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FGF10/FGFR2 Signaling: Therapeutically Targetable Vulnerability in Ligand-responsive Cholangiocarcinoma Cells

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Abstract. *Background/Aim: Increasing evidence has revealed FGFR2 as an attractive therapeutic target for cancer including cholangiocarcinoma (CCA). The present study investigated the oncogenic mechanisms by which FGF10 ligand activates FGFR2 in CCA cells and determined whether FGFR inhibitors could suppress FGF10-mediated migration of CCA cells. Materials and Methods: Effects of FGF10 on the proliferation, migration, and invasion of KKU-M213A cells were assessed using clonogenic and transwell assays. Protein expression levels of FGFR2 and pro-angiogenic factors were determined via immunoblotting and antibody array analysis. FGFR2 knockdown using a small interfering RNA was used to validate the role of FGF10 in promoting cell migration via FGFR2. The effects of infigratinib (FGFR inhibitor) on cell viability, were determined in KKU-100, KKU-M213A, KKU-452 cells. Moreover, the efficacy of the FGFR inhibitor in suppressing migration via FGF10/FGFR2 stimulation was assessed in KKU-M213A cells. Results: FGF10 significantly increased the expression of phospho-FGFR/FGFR2 and promoted the proliferation, migration, and invasion of KKU-M213A cells. FGF10 increased the expression levels of p-Akt, p-mTOR, VEGF, Slug, and pro-angiogenic proteins related to metastasis. Cell migration mediated by FGF10 was markedly decreased in FGFR2-knockdown cells. Moreover, FGF10/FGFR2 promoted the migration of cells, which was suppressed by the FGFR inhibitor. Conclusion: FGF10/FGFR2 activates the Akt/mTOR and VEGF/Slug pathways, which are associated with the stimulation of migration and invasion in CCA. Moreover, the FGF10/FGFR2 signaling was inhibited by an FGFR inhibitor resulting suppression of cell migration, which warrants further studies on their clinical utility for CCA treatment.*

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Cholangiocarcinoma (CCA) is a malignancy of the biliary duct with a 5-year survival rate of less than 10% and very poor response to standard chemotherapy (1). In recent years, oncological therapy tailored to the features of individual tumors has emerged as a promising approach for CCA treatment. One of the most promising targets for CCA is the fibroblast growth factor receptor 2 (FGFR2), and its aberrations have been detected in 10-15% of intrahepatic CCA cases (2, 3). At least two FGFR inhibitors have been approved by the United States Food and Drug Administration (USFDA) for the treatment of advanced stage CCA with FGFR fusion (4, 5). *FGFR2* can be expressed as multiple transcript variants *via* tissue-specific splicing. FGFR2-IIIb is one of FGFR2 isoforms (6, 7) which binds specifically to FGF7 and FGF10 ligands (8).

FGF10, a member of the FGF7 ligand subfamily, is secreted by mesenchymal cells and stromal cells surrounding cancer cells. FGF10 has been reported to orchestrate epithelial–mesenchymal interactions during gastrointestinal tract development (9), possesses broad mitogenic and cell survival activities and is involved in various biological processes, including cell growth, morphogenesis, tissue repair, and tumor growth and invasion (10). FGFRs are involved in multiple signaling pathways, including Ras/mitogen-activated protein kinase, phosphoinositide 3-kinase (PI3K)/Akt, Janus kinase/signal transducer and activator of transcription 3, and phospholipase Cγ pathways (8). FGF10 acts as a canonical FGFR ligand *via* the activation of FGFR2-IIIb. Upon FGF10 binding, FGFRs dimerize,

leading to tyrosine residue phosphorylation, subsequently activating key intracellular signaling pathways in various cell types. Notably, the interplay between FGF10 and FGFR2 is necessary for the development of branches and tubules in the biliary tract and for pancreatic organogenesis (9).

The tumor-promoting role of FGF10 has been substantiated in many malignant models and cancers, including pancreatic cancer (11), small cell lung cancer (12), and prostate adenocarcinoma (13). FGF10 was identified as a poor prognostic biomarker in gastric adenocarcinoma, wherein the expression of FGF10 was significantly associated with lymph node invasion and distant metastasis (14). In a CCA model, FGF10 induced malignancy in intraductal papillary neoplasm of the bile duct with time that changed from low grade disease to high grade disease *via* the FGF10/FGFR2/RAS/extracellular signal-regulated protein kinase (ERK) signaling pathway (15).

FGFR inhibitors are small-molecule tyrosine kinase inhibitors that competitively inhibit ATP binding to tyrosine kinase domain of FGFR, leading to the suppression of its downstream signaling (16). Pemigatinib, the first FGFR inhibitor, and infigratinib (BGJ398), an orally active pan-FGFR inhibitor acting against FGFR1-3, are approved by the USFDA for advanced or metastatic CCA (4). In CCA treatment, most FGFR inhibitors show beneficial effects on CCA subgroups with FGFR fusion, rather than other FGFR aberrations (2).

In this study, we comprehensively explored the oncogenic role of FGF10/FGFR2 in CCA cells. We determined the underlying mechanisms of FGF10/FGFR2-mediated proliferation, migration, and invasion of CCA cells *via* the mammalian target of rapamycin (mTOR) and vascular endothelial growth factor (VEGF)/Slug pathways. In addition, the inhibitory effect of a pharmacological inhibitor of FGFR on FGF10/FGFR2-mediated migration of cells in a CCA model was investigated. In this study we demonstrated that FGF10/FGFR2 signalling could be a therapeutic target in ligand-responsive cancer cells including CCA cells.

