

## Review

# FXR and liver carcinogenesis

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Farnesoid X receptor (FXR) is a member of the nuclear receptor family and a ligand-modulated transcription factor. In the liver, FXR has been considered a multi-functional cell protector and a tumor suppressor. FXR can suppress liver carcinogenesis via different mechanisms: 1) FXR maintains the normal liver metabolism of bile acids, glucose and lipids; 2) FXR promotes liver regeneration and repair after injury; 3) FXR protects liver cells from death and enhances cell survival; 4) FXR suppresses hepatic inflammation, thereby preventing inflammatory damage; and 5) FXR can directly increase the expression of some tumor-suppressor genes and repress the transcription of several oncogenes. However, inflammation and epigenetic silencing are known to decrease FXR expression during tumorigenesis. The reactivation of FXR function in the liver may be a potential therapeutic approach for patients with liver cancer.

**Keywords:** FXR; bile acid; liver cancer; nonalcoholic fatty liver disease; carcinogenesis; liver regeneration; inflammation

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## Introduction

The farnesoid X receptor (FXR) is a ligand-modulated transcription factor and a member of the nuclear receptor family. FXR was originally cloned by Seol *et al*<sup>[1]</sup> and Forman *et al*<sup>[2]</sup> in 1995, and the subsequent reports from three labs revealed that bile acids (BAs) were endogenous agonists of FXR<sup>[3–5]</sup>. In the following years, FXR was found not only to participate in the regulation of the BA levels<sup>[6,7]</sup> and lipid and glucose metabolism<sup>[8]</sup>, but also play important roles in regulating liver regeneration<sup>[9]</sup>, hepatic fibrosis<sup>[10,11]</sup>, cholestasis<sup>[12]</sup>, hepatic inflammation<sup>[13,14]</sup>, and immune responses<sup>[15–18]</sup>. Therefore, FXR is a multi-functional cell protector in the liver.

In 2007, FXR knockout (FXR<sup>-/-</sup>) mice were found to spontaneously develop liver tumors when they aged<sup>[19,20]</sup>. Interest in the potential function of FXR in cancer surged thereafter. Compared with matched normal tissues, FXR expression is significantly reduced in human tumor specimens<sup>[21–25]</sup>, and the downregulation of FXR is associated with malignant clinicopathological characteristics<sup>[23,26]</sup>. Loss of FXR leads to early mortality and/or promotes intestinal carcinogenesis in the mice with either a mutated APC gene (APC<sup>min/+</sup>) or chronic colitis<sup>[26–28]</sup>. FXR deficiency also facilitates the progression of colorectal adenocarcinoma in C57BL/6 mice treated with the

colon carcinogen azoxymethane<sup>[28]</sup>. A gain-of-function study using a xenograft mouse model showed that overexpression of FXR or treatment with FXR agonists represses cancer cell proliferation *in vitro* and xenograft growth in nude mice *in vivo*<sup>[23,27,29]</sup>. Interestingly, long-lived little mice have high levels of FXR and do not develop liver cancer after treatment with the chemical carcinogen diethylnitrosamine (DEN)<sup>[24]</sup>. These studies strongly suggest that FXR is a tumor suppressor and acts as an intriguing bridge between metabolic regulation and tumor development. The structure of FXR and the roles of FXR in the regulation of various metabolic processes have been previously described in detail<sup>[8,30–34]</sup>; here, we will summarize the findings related to the roles of FXR in liver carcinogenesis, which remains a major burden of cancer morbidity around the world.

## FXR, bile acid and liver cancer

Our understanding of the role of BAs has evolved from physiological detergents essential for lipid absorption to hormone-like signaling molecules<sup>[30,31]</sup>. Excessive accumulation of BAs has a cytotoxic effect and is considered an important etiology of tumorigenesis<sup>[35]</sup>. As an endogenous BA sensor, FXR is abundantly expressed in tissues participating in the BA enterohepatic circulation, such as the liver and lower digestive tract<sup>[31]</sup>. The primary roles of FXR are to maintain BA homeostasis and prevent BA-induced toxicity by eliciting transcriptional alterations of genes involved in BA synthesis, transpor-

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tation, conjugation and detoxification<sup>[36]</sup>.

