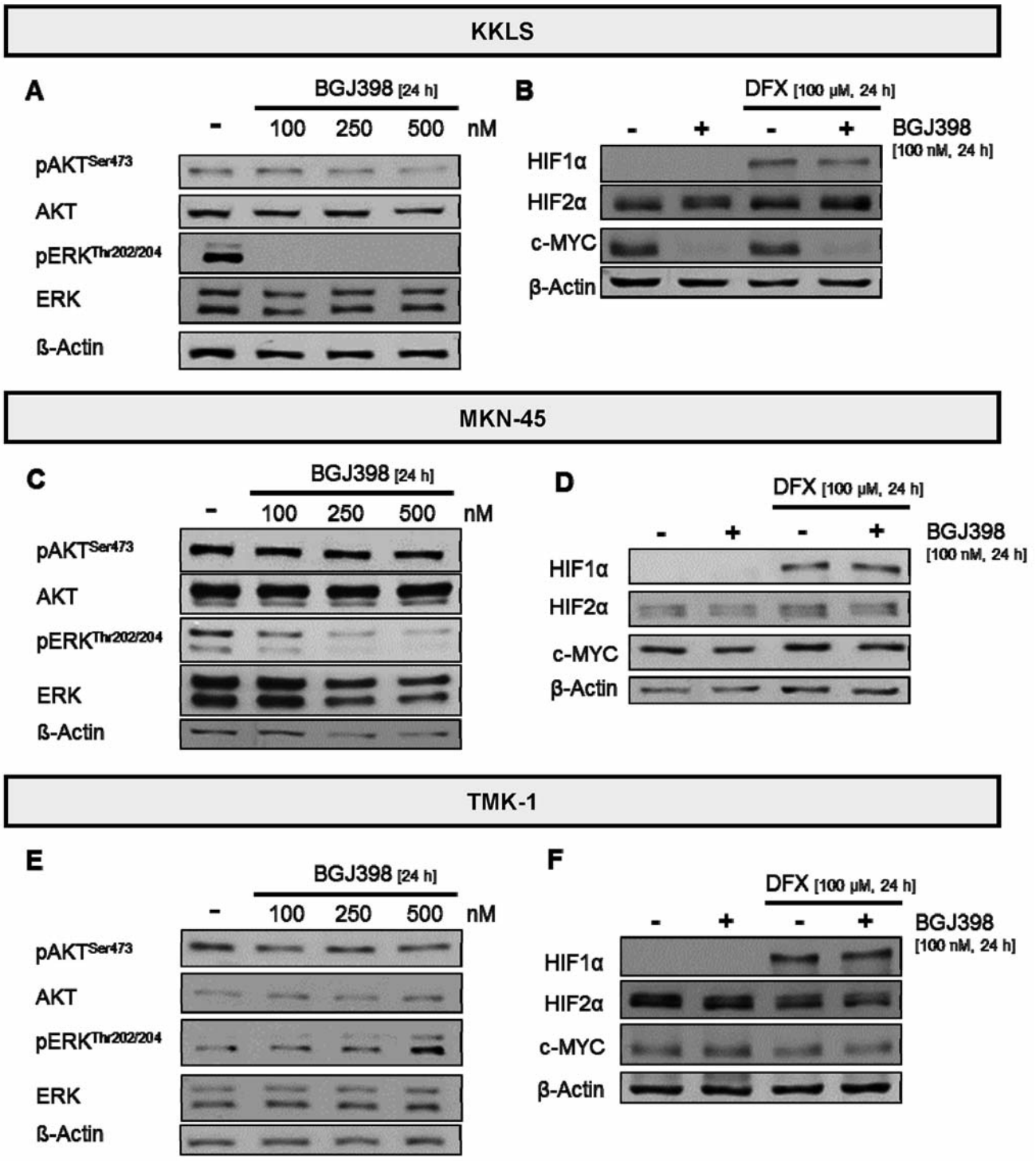

Figure 4. *Effects on activation of signaling intermediates and expression of transcription factors in vitro by fibroblast growth factor receptor (FGFR) blockade with BGJ398. A: In KKLS cells, targeting FGFR abrogated extracellular-signal-regulated kinase (ERK) phosphorylation and impaired protein kinase B AKT phosphorylation in a dose-dependent manner. B: BGJ398 abolished c-MYC expression but had no effect on hypoxia-inducible factor-1α (HIF1α) nor on hypoxia-inducible factor-2α (HIF2α) expression upon induction with desferroxamine (DFX). C: In MKN-45 cells, FGFR inhibition induced a dose-dependent reduction of ERK phosphorylation but had no effects on AKT. D: FGFR inhibition did not affect c-MYC, HIF1α or HIF2α expression in these cells. E and F: In TMK-1 cells, BGJ398 had no effects on the tested signaling intermediates (ERK, AKT) or transcription factors (c-MYC, HIF1α, HIF2α).*