Materials and Methods

Cell lines and cell culture. The human CCA cell lines KKU-452 (JCRB1772) (17), KKU-213A (JCRB1557) (18) and KKU-100 (JCRB1568) (19) were developed at the Cholangiocarcinoma Research Institute, Khon Kaen University, and deposited in the Japanese Cancer Research Resources Bank (JCRB, Ibaraki, Japan). CCA cells were cultured and maintained as previously described (20). Briefly, CCA cells were cultured in Ham's F12 media containing 10% fetal bovine serum (HiMedia Laboratories, Mumbai, India) and maintained in an atmosphere of 5% CO_2 at 37˚C and subcultured every 2 or 3 days using 0.25% trypsin-EDTA (Gibco BRL Life Technologies, Grand Island, NY, USA).

Sulphorhodamine B (SRB) assay. KKU-100, KKU-M213A, and KKU-452 cells were seeded into a 96-well plate at a density of 3-5×10^3 cells/well and incubated overnight. For the treatment, the culture medium was replenished with various concentrations of FGF10 in culture medium containing 2%FBS or infigratinib in culture medium containing 10% FBS and incubated for 48 and 72 h. After incubation, the cells were fixed with 10% trichloroacetic acid for 1 h and stained with 0.4% SRB in 1% acetic acid solution for 30 min. The absorbance was measured at a wavelength of 540 nm using a microplate reader.

Clonogenic assay. KKU-M213A cells were seeded into a 6-well plate (1×10^5 cells/well) and treated with 1, 10, and 100 ng/ml FGF10 for 48 h. After treatment, cells were subcultured into a 6-well plate at a density of 400 cells/well with fresh media and further incubated for 7 d. Then, the colonies were stained as previously described (20). The number of colonies in each well was measured using Image-Pro Plus software (Media Cybernetics, Rockville, MA, USA).

Cell migration and invasion assays. KKU-M213A cells were seeded into the transwell inserts at the density of 20,000 cells/well. Ham's F12 medium with 10% (v/v) FBS (700 μl) was added to the lower chamber of the transwell. Then, 100 μl of FGF10 in serum-free media was added to the insert before seeding cells. The assay of cell migration and cell invasion were determined using a transwell chamber (Corning®Transwell®, Corning, NY, USA) as previously described (20). The invading cells were captured and analyzed by ImagePro Plus software (Media Cybernetics).

Small interfering RNA (siRNA) transient transfection. siRNA transient transfections were performed to silence *FGFR2* gene expression. Briefly, 1×10^5 KKU-M213A cells were seeded into a 6-well plate overnight. After transfection, the cells were transfected with 100 pmol of siRNA against FGFR2 (si-FGFR2) (J-003132-19-0010; Dharmacon, Lafayette, CO, USA) and non-targeting siRNA (si-NT) (D-001810-10-20; Dharmacon) for 72 h using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA), as previously described (20). After transfection, knockdown efficiency of *FGFR2* gene expression was examined *via* western blotting analysis.

Western blotting analysis. Whole cell extracts were applied onto a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel composed of 1.5 M Tris buffer (pH 8.8), 10% SDS, 10% ammonium persulfate, and 0.04% TEMED (Bio-Rad, Hercules, CA, USA). Proteins were then transferred onto a polyvinylidene fluoride membrane. Then, blotting membranes were blocked with 5% bovine serum albumin in Tris-buffered saline consisting of 0.1% Tween-20 (TBST) for 2 h. Blotting membranes were incubated overnight at 4˚C with the following primary antibodies: phospho-FGFR (3471s), phospho-mTOR (5536s), mTOR (2983s), Slug (9585s; Cell Signaling, Danvers, MA, USA), FGFR2 (ab75984; Abcam, Cambridge, UK), VEGF (sc-7269), and actin (sc-1616; Santa Cruz Biotechnology, San Diego, CA, USA). Next, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) for 3 h at room temperature. Then, proteins were detected using Luminata Forte Western HRP substrate (Merck Millipore Corporation, Billerica, MA, USA). The protein bands were photographed using a ChemiDoc MP Imaging System, and the intensity of the target bands was analyzed using the Image Lab software (version 6.0; Bio-Rad).

Angiogenesis antibody array. Protein lysates from KKU-M213A cells treated with 10 ng/ml FGF10 for 48 h were hybridized with a RayBio human angiogenesis antibody array (AAH-ANG-1000-8;

