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Review

Farnesoid X Receptor (FXR) from normal to malignant state

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Summary. The Farnesoid X Receptor (FXR) is a member of the nuclear receptor superfamily of ligand-activated transcription factors, which plays crucial role in bile acid, cholesterol, lipid and glucose metabolism, as well as in the development of atherosclerosis, intestinal bacterial growth and liver regeneration. FXR is also involved in the pathogenesis of cholestatic diseases, non-alcoholic fatty liver disease and inflammatory bowel disease. Recent evidence further suggests a key role for FXR in apoptosis and cancer. Notably, FXR deficiency promoted intestinal inflammation and tumorigenesis, suggesting that FXR activation might be a promising strategy in the treatment of colon cancer. FXR deficiency in mice led to the development of spontaneous hepatocarcinomas, while FXR inhibition might represent a novel therapeutic approach in Barett's esophagus. In breast cancer cell lines, FXR agonists down-regulated the breast cancer target gene aromatase. FXR inhibited Leydig tumor growth and progression, supporting evidence that FXR may be an important regulator of androgen homoeostasis. Further studies are required in order to establish possible antitumor effects of this nuclear receptor. Either reactivating or inhibiting FXR expression may represent promising therapeutic strategies in the treatment of certain types of human cancer.

Key words: Farnesoid X Receptor, Bile acids, Inflammation, Cholestasis, Carcinogenesis

Introduction

Nuclear receptors (NRs) are ligand-activated transcription factors with important roles in different aspects of human physiology and development (Chawla et al., 2001). The Farnesoid X Receptor (FXR) was initially cloned in 1995, and belongs to a group of metabolic receptors including Vitamin D Receptor (VDR), Pregnane X Receptor (PXR), Liver X Receptor alpha and beta (LXRα and LXRß, respectively) and Constitutive Androstane Receptor (CAR). FXR regulates the expression of various genes involved in bile acid, lipid and glucose metabolism, by binding to DNA either as a monomer or an heterodimer with a common partner for NRs, Retinoid X Receptor (RXR). FXR is highly expressed in the liver, intestine, kidney and adrenals (Zhang et al., 2003). Two known FXR genes exist, the *Fxrα* and *Fxrβ*. *Fxrα* gene in humans encodes four FXRα isoforms (FXRα1, FXRα2, FXRα3 and FXRα4) as a result of the use of different promoters and alternative RNA splicing. The second FXR gene, *Fxrβ*, is a pseudogene in humans and its role remains uncertain. Most FXR target genes are regulated independently by all FXRa isoforms, while other target genes, including those encoding intestinal bile acid binding protein (IBABP), syndecan-1, αA-crystallin and fibroblast growth factor 19 (FGF19), are more isoform-specific and mainly response to the FXRα2 and FXRα4 isoforms. FXRα binds to specific DNA response elements as a heterodimeric complex with the RXR (Lee et al., 2006).

Bile acids (BA) are endogenous ligands for FXR. Chenodeoxycholic acid (CDCA) is the most effective activator, while deoxycholic acid (DCA) and lithocholic acid (LCA) are weaker FXR activators. Ursodeoxycholic (UDCA) and muricholic acids cannot activate FXR. Other natural FXR ligands include oxysterol 22(R)-hydroxycholesterol and androsterone, and poly-unsaturated fatty acids such as arachidonic acid and decosahexaenoic acid, while BA metabolites such as 26- or 25-hydroxylated bile alcohols are considered as weak FXR ligands. Additionally, several synthetic FXR ligands have been generated, such as GW4064, 6ECDCA, AGN29, and AGN31, with GW4064 being the most widely used (Wang et al., 2008a) (Fig. 1).

FXR regulates the expression of a large number of

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target genes by binding either as a monomer or as an heterodimer with RXR to FXR response elements (FXREs). FXR regulates human intestinal bile acid binding protein (IBABP), small heterodimer partner (SHP), bile salt export pump (BSEP), BA-CoA:amino acid N-acetyltransferase (BAT) and phospholipid transfer protein (PLTP) via IR-1 elements in the promoters of these genes, which present different affinity for FXR (Laffitte et al., 2000).

In the present review, the role of FXR in different metabolic processes, and its involvement in cancer, as well as the underlying mechanisms are discussed. FXR and its multiple targets in humans are briefly presented in Figure 2.

FXR and physiology

FXR and bile acid metabolism

FXR and its specific target genes have a crucial role in regulating BA metabolism, via a negative feedback pathway (Neimark et al., 2004; Gadaleta et al., 2010). A rise in intracellular BA levels results in an increase in BA-induced FXR activation and enhances transcription of its target genes. One such hepatic FXR target gene is SHP, an atypical member of the NR superfamily that lacks a DNA-binding domain. SHP can dimerize with and inactivate both LRH1 and LXRα, resulting in a decrease in Cyp7a1 expression. Another pathway that regulates BA acid production is initiated after activation of FXR in enterocytes. This activation results in enhanced transcription and secretion of fibroblast growth factor 15 (FGF15) (Inagaki et al., 2005). Subsequent binding of FGF15 to fibroblast growth factor receptor 4 (FGFR4), a transmembrane tyrosine kinase receptor localized on the hepatocyte cell surface, results in repression of cytochrome P450 7a1 and 8b1 (Cyp7a1, Cyp8b1) through a JNK-dependent signaling cascade (Holt et al., 2003). In addition to Cyp7a1 and Cyp8b1, FXR also regulates genes involved in BA and phospholipid secretion across the bile canalicular membrane, BA transport and finally BA conjugation and detoxification (Ananthanarayanan et al., 2001; Jung et al., 2002; Kast et al., 2002; Huang et al., 2003).

FXR and lipid metabolism

FXR affects cholesterol metabolism through the repression of *CYP7A1*, the main rate-controlling enzyme for cholesterol catabolism into BAs. *CYP7A1* mediates the conversion of cholesterol to BAs, resulting in a relative deprivation of hepatic microsomal cholesterol content and finally in upregulation of LDL-receptor (LDL-R) expression and activity which consequently reduces plasma LDL-cholesterol (LDL-C) levels (Insull, 2006). Moreover, human CYP7A1 deficiency results in a statin-resistant hypercholesterolemia (Pullinger et al., 2002). On the other hand, CDCA, as well as GW4064, have been shown to potentiate LDL-R activity in response to statin treatment in human hepatocytes (Langhi et al., 2008). FXR also controls intestinal cholesterol absorption since *FXR*$^{-/-}$ mice have been recently reported to exhibit increased dietary cholesterol absorption (Wang et al., 2006; Lefebvre et al., 2009). Additionally, FXR has a profound effect on HDL metabolism and remodeling. *FXR*$^{-/-}$ mice display elevated plasma HDL-C and apolipoprotein (apo) A-I concentrations (Sinal et al., 2000). FXR represses human and murine apoA-I gene expression both *in vitro* and *in vivo*, via a monomeric FXR binding site in the promoter

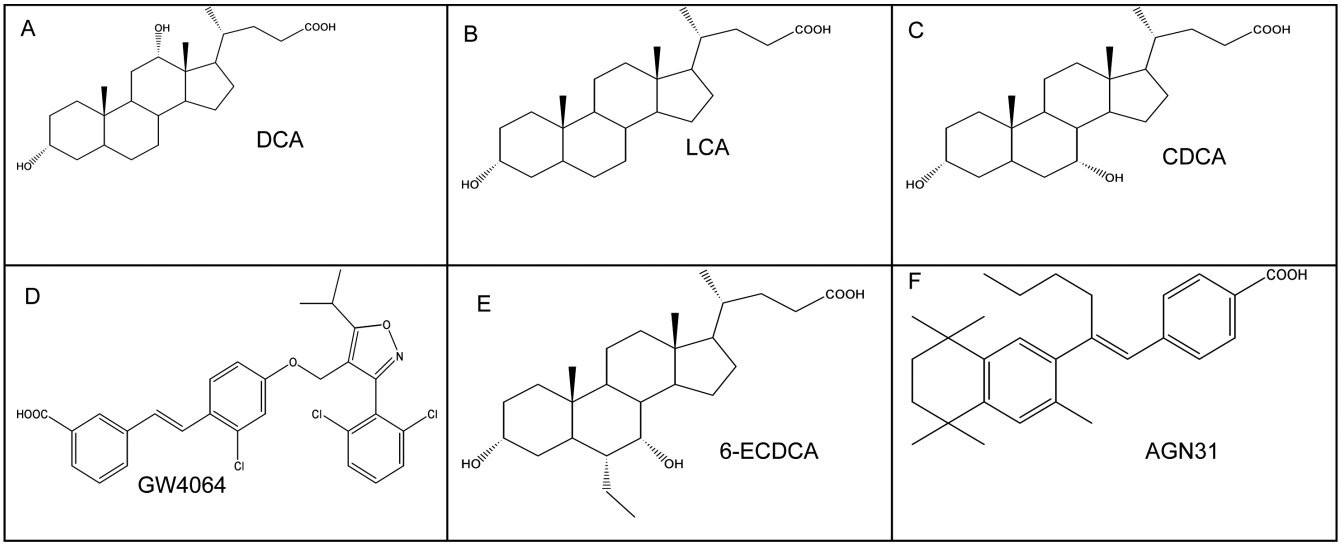

Fig. 1. Chemical structures of naturally occurring (A-C) and synthetic (D-F) FXR ligands.

(Claudel et al., 2002). FXR-mediated repression may also involve a FXR-induced SHP-mediated inhibition of LRH-1 transcriptional activity (Lambert et al., 2003; Watanabe et al., 2004). Finally, FXR activation affects triglyceride metabolism at different levels. FXR is involved in the control of hepatic de novo lipogenesis. A recent study demonstrated that FXR enhances the transcription of fatty acid synthase (*FAS*), a key lipogenic enzyme, through direct binding to an IR-1 site in the *FAS* promoter. Data suggest that FXR activation may control de novo lipogenesis through both SREBP-1c-dependent and -independent mechanisms (Matsukuma et al., 2006).

