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Original Article

Chenodeoxycholic acid suppresses colorectal cancer by activating Farnesoid X receptor

Jun Xie[1*], Yiding He[2*], Chenhao Xu[2], Sihao Wang[2], Guangchuan Qu[2], Yilin Ren[3], Tong Wang[2], Yuzheng Xue[3]

[1]Department of Internal Medicine, Affiliated Children's Hospital of Jiangnan University (Wuxi Children's Hospital), Wuxi 214023, Jiangsu, China; [2]Department of General Surgery, Wuxi People's Hospital Affiliated to Nanjing Medical University, Wuxi 214023, Jiangsu, China; [3]Department of Gastroenterology, Affiliated Hospital of Jiangnan University, Wuxi 214122, Jiangsu, China. [*]Equal contributors.

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Abstract: Colorectal cancer (CRC) is a major cause of cancer-related mortality worldwide. Therefore, it is critical to identify accurate biomarkers for improving early diagnosis and clinical outcomes. In our study, serum was collected from 90 healthy controls and 98 CRC patients after 8-hour fasting, followed by the measurement of chenodeoxycholic acid (CDCA) levels using ELISA. Cell proliferation and metastasis were assessed using MTT, colony-forming, and transwell assays. Farnesoid X receptor (FXR) was silenced with shFXR lentivirus, and its expression in tumors was analyzed by RT-qPCR. The result revealed that serum CDCA levels were significantly lower in CRC patients compared to healthy controls. Lower CDCA levels were associated with advanced TNM stage, differentiation status, and lymph node metastasis. FXR mRNA was significantly downregulated in CRC tumors and was negatively correlated with tumor aggressiveness. Serum CDCA levels were positively correlated with tumor FXR mRNA expression, and higher FXR expression was associated with better 5-year overall survival. CDCA treatment significantly suppressed CRC cell proliferation and migration in a dose- and time-dependent manner, whereas FXR knockdown attenuated these inhibitory effects. These findings indicated that CDCA suppressed CRC progression by activating FXR and inhibiting Wnt/β-catenin signaling. This highlights the potential of CDCA and FXR as therapeutic targets for CRC.

Keywords: Colorectal cancer, chenodeoxycholic acid, Farnesoid X receptor, Wnt3a, β-catenin

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy worldwide and remains a major cause of cancer-related mortality [1]. In 2019, CRC caused approximately 690,000 deaths globally, and its incidence has continued to rise among individuals under 55 years of age [2, 3]. By 2040, annual CRC cases worldwide are expected to double, rising from 1.6 million to 3.2 million [4]. Effective screening is critical for the early detection and management of CRC, because it significantly improves patient survival rates and clinical outcomes [5]. Currently, colonoscopy is the most widely used screening method and the gold standard for CRC diagnosis due to its superior visualization and diagnostic accuracy [6]. However, colonoscopy remains an invasive procedure with practical limitations, including high technical and financial demands, limited patient acceptance, and procedure-related risks, highlighting the need for convenient and non-invasive screening approaches [7]. Therefore, minimally invasive, convenient, and reliable biomarker-based screening methods are needed to support the early diagnosis of CRC and improve clinical outcomes.

Bile acids are cholanic acid derivatives produced mainly from cholesterol metabolism in the liver [8]. They are classified as primary or secondary bile acids based on their biosynthetic origin. Primary bile acids are synthesized through the classical and alternative pathways. The classical pathway produces cholic acid (CA) and chenodeoxycholic acid (CDCA), while the alternative pathway primarily generates CDCA [9]. Dysregulated bile acid metabolism has been increasingly associated with CRC risk, suggesting a critical role in CRC pathogenesis [10].

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CDCA has multiple pharmacological activities, including reducing biliary cholesterol saturation and exerting antimicrobial, anti-inflammatory, antitussive, and bronchodilatory effects [11]. Recent studies have highlighted the anticancer potential of bile acids and their derivatives, suggesting that bile acid-related compounds may regulate tumor cell proliferation, apoptosis, and tumor-associated signaling pathways. However, the mechanisms underlying CDCA's biological functions, particularly its anti-tumor effects, remain unclear and require further investigation [12].

CDCA is one of the endogenous bile acids capable of activating farnesoid X receptor (FXR), a bile acid-activated nuclear receptor that plays a central role in bile acid homeostasis and metabolic regulation [13, 14]. Research has shown that FXR expression levels are significantly reduced in colorectal cancer patients and tumor cell lines. Moreover, low level of FXR is associated with several critical clinical factors, including tumor stage, differentiation, local recurrence, distant metastasis, and patient prognosis [15]. FXR knockout in mice increased intestinal tumor incidence and tumor burden whereas FXR restoration reduced tumor volume in animal models. These findings highlight the critical role of FXR in suppressing the initiation and progression of colorectal cancer [16].