FXR tightly controls BA synthesis through the induction of the hepatic small heterodimer partner (SHP, NR0B2)<sup>[6]</sup> and ileal fibroblast growth factor 15/19 (FGF15/19, mouse FGF15 or human homolog FGF19)<sup>[7]</sup>. In the liver, both SHP and FGF15/19 trans-repress the expression of cholesterol 7 $\alpha$ -hydroxylase (*CYP7A1*), which is the rate-limiting enzyme in the classic BA synthesis pathway<sup>[6]</sup>.

BA synthesis is mainly under the control of the FXR and SHP axis; however, FXR is not exclusively epistatic to SHP in a linear regulatory pathway<sup>[37–39]</sup>. Mice with a combined loss of FXR and SHP demonstrate more severe liver injury and much greater BA overload than that in either single knockout mouse<sup>[37]</sup>. This study exhibits that the two nuclear receptors act synergistically to maintain BA homeostasis.

Disruption of BA metabolism is the major defect discovered in FXR<sup>-/-</sup> mice with spontaneous hepatocarcinogenesis<sup>[19, 20]</sup>. The BAs in both serum and liver were elevated in these mice<sup>[19]</sup>. When the BA pool was lowered by 2% cholestyramine food, the malignant lesions were statistically reduced<sup>[19]</sup>. Overload of BAs due to the depletion of the FXR gene is the causative factor for injury of liver cells, induction of chronic inflammation, enhancement of cell proliferation, and development of liver tumors<sup>[19, 20, 40]</sup>. In FXR<sup>-/-</sup>SHP<sup>-/-</sup> double-knockout (DKO) mice, the sharply elevated BA levels lead to the activation of the Yes-associated protein (YAP)<sup>[41]</sup>, which is a core component of the Hippo pathway and has recently been considered a crucial promoter of hepatocarcinogenesis<sup>[42–44]</sup>. The activation of YAP by BAs is concentration dependent. The physiological concentration or the modest elevation of BA levels, such as in exclusively FXR or SHP individual knockout mice, cannot lead to YAP activation<sup>[41]</sup>.

Consistent with the observation that elevated BA levels in mice fed with a 0.2% cholic acid diet significantly promoted N-nitrosodiethylamine-initiated hepatocellular carcinoma (HCC)<sup>[19]</sup> formation, Lozano *et al*<sup>[45]</sup> revealed that intrahepatic BA accumulation in bile-duct-ligated rats facilitated the carcinogenic effects of thioacetamide metabolites in cholangiocarcinoma development. The persistently high levels of BA enhanced the inflammation and bile duct proliferation, and led to the downregulation of FXR expression. Those data indicate that during hepatocarcinogenesis, BAs may function as tumor promoters as well as DNA-damaging initiators<sup>[19, 45]</sup>. The imbalanced ratio of free and conjugated BAs also promotes the growth of human cholangiocarcinoma via FXR<sup>[46]</sup>. Further investigations of human clinical samples are needed to reveal the role of BA-mediated FXR signaling in the tumorigenesis of the human liver.

### FXR, nonalcoholic fatty liver disease (NAFLD) and liver cancer

NAFLD is a spectrum of chronic, progressive liver diseases characterized by hepatic steatosis and is closely related to obesity and metabolic syndrome<sup>[47, 48]</sup>. NAFLD is globally prevalent now due to the obesity epidemic and has become a major public health problem because a significant portion of obese

patients will progress to having nonalcoholic steatohepatitis (NASH), and no specific therapies are currently approved for NAFLD or NASH<sup>[47–50]</sup>. NASH individuals demonstrate serious liver injuries including hepatocyte damage, inflammation and fibrosis<sup>[47–50]</sup>. Moreover, epidemiological evidence has proved that NASH and its two major comorbidities, obesity and diabetes mellitus (DM), increase the risk of HCC, especially when NASH-related cirrhosis has developed<sup>[47]</sup>. Some metabolic or stress-response pathways including one-carbon metabolism, NF- $\kappa$ B, PTEN, and microRNAs may be involved in the regulation of NASH-mediated hepatocarcinogenesis<sup>[47]</sup>.