FXR and glucose homeostasis

Changes in the BA pool size and BA secretion are directly dependent on the insulin-deficient state, as BA sequestrants have been shown to reduce blood glucose levels in animal and human studies (Staels and Kuipers, 2007). FXR might be one of the molecular links between altered BA metabolism and diabetic states (Duran-Sandoval et al., 2004). The molecular mechanisms behind the insulin-sensitizing effect of FXR remain not well defined. On the other hand, a role for FXR in the induction of gluconeogenic gene expression can be inferred from several observations. Fasting markedly induces hepatic expression of PPARγ coactivator 1α (PGC1α), which subsequently stimulates the entire program of genes involved in hepatic gluconeogenesis by acting as a coactivator for glucorticoid receptor (GR) and hepatocyte nuclear factor 4 (HNF-4). FXR affects glucose homeostasis by regulating PGC1α activity. The end result is FXR-dependent decreased gluconeogenesis (Cariou et al., 2006; Ma et al., 2006).

FXR and atherosclerosis

Mice lacking both FXR and apolipoprotein E (*Fxr*$^{-/-}$, *apoE*$^{-/-}$ mice) had increased levels of atherosclerosis compared with *apoE*$^{-/-}$ mice, consistent with their hyperlipidemia (Hanniman et al., 2005). Additionally, mice lacking LDL-R and FXR (*Fxr*$^{-/-}$, *Ldlr*$^{-/-}$ mice) had less atherosclerosis than *Ldlr*$^{-/-}$ ones (Zhang et al., 2006). Recent *in vitro* studies demonstrated that FXR is expressed in both vascular smooth muscle cells (VSMCs) and endothelial cells (Zhang et al., 2008a,b). Finally, FXR seems to be involved in the BA-mediated repression of paraoxonase-1 (PON1) (Shih et al., 2006).

FXR and liver regeneration

BAs have a novel role in liver regeneration. A diet containing 0.2% cholic acid stimulated liver regeneration in partially hepatectomized mice, while diets containing the BA sequestrant cholestyramine impaired liver regeneration. Furthermore, *Fxr*$^{-/-}$ mice had impaired liver regeneration ability (Huang et al., 2006). Additionally, BAs were shown to be required for the induction of the proliferation factor FoxM1b, important for liver regeneration, that was recently identified as an FXR target gene (Chen et al., 2010).

FXR and liver-biliary tract diseases

Cholestasis

FXR agonist GW4064 markedly reduced liver injury in animal models representing an acute form of cholestasis of short duration (Liu et al., 2003). The FXR agonist 6-ethyl chenodeoxycholic acid (6-ECDCA) protected from cholestasis by increasing the expression of SHP, apical bile acid transporter ABCB11 and apical phospholipid transporter ABCB4, while reducing the expression of CYP7A1 and CYP8B (BA neosynthesis) or NTCP (basolateral BA uptake) in a more chronic cholestasis animal model (Fiorucci et al., 2005). Additionally, it has been proposed, based on analysis of

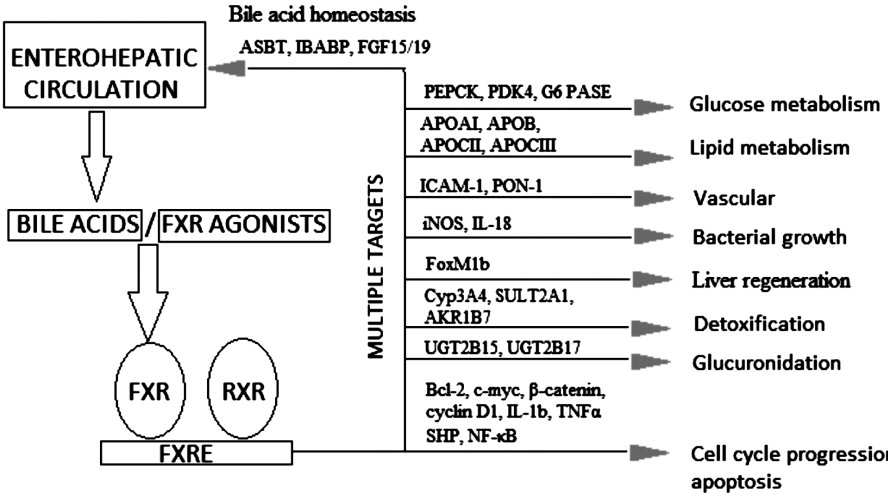

Fig. 2. FXR and its multiple target genes involved in different metabolic processes.

a limited number of ileal tissue samples of patients with progressive familial intrahepatic cholestasis (PFIC1) and complementary *in vitro* studies, that ATP8B1 function (encoding aminophospholipid flippase in the hepatocytic canalicular membrane) may normally activate PKCζ with subsequent phosphorylation, nuclear localization and activation of FXR, and that this cascade is deficient in PFIC1 patients. The resulting decreased expression of downstream targets of FXR, such as apical sodium-dependent bile salt transporter (ASBT) and ABCB11, could underlie the cholestasis in these patients (Frankenberg et al., 2008). Finally, four new heterozygous functional variants of FXR in patients with intrahepatic cholestasis of pregnancy were recently described (Van Mil et al., 2007).

Primary cholestatic diseases

The healthy biliary tract generally remains sterile and tolerant to pro-inflammatory stimuli of the pathogen associated molecular patterns (PAMPs) (Harada and Nakanuma, 2010). FXR appears to be involved in maintaining sterility of the biliary tract: FXR activation by BAs facilitates vasoactive intestinal peptide receptor-1 (VPAC1)-induced bile secretion (choleresis), promotes expression of α-crystalline (a putative defence molecule against oxidative stress) and induces expression of the antimicrobial peptide cathelicidin in biliary epithelial cells (Chignard and Poupon, 2009; D'Aldebert et al., 2009). These processes appear deficient in primary biliary cirrhosis and primary sclerosing cholangitis, with the result that PAMPs accumulate in the biliary tract and induce inflammation. The potent synthetic FXR agonist 6-ethyl chenodeoxycholic acid (6-ECDCA) is currently evaluated in clinical trials of patients with primary biliary cirrhosis (Fickert et al., 2002; Wagner et al., 2003).

Cholesterol gallbladder stones

FXR knockout mice on lithogenic diet are highly susceptible to cholesterol gallstone formation. In wild type mice on lithogenic diet, gallstone formation can be prevented by the synthetic FXR agonist GW4064, because increased amounts of solubilizing BAs and phospholipids prevent cholesterol supersaturation and nucleation of cholesterol crystals under these circumstances (Moschetta et al., 2004). Decreased enterocytic expression of FXR and its target genes ASBT, ileal lipid binding protein (ILBP) and OSTαOSTß, all involved in BA transport, have been described in gallstone-patients (Bergheim et al., 2006; Renner et al., 2008).

Non-alcoholic fatty liver disease

FXR activation has a beneficial impact on triglyceride and glucose metabolism, which participate in the pathogenesis of NAFLD. Additionally, FXR activation by 6-ECDCA protected against liver steatosis in animal models (Cipriani et al., 2010). Experimental data in animal models suggest that FXR inhibited inflammation and fibrosis in NAFLD (Zhang et al., 2009). Apart from their anti-inflammatory effects and effects on lipid and glucose metabolism, FXR agonists may also inhibit fibrosis progression by inhibiting hepatic stellate cell activation, through FXR-PPARγ-dependent or FXR-SHP dependent mechanisms (Fiorucci et al., 2004; Mencarelli et al., 2009).

Intestinal bacterial growth and inflammatory bowel disease

Obstruction of bile flow in humans causes intestinal bacterial growth, mucosal injury and bacterial translocation. BA administration can prevent intestinal bacterial growth. FXR is critical for controlling intestinal bacterial growth and maintaining a competent barrier by inducing a number of intestinal genes, including angiogenin, inducible nitric oxide synthase (iNOS) and IL-18, all of which are involved in enteroprotection. *Fxr*$^{-/-}$ mice have bacterial overgrowth in their ileum. The development of FXR agonists could provide a novel mechanism for controlling intestinal bacterial growth (Inagaki et al., 2006).

The importance of host-microbe interactions in pathogenesis of inflammatory bowel disease is today established. Recently, FXR has been implicated in pathogenesis of idiopathic inflammatory bowel disease, Crohn's disease and ulcerative colitis. FXR activation by the synthetic ligand GW4064 offered strong protection against bacterial overgrowth in wild type, but not in FXR knockout mice. Interestingly, the FXR ligand increased mRNA expression of iNOs, Angiogenin 1 (ANG1) and Carbonic anhydrase 12 (CAR12) (Inagaki et al., 2006). Additionally, administration of the potent synthetic FXR ligand 6-ECDCA protected against the colitis in wild type mice, with reduced expression of various pro-inflammatory cytokines in colonic homogenates and colon-derived macrophages, but not in FXR knockout mice. Both in animal models and in Crohn's disease patients, intestinal inflammation was associated with decreased FXR mRNA expression (Vavassori et al., 2009).

FXR activation was inhibited by pro-inflammatory stimuli in different model systems, probably via NF-κB-dependent tethering of FXR. The pro-inflammatory cytokine TNFα decreased FXR target gene expression in enterocyte-like differentiated HT29 cells. Moreover, in mice with severe intestinal inflammation induced by dextran sodium sulfate (DSS), expression of FXR target genes IBABP and FGF15 was similarly reduced in ileum as well as in colon. FXR KO mice had severely impaired intestinal integrity compared to WT mice at baseline, suggesting that they are probably more susceptible to chronic inflammation. This study showed that FXR is not only an active player in the inhibition of inflammation, but also a target of the inflammatory

response itself (Gadaleta et al., 2011a). In a similar study, colitis was induced in WT and FXR KO mice using DSS, and in WT mice using trinitrobenzenesulfonic acid (TNBS). WT mice treated with the FXR agonist INT-747 were protected from DSS- and TNBS-induced colitis. Furthermore, FXR activation downregulated expression of key proinflammatory cytokines in different immune cell populations, and preserved epithelial barrier function in intestines of WT mice. According to the authors, FXR agonists could represent a novel therapeutic strategy for inflammatory bowel disease (Gadaleta et al., 2011b).