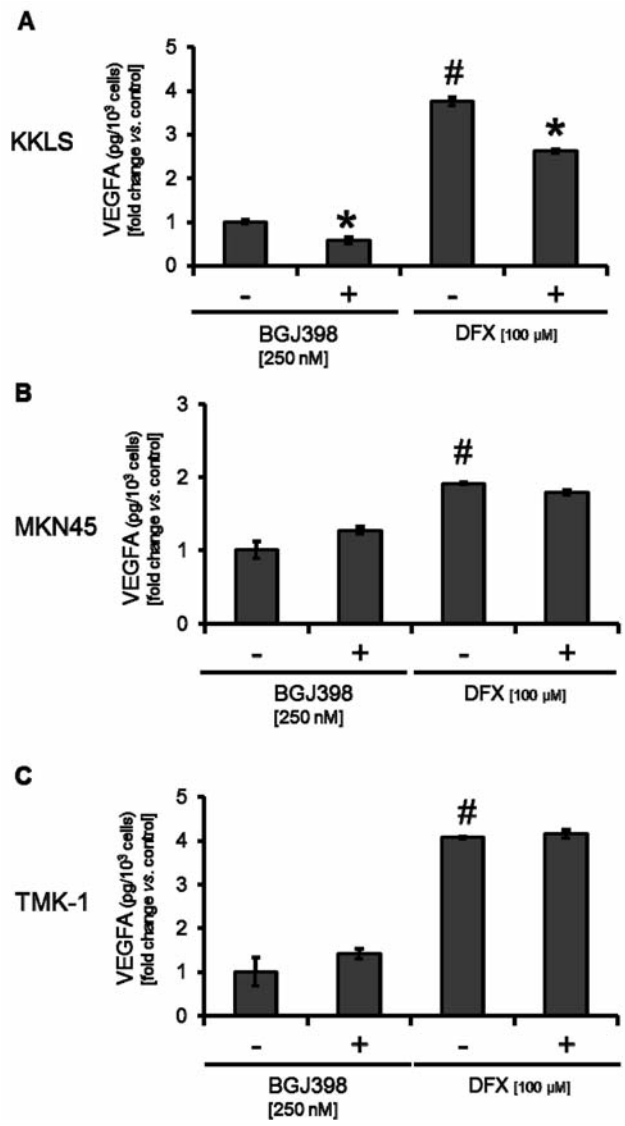

Figure 5. *Modulation of vascular endothelial growth factor-A (VEGFA) secretion upon fibroblast growth factor receptor (FGFR) blockade. Treatment with DFX led to significant induction of VEGFA secretion from all tumor cell lines (#p<0.05) A: Treatment with the FGFR inhibitor BGJ398 significantly diminished constitutive and DFX-induced VEGFA secretion from KKLS cells (*p<0.05). B, C: In contrast, no impact was observed on VEGFA secretion in MKN-45 and TMK-1 cells upon FGFR blockade. Data are the mean±SEM of n=3.*

no changes were detected in MKN-45 (Figure 4D) and TMK-1 (Figure 4F) cells. Taken together, FGFR blockade with BGJ398 suppressed oncogenic signaling in KKLS and to a lesser extent in MKN-45 cells, whereas TMK-1 cells were relatively unaffected.

Impact on VEGFA secretion under FGFR inhibition. We recently showed that FGFR inhibition with BGJ398 impairs VEGFA secretion from HCC cell lines *in vitro* (16). Therefore, we assessed whether this effect also takes place in GC cell lines. The results show that incubation with DFX led to a significant induction of VEGFA secretion from all three tumor cell lines after 24 h (Figure 5). However, only in KKLS cells FGFR inhibition by BGJ398 impair constitutive and DFX-induced VEGFA secretion (Figure 5A). In MKN-45 and TMK-1 cells, no effect of BGJ398 was observed (Figure 5B and C).

