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Review

The farnesoid X receptor and colon cancer

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ABSTRACT

Worldwide, colorectal cancer (CRC) is a leading cause of cancer death, primarily because of limited therapeutic options for those with advanced disease. The farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily of ligand-activated transcription factors. Besides its prominent role in bile acid synthesis, and lipoprotein and glucose metabolism, recent data indicate that FXR also plays a key role in regulating intestinal cell proliferation and carcinogenesis. Here, we review the role of FXR as a tumor suppressor in CRC, with particular emphasis on the molecular mechanisms underlying FXR-dependent tumorigenesis and its regulation, FXR-bile acid relationships and FXR-targeted drugs as potential therapeutic agents.

Key words: Colon cancer; farnesoid X receptor; nuclear receptor; tumor suppressor

INTRODUCTION

Despite advances in screening and treatment, colorectal cancer (CRC) results in over 50,000 deaths yearly and may soon surpass lung cancer as the overall leading cause of cancer-related death in the USA alone.[1,2] Despite increased efforts to improve access and compliance, many people neglect CRC screening. In addition, the efficacy of colon cancer screening is limited by the limited sensitivity of tests, "miss" rates on colonoscopy and other factors. Chemoprevention using non-steroidal anti-inflammatory drugs is marginally effective[3,4] but limited by gastrointestinal (GI)[5] and cardiovascular[6] toxicity that led to the withdrawal of rofecoxib.[7] Non-surgical treatments (e.g. chemotherapy and radiation) for advanced colon cancer have limited efficacy. Although the use of biologicals that target vascular endothelial growth factor and epidermal growth factor receptor (EGFR) (i.e. bevacizumab, cetuximab and panitumumab) may increase survival with advanced CRC by several months, these agents have a limited impact on 5-year survival, on the order of only 10%.[8-10] Moreover, their use is limited by off-target toxicity that commonly reduces patient tolerance; EGFR, which is expressed widely in non-intestinal epithelial cells (e.g. dermal epithelial cells),[11] causes skin reactions that may force discontinuation of treatment.

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FARNESOID X RECEPTOR AND ITS LIGANDS

Farnesoid X receptor (FXR) [nuclear receptor subfamily 1, group H, member 4 (NR1H4)] is a member of the nuclear receptor superfamily of ligand-activated transcription factors and acts as a bile acid sensor.[12-14] FXR regulates the expression of genes involved in bile acid synthesis, and cholesterol and triglyceride metabolism by binding to their promoters as a homo- or hetero-dimer with a common partner of nuclear receptors, retinoid X receptor. FXR agonists include naturally-occurring bile acids (e.g. chenodeoxycholic acid [CDCA; EC50 of 10-50 μmol/L]),[15] synthetic compounds GW4064 (EC50 of 15 nmol/L),[16] 6E-CDCA (EC50 of 99 nmol/L),[17] WAY-362450 (EC50 of 4 nmol/L)[18] and fexaramine (EC50 of 25 nmol/L);[19] FXR antagonists include plant-derived guggulsterone[20] and synthetic AGN34.[21] The FXR agonist fexaramine is poorly absorbed following oral administration; thus, it acts as an intestine-restricted FXR agonist without systemic side-effects.[19] Oral administration of fexaramine results in serum levels that are an order of magnitude lower than those obtained following intraperitoneal injection of the drug, and it activates FXR target genes only in the GI tract.[19]

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FXR EXPRESSION AND REGULATION IN NORMAL INTESTINAL MUCOSA

FXR is expressed primarily in the GI tract, liver and kidney.[22,23] Modica *et al*.[24] showed that murine FXR (Nr1h4) is expressed at high levels in the small intestine and colon, whereas human FXR (NR1H4) is expressed at moderate levels in the colon. FXR expression is localized primarily to fully differentiated cells lining the intestinal epithelium of the ileum and colon.[24] In the *Apc*$^{min/+}$ murine model of CRC, FXR messenger RNA (mRNA) levels were down-regulated in tumor tissue compared with adjacent normal mucosa. Likewise, in patients with familial adenomatous polyposis (FAP) syndrome, FXR mRNA expression was decreased in normal and neoplastic tissues. In a human CRC cell line, HT-29 cells, restoring wild-type APC protein induced FXR expression, suggesting that APC may directly or indirectly regulate FXR expression.[24] FXR can also be regulated at the transcriptional level by the caudal-related homeobox 2.[25] Moreover, Bailey *et al*.[26] showed that DNA methylation and KRAS signaling silence FXR in human CRC. In approximately, 12% of human colon cancers and in several colon cancer cell lines, including SW620, FXR promoter methylation of a CpG island results in very low FXR expression.[26] Cabrerizo *et al*.[27] found FXR promoter methylation at two additional CpG islands (-358 and -1890 bp). Furthermore, functional analysis of the 5'-promoter region of the human FXR gene in HepG2 cells suggests that hepatic nuclear factor 1a may be a transcription factor for FXR.[28]

FXR IS AN INTESTINAL TUMOR SUPPRESSOR

In addition to its essential role in regulating lipid metabolism, emerging evidence supports a key role for FXR as an intestinal tumor suppressor. In two mouse models of CRC, *Apc*$^{min/+}$ and chronic colitis, Modica *et al*.[29] showed that FXR deficiency increased adenoma size and number. In a xenograft model, they showed that FXR reactivation via adenoviral infection blocked tumor growth. Using *Apc*$^{min/+}$ and azoxymethane-induced mouse models of CRC, Maran *et al*.[30] confirmed that FXR was an intestinal tumor suppressor. Smith *et al*.[31] showed that activating FXR with sodium taurocholate markedly reduced adenoma formation in *Apc*$^{min/+}$ mice.