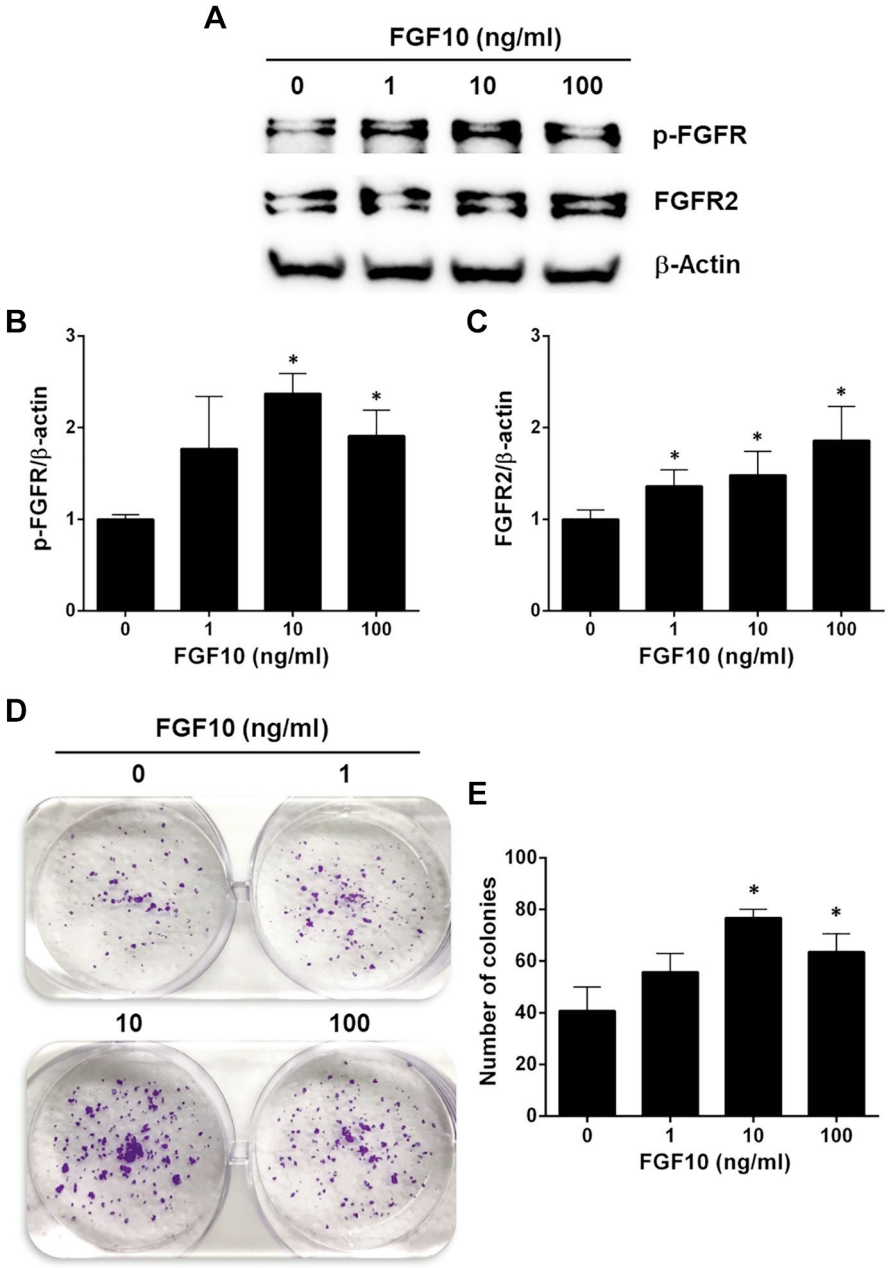

Figure 1. *Fibroblast growth factor 10 (FGF10) stimulates fibroblast growth factor receptor 2 (FGFR2) expression and promotes proliferation of KKU-M213A cells. (A) KKU-M213A cells were treated with 1, 10, and 100 ng/ml of FGF10 for 48 h. Protein expression was determined via western immunoblotting. Bars represent the relative expression of p-FGFR (B) and FGFR2 (C) normalized to β-actin expression. (D) Clonogenic assay was performed in cells treated with 1, 10, and 100 ng/ml FGF10 for 48 h. (E) Bars represent the number of colonies in the treatment group compared to the untreated group. Data are presented as the mean±standard deviation (SD) from three independent experiments. *p<0.05 compared to the untreated control group.*

RayBiotech, Peachtree Corners, GA, USA). The levels of protein expression were analyzed according to the manufacturer's instructions. The signal intensities of individual spots were captured using the ChemiDoc MP Imaging System and were analyzed using the Image Lab software (version 6.0; Bio-Rad).

Statistical analysis. All values are presented as the mean±standard deviation of three experiments. Statistical differences among treatment groups were analyzed using one-way analysis of variance with the Student–Newman–Keuls post-hoc test. Statistical significance was set at $p<0.05$.

Results

FGF10 stimulated FGFR2 expression and promoted proliferation of KKU-M213A cells. We investigated whether exogenous FGF10 promoted FGFR2 activation and CCA cell proliferation. KKU-M213A cells were selected to perform the immunoblotting and clonogenic assays because a preliminary experiment revealed that the KKU-M213A cells exhibit constitutive phosphorylated FGFR expression. Treatment with FGF10 for 48 h caused a dose-dependent increase in FGFR2 expression (Figure 1A and C). FGF10 induced a remarkable increase in FGFR phosphorylation compared to basal expression (Figure 1A and B). Furthermore, treatment with FGF10 increased cell renewal, as the number of colonies was increased by 50% compared to the control (Figure 1D and E).

FGF10/FGFR2 promoted migration and invasion of KKU-M213A cells. FGF10 plays roles in the initiation and progression cancer in various models (15). To determine whether exogenous FGF10 promotes metastasis *in vitro*, we performed invasion and migration assays using the transwell chamber. KKU-M213A cells were treated with different concentrations (1, 10, and 100 ng/ml) of FGF10. Treatment with FGF10 significantly increased the number of migrating and invading cells compared to that in the untreated group (Figure 2A-D). FGF10 showed a trend of increasing the number of migrating and invading cells, even at low concentrations.

