CHAPTER 12

FGF19 AND CANCER

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Abstract: Fibroblast growth factors (FGFs) and their cognate receptors, FGF receptors (FGFRs), play critical roles in a variety of normal developmental and physiological processes. Numerous reports support a role for deregulation of FGF-FGFR signaling, whether it is at the ligand and/or receptor level, in tumor development and progression. The FGF19-FGFR4 signaling axis has been implicated in the pathogenesis of several cancers, including hepatocellular carcinomas in mice and potentially in humans. This chapter focuses on recent progress in the understanding of the molecular mechanisms of FGF19 action and its potential involvement in cancer.

INTRODUCTION

Fibroblast growth factors (FGFs) comprise a family of 22 structurally related polypeptides with diverse biological activities.[1] Most of these signaling molecules bind to and activate members of the FGF receptor (FGFR) family. The FGFR family is composed of four receptor tyrosine kinases, designated FGFR1- FGFR4, and one receptor which lacks a cytoplasmic tyrosine kinase domain, designated FGFR5.[2,3] The interaction of FGFs with FGFR1-4 results in receptor dimerization and autophosphorylation, recruitment of membrane-associated and cytosolic accessory proteins, and initiation of multiple signaling cascades.[4] The FGF-FGFR signaling network plays important roles in development and tissue repair by regulating cellular functions/processes such as growth, differentiation, migration, morphogenesis, and angiogenesis. Not surprisingly, dysregulation of this signaling system appears to be important for tumor development and progression.

Endocrine FGFs and Klothos, edited by Makoto Kuro-o.

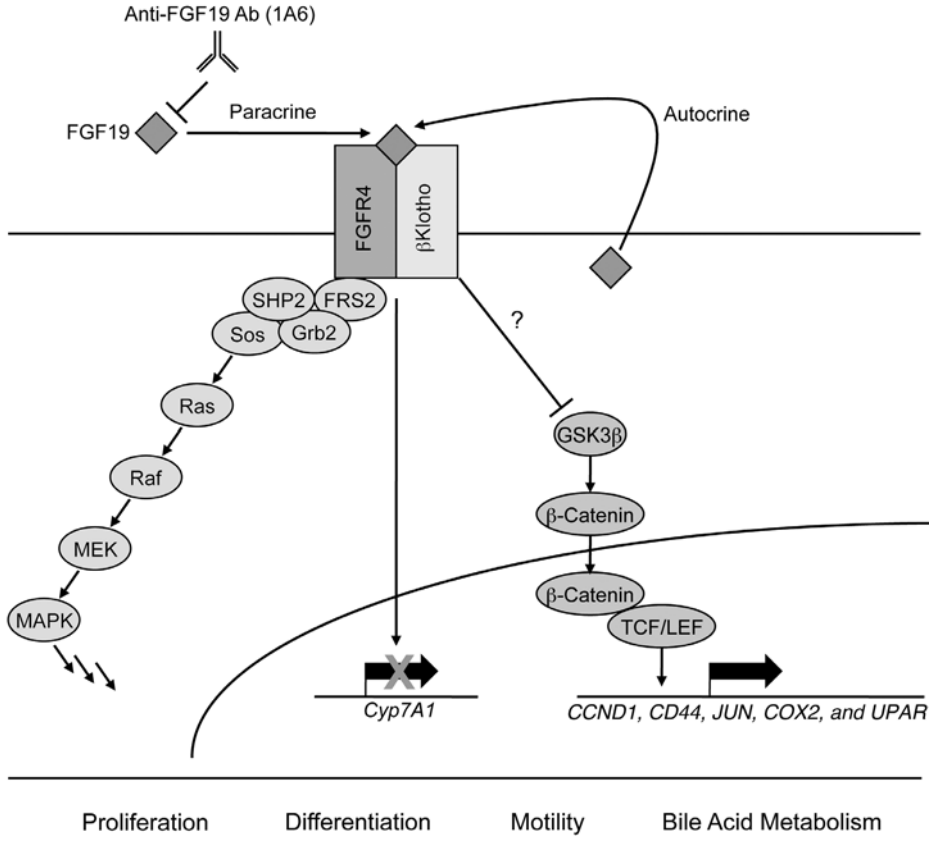

Figure 1. FGF19-FGFR4 Signaling Pathway. The growth factor FGF19 regulates a variety of cellular/physiologic functions in normal and possibly neoplastic states (e.g., bile acid metabolism, proliferation, differentiation, and cellular motility). Signaling by this ligand is mediated by its cognate receptor FGFR4 and a recently identified coreceptor βKlotho. FGF19-mediated activation of mitogen-activated protein kinase (MAPK) and β-catenin pathways may be involved in the development and progression of cancers, such as hepatocellular carcinoma. Blocking the interaction of FGF19 with FGFR4 using a high affinity, high specificity anti-FGF19 antibody (1A6) inhibits FGF19 signaling and tumor growth in vivo.

