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Fibroblast growth factor receptor 2 fusions as a target for treating cholangiocarcinoma

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Abstract

Purpose of review—This review will cover the role of the fibroblast growth factor pathway in the pathogenesis, targeted therapy potential and prognostic value in patients with cholangiocarcinoma (CCA).

Recent findings—Recent studies that have identified fibroblast growth factor receptor 2 (FGFR2) fusions, prognostic implications of FGFR2 fusions, treatment strategies that target FGFR2 in CCA and future directions for understanding and targeting the FGFR2 pathway in this disease, will be discussed.

Summary—Understanding the role of the FGFR2 pathway as a disease pathogenetic mechanism and the ability to develop targeted therapies and diagnostics surrounding this concept are critical elements toward developing novel targeted approaches in CCA.

Keywords

cholangiocarcinoma; fibroblast growth factor receptor 2 signaling; fibroblast growth factor receptor 2 targeting

INTRODUCTION

Cholangiocarcinoma (CCA) is a malignancy of the biliary tract and arises in the intrahepatic or extrahepatic bile ducts. The liver flukes, *Opisthorchis viverrini* and *Camellia sinensis*, hepatitis B and C viruses, obesity, hepatolithiasis, thorotrast exposure and conditions such as primary sclerosing cholangitis and Caroli's disease, have been identified as risk factors [1].

Currently, surgical resection or liver transplantation represents the only curative therapeutic modalities. In patients with advanced disease, gemcitabine and cisplatin combination conferred a survival advantage over gemcitabine and currently serves as a practice standard [2]. With the advent of Next-Gen sequencing and its application toward molecular profiling

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Conflicts of interest

There are no conflicts of interest.

of tumors, a great deal of excitement has been generated toward its application for novel therapeutic target discovery. Early efforts of whole exome sequencing in patient cohorts with liver fluke associated CCA and nonliver fluke associated CCA have identified an array of recurrent genetic aberrations such as IDH1, IDH2 and KRAS mutations [3–6]. These studies have provided the impetus to continue investigation of the CCA genome to elucidate additional disease pathogenesis and therapeutically relevant targets.

FIBROBLAST GROWTH FACTOR RECEPTOR 2 FUSIONS AND OTHER FIBROBLAST GROWTH FACTOR PATHWAY GENOMIC ALTERATIONS IN CHOLANGIOCARCINOMA

The fibroblast growth factor receptor (FGFR) pathway is involved in cellular migration, proliferation, survival and differentiation. It has been implicated in normal physiological processes such as mesodermal patterning of the embryo, wound healing and angiogenesis [7].

Gene fusions of fibroblast growth factor receptor 2 (FGFR2) with multiple partners have been uncovered in a number of recent studies in patients with CCA as well as other cancers ([8⁹,10¹¹,12] Table 1).

The relative oncogenic potential of the disparate FGFR2 fusions on a comparative basis with regards to prognosis, prediction to response and outcomes when treated with agents that target the FGFR pathway, are currently unknown and should be evaluated rigorously in larger, prospective studies.

DEMOGRAPHIC, HISTOLOGICAL, ETIOLOGIC AND PROGNOSTIC CHARACTERISTICS OF CHOLANGIOCARCINOMA PATIENTS WITH FIBROBLAST GROWTH FACTOR RECEPTOR 2 FUSIONS

In addition to Next-Gen sequencing-based approaches that have been predominantly utilized in the context of discovery projects, more traditional diagnostic platforms such as fluorescent in-situ hybridization (FISH), have been pursued as molecular profiling approaches to identify CCA patients with FGFR2 fusions [10¹³]. Using break-apart FISH assays Graham *et al.* [13] evaluated patients with CCA ($n = 152$) and intraductal papillary neoplasm of the bile duct ($n = 4$) in a North American cohort. FGFR2 fusions were observed in 13% (12 of 96) of intrahepatic CCA and none was found in extrahepatic or perihilar CCA [13]. From a histological perspective, FGFR2 fusion-positive tumors had either prominent intraductal growth or anastomosing tubular glands with desmoplasia. Weak and patchy expression of CK19 was suggestive of a hepatic progenitor cell phenotype. Intriguingly, the survival of CCA patients with FGFR2 fusions was significantly higher (123 vs. 37 months), suggesting the potential utility of FGFR2 fusion identification as a prognostic marker. The median age of 52 years is lower than the reported median age of CCA (~65 years). A female preponderance (13 vs. 4%) was also noted. No relation with hepatitis B or C viruses or associated cirrhosis was noted and none of the primary sclerosing cholangitis patients had FGFR2 fusions.

