Farnesoid X receptor represses matrix metalloproteinase 7 expression, revealing this regulatory axis as a promising therapeutic target in colon cancer

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Abstract

The farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily of bile acid-activated transcription factors and an important regulator of cell proliferation, apoptosis, and Wnt signaling. Down-regulated expression of FXR plays an important role in some malignancies such as colon cancer, and in rodent models of intestinal neoplasia, FXR knockout increases the size and number of colon tumors. These previous observations implicate FXR as a tumor suppressor, but the underlying molecular mechanisms are unclear. Employing complementary experimental approaches and using human colon cancer specimens, human and murine colon cancer cell lines, and FXR transgenic mice, here we identified an additional, potentially important role for FXR. We observed an inverse relationship between the expression of FXR and matrix metalloproteinase-7 (MMP7), a collagenase and signaling molecule consistently associated with colon cancer progression. We noted that FXR gene ablation increases MMP7 expression. Consistent with this finding, FXR overexpression and a dominant-negative FXR mutation reduced and augmented, respectively, MMP7 expression. Of note, MMP7 was the only MMP gene family member whose expression was down-regulated after FXR activation. FXR-mediated regulation of MMP7 transcription did not require heterodimerization with the retinoid X receptor (RXR), indicating that FXR represses MMP7 expression independently of RXR. Lastly, we uncovered that FXR suppresses MMP7 transcription by binding to a negative FXR-responsive element in the 5′ MMP7 promoter, an event that inhibited colon cancer cell proliferation and invasion. These findings identify the FXR–MMP7 axis as a potential therapeutic target for managing colon cancer.

Introduction

The farnesoid X receptor (FXR; encoded by NR1H4), a member of the nuclear receptor superfamily of ligand-activated transcription factors, is expressed at high levels in the mammalian liver and intestines. An important function of FXR is as a bile acid sensor that regulates feedback repression of hepatic bile acid synthesis (1-3). As a homodimer or heterodimer with the retinoid X receptor (RXR), FXR binds to the promoter regions of several genes involved in bile acid synthesis and transport thereby regulating their expression and actions, as well as modulating cholesterol and triglyceride metabolism. A key mechanism whereby FXR mediates feedback suppression of bile acid synthesis is by repressing the expression of cholesterol 7 alpha-hydroxylase (CYP7A1), the rate-limiting enzyme in the metabolic pathway from cholesterol to bile acids.

In addition to naturally-occurring bile acids [most potently chenodeoxycholic acid (CDCA; EC50, 10-50 µM) (4), more potent FXR agonists were developed [e.g. GW46064 (EC50, 15 nM) (5), 6E-CDCA (EC50, 99 nM) (6) and WAY-362450 (EC50, 4 nM)] (7)]. The U.S. Food and Drug Administration approved a highly potent synthetic FXR agonist, obeticholic acid (EC50, 99 nM), for the treatment of primary biliary cholangitis, a progressive disease associated with increased circulating and hepatic bile acid levels. FXR antagonists were also identified, including the plant-derived molecule, guggulsterone (IC50, 15 µM) (8) and a synthetic molecule, AGN34 (IC50, <10 nM) (9). These agents provide useful experimental tools to parse the actions of FXR.

In addition to its essential role in regulating bile acid synthesis and lipid metabolism, emerging evidence supports an important role for FXR as an intestinal tumor suppressor (10). In comparison to adjacent normal epithelium, colon adenomas express reduced levels of FXR; FXR levels are even further reduced in colon adenocarcinomas (11,12). Diminished FXR expression in colon cancer is associated with advanced tumor stage and a worse prognosis (12,13). In mouse models of colon neoplasia, FXR deficiency results in increased adenoma size and number (14,15). Although these findings strongly implicate FXR as a tumor suppressor, the underlying molecular mechanisms are uncertain.

The 28-member matrix metalloproteinase (MMP) family is comprised of zinc-dependent endopeptidases (collagenases, stromelysins, gelatinases) that are secreted or membrane-bound proenzymes activated by proteolytic cleavage.
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(16,17). Besides degrading components of the extracellular matrix, including collagens and gelatins, MMPs, including MMP7 may regulate the release of cell surface molecules including growth factors, growth factor receptors, cytokines and cell adhesion molecules (18). Experimental data from several laboratories support a pivotal role for MMP7 in promoting cell proliferation and the development of intestinal tumors (19). Prenzel et al. showed that MMP7 activity is critical for cross-talk between G-protein-coupled and epidermal growth factor receptors (EGFR) (20).

In human colon cancer cells, we found MMP7 mediates transactivation of EGFR by catalyzing the release of heparin-binding-EGF-like growth factor (HB-EGF), one of seven known EGFR ligands (21). In mouse models, Wilson et al. showed MMP7 deficiency reduces intestinal tumor formation (22). Others used animal models to show MMP7 expression and activation is an important contributor to colon cancer metastasis (23-25). Notably, the stage and prognosis of human colon cancer correlates with increased MMP7 expression (26,27), the inverse of what is reported for FXR expression (12,13).

Previously, we reported Src-mediated cross-talk between FXR and EGFR modulates ERK phosphorylation, providing one mechanism whereby FXR may regulate cell proliferation and tumorigenesis (28). Now, based on the wide range of transcriptional targets for FXR and the findings summarized above, primarily the inverse actions of FXR and MMP7 on colon neoplasia, we hypothesized that FXR directly regulates MMP7 expression. Herein, we report experimental findings supporting this hypothesis.

Results

Correlation between reduced levels of FXR and increased MMP7 expression in colon cancer

Lian et al. used gene microarray to show that treating human AGS gastric cancer cells with CDCA substantially reduced levels of MMP7 mRNA (29). Nonetheless, the molecular basis of this FXR-mediated action, and whether it extended to other types of gastrointestinal cancer was unexplored. To fill these gaps in knowledge and pursue this interesting observation, we examined the correlation between FXR and MMP7 expression in paired, fresh-frozen surgical specimens of colon cancer and adjacent normal colon tissues obtained from the same persons.

