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# Pleiotropic roles of FXR in liver and colorectal cancers

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## Abstract

Nuclear receptor farnesoid X receptor (FXR) is generally considered a cell protector of enterohepatic tissues and a suppressor of liver cancer and colorectal carcinoma (CRC). Loss or reduction of FXR expression occurs during carcinogenesis, and the FXR level is inversely associated with the aggressive behaviors of the malignancy. Global deletion of FXR and tissue-specific deletion of FXR display distinct effects on tumorigenesis. Epigenetic silencing and inflammatory context are two main contributors to impaired FXR expression and activity. FXR exerts its antitumorigenic function via the following mechanisms: 1) FXR regulates multiple metabolic processes, notably bile acid homeostasis; 2) FXR antagonizes hepatic and enteric inflammation; 3) FXR impedes aberrant activation of some cancer-related pathways; and 4) FXR downregulates a number of oncogenes while upregulating some tumor suppressor genes. Restoring FXR functions via its agonists provides a therapeutic approach for patients with liver cancer and CRC. However, an in-depth understanding of the species-specific pharmacological effects is a prerequisite for assessing the clinical safety and efficacy of FXR agonists in human cancer treatment.

### Keywords

FXR; Bile acid; liver cancer; colorectal cancer

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## 1. Introduction

Nuclear receptor farnesoid X receptor (FXR) was originally cloned by Seol W et al. and Forman BM et al. [1, 2] in 1995. Four years later, FXR was deorphanized when bile acids were identified as its bona fide physiological ligands[3–5]. Since then, in addition to being a key "metabolic nuclear receptor" to maintain the homeostasis of enterohepatic circulation of bile acids[6–8], multiple functions of FXR have been revealed, among which increasing attention is given to the roles of FXR in cancer [9–16].

The structural and genetic analysis of FXR[17] and its pathophysiological function in metabolic diseases, such as cholestasis, nonalcoholic fatty liver disease (NAFLD) and diabetes, have been well described in many excellent review articles [6, 8, 18, 19]. In this review, we will focus on the emerging roles of FXR in cancer, particularly tumorigenesis of the liver and colon, because of the abundant expression levels of FXR in these two organs, where FXR acts as a master regulator of multiple metabolic processes, notably bile acid homeostasis[20–26], as well as an important modulator of hepatic and intestinal inflammation and the immune response[27–30]. The potential underlying molecular mechanisms of FXR in cancer will be summarized. The advantages and possible pitfalls of FXR as a target of cancer treatment will also be discussed in this review.

## 2. FXR and liver cancer

### 2.1. FXR downregulation in liver cancer

Hepatocellular carcinoma (HCC) is the most common type of primary liver carcinoma, ranking as the sixth most prevalent type of cancer and the third leading cause of cancer-related death[31]. The high mortality of HCC is partly attributed to the lack of good biomarkers for early diagnosis and treatment response. Metabolic disorders, including bile acid-induced liver injury and NAFLD, are becoming the causative liver diseases of HCC [9, 31, 32]. As a multifaceted metabolic regulator, FXR represents a bridge between metabolic defects and hepatocarcinogenesis [9]. Despite the controversial debate in terms of the negative[33–35] or positive[36] association between hepatic FXR expression level and HCC progression, increasing evidence supports that FXR signaling is compromised during the pathogenetic process of HCC, especially in the advanced stages of this devastating malignancy.

In 2007, two reports simultaneously demonstrated that global FXR null ($FXR^{-/-}$) mice developed spontaneous liver tumors when aged[9, 37]. Later, to investigate whether there exists similarity between aged $FXR^{-/-}$ mice and HCC human patients, a systematic analysis of $FXR^{-/-}$ mice was performed [34]. The results suggest that hepatocarcinogenesis in $FXR^{-/-}$ mice can largely mimic the progress of human HCC development[34]. As expected, FXR expression levels were decreased in human HCC specimens compared to normal liver tissues[34]. In the same year, Su et al. published the results of their clinical investigation that downregulation of FXR was associated with multiple malignant clinicopathological features in human HCC[33]. Concurrent reduction of another nuclear receptor, small heterodimer partner (SHP, NR0B2), which is a primary target gene of FXR [38], was observed in HCC tissues in this study[33]. A number of reports suggest that SHP may act as a tumor

suppressor of HCC [39–41] in addition to its original function as a suppressor of bile acid synthesis[38].

Although growing experimental data from other labs provide compelling evidence that FXR expression is lost during the development of HCC[42–45], the potential underlying molecular mechanisms accounting for the loss of FXR expression during hepatic tumorigenesis remain elusive. Some evidence indicates that aberrant epigenetic regulation may be a key contributor to diminished hepatic FXR gene transcription. Minle Li et al. [46] observed that the metabolic enzyme transketolase (TKT), which is upregulated in HCC, inhibited FXR promoter activity in HCC cells by facilitating the binding of histone deacetylase 3 (HDAC3) to the FXR promoter, eventually leading to suppressed FXR expression.

Other potential players in FXR regulation are microRNAs (miRs). MiRNAs are noncoding short RNAs capable of degrading their target mRNAs and leading to the silencing of tumor suppressor genes [47]. Two studies investigating the role of miR-421 in the inhibition of FXR transcription were published in 2012[48]. In HCC cells, miR-421 reduces FXR transcription by targeting the 3′UTR of FXR mRNA, and the resulting downregulation of FXR promotes the proliferation and migration of HCC cells. Working through a similar mechanism, miR-421 contributes to the decreased FXR protein levels in biliary tract cancer (BTC) cells and acts as an oncogenic miRNA in the development of BTC[49]. Recently, in human colonic adenocarcinoma, a significant negative association between the expression levels of FXR and miR-192 was also observed[50]. In Huh-7 HCC cells and Caco-2 colon adenocarcinoma cells, miR-192-dependent suppression of endogenous FXR mRNA and protein expression was observed[50]. As expected, the 3′UTR of FXR mRNA was shown to contain the binding site of miR-192[50].