We further evaluated whether FGFR2 is essential for FGF10 to promote the migration of CCA cells. FGFR2 expression in KKU-M213A cells was knocked down using siRNA. Immunoblotting confirmed that FGFR2 expression was significantly suppressed compared to that in the non-target knockdown group (Figure 2E and 2F). Migration of FGFR2 knockdown cells was reduced by more than 50% compared to non-targeted or parental groups, and their migratory activity was comparable to the parental group without FGF10 treatment (Figure 2G and H). Consistently, treatment with FGF10 significantly stimulated p-FGFR and FGFR2 expression in non-targeted cells, whereas the expression levels of both were unchanged in FGFR2 knockdown cells (Figure 2I-K). These results confirm the crucial role of FGFR2 in FGF10-mediated cell migration and invasion.

FGF10/FGFR2 stimulated key metastatic proteins. To determine the molecular mechanism underlying FGF10/FGFR2-mediated metastasis, an immunoblotting assay was used to determine the expression levels of key metastatic proteins. KKU-M213A cells were treated with 10 ng/ml FGF10 for various time periods (6, 24, and 48 h). FGF10 showed a trend to increase expression of p-AKT within 24 h that was significantly increased at 48 h (Figure 3A-C). The expression of p-mTOR was increased at 24 and 48 h (Figure 3A-C). Furthermore, FGF10 significantly increased the expression levels of the pro-angiogenic regulator VEGF (Figure 3A and D) and the mesenchymal marker Slug after 48 h of incubation (Figure 3A and E).
To further investigate the signaling pathways involved in tumor progression, a protein array assay containing antibodies against pro-angiogenic factors was used to screen for proteins related to angiogenesis. KKU-M213A cells were treated with FGF10 for 48 h. FGF10 increased the levels of angiogenic platelet-derived growth factor (PDGF)-BB, interleukin (IL)-1α, IL-1β, IL-8, and uPAR, while it reduced the levels of the endogenous inhibitor of angiogenesis TIMP1 (Figure 3F and G).

FGFR inhibitor suppressed FGFR2 expression. As FGFR plays a significant role in promoting cancer cell survival, we evaluated the effects of pharmacological FGFR inhibitors on CCA cells. Three CCA cell lines were treated with various concentrations of infigratinib for 48 and 72 h. The drug suppressed proliferation in KKU-100, KKU-M213A, and KKU-452 cells in concentration- and time-dependent manner (Figure 4A-C). As shown in Table I, the IC_{50} values of infigratinib in CCA cells were in the range of 4.23-4.98 μM for KKU-100, 4.53-5.28 μM for KKU-M213A, and 4.92-5.60 μM for KKU-452 cells. Among all CCA cell lines, KKU-100 cells were slightly more sensitive to infigratinib than KKU-M213A or KKU-452 cells (Table I).

Infigratinib is a selective FGFR inhibitor that binds to the kinase domain and prevents the autophosphorylation of the receptor and blocks the downstream signaling cascades of FGFR. We examined the suppressive effect of infigratinib on p-FGFR/FGFR2 expression in KKU-M213A cells. Protein expression was determined *via* western blotting analysis. The results showed that infigratinib significantly decreased the p-FGFR levels at 5 and 7.5 μM to approximately 40% to 50% of controls (Figure 4D and E). In addition, three concentrations of infigratinib (2.5, 5, and 7.5 μM) significantly suppressed the expression levels of FGFR2 (Figure 4D and F).

The FGFR inhibitor infigratinib suppressed cell migration mediated by FGF10/FGFR2. To explore whether infigratinib could suppress FGF10/FGFR2-mediated migration we performed transwell migration assays using KKU-M213A cells treated with infigratinib and FGF10. Treatment with FGF10 alone significantly increased cell migration. In contrast, infigratinib inhibited cell migration promoted by FGF10. Notably, the inhibition of cell migration by infigratinib was significantly stronger in the presence of FGF10 at both lower (2.5 μM) and higher concentrations (5 μM) (Figure 5A and B). This indicates that infigratinib suppresses cell migration mediated by FGF10/FGFR2 signaling.