In a complementary study conducted by Arai *et al.* [10¹¹], FGFR2 fusions involving FGFR2-BICC1 and FGFR2-AHCYL1 were identified in 13.6% of patients with intrahepatic CCA. Akin to the anatomic restriction to intrahepatic CCA cases in the North American study by Graham *et al.*, no FGFR2 fusions were found in patients with extrahepatic CCA. FGFR2-BICC1 fusions were also seen in one colorectal cancer patient (one of 149) and in one hepatocellular cancer patient (one of 96). None was seen in patients with gastric cancer. KRAS and BRAF mutations were not seen in any of the FGFR2 fusion-positive CCA cases, suggesting its potential role as a driver event. Unlike the North American study by Graham *et al.* there was not a female preponderance and no survival differences between fusion-positive and fusion-negative patients were noted. There was however an association that was noted between fusion-positive cases with underlying hepatitis B and C virus infection. These differences in patient characteristic and outcomes between the Graham and Arai studies could be attributed to differing CCA etiologic distribution between the studies and exposure differences pertaining to diet and environment.

Thus far, FGFR2 fusions have been identified only in patients with intrahepatic CCA and been completely absent in extrahepatic CCA and perihilar CCA across the multiple studies described earlier [8⁹,10¹¹,11¹²,13]. The anatomic restriction is suggestive of not only differing genomic causes of CCA based on primary site of origin, but also points to the possibility of an association to differing exposures including viruses such as hepatitis B/C and environmental toxins with predilection for liver injury. Larger, more rigorous epidemiological and toxicological studies could help elucidate the aforementioned anatomic restriction of FGFR2 fusions to intrahepatic CCA.

In addition to whole transcriptome sequencing and Next-Gen sequencing panel approaches, FISH using a break apart assay strategy could be employed to rapidly identify patients who have FGFR2 fusions [10¹¹,13].

The identification of FGFR2 fusions in patients with surgical resections (i.e. at a nonadvanced, early clinical stage) and in one instance, in a patient with intraductal papillary neoplasm of the bile duct, suggests that FGFR2 fusions are early oncogenic events. This would imply that FGFR2 fusions would have potential for serving as driver events in CCA and would be present in a substantial proportion/majority of the tumor cells.

Mutations in FGFR2 have been identified in whole exome sequencing efforts conducted thus far [4]. However, these mutations have not been in the kinase domain, and as such the functional relevance has been unclear. Both in-vitro/in-vivo functional studies and clinical studies using FGFR inhibitors in patients with FGFR2 mutations will provide additional perspective with regards to the role of FGFR2 mutations as therapeutic targets.

In a study by Wang *et al.* focusing on methylation patterns of CCA patients with IDH1/IDH2 mutations, it was noted that IDH1/IDH2 mutant patients had significant overexpression of FGFR2, FGFR3 and FGFR4 in the absence of FGFR mutations or FGFR2 fusions. The basis for the association is unknown and would certainly be of significant interest to understand in greater detail [14].

TARGETING FIBROBLAST GROWTH FACTOR RECEPTOR 2 FUSIONS IN CHOLANGIOCARCINOMA WITH SMALL MOLECULE FIBROBLAST GROWTH FACTOR KINASE INHIBITORS

Given that the kinase domain has been found to be intact in FGFR2 fusions identified thus far, small molecule kinase inhibitors (SMKIs) have emerged as a logical strategy for the treatment of CCA patients with FGFR2 fusions. Preliminary evidence of anti-tumor activity has been observed in a CCA patient with FGFR2-MGEA5 fusion treated with ponatinib and similarly in an FGFR2-TACC3 fusion-positive CCA patient treated with pazopanib and subsequently ponatinib [8[¶]]. Based on these encouraging data and supporting preclinical evaluations, a number of clinical studies prospectively investigating the activity of FGFR small molecule inhibitors in CCA have been initiated (Table 2). In a similar realm, a number of additional FGFR small molecule inhibitors, that are in clinical studies, could be positioned for evaluation in FGFR fusion-positive CCA and possibly also in CCA patients who do not have FGFR2 fusions, but who do have other alterations in FGFR2 (such as mutations or amplifications) or who have genomic alterations in other FGFR2 pathway members (Table 2).

ISOFORM SELECTIVITY AND ROLE FOR ANTIBODY THERAPY IN FIBROBLAST GROWTH FACTOR RECEPTOR 2 FUSION-POSITIVE CHOLANGIOCARCINOMA PATIENTS

Isoform selectivity to the FGFR2-IIIb isoform has been observed in patients with FGFR2 fusions [8[¶],11[¶]]. Restriction to FGFR2-IIIb isoforms has multiple implications. Prior studies have shown that FGFR2-IIIb exhibits selectivity to binding to the FGF7 and FGF10 ligands. From this perspective, antibodies with specificity for the FGFR2-IIIb isoforms could constitute attractive therapeutic targets in FGFR2 fusion-positive CCA as there would be potential to avoid the off target toxicities of FGFR SMKIs [15]. FGFR2-IIIb antibodies could also be positioned in combination with FGFR SMKI to achieve more complete blockade of the FGFR2 signaling axis.