Most FGFs function primarily in a paracrine and/or autocrine fashion. However, the FGF19 subfamily members (FGF19/Fgf15, FGF21, and FGF23) can function as endocrine factors or hormones. FGF19 is an important regulator of metabolism under normal physiological conditions. In disease states, FGF19 may be critical for the development and progression of a number of cancers. Of particular note, FGF19 signaling has been proposed to be important in promoting hepatocellular carcinomas (HCCs) in mice and potentially in humans.[5-7] As illustrated in Figure 1, the effects of FGF19 on downstream signaling (e.g., mitogen-activated protein kinase and β-catenin pathways) are mediated by its cognate receptor FGFR4 and a recently identified coreceptor βKlotho (KLB).[8,9] Thus, FGF19 may serve as an important therapeutic target in the treatment of relevant cancers.

FGF19/FGF15

FGF19 was cloned by homology to its mouse ortholog *Fgf15*.[10,11] *FGF19* messenger RNA (mRNA) is found in brain, skin, cartilage, retina, gall bladder, small intestine, and kidney.[10,11] The expression of FGF19/Fgf15 is induced in the ileum in response to bile acids that are released into the intestinal lumen after feeding. FGF19/Fgf15 then circulates to the liver to suppress the expression of CYP7A1, the rate-limiting enzyme for bile acid synthesis.[12] FGF19/Fgf15 also limits bile acid release into the intestine by triggering gall bladder filling.[13] In this manner, FGF19/Fgf15 serves as a key regulator in a postprandial negative feedback loop modulating bile acid synthesis and release.

FGF19 BINDING SPECIFICITY: FGFR4 AND THE βKLOTHO CORECEPTOR

Among the FGF family of ligands, FGF19 has a unique receptor binding specificity. Initially, FGF19 was shown to bind exclusively to FGFR4.[11] Using co-immunoprecipitation, Xie et al assessed the binding of FGF19 to immunoadhesin chimeric constructs of a subset of the human FGFRs (i.e., FGFR1, FGFR2-3 IIIb and IIIc isoforms, and FGFR4). These authors observed a heparin-dependent interaction of FGF19 that was restricted to FGFR4.[11] Utilizing a panel of FGFR immunoadhesin chimeric constructs that included all of the known splice variants of FGFR1-3 (i.e., IIIb and IIIc isoforms) and FGFR4, we also demonstrated that FGF19 bound exclusively to FGFR4 using both co-immunoprecipitation and solid phase receptor binding assays.[9] Although FGF19 exhibits little or no binding to glycosaminoglycan (the endocrine FGFs as a whole have a low affinity for heparin),[14] its interaction with FGFR4 is exquisitely dependent on the presence of heparin.[11] This interaction could be explained by the unique heparin binding ability that FGFR4 has among the FGFR family of receptors.[15,16] Zhang et al demonstrated a different receptor specificity for FGF19 in proliferation assays using the BaF3 murine pro-B-cell line. Although FGF19 had the greatest activity with the BaF3 cells stably transfected with mouse Fgfr4, this ligand also promoted the proliferation of the mouse Fgfr1 IIIc, Fgfr2 IIIc, and Fgfr3 IIIc expressing cells.[17] The activity of FGF19 in this context was also dependent on the presence of heparin.[17] FGF19 is not expressed in mice and it shares only 51% to 53% amino acid sequence identity with its closest mouse ortholog, Fgf15.[10,11] In contrast, all of the remaining human FGFs, with the exception of FGF19, share 75% to 100% amino acid sequence identity with their corresponding mouse orthologs. Given the reduced sequence identity between the human and mouse orthologs, it is possible that FGF19 binds with a different specificity to the human FGFRs as compared to the mouse Fgfrs.

While FGF19 may be able to bind and function through a number of other FGFRs, its activity is primarily transmitted through the binding and activation of FGFR4. FGFR4 is the most widely distributed member of the FGFR family. Under normal circumstances, FGFR4 is expressed in liver, lung, gall bladder, small intestine, pancreas, colorectal, lymphoid, ovary, and breast tissues.[9] It has recently been demonstrated that endocrine FGFs, such as FGF19, also require the Klotho family of proteins as coreceptors to promote the binding of these ligands to their corresponding FGFRs.[8,9,18,19]

The Klotho family of proteins, comprised of Klotho (KL) and KLB, are Type I transmembrane glycoproteins containing extracellular regions that contain two beta-glycosidase-like repeats. FGFR4 binds to both KL and KLB.[8,9,19] We and others

have recently identified KLB as a coreceptor for FGFR4 that is required for the high affinity binding and activity of FGF19.[8,9] Using biochemical and cell based assays we demonstrated that human KLB promotes the exclusive interaction of FGF19 with FGFR4.[9] As previously mentioned, FGF19 has low affinity for heparin. KLB appears to stabilize the interaction of this ligand with its receptor, perhaps acting as a surrogate for heparin. Intriguingly, Kurosu et al used cells stably transfected with the various Fgfr isoforms to show that mouse Klb promotes the binding of FGF19 not only to mouse Fgfr4, but also to mouse Fgfr1 IIIc, Fgfr2 IIIc, and Fgfr3 IIIc.[8] This role of KLB in FGF19 binding and activity may in part explain the ability of FGF19 to act through other FGFRs. Previous binding studies demonstrating exclusive binding of FGF19 to FGFR4 were done in the absence of KLB.[11] It has been reported that FGF19 can bind and activate FGFR4 in the absence of KLB.[20] However, KLB significantly increased FGF19 potency on FGFR4 activation.[20] The authors speculated that due to the low normal plasma concentration of FGF19/Fgf15, KLB is critical in conferring FGFR4 activation under physiological conditions; the experiments reported by Wu et al were performed using supraphysiological levels of recombinant FGF19. Although these studies showed differential receptor binding specificities they reach identical conclusions. KLB reduces or alleviates the need of FGF19 for heparin to bind to the FGFRs, KLB promotes the interaction of FGF19 with the FGFRs without altering its binding specificity, and FGF19 binds preferentially to FGFR4.