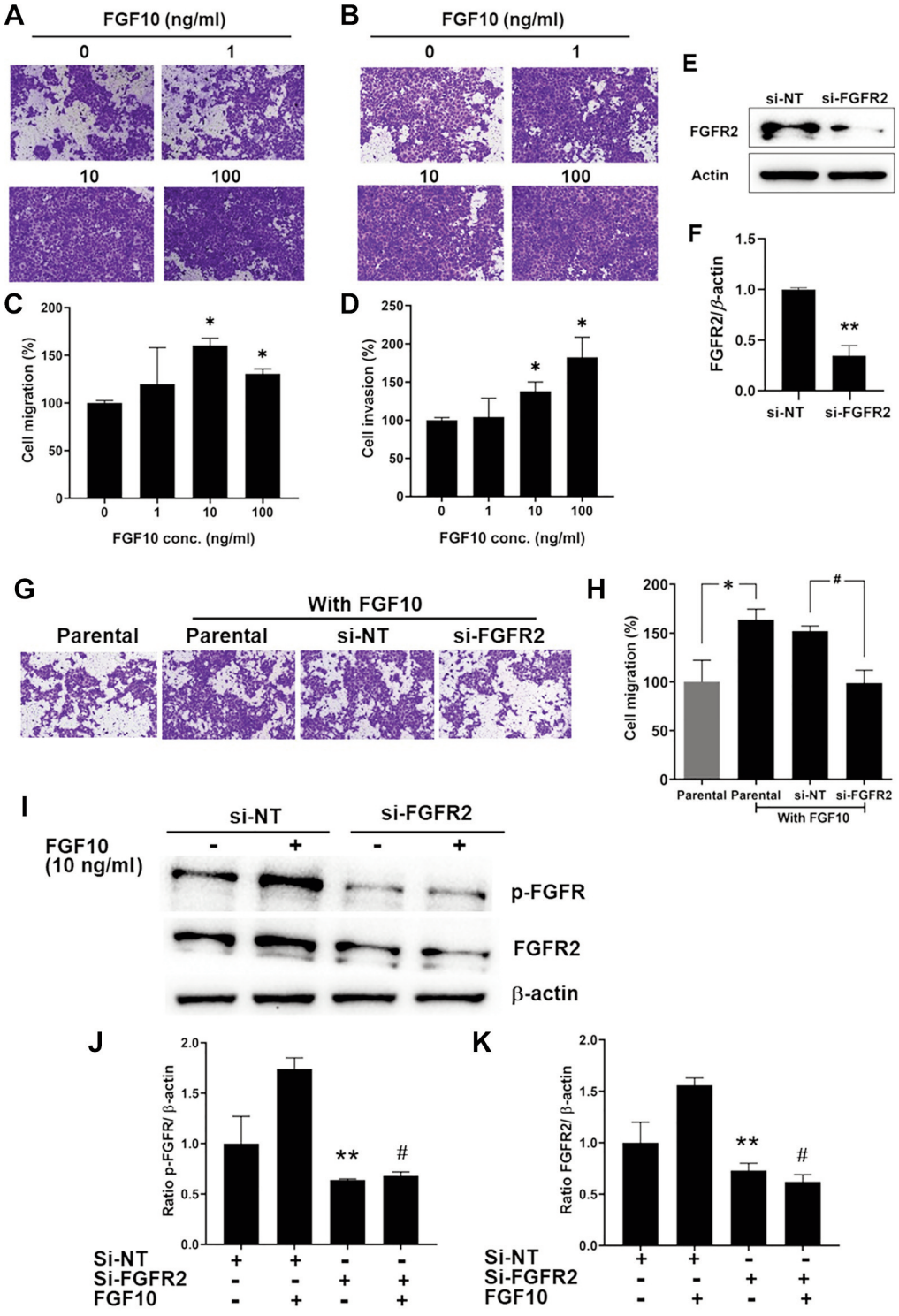

Figure 2. *FGF10/FGFR2 mediates migration and invasion of KKU-M213A cells. KKU-M213A cells were treated with 1, 10, and 100 ng/ml FGF10 48 h before the cells were used for migration and invasion assays using the transwell chamber (A-D). The cells were transiently transfected with si-FGFR2 or non-target control (si-NT) for 72 h, and immunoblots of FGFR2 expression were analyzed (E and F). Knockdown cells were treated with FGF10 for 48 h before migration assay (G-H). The effect of FGF10 on the activation of FGFR2 was analyzed via immunoblotting assay (I-K). Bars represent the relative expression of specific protein normalized to β-actin expression as the mean±SD (F, J, and K). *p<0.05 compared to the untreated parental group, **p<0.05 compared to the non-target siRNA alone group, #p<0.05 compared to the non-target siRNA treated with FGF10 group.*