As a multipurpose metabolic regulator, FXR activation inhibits the transition of NAFLD to NASH via maintaining the homeostasis of glucose, lipid and energy metabolism as well as by antagonizing hepatic inflammation and fibrogenesis, two key pathological features of NASH<sup>[11, 50, 51]</sup>. Evidence from various mouse models and clinical studies have shown that FXR activation by its agonists have beneficial effects on the treatment of NASH.

Type 2 DM has been considered an independent risk factor for HCC<sup>[52]</sup>. Insulin resistance is a key player in the pathogenesis of type 2 DM and a main driver of NASH<sup>[51]</sup>. Both administration of the FXR agonist GW4064 or exogenous overexpression of FXR significantly increased insulin sensitivity and improved glucose tolerance in *db/db* or *ob/ob* mice<sup>[53, 54]</sup>. In contrast, FXR<sup>-/-</sup> mice demonstrated insulin resistance in the liver and peripheral tissue<sup>[53]</sup>. FXR deficiency also increases the susceptibility to developing NASH in a low-density lipoprotein receptor-knockout mouse fed with a high-fat diet<sup>[55]</sup>. The FXR agonist WAY362450 has been shown to protect against NASH by reducing hepatic inflammation and fibrosis in mice fed a methionine and choline-deficient (MCD) diet<sup>[56]</sup>. MCD-fed mice share a similar hepatic manifestation as human NASH<sup>[57]</sup>. Obeticholic acid (OCA; INT-747) is a 6 $\alpha$ -ethyl derivative of CDCA. The results from several animal models indicate that OCA treatment ameliorates hepatic steatosis, inflammation and fibrosis<sup>[49]</sup>. In leptin receptor mutated Zucker (*fa/fa*) rats, which display similarities to the clinical features of NAFLD/NASH patients<sup>[58]</sup>, OCA reverses insulin resistance, alleviates lipid abnormalities and reduces the severity of the liver steatosis<sup>[58]</sup>. At present, OCA is the first selective FXR agonist to enter phase 2 clinical trials<sup>[51]</sup>. OCA mediated FXR activation has been shown to enhance insulin sensitivity in patients with type 2 DM and NAFLD<sup>[51]</sup>. Consistent with these data, hepatic FXR expression is significantly downregulated in NAFLD patients<sup>[59]</sup>. Activation of FXR may be effective to retune NAFLD-related metabolic disorders and impede the progress of NAFLD-NASH-HCC.

### FXR, liver regeneration/repair and liver cancer

Liver regeneration (LR) after partial hepatectomy (PH) is a complex process of compensatory hyperplasia driven by the replication of remaining hepatocytes and is regulated by a well-cooperated network of signaling pathways, such as growth factors, cytokines and transcription factors<sup>[60]</sup>. Huang *et al* found that FXR-dependent BA signaling was required for

normal LR<sup>[9]</sup>. In response to the increased BA flux after 70% PH, FXR activates hepatic SHP and intestinal FGF15, which results in the suppression of Cyp7A1 and BA synthesis<sup>[9, 61, 62]</sup>. Another FXR target gene, the bile salt export pump (BSEP), a canalicular BA effluxer<sup>[63]</sup>, can also be induced to enhance BA export<sup>[9]</sup>. In parallel, FXR directly promotes liver regrowth by activating the proliferative transcription factor FoxM1b<sup>[9]</sup>. Consistent with this result, FXR is able to alleviate age-related proliferation defects by transcriptional activation of FoxM1b in the mouse regenerating livers<sup>[64]</sup>. However, the impaired FXR activities in SIRT1 (a histone deacetylase) transgenic mice due to the persistent deacetylation and lower protein expression of FXR result in defective hepatocyte proliferation in the regenerating liver<sup>[65]</sup>.

FXR deficient mice not only exhibit delayed LR after 70% PH<sup>[9, 66]</sup> but also demonstrate defective repair ability in the damaged liver. When FXR is knocked down, the effect of anti-apoptosis on liver cells is compromised under the condition of serum deprivation or food withdrawal<sup>[67]</sup>. Meng *et al*<sup>[17]</sup> reported that FXR<sup>-/-</sup> mice suffered from more severe CCl<sub>4</sub>-induced liver damage, marked by increased hepatocyte death and intrahepatic cholestasis. CYP27<sup>-/-</sup> mice that underwent 70% PH or CCl<sub>4</sub> treatment displayed impaired LR or liver repair capacity due to the low BA levels in these animals, which indicates that sufficient FXR activities are required for normal LR or liver repair<sup>[9, 68]</sup>. An injured liver will be unable to complete normal regeneration if FXR is deleted, thereby resulting in repeated cycles of cell death and compensatory liver regeneration. A previous report suggests that irregular proliferation of hepatocytes is a risk factor in promoting hepatocarcinogenesis<sup>[69]</sup>.