Given that FGF19 activity requires the co-expression of FGFR4 and KLB, the distribution and the relative levels of these proteins will dictate the target organ site of FGF19 endocrine action. Unlike the broad distribution of *FGFR4* expression, the distribution of *KLB* is more restricted. *KLB* is expressed in adipose, liver, pancreas, and breast tissues.[9] *FGFR4* and *KLB* are both highly co-expressed in the liver and, not surprisingly, mediate the specific activities of FGF19 in this tissue.[9,21] Much lower levels of *FGFR4* and *KLB* co-expression are also found in pancreas and breast tissues.[9] However, FGF19 activity has not been described in these tissues thus far. *KLB* is highly expressed in adipose tissues, but the absence of *FGFR4* precludes the activity of FGF19 in this tissue.[9] These findings taken together indicate that the liver is expected to be the main target organ for the endocrine actions of FGF19.

FGF19 AND CANCER

FGF19 Activity

FGF-FGFR signaling networks play important roles in cell proliferation and the activities stimulated by some of these ligand-receptor combinations have been linked to the development and progression of cancer.[22,23] FGF19 may promote hepatocellular carcinoma by utilizing a number of potential molecular mechanisms (summarized in Table 1). The ectopic expression of *FGF19* in transgenic mice led to tumor formation in the liver.[6] These *FGF19* transgenic mice developed liver tumors by 10 to 12 months of age.[6] Tumors arose from pericentral hepatocytes after increased proliferation and dysplasia.[6] This increase in hepatocellular proliferation is believed to be a prerequisite for neoplastic transformation.[24] Consistent with these findings, the injection of wild-type mice with recombinant FGF19 also promoted hepatocyte proliferation.[6] Elevated expression of α-fetoprotein (*AFP*) mRNA, an oncofetal protein used as a marker for the detection of liver cancer,[25] accompanied the increased hepatocellular proliferation in the *FGF19*

Table 1. Effects of FGF19 Signaling in Cancer

Effect	Reference
Proliferation	5,6,32,57-59
Survival	58,59
Chemotaxis/Motility	5
Adhesion	7

transgenic mice.[6] The nuclear accumulation of β-catenin observed in neoplastic cells from a subset of the liver tumors found in the *FGF19* transgenic mice potentially implicates activation of the Wingless/Wnt signaling pathway in this tumorigenic process.[6] In addition to this immunohistochemical evidence, cloning and sequencing of DNA from these tumor tissues revealed point mutations resulting in amino acid substitutions in and around the glycogen synthase-3β (GSK-3β) phosphorylation site of β-catenin that were suggestive of β-catenin activation.[6] Taken together, these studies demonstrated that FGF19 promotes hepatocellular carcinoma in mice.

To investigate the role of FGF19 in human cancer, we evaluated its expression in primary human tumors and analyzed the consequence of therapeutic FGF19 neutralization in relevant tumor models. *FGF19* and *FGFR4* were coexpressed in primary human hepatocellular carcinomas, lung squamous cell carcinomas, and colon adenocarcinomas.[5] Relative to the corresponding normal tissues, *FGF19* was found to be overexpressed in these cancers, as well as in a subset of human colon cancer cell lines.[5] *FGF19* was also strongly expressed in livers that had undergone cirrhosis, a preneoplastic condition that often leads to liver carcinoma.[5] To assess the importance of FGF19 in tumor growth and development, an anti-FGF19 blocking antibody (1A6) that selectively inhibits the interaction of FGF19 with FGFR4 was generated. 1A6 inhibited FGF19-modulated fibroblast growth factor receptor substrate 2 (FRS2) and mitogen-activated protein kinase (MAPK) signaling in Hep3B HCC cells and completely relieved repression of *CYP7A1* gene expression in HepG2 HCC cells.[5] To further determine whether FGF19 neutralization could inhibit tumor growth in vivo, HCT116 and Colo201 colon cancer xenograft mouse models were utilized. In comparison to xenograft mice treated with a control antibody, 1A6 treated mice showed a statistically significant growth inhibition of both HCT116 (60% inhibition, $P = 0.01$, $n = 5$ at 15 mg/kg 1A6, twice weekly treatment) and Colo201 (64% inhibition, $P = 0.03$, $n = 5$ at 30 mg/kg 1A6, twice weekly treatment) tumors.[5] In both tumor models, analysis of excised tumors showed that 1A6 significantly inhibited FGFR4, FRS2, MAPK, and β-catenin activation.[5] In order to evaluate 1A6 for in vivo activity, 1A6 was tested in the *FGF19* transgenic mice that had previously been demonstrated to develop liver tumors. In this study, *FGF19* transgenic mice were first treated with diethylnitrosamine (DEN), which accelerated tumor formation by 50%. All of the mice treated with control antibody had multifocal, large liver tumors throughout the liver lobes; with the exception of one 1A6 treated mouse that had a single small tumor, the 1A6 treated animals had no liver tumors.[5] Liver weights and tumor volumes from 1A6 treated transgenic mice were significantly lower than in control antibody treated mice.[5] These findings suggest that FGF19 may be involved in human tumor pathogenesis, notably in the liver, in early neoplastic progression.