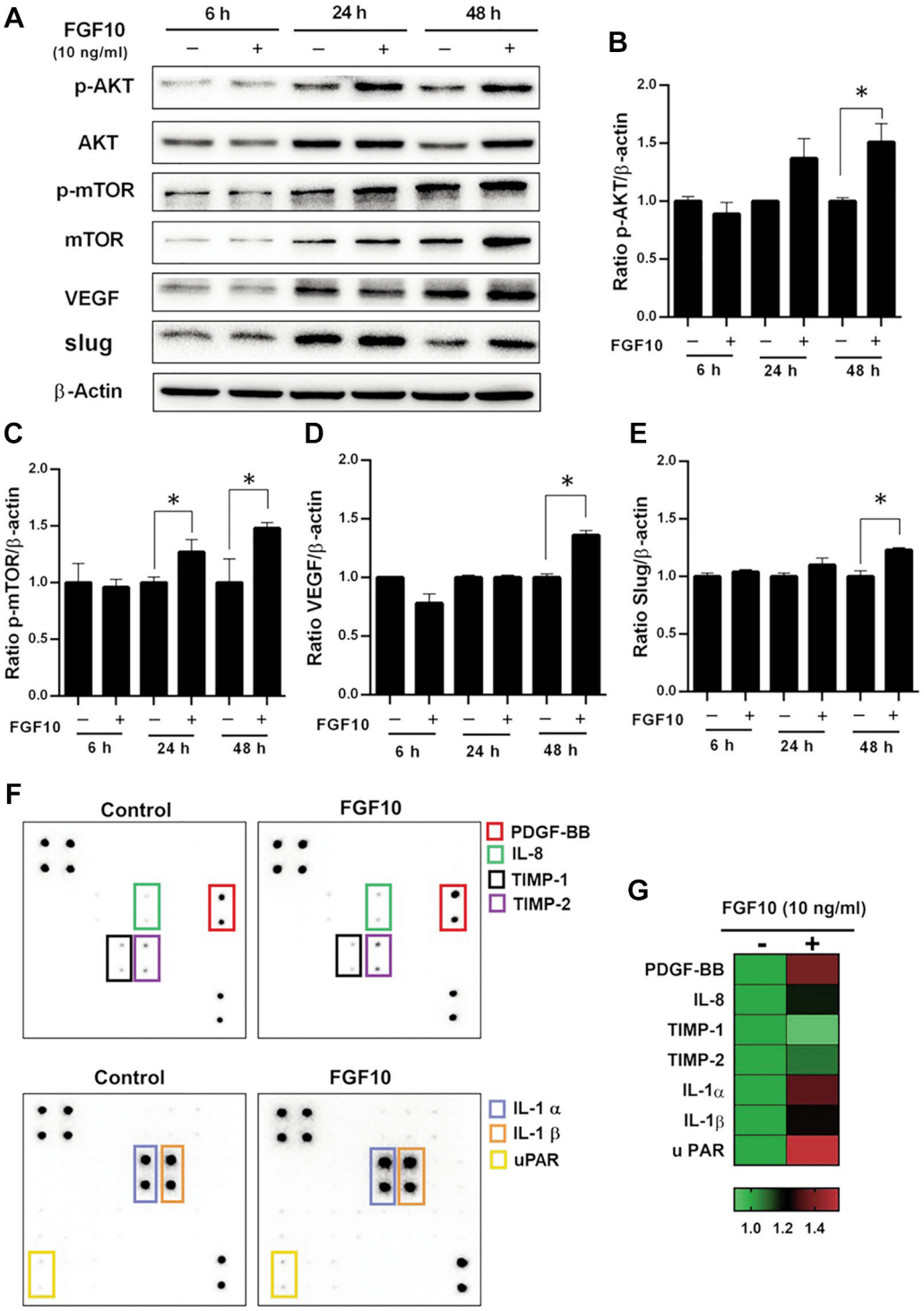

Figure 3. *Effect of FGF10/FGFR2 on downstream signaling proteins related to cancer metastasis and angiogenesis. (A-E) KKU-M213A cells were treated with FGF10 (10 ng/ml) for 6, 24, and 48 h. Protein expression levels of p-AKT, AKT, mammalian target of rapamycin (mTOR), p-mTOR, VEGF, and Slug were determined via western blotting analysis. Bars represent the relative expression of specific protein normalized to β-actin expression as the mean±SD from three independent experiments. *p<0.05 compared to the untreated control group. (F-G) KKU-M213A cells were treated with or without FGF10 (10 ng/ml) for 48 h. The cells were harvested and subjected to human angiogenesis antibody analysis. The positions of selected pro-angiogenic factors on the membranes are marked with colored boxes. (G) The protein expression profiles, are presented in a heatmap. Rows indicate the protein expression, while columns represent the condition of the treatment. Double gradient color indicates the expression value: light green, lowest; red, highest.*