FXR and cancer

FXR and gastrointestinal cancer

Esophageal cancer

FXR expression in esophageal cancer. Esophageal cancer carries a poor prognosis with overall 5 year survival rate of approximately 10%. Barrett's esophagus, which is associated with reflux disease, is considered as a risk factor for esophageal adenocarcinoma. The results of a study indicated that the expression of FXR was increased in esophagitis, Barrett's esophagus and adenocarcinoma compared to normal mucosa. In adenocarcinoma, the expression of FXR was inferior to that measured in esophagitis and Barrett's esophagus. This difference suggested an almost complete loss of expression of FXR in esophageal adenocarcinoma cells (De Gottardi et al., 2006). Additionally, the mRNA levels of FXR and the FXR-regulated genes IBABP, SHP, and chemokines IL-8 and macrophage inflammatory protein 3 alpha (MIP3a), were found increased in Barett's epithelium cells (Capello et al., 2008). Mucins protect normal esophagus mucosa. In high-grade dysplasia and adenocarcinoma of Barrett's esophagus, expression of MUC1 and MUC4 mucin genes is increased. MUC4 is a transmembrane protein involved in ErbB2 signalling. In a study, BAs taurocholate acid (TC), taurodeoxycholate acid (TDC), taurochenodeoxycholate acid (TCDC), glycocholate acid (GC) and GNa were identified as strong inducers of MUC4 mucin expression at the transcriptional level, suggesting that these conjugates may be considered as important factors in the bile to mediate MUC4 upregulation in esophageal cancer. According to this study, as BAs are known to interact with FXR and pregnane X receptor (PXR) to regulate transcription of their target genes, exploring the role of FXR and PXR in the regulation of MUC4 expression may show their direct or indirect involvement (Mariette et al., 2004).

In vitro studies. Suppression of apoptosis is an important mechanism in the progression of Barrett's esophagus towards adenocarcinoma. Treatment of Barrett's esophagus-derived cells with the FXR agonist GW 4064 did not significantly affect the percentage of apoptotic cells compared to untreated. On the other hand, *in vitro* treatment with the FXR antagonist guggulsterone resulted in an increased proportion of cells undergoing apoptosis. This was suggested to be related to an increased expression of antiapoptotic proteins such as Bclx-l and Bcl-2, as well as a decrease in proapoptotic factors such as Bax. This study suggested that the stimulation of apoptosis might be an effect of guggulsterone-mediated FXR inhibition, as presented in Figure 3A, although it could not be excluded that apoptosis was induced by other mechanisms (De Gottardi et al., 2006). Exposure of esophageal cell line TE7 to DCA resulted in an in vitro induction of the former, which was abolished by the FXR antagonist guggulsterone (Capello et al., 2008).

In another study, no significant changes in proliferation were seen after treatment with ECDCA in either Barrett's or adenocarcinoma cell lines. However, in Barrett's cells, ECDCA increased apoptosis compared to control, in a dose dependent manner, while failed to increase apoptosis in adenocarcinoma cell lines. This study claimed that the loss of FXR expression during the progression of carcinogenesis may down-regulate anti-carcinogenic signaling and induce expression of anti-apoptotic and proliferation related genes. Although FXR agonists, such as ECDCA, cause increased apoptosis in premalignant Barrett's cells, due to the loss of FXR in adenocarcinoma, these agonists cannot affect proliferation or apoptosis in cancer cells (Demars et al., 2005).

It was shown that CDCA may activate IKK,/mTOR signaling in SEG-1 and BE3 esophageal adenocarcinoma cell lines, thereby increasing cell proliferation rate and transforming phenotype associated with tumorigenic potential. In Barrett's esophagus CPC-A and CPC-C cell lines, the inflammatory cytokine TNF· and CDCA also resulted in up-regulation of the IKK,/TSC1/mTOR pathway and in turn enhanced proliferation of Barrett's related cells (Yen et al., 2008).

Future perspectives. The expression of FXR and PXR as a diagnostic tool for grading of dysplasia in Barrett's oesophagus patients was examined in a study. On a total of 192 oesophageal tissue samples, 22 of them with no dysplasia (ND), 17 with low-grade dysplasia (LGD), 20 with high-grade dysplasia (HGD) and 24 with adenocarcinoma (AC), nuclear FXR expression was observed in 68% of ND cases, 0% of HGD, 18% of LGD and 8% of AC. Additionally, PXR was highly expressed in HGD and AC samples. According to the authors, the combination of both FXR and PXR expression might be a useful tool in distinguishing the different stages of neoplastic progression in Barret's oesophagus, while its possible prognostic value remains to be determined (van de Winkel et al., 2011).

Gastric cancer

The reflux of bile into the stomach seems to be

involved in intestinal metaplasia and gastric carcinogenesis. It was shown that the BAs CDCA and DCA could induce the expression of caudal type homeobox protein 2 (Cdx2) and mucin 2 (MUC2), which are promoters of intestinal metaplasia, in normal rat gastric epithelial RGM-1 cells. Especially CDCA induced expression of Cdx2 and MUC2 at the mRNA and protein level in a dose-dependent manner, suggesting that BAs likely cause gastric intestinal metaplasia by inducing the ectopic expression of Cdx2 in normal epithelial cells. Additionally, this study demonstrated that FXR is expressed in RGM-1 cells. The FXR agonist GW4064 also induced the expression of Cdx2 and MUC2 and this up-regulation was abolished by guggulsterone. Taken together, these results showed that the activation of FXR may play an important role in the induction of intestinal metaplasia and gastric carcinogenesis by BAs (Xu et al., 2010).

Intestinal and colon cancer

FXR expression in intestinal and colon cancer: in vitro studies. Globally, colorectal cancer is the third most commonly diagnosed cancer in males and the second in females, with over 1.2 million new cases and 608,700 deaths estimated to have occurred in 2008 (Jemal et al., 2011). BAs have been implicated in colorectal cancer, although the mechanisms by which they promote carcinogenesis are not well understood. The expression of different NRs in tissue samples of surgically removed colon cancer was studied, and FXR mRNA was reduced to 17.2% in adenomas compared to normal colorectal mucosa, while an even more pronounced decrease was observed in carcinomas (10 fold average). It was shown by De Cottardi et al., that IBABP was up-regulated in the neoplastic colon mucosa, with adenomas presenting a 4.9-fold increase compared to normal colon, stage I carcinomas a 17.4-fold increase, while a 38.6-fold increase in IBABP was measured in stage IV carcinomas. As it was noted, the absence of a parallel down-regulation of IBABP and FXR was unexpected since FXR has been shown to up-regulate IBABP expression. Additionally, the metastastic adenocarcinoma cell lines SW-480 and SW-620 expressed non detectable FXR levels, while better differentiated Caco-2 and HT-29 cells displayed a significant FXR expression level. Consequently, FXR expression seems to be inversely correlated with tumor cells degree of differentiation. According to this study, FXR silencing may be important for survival and decreased apoptosis of cancer cells, thus leading to colon carcinogenesis (De Gottardi et al., 2004).

A new transcript of the gene encoding IBABP, called IBABP-L, which provides a missing mechanistic link

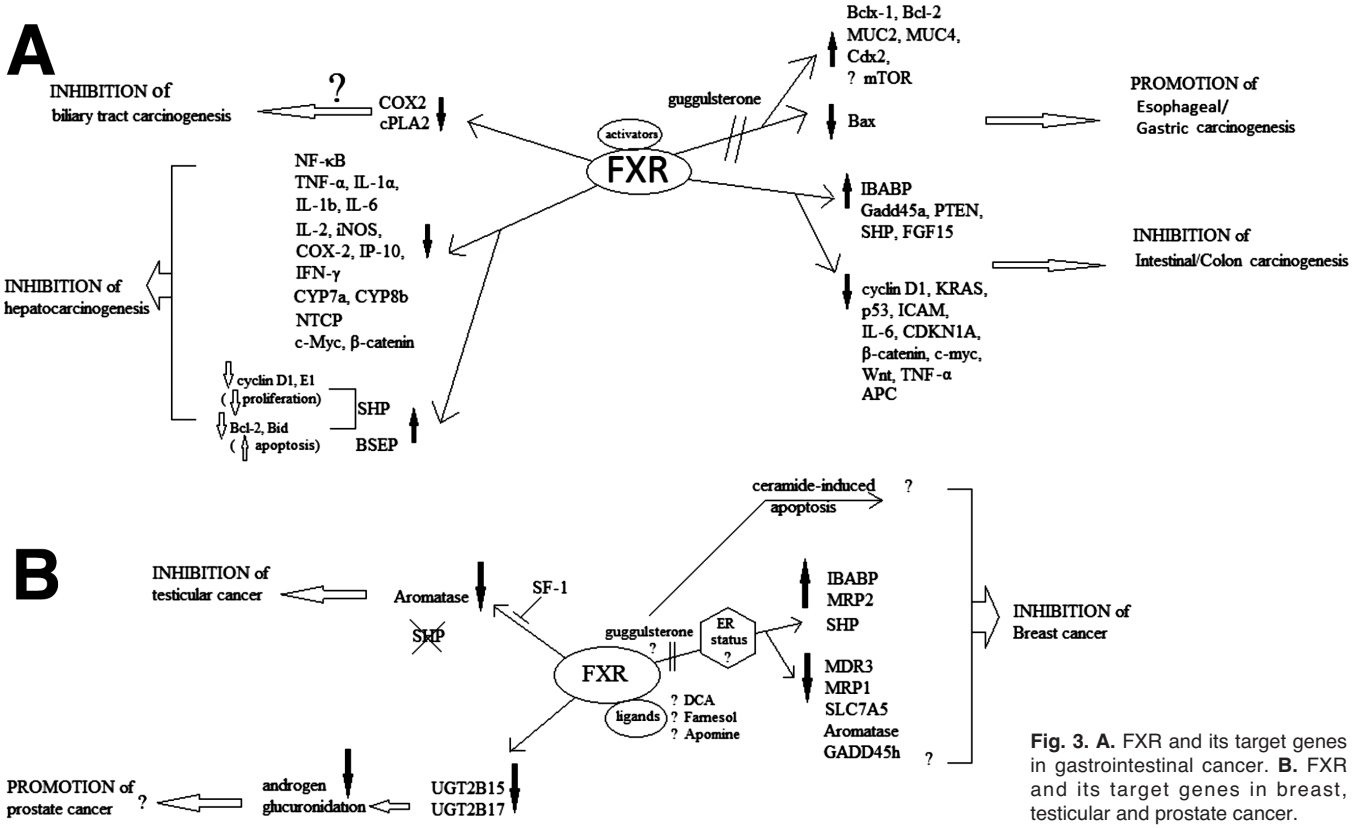

Fig. 3. A. FXR and its target genes in gastrointestinal cancer. **B.** FXR and its target genes in breast, testicular and prostate cancer.