Using QPCR to measure mRNA and immunoblotting to measure protein levels, we detected an apparent inverse relationship between FXR and MMP7 gene and protein levels. Both mRNA (Fig. 1A) and protein (Fig. 1B) levels of FXR were substantially lower in tumors compared to normal mucosae obtained from the same patients. In contrast, compared to normal mucosae from the same patients, MMP7 mRNA and protein levels were substantially greater in the same tumors (Fig. 1A and 1B). Moreover, when tumor levels of MMP7 protein were elevated we detected overexpression of both pro-MMP7 (latent form) and cleaved MMP7 (active form) (Fig. 1B; this is most evident in cancer tissue samples from patients 2 and 3 that had the highest levels of MMP7 mRNA and protein expression). To measure the relationship between FXR and MMP7, we performed a correlation analysis. As shown in Fig. 1C and D, coefficients of determination for FXR vs. MMP7 mRNA and protein were negatively correlated. Due to intratumor heterogeneity, we were not surprised to find small differences between mRNA and protein coefficients. Reassuringly, in control experiments, mRNA levels of MMP1, MMP10, and the retinoid X receptor (RXR) in tumor tissues compared to normal mucosa were not significantly different ($P>0.05$; Fig. 1E-G). These findings suggested a specific relationship between FXR and MMP7 expression levels.

To pursue these in vivo findings, we examined the relationship between levels of FXR and MMP7 expression in several human CRC cell lines, including HT-29 cells that were stably transfected with human FXR. In each tested cell line, we detected an inverse relationship between FXR and MMP7 expression (Fig. 1H).

To provide further evidence of an inverse relationship between FXR and MMP7 expression in colon cancer, we performed immunohistochemical staining (IHC) using an additional 10 sets of paraffin-embedded formalin-fixed cancers, adjacent normal mucosae, and liver metastases from the same individuals with stage...
IV colon cancer. A senior gastrointestinal pathologist (CBD) masked to experimental details assessed staining intensity (see Experimental Procedures). As shown in Fig. 2A and B, IHC revealed significantly diminished FXR expression in primary and liver lesions. In contrast, MMP7 protein levels were increased significantly in primary and metastatic tumors (Fig. 2A and C). These findings provide further evidence of an inverse relationship between FXR and MMP7 expression, consistent with a role for FXR as a potential regulator of MMP7 gene transcription.

**FXR gene ablation increases MMP7 expression**

To determine whether ablation of FXR increased MMP7 expression in vivo, we measured MMP7 expression in FXR knockout mice. As shown in Fig. 3, we detected increased MMP7 protein and mRNA levels in intestinal tissues and liver homogenates from FXR knockout (KO) mice (B6.129X1(FVB)-Nr1h4tm1Goz/J). Compared to tissues from WT mice, MMP7 protein levels were robustly increased in intestinal tissues (Fig. 3A and B) and liver homogenates (Fig. 3C and D) from FXR KO mice. Both Pro-MMP7 (latent form) and cleaved MMP7 (active form) were increased in tissues from FXR KO mice (Fig. 3A). In addition, MMP7 mRNA levels were increased in intestinal tissues from FXR KO mice (Fig. 3E). Collectively, these findings supported an inverse relationship between FXR and MMP7 expression.

**FXR overexpression and dominant-negative FXR mutation alters MMP7 expression**

When overexpressed, the W469A mutation in the FXR DNA-binding domain results in dominant-negative effects similar to those observed with FXR gene ablation (31). As previously described, after transfecting HT-29 human colon cancer cells with these plasmids, we successfully generated several stably-transfected clones overexpressing either FXR or W469A (28). Overexpressing FXR in HT-29 cells (Fig. 4A) robustly reduced MMP7 protein (Fig. 4A) and mRNA levels (Fig. 4B), and diminished cell proliferation (Fig. 4C). In contrast, overexpressing dominant-negative FXR in HT-29 cells (Fig. 4D) increased MMP7 protein (Fig. 4D) and mRNA (Fig. 4E) levels, and augmented cell proliferation (Fig. 4E). Moreover, as shown in Fig. 4G, using ELISA to measure MMP7 levels revealed that over-expressing FXR reduced the levels of secreted MMP7 in cell supernatants, whereas overexpressing dominant-negative FXR W469A increased levels of active MMP7 in supernatants. Moreover, siRNA knockdown of FXR in HT-29 cells increased MMP7 protein expression (Fig. 4H). As a negative control, siRNA knockdown of MMP7 in HT-29 cells did not alter FXR expression (Fig. 4I).

To determine whether FXR activation affects enzymatic activity, we performed casein zymography (32) (see Experimental Procedures) using HT-29 whole cell lysates and supernatants. As a negative control, we used MMP7 siRNA-transfected HT-29 cells with drastically reduced MMP7 expression (Fig. 4I). As shown in Fig. 4I, activation of FXR with CDCA dose-dependently reduced hydrolysis of casein, providing direct evidence of reduced MMP7 enzymatic activity in both HT-29 whole cell lysates (Fig. 4J; left panel) and HT-29 cell media (Fig. 4J; right panel). As an additional demonstration that activating FXR reduces active MMP7, we used ELISA to measure active secreted MMP7 in cell media. As shown in Fig. 4K, treating HT-29 cells with the FXR agonist CDCA significantly reduced levels of active MMP7 in cell supernatants.

Lastly, we used xenograft models in nude mice to explore whether FXR overexpression in vivo increased MMP7 expression (28). We found that MMP7 protein levels were strikingly reduced in xenografts derived from HT-29 cells stably transfected with human FXR (Fig. 4L). Collectively, the experiments shown in Fig. 4 provide compelling evidence that FXR expression governs MMP7 expression in colon cancer.

**FXR activation down-regulates MMP7 gene expression at the transcriptional level**

To explore the mechanism underlying this regulation of MMP7 by FXR, we examined the effects of FXR activation on MMP7 in human
colon cancer cell lines. In HT-29 cells, the FXR agonist CDCA dose-dependently decreased MMP7 mRNA (Fig. 5A) and protein (Fig. 5B) levels. Likewise, FXR activation with another agonist GW4064 also dose-dependently reduced MMP7 mRNA (Fig. 5 C) and protein levels (Fig. 5D). In contrast to the findings in HT-29 cells, in SW620 human colon cancer cells that lack FXR expression (Supplemental Fig. 1B), treating cells with CDCA and GW4064 did not alter MMP7 mRNA or protein levels (Fig. 5, E-H). As a positive control, in HT-29 cells the expression of an FXR target gene, ileal bile acid-binding protein (IBABP), was dose-dependently up-regulated by the FXR agonists CDCA (Fig. 5I) and GW4064 (Fig. 5J). In contrast, in SW620 cells, CDCA (Fig. 5K) and GW4064 (Fig. 5L) did not alter levels of IBABP mRNA levels. Moreover, CDCA and GW4064 did not alter MMP1 or MMP10 mRNA levels in HT-29 cells (Supplemental Fig. 1C-F), suggesting that this action of FXR is specific to MMP7.