FXR expression is downregulated when hepatic inflammation occurs. In both $FXR^{-/-}$ mice[9, 37] and in human HCC patients[34, 51], liver proinflammatory cytokines, including TNFα, IL-1β, and IL-6, are upregulated, thereby attenuating the binding of hepatic nuclear factor 1α (HNF1α) to the FXR promoter, which results in the reduction of FXR expression in HCC tissues[34]. Taken together, both the inflammatory microenvironment and aberrant epigenetic regulation may contribute to the reduced expression and activity of FXR during liver tumorigenesis.

### 2.2. FXR, bile acid synthesis and liver cancer

Bile acids are a group of amphipathic steroids with structural diversity[52, 53]. They are both physiological detergents indispensable to dietary lipid absorption [17] and signaling molecules implicated in distinct pathways relevant to metabolic regulation and carcinogenesis [53, 54]. Therefore, the levels and composition of bile acids are tightly controlled to prevent aberrant bile acid levels. Abnormally high levels of bile acids exhibit systemic inflammatory, cytotoxic and protumorigenic effects [55, 56].

FXR is highly expressed in the liver and intestine[57], where it acts as a major endogenous bile acid sensor and regulator [53, 58] by regulating the transcriptional responses of genes involved in bile acid synthesis and enterohepatic circulation [6, 8, 20, 59]. To maintain

bile acids within the physiological concentration in the liver, FXR finely controls powerful negative feedback of bile acid synthesis through hepatic SHP [38] and enteric endocrine hormone fibroblast growth factor 15/19 (FGF15/19, mouse FGF15 or human homolog FGF19) [55]. In the liver, SHP and FGF15/19 mediate different regulatory cascades to suppress the expression of cholesterol 7α-hydroxylase (CYP7A1), a rate-limiting enzyme of the classic route of bile acid synthesis [14, 38, 55, 60].

One of the principal culprits of FXR inactivation is the alteration in bile acid pool size. Hepatic chronic accumulation of bile acids in $FXR^{-/-}$ mice is identified as a vital contributor to liver tumor formation when mice age [9, 37]. Elevated bile acid levels in 0.2% cholic acid-fed mice significantly facilitate N-nitrosodiethylamine-initiated HCC formation [9]. In contrast, spontaneous malignant foci are significantly diminished when $FXR^{-/-}$ mice are fed 2% cholestyramine food to lower bile acid pool [9]. Moreover, a panel of FXR target genes, including SHP and CYP7A1, is dysregulated in $FXR^{-/-}$ mice with age [9, 37]. Long-standing hepatic bile acid overload due to the deletion of FXR results in chronic liver inflammation, aberrant death and compensatory proliferation of hepatocytes, which leads to eventual hepatic carcinoma [9, 37].

Although SHP is a well-known downstream gene of FXR, FXR is not uniquely epistatic to SHP in the feedback inhibition of Cyp7A1[55, 61]. FXR and SHP may act synergistically to tightly control the systemic bile acid level. Mice with double-knockout of FXR and SHP ($FXR^{-/-}SHP^{-/-}$) exhibit more severe liver damage and earlier onset of cholestasis than those in either FXR or SHP single knockout mice [61]. Consistent with this study, Yes-associated protein (YAP), a core molecular component of the Hippo pathway that functions as a promoter during hepatocarcinogenesis[62], was strongly activated in $FXR^{-/-}SHP^{-/-}$ mice by dramatically higher concentrations of bile acids. On the other hand, YAP activation has not been found in $Fxr^{-/-}$ or $Shp^{-/-}$ mice because the bile acid level has not reached a threshold value in these animals[62]. Consistently, Ji Su et al. also observed the reciprocity between bile acids and Yap[63]. They demonstrated the role of the FXR-FGF15/19-Hippo pathway in the regulation of bile acid synthesis during liver tumorigenesis[63].

In addition to the inhibitory roles of bile acid synthesis[55, 64], the bile acid-FXR-FGF15/19 gut-liver axis has beneficial effects on regulating diverse enterohepatic responses, including carbohydrate, lipid and protein homeostasis [60, 65, 66]. Therefore, this pathway provides new targets for the pharmacological treatment of bile acid-related diseases or other metabolic disorders[60, 64, 65]. Although clinical trials of FXR agonists and FGF19 analogs are currently underway [60, 65], accumulating evidence from animal investigations suggests that chronic exposure to FGF19 is implicated in driving liver cancer formation [14, 66–70]. Therefore, developing variants of FGF19 that uncouple the benefit of correcting metabolic dysregulation from liver tumorigenic potential is challenging. In recent studies, novel FGF19 analogs, including M70[68] and M52[71], display noncarcinogenetic features while retaining the function of regulating bile acid metabolism [68, 71]. Experimental data from Zhou M et al. indicate that human FGF19 and mouse FGF15 exhibit markedly different biological effects, including tumorigenicity, in three mouse models[66], which may raise the concern of clinical safety assessment of FXR agonists counting on rodent models but ignore the species-specific characteristics of FGF19 and FGF15[66]. Taken together, selectively

targeting the FXR-FGF19 axis provides a pharmacological option for patients with chronic liver diseases or liver malignancy [68]. In-depth knowledge of the mechanisms underlying the bile acid-induced liver-gut crosstalk of FXR modulation is important to develop new drugs with both efficacy and safety[66].