While the aforementioned studies implicate FGF19 in tumorigenesis, the molecular mechanism by which this endocrine ligand functions in this manner is unclear. As

previously mentioned, evidence of β-catenin activation was observed in neoplastic cells from *FGF19* transgenic mice. β-Catenin plays a key role in maintaining cell-cell adhesion and as a downstream effector in the Wnt signaling cascade. Interaction of E-cadherin with α- and β-catenin is essential for stable cell-cell adhesion and association of these proteins is regulated by tyrosine phosphorylation of β-catenin.[26,27] A complex of proteins containing adenomatous polyposis coli, axin, conductin, and GSK-3β regulates the stability of cytoplasmic β-catenin by targeting β-catenin for ubiquitination and proteasomal degradation. Activation of Wnt signaling results in GSK-3β inactivation and subsequent nuclear accumulation of β-catenin where β-catenin can then interact with TCF/LEF transcription factors to activate corresponding target gene expression.[28-30] Deregulation of Wnt signaling may be important for the initiation and/or progression of several malignancies (e.g., HCC, colorectal, ovarian, endometrial and prostate cancers, and melanoma).[28-31] Furthermore, the anti-FGF19 antibody 1A6 repressed β-catenin activation in colorectal cancer xenograft models. The effect of FGF19 on the β-catenin signaling pathway was recently assessed. The treatment of HCT116 colon cancer cells with FGF19 resulted in a dose-dependent increase in β-catenin phosphorylation, accompanied by a concomitant loss in E-cadherin binding.[7] FGF19 also increased GSK-3β phosphorylation and active β-catenin, and led to the activation of β-catenin/TCF4-regulated transcription.[7] Treatment of HCT116 cells with 1A6 reduced these effects; phospho-GSK-3β and active β-catenin levels were significantly reduced and FGF19-induced β-catenin/TCF4-regulated gene expression (i.e., *CCND1*, *CD44*, *JUN*, *COX2,* and *UPAR*) was consistently repressed.[7]

The Role of FGFR4 in Modulating FGF19 Activity

The normal activity of FGF19 is primarily modulated through the binding and activation of FGFR4. FGFR4 has recently been reported to uniquely mediate FGF19-induced hepatocyte proliferation.[32] Using a series of FGF19 truncation and chimeric constructs, Wu et al identified that amino acid residues 38 to 42 of FGF19 are sufficient to confer FGFR4 activation and increased hepatocyte proliferation, a process that is believed to be a prerequisite for HCC formation.

Contrary to the other FGFR family members, FGFR4 does not have alternatively spliced variants. Although FGFR4 is not alternatively spliced, its function appears to be altered by polymorphisms (summarized in Table 2). The contribution of FGFR4 polymorphisms to hepatocellular carcinoma was recently evaluated. A comprehensive sequence analysis of FGFR4 was conducted on 57 pairs of matched HCC and normal tissue samples.[33] Three known single nucleotide polymorphisms (i.e., Val10Ile, Leu136Pro, and Gly388Arg) and five previously unreported amino acid sequence alterations (i.e., Asp126Asn, Thr179Ala, Gly426Asp, Asp709Gly, and truncation at amino acid residue 450) were identified.[33] These polymorphisms appear to be germ line alterations as they were found in both the tumor and corresponding normal tissues of the respective samples. The Gly388Arg polymorphism was the most highly distributed variant.[33] Compared to the Gly/Gly genotype patients, homozygous Arg/Arg individuals had increased circulating levels of AFP, the embryonic tumor cell marker characteristic for liver cancer.[33] *FGFR4* mRNA expression was also found to be increased by at least 2-fold in 31.6% (18 of 57) of the HCC samples compared to the matched normal liver samples.[33]

Increased expression and genotype distribution of FGFR4 in the liver provides a potential link between FGFR4 and progression of hepatocellular carcinoma. To further investigate this potential functional role of FGFR4 in HCC, tumor responses were measured