As the active form of MMP7 is largely secreted into the culture media, we detected almost exclusively Pro-MMP7 (latent form) after immunoblotting HT-29 cell lysates. Adding guggulsterone, a specific FXR antagonist, abolished FXR agonist-induced repression of MMP7 expression (Supplemental Fig. 1A). Taken together, these results are compatible with the hypothesis that FXR represses MMP7 expression at the transcriptional level.

To investigate whether FXR alters mRNA stability, we treated HT-29 cells with actinomycin D (1 µg/mL) and used QPCR to measure whether CDCA altered MMP7 levels. As shown in Fig. 5M, CDCA treatment did not alter MMP7 mRNA levels in human colon cancer cells (half-life of ~16 h) (33). This result indicates activating FXR does not alter the stability of MMP7 mRNA and supports the conclusion that FXR acts as a transcriptional repressor for MMP7.

**FXR regulation of MMP7 transcription does not require RXR**

FXR can bind to its target promoters as either a homo- or hetero-dimer with another nuclear factor, retinoid X receptor (RXR). To determine whether RXR is required for the actions of FXR as a repressor of MMP7 expression, we treated cells with three RXR antagonists, K00083, HX531, and UV13003. None of these RXR antagonists altered the effects of CDCA on MMP7 expression (Fig. 5N and O). Based on these findings, we conclude that FXR most likely binds to the MMP7 promoter as a homodimer and does not require RXR. Our finding that RXR antagonists (retinoid acid and SR11237) did not reduce MMP7 expression (Supplemental Fig. 1G) support this conclusion.

**MMP7 is the only MMP gene downregulated by FXR activation**

To determine whether FXR down-regulates other MMP genes, we examined the effects of activating FXR in HT-29 cells on mRNA levels for all the known MMP genes. Of 28 known MMP genes, HT-29 cells only expressed MMP1, 7, 8, 9, 10, 14, 19, 21, 26 and 28 at levels that were reliably detected by QPCR. Activating FXR did not alter the expression levels of any of these genes (Supplemental Fig. 1H). In addition, activating FXR did not alter the expression of Myc, COX2 or cyclin D1 (CCND1) (Supplemental Fig. 1H). These data provide strong evidence that FXR is a specific transcriptional repressor of MMP7.

**Absence of traditional MMP7 FXR-responsive elements (FXRE)**

Traditional nuclear hormone receptor binding sequences consist of 6-bp half-sites, arranged as direct, inverted, or everted repeats. Several computational tools, including NHR-Scan, can be used to predict in silico the presence of nuclear hormone receptor binding sequences in the human genome (34). To identify possible FXR-biding elements in the MMP7 promoter, we performed NHR-Scan (http://www.cisreg.ca/cgi-bin/NHR-scan/nhr_scan.cgi) using the 5.0-kb MMP7 promoter sequence. The scan revealed seven potential traditional FXREs in the MMP7 promoter upstream of the transcription start site. Yet, chromatin immunoprecipitation (ChIP) analyses of these sites in HT-29 cells did not
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Reduced length sequences from this 30-bp element [16-bp oligo (5'-ATTGTGCTTCCCTGC), 20-bp oligo (5'-ATTGTGCTTCCCTGCAAT, or 21-bp oligo 5'TTCTTGCAATAACGATGTA)] failed to cause gel shifts with either recombinant human FXR or HT-29 nuclear extracts (data not shown). These results indicate the entire 30-bp MMP7 FXRE is required for FXR binding.

Identification of a non-traditional FXR binding element in the MMP7 promoter

In addition to traditional FXREs, non-traditional negative FXREs are increasingly recognized, the majority located in the 5' promoter region (35). To seek a potential non-traditional FXR binding element on the MMP7 promoter, we performed analyses using MMP7 promoter-luciferase constructs containing either 2.3 kb or 296 bp of MMP7 promoter sequences upstream of the transcription start site (see Experimental Procedures). We found this negative FXRE in HT-29 cells is located between 141 and 171 bp of the MMP7 5' promoter (Fig. 6A). CDCA-induced MMP7 inhibition was absent when we reduced the size of the promoter sequence from 171 to 141 bp (Fig. 6B). The sequence between 141 and 171 bp is 5’ATTGTGCTTCCCTGCAATAACGATGTAA. The increase in luciferase activity induced by the FXR antagonist, guggulsterone, requires the same 30-bp promoter element (Fig. 6C). Although the presence of antagonists alone should not increase gene expression, endogenous FXR agonists appear to maintain basal levels of FXR activation (36).

To determine whether human FXR can bind to this 30-bp negative MMP7 FXRE, we performed gel shift assays. Using double-stranded 3' end biotin-labeled probes in electrophoretic mobility shift (EMSA) assays that contained this 30-bp fragment, we detected strong binding of the probe by both HT-29 nuclear extracts and recombinant human FXR protein, an effect abolished by specific competitors (Fig. 6D). Anti-FXR antibody caused a super-shift of this complex, also abolished with addition of a specific competitor. Although this 30-bp element also contains a PEA3-binding site, i.e., 5’GCTTCCTG-3’ (Fig. 6A), anti-PEA3 antibody did not induce a super-shift, suggesting FXR binds to this 30-bp element and displaces PEA3.

Next, we used EMSA assays to identify the minimal sequence required for FXR binding.

Overexpressing FXR inhibits colon cancer cell invasion in vitro

To determine whether FXR activation or overexpression affects tumor cell invasion, a functional outcome involving MMP7, we performed collagen-based Boyden chamber cell invasion assays. Overexpressing FXR strongly inhibited HT-29 cell invasion (Fig. 7A). In contrast, overexpressing dominant-negative FXR W469A increased cell invasion (Fig. 7A). Likewise, as shown in Figs. 7B and 7C, FXR activation with an agonist, 6E-CDCA, inhibited invasion of the murine colon cancer cell line MC38. In addition, as shown in Fig. 7A, treating cells with neutralizing anti-MMP7 antibody significantly reduced the invasion of FXR W469A-transfected HT-29 cells, a finding that confirmed MMP7 plays a role in FXR-dependent cell invasion.
Discussion

A strong body of epidemiological and animal data supports the concept that bile acids are risk factors for colon cancer. Colon cancer risk is also associated with the consumption of a so-called ‘Western diet’, rich in carbohydrates and saturated fatty acids (37-39). Studies show that individuals consuming a Western diet and those with colon cancer both have elevated fecal levels of secondary bile acids, primarily lithocholic and deoxycholic acids (40-43). Provocative but controversial epidemiological studies identified a relationship between the increase in intestinal bile acid levels following cholecystectomy and an elevated risk of colon cancer; other findings dispute such an association (44,45). Nonetheless, in rodent models of colon cancer, either instilling exogenous bile acids into the intestines or increasing the spillage of endogenous bile acids into the colon consistently promotes advanced neoplasia (46-48). Despite this incriminating body of literature, a clear understanding of the molecular mechanisms underlying the association between increased intestinal bile acid levels and colon neoplasia has remained elusive.