### 2.3. Tissue-specific FXR modulation and liver cancer

With the technological advances of tissue-specific gene deletion, interest in exploring the influence of either hepatocyte- or enterocyte-specific FXR deficiency on carcinogenesis has surged. Shogo T et al. observed that in aged $FXR^{-/-}$ mice, a potential mechanism of hepatocarcinogenesis is the elevated taurocholate (TCA)-induced overexpression of the oncogene Myc [72], a common occurrence during HCC development[73]. FXR deletion in either hepatocytes or enterocytes alone is unable to significantly increase TCA, thus leading to a much lower incidence of spontaneous age-dependent hepatic tumors than global FXR deficiency[72]. Consistent with this study, experimental data from Kong B et al. found that mice with unique hepatocyte-specific FXR deletion did not spontaneously develop liver tumors as they aged but were susceptible to cholic acid-induced hepatocarcinogenesis. Both studies indicate that singular silencing of FXR in hepatocytes is insufficient to strongly disturb bile acid homeostasis or facilitate liver tumor formation. The synergistic effect of a lack of hepatocyte FXR and elevated bile acid levels is required for dysregulated cell proliferation and subsequent liver tumorigenesis[72, 74]. On the other hand, in $FXR^{-/-}$ mice, intestinal-specific reactivation of FXR is able to restore bile acid homeostasis via FGF15-Cyp7A1 signaling and prevent bile acid overload-mediated liver damage and age-related hepatic tumors[75]. Collectively, further understanding the mechanisms of the tissue-specific pattern of FXR regulation will be very helpful for the discovery of tissue-selective FXR modulators to impede the progression of hepatic malignancy, which is associated with FXR inactivation.

### 2.4. FXR, bile acid transporters and liver cancer

In addition to the repression of bile acid synthesis, FXR also participates in regulating other aspects of bile acid enterohepatic circulation. In the liver, bile acid-dependent FXR activation induces the expression of the transporter bile salt export protein (BSEP; encoded by ABCB11) to promote the efflux of bile acids into the biliary canaliculi[15, 76]. Two FXR genes are named FXRα (NR1H4, referred to as FXR in this paper) and FXRβ (NR1H5)[6]. In humans, FXRβ is a pseudogene[77], while FXRα has four isoforms in both humans and mice[6]. FXRα1 and FXRα2 are two main human hepatic FXR isoforms[78]. Alterations in the relative ratio of the FXR isoforms have a significant impact on the transcription of some of the FXR target genes [79]. For example, human BSEP was induced by FXR in an isoform-specific manner. FXR-α2 shows a much higher capability than FXR-α1 in transactivating BSEP both *in vitro* and *in vivo*. BSEP deficiency may facilitate severe cholestasis and HCC in young children[80, 81]. Loss of BSEP expression in human HCC tissues is associated with increased FXR-α1/FXR-α2 ratios due to inflammation in the liver[51]. Restoration of BSEP expression to reestablish bile acid homeostasis by suppressing chronic hepatic inflammation may provide another potential therapy for HCC[51].

### 2.5. FXR and cholangiocarcinoma (CCA)

Cholangiocarcinoma (CCA) represents the second most frequent primary liver carcinoma, originating from cholangiocytes and characterizing aggressive behavior and poor prognosis[82]. FXR is downregulated in human CCA specimens [83, 84] and CCA cells [84, 85]. The FXR agonists obeticholic acid (OCA) and GW4064 display inhibitory functions on CCA cell proliferation both *in vitro* and *in vivo*[83–85]. Intrahepatic accumulation of bile acids in bile duct-ligated rats is incapable of initiating tumor formation but shows a cocarcinogenic effect via enhanced bile duct proliferation, increased inflammation, and impaired FXR-dependent activity during cholangiocarcinoma development induced by thioacetamide metabolites [86]. In addition to persistently high bile acid concentrations, abnormal bile acid compositions, for example, imbalanced proportions of free and conjugated bile acids, also favor the growth of human cholangiocarcinoma in an FXR-dependent manner[83]. Together, these results indicate that during liver tumorigenesis, bile acid may function as a tumor promoter. Further clinical research is required to determine the role of bile acid-FXR signaling in human cholangiocarcinoma.

In summary, FXR acts as a primary regulator of bile acid homeostasis in enterohepatic tissues. By alternating its target genes involved in bile acid circulation and metabolism, FXR maintains the bile acid concentration and composition appropriate for lipid absorption while simultaneously limiting bile acid toxicity. Loss of FXR activity will result in aberrant bile acid levels and subsequent protumorigenicity due to increased inflammation and cell proliferation. Restoring FXR function via its agonists or exogeneous FXR expression may impede the process of liver cancer development.

### 2.6. FXR, hepatic inflammation and liver cancer

Chronic liver diseases caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, metabolic disorders such as obesity-associated NAFLD, and other etiologic factors are characteristic of unresolved hepatic inflammation, which is closely associated with the development of HCC [31, 87]. Experimental data support the anti-inflammatory effect of FXR in the liver. For example, FXR is able to alleviate the injury of hepatitis induced by con-canavalin A [88] and LPS[27]. Moreover, persistent activation of signal transducer and activator of transcription 3 (STAT3), a crucial mediator of tumor-promoting inflammation[89, 90], is observed in $FXR^{-/-}$ mouse livers[91]. High levels of bile acids due to FXR deletion give rise to upregulation of the proinflammatory cytokine IL-6, a strong inducer of STAT3[89], and downregulation of suppressor of cytokine signaling 3 (SOCS3), a direct target gene of FXR[92] and a feedback inhibitor of STAT3, which collectively lead to the constitutive activation of STAT3[91]. In parallel, the FXR agonist OCA inhibits the proliferation and metastasis of HCC cells by blocking the IL-6/STAT3 signaling pathway[93]. On the other hand, IFN$\gamma$ is an upregulated proinflammatory cytokine in $FXR^{-/-}$ livers and retards the process of hepatocarcinogenesis by preventing STAT3 activation and inducing p53 expression[94].

The transcription factor nuclear factor-$\kappa$B (NF-$\kappa$B) is a critical participator during the disease progression of hepatic inflammation, fibrosis and HCC. It is considered a promoter of liver carcinogenesis[95]. Wang et al. showed that there is a reciprocal inhibition between

FXR and the NF-κB signaling pathway. Activation of FXR specifically inhibits the NF-κB-induced inflammatory response by reducing the binding of NF-κB to its DNA binding sequences. On the other hand, the transcriptional activity of FXR is antagonized by LPS-induced NF-κB activation[27]. Both NF-κB and STAT3 are important regulators of hepatic inflammation and cancer. They may cooperate and crosstalk to create a dangerous liaison that drives the development of liver cancer [96].