Table 2. Effect of FGF19 on FGFR4 Polymorphisms in Cancer

Polymorphism	Indication	Effect of Polymorphism/Effect of FGF19	Reference
Arg388	Hepatocellular carcinoma	Increased circulating levels of AFP/FGF19 treatment increased AFP secretion and FRS2α phosphorylation in cultured cells	33
	Breast cancer	• Increased cell motility/Unknown	38
		• Promote elongated morphology in cell line/Unknown	38
		• Associated with resistance to therapy/ Unknown	60
		• Decreased disease free survival time/ Unknown	38
		Not indicative of disease free survival time/Unknown	51
	Colon cancer	Decreased disease free survival time/ Unknown	38,40
		Not predictive of disease progression/ Unknown	40
	Lung cancer	• Decreased age at onset and survival; Increased stage of disease/Unknown	40,44
		• Shorter survival in node positive patients/Unknown	45
		No association with disease prognosis/ Unknown	54
	Prostate cancer	• Increased receptor stability and sustained activity/Unknown	43
		• Increased incidence and/or occurrence of aggressive disease/Unknown	41,43
		No association with survival differences/ Unknown	33,53
	Soft tissue sarcomas	Decreased cumulative overall and metastasis-free survival/Unknown	50
	Bone sarcomas	No significant difference/Unknown	50
	Malignant gliomas	No association with risk of survival differences/Unknown	52
	Melanoma	No association with risk of survival differences/Unknown	55,60
	Squamous and basal cell carcinomas	No association with risk to disease development/Unknown	55
	Head/neck squamous cell carcinomas	Associated with reduced overall survival and with advanced tumor stage/Unknown	47,48
Gly388	Head/neck squamous cell carcinomas	Increased risk of developing disease/ Unknown	46
Tyr367Cys	Breast Cancer	Enhanced ERK activation and cell proliferation/No effect	39

in HuH7 and HepG2 HCC cell lines, which are both homozygous for the Arg388 allele, stimulated with FGF19.[33] The stimulation of these cell lines with FGF19 increased AFP secretion in culture, as compared to untreated cells.[33] Phosphorylation of the FRS2α adaptor protein was also stimulated by FGF19 treatment of HuH7 and HepG2 cells, as compared to untreated cells, indicating activation of the FGFR4 signaling pathway.[33] Conversely, the suppression of FGFR4 expression using siRNA reduced the secretion of AFP.[33] The treatment of HCC cell lines with an FGFR inhibitor reduced the FGF19- and serum-stimulated AFP secretion, blocked proliferation and invasion, and induced apoptosis in vitro.[33] Based on these findings the authors associated the activity of the FGF19-FGFR4 axis with the progression of liver cancer. Furthermore, the potential role of FGFR4 mutants in FGF19 signaling is highlighted here, given that the HCC cell lines utilized in this study were homozygous for the Arg388 allele.

The role of FGFR4 in liver cancer progression was also studied in genetically engineered mice. It was shown that the repression of *Fgfr4* expression in mice increased liver injury and fibrosis induced by carbon tetrachloride.[34] Also, the induction of hepatocarcinogenesis by DEN was accelerated in *Fgfr4-null* mice compared to their wild-type littermates.[35] These findings suggested that Fgfr4 protects the hepatocytes against acute and chronic injury and plays a cancer suppressor function in liver.[34,35] It is unclear why Fgfr4 was found to have a protective effect in these reports. As already described, *FGF19* transgenic mice, as well as wild-type mice injected with recombinant FGF19, were shown to develop HCC.[6] Furthermore, inducible knockdown of *Fgfr4* expression in a xenograft model showed reduced tumor growth.[7] The studies in which Fgfr4 showed protective effects utilized endogenous mouse Fgf15. Thus, the potential differential effects of Fgf15 and FGF19 on the FGFR signaling pathway may in part explain this difference in observations.

FGFR4 is also associated with breast cancer. *FGFR4* was found to be amplified 2- to 4-fold in 10% of primary breast tumors.[36] A recent study suggests that resistance to chemotherapy, such as doxorubicin, is associated with *FGFR4* upregulation.[37] To address this potential link, the authors generated an anti-FGFR4 antibody (10F10) and tested it against various breast cancer cell lines that endogenously express FGFR4. Administration of 10F10 reduced phospho-ERK levels that were stimulated by FGF19 treatment in the breast cancer cell lines, as compared to untreated cells.[37] Treatment of the breast cancer cell lines with 10F10 resulted in increased rates of apoptosis following doxorubicin induction, as compared to control antibody treatment, correlating with inhibition of FGF19 signaling through FGFR4.[37]

FGFR4 polymorphisms have also been identified in breast carcinomas. As is the case for HCC, a Gly388Arg mutation was identified in the MDA-MB-453 breast cancer cell line.[38] In a panel of breast cancer cell lines the genotype frequency was 58% for homozygous Gly alleles, 31.5% for heterozygous Gly/Arg alleles and 10.5% for homozygousArg alleles.[38] Among 145 breast cancer patients 46% were found to be homozygous Gly/Gly, 43% heterozygous Gly/Arg, and 11% homozygous Arg/Arg.[38] An analysis of the genomic DNA from the circulating white blood cells from a subset of these patients demonstrated the same genotype as seen in the tumor tissue demonstrating that the Gly to Arg conversion was a germ line polymorphism.[38] The distribution of the allele frequency was also shown to be similar in healthy individuals.[38] The authors showed that the Gly/Arg388 genotype was significantly prevalent among patients with metastatic breast cancer that had recurrence within 62 months.[38] However, no Gly388 patients had suffered from metastatic disease at the time of the study.[38] The authors suggested that the Arg388 allele

represents a determinant that is innocuous in healthy individuals but predisposes cancer patients to significantly accelerated disease progression. In addition, the presence of the Arg allele in breast cancer patients was associated with resistance to adjuvant therapy.[38] Infection of breast cancer cells with a recombinant retrovirus encoding *FGFR4* with the Gly allele decreased cell migration compared to the parental cell line.[38] However, upon infection with a recombinant retrovirus encoding *FGFR4* with the Arg allele the cells adopted an elongated morphology associated with a mesenchymal phenotype and scattered distribution.[38] The Gly388Arg polymorphism was reported to modulate cancer cell migration in vitro and to be associated with breast cancer prognostic parameters.[38]

Although a potential link between the Gly388Arg mutant and breast cancer progression has been identified, there have not been any reports addressing the possible effects of FGF19 on this receptor polymorphism. However, given that FGF19 stimulation of HCC cell lines containing the Gly388Arg mutant leads to activation of the FGFR4 signaling pathway, FGF19 may have similar effects on this receptor polymorphism in breast cancers.