It seemed reasonable to assume studies would find FXR, a master regulator of bile acid metabolism and transport, plays an important role in bile acid-induced promotion of colon neoplasia. Instead, the preponderance of evidence suggests FXR inhibits intestinal tumorigenesis by bile acid-independent mechanisms. For example, work by Degirolamo et al. suggested FXR deficiency, not elevated bile acid levels, mediates susceptibility to intestinal tumorigenesis; the tumor-promoting activity of bile acids were independent of their ability to activate intestinal FXR (15,49). We, and others, showed that several bile acids, including native and conjugated DCA and LCA, promote colon neoplasia by functional interaction with M3 muscarinic receptors, which are overexpressed in the majority of colon cancers; this mechanism is independent of FXR (50-54). Overall, the prevailing evidence favors the conclusion that bile acids have no direct bearing on the effects of FXR as an intestinal tumor suppressor, a role that is also independent of FXR acting as a regulator of bile acid metabolism.

Bile acid-independent action of FXR as a suppressor of intestinal tumorigenesis and progression appear to involve multiple signaling pathways (28,55). In animal models, Modica et al. showed FXR activation promotes intestinal epithelial cell apoptosis and Wnt signaling (15). In previous in vitro and in vivo work, we showed FXR activation reduced human colon cancer cell proliferation by inhibiting Src-mediated cross-talk between FXR and EGFR, which reduced ERK activation (28). In the present study, we employed multiple complementary experimental approaches and used colon cancer tissue, human and murine colon cancer cell lines, human xenografts and an FXR transgenic mouse, to identify a potentially important novel role for FXR. We found FXR directly regulates MMP7 expression by acting as a transcriptional repressor.

Starting by consistently demonstrating the relationship between reduced FXR levels and increased MMP7 expression in colon cancer, we showed FXR gene ablation increases MMP7 expression. FXR overexpression and dominant-negative FXR mutation respectively reduced and augmented MMP7 expression. We also provided evidence that regulation of MMP7 gene transcription by FXR does not require it to partner with RXR. Finally, MMP7 is the only member of the large MMP gene family down-regulated by activating FXR. Notably, in the course of these studies, we identified a novel non-traditional negative FXR-responsive element in the MMP7 5’ promoter.

Although transcriptional co-regulator complexes are often composed of both activators and co-repressors (56), others have shown that FXR acts as a transcriptional repressor via a distinct mechanism; FXR binds to negative FXREs as a monomer and competes with transcriptional activators to suppress transcription of multiple genes, including ApoAI, ApoCII, OAT2, and UGT2B4 (57-60). Similarly, by binding to a PEA3-binding site, FXR transcriptionally represses MMP7 expression by displacing the transcriptional activator PEA3. Collectively, these findings fill key gaps in knowledge regarding the mechanisms whereby FXR modulates colon cancer progression, and
affirm FXR as a promising therapeutic target in colon cancer.

Classical nuclear hormone receptor binding sequences consist of repeats with the core sequence AGGTCA separated by one or more nucleotides (n). The traditional FXREs include direct repeats separated by n number of nucleotide (DRn), inverted repeats (IRn) and everted repeats (ERn). However, non-traditional FXRE promoter elements were also identified in several human genes (35); these include FXR-binding sites in the 5’ promoters of ApoAI and UGT2B7 (35). In this study, we demonstrated a novel negative FXRE in the MMP7 gene promoter. Although the majority of traditional FXREs are located in the 5’ promoter regions upstream from the transcription start sites (35), some FXREs are located in either distant 5’ promoter region, in introns, or in the 3’ region (35). Although we did not identify traditional FXREs within the 5.0-kb MMP7 5’ promoter sequence, it is possible additional FXREs exist in other regions of the MMP7 gene.

It is noteworthy that FXR appears to regulate MMP gene expression differentially. In the liver which predominantly expresses MMP2 and MMP9, but not MMP7, activating FXR in hepatic stellate cells increased MMP2 expression two-fold, possibly by reducing levels of TIMP1 and TIMP2 (61). Yet, in gastric tissue, activating FXR downregulated MMP7 expression (29). In our study, activating FXR in human colon cancer cells that express high levels of MMP7, but very low levels of MMP9 and MMP2, resulted in suppression of MMP7 expression and activity. Although the molecular mechanisms underlying this divergence in FXR-mediated MMP gene regulation is poorly understood, we speculate it may be related to MMP7 being the only member of the MMP family that functions as both a signaling molecule/growth factor and as an enzyme.

Currently, there are limited therapeutic options for advanced colon cancer; although approaches targeting EGFR and vascular endothelial growth factors may be effective initially, their efficacy commonly wanes within months. These agents do not meaningfully alter five-year survival rates (~10%) (62-64). Moreover, use of these agents is frequently limited by toxicity resulting from off-target side-effects; EGFR is expressed widely in non-intestinal epithelial cells (e.g. dermal epithelial cells) (65). These comments highlight the urgent need for novel therapeutic approaches.

In addition to colon cancer, matrix metalloproteinases play important roles in the progression of a variety of solid tumors and have been considered potential therapeutic targets for at least 20 years (19,66). Interest in targeting MMPs was diminished when several synthetic MMP inhibitors failed to improve colon cancer survival in late-stage clinical trials. Reappraisal of these trials attributed failure to non-selective targeting that also activated MMPs that promoted neoplasia; given the large MMP family and the myriad cell functions they impact, it is perhaps not surprising that some MMPs are tumor suppressors (67). Although recent headway was achieved in developing selective MMP inhibitors (e.g. MMP9) (68,69), MMP7 is not presently a selectively druggable target.