Interestingly, liver transplant is an optional therapy for liver cancer patients in advanced stages and follows the same principles as those that regulate LR after PH in the laboratory animals<sup>[60]</sup>. Understanding the mechanisms of LR, such as the roles of BA-FXR signaling in this complicated course, is helpful for the development of new therapeutic strategies for many severe liver diseases, including cirrhosis and cancer.

### FXR, hepatic inflammation and liver cancer

HCC is the most common primary liver cancer, and the incidence is rising worldwide largely due to hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, alcohol abuse, and obesity-associated NASH<sup>[50, 70]</sup>. Overall, 90% of HCC cases occur in the setting of unresolved inflammation and subsequent severe fibrosis, regardless of the etiology<sup>[70]</sup>.

Evidence suggests that FXR demonstrates anti-inflammatory properties in the liver. Activation of FXR protects against concanavalin A-induced autoimmune hepatitis<sup>[16]</sup> and alleviates LPS-mediated hepatic inflammation<sup>[13]</sup>. The proinflammatory cytokines, such as IL-6, are strong inducers of the activation of signal transducer and activator of the transcription 3 (STAT3) signaling pathway<sup>[71-73]</sup>. STAT3 protein is considered an indispensable participant in fostering proliferation and resisting apoptosis of tumor cells<sup>[74, 75]</sup>. He *et al* analyzed 52 human HCC clinical samples and found that approximately 60% of

the specimens had increased nuclear phospho-STAT3 and that the activation of STAT3 was associated with adverse characteristics of the tumor<sup>[76]</sup>. In FXR<sup>-/-</sup> mice, increased BAs mediate upregulation of cytokine IL-6 and the reduction of suppressor of cytokine signaling 3 (SOCS3, a feedback inhibitor of STAT3), which collectively lead to the constitutive activation of STAT3<sup>[72]</sup>.

Another essential contributor of hepatocarcinogenesis is the transcription factor nuclear factor-κB (NF-κB). NF-κB is a master regulator of inflammatory signaling pathway, and it can also be modulated by proinflammatory cytokines. In the liver, NF-κB provides a central link between hepatic damage, fibrosis and HCC. It is also considered a promoter of liver carcinogenesis<sup>[77, 78]</sup>. Wang *et al* revealed negative crosstalk between FXR and the NF-κB signaling pathway. On the one hand, activation of FXR inhibits NF-κB transcriptional activity via decreasing the binding between NF-κB and DNA. On the other hand, FXR transactivity is antagonized by LPS-induced NF-κB activation<sup>[13]</sup>. As the two key players in liver inflammation and cancer, NF-κB and STAT3 cooperate to respond to various stimuli including proinflammatory cytokine IL-6 and upregulation of pro-proliferative and anti-apoptotic genes. This will drive the development of liver cancer<sup>[74]</sup>. Together, these data suggest that the inhibition of NF-κB and STAT3 pathways may be another possible mechanism contributing to the FXR function as a liver tumor suppressor.

### FXR-regulated target genes and liver cancer

SHP is one of the most strongly induced genes by FXR. SHP is an atypical orphan nuclear receptor, as it lacks a DNA binding domain and serves as a pivotal co-repressor via inhibiting transactivation of specific nuclear receptor partners<sup>[79]</sup>. SHP is considered a tumor suppressor<sup>[80]</sup> in addition to a metabolic regulator<sup>[81]</sup>. SHP null mice spontaneously develop HCC at 12 to 15 months of age<sup>[82]</sup>, and SHP expression is diminished in human HCC samples and cell lines<sup>[23, 83]</sup>. SHP represses tumor growth via the inhibition of cellular proliferation<sup>[82, 83]</sup> and the activation of apoptotic signals<sup>[84, 85]</sup>. He *et al* observed that SHP was downregulated by promoter hypermethylation, which is an important epigenetic event during HCC development<sup>[83]</sup>.