between Nuclear Factor Kappa Beta (NF-κB) and BAs, was identified. IBABP is part of the FXR transcription pathway that responds to BAs. In contrast, IBABP-L is not regulated by FXR, but regulated by NF-κB. IBABP-L is necessary for the survival of colon cancer cells in the presence of physiologic levels of DCA. Colon cancer cells are resistant to cell death induced by normal levels of DCA. When the expression of IBABP-L is knocked down, the cells undergo apoptosis. These observations verified that IBABP-L promotes survival of colorectal cancer cells in the presence of toxic BAs and is likely to contribute to colon tumorigenesis (Fang et al., 2007).

In another study, it was showed that the aldo-keto reductase 1B7 (AKR1B7) catalyzes the reduction of 3-keto bile acids to their 3ß-hydroxy derivatives, which are less cytotoxic than their 3α-hydroxy epimers. Furthermore, AKR1B7 was showed to be directly and robustly induced by FXR in murine small intestine, colon, and liver. Although it remains to be determined whether 3ß-hydroxy-DCA and other 3ß-hydroxy bile acids are less potent tumor promoters than their 3α-hydroxy and 3-keto precursors, the authors speculate that the induction of AKR1B7 may contribute to the antineoplastic actions of FXR (Schmidt et al., 2011).

Colon PC/AA/C1, PCmsrc and HCT-8/E11 cells were studied with the PC/AA/C1 cells used as a model for colorectal cancer development. BAs CDCA, LCA, CA and DCA, but not UDCA, were acting as stimulators of invasion in PC/AA/C1 cells transformed by the SRC oncogene in PCmsrc cells, and of HCT-8/E11 cells originating from a sporadic tumor. Src kinase activation is considered as a frequent and early event in colon cancer progression. BA-induced invasion occurred through stimulation of haptotaxis and was dependent on the RhoA/Rho-kinase pathway and signaling cascades using protein kinase C, MAP kinase, and COX-2. Additionally, BA-induced invasion was associated with activation of the Rac1 and RhoA GTPases and expression of FXR. FXR expression in invasion-competent PCmsrc and HCT-8/E11 cells led the authors to assert that other transcriptional mechanisms might be implicated in the stimulation of invasion by BA (Debruyne et al., 2002).

FXR mutation and activation: in vivo studies. FXR deficiency affected murine ileum and colon morphology in mice examined at 2 and 12 months of age. Two-month-old FXR KO mice had similar colon morphology compared with those of 2-month-old wild type ones, but 12-month-old FXR KO colon showed tall and almost villi-form papillary folds. In addition, FXR KO mice, regardless of age, had moderately increased colon crypt height, while loci of lymphoid cells and decreased number of goblet cells were detected. The current study showed that FXR deficiency enhanced colon cell proliferation *in vivo*. Paradoxically, FXR deficiency also increased the number of cells undergoing apoptosis, evaluated by TUNEL test. FXR deficiency in female APC^{min} mice increased the adenoma multiplicity and average size. FXR deficiency significantly increased adenocarcinoma prevalence, with 57% in male and 43% in female mice, while the average adenocarcinoma size was larger in FXR KO mice. Furthermore, in the colon of 2-month-old mice, FXR deficiency did not affect mRNA levels of ß-catenin, c-myc, cyclin E1, cyclin A2, CDKN1A, mdm2, APC, Gadd45a, NFκB, IL-1ß, or TNF· but increased mRNA expression of cyclin D1 (1.6-fold), K-ras (2.1-fold), p53 (1.6-fold), ICAM (1.5-fold), and IL-6 (2.0-fold). In 12-month-old mice, FXR deficiency did not alter mRNA levels of cyclins D1, E1, and A2, mdm2, p53, NFκB, ICAM, IL-1ß, or TNFα but increased mRNA expression of CDKN1A (1.5-fold) and reduced mRNA levels of APC, Gadd45a, PTEN, and IL-6 compared to wild type mouse. Protein levels were in concert with mRNA expression, although ß-catenin and c-Myc protein levels were increased in contrast with their mRNA levels in the colon of 2-month-old FXR KO mice. Additionally, protein levels of APC were increased at both ages in the colon of FXR KO mice. In summary, this study showed that FXR deficiency promotes intestinal carcinogenesis and suggested that FXR activation by non–BA ligands might be a novel target to prevent or reduce colon carcinogenesis (Maran et al., 2009).

In a similar study, FXR expression was shown to be low in mouse and human intestinal tumors compared with normal intestinal mucosa. A significant increase was observed in the number and size of tumors of $FXR^{+/-}$ $APC^{Min/+}$ mice relative to $FXR^{+/+}$ $APC^{Min/+}$ ones. The intestine of 7- to 8-week-old $FXR^{-/-}$ APCMin/+ mice already contained numerous precursor lesions and adenomalike lesions. Massive neutrophils and macrophages infiltration in the mucosa of $FXR^{-/-}$ $APC^{Min/+}$ mice was found, with increased nuclear b-catenin accumulated and Ki67-positive epithelial cells. According to this study, the promotion of Wnt signaling caused by increased TNFα production and mucosal infiltration of activated neutrophils and macrophages may play a contributing role to tumor susceptibility. Cholestyramine is able to significantly reduce the circulating BA levels. One-month-old male $FXR^{+/+}$ $APC^{Min/+}$ and $FXR^{-/-}$ $APC^{Min/+}$ mice were treated with cholestyramine for 4 weeks. No significant difference in the number of intestinal or colon precursor lesions of the $FXR^{-/-}$ APCMin/+ mice treated with cholestyramine containing diet were found. Data from this study suggested that the absence of FXR from the intestinal epithelium and not merely elevated BA concentrations per se increased susceptibility to tumorigenesis. Additionally, reactivation of FXR markedly increased apoptosis, reduced proliferation, and significantly blocked tumor growth in xenograft mouse models, in which LS174T and HT29 colon cancer cell lines were injected. The synthetic FXR ligand GW4064 was also able to activate apoptosis both in the cellular model and in the normal intestinal mucosa in vivo. According to this study, activation of FXR seems to block tumor growth and to induce a network of proapoptotic genes

that are downregulated in tumors. According to the authors, it is unlikely that the tumor promoting activity of BAs occurs as a function of their ability to activate FXR in the intestine (Modica et al., 2008).

Mice fed with sodium taurocholate (NaTC) for 8 weeks exhibited increased ileal expression of SHP, as well as other FXR-dependent genes, FGF15 and IBABP, confirming that oral taurocholate activates FXR in the murine small intestine. It was also shown that NaTC-fed mice exhibited reduced intestinal cyclin D1 expression and cellular proliferation. These findings were consistent with the hypothesis that taurocholate inhibits cell cycling through SHP-dependent downregulation of cyclin D1. Administering NaTC to $APC^{Min/+}$ mice caused a marked reduction in intestinal adenomas, an effect mediated through FXR activation (Smith et al., 2010).

Future perspectives. BAs play an important role in regulating cell survival and cell death in colon adenocarcinoma cells. Several natural or synthetic BAs have been shown to induce toxicity and apoptosis in colon cancer cell lines (Park et al., 2004; Wachs et al., 2005; Yui et al., 2005; Kim et al., 2006; Katona et al., 2009). BAs are endogenous ligands for FXR and beyond the certain mechanisms that have been studied, their interaction with FXR as a potent cytotoxic mechanism, although still hypothetic, remains to be determined. Based on these data, these synthetic BA derivatives may serve as potential compounds in the treatment of colon cancer.

Guggulsterone has several antitumour effects and acts as an FXR antagonist. Guggulsterone reduced BA-induced and constitutive CdX2 expression in human gut-derived adenocarcinoma, Bic-1 cells, but did not affect cell viability or the cell cycle and did not attenuate BA-induced or constitutive NF-κB activation. It was suggested that guggulsterone may be used as a novel drug to target CdX2 expression in certain gut adenocarcinomas (Yamada et al., 2010).

Modica et al. (2010) mapped the mRNA abundance and the epithelial localization of the entire nuclear receptor family in mouse and human intestine, which indicated that the localization pattern of FXR in normal intestine predicts the modulation of its expression in tumors. According to the authors, this nuclear receptor could be useful as early diagnostic marker or targeted for clinical intervention in intestinal polyposis and cancer.

Several FXR target genes involved in FXR-induced protection of intestinal tumorigenesis, are presented in Figure 3A.