Our finding of a novel molecular mechanism whereby FXR acts as a transcriptional repressor of MMP7 provides the rationale for an entirely new therapeutic approach to targeting MMP7 via the regulatory role of FXR. Selective FXR agonists with favorable safety profiles show great promise in treating chronic liver diseases. These include obeticholic acid (Ocaliva®), approved by the U.S. Food and Drug Administration in 2016 to treat primary biliary cholangitis (70), and EDP-305, successful in late-stage clinical trials to treat non-alcoholic steatohepatitis with liver fibrosis. Moreover, to minimize potential systemic toxicity, an intestine-specific FXR agonist, fexeramine, may prove to be a useful therapeutic agent specifically in colon cancer (71).

In conclusion, we report our finding that FXR transcriptionally represses MMP7 expression by interacting with a novel non-traditional negative FXR-responsive element in the MMP7 5’ promoter. Given the current limited options for treating advanced colon cancer, our findings identify the FXR-MMP7 axis as a new promising therapeutic target. Because combination therapy is commonly required to prevent or treat cancer effectively, identifying additional genes that
interact with the FXR-MMP7 axis is also warranted.

**Experimental procedures**

**Materials**

Chemicals: We purchased GW4064 from Calbiochem Inc., and all other chemicals from Sigma-Aldrich or Thermo Fisher Scientific.

Human samples: De-identified paraffin and frozen sections from surgically resected human colon cancers and adjacent normal colon tissue, and metastatic liver lesions from the same persons were obtained from the Department of Pathology at the University of Maryland Medical Center. This study was approved by the Institutional Review Board at the University of Maryland Baltimore and abides by the Declaration of Helsinki principles.

**Cell lines and cell culture**

Human colon cancer cell lines HT-29, SW620, HCT116, SNU-C4, Caco-2 and mouse colon cancer cell line MC38 were purchased from the American Type Culture Collection (ATCC) and maintained in growth media supplemented with 10% fetal bovine serum. HT-29, HCT116 and SNU-C4 were grown in McCoy’s 5A media. SW620, Caco-2 and MC38 were grown in Leibovitz’s L-15, Eagle’s Minimum Essential and Dulbecco’s modified MEM growth media, respectively. We passaged adherent cells weekly at sub-confluence after trypsinization, and maintained cultures in incubators at 37°C in an atmosphere of 5% CO₂ and 95% air.

**Experimental animals**

Wild-type (WT) C57BL/6J mice and FXR knockout mice (B6.129X1-Nr1h4tm1Goz/J) were purchased from the Jackson Laboratory. For all experiments, we used six-week-old male mice. We housed mice under identical conditions in a pathogen-free room with free access to commercial rodent chow and water; we allowed mice to acclimatize in the vivarium for two weeks prior to experiments. This study was approved by the Office of Animal Welfare Assurance from the University of Maryland School of Medicine and the Research Development Committee at the VA Maryland Health Care System.

**Plasmids and stable transfections**

Generation of stably-transfected human HT-29 colon cancer cell lines using the plasmid pCDNA3.1hFXR was described previously(28). To generate cell lines stably overexpressing either full-length human FXR or a dominant-negative FXR mutant, we first constructed plasmids pcDNA3.1hFXR and pcDNA3.1FXRW469A using pCMX-hFXR and pCMX-hFXR W469A (a gift from Dr. David Mangelsdorf, UT Southwestern School of Medicine) that contain the full-length human FXR cDNA and the dominant-negative FXR mutant protein W469A, respectively.

**Quantitative real-time PCR (QPCR)**

We performed QPCR and quantification of mRNA levels as described previously (33). We confirmed the specificity of amplifications by melting-curve analysis and calculated relative levels of mRNA according to the standard ∆∆Ct method. We normalized expression values by comparison with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). We listed QPCR primer sequences used in this study in Table 1.

**FXR and MMP7 siRNA transfections**

We purchased FXR (FXR SMARTpool: ON-TARGETplus L-003414-00) and Non-targeting siRNA #1 D-001810-01 and MMP7 siRNA (MMP7 Silencer Select assay ID S8856 and Silencer Select negative control #1) from Dharmacon and Invitrogen, respectively, and performed siRNA transfections using Lipofectamine as per the manufacturer’s instructions (Thermo Fisher Scientific). HT-29 cells were transfected for 24 to 48 h before preparation of RNA and protein extracts for QPCR and immunoblotting, respectively.

**Subcloning and luciferase assays**
MMP7 5' promoter plasmids were constructed using PCR-generated fragments using the pGL4.14 plasmid (Promega) and pGL2.hmat-2.3 and pGL2.hmat-296 that contains 2.3 kb and 296 bp of MMP7 promoter (gifts from Dr. Lynn Matrisian, Vanderbilt University). For all constructs, we used reverse PCR primer GTGAGAAGATCTTGCTAGTGACTGCGA AATT; for the 191 bp construct, we used the forward PCR primer GTGAGACTCGAGATCCTTTGAAAGACAA ATAC; for the 141 bp construct, we used PCR primer GTGAGACTCGAGTACTTCCTCGTTTTAGT T; for the 111 bp construct, we used PCR primer GTGAGACTCGAGACACATACTTTCACGGT TCTG. We confirmed constructs by DNA sequencing. Vectors were transfected into HT-29 cells for 24 h, followed by addition of either CDCA (200 µM) or guggulsterone (20 µM) for an additional 24 h. Luciferase activity was measured using a SpectraMax spectrophotometer with a 96-well microplate reader.

**Antibodies and immunoblotting**

Immunoblotting were performed as described previously (72). To ensure equal loading of protein samples, protein concentrations of cell lysates were determined using the BCA Protein Assay kit (catalog # 23227; Thermo Scientific, Inc). Ten to 20 µg protein was loaded into each lane. We purchased rabbit anti-human FXR (Santa Cruz Biotechnology catalog # SC-1205, lot # K3009, H1904 for immunoblotting and SC-1204, lot # C2404 for immunoprecipitation), goat anti-human MMP7 polyclonal antibody (R&D Systems catalog # AF907, lot # DPS0910111, DPS091308, DPS0710081), rabbit anti-mouse MMP7 monoclonal antibodies (Cell Signaling catalog # 3801), mouse monoclonal anti-β-actin antibodies from (Sigma-Aldrich catalog # A1978, lot # 087M4850V). After probing with the primary antibodies, immunoblots were incubated with horseradish peroxide-conjugated secondary antibodies and visualized by chemiluminescence (Pierce) the ChemiDoc™ Touch Imaging System (BIO-RAD). To avoid oversaturation of bands and to work within the linear ranges only, we performed semi-quantifications using Quantity One software (BIO-RAD).