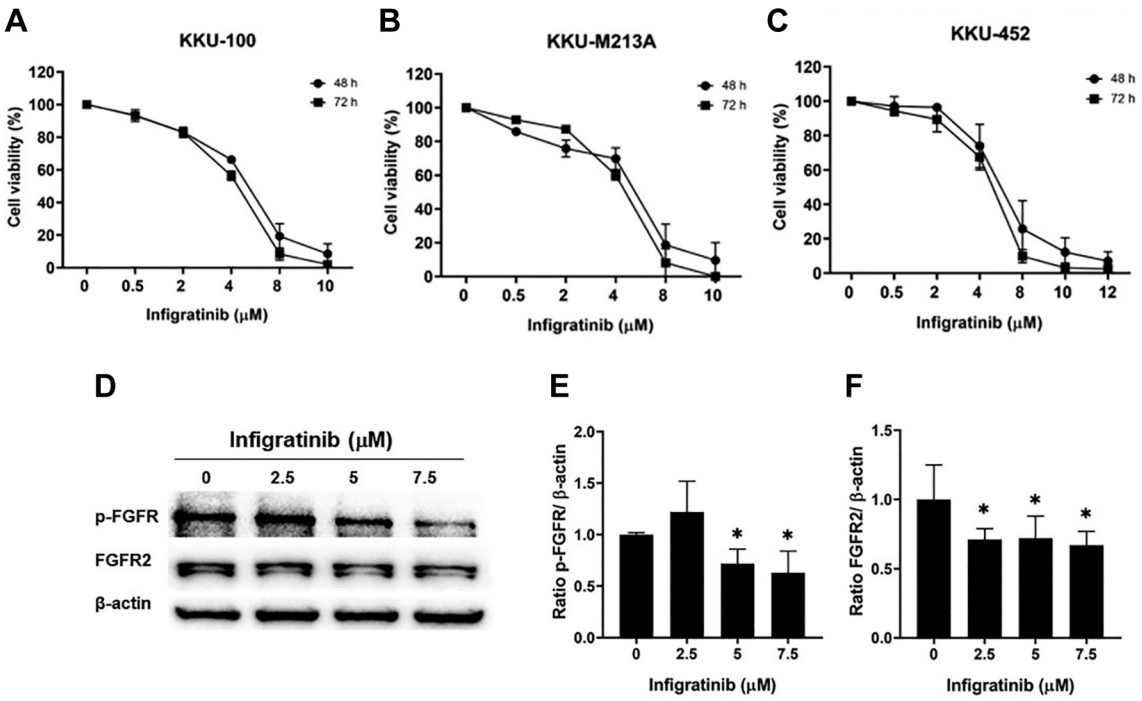

Figure 4. *Suppressive effect of infigratinib on viability and FGFR2 expression in cholangiocarcinoma (CCA) cells. KKU-100 (A), KKU-M213A (B), and KKU-452 cells (C) were treated with 0-12 μM of infigratinib for 48 and 72 h. Cell viability was analyzed using the sulphorhodamine B (SRB) assay and presented as % cell viability. Bars represent the mean±SD from three independent experiments. (D-F) Infigratinib suppressed the expression levels of p-FGFR and FGFR2 in KKU-M213A cells. The cells were treated with 0-7.5 μM of infigratinib. Expression levels of p-FGFR and FGFR2 were determined via western blotting analysis. Representative figures of protein expression from one experiment (D). Bars represent the relative expression of specific protein normalized to β-actin expression as the mean±SD from three independent experiments (E and F).*

Discussion

FGF10 is a multifunctional mesenchymal-epithelial signaling growth factor that plays a crucial role in the development of multiple organs and tissue homeostasis (21). However, FGF10 deregulation has been shown to be associated with human genetic disorders as well as carcinogenesis in various cancer types (22). FGF10 promotes the premalignant lesion of CCA in a mouse model. However, the role of FGF10 in CCA oncogenesis remains unclear. The present study showed that exogenous FGF10 stimulates oncogenic phenotypes, including proliferation, migration, and invasion of ligand-responsive CCA cells. Moreover, FGF10 mediated oncogenic stimulation *via* FGFR2 activation and increased the activation of AKT/mTOR signaling, together with the up-regulation of pro-angiogenic VEGF, Slug, PDGF-BB, IL-1α, IL-β, and IL-8. This study also showed that the pan FGFR inhibitor infigratinib could suppress the FGF10/FGFR2-mediated CCA growth and migration.

Ligand-dependent FGFR signaling plays a role in carcinogenesis through either autocrine production by cancer cells or paracrine production of ligands by stromal cells (23). In the present study, we examined the role of exogenous FGF10 in a CCA model. We found that FGF10 stimulated FGFR signaling *via* the up-regulation of p-FGFR and FGFR2 expression in KKU-M213A cells. It also promoted CCA cell proliferation as shown using the clonogenic assay. Our result are consistent with previous reports in breast cancer models where recombinant FGF10 protein promoted colony formation by MCF-7 breast cancer cells (24). Another study has demonstrated the oncogenesis of pancreatic cancer was mediated by paracrine FGF/FGFR activation. FGF10 secreted from stromal cells surrounding cancer cells promotes the migration and invasion of pancreatic cancer cells expressing *FGFR2b* (11).

Table I. *Half-maximal inhibitory concentration (IC50) values (μM) of infigratinib in cholangiocarcinoma cells.*

CCA cells	IC_{50} for 48 h (Mean±SD)	IC_{50} for 72 h (Mean±SD)
KKU-100	4.98±0.52 μM	4.23±0.20 μM
KKU-M213A	5.28±0.89 μM	4.53±0.19 μM
KKU-452	5.60±0.51 μM	4.92±0.19 μM

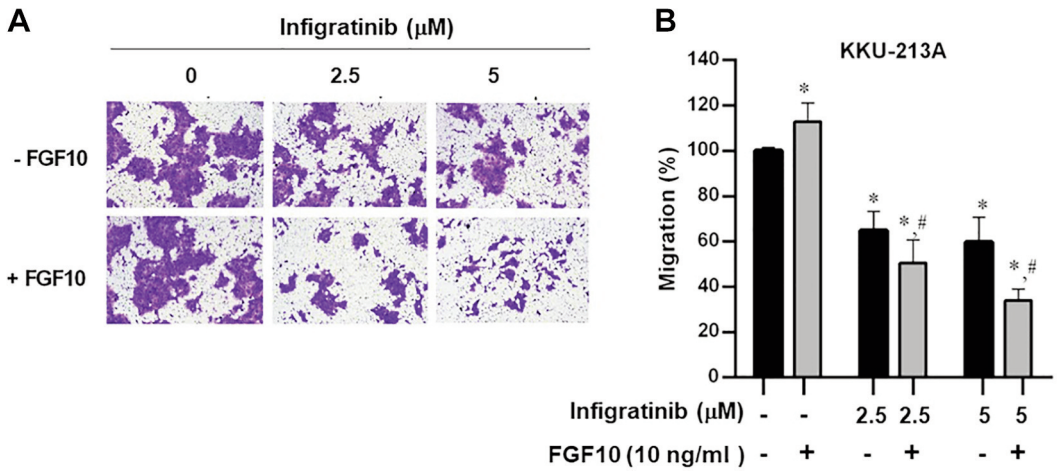

Figure 5. *Infigratinib suppresses cell migration mediated by FGF10/FGFR2. KKU-M213A cells were treated with infigratinib for 24 h, subcultured, and reseeded onto a transwell chamber in a serum-free medium containing infigratinib with/without 10 ng/ml FGF10 for another 48 h. (A) KKU-M213A cells were photographed using fluorescence microscope (magnification, 10´). (B) Data present the percentage of migrating cells in each treatment group as the mean±SD from three independent experiments. *p<0.05 compared to the control group, #p<0.05 compared to the infigratinib alone group.*