Biliary tract and liver cancer

FXR expression in biliary tract and liver cancer. Significant progress has been made in defining the link between COX-2 and cholangiocarcinogenesis. Activation of EGFR has been proposed as an important mechanism for upregulation of cyclooxygenase 2 (COX-2) and prostaglandin E2 (PGE2) production in human cholangiocarcinomas cells. Moreover, the expression of COX-2 is positively correlated with the level of erbB-2. Additionally, IL-1ß, TNF-α and IFN-γ induce the expression of COX-2 in human cholangiocarcinoma cells (Wu, 2005). Bile acids have been shown to enhance COX-2 expression in cholangiocarcinoma cells through MAPK-dependent transactivation of EGFR (Yoon et al., 2002). The existence of FXR in immortalized mouse cholangiocytes was confirmed. GCDC induced COX-2 expression and PG production (PGE2, 6.3-fold; PGF2·, 8.5-fold), whereas cytosolic phospholipase A2 (cPLA2) expression and activity were reduced (Komichi et al., 2005).

In another study, hierarchical clustering analysis was applied to expression data of NR genes, and liver specific NRs (GR, TR,, ROR·, SHP, TR4, PXR, LXRα, HNF4α, PPARα and FXR) were shown to be highly expressed in normal liver while their expression was deceased in liver cancer tissues. Of liver specific NRs, SHP was dominantly expressed in normal tissue whereas its expression was diminished in liver cancer patients. The possibility that expression patterns of SHP might reflect different clinical outcome of liver cancer patients was examined. Kaplan-Meier plot showed that SHP expression significantly associated with patient survival. Patients with lower SHP expression showed significantly poorer survival than those with higher, suggesting that SHP may function as a tumor suppressor in human liver. SHP gene expression was high in normal liver and SHP negatively correlated genes were enriched in liver cancer. Of the negatively correlated genes, those regulating cell growth and proliferation in cancer cells were included (CDK4, MCM5, EXOCS1, CCNB1, BUB3 and BCL2L2), suggesting that SHP might modulate cell proliferation in cancer cells by negatively regulating those genes either directly or indirectly. Since SHP negatively regulates cell proliferation related gene sets, poorer survival of liver cancer patients with low expression of SHP might be due to higher proliferation of cancer cells. SHP governs FXR, PXR, LXRα and HNF1B which are key regulators maintaining glucose levels in liver. According to this study, SHP is a good prognostic factor by possibly inhibiting a subset of genes involved in cell proliferation (Park et al., 2010).

It has been shown that UDCA is able to inhibit CDCA activation of FXR in a manner parallel to its ability to antagonize other CDCA-induced signalling cascades, such as that leading to apoptosis. Thus an enhanced proportion of UDCA in the nucleus of normal hepatocytes would be expected to cause an up-regulation of Cyp7a1 and Cyp8b1 by lowering the BA/FXR-mediated repression of Cyp7a1 and Cyp8b. According to this study, during hepatocarcinogenesis Cyp8b1, but not Cyp7a1, was up-regulated, whereas Cyp27, whose expression is not believed to be controlled directly by FXR/SHP, was significantly down-regulated. Thus, maintenance of the expression levels of Cyp7a1, together with down-regulation of Cyp27 and increased expression of Cyp8b1, probably accounted for the

increased proportion of CA, whereas that of CDCA and other non-C12-hydroxylated BAs, such as ·-muricholic acid, was decreased. This study indicated that mechanisms for the control of the expression of key enzymes in BA synthesis involving FXR, SHP or FTF play a minor role during hepatocarcinogenesis (Monte et al., 2005).

In vitro studies. The synthetic FXR agonist GW4064 reduced cPLA2 expression in a concentration-dependent manner in immortalized mouse cholangiocytes, although COX-2 expression was unchanged. Furthermore, cPLA2 activity was reduced about 30% by both GCDC and GW4064. It was found that GW4064 suppressed PG production in a concentration-dependent manner. The results of this study showed that a synthetic FXR ligand down-regulated cPLA2 expression and activity to reduce GCDC-induced COX-2 activity, defined as PG production, in immortalized mouse cholangiocytes. GCDC induced COX-2 expression via the EGFR/MAPKs cascade, while it reduced cPLA2 expression via FXR signaling in cholangiocytes. According to this study, a proposed intracellular pathway for BA-induced PG production in cholangiocytes includes FXR activation after GCDC is taken up by ASBT. At the same time, GCDC induced COX-2 expression via the EGFR/MAPKs cascade. In contrast, GW4064 selectively activated FXR, and it down-regulated cPLA2 to decrease arachidonic acid release from membrane phospholipids and reduce COX-2 activity (defined as PG production). BAs are considered to be involved in biliary tract carcinogenesis, although the underlying mechanisms are not well understood. In this study, the effects of GW4064 on immortalized mouse cholangiocytes showed that GW4064 could protect cholangiocytes against BA toxicity, suggesting that administration of this FXR agonist might be effective to prevent biliary tract carcinoma, especially in patients with cholestatic liver disease (Komichi et al., 2005).

In another report, FXR was shown to be a negative modulator of NF-κB-mediated hepatic inflammation. Activation of FXR by its agonist ligands inhibited the expression of inflammatory mediators in response to NF-κB activation in both human hepatoma HepG2 cells and primary hepatocytes cultured *in vitro*. Additionally, FXR activation suppressed NF-κB transcriptional activity by decreasing the binding between NF-κB and DNA sequences. In addition to its roles in regulating proinflammatory genes, another major function of NF-κB is to regulate many antiapoptotic genes, including members of Bcl-2 family such as Bcl-xL and Bfl-1/A1 as well as the cellular inhibitors of apoptosis, cIAP1 and cIAP2, TRAF1, TRAF2, and GADD45ß. The results suggested that FXR activation selectively inhibited NF-κB target genes for hepatic inflammation but not antiapoptotic genes. In addition, NF-κB activation induced by TNF-α, repressed FXR activation induced by its ligand GW4064 in hepatocytes of wild-type mice. Treatment with GW4064 dramatically induced FXR reporter activity in the presence of FXR/RXR in HepG2 cells. TPA and LPS strongly repressed GW4064-induced FXR reporter activity. The transactivation of NF-κB by overexpression of p65 also repressed GW4064-induced FXR reporter activity in a dose-dependent manner, suggesting that activation of NF-κB antagonizes FXR activity. Collectively, based on the fact that hepatic inflammation is closely linked to hepatocarcinogenesis, the authors suggested that the mutual suppression between FXR and NF-κB may be an important mechanism for preventing tumorigenesis. Such findings support the role of FXR as a central hepatoprotector and suppressor of hepatocarcinogenesis (Wang et al., 2008b).

In vivo studies. It was shown that TNF-α, IL-1α, and IL-2 were up-regulated in $FXR^{-/-}$ livers from 12-month-old mice compared with wild-type ones. Induction of hepatic iNOS, COX-2, interferon-inducible protein 10 (IP-10) and IFN-γ expression, in response to LPS, was significantly greater in $FXR^{-/-}$ mice compared with wild-type. The levels of alanine transaminase (ALT), a marker of liver damage, were also significantly increased by treatment with LPS in $FXR^{-/-}$ compared with wild-type mice. Both *in vitro* and *in vivo* results showed that NF-ÎB activation suppressed the expressions of FXR and its target genes SHP and BSEP. These results demonstrated that FXR is a negative regulator of the hepatic inflammation *in vivo* (Wang et al., 2008b).

In another study, both male and female FXR-/- mice spontaneously developed liver tumors. In contrast, no liver tumors were observed in wild-type mice of the same age. Pathological data confirmed that tumors were hepatocellular adenoma and carcinoma (Yang et al., 2007). In a similar study, mice lacking expression of FXR were found to develop toxic liver lesions and liver tumors at 12 months of age, with an incidence of 38% of mice having liver tumors. In this experiment, two major histological types of tumor were identified, hepatocellular adenoma or carcinoma and hepatocholangiocellular carcinoma, a very rare tumor in mice (Kim et al., 2007). Yang et al. (2007) showed that the levels of ALT in aging $FXR^{-/-}$ mice were much higher than those in wild-type mice of the same age. The total BA levels in serum and livers from aging $FXR^{-/-}$ and wild-type mice were measured and both serum and liver BAs were found significantly higher in $FXR^{-/-}$ mice compared with the wild-type controls. Genes involved in inflammation and cell cycle were up-regulated in aging $FXR^{-/-}$ mice but not in wild-type controls. In the absence of FXR, CYP7a and CYP8b were moderately increased in young $FXR^{-/-}$ mice. NTCP is a basolateral BA transporter that pumps BAs into liver, and its expression is inhibited by FXR activation. In the absence of FXR in aging mice, NTCP expression increased. SHP is a primary target of FXR and is a key factor that mediates the down-regulation of CYP7a gene. The level of SHP expression was much lower in aging $FXR^{-/-}$ mice. Additionally, the results indicated that both cyclins D1

and E1 expression was strongly increased in the liver of aging FXR$^{-/-}$ mice compared with the wild-type controls. As it was shown, mRNA levels of proinflammation factors IFNγ, TNFα and IL-6, were significantly higher than those in wild-type controls. In contrast, the expression of COX-2 did not change. Compared with the standard diet, cholestyramine feeding significantly reduced the number and size of liver malignant lesions in aging FXR$^{-/-}$ mice. These findings indicated that metabolic defects such as chronically higher levels of BAs can promote liver tumor formation, thus suggesting an intriguing link between metabolic regulation and hepatocarcinogenesis. Kim et. al., in order to further investigate the mechanism contributing to the formation of liver tumors in FXR$^{-/-}$ mice, analyzed the expression of genes involved in inflammation and cell proliferation. Only IL-1, mRNA levels were increased in 3- and 12-month-old FXR$^{-/-}$ mice as compared to wild-type ones. The oncogene c-myc mRNA was elevated in FXR$^{-/-}$ mice with higher levels noted in younger mice. The protein level of c-myc was accordingly increased in FXR$^{-/-}$ mice at both ages examined. ß-Catenin was increased about 30% over controls in 3-month-old mice, and more than double in 12-month-old FXR$^{-/-}$ ones as compared to wild-type, but the protein level of ß-Catenin did not show detectable differences. Proliferating cell nuclear antigen (PCNA) mRNA was markedly elevated in 3-month-old FXR$^{-/-}$ mice in non-neoplastic hepatic parenchyma. In 12-month-old FXR$^{-/-}$ mice, PCNA mRNA expression was signficantly decreased. In conclusion, it was suggested that the increased BA levels could induce IL-1ß, which mediates cell proliferation, differentiation, and apoptosis. Probably, IL-1, indirectly results in expression of ß-catenin and c-myc, which eventually lead to tumorigenesis. Consequently, according to these studies, FXR deficiency and hepatic BAs play a role as key mediators of inflammation-induced hepatocarcinogenesis.