Chronic infection with HBV is one of the major risk factors for HCC and is largely responsible for the high morbidity and mortality of this deadly malignancy[31]. It is well established that hepatitis B virus X protein (HBx) is a modulator of HBV-relevant HCC, although the pathogenic mechanism remains controversial[97, 98]. Random HBV genome integration may result in truncations of the HBx protein at the C-terminus. A recent report raises an interesting concept that FXR and full-length HBX interact to prevent HCC development[97, 98]. One study showed that full-length HBx, rather than truncated HBx, facilitates FXR binding to its response element and enhances FXR transcriptional induction of its target genes[97, 98]. Full-length HBX-FXR signaling may serve as a liver protector by inhibiting hepatic inflammation and tumorigenesis[97, 98]. Conversely, a lack of FXR drives HBX-induced HCC development[98].

Together, these new findings suggest that FXR may execute its anti-inflammatory properties by antagonizing the NF-κB and STAT-3 pathways or by interacting with full-length HBX, thus preventing the progression of inflammation-associated HCC.

### 2.7. FXR- regulated signaling pathways and liver cancer

The Wnt/β-catenin pathway participates in diverse biological processes and cancer pathogenesis in the liver[99]. Aberrant activation of this signaling pathway has been observed in the majority of HCC patients and is positively associated with HCC metastasis and poor prognosis[100, 101]. In $FXR^{-/-}$ mice, persistent activation of the Wnt/β-catenin pathway and a concomitant decrease in E-catenin are relevant to spontaneous HCC [42]. By impeding the nuclear translocation of β-catenin, FXR is capable of impairing the migration and invasion of the liver cancer cell line SK-Hep-1[102]. In HCC cells, FXR blocks β-Catenin/TCF4 complex formation by direct interaction with β-Catenin, which results in decreased cyclin D1 expression [43]. Therefore, inhibition of the Wnt/β-catenin pathway is one of the possible mechanisms through which FXR exerts its anti-HCC function [43].

Both FXR agonists and exogenous FXR overexpression repress *in vitro* liver cancer cell proliferation and *in vivo* xenograft growth[33, 103]. The mammalian target of rapamycin (mTOR) is a well-conserved serine/threonine kinase implicated in the signaling network relevant to cancer metabolism and cell growth [104]. FXR prolongs the G0/G1 phase of liver cancer cells potentially via the suppression of mTOR/S6K signaling[103]. A recent study suggests that FXR is involved in the regulation of liver cancer stem cells (CSCs). Activation of FXR retards the expression and activity of Notch1, a critical regulator of liver CSCs, and directs asymmetrical cell division of $Sox9^{+}$ cells to impede liver cancer development[105].

The c-Jun N-terminal kinase (JNK) pathway is constitutively activated in most HCCs[106]. FXR activation reduces reactive oxygen species (ROS) production by transcriptional

induction of superoxide dismutase 3 (SOD3), an antioxidant defense enzyme, leading to reduced JNK activity in liver carcinogenesis[107]. In carbon tetrachloride (CCl4)-treated $FXR^{-/-}$ mice, bile acid TCA directly amplifies acute liver injury mainly mediated by JNK in FXR-deficient mice[108].

The antitumor efficacy of FXR may also be mediated by modulation of tumor suppressor genes. N-myc downstream-regulated gene 2 (NDRG2) is regarded as a suppressor of HCC metastasis [109]. NDRG2 mRNA is diminished in the liver of human HCC and $FXR^{-/-}$ mice[45]. FXR activation or ectopic overexpression induces NDRG2 expression by binding to the FXR response element of the NDRG2 gene, resulting in inhibition of liver tumor growth and metastasis [45, 110]. Histidine-rich glycoprotein (HRG) is an abundant plasma protein derived from the liver and is regarded as a tumor suppressor[111]. HRG is a transcriptional target gene of FXR, and HRG expression is reduced in human HCC. Administration of the FXR agonist PX20606 increases plasma HRG levels, which might be beneficial to HCC patients [111].

Neutralization of tumor suppressor proteins (TSPs), including Rb, p53, hepatocyte nuclear Factor 4a (HNF4a), and CCAAT/enhancer binding protein (C/EBP)α, is one of the early events of hepatocarcinogenesis[44]. This process is mediated by the oncoprotein gankyrin, a small subunit of the proteasome[44]. FXR exerts an antitumorigenic effect by epigenetically silencing the gankyrin promoter, which consequently prevents tumor suppressor proteins from degrading[44]. In contrast, by triggering the degradation of the abovementioned four TSPs, gankyrin is implicated in the inhibition of differentiation of hepatic stem cells, an etiology of hepatoblastoma[112]. Valanejad L et al. suggested that the FXR-Gank-TSPs-Stem cells pathway is a key determinant in DEN-initiated mouse liver cancer and in pediatric hepatoblastoma[112].

In summary, FXR displays a variety of antihepatocarcinogenetic effects mainly in the following ways: 1) FXR maintains bile acid homeostasis by regulating bile acid synthesis and circulation; 2) FXR suppresses liver inflammation by antagonizing NF-κB and STAT-3 signaling or by interacting with full-length HBX; 3) FXR impedes several oncogenic pathways, including Wnt/β-catenin, mTOR/S6K, Notch and JNK; and 4) FXR downregulates the oncogene gankyrin but upregulates the tumor suppressor genes NDRG2 and HRG (Fig. 1).

## 3. FXR and colorectal carcinomas (CRC)

Colorectal cancer (CRC) ranks as the fourth most deadly cancer globally [113], with the highest morbidity in developed countries, where more people prefer Western diets and lifestyles[113, 114]. Recent studies show that both environmental and hereditary etiologic agents of CRC may converge to FXR [115, 116]. Disruption of intestinal FXR activity is considered a protumorigenic phenotype as the putative protective role of FXR during the development of CRC has been well established [10]. Loss of FXR expression is a primary factor contributing to colorectal FXR inactivation.