Targeting FGFR impairs tumor growth in vivo. Finally, FGFR blockade with BGJ398 was assessed in subcutaneous tumor models *in vivo*. Treatment with BGJ398 (10 mg/kg/d) caused a significant inhibition of KKLS tumor growth (Figure 6A and B). These results were also confirmed in mice bearing MKN-45 and TMK-1 tumors (Figure 6C and D). However, impairment of tumor growth with regard to tumor weight (Figure 6D) was relatively less in MKN-45 (Figure 6C) and TMK-1 tumors (Figure 6D) compared to KKLS tumors (Figure 6B). Nonetheless, these data suggest that treatment with the FGFR inhibitor BGJ398 has the potential to impair tumor growth of GC xenografts *in vivo*.

Inhibition of tumor vascularization in vivo. Finally, vascularization of the excised subcutaneous tumors was assessed by CD31 staining. In KKLS tumors, the CD31-positive vessel area was significantly reduced upon treatment with BGJ398 (Figure 7A). However, TMK-1 tumors showed no difference from the controls upon BGJ398 treatment (Figure 7B). Similar results were found in MKN-45 tumors (data not shown).

Discussion

The FGF/FGFR signaling network is involved in a wide range of cancer-associated processes, including cell proliferation, survival, motility and angiogenesis [reviewed in (5-7)]. With regard to GC, overexpression of FGFR1, FGFR2 and FGFR4 has been reported [reviewed in (11)]. In particular, four studies have focused on FGFR2, or the FGFR2 homolog K-sam, and found an association with higher TNM stage and reduced patient survival (9, 10, 21, 22). Moreover, cancer cells have been observed to switch expression of FGFRs from the more epithelial isoform (IIIb) to the more mesenchymal isoform (IIIc) (the epithelial–mesenchymal transition) enabling them to receive signals usually restricted to the connective tissue [reviewed in (23)]. Some researchers, therefore, proclaim that the FGFR2IIIc isoform is more oncogenic (24, 25). Recently, FGFR2IIIc has been linked to a more advanced tumor stage in colorectal cancer (26) and increased tumor cell proliferation *in vitro* and *in vivo* (pancreatic cancer) (25). No data regarding the role of FGFR2IIIb and FGFR2IIIc in GC are currently available.