FXR activation by its ligands, CDCA and GW4064, rescued the serum deprivation-induced apoptosis in human hepatoma HepG2 cells, in a dose dependent manner, by activating the MAPK/ERK1/2 pathway. In this study, FXR$^{-/-}$ mice exhibited an exacerbated liver apoptosis and lower levels of phosphorylated-ERK1/2 compared to wild type mice after starvation. The authors suggested that the previously observed beneficial effects of FXR activation might result from maintaining BA homeostasis and suppressing cell death (Wang et al., 2008c).

SHP, which is an FXR target, negatively regulated tumorigenesis both *in vivo* and *in vitro*. SHP$^{-/-}$ mice aged 12 to 15 months old developed spontaneous hepatocellular carcinoma, which was found to be strongly associated with enhanced hepatocyte proliferation and increased cyclin D1 expression. In contrast, overexpressing SHP in hepatocytes of SHP transgenic mice reversed this effect. These results provided the first evidence, according to the authors, that SHP plays tumor suppressor role in hepatocellular carcinoma (Zhang et al., 2008a,b). In another study, the expression of SHP was diminished in HCC pathologic specimens and cell lines by epigenetic silencing owing to SHP promoter hypermethylation, while *in vitro* methylation decreased SHP promoter transactivation, an event that was reversed by demethylation. Over-expression of SHP inhibited HCC tumor growth in xenografted nude mice, and increased the sensitivity of HCC cells to apoptotic stimuli (He et al., 2008).

Chemopreventive activities of farnesol and geraniol were evaluated in a study, during the initial phases of hepatocarcinogenesis, in which rats received during eight consecutive weeks 25 mg/100 g body weight farnesol, geraniol, or corn oil (control). Compared to controls, farnesol and geraniol fed rats showed reduced preneoplastic lesions cell proliferation and DNA damage, but only geraniol fed rats showed increased preneoplastic lesions apoptosis. No differences were observed in this study, between the different groups, regarding hepatic levels of FXR. Results indicated that farnesol and geraniol could represent promising chemopreventive agents against hepatocarcinogenesis. According to this study, FXR did not seem to be involved in the isoprenoids' chemopreventive activities (Ong et al., 2006).

The main mechanisms affecting cell proliferation and apoptosis that are involved in FXR-induced suppression of hepatocarcinogenesis, are schematically presented in Figure 3A.

Pancreatic cancer

Pancreatic cancer is one of the most aggressive human cancers with more than 200,000 deaths worldwide every year (Raimondi et al., 2009). In a recent study, the baseline expression of FXR was determined in a panel of human pancreatic cancer cell lines including AsPC-1, Capan-1, Capan-2, MIA-PaCa2 and PANC-1. It was found that 70.6% of pancreatic cancer tissues with lymph node metastasis were positive for FXR expression, while in pancreatic cancer tissues without lymph node metastasis, positive expression of FXR was found only in 17.6% of them, thus, revealing that the prognosis of patients with FXR-expressing tumours was significantly poorer than that of those with non-expressing ones (Lee et al., 2011).

In vitro data from this study showed that the down-regulation of FXR expression in MIA-PaCa2 and PANC-1 cells inhibited cell proliferation and decreased cell migration in both cancer cell lines. Down-regulation of FXR by siRNA transfection decreased NF-κB DNA-binding activity and VEGF levels. Additionally, in both cell lines, treatment with guggulsterone inhibited cell proliferation in a dose-dependent manner and decreased cell migration and invasion. Both MIA-PaCa2 and PANC-1 cells increased cell migration and invasion by treatment with the FXR agonist GW4064, while post-treatment SHP mRNA levels were not altered.

The naturally derived isoprenoids perillyl alcohol,

farnesol, and geraniol exhibited an additive anti-proliferative effect against MIA PaCa-2 human pancreatic cancer cells and induced a G0/G1 cell cycle arrest that coincided with an increase in the expression of the cyclin kinase inhibitor proteins p21Cip1 and p27Kip1 and a reduction in cyclin A, cyclin B1, and cyclin-dependent kinase (Cdk) 2 protein levels. Similar findings were given in BxPC-3 human pancreatic adenocarcinoma cells. Although farnesol is a weak activator of FXR and such activation could not be considered as a general mechanism in farnesol-induced apoptosis, the exact mechanisms of those compounds antiproliferative effect is not yet well defined and the possibility of their interaction with farnesoid receptors cannot be excluded (Wiseman et al., 2007). In another study, after 48 hours of treatment with farnesol, geraniol or perillyl alcohol, BxPC3 pancreatic cancer cells exhibited a 3 to 10-fold increase in apoptosis and higher Bak expression than the controls (Burke et al., 2002).

Finally, *in vivo* results of the former study showed that pancreatic carcinoma incidence was decreased in animal models fed with perillyl alcohol and farnesol diets, while higher apoptotic rates and diminished expression of the antiapoptotic protein BCL-XL were found compared to control.

FXR and other types of cancer

Breast cancer

FXR expression in breast cancer. Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths (Jemal et al., 2011). In a study, immunohistochemical analysis identified the expression of FXR in the ductal epithelial cells of normal breast tissue. FXR was also present at high levels in infiltrating ductal adenocarcinoma cells (Swales et al., 2006). FXR expression was shown in human breast tumor samples and in breast carcinoma cell lines MCF-7 (ER-positive) and MDA-MB-231 (ER-negative). In addition, immunohistochemical analyses on 65 breast carcinoma samples established significant correlations between FXR expression and ER, Ki-67, and topoisomerase-II alpha expressions. Data from this study suggested that FXR expression could be associated with a poor prognosis subgroup of ER-positive breast carcinomas (Journe et al., 2008). In a similar study, a higher FXR expression in ER-positive luminal-like tumors, as compared to ER-negative breast tumors was revealed. These data support the hypothesis of a lower FXR expression in less differentiated tumor cells, such as ER-negative breast carcinoma cells. Moreover, in the ER-positive subgroup of breast tumors, FXR expression was highly correlated with cyclin D and p27 protein expression. On the other hand, no association was observed between FXR expression and neoplasm recurrence in the total population or in the ER-positive subgroup. Interestingly, in patients presenting good prognosis, FXR expression was correlated with several proliferation factors, as cyclin D and p27, whereas in those presenting poor outcome, it was exclusively correlated with c-myc. Notably, the association between FXR expression and cell proliferation (Ki-67) was only noted in ER-positive tumors from postmenopausal patients (Journe et al., 2009). The mean plasma DCA concentrations were found 52% higher in patients with breast cancer compared to controls, supporting the hypothesis that DCA may be involved in the aetiology of this type of neoplasia (Costarelli and Sanders, 2002). On the other hand, according to a recent conference publication, FXR expression was upregulated in invasive cancer and progressively increased from normal breast tissue to malignancy. Additionally, FXR expression correlated with BMI in normal breast tissue, suggesting that FXR may be a molecular link between obesity and the development and progression of breast cancer (Kounalakis et al., 2009).

In vitro studies. Urokinase-type plasminogen activator (uPA) and receptor (uPAR), as well as F-actin, are considered as factors critical for the migration in human breast cancer (Rabbani and Xing, 1998; Kjoller and Hall, 2001). The BA DC or CDC at physiological concentrations induced the nuclear translocation of FXR and enhanced the gene expression of uPA and uPAR, as well as F-actin formation in human breast cancer cells MDA-MB-231. Only low levels of FXR gene expression were detectable unless MDA-MB-231 cells were incubated with DC. The significance of FXR activation for DC induced cell survival and migration was supported by the observation that the FXR antagonist Z-guggulsterone induced apoptosis, which could be counteracted by DC. The concentration of Z-guggulsterone required to induce apoptosis was significantly higher than that predicted by its affinity to FXR. This suggested that Z-guggulsterone triggered apoptosis by other cell-signaling pathways in addition to antagonizing the activation of FXR. The results of this study suggested that DC first binds to FXR, which triggers its own gene expression and that of uPA. Secreted uPA translocates to the cell surface by binding to uPAR in lipid membrane domains, which stimulates F-actin formation and cancer cell migration. According to the authors, nutritional strategies that decrease the systemic level of DC, or natural FXR antagonists such as guggulsterone, might be useful as breast cancer treatments (Silva et al., 2006).

The FXR activators CDCA and GW4064 induced apoptosis in the FXR-expressing breast cancer cell lines MCF-7 and MDA-MB-468 irrespective of ER status. CDCA had no effect on cell proliferation in this study. Although higher concentrations of GW4064 were required to induce cell death than to induce gene expression, the FXR dependency of this cell death was confirmed. Such results contrast to the previous mentioned study which suggested that BAs promote the

growth and metastasis of breast cancer through FXR. The effects of FXR ligands on the expression of transporters known to be FXR target genes were also examined. The FXR ligand GW4064 induced the expression of IBABP and MRP2. Many of the other known FXR target genes were not expressed in the MCF-7 cells irrespective of treatment, including BSEP, ASBT, OATP8 and SLC21A6. The expression of MDR3 was surprisingly reduced by GW4064. Both MRP1 and SLC7A5 were down-regulated by FXR ligand in MCF-7 cells. According to this study, MRP1 reduction may be beneficial in prevention of drug resistance to chemotherapeutic drugs. The expression of breast cancer resistance protein ABCG2 and the major vault protein LRP were unchanged. Additionally, FXR activation had no effect on the anticancer agent paclitaxel–induced MCF-7 cell death, a drug often susceptible to resistance. It was also shown that MCF-7 and MDA-MB-468 cell lines express both SHP and LRH-1 and that SHP was induced by treatment with GW4064. Aromatase expression was inhibited at both mRNA and protein levels by GW4064, suggesting that the FXR-SHP-LRH-1 cascade is active in the breast cancer cell lines and can be used to inhibit aromatase expression. Similarly, in MCF-7 cells, GW4064 suppressed the antiapoptotic GADD45h. According to the authors, FXR could represent a promising new therapeutic target for ductal breast cancer (Swales et al., 2006).