### 3.1. FXR downregulation in CRC

In human normal intestinal mucosal tissues, FXR is strongly expressed in well-differentiated surface epithelia[10]. In line with the proximal-distal gradient of declining exposure to bile acid along the colon, FXR is dramatically downregulated from the terminal ileum to the rectosigmoidal junction [117, 118]. The rare occurrence of carcinoma in the ileum and the highest incidence of carcinoma in the distal parts of the colon are negatively correlated with FXR expression levels [118]. Ulcerative colitis (UC) patients with concomitant primary sclerosing cholangitis (PSC) have a higher risk of developing proximal colon neoplasia than UC-only patients, which is in parallel with the lower level of FXR in the proximal colon in UC-PSC patients. [119].

Decreased FXR expression is an early molecular event during human colon cancer development[117]. Compared to the adjacent nonneoplastic mucosa, progressively diminished FXR mRNA and protein are observed in human and murine intestinal adenomas[10, 117, 120] and carcinomas. [117, 120–122]. An inverse correlation is also found between the FXR expression levels and the subsequent neoplastic transformation from low degree dysplasia and high degree dysplasia to adenocarcinoma[119], whereas another report implies that the reduction of FXR level may only occur during the malignant transformation from adenoma to adenocarcinoma[118].

Several studies indicate that FXR abundance in human colon carcinomas is inversely associated with adverse clinical outcomes [118, 121]. Consistent with these studies, the disparity in the FXR expression level is large among colon carcinoma cells with different degrees of behavioral malignancy. For example, undifferentiated SW480 cells lack FXR and display aggressive growth potential, while significant FXR mRNA levels are detected in two less aggressive cell lines, Cao-2 and HT-29[120].

A number of animal models further confirm the relevance between FXR deficiency and intestinal carcinogenesis. In mice with adenomatous polyposis coli (APC) gene silencing mutation (Apc $^{min/+}$) or those treated with the colon chemical carcinogen azoxymethane (AOM)-dextran sulfate sodium (DSS), FXR loss promotes intestinal tumor progression, which leads to early mortality [10]. A study using similar mouse models also provided evidence that FXR deletion demonstrates more susceptibility to the development of intestinal adenocarcinoma [123].

### 3.2. Molecular mechanism of FXR inactivation in CRC

Recent studies aim to explore the mechanical links between FXR silencing and CRC initiation or progression [115, 116, 121, 122, 124]. Long-term exposure to overloaded deleterious bile acids is one of the major risks for CRC [56, 125]; therefore, disturbance of bile acid metabolism resulting from impaired FXR functions has gained significant attention. FXR inactivation not only causes a higher yield of bile acids due to the derepressive effect on hepatic de novo synthesis of bile acids [126] but also loses the transcriptional control of bile acid transporters in the intestine[127]. Genetic instability, epigenetic aberrance, a high-fat diet (HFD) with low fiber and dysbiosis of gut microbiota are important causative factors contributing to disabled intestinal FXR signaling[116].

Mutations within the FXR gene per se have not been clinically identified to illustrate the reduced FXR expression or function in CRC patients[117]. However, loss of FXR expression is observed under conditions of genetic abnormality or with concomitant epigenetic dysregulation. Silencing mutation of the tumor suppressor gene APC, a crucial inhibitor of Wnt signaling [128], occurs early during malignant transformation of colonic mucosa[129, 130]. Approximately 85% of colorectal cancer patients carry mutated APC genes[113]. The Apc $^{min/+}$ mouse is an animal model of human familial adenomatous polyposis (FAP)[10]. FXR expression is robustly decreased in patients with FAP[10, 131]. A study from Selmin O.I. et al. demonstrated that in colonic normal mucosa and tumor tissues of Apc $^{min/+}$ mice as well as in human colon cancer cells, APC deficiency leads to CpG hypermethylation of the FXR promoter and then a reduction in FXR expression [129]. In accord with these data, transcriptional silencing of FXR by promoter methylation in human colon cancer has also been observed [117]. Meanwhile, inhibition of the KRAS mutation-mediated signaling pathway, a well-known oncogenic route of colon cancer, leads to markedly upregulated FXR expression levels[117].

The transcription factor caudal-related homeo-box 2 (CDX2) also participates in the transcriptional regulation of FXR. CDX2 exerts its function by directly binding to the FXR promoter and facilitating FXR expression [131]. Commensurate reductions in FXR and CDX2 resulting from inactivating APC mutations are observed in the tumor tissues of Apc$^{min/+}$ mice and FAP patients[131]. Therefore, modulation of the APC-CDX2-FXR axis could be a potential approach for the inhibition of colonic cancer development[131].

Dietary modulation is another contributor to epigenetic alterations in FXR expression. When weaned male C57BL/6 mice are fed a high-fat diet rich in n-6 linoleic acid, reduced CpG methylation of the FXR promoter and corresponding elevated FXR mRNA are found in the colonic mucosa[132]. Taken together, mutation of CRC-associated key genes and epigenetic dysregulation are two etiologic agents leading to the silencing of intestinal FXR.

### 3.3. HFD, microbial bile acid cometabolism, FXR and CRC

A diet with excessive fat and scarce fiber is considered a major risk factor for CRC[114, 133–135]. Accumulated evidence from animal models or clinical studies has demonstrated that a HFD links dysregulation of gut microbial and bile acid metabolism to the risk of developing CRC[114, 116, 125, 135, 136]. A high intake of dietary fat stimulates de novo hepatic synthesis of bile acids, and more bile acids are secreted into the intestinal lumen [116, 137], resulting in alterations of gut microbiota composition and metabolism[116]. This leads to an increased amount of primary bile acids being deconjugated by ileal microbial bile salt hydrolases (BSH) and delivered to the colon, where they favor the growth of 7α-dehydroxylating bacteria and stimulate their activity to convert more primary bile acids into secondary bile acids[116], mostly deoxycholic acid (DCA) and lithocholic acid (LCA) in humans[116, 137]. Indeed, numerous experimental studies strongly support an etiologic role of DCA in promoting CRC[138, 139], and increased levels of serum or fecal DCA are observed in CRC patients or in populations at high risk of developing CRC[136, 137, 140, 141].