**Immunohistochemical analyses**

We performed immunohistochemistry using paraffin-embedded blocks from paired human colon cancer surgical specimens. Quantification of staining intensities was performed as described previously (73) by an expert pathologist (CD) masked to experimental groups. IgG and secondary antibodies were used as negative controls. Both anti-FXR and anti-MMP7 antibodies used in IHC detected predominantly one band in HT-29 and human colon tumors (Supplemental Fig. 1 J and K). In addition, non-specific IgG and secondary antibodies alone did not show any specific staining (Supplemental Fig. 1L).

**Casein Zymography**

MMP7 enzymatic activity was analyzed by casein gel zymography as described (32). Briefly, whole cell lysates or supernatants from HT-29 cells grown in 10-cm culture dishes were prepared after CDCA treatment for 24 h and subjected to electrophoresis using 12% SDS-PAGE containing casein. Fifty µg protein was loaded into each lane. The gels were washed in the Zymogram Renaturing Buffer (Novex catalog # LC2670) for 30 min to remove SDS, incubated in the Zymogram Developing Buffer (Novex catalog # LC2671) for 24 h. The gels were then stained with 0.25% Coomassie Brilliant Blue and photographed.

**MMP7 ELISA**

HT-29 cells were seeded at 10⁵ cells per 2 mL of medium per well in six-well plates. Twenty-four h after addition of different concentrations of CDCA, supernatants were collected and centrifuged at 500 rpm for 5 min. As described previously, MMP7 enzymatic activity was measured using the Human Total MMP7 Quantikine Elisa Kit (R & D System catalog #RND-DMP700) following the manufacturer’s instructions (33).
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**Chromatin immunoprecipitation (ChIP) assays**

We performed ChIP assays in human HT-29 colon cancer cells using the the Pierce Magnetic ChIP Kit (catalog # 26157; Thermo Scientific Inc.) per the manufacturer’s instructions (MAN0016150_PierceMagneticChIPKit_PI.pdf). Briefly, in vivo crosslinking was performed using 4 million cultured HT-29 cells using 1% formaldehyde. Cells lysis was performed using buffers containing proteinase inhibitor cocktails. Lysates were then digested with Micrococcal Nuclease (MNase; Pierce Kit) to generate random DNA fragments from 160 to 640 bp with an intense ladder of bands at approximately 160, 320 and 480 bp, which correspond to the 1, 2 and 3 nucleosome units and 480 bp, which correspond to the 1, 2 and 3 nucleosome units (Fig. 6F, left panel). Chromatin was obtained after brief sonication to rupture nuclei. Five µl aliquot was removed for agarose gel analysis. Immunoprecipitation of crosslinked protein/DNA were performed overnight at 4 °C using antibodies against human FXR, RNA Polymerase II (positive control) and rabbit or goat IgG (negative controls). Elution and reverse crosslinks of protein/DNA complexes to free DNA were performed using the ChIP Elution Buffer without protein kinase K using a magnet. Eluted DNA were purified using DAN spin columns. Real-time quantitative PCR was performed using QPCR primers listed in Table 1 using eluted DNA and 1% input. For FXR activation, monolayer HT-29 cells were treated with 200 µM CDCA for 2 h prior to crosslinking. We used goat anti-human FXR antibody (Santa Cruz Biotechnology, catalog # SC-1204, lot #C2404), rabbit anti-RNA Polymerase II (Thermo Scientific, Kit component, catalog # not specified), rabbit IgG (Thermo Scientific, Kit component # not specified) and goat IgG (Calbiochem, catalog # N102, lot # D00067506). GAPDH was used as a negative control.

**Electrophoretic mobility shift assay (EMSA)**

We performed EMSA using the LightShift Chemiluminescent EMSA kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. We purchased recombinant human full-length FXR protein form Sigma. Binding reactions contained 10 mM Tris, 50 mM KCl, 1 mM DTT, 2.5% glycerol, 5 mM MgCl₂, 50 ng/µL poly (dI-dC), 0.05% NP-40, 5 µM zinc sulfate, 1x of proteinase inhibitor cocktail (Sigma) and 5 pmol 3′-end biotin-labeled DNA. Protein-DNA complexes were resolved on a 6% DNA retardation gel (Invitrogen). We labeled double-stranded oligonucleotides (forward sequence 5′-ATTGTGTGCTTCCGGCAGATGTA A-3′; reverse sequence 5′-TTACATCGTTATGGCAGGAAACACACA AT-3′) with biotin using the Biotin 3′ end DNA Labeling Kit (Thermo Scientific) using 20 fmol of each probe. Briefly, we performed incubations in a total volume of 20 µl of binding buffer (1x bind buffer, 2.5% glycerol, 5 mM MgCl₂, 50 ng/µl polydI-dC, 0.05% NP-40, 5 µM zinc sulfate, 1 µg nuclear extract and 5 µl labeled-probe) for 20 min at room temperature. For competitive inhibition, we used more than 100X excess of specific oligos before adding labeled probes. For the supershift experiments, 1 µg nuclear extract or 0.5 µg FXR recombinant protein (Sigma, catalog # SRP3029, lot #037298123) were pre-incubated for 15 min at room temperature with 1 µg anti-FXR antibody (Santa Cruz Biotechnology, catalog # SC-1204, lot # C2404) or anti-PeA3 antibody (Santa Cruz Biotechnology, catalog # SC-166629, lot #L0516 for western blot and catalog # SC-166629X, lot #L0302 for immunoprecipitation) before adding biotin-labeled probes. The reactions were terminated by adding 5X loading buffer. Twenty µl mixed solution was loaded on a 6% DNA retardation gel (Invitrogen) and electrophoresed in 0.5X Tris-borate-EDTA buffer at 100 volts for 30 min. We transferred the protein/DNA complexes to positively charged nylon membranes (Invitrogen) and we detected gel shifts using the chemiluminescent nucleic acid detection module (Thermo Scientific).