FXR is down-regulated drastically in colon tumors from both murine (*Apc*$^{min/+}$) and human FAP models of CRC.[24] FXR mRNA expression is reduced in colon adenomas and even more profoundly in colon adenocarcinomas.[32,33] Diminished FXR expression is associated with advanced CRC stage and an adverse prognosis.[26,33]

Colon cancer risk increases substantially with chronic intestinal inflammation as in inflammatory bowel disease, including both Crohn's and ulcerative colitis (UC).[34,35] FXR activation decreases the production of pro-inflammatory cytokines, such as interleukin (IL) 1-beta, IL-2, IL-6, tumor necrosis factor-alpha and interferon-gamma, thereby reducing inflammation and intestinal permeability.[36] Torres *et al*.[37] showed that FXR expression was inversely correlated with neoplastic progression and the severity of colonic inflammation in UC. FXR expression is also reduced in colonic mucosa from patients with primary sclerosing cholangitis (PSC) and UC-associated neoplasia. Compared to patients with UC alone, those with PSC-UC have diminished FXR expression in the right colon suggesting they are at a higher risk of proximal colon neoplasia.[37]

FXR AND COLON CARCINOGENESIS

Although the above observations strongly implicate FXR as a tumor suppressor, the underlying mechanism is incompletely understood. No mutations have been identified in the FXR gene in CRC.[26] Several studies suggest the role of FXR in colon carcinogenesis is multifactorial. Modica *et al*.[29] showed the importance of Wnt signaling and apoptosis downstream of FXR. FXR promotes Wnt signaling with the expansion of basal proliferative intestinal cells, and a concomitant reduction in the apoptosis-competent apical epithelium. When FXR is activated in CRC cells, induction of apoptosis results in the removal of genetically altered tumor cells. The same investigators showed that FXR activation increased expression of several pro-apoptotic genes, including FAS, BAK1, P21, KLF4, FADD, CAS9 and P27. Maran *et al*.[30] showed that FXR deficiency increases intestinal cell proliferation, accompanied by up-regulation of cyclin D1 and IL-6. In addition, it was shown that sodium taurocholate inhibits intestinal tumorigenesis by activating FXR, leading to increased Shp expression and consequent down-regulation of cyclin D1.[31]

Several other potential mechanisms may account for FXR inhibition of intestinal tumor genesis. Peng *et al*.[38] showed that Src-mediated cross-talk between FXR and the EGFR inhibited human intestinal cell proliferation *in vitro* and growth of human colon cancer xenografts in nude mice. Yang *et al*.[39] has showed that FXR is a transcription factor for microRNA-22, and also a tumor suppressor which silences cyclin A gene expression in colon cancer cells. In inflammation-associated intestinal neoplasia, activation of FXR is repressed by pro-inflammatory cytokines that activate intestinal nuclear factor-kB signaling;[40] the investigators concluded that FXR not only inhibits inflammation, but also is targeted by the inflammatory response, resulting in a vicious cycle where reduced FXR activity causes less repression of inflammation. Zhou *et al*.[41] also showed that activation of the PPARα-UGT axis repressed intestinal FXR-FGF15/19 feed-back and exacerbates experimental colitis, thereby possibly promoting intestinal tumorigenesis. In mice, both PPARα knockout and treatment with recombinant FGF19 strongly attenuated dextran sulfate sodium-induced colitis.[41]

ROLE OF BILE ACIDS IN FXR-MEDIATED INHIBITION OF TUMORIGENESIS

Colon cancer is often linked to a Western diet, rich in carbohydrates and saturated fatty acids.[42-44] Subjects who consume a Western diet and patients with CRC have elevated levels of fecal secondary bile acids, mostly lithocholic acid (LCA) and deoxycholic acid (DCA), implicating bile acids as contributing factors in colon carcinogenesis.[45-48] Although controversial, cholecystectomy, which increases intestinal bile acid levels, may predispose persons to CRC.[49,50] Nonetheless, recent evidence suggests that FXR inhibits intestinal tumorigenesis through a bile acid-independent mechanism. Degirolamo *et al*.[51] showed that FXR deficiency, not elevated bile acid levels, mediated susceptibility to intestinal tumorigenesis. The tumor-promoting activity of bile acids does not occur as a function of their ability to activate FXR in the intestines.[29,51] Raufman *et al*. showed that several bile acids, including DCA and LCA, promoted colon carcinogenesis and cell proliferation by interacting with M3 muscarinic receptors that are overexpressed in a majority of colon cancers and human colon cancer cells through transactivation of EGFR.[52-55] Although the role of FXR as an intestinal tumor suppressor might not be directly mediated by bile acids, FXR activation can have tumor-suppressive effects by transcriptional induction of detoxifying enzymes that mediate transformation and excretion of toxic bile acids.[51] Interestingly, FXR's role in liver cancer (hepatocellular carcinoma) as a tumor suppressor may be mediated by bile acids.[56-59]

FUTURE DIRECTIONS

In addition to being a master regulator of bile acid synthesis, and glucose and fat metabolism, recent research data reveal a novel and important role for FXR as a tumor suppressor in intestinal carcinogenesis, cell proliferation and tumor growth. Because FXR is considerably down-regulated in colon tumor cells, restoring or reactivating FXR expression may offer a therapeutic strategy. In addition, because normal intestinal epithelial cells express high levels of FXR, pharmacological FXR agonists might be effective chemopreventive agents, particularly in high-risk populations, including those with hereditary CRC (e.g. FAP and Lynch syndrome). To avoid systemic toxicity associated with FXR activation (e.g. 6E-CDCA can cause pruritus)[60] intestine-specific FXR agonists, like fexaramine may be especially useful.[19]

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Conflicts of interest

There are no conflicts of interest.

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