Interestingly, an FGFR4 Tyr367Cys mutant has also been identified in MDA-MB-453 breast cancer cells.[39] This mutant is reported to be constitutively active due to spontaneous dimerization of the mutant receptor and independent of ligand stimulation.[39] When MDA-MB-453 cells were treated with FGF19, enhanced ERK activation and cell proliferation were not observed.[39] In contrast, when MDA-MB-361 cells which express wild-type FGFR4 were administered FGF19, ERK phosphorylation and cell proliferation increased.[39] Inhibition of FGFR4 Tyr367Cys expression in MDA-MB-453 cells by small interfering RNAs (siRNAs) reduced MAPK signaling (i.e., ERK phosphorylation), reduced proliferation rate, and depleted cell viability, as compared with control siRNA.[39] The ligand independence of the FGFR4 Tyr367Cys mutant was confirmed by the inability of 10F10, the anti-FGFR4 antibody that was raised against the extracellular domain of FGFR4, to downregulate MAPK signaling.[39]

FGFR4 overexpression and polymorphisms (i.e., Gly388Arg) have been associated with a number of other cancers, including colorectal carcinoma,[38,40] prostate cancer,[41-43] lung cancer,[40,44,45] squamous cell carcinoma,[46-48] melanoma,[49] and soft tissue sarcomas.[50]The utility of this FGFR4 Gly388Arg polymorphism as a predictive marker for disease free survival time has been debated. After independent analysis of cancer patient populations, several groups have reported that the Gly388Arg status cannot be used to predict disease free survival time for patients with breast carcinoma,[40,51] colon carcinoma,[40] malignant gliomas,[52] prostate cancer,[33,53] lung cancer,[54] basal cell carcinoma, squamous cell carcinoma, and melanoma.[55] Despite these disparate findings, little to no role for FGF19 signaling has been reported in any of these additional cancer types thus far. KLB, a key component required for FGF19 signaling through FGFR4, is not expressed at significant levels in these tissues. However, KLB may be able to function in a similar manner to KL, in which, in addition to being cell-associated, the extracellular portion of KL is secreted,[56] thus facilitating FGF19 activity in tissues where it is not normally expressed. Overexpression of FGF19 by these tumors may also facilitate FGF19/FGFR4 signaling in an autocrine manner by acting to increase the local concentration of ligand. FGF19 is overexpressed in colon adenocarcinoma.[5,11] It may also be possible that FGF19 does not have a substantive role in the genetic background of these cancers. Further investigation is required to address these issues.

CONCLUSION

As previously described, the FGF-FGFR signaling pathway is thought to play important roles in a number of human cancers. The FGF19-FGFR4 signaling axis in particular promotes hepatocyte proliferation and the development and progression of HCC in vivo.[6] Thus, FGF19 is an attractive target for the treatment of liver cancers and other potential neoplasms. As such, a neutralizing antibody against FGF19 has been shown to inhibit FGF19-mediated signaling and tumor growth in mice.[5] The recent identification of KLB as a critical player in the FGF19/FGFR4 signaling complex adds another level of regulation to this endocrine FGF signaling pathway. The relative expression levels and tissue distribution of FGFR4 and KLB are key in determining the effects of FGF19. This may provide additional targets for therapeutic intervention in cancer, especially given the recent findings that FGF19 may affect multiple FGFR signaling pathways[8,17] and certain FGFR4 polymorphisms are constitutively active (i.e., signaling in a ligand-independent manner).[39] Targeting FGFR4 and/or KLB with antibody therapeutics and/or small molecule inhibitors may also prove to be beneficial in the treatment of appropriate human cancers.