A Western-style diet (WD) is characterized by high fat and a lack of fiber and vitamin D[142]. A long-term study of WD-fed mice depicts the protumorigenic effect of WD on the colonic mucosa[142]. The underlying mechanism is the enhancement of cell proliferation resulting from WD-induced disturbance of colonic bile acid homeostasis, which is due to impaired FXR function rather than decreased FXR expression[142]. Although elevated fecal bile acid concentrations are observed in WD-fed mice, the reduction in intracellular levels of secondary bile acids such as DCA and LCA after WD feeding contributes to decreased FXR activity[142]. In contrast, a recent report showed that under conditions of a high-fat diet and APC mutation, DCA may act as an antagonist of FXR, which induces the proliferation of intestinal cancer stem cells and chromosomal aberrations[115]. In human colon cancer cells, APC deficiency attenuates the stimulation of FXR expression by DCA, which may be attributed to the upregulation of the c-MYC oncogene, a downstream target of the Wnt/β-catenin pathway[129].

### 3.4. FXR, intestinal bile acid transporters and CRC

Efficient enterohepatic circulation is one of the master determinants of the composition and size of the bile acid pool [126, 127]. Approximately 95% of bile acids are resorbed in the distal ileum through apical sodium-dependent bile salt transporter (ASBT, encoded by SLC10A2). Bile acids are then distributed in the cytosol by intestinal bile acid-binding protein (IBABP, encoded by FABP6) and finally exported across the basal membrane for active reuptake by organic solute transporter-α and -β (OSTα/OSTβ, encoded by SLC1A/B)[53, 76]. FXR regulates the intestinal resorption of bile acid by inducing the expression of IBABP and OSTα/OSTβ while inhibiting ASBT[143, 144]. FXR inactivation-dependent dysregulation of these bile acid transporters is one of the primary etiologic factors for abnormal intestinal structure and concentration of bile acids, which are linked to CRC risk[129, 142]. Concurrent reductions in FXR and IBABP are observed in colon tumors of $Apc^{Min/+}$ mice[129]. On the other hand, the robustly lowered expression of IBABP and OSTα/OSTβ is due to retarded FXR function rather than decreased FXR abundance in WD-fed mice [142]. Furthermore, Gottardi et al. displayed the absence of parallel IBABP downregulation with lowered FXR expression in both colorectal tumor tissues and colon cancer cells, which suggests that an FXR-independent pathway, such as the transcription factor liver X receptor (LXR)-mediated pathway, may be involved in the regulation of IBABP expression in colon cancer[120, 145]. Several FXR-independent mechanisms elucidate the downregulation of ASBT in WD-fed mice[142]. Therefore, although FXR plays a crucial role in the transcriptional control of bile acid transporters, other regulators function on these transporters in an FXR-independent manner.

### 3.5. FXR, Wnt/β-catenin pathway and CRC

FXR may exert its inhibitory effect on colonic tumorigenesis by modulating different signaling pathways in addition to the bile acid metabolic routes. Elevated bile acid levels per se are incapable of increasing susceptibility to intestinal tumorigenesis in $FXR^{-/-}$ mice despite the well-known function of bile acids as CRC promoters[10]. However, loss of FXR activity rapid intestinal tumor progression by upregulating Wnt/β-catenin signaling[10]. Both *in vitro* and *in vivo* experimental data support that FXR has a suppressive effect on colon cancer cell behaviors such as growth, invasiveness and metastasis by antagonizing

Wnt/β-catenin signaling [121]. The interaction between FXR and β-catenin leads to impaired assembly of the β-catenin/TCF4 complex and a consequent reduction in β-catenin-regulated genes [121]. Matrix metalloproteinase-7 (MMP7), a collagenase and a target gene of β-catenin [146], is correlated with colon cancer progression[147]. Peng Z et al. observed that FXR transcriptionally represses MMP7 expression by binding to a negative FXR-responsive element in the MMP7 promoter with a consequence of inhibition of proliferation and invasion of colon cancer cells[122].

### 3.6. FXR, inflammation and CRC

Currently, it is well accepted that an inflammatory microenvironment is an essential constituent of various tumors at different stages[148]. Chronic intestinal inflammation is associated with a greater risk of CRC in patients who suffer from inflammatory bowel disease (IBD), including chronic ulcerative colitis and Crohn's disease [148–151]. Disordered mucosal immune function and compromised epithelial barrier-related inflammation are two primary factors contributing to the progression of IBD[151]. As a multifunctional participator in maintaining intestinal health, FXR may act as a modulator of the intestinal innate immune system and counterregulator of intestinal inflammation[29]. The colon of $FXR^{-/-}$ mice displays a proinflammatory phenotype and predisposes to uncontrolled immune reactions[29]. Experimental data from a mouse model of bile duct ligation indicate that FXR plays a role as an intestinal protector by blocking bacterial overgrowth and maintaining the integrity of the epithelial barrier[30]. A recent report demonstrated that nelumal A, a novel FXR agonist, markedly reduces DSS-induced colonic inflammation and slows AOM/DSS-induced colitis-related CRC development due to its suppressive effect on bile acid synthesis, oxidative stress and cell proliferation[124]. Consistent with this study, in mice with chemically induced colitis, activation of FXR by its intestinal agonist INT-747 improves colitis symptoms, impedes epithelial permeability and alleviates goblet cell loss. Mechanistically, FXR curtails hyperactivation of NF-kB signaling, a key etiologic factor of the pathophysiology of IBD[152], therefore reducing proinflammatory cytokine production in the colonic mucosa of colitis mice and in immune cells from IBD patients[28]. Experimental evidence supports mutual antagonism between FXR and NF-κB in the colon[152]. Despite the inhibitory effect of FXR on intestinal inflammation, FXR activation is robustly reduced by NF-κB-induced proinflammatory reactions, which result in impaired suppression of inflammation and eventually accelerate the progression of IBD- and IBD-associated CRC[152]. Taken together, FXR inhibits the development of chronic intestinal inflammation by antagonizing NF-κB signaling, modulating intestinal innate immunity, inhibiting bacterial overgrowth, and protecting the integrity of the epithelial barrier.