In vitro data demonstrated that farnesol-induced FXR activation caused mitogenicity in MCF-7 cells through a positive crosstalk with ER. The growth stimulation was completely suppressed by antiestrogens. In contrast, MDA-MB-231 cells appeared farnesol-insensitive, suggesting an involvement of ER in farnesol mitogenicity. In addition, farnesol exposure resulted in an increase of FXR protein level, suggesting activation-induced receptor upregulation. Evidence was provided that farnesol stimulated MCF-7 cells and that this stimulatory effect most probably occurred through an FXR-mediated activation of ER. ER downregulation induced in MCF-7 cells by farnesol exposure was accompanied by a proliferative response similar to that induced by estrogen agonists. In addition, farnesol increased PgR expression (used as a marker of ER-mediated gene transactivation) in MCF-7 cells. This demonstration of potential FXR crosstalk with ER in breast cancer cells calls for some caution regarding the clinical use of FXR ligands, because of possible mitogenic effects on breast cancer tissue (Journe et al., 2008).

Additionally, *in vitro* data indicated that FXR activation by CDCA had no effect in breast cancer cell lines in estrogen-containing medium, while it stimulated the proliferation of the ER-positive cell line MCF-7 in steroid free medium (an experimental condition mimicking low levels of circulating estrogens in postmenopausal women). According to this study, the correlation between FXR expression and tumor cell proliferation in postmenopausal patients could be extended to the case of breast cancer patients treated with aromatase inhibitors, as these drugs markedly decrease plasma estrogen level by inhibiting or inactivating aromatase enzymes. Thus, aromatase inhibitor treatments could disclose a possible crosstalk between FXR and ER, which may induce proliferative responses and lead to some forms of resistance to hormone therapy. The authors suggested that FXR could be used as a valuable biomarker to further characterize high and low proliferating tumors in ER-positive breast cancer cases, especially in estrogen deprived patients such as postmenopause women or others treated with aromatase inhibitors (Journe et al., 2009).

Murine breast carcinoma 4T1 cells were used to determine apoptosis and alteration of sphingolipid ceramide metabolism by DCA *in vitro*, while a syngeneic mouse model for breast cancer metastasis was used to quantify the effect of DCA on metastasis, *in vivo*. DCA reduced ceramide-induced apoptosis in breast cancer cells, and according to this study, VEGF/Flk-1 was a key factor in DCA-induced reduction in ceramide. The effect of the FXR antagonist Z-guggulsterone on the protein level of Flk-1 suggested that DCA promoted cell survival via a novel FXR-to-Flk-1 cell signaling pathway reducing pro-apoptotic ceramide. According to the authors, a mechanistic explanation was provided in that lowering DCA serum levels or blocking FXR with Z-guggulsterone would increase ceramide-induced apoptosis, thus reducing growth or spread of breast tumors (Krishnamurthy et al., 2008).

Future perspectives: apomine. Apomine (SR-45023A) has been identified as an FXR activator (Howard et al., 2000; Niesor et al., 2001). In a study, the effect of apomine on the growth of two breast cancer cell lines, MCF-7 and MDA-MB-231 was determined. Apomine caused significant growth inhibition of both cell lines after 72 h of treatment. Apomine-induced growth inhibition was associated with caspase and p38 MAPK activation, while this drug had no effect on Ras localisation, and addition of mevalonate failed to prevent apomine-induced apoptosis. According to this study, apomine induced apoptosis in breast cancer cells independentently of ER status and not via inhibition of the mevalonate pathway, thus raising the possibility that apomine induces apoptosis in breast cancer cells via FXR activation, to which it is known to bind (Lowe et al., 2005). In another study, apomine inhibited the growth of the majority of tested tumor cell lines derived from leukemia, colon, liver, ovary, among them the breast cancer cell line MCF-7. According to the authors, the overall profile on mevalonate synthesis inhibition, cell growth inhibition, and apoptosis suggested that apomine acted as a synthetic mimetic of farnesol, yet a direct mechanism of apoptosis implicating FXR was not demonstrated (Flach et al., 2000).

The main FXR target genes involved in breast tumorigenesis, and whether they are up- or down regulated, are presented in Figure 3B.

Testicular cancer

Overexpression of aromatase plays a significant role in excessive estrogen production sustaining tumorigenesis in Leydig cells. It was shown that FXR was expressed in tissues of normal and tumor Fisher rat testis and in Leydig normal and tumor cell lines. In R2C rat Leydig tumor cells, FXR activators CDCA and GW4064 down-regulated aromatase expression at both mRNA and protein levels, together with the inhibition of its enzymatic activity. Additionally, FXR activation did not induce SHP expression in Leydig tumor cells in which inhibition of the aromatase protein by CDCA occured even when this NR was knocked down. These results suggested that SHP is not required for the effect of the FXR ligand to downregulate aromatase expression, at least in R2C cells. Transient transfection experiments, using vector containing rat aromatase promoter PII, evidenced that CDCA reduces basal aromatase promoter activity. Data from this study suggested that FXR is able to compete with steroidogenic factor 1 (SF-1) in binding to a common sequence within the PII promoter of aromatase interfering negatively with its activity. Finally, CDCA induced growth inhibition in R2C cells, which was reversed in the presence of FXR dominant negative as well as after knocking down FXR with a specific siR. NA, thus addressing an FXR-dependence of this event. Knocking down aromatase enzyme reduced estradiol production by R2C cells upon aromatizable androgen exposure and exhibited as biological counterpart a decreased cell proliferation. The addition of CDCA could only slightly decrease cell growth demonstrating that the FXR activator through an inhibition of aromatase expression exerts an important role in reducing R2C cell proliferation. Consequently, these results demonstrated a new molecular mechanism through which FXR antagonizes estrogen signaling and inhibits Leydig tumor growth and progression (Catalano et al., 2010) (Fig. 3B).

Prostate cancer

Prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males, accounting for 14% (903,500) of the total new cancer cases and 6% (258,400) of the total cancer deaths in males in 2008 (Jenal et al., 2011). In the human prostate, androgens are inactivated in the form of hydrophilic glucuronide conjugates, and these metabolites are formed by the two human UGT2B15 and UGT2B17 enzymes. The present study demonstrated the presence of FXR in epithelial cells of the human prostate and established its activators as important regulators of androgen metabolism in prostate cancer LNCaP cells. FXR activation caused a dramatic reduction of UGT2B15 and UGT2B17 gene expression, resulting in a decreased glucuronide conjugation of the active androgen DHT and its reduced metabolites ADT and 3α-DIOL. Considering the importance of activator protein-1 (AP-1) factors in the pathogenesis of prostate cancer, it would be possible that the FXR-dependent regulatory pathway of UGT2B genes in LNCaP cells involves AP-1 factors. It was also observed that Ugt2b expression seemed to increase in the prostate from FXR-null compared with wild-type mice. Such results provided evidence for a likely physiological role of ADT as an FXR ligand in the human prostate. Additionally, during cholestasis plasma levels of BAs are drastically increased, and based on the present study, in such patients, glucuronidation of androgen may be reduced in the prostate tissue. The authors noted that, as GW4064 is considered as a promising treatment for cholestasis, it would be of importance to monitor circulating markers of prostate cancer in GW4064-treated patients. Overall, it is suggested that FXR may be an important regulator of androgen homoeostasis, as seen in Figure 3B (Kaeding et al., 2008; Verreault et al., 2010).

Clinical trials and other reports

Apomine induced cell death in the A375 human melanoma cell line through a novel membrane-mediated mechanism that is independent of caspase-3 activation. Apomine-mediated cell death in the A375 and UACC 3093 human melanoma cell lines was also independent of N-Ras farnesylation, which is a general described mechanism of action for apomine in other cancer cell types (Flach et al., 2000). Data from this study suggested a novel plasma membrane-mediated cytolytic pathway for apomine-induced cell death. Apomine is a known synthetic activator of FXR, but the authors claimed that the A375 human melanoma cell line was found containing very low levels of this NR (Pourpak et al., 2007). A phase II open-label trial of apomine in patients with refractory melanoma took place so as to evaluate the efficacy and safety of this drug. Patients received apomine 100 mg orally, twice daily (total dose 200 mg per day), continuously for 28 days. Stable disease was achieved in 2 patients (5%), while no complete or partial responses were observed. Progression free survival of at least 16 weeks was observed in 6 patients (14%). The median overall survival was 6.1 months. Abdominal pain was the most frequent adverse event occurring in 26% of patients. In this study, apomine failed to produce a 30% progression free survival rate at 16 weeks, which was considered a meaningful benefit for further development. According to the authors, it cannot be excluded that through the activation of FXR apomine induces its antineoplastic effects (Lewis et al., 2006).

Additionally, in another phase I pharmacokinetic trial and correlative *in vitro* phase II tumor kinetic study, apomine proved remarkably active against 35 fresh ovarian cancer samples with 63–91% of ovarian tumors being sensitive. This activity level was comparable with that determined for cisplatin, carboplatin, and topotecan

and was considerably higher than that for paclitaxel, at clinically achievable concentrations. According to the authors, apomine-induced loss of cell survival could be mediated through an FXR activation mechanism (Alberts et al., 2001).

