Chronic elevation of plasma fibroblast growth factor 19 in long-term farnesoid X receptor agonist therapy, a happy marriage or cause for oncological concern?

Chronic elevation of plasma FGF19 in long-term FXR agonist therapy, a happy marriage or cause for oncologic concern?

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An estimated 782,000 cases of primary liver cancer were diagnosed globally in 2012, making it the fifth and ninth most frequent neoplasm in males and females, respectively. Liver cancer is the second leading cause of cancer-related mortality worldwide, and estimated to account for 746,000 deaths in 2012[1]. Hepatocellular carcinoma (HCC) represents the majority of liver cancer cases, and typically develops on a background of chronic liver disease. The main risk factor for development of HCC is cirrhosis due to alcohol abuse or chronic infection with HBV or HCV. Non-alcoholic fatty liver disease (NAFLD) is already the most common chronic liver disease in developed countries and its incidence is increasing. The progressive form non-alcoholic steatohepatitis (NASH) is considered an important cause of cryptogenic cirrhosis. NAFLD-associated HCC can however develop in the absence of cirrhosis, and is expected to become more frequent as a consequence of the global obesity epidemic[2].

At present, there is no approved pharmaceutical therapy for NAFLD, although new treatment options are in different phases of (pre)clinical testing[3]. One such approach, currently evaluated in phase II/III trials, targets the metabolic and fibro-inflammatory components in NASH and comprises agonistic activation of the Farnesoid-X Receptor (FXR)[4]. FXR is a ligand-activated transcription factor that -along with its target Fibroblast Growth Factor 19 (FGF19)- mediates signaling activities of bile salts. FXR is expressed in the intestines and liver, and is activated in the postprandial state by bile salts in enterohepatic circulation. FXR and ileum-derived FGF19 regulate a number of metabolic processes in the liver (*e.g.* bile salt homeostasis, gluconeogenesis, lipogenesis), and exert an anti-inflammatory action. FXR agonists are being developed by several pharmaceutical companies. The FXR agonist obeticholic acid improved liver histology (reduction of NAFLD activity score) and halted fibrotic progression during a 72-weeks intervention study in patients with biopsy-proven NASH[4]. An initial report indicates that daily subcutaneous administration of an FGF19-based biologic (NGM282) led to a rapid reduction -and is some cases complete normalization- of hepatic fat content in patients with NASH[5].

Successful therapy for NAFLD is expected to have several health benefits including the prevention of NAFLD-associated HCC. However, at present it is unclear if chronic elevation of circulating FGF19 in patients treated for long periods with an FXR agonist, poses an oncological risk. The tumorigenic potential of FGF19 was first uncovered in 2002, when it was shown that overexpression of FGF19 in mice results in HCC, with mice of either gender developing one or multiple liver tumors after 10 months of age. FGF19-induced hepatocarcinogenesis appears to be accelerated on a background of fatty or cholestatic liver disease[6]. Of note, FGF19 does not occur naturally in mice, and overexpression of Fgf15 (the endogenous murine equivalent of FGF19) does

not induce tumor formation in normal or diseased liver[6]. In fact, Fgf15 is held responsible for the prevention of liver tumor formation in mice that lack *Fxr*[7]. In the absence of Fxr, deranged bile salt homeostasis leads to a chronic inflammatory environment in which liver tumors develop after >15 months. In this background of complete genetic *Fxr* deficiency, re-expression of constitutively active Fxr in the intestines, which results in upregulation of ileal *Fgf15*, is sufficient to prevent hepatocarcinogenesis. It is currently unknown whether intestinal Fxr/Fgf15 signaling is protective in other experimental models of HCC. Hepatic Fxr regulates expression of multiple tumor suppressor genes, and agonistic activation of Fxr reduces growth of liver tumors in xenografted nude mice[8]. Potential beneficial actions of FXR agonists on human tumor biology are unexplored.