In summary, dietary, genetic and microbial factors may converge to have a compounding effect on bile acid metabolism and alter the composition and size of the bile acid pool, resulting in impaired intestinal FXR activity. This in turn aggregates the dysregulation of bile acids, increases inflammation, causes dysbiosis of gut microbiota and dysregulation of cancer-related cell signaling pathways, thereby promoting the malignant transformation or progression of CRC[116] (Fig. 2).

## 4. Conclusions and perspective

FXR is currently viewed as a suppressor of liver cancer and CRC. By modulating the bile acid-induced transcriptional response of its target genes, FXR acts as a protector of enterohepatic tissues through the following potential mechanisms: 1) FXR regulates multiple cellular metabolic pathways, particularly bile acid homeostasis; 2) FXR reduces hepatic and enteric inflammation; 3) FXR inhibits constitutive activation of cancer-related pathways; and 4) FXR silences oncogenes and induces tumor suppressor genes (Table 1). Given space limitations, other beneficial effects of FXR are not described here, which may also play important roles in antitumorigenesis.

A reduction or loss of FXR expression is detected in cancerous tissues, and downregulation of FXR is positively associated with multiple malignant clinicopathological features. Hepatocyte- or enterocyte-specific or global absence of FXR has a distinct influence on carcinogenesis. Epigenetic aberrance contributes significantly to diminished FXR expression. Similarly, an inflammatory microenvironment is responsible for decreased FXR expression and activity.

Restoration of FXR activity by administration of FXR agonists may represent a therapeutic option for patients with liver or colorectal malignancies. However, in-depth knowledge of the pharmacological effects as well as the species variation between humans and animals is important when assessing the clinical safety and efficacy of FXR agonists.

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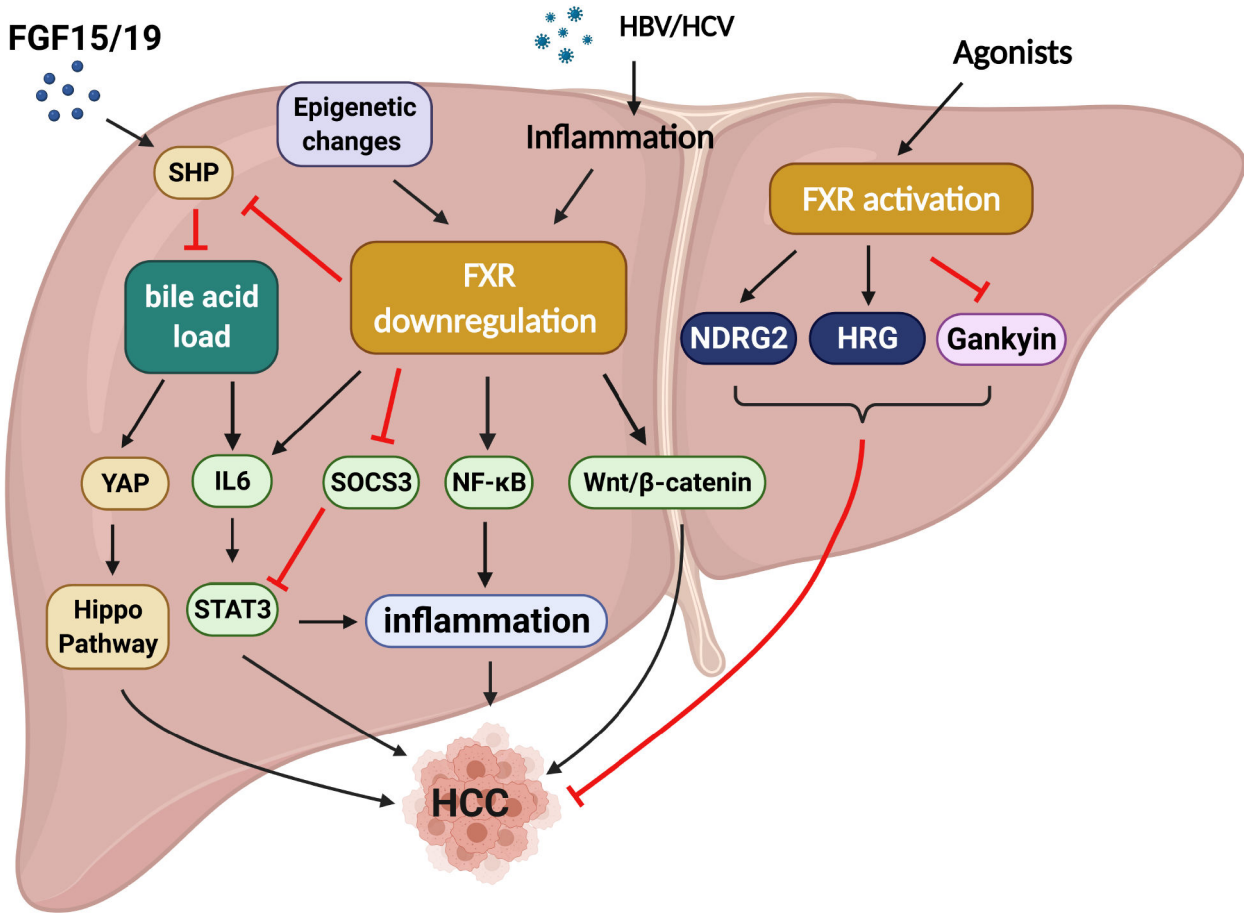


**Fig. 1. FXR and liver cancer.**

Epigenetic silencing and hepatic inflammation are two major contributors to FXR inactivation in the liver. Compromised FXR expression or signaling leads to derepression of bile acid synthesis and decreased bile acid efflux. Elevated bile acid levels give rise to enhanced inflammation and aberrant cellular behaviors. Moreover, impaired FXR function leads to less inhibition of NF-κB- and IL-6/STAT3-mediated hepatic inflammation, which in turn diminishes FXR expression levels. Decreased interaction with full-length HBX is also responsible for FXR inactivation-induced inflammation. Finally, disabled FXR signaling results in loss of control of either oncogenic genes (such as Gankyrin and Wnt/β-catenin) or tumor suppressor genes (such as SOCS3, NDRG2 and HRG). Collectively, FXR downregulation promotes hepatocarcinogenesis by disturbing bile acid metabolism, enhancing inflammation and deregulating cancer-related oncogenic pathways.