**Matrigel chamber invasion assay**

We performed invasion assays using the Biocoat Matrigel Invasion Chamber Kit (BD Biosciences, Bedford, MA) as described previously (72). Briefly, we trypsinized and resuspended HT-29 human colon cancer cells in serum-free McCoy’s 5A medium, placed cells in the upper chamber (5 x 10⁴ cells/chamber), and then added the indicated agents or vehicle (control). McCoy’s 5A medium containing 10% FBS was placed in
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the lower chamber. We incubated cells for 48 h in a humidified atmosphere with 95% air and 5% CO2 at 37°C. We fixed and stained invasive cells using Diff-Quick™ stain. We removed non-invasive cells in the upper chamber by wiping with a cotton swab and counted all cells on the lower surface of the insert that had penetrated Matrigel using a light microscope. We performed each experiment in triplicate.

Statistical analysis

Data are expressed as mean ± SE of at least three independent experiments. We performed one-way ANOVA, Tukey’s HSD post hoc analyses and Student’s un-paired t-tests (SigmaPlot, Systat Software, Inc., San Jose, CA) and considered a $p$ value $< 0.05$ to be statistically significant.

Conflict of interest. The authors declare that they have no conflicts of interest with the contents of this article.

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Table 1. QPCR Primers and MMP7 promoter positions for ChIP experiments

| Genes  | Forward primer sequence 5' to 3' | Reverse primer sequence 5' to 3' |
|--------|----------------------------------|----------------------------------|
| MMP1   | AACTGCCAAATGGGCTTG               | CGGTGAGCATCTGCTCC               |
| MMP2   | CTGAGGGCCTTCTCTTG                | TCACGCTTCCAGACTTTTG            |
| MMP3   | CCAAGCAATAGCTGAGACTTT           | TTCTTGTAGATTTGGGCAAA          |
| MMP7   | GGAGATGCTCACTTCACTGA            | ATACCAAGAGATGGGCAAG           |
| MMP8   | AGCAATTGACCGACGTTT             | GGAAAGGGGACTGATGCT            |
| MMP9   | GAGGTGGACCGAGATTTC            | CCTGAGAGATAGGCTTTC           |
| MMP10  | TGAACCTAAGGTTGATGCTG          | GCTCAGCATCCTGCGCCATG          |
| MMP12  | TGGAGGTATGATGAAAGGAGA        | TTAGGCCGATTCTGTT             |
| MMP13  | GAAGACTTCCAGGAATTTG           | AAATGGAATTGCTGGCAGAT         |
| MMP14  | ACATCAAGATGAGGGCAAGG         | CGGTGTTACCTCAGACTCC          |
| MMP19  | TTAAGCTGACCCAGAGTATG         | AGTGGGAGCTCTGCTCTTT         |
| MMP21  | CTGGAATCCAACCAGACACAC         | ATCTCTGTGCAGGGCCTGTAT         |
| MMP26  | GTCAAGCTTCAGACACTGGA         | AGCTCTGATTCCAGAGACTG         |
| MMP28  | CATGCAGGAAGAGATGGGT        | GGAACACTCCAGCATGAC          |
| Mmp7 (m) | CTGTTCCCGGTACTGTGATG    | TCAGACGCCTGCTTTCCTT          |
| I-BABP | CCAACCAATGTCTCTGCTCTTCTTGTCTC | ACCAAGTGGAAGTCTGCCCCATCTCTG |
| FXR    | CAGCAATGGTTATTATCTGCTCC     | CAGGCTGTTGAATTTACACAGCC     |
| RXRα   | TTTAACCTGACTTCCAAAGG         | CTCCAAGGAGCCATAGACTT        |
| Fxr (m) | AGGAGTACGGTTCTGTCTCACA     | CGCCTCTGTGCTTGTGATG          |
| GAPDH  | CCCATGGTGCTGCTGAGG         | CGACAGTCGGGACTTGT          |
| Gapdh (m) | GGTGAAAGTCCGTTGGAAC | TGAATGACAAACACTCCACT       |
| ChIP 1 (1-111) | CATCAACTTTTAAGTGTCTC | TGCTAGCAGCTGAGAAATTT         |
| ChIP 2 (49-171) | CATTGTTGCTTCTCGCCAAT     | TCTCAAGCCTGGAATGTTGGAAA     |
| ChIP 3 (202-281) | CCTCAATGGATACATGAGCCA | TCTGCTGATAAATTGACTCCA       |
| ChIP 4 (965-1072) | CACTGCACTGCTTATCTTCA | TAAGAATTGACGATTGCTGTG       |
| ChIP 5 (1523-1678) | GAAACAGGTTTTGCCATGT | GCCACAAATGTTGGTGAAAAT       |
| ChIP 6 (1708-1810) | TGAGTGAGATGTCTGAGC  | GGTGAGATGCTGCTTGAGT         |
| ChIP 7 (1912-2038) | GTATGTTTAAAATCTTCAAC | GTGAAATATTTATTTGATAG        |
| ChIP 8 (2805-2955) | TACAGCACCTGCGACCAAC | TTCGAGATGCTGCTGTTAATC       |
| ChIP 9 (3765-3935) | CAGGTTTGTAGGAGCAGCAGGCC | CTCTAGAGGCACATCACTC         |
| ChIP 10 (4278-4429) | TTTTCTAATATCTTCTTCCAGAGC | AGGGGTACGACTGGGAGCAT        |
FXR transcriptionally represses MMP7 expression

Figure 1. Inverse relationship between FXR and MMP7 mRNA and protein levels. A, mRNA levels were determined by QPCR in eight sets of paired human surgical specimens of colon tumors and adjacent normal mucosae. See Experimental Procedures. B, protein levels were determined by immunoblotting in the same eight sets of paired human surgical specimens of colon tumors and adjacent normal mucosae. C and D, the levels of FXR mRNA (C; N=16) and protein (D; N=16) were negatively correlated with the levels of MMP7 mRNA (p = 0.0372) and protein (p = 0.0306). E, F and G, mRNA levels of MMP1 (E), MMP10 (F) and RXR (G) in paired human CRC surgical specimens (N=10) were not significantly different in colon cancer vs. adjacent normal tissue. H, proteins levels of MMP7 and FXR in human CRC cell lines and HT-29 cells stably transfected with human FXR. We measured mRNA levels by QPCR. Values represent mean ± SE. Quantification of relative protein levels was performed by Image software.
Figure 2. Negative correlation between FXR and MMP7 expression levels. A, immunohistochemical staining of paired human surgical specimens of adjacent normal mucosa, primary colon tumors and metastatic liver lesions from the same person. B and C, graded staining intensities of FXR (B) and MMP7 (C) proteins in paired human surgical specimens of adjacent normal mucosa, primary colon tumors and metastatic liver lesions from the same persons. Values represent means ± SE from 10 samples. a.u., arbitrary units. Size bars = 50 micrometers.
FXR transcriptionally represses MMP7 expression