The mechanism behind the tumor-inducing effect of FGF19 has remained obscure for a long period of time, with little more known than a requirement for FGF Receptor 4 (FGFR4). Using an elegant series of experiments, Zhou and colleagues fill this knowledge gap, and address the relevance of the unraveled pathway for human HCC[9]. Zhou *et al.* observed that FGF19-induced hepatocarcinogenesis depends on the presence of Stat3 in hepatocytes. FGF19 induces phosphorylation of this transcription factor, resulting in its activation and enhanced transcription of genes involved in proliferation and cell survival. Intriguingly, this is not a direct effect but depends on the presence of non-parenchymal liver cells. Screening for a number of cytokines and growth factors known to cause Stat3 activation, led to the identification of Interleukin-6 (Il6) as the mediator of FGF19-driven HCC formation. This is supported by lack of FGF19-induced tumorigenesis in mice nullizygous for *Il6*, and lack of tumors in (wild-type) mice treated with anti-Il6 antibodies. Further delineation of the cellular/molecular events led Zhou *et al.* to propose the following model for FGF19-driven hepatocarcinogenesis. FGF19 induces *Il6* expression in innate immune cells of the liver. Released Il6 activates the gp130 receptor on the cell surface of hepatocytes, leading to recruitment of the effector kinase Jak that in turn phosphorylates and activates Stat3. The importance of the Il6/Jak/Stat3 axis in FGF19-mediated tumorigenesis was studied in clinically relevant models of chronic liver disease. *db/db* mice served as model for NAFLD, and *Mdr2* knock-out mice as model of primary sclerosing cholangitis (PSC). The pan-JAK inhibitor tofacitinib (approved for treatment of rheumatoid arthritis) prevents FGF19-driven HCC in *db/db* mice, and anti-Il6 antibodies block tumor formation in mice lacking *Mdr2,* with additional improvement of cholestatic liver tests and liver histology in the PSC model.

The genomic and mutational landscape in human HCC is heterogeneous. Amplification of a gene cluster (*CCND1-ORAOV1-FGF19-FGF4-FGF3*) on chromosome 11q13.3 that includes *FGF19* is observed in up to 14% of HCC cases, as well as in a variety of other human cancers including

cholangiocarcinoma[9,10]. *FGF19* gene amplification appears mutually exclusive with inactivating mutations in the tumor suppressor gene *TP53*[9]. In HCC, *FGF19* gene amplification is accompanied by increased *FGF19* expression[9,10], and *in vitro* studies indicate that *FGF19* is an important driver gene in HCC[10]. FGF19-positive HCC is associated with a more aggressive tumor type and poor prognosis. Although these observations do not prove a causal relation between FGF19 and HCC formation in humans, it is conceivable that autocrine or paracrine FGF19 signaling via the FGFR4/βKlotho receptor complex provides a mitogenic signal in a subset of human HCCs. Zhou *et al.* found no evidence for a direct mitogenic effect of FGF19 in isolated human hepatocytes, but noted elevated expression of STAT3 target genes in FGF19-positive human HCCs in comparison with FGF19-negative HCCs or control livers[9]. This human data is in support of the concept of Zhou and colleagues, that FGF19 promotes HCC in a non-cell autonomous manner.

The observation that exogenous human FGF19 induces HCC in mice while endogenous murine Fgf15 is devoid of such activity, raises questions on the relevance of FGF19 in human hepatocarcinogenesis. This doubt was further fostered by the supraphysiological levels (>20 ng/mL) of FGF19 in the systemic circulation that were attained in mice with transgenic or adenoviral overexpression of *FGF19*. However, in a recent study it was demonstrated that FGF19 levels as low as 0.6 ng/mL induce liver tumors in diabetic *db/db* mice[9]. Although a direct comparison is not substantiated, these levels are in the range that is observed in patients with cholestasis and in patients treated with FXR agonist. Hence, the study of Zhou *et al*. asks for close monitoring of patients receiving long-term therapy with agents that cause systemic elevation of FGF19, such as FXR agonists. Surveillance screening for HCC by frequent ultrasonography, as already implemented in the management of at-risk patients[11], may be considered in future clinical trials of FXR agonists. A further merit of the study of Zhou *et al*. is that it provides a rationale for personalized treatment of FGF19-positive liver tumors with compounds that target the IL6/IL6R/JAK axis.

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