The role of FGF ligands in cancer progression, including migration and invasion, has been investigated. We demonstrated that FGF10 induced migration and invasion of CCA cells. The effects of FGF10 were mediated *via* the FGFR2 activation, which is consistent with previous reports that FGF10 is the specific ligand of FGFR2 (22). The result was confirmed by the knockdown of FGFR2 where the cell migration mediated by FGF10 was suppressed.

Akt/mTOR signaling is the hallmark of metastasis in many cancers, including CCA. This pathway is one of the downstream effectors of FGFR signaling. Our results are consistent with previous findings by Lau *et al.*, who demonstrated that FGF2 promoted cell invasion of ovarian cancer cells through PI3K/Akt/mTOR activation (25). Our results demonstrated that FGF10 increased the expression of p-AKT, p-mTOR, and their downstream VEGF in KKU-M213A cells. Signal activation induced by FGF10/FGFR2 in CCA cells is mediated by the Akt/mTOR pathway. However, a recent study has shown that in premalignant lesions, intraductal papillary neoplasm of the bile duct (IPNB) and FGF10 induced human IPNB phenotypes *via* the RAS/ERK signaling pathway (15). Indeed, FGF10 may exert its effects *via* both pathways. In addition, there is evidence supporting that FGF10 could be regulated at the translational level by mTORC1 in skin tumors, where an increase in FGF10 expression was found to be dependent on PTEN-PI3K-mTOR signaling in squamous cell carcinoma (SCC). These studies also revealed that the FGF10-FGFR2 autocrine feedback loop greatly amplifies FGF signaling (26).

The FGF-VEGF axis is a potentiating factor contributing to cancer metastasis (27). We determined the expression of VEGF, which acts as a pro-angiogenic regulator in tumorigenesis and indicator of poor survival outcome (28). Our results showed that FGF10 increased the expression levels of VEGF and Slug in KKU-M213A cells. Likewise, FGF2 induces angiogenesis by activating the SRSF1/SRSF3/SRPK1 pathway that regulates VEGFR1 alternative splicing in endothelial cells (29). In addition, VEGF-C has been implicated in epithelial–mesenchymal transition (EMT) through Slug up-regulation in skin cancer (30). Notably, Slug expression is a crucial factor in EMT and a prognostic marker for intrahepatic CCA with lymph node metastasis (31). Other pro-angiogenic factors were also investigated following FGF10 treatment. The result of protein array showed that FGF10 induced the up-regulation of PDGF-BB expression, which plays roles in the regulation of survival and apoptosis in CCA (32). Moreover, FGF10 also increased the expression levels of IL-1α and IL-1β, which are required for tumor invasiveness and angiogenesis (33). IL-1β can activate VEGF expression through the PI3-K/mTOR pathway (34). This is consistent with our finding of up-regulated levels of VEGF in CCA cells after exposure to FGF10. Additionally, a study on breast cancer cells showed that FGF10 induction enhanced matrix metalloproteinase-9 expression and decreased TIMP1 expression, which is an inhibitor of angiogenesis (24). This result is consistent with our study that found the expression levels of TIMP1 to be decreased in CCA.

Pemigatinib, the first FGFR inhibitor, was approved by the USFDA in April, 2020 for the treatment of CCA patients with FGFR2 fusion or rearrangement. Among all the FGFR inhibitors, infigratinib was used to suppress FGFR2 activity in our CCA model. Immunoblotting analysis confirmed that infigratinib suppressed p-FGFR and FGFR2 expression levels

in KKU-M213A cells. The drug also decreased the proliferation and migration of CCA cells. Notably, infigratinib interrupted cell migration mediated by FGF10 through FGFR2 and probably *via* AKT/mTOR signaling. This finding is consistent with a previous report in a gastric cancer model. Huang *et al*. reported that exogenous FGF7 promotes the invasion and migration of human gastric cancer cells *via* FGFR2 and thrombospondin 1. Moreover, this signal activation can be suppressed using FGFR and mTOR inhibitors (35).

In conclusion, the present study demonstrated that FGF10/FGFR2 promotes the up-regulation of the Akt/mTOR and VEGF/Slug pathways, which are associated with the regulation of migration and invasion in CCA. The role of FGFR inhibitors in suppressing cell migration mediated by FGF10 was also highlighted. Our findings warrant further elucidation of FGF10/FGFR2/AKT/mTOR as a potential target for metastatic CCA.

Conflicts of Interest

All Authors declare that they have no conflicts of interest in relation to this study.

Authors' Contributions

S.K. and A.J. conceived and designed the study. K.O. and R.J. conducted experiments. A.P. and L.S. contributed new reagents or analytical tools. S.K., K.O. and V.K. analyzed data. S.K. and V.K. wrote the manuscript. S.K. conducted funding acquisition. All Authors read and approved the manuscript.

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