REFERENCES

1. Ornitz DM, Itoh N. Fibroblast growth factors. Genome Biol 2001; 2:REVIEWS3005.
2. Eswarakumar VP, Lax I, Schlessinger J. Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 2005; 16:139-149.
3. Sleeman M, Fraser J, McDonald M et al. Identification of a new fibroblast growth factor receptor, FGFR5. Gene 2001; 271:171-182.
4. Powers CJ, McLeskey SW, Wellstein A. Fibroblast growth factors, their receptors and signaling. Endocr Relat Cancer 2000; 7:165-197.
5. Desnoyers LR, Pai R, Ferrando RE et al. Targeting FGF19 inhibits tumor growth in colon cancer xenograft and FGF19 transgenic hepatocellular carcinoma models. Oncogene 2008; 27:85-97.
6. Nicholes K, Guillet S, Tomlinson E et al. A mouse model of hepatocellular carcinoma: ectopic expression of fibroblast growth factor 19 in skeletal muscle of transgenic mice. Am J Pathol 2002; 160:2295-2307.
7. Pai R, Dunlap D, Qing J et al. Inhibition of fibroblast growth factor 19 reduces tumor growth by modulating beta-catenin signaling. Cancer Res 2008; 68:5086-5095.
8. Kurosu H, Choi M, Ogawa Y et al. Tissue-specific expression of betaKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21. J Biol Chem 2007; 282:26687-26695.
9. Lin BC, Wang M, Blackmore C et al. Liver-specific activities of FGF19 require Klotho beta. J Biol Chem 2007; 282:27277-27284.
10. Nishimura T, Utsunomiya Y, Hoshikawa M et al. Structure and expression of a novel human FGF, FGF-19, expressed in the fetal brain. Biochim Biophys Acta 1999; 1444:148-151.
11. Xie MH, Holcomb I, Deuel B et al. FGF-19, a novel fibroblast growth factor with unique specificity for FGFR4. Cytokine 1999; 11:729-735.
12. Inagaki T, Choi M, Moschetta A et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell Metab 2005; 2:217-225.
13. Choi M, Moschetta A, Bookout AL et al. Identification of a hormonal basis for gallbladder filling. Nat Med 2006; 12:1253-1255.
14. Asada M, Shinomiya M, Suzuki M et al. Glycosaminoglycan affinity of the complete fibroblast growth factor family. Biochim Biophys Acta 2009; 1790:40-48.
15. Goetz R, Beenken A, Ibrahimi OA et al. Molecular insights into the klotho-dependent, endocrine mode of action of fibroblast growth factor 19 subfamily members. Mol Cell Biol 2007; 27:3417-3428.
16. Harmer NJ, Pellegrini L, Chirgadze D et al. The crystal structure of fibroblast growth factor (FGF) 19 reveals novel features of the FGF family and offers a structural basis for its unusual receptor affinity. Biochemistry 2004; 43:629-640.

17. Zhang X, Ibrahimi OA, Olsen SK et al. Receptor specificity of the fibroblast growth factor family. The complete mammalian FGF family. J Biol Chem 2006; 281:15694-15700.
18. Kuro-o M. Endocrine FGFs and Klothos: emerging concepts. Trends Endocrinol Metab 2008; 19:239-245.
19. Kurosu H, Ogawa Y, Miyoshi M et al. Regulation of fibroblast growth factor-23 signaling by klotho. J Biol Chem 2006; 281:6120-6123.
20. Wu X, Ge H, Lemon B et al. Selective activation of FGFR4 by an FGF19 variant does not improve glucose metabolism in ob/ob mice. Proc Natl Acad Sci USA 2009; 106:14379-14384.
21. Tomiyama K, Maeda R, Urakawa I et al. Relevant use of Klotho in FGF19 subfamily signaling system in vivo. Proc Natl Acad Sci USA 2010; 107:1666-1671.
22. Grose R, Dickson C. Fibroblast growth factor signaling in tumorigenesis. Cytokine Growth Factor Rev 2005; 16:179-186.
23. Korc M, Friesel RE. The role of fibroblast growth factors in tumor growth. Curr Cancer Drug Targets 2009; 9:639-651.
24. Fausto N. Mouse liver tumorigenesis: models, mechanisms and relevance to human disease. Semin Liver Dis 1999; 19:243-252.
25. Spangenberg HC, Thimme R, Blum HE. Serum markers of hepatocellular carcinoma. Semin Liver Dis 2006; 26:385-390.
26. Lilien J, Balsamo J. The regulation of cadherin-mediated adhesion by tyrosine phosphorylation/dephosphorylation of beta-catenin. Curr Opin Cell Biol 2005; 17:459-465.
27. Nelson WJ, Nusse R. Convergence of Wnt, beta-catenin, and cadherin pathways. Science 2004; 303:1483-1487.
28. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell 2006; 127:469-480.
29. Peifer M, Polakis P. Wnt signaling in oncogenesis and embryogenesis—a look outside the nucleus. Science 2000; 287:1606-1609.
30. Polakis P. Wnt signaling and cancer. Genes Dev 2000; 14:1837-1851.
31. Morin PJ. beta-catenin signaling and cancer. Bioessays 1999; 21:1021-1030.
32. Wu X, Ge H, Lemon B et al. FGF19-induced hepatocyte proliferation is mediated through FGFR4 activation. J Biol Chem 2010; 285:5165-5170.
33. Ho HK, Pok S, Streit S et al. Fibroblast growth factor receptor 4 regulates proliferation, anti-apoptosis and alpha-fetoprotein secretion during hepatocellular carcinoma progression and represents a potential target for therapeutic intervention. J Hepatol 2009; 50:118-127.
34. Yu C, Wang F, Jin C et al. Increased carbon tetrachloride-induced liver injury and fibrosis in FGFR4-deficient mice. Am J Pathol 2002; 161:2003-2010.
35. Huang X, Yang C, Jin C et al. Resident hepatocyte fibroblast growth factor receptor 4 limits hepatocarcinogenesis. Mol Carcinog 2009; 48:553-562.
36. Jaakkola S, Salmikangas P, Nylund S et al. Amplification of fgfr4 gene in human breast and gynecological cancers. Int J Cancer 1993; 54:378-382.
37. Roidl A, Berger HJ, Kumar S et al. Resistance to chemotherapy is associated with fibroblast growth factor receptor 4 up-regulation. Clin Cancer Res 2009; 15:2058-2066.
38. Bange J, Prechtl D, Cheburkin Y et al. Cancer progression and tumor cell motility are associated with the FGFR4 Arg(388) allele. Cancer Res 2002; 62:840-847.
39. Roidl A, Foo P, Wong W et al. The FGFR4 Y367C mutant is a dominant oncogene in MDA-MB453 breast cancer cells. Oncogene 2010; 29:1543-1552.
40. Spinola M, Leoni V, Pignatiello C et al. Functional FGFR4 Gly388Arg polymorphism predicts prognosis in lung adenocarcinoma patients. J Clin Oncol 2005; 23:7307-7311.
41. Ma Z, Tsuchiya N, Yuasa T et al. Polymorphisms of fibroblast growth factor receptor 4 have association with the development of prostate cancer and benign prostatic hyperplasia and the progression of prostate cancer in a Japanese population. Int J Cancer 2008; 123:2574-2579.
42. Wang J, Stockton DW, Ittmann M. The fibroblast growth factor receptor-4 Arg388 allele is associated with prostate cancer initiation and progression. Clin Cancer Res 2004; 10:6169-6178.
43. Wang J, Yu W, Cai Y et al. Altered fibroblast growth factor receptor 4 stability promotes prostate cancer progression. Neoplasia 2008; 10:847-856.
44. Falvella FS, Frullanti E, Galvan A et al. FGFR4 Gly388Arg polymorphism may affect the clinical stage of patients with lung cancer by modulating the transcriptional profile of normal lung. Int J Cancer 2009; 124:2880-2885.
45. Sasaki H, Okuda K, Kawano O et al. Fibroblast growth factor receptor 4 mutation and polymorphism in Japanese lung cancer. Oncol Rep 2008; 20:1125-1130.
46. Ansell A, Farnebo L, Grenman R et al. Polymorphism of FGFR4 in cancer development and sensitivity to cisplatin and radiation in head and neck cancer. Oral Oncol 2009; 45:23-29.
47. da Costa Andrade VC, Parise O, Jr., Hors CP et al. The fibroblast growth factor receptor 4 (FGFR4) Arg388 allele correlates with survival in head and neck squamous cell carcinoma. Exp Mol Pathol 2007; 82:53-57.
48. Streit S, Bange J, Fichtner A et al. Involvement of the FGFR4 Arg388 allele in head and neck squamous cell carcinoma. Int J Cancer 2004; 111:213-217.