HEAT SHOCK PROTEIN INHIBITORS AS A STRATEGY FOR TARGETING FIBROBLAST GROWTH FACTOR RECEPTOR 2 FUSION-POSITIVE CHOLANGIOCARCINOMA

Heat shock proteins, particularly Hsp90 along with CDC37, have been shown to serve as chaperones to a wide array of oncogenic client proteins including FGFR family members [16–18]. Targeting approaches combining FGFR SMKI with Hsp90/CDC37 inhibitors would constitute a promising therapeutic strategy from this perspective.

COMBINATORIAL APPROACHES FOR TARGETING FIBROBLAST GROWTH FACTOR RECEPTOR 2 SIGNALING

In-vivo model system evaluation of FGFR SMKIs has thus far not shown overt tumor regressions. Although worthy of exploration, it is somewhat unlikely that the reduction in tumor growth rate alone will manifest as clinically meaningful responses. Downstream, escape pathways that can bypass upstream FGFR signaling or be amplified in the context of inhibition of the FGFR2 pathway at the FGFR2 node, constitute a rational strategy that should be pursued from a combinatorial standpoint. In an endometrial cancer FGFR2 mutation model, combination of ponatinib and ridaforolimus resulted in superior in-vivo efficacy compared with either agent alone [19]. Similar strategies should be pursued in the context of targeting FGFR2 fusions in CCA.

CONCLUSION

Future efforts should focus on developing FGFR2 specific kinase inhibitors to avoid/limit off target toxicities because of targeting of other kinases, particularly angiogenic kinases such as VEGFR1, VEGFR2 and PDGFRA, that have manifest as treatment related toxicities in the FGFR inhibitors utilized thus far. As described previously, other aspects of FGFR2 fusion targeting include more complete blockade of the signaling axis through combination antibody/small molecule inhibition, combinatorial targeting of upstream (FGFR2) and downstream (e.g. PI3K-Akt-mTOR) pathways and combination approaches with Hsp90/ CDC37 inhibitors. Elucidation of mechanisms of resistance to FGFR inhibitors in FGFR2 fusion CCA patients will also be an area of heightened interest, particularly from standpoint of convergent or divergent tumor evolution. These mechanisms will be defined through collection and analysis of serial samples in FGFR2 fusion CCA patients treated with FGFR inhibitors.

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KEY POINTS

- FGFR2 aberrations, particularly FGFR2 fusions have been identified as a novel oncogenic, druggable target in patients with CCA.
- Both small molecular inhibitors and isoform specific FGFR2 antibodies would serve as suitable therapeutic interventions in this patient population.
- Further studies of FGFR2 signaling and intervention should be pursued.

Table 1

Studies that have identified fibroblast growth factor receptor 2 fusions in cancer patients and cancer cell lines

Fusion	CCA	Breast cancer	Lung cancer (squamous cell)	Thyroid cancer	Colon cancer	Hepatocellular cancer	Prostate cancer
FGFR2 fusions identified in patient samples (numbers in parentheses correspond to references)							
<i>FGFR2-BICC1</i>	+ (8,10,11,12)				+ (10)	+ (10)	
<i>FGFR2-AHCYL1</i>	+ (10)						
<i>FGFR2-MGEA5</i>	+ (8)						
<i>FGFR2-TACC3</i>	+ (8,12)						
<i>FGFR2-KIAA1598</i>	+ (12)						
<i>FGFR2-CREB5</i>	+ (9)						
<i>FGFR2-KIAA1967</i>			+ (11)				
<i>FGFR2-CCDC6</i>		+ (11)					
<i>FGFR2-AFF3</i>		+ (11)					
<i>FGFR2-CASP7</i>		+ (11)					
<i>FGFR2-OFD1</i>				+ (11)			
<i>SLC45A3-FGFR2</i>							+ (11)

CCA, cholangiocarcinoma; FGFR2, fibroblast growth factor receptor 2.

Table 2

Ongoing clinical trials of fibroblast growth factor receptor 2 targeting agents

Agent	Status	Therapeutic class	NCT number
Ponatinib	Phase II in CCA	FGFR SMKI	NCT02265341
Ponatinib	Phase II in FGFR aberrant patients	FGFR SMKI	NCT02272998
BGJ398	Phase II in CCA	FGFR SMKI	NCT02150967
CH5183284/Debio 1347	Phase I	FGFR SMKI	NCT01948297
ARQ 087	Phase I	FGFR SMKI	NCT01752920
TAS-120	Phase I	FGFR SMKI	NCT02052778
AZD4547	Phase I	FGFR SMKI	NCT00979134
JNJ-42756493	Phase I	FGFR SMKI	NCT01703481
BAY1163877	Phase I	FGFR SMKI	NCT01976741
Pazopanib + trametinib	Phase I expansion in CCA	FGFR SMKI	NCT01438554
FPA144	Phase I	FGFR2-IIIb Ab	NCT02318329

CCA, cholangiocarcinoma; FGFR2, fibroblast growth factor receptor 2; FGFR SMKI, fibroblast growth factor receptor small molecule kinase inhibitor.