Abbreviations: HCC, Hepatocellular carcinoma; FXR, Farnesoid X receptor; FGF15/19, Fibroblast Growth Factor 15/19; SHP, Small Heterodimer Partner; YAP, Yes-associated protein; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; IL-6, Interleukin- 6; STAT3, Signal Transducer and Activator of Transcription 3; SOCS3, Suppressor of Cytokine Signaling 3; NF-κB, Nuclear Factor-κB; NDRG2, N-myc Downstream-Regulated Gene 2; HRG, Histidine-Rich Glycoprotein.

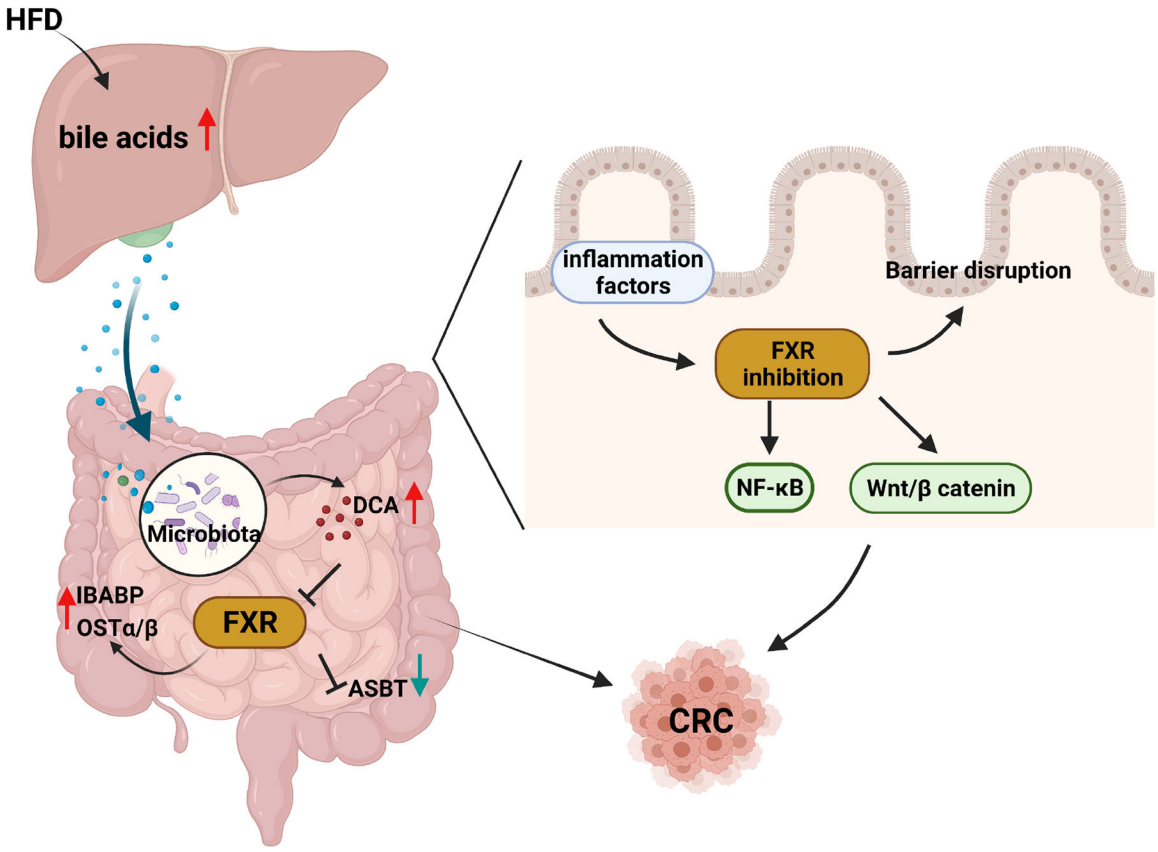


**Figure 2. FXR and Colorectal cancer.**

Gene mutations, epigenetic silencing, inflammation and high-fat dietary intake converge to have a compounding effect on altering the composition and size of the bile acid pool as well as impaired intestinal FXR activity, which in turn aggregates bile acid dysregulation, inflammation, activation of oncogenic signaling pathways and dysbiosis of gut microbiota, thereby promoting the malignant transformation and progression of CRC.

Abbreviations: CRC, Colorectal Cancer; HFD, High-Fat Dietary; DCA, Deoxycholic Acid; IBABP, Intestinal Bile Acid-Binding Protein; OSTα/OSTβ, Organic Solute Transporter-α and -β; ASBT, Apical Sodium-dependent Bile Salt.

**Table 1.**

FXR downstream target genes during the development of HCC and CRC.

| Cancer | Genes | Function | change | References |
|---|---|---|---|---|
| HCC | SHP | inhibit BA synthesis; tumor suppressor | up | [33] , [38] |
| | BSEP | BA efflux; tumor suppressor | up | [51] , [76] |
| | NDRG2 | tumor suppressor | up | [45] |
| | HRG | tumor suppressor | up | [110] |
| | FGF19 | inhibit BA synthesis; tumor promoter | up | [55], [66] |
| | STAT3 | pro-inflammation; tumor promoter | down | [92] |
| | NF-κB | pro-inflammation; tumor promoter | down | [27] |
| | β-catenin | tumor promoter | down | [42] |
| | JNK | tumor promoter | down | [106] |
| | GANKYRIN | tumor promoter | down | [44] |
| | mTOR | tumor promoter | down | [102] |
| | Notch1 | tumor promoter | down | [104] |
| CRC | NF-κB | pro-inflammation; tumor promoter | down | [152] |
| | β-catenin | tumor promoter | down | [120] |
| | MMP7 | tumor promoter | down | [121] |