As mentioned above, both FXR and SHP are liver tumor inhibitors. Although the role of the FXR-SHP axis in the BA metabolism has been well documented, the interplay between FXR and SHP in liver carcinogenesis remains unclear. The level of SHP expression was much lower in aging FXR<sup>-/-</sup> mice with HCC compared to young FXR<sup>-/-</sup> mice<sup>[19]</sup>. In human HCC specimens, expression of FXR and SHP are both markedly decreased<sup>[23, 25]</sup>, and the FXR mRNA level was significantly and positively correlated with the SHP mRNA level in HCC tissues<sup>[23]</sup>. Those studies demonstrate that loss of SHP may contribute to liver carcinogenesis in livers deficient in FXR. Although hepatocyte-specific SHP overexpression does not reduce the liver tumor incidence and size or blunt the activation of the IL-6/STAT3 signaling pathway in the FXR<sup>-/-</sup> mice, the increased SHP expression can lower the hepatocellular dysplasia, reduce the inflammatory cell infiltration and

enhance apoptosis<sup>[71]</sup>. SHP may partially protect against HCC development in FXR null mice<sup>[71]</sup>. Overall, these findings imply that FXR and SHP may act coordinately to perform their liver tumor inhibitory functions, but limited information is available regarding the exact molecular mechanism of interaction between the two nuclear receptors during liver carcinogenesis.

BSEP is another FXR key target gene that is vital to keep the normal BA levels in the hepatocytes by acting as a canalicular BA effluxer<sup>[63]</sup>. The absence of BSEP can cause severe cholestasis and HCC in young children<sup>[86, 87]</sup>. However, BSEP expression is dramatically reduced in human HCC samples<sup>[88]</sup>. BSEP is transcriptionally induced by FXR in an isoform-specific manner<sup>[88]</sup>. FXR $\alpha$ 1 and FXR $\alpha$ 2 are two main isoforms of FXR expressed in normal human liver<sup>[89]</sup>, and FXR $\alpha$ 2 has a greater ability to transactivate BSEP<sup>[88]</sup>. The increase in the FXR $\alpha$ 1/FXR $\alpha$ 2 ratio due to the stimulation of proinflammatory cytokines results in significant downregulation of BSEP<sup>[88]</sup>.

Another FXR targeted tumor suppressor gene is N-myc downstream-regulated gene 2 (NDRG2). NDRG2 mRNA is diminished in livers of FXR<sup>-/-</sup> mice and human HCC patients. FXR agonists or ectopic overexpression of FXR leads to the transcriptional induction of the NDRG2 gene. Furthermore, FXR can bind to the intronic IR1-type element(s) of the NDRG2 gene in mouse liver and human liver cancer cells<sup>[29]</sup>.

FXR also modulates the function of oncogenes. Several mouse models and clinical investigations provide direct evidence that during the development of mouse and human liver cancer, loss or reduction of FXR expression levels will cause the de-repression of the promoter of the oncoprotein gankyrin, which is a small subunit of the proteasome and mediates the degradation or elimination of activities of four tumor suppressor proteins: Rb, p53, hepatocyte nuclear factor 4a (HNF4a), and CCAAT/enhancer binding protein (C/EBP)  $\alpha$ <sup>[24]</sup>.

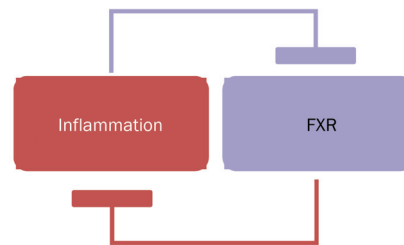
Hepatocarcinogenesis is a multistep process with accumulation of sequential genetic and epigenetic alterations. FXR may exert its tumor inhibitory function partially via direct inactivation of oncogenes and activation of tumor-suppressor genes. More FXR target genes related to the liver carcinogenesis may be identified in the future.

### Downregulation of FXR expression in liver cancer

As mentioned above, the expression of FXR is lost during the development of liver cancer. However, the precise molecular mechanism of FXR downregulation remains elusive in the current field.