49. Streit S, Mestel DS, Schmidt M et al. FGFR4 Arg388 allele correlates with tumour thickness and FGFR4 protein expression with survival of melanoma patients. Br J Cancer 2006; 94:1879-1886.
50. Morimoto Y, Ozaki T, Ouchida M et al. Single nucleotide polymorphism in fibroblast growth factor receptor 4 at codon 388 is associated with prognosis in high-grade soft tissue sarcoma. Cancer 2003; 98:2245-2250.
51. Jezequel P, Campion L, Joalland MP et al. G388R mutation of the FGFR4 gene is not relevant to breast cancer prognosis. Br J Cancer 2004; 90:189-193.
52. Mawrin C, Kirches E, Diete S et al. Analysis of a single nucleotide polymorphism in codon 388 of the FGFR4 gene in malignant gliomas. Cancer Lett 2006; 239:239-245.
53. Yang YC, Lu ML, Rao JY et al. Joint association of polymorphism of the FGFR4 gene and mutation TP53 gene with bladder cancer prognosis. Br J Cancer 2006; 95:1455-1458.
54. Matakidou A, El Galta R, Rudd MF et al. Further observations on the relationship between the FGFR4 Gly388Arg polymorphism and lung cancer prognosis. Br J Cancer 2007; 96:1904-1907.
55. Nan H, Qureshi AA, Hunter DJ et al. Genetic variants in FGFR2 and FGFR4 genes and skin cancer risk in the Nurses' Health Study. BMC Cancer 2009; 9:172.
56. Liu H, Fergusson MM, Castilho RM et al. Augmented Wnt signaling in a mammalian model of accelerated aging. Science 2007; 317:803-806.
57. Engstrom W, Granerus M. Effects of fibroblast growth factors 19 and 20 on cell multiplication and locomotion in a human embryonal carcinoma cell line (Tera-2) in vitro. Anticancer Res 2006; 26:3307-3310.
58. Miyake A, Nakayama Y, Konishi M et al. Fgf19 regulated by Hh signaling is required for zebrafish forebrain development. Dev Biol 2005; 288:259-275.
59. Siffroi-Fernandez S, Felder-Schmittbuhl MP, Khanna H et al. FGF19 exhibits neuroprotective effects on adult mammalian photoreceptors in vitro. Invest Ophthalmol Vis Sci 2008; 49:1696-1704.
60. Thussbas C, Nahrig J, Streit S et al. FGFR4 Arg388 allele is associated with resistance to adjuvant therapy in primary breast cancer. J Clin Oncol 2006; 24:3747-3755.