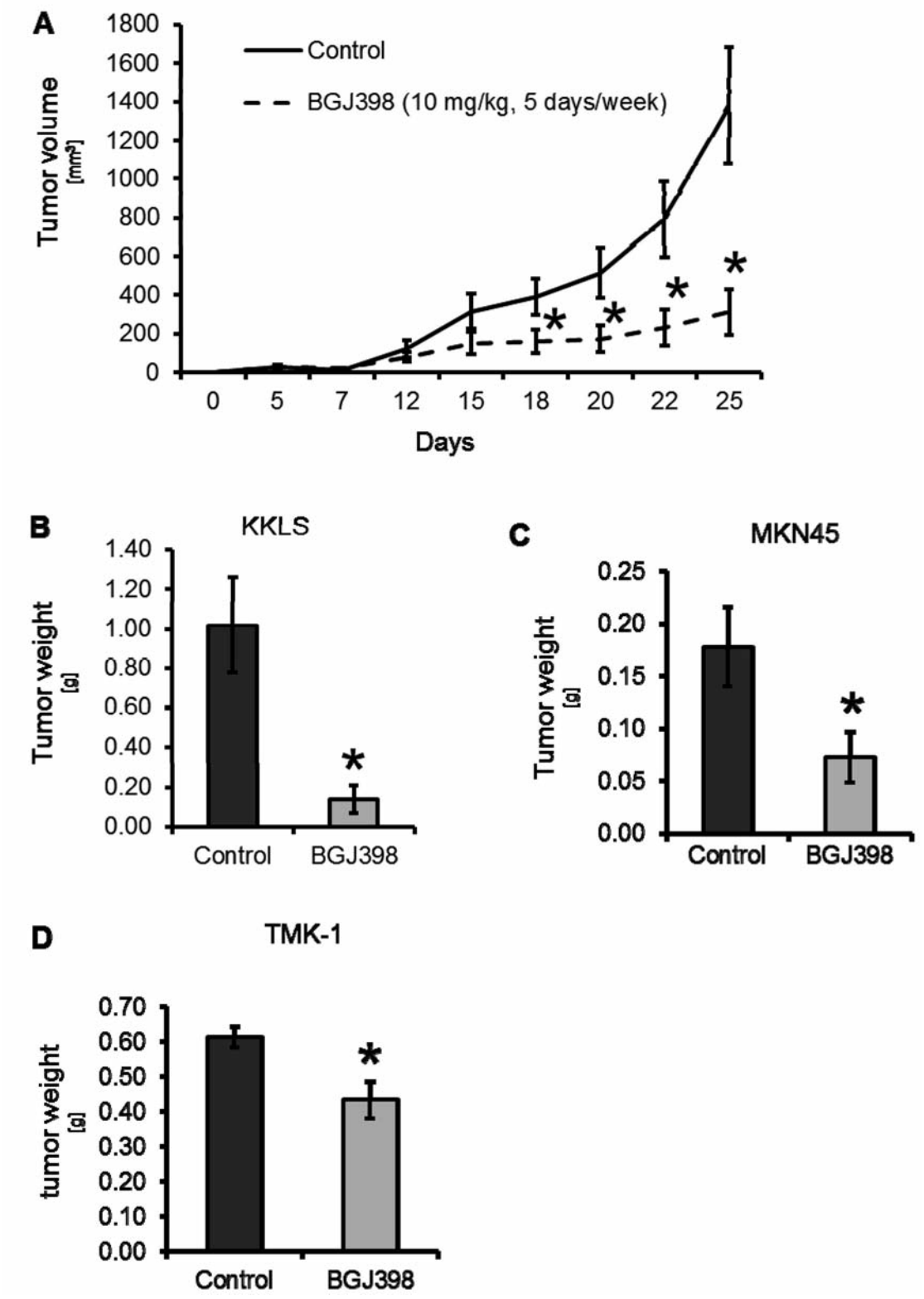

Figure 6. *Targeting fibroblast growth factor receptor (FGFR) with BGJ398 in subcutaneous tumor models. A: Treatment with BGJ398 led to a significant reduction in KKLS tumor growth in vivo (*p<0.05). B: This was also reflected by final tumor weight (*p<0.05). C, D: Similarly, final tumor weight was significantly reduced in the subcutaneous MKN-45 and TMK-1 tumor model (*p<0.05). Data are the mean±SEM of n=6-8 mice/group.*

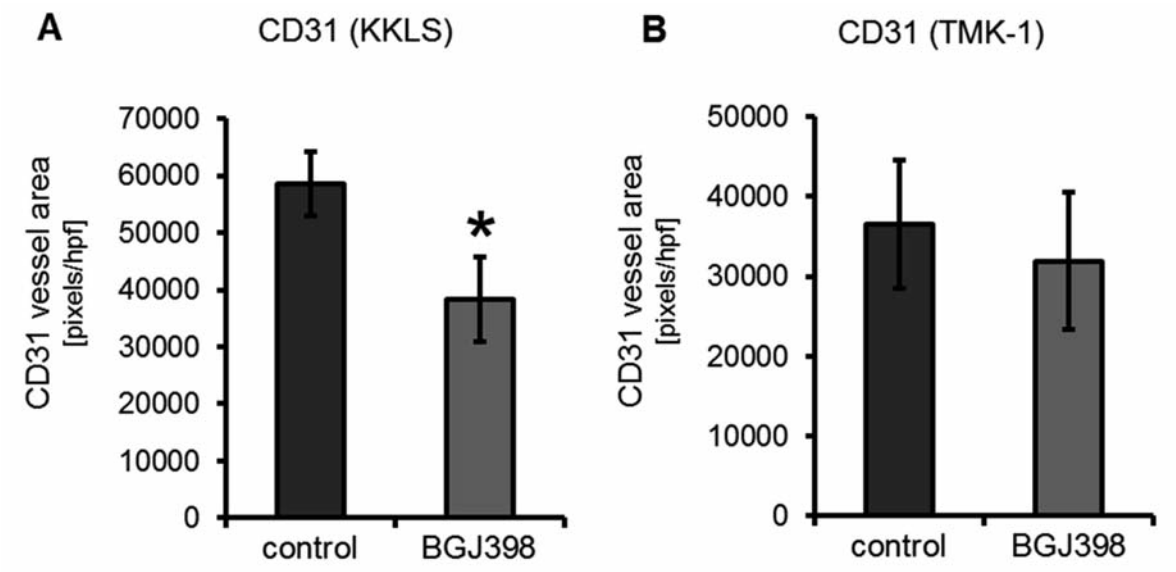

Figure 7. *Effects on vascularization in vivo as shown by staining for CD31. In KKLS tumors (A), vascularization was significantly impaired by fibroblast growth factor receptor (FGFR) blockade (*p<0.05), whereas no difference was detected in the TMK-1 tumor model (B). hpf: High-power field. Data are the mean±SEM of n=6-8/group.*