A number of studies have demonstrated that farnesol and related isoprenoids, including geraniol and perillyl alcohol, inhibit cell proliferation and induce apoptosis in a broad range of malignant cell types including pancreatic adenocarcinoma, lung adenocarcinoma, hepatoma, melanoma, leukemia, colorectal carcinoma and oral squamous carcinoma (Yu et al., 1995; Sahin et al., 1999; Joo et al., 2007; Wiseman et al., 2007; Au-Yeung et al., 2008; Scheper et al., 2008). Although farnesol is a weak activator of FXR, it is able to inhibit cell proliferation and induce apoptosis in a number of cell types that do not express FXR, suggesting that FXR activation is not a general mechanism in farnesol-induced apoptosis (Joo and Jetten, 2010).

Conclusion

In this study, evidence was provided about the role of FXR in crucial biochemical and cellular processes, and its involvement in the pathogenesis of several diseases, including cancer. FXR affects several metabolic pathways through its specific target genes, regulating BA synthesis and homeostasis, glucose and lipid metabolism, development of atherosclerosis, intestinal bacterial growth and liver regeneration. Additionally, FXR is involved in the pathogenesis of different cholestatic diseases, as well as non-alcoholic fatty liver disease and inflammatory bowel disease.

FXR deficiency resulted in increased intestinal tumorigenesis and colon cell proliferation, which was accompanied by an up-regulation in the expression of genes involved in cell cycle progression and inflammation, including cyclin D1 and IL-6, and a promotion of Wnt signaling via increased infiltrating neutrophils and TNFα production. Consequently, FXR deficiency promoted inflammation, cell proliferation, and tumorigenesis in the intestine, suggesting that activation of FXR might be a promising strategy in the treatment of colon cancer. Additionally, FXR deficiency in mice led to the development of spontaneous hepatocarcinomas. Increased BA levels induced IL-1ß

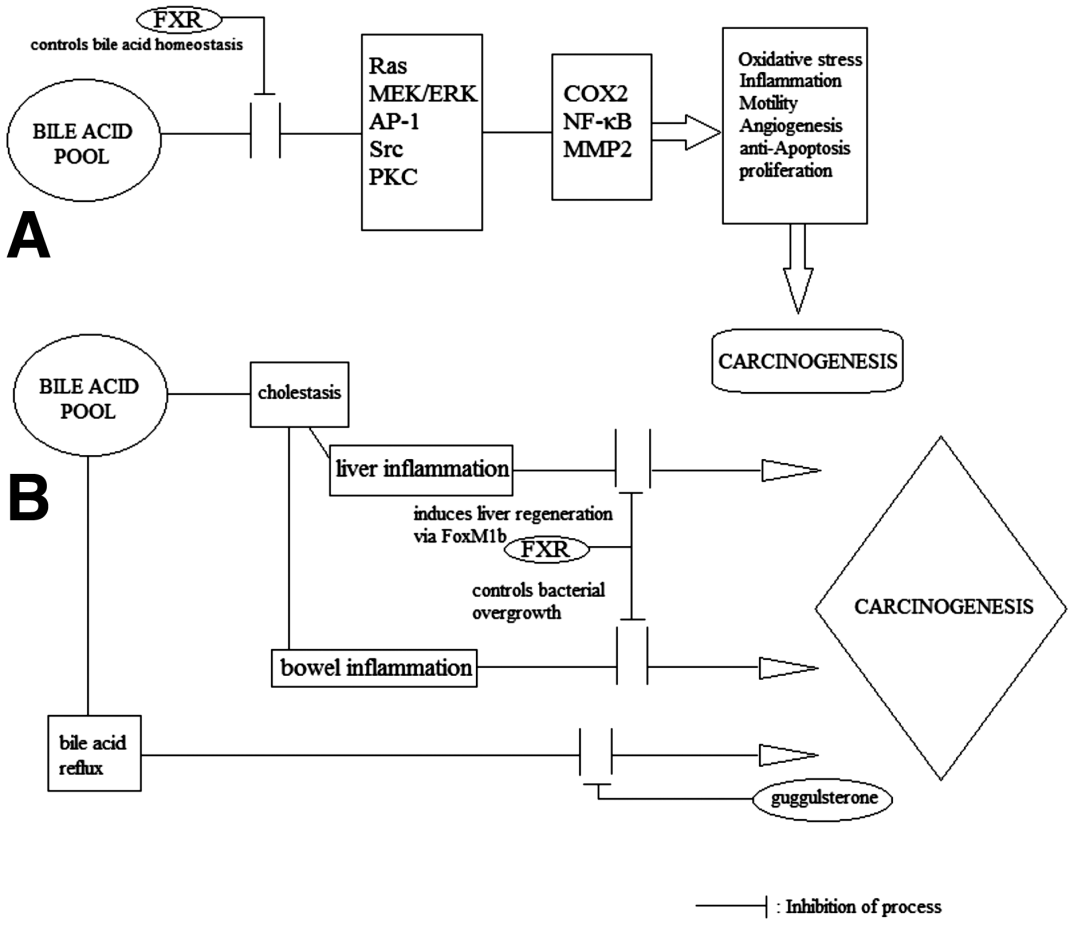

Fig. 4. A. BAs are involved in cancer of the gastrointestinal tract. Pathways possibly implicated in BA signaling in cancer include AP-1 proto-oncogene which is activated through PKC and ERK signaling, while COX-2 might be responsible for biological effects such as antiapoptosis, motility and invasion. **B.** BA reflux in esophagus, cholestasis and liver inflammation, bacterial overgrowth and bowel inflammation are possibly implicated in tumorigenesis. FXR maintains BA homeostasis, controls intestinal bacterial growth and has a role in liver regeneration through FoxM1b. Thus, FXR seems to protect against carcinogenic effects of BAs

expression, which is considered a mediator of cell proliferation, differentiation, and apoptosis. IL-1ß indirectly resulted in expression of ß-catenin and c-myc, which eventually led to tumorigenesis. Furthermore, activation of FXR by its agonist ligands inhibited the expression of hepatic inflammatory mediators in response to NF-κB activation, supporting the role of FXR as a central hepatoprotector and suppressor of hepatocarcinogenesis. It was shown that FXR was focally overexpressed in Barrett's esophagus and that treatment with the FXR antagonist guggulsterone, significantly enhanced apoptosis in a human BE cell line. This was related to an increased expression of antiapoptotic proteins such as Bclx-l and Bcl-2, and a decrease in proapoptotic factors such as Bax. Thus, FXR inhibition might represent a novel therapeutic approach in BE (Fig. 3A).

In breast cancer cell lines, FXR agonists down-regulated the breast cancer target gene aromatase and the transporters MDR3, MRP-1, SLC7A5, and inhibited cell proliferation. Several FXR target genes such as SHP, IBABP and MRP2 were up-regulated. FXR seemed to play a protective role in breast cancer development, and might be considered as an antineoplastic drug for future development. Other studies showed that ER-positive breast tumors could be stimulated to proliferate via a crosstalk between FXR and ER, particularly in a state of estrogen deprivation. Additionally, the FXR activator farnesol, exerted a mitogenic effect on MCF-7 cells, while MDA-MB-231 cells appeared farnesol-insensitive, suggesting an involvement of ER in farnesol mitogenicity. Finally, FXR antagonized estrogen signaling and inhibited Leydig tumor growth and progression, while it was also suggested that FXR may be an important regulator of androgen homoeostasis (Fig. 3B).

BAs have been considered as etiologic agents in cancer, especially of the gastrointestinal tract. Carcinogenesis-related BA exposure includes induction of reactive oxygen (ROS) and reactive nitrogen species (RNS) and induction of DNA damage, thus stimulating mutations that lead to induction of apoptosis. These effects have been mainly reported in relation to esophageal and colorectal cancer, but also to some extent in relation to other types of cancer (Bernstein et al., 2009). Pathways possibly implicated in BA signaling in cancer include the activator protein-1 (AP-1) proto-oncogene which is activated through protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) signaling. COX-2 is a target of AP-1 and might be responsible for biological effects such as antiapoptosis, motility and invasion. PKC is activated through the Src tyrosine kinase, while Ras and caveolins are upstream regulators of PI3K and Raf (Debruyne et al., 2001) (Fig. 4A).

Chronic exposure to BAs may play an important role in carcinogenesis. BA reflux in esophagus, cholestasis and liver inflammation, as well as bacterial overgrowth and bowel inflammation are possibly implicated in tumorigenesis. FXR has a key role in maintaining BA homeostasis and activation of FXR induces the expression of IBABP and ileal BA transporters, thus regulating BA metabolism via a negative feedback pathway. Additionally, FXR is critical for controlling intestinal bacterial growth and maintaining a competent barrier by inducing a number of intestinal genes, including iNOS and IL-18, which are involved in enteroprotection, while FoxM1b, important for liver regeneration, was recently identified as an FXR target gene. Based on this evidence, FXR seems to play an important role in protecting against carcinogenic effects of BAs (Fig. 4B).

The role of FXR in growth regulation, apoptosis, and cancer is still under evaluation, as separate controversial studies have established both positive and negative correlations between FXR expression and cancer. The mechanisms through which FXR mediates its effects on carcinogenesis are not well understood. Additionally, although apomine has been identified as an FXR agonist, apomine-induced cancer cell growth inhibition and apoptosis cannot be exclusively related to FXR activation. Similarly, FXR activation is not a generally accepted mechanism in farnesol-induced apoptosis via inhibition of the mevalonate pathway. At least two phase I clinical trials take place so as to study the effectiveness of the synthetic FXR agonist apomine in treating patients who have advanced or metastatic solid tumors and have not responded to previous treatment. The aim of these trials is to determine the maximum tolerated dose, the toxic effects, the pharmacokinetic profile, and the antitumor activity of apomine administered orally in these patients (National Institutes of Health (NIH), Clinical Trials. Available at http://www.clinicaltrials.gov).

Further studies are required in order to establish growth-inhibitory and antitumor effects of this nuclear receptor. From a therapeutic point of view, future strategies aimed at reactivating or inhibiting FXR expression may be useful in the treatment of certain types of human cancer.

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