Figure 3. Increased MMP7 expression in FXR knockout mice. A, MMP7 immunoblots from intestines of WT and FXR KO mice (N = 3 each). B, MMP7/β-actin ratios in the mouse intestines (N=3). C, MMP7 immunoblots from livers of WT and FXR KO mice (N = 2 each). D, MMP7/β-actin ratios in the mouse livers (N=3). E, MMP7 mRNA levels in the mouse intestines. mRNA and protein levels were determined by QPCR and immunoblotting, respectively. Quantification of relative protein levels was performed by Image software. Band density was quantified and normalized to β-actin levels. Values represent means ± SE. *p < 0.05 vs. control.
Figure 4. Effects of FXR overexpression or inhibition on MMP7 gene expression. A, B and C, FXR and MMP7 protein (A), MMP7 mRNA (B) and cell proliferation (C) in HT-29 cells stably transfected with human full-length FXR. D, E and F, FXR and MMP7 protein (D), MMP7 mRNA (E), and cell proliferation (F) in HT-29 cells stably transfected with human dominant-negative mutant FXR W469A. G, effect of CDCA on secreted MMP7 levels in vector-transfected HT-29 cells or FXR-overexpressed or W469A-overexpressed HT-29 cells. H, FXR and MMP7 protein levels from FXR siRNA-transfected HT-29 cells for 24 or 48 h. I, MMP7 and FXR protein levels from MMP7 siRNA-transfected HT-29 cells for 24 h. J, dose responses in HT-29 cells for CDCA-induced reduction in MMP7 activity measured in whole cell lysates (left panel) or supernatants (right panel) using casein zymography. MMP7 siRNA (SiR)-transfected HT-29 cells were used a negative control. K, active (secreted) MMP7 levels in the supernatants of HT-29 cells treated with CDCA measured by ELISA. L, MMP7 protein levels in xenografts generated from either vector alone or human full-length FXR. Values represent means ± SE from three experiments. For B, C, E and F, *p < 0.05 vs. vector alone or DMSO control in unpaired t-tests. One-way ANOVA identified significant variance in G (p < 0.001) and K (p < 0.001). Tukey’s HSD post hoc tests showed significant differences (p < 0.05) between any two groups except 1 µM vs. 10 µM, 1 µM vs. 50 µM and 10 µM vs. 50 µM.
FXR transcriptionally represses MMP7 expression

Figure 5. Effects of FXR activation on MMP7 expression. A and B, dose responses in HT-29 cells for CDCA-induced reduction in MMP7 mRNA (A) and protein (B). C and D, dose responses in HT-29 cells for GW4064-induced decrease in MMP7 mRNA (C) and protein (D). E and F, absence of dose response for CDCA-induced changes in MMP7 mRNA (E) and protein (F) levels. G and H, absence of dose response for GW4064-induced changes in MMP7 mRNA (E) and protein (F) levels. I and J, dose responses in HT-29 cells for CDCA-induced (I) or GW4064-induced (J) increases in IBABP mRNA. K and L, absence of dose response in SW620 cells for CDCA-induced (K) and GW4064-induced (L) changes in IBABP mRNA levels. M, MMP7 mRNA levels with actinomycin D (1µg/ml) treatment in the presence or absence of 200 µM CDCA. N and O, effects of RXR inhibitor K00083 (N), HX531 (O) and UVI3003 (O) on CDCA (200 µM)-induced decrease of MMP7 protein level in HT-29 cells. We measured mRNA and protein levels by QPCR and immunoblotting, respectively. We harvested cells at 6 h (for IBABP mRNA), 24 h (for MMP7 mRNA and immunoblotting) after addition of test agents. Values represent mean ± SE from three experiments. One-way ANOVA analysis: A. p < 0.0001; C. p < 0.0001; D. p < 0.0001; F. p < 0.0001; G. p = 0.2263; H. p = 0.1380; J. p = 0.2661; L. p = 0.0899. Tukey’s HSD post hoc tests showed significance (p < 0.05) between any two groups in A, C, I and J, except in A, 1 µM vs. DMSO, 10 µM vs. DMSO, 50 µM vs. DMSO, 10 µM vs. 1 µM, 50 µM vs. 1 µM and 50 µM vs. 10 µM; C, 0.1 µM vs. 0.01 µM, 10 µM vs. 0.01 µM and 10 µM vs. 1 µM; I, 1 µM vs. DMSO; J, 0.01 µM vs. DMSO.
Figure 6. Negative FXRE in MMP7 promoter. A, Schematic of the MMP7 promoter constructs and the negative response element marked by the small black rectangle. PEA-3-binding sequence is underlined. B, Luciferase assay of MMP7 promoter with CDCA (200 µM) treatment in HT-29 cells. C, Luciferase assay of MMP7 promoter with guggulsterone treatment in HT-29 cells. CDCA was added to cells (for an additional 24 h) transiently transfected for 24 to 48 h with MMP7-luc plasmids with control pGL4.14-Renilla luciferase vectors. D, we performed EMSA using 3'-biotin-labeled probes and nuclear extracts from HT-29 cells. E, ChIP assay using random DNA fragments generated by Micrococcal Nuclease digestion in HT-29 cells without CDCA treatment or after treatment with 200 µM CDCA for 24 h. F, left panel, fragmentated genomic DNA (M: molecular marker; lane 1, DNA after Micrococcal Nuclease (MNase) digestion (see Experimental Procedures) from HT-29 cells; right panel, agarose gel images of QPCR products from ChIP assays using MNase-digested DNA. GAPDH was used as a control. MMP7 promoter positions and DNA sequences of QPCR primers are listed in Table 1. *p < 0.05 vs. control.
Figure 7. FXR overexpression inhibits colon cancer cell invasion. A. We performed Boyden chamber invasion assays using either HT-29 cells stably-transfected with vector alone, FXR, FXR W469A or FXR W469A treated with neutralizing anti-MMP7 antibody. B, Boyden chamber invasion assay using mouse colon cancer cell line MC38Luc1 with or without FXR activation by 6E-CDCA. C, representative microscopic images of invaded MC38Luc1 cells with and without FXR activation by 6E-CDCA (magnification 100X). Values represent mean ± SE. * p < 0.05 compared to W469A. ** p < 0.05 compared to control.
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