Inflammation may provide a microenvironment to reduce the expression of FXR. The elevated levels of proinflammatory cytokines, such as TNF $\alpha$ , IL-1 $\beta$ , and IL-6, in both FXR<sup>-/-</sup> mice<sup>[19, 20]</sup> and in most human HCC patients<sup>[25, 88]</sup>, may reduce the FXR expression via inhibiting the transactivity of hepatic nuclear factor 1 $\alpha$  (HNF1 $\alpha$ ) on the FXR gene promoter<sup>[25]</sup>. TNF $\alpha$  and IL-1 $\beta$  alter the relative expression of FXR $\alpha$ 1 and FXR $\alpha$ 2, which leads to an increase in the FXR $\alpha$ 1/FXR $\alpha$ 2 ratio and subsequent reduction of BSEP, indicating a potential interaction between FXR alternative splicing procedure and the

inflammatory-cytokine mediated signal pathway<sup>[88]</sup>. Gadaleta *et al*<sup>[90]</sup> demonstrated that a vicious cycle was formed when NF- $\kappa$ B-dependent reduction of FXR expression led to less inhibition of NF- $\kappa$ B-mediated intestinal inflammation (Figure 1).



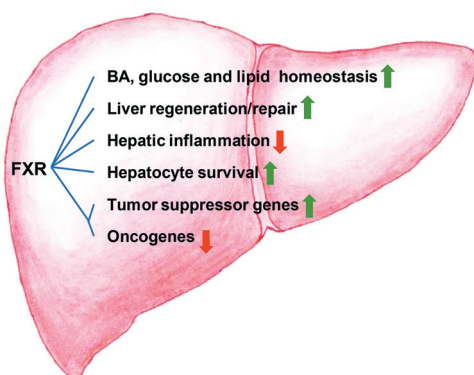
**Figure 1.** A vicious cycle of FXR and liver inflammation. FXR antagonizes NF- $\kappa$ B activity and suppresses hepatic inflammation. Conversely, hepatic inflammation decreases FXR expression.

Epigenetic silencing is another important contributor to the reduction of FXR expression. Two studies have demonstrated a role of miR-421 in the suppression of FXR transcription by targeting the 3'UTR of FXR mRNA in HCC cells and biliary tract cancer cells<sup>[91, 92]</sup>. However, overexpression of SIRT1 in transgenic mice results in persistent deacetylation and decreased protein expression of FXR. In human HCC, elevated SIRT1 is correlated with the absence of FXR<sup>[65]</sup>. Recently, Bailey *et al*<sup>[21]</sup> reported that diminished FXR expression in human colon cancer was partly due to both DNA methylation of the FXR promoter and increased KRAS signaling. Therefore, activation of oncogenic pathways may also be responsible for the reduced expression of FXR in liver cancer.

### Conclusion and perspectives

Physiologically, FXR displays its hepatoprotective roles in the following ways: 1) Maintains normal liver homeostasis and metabolism of BAs, glucose and lipid; 2) Participates in LR and acts as an important factor to reestablish BA homeostasis and promote liver regeneration; 3) Protects liver cells from further damage by inhibiting cell death; 4) Counter-regulates hepatic inflammation through suppression of NF- $\kappa$ B- and STAT3-mediated signaling pathways; and 5) Induces the expression of tumor-suppressor genes and represses the transcription of oncogenes (Figure 2). In addition, FXR may function in other metabolic processes, such as cholestasis and fibrosis, to suppress liver carcinogenesis, although these roles are not covered in this review.

Loss of FXR activity could be an important molecular event in the initiation and progression of liver cancer. Inflammation and epigenetic silencing are the two main contributors to the downregulation of FXR expression. Due to the deficiency of FXR function, hepatocytes will be exposed to a microenvironment that favors malignant transformation and cancer development. Interestingly, changing the FXR silencing or activation of remnant FXR in the healthy tissues adjacent to the



**Figure 2.** FXR and anti-liver carcinogenesis. FXR suppresses liver carcinogenesis via several diverse mechanisms: 1) FXR maintains the normal liver homeostasis and metabolism of BAs, glucose, and lipid. 2) FXR promotes liver regeneration and repair after injury; 3) FXR suppresses hepatic inflammation; 4) FXR enhances hepatocyte cell survival; and 5) FXR increases the expression of some tumor suppressor genes and represses the transcription of several oncogenes.

tumor may be a potential therapeutic strategy for liver cancer patients.

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