Only Zhao and co-workers examined monoclonal antibodies against FGFR2IIIb and FGFR2IIIc in GC cell lines SMU-16 and OCUM-2M *in vitro* and *in vivo*, showing similar growth inhibition upon blockade of either FGFR2IIIb or combined blockade of FGFR2IIIb/FGFR2IIIc (27). Nonetheless, in order to address this issue, we did not only determine the expression of FGFR2 in cancer cell lines, but also differentiated the two isoforms, FGFR2IIIb and FGFR2IIIc. Results from our study show that FGFR2IIIb is not detectable in TMK-1 and KKLS GC cell lines but is expressed in MKN-45 cells. Concerning FGFR2IIIc, high expression is found in KKLS cells, with lower expression in MKN-45 and no expression in TMK-1 cells. Interestingly, cell-growth assays revealed substantial efficacy of the FGFR inhibitor BGJ398 in KKLS cells, and lesser effects in MKN-45 and TMK-1 cells. Since all other FGFRs are expressed in these cell lines, our results suggest that the efficacy of targeting FGFR with the small molecule BGJ398 is linked to FGFR2 expression, as previously suggested by Guagnano *et al*. (24). However, our results indicate that FGFR2IIIc expression in GC cells is more important than total FGFR2 expression and might help identify patients who might benefit from FGFR-directed targeted therapy with small molecule inhibitors.

The formation of new blood vessels plays a key role in tumor growth, progression, and metastasis. Beyond VEGF, the FGF/FGFR system is a potent stimulator of angiogenesis both under normal physiological conditions and in cancer [reviewed in (23, 28, 29)]. Assessment of the antiangiogenic efficacy of treatment with the FGFR inhibitor BGJ398 revealed suppression of constitutive and DFX-induced VEGFA secretion from KKLS GC cells but no impact on TMK-1 and MKN-45 cells. Moreover, impairment of oncogenic signaling (AKT, ERK), as well as c-MYC expression, was found in KKLS cells, with only minor effects observed in MKN-45 (ERK) cells; no impairment of signaling or c-MYC expression was detectable in TMK-1 cells. These findings are somewhat surprising since we recently described an inhibition of VEGFA secretion upon FGFR inhibition in four HCC cell lines (16). However, c-MYC is a major driver of tumor angiogenesis (30) and the differences between the three cell lines regarding VEGFA secretion in response to FGFR inhibition might be explained by the diverse effects on expression of this transcription factor. Regarding the effects of FGFR inhibition on c-myc expression, Malchers *et al*. recently demonstrated that coexpression of FGFR1 and c-MYC makes tumors more sensitive to FGFR inhibition in squamous lung cell carcinomas (31). This observation provides an explanation for our results, since we found the highest FGFR1 expression in KKLS, which is the only GC line where c-MYC expression was diminished upon BGJ398 treatment. Taken together, our results suggest that treatment with the FGFR inhibitor has anti-angiogenic properties in GC cell lines but only in conjunction with FGFR1 expression.

Finally, we evaluated our results *in vivo* using subcutaneous tumor models and demonstrated significant growth inhibition in all GC tumors we tested. However, a detailed analysis reveals that the efficacy of FGFR inhibition was far better in KKLS than in TMK-1 tumors, with an intermediate effect on MKN-45 tumor growth. Interestingly, this observation

emphasizes our findings from the *in vitro* experiments regarding the efficacy of BGJ398 on KKLS, MKN-45 and TMK-1 tumor cell growth. Moreover, tumor vascularization was only impaired in KKLS tumors. This result confirms our findings from the *in vitro* experiments regarding the antiangiogenic impact of FGFR inhibition with BGJ398. Nonetheless, FGFR inhibition clearly had a slight effect on the growth of TMK-1 tumors *in vivo*. One might speculate that effects on tumor growth may be mediated by non-tumor cells such as pericytes. Furthermore, although BGJ398 is a specific FGFR inhibitor, some activity against other kinases such as VEGFR2, KIT and Lck/Yes novel tyrosine kinase (LYN) at sub-micromolar concentrations has been reported (12). This might also explain our observed effects on TMK-1 tumor growth *in vivo*. In general, results from our *in vivo* studies confirmed the *in vitro* results regarding the differential efficacy of BGJ398-mediated FGFR inhibition on human GC cell lines.

From our finding, we propose that both FGFR1 and FGFR2IIIc mediate the inhibitory effect of BGJ398 on GC cells. Presumably, effects on growth are more likely mediated *via* FGFR2IIIc blockade, while angiogenesis is mainly suppressed through FGFR1/c-myc inhibition. We, therefore, conclude that treatment with BGJ398 impairs tumor growth *in vitro* and *in vivo* by effects on oncogenic signaling and angiogenesis, in particular when tumor cells express FGFR1 or FGFR2IIIc.

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