This study aims to evaluate serum CDCA levels in CRC patients and investigate their correlation with CRC pathology. Additionally, we also assessed FXR expression in CRC tissues, its relationship with serum CDCA levels, and its prognostic relevance. Finally, we investigated whether CDCA regulates CRC cell behavior through FXR-related signaling mechanisms.

Materials and methods

Serum collection

Serum samples were obtained from 90 healthy individuals and 98 CRC patients at the Affiliated Children's Hospital of Jiangnan University, following approval from the Ethical Committee of Affiliated Children's Hospital of Jiangnan University (Wuxi Children's Hospital) (#WXCH2025-02-067). Written informed consent was secured from all participants. CRC patients were pathologically diagnosed and had not undergone surgery, chemotherapy, or radiotherapy prior to enrollment. Additionally, these patients had no history of other malignant tumors, severe metabolic disorders, or inflammatory bowel diseases.

Healthy controls underwent gastrointestinal endoscopy to confirm the absence of intestinal tumors and had no history of metabolic or inflammatory bowel diseases. Serum samples were obtained from all participants after an 8-hour fast using coagulation-promoting blood collection tubes.

Serum CDCA detection

Serum CDCA levels were measured using the Chenodeoxycholic Acid enzyme-linked immunosorbent assay (ELISA) kit (ARG81203, Arigo Biolaboratories Corporation, Hangzhou, China). The assay used an HRP-conjugated antibody-based ELISA detection system, and absorbance was measured at 450 nm. The detection sensitivity was 30 nM, with a dynamic range of 31.25 to 2000 nM. A sample volume of 50 μL was required for each assay, and all procedures were conducted following the manufacturer's instructions.

Cell culture

Human colorectal cancer cell lines SW620 and HCT116 were obtained from the American Type Culture Collection (ATCC). The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin and streptomycin, p/s). Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Cells were passaged every 2 to 3 days using 0.25% trypsin-EDTA. CDCA was purchased from Sigma-Aldrich (C9377).

Lentivirus infection

Lentiviral vectors containing shRNA sequences targeting the FXR gene (shFXR) were designed and produced by GeneChem Co., Ltd. (Shanghai, China). The target sequence was 5'-GGACCATGAAGACCAGATT-3'. A scrambled shRNA sequence, 5'-TTCTCCGAACGTGTCACGT-3', was used as a negative control. Colorectal cancer cell lines were seeded at a density of 5×10^5 cells per well. After overnight incubation, 20 μL of lentivirus and polybrene were added, and cells were selected with puromycin 48 h after infection.

Quantitative real-time PCR

Total RNA was extracted from tumor specimens using Trizol reagent (Invitrogen, USA). The RNA was then reverse-transcribed into complementary DNA (cDNA) using the cDNA Synthesis SuperMix kit (Yeason, Shanghai, China). Gene expression was quantified using the LightCycler 480 II system (Roche, Switzerland). The qRT-PCR was performed in a total volume of 20 μL, containing 10 μL of 2× SYBR Green Premix Ex Taq II (Takara, Japan), 2 μL of 10 μM forward primer, 2 μL of 10 μM reverse primer, 2 μL of cDNA template, and 4 μL of nuclease-free water. The amplification conditions were as follows: initial denaturation at 95°C for 30 s, followed by 39 cycles of denaturation at 95°C for 10 s and annealing/extension at 60°C for 30 s. Melting curve analysis was performed from 65°C to 95°C, with fluorescence signals collected at 0.5°C intervals. Primer sequences for the FXR gene were: forward 5'-GACTTTGGACCATGAAGACCAG-3' and reverse 5'-GCCCAGACGGAAGTTTCTTATT-3'. GAPDH was used as the reference gene, with the following primers: forward 5'-TGTGGGCATCAATGGATTTGG-3' and reverse 5'-ACACCATGTATTCCGGGTCAAT-3'. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with GAPDH used for normalization.

Western blot

Cells were lysed using radioimmunoprecipitation assay (RIPA) buffer supplemented with a protease inhibitor cocktail (Roche, Penzberg, Germany). Protein concentrations were determined using the BCA Protein Assay Kit (Yeason, Shanghai, China). Equal amounts of denatured protein were separated by SDS-PAGE and transferred to PVDF membranes. After blocking with 5% non-fat milk, the membranes were incubated overnight at 4°C with primary antibodies against Wnt3a (1:1000, 26744-1-AP, Proteintech, Wuhan, China), FXR (1:1000, 81820-2-PBS, Proteintech), β-catenin (1:1000, 51067-2-AP, Proteintech), c-Myc (1:1000, 67447-1-Ig, Proteintech), Cyclin D1 (1:1000, 60186-1-Ig, Proteintech) and β-actin (1:5000, 66009-1-Ig, Proteintech). The membranes were then incubated with HRP-conjugated goat anti-rabbit (RGAR001, Proteintech) or goat anti-mouse (RGAM001, Proteintech) secondary antibodies for 1 hour at room temperature, followed by three washes with TBST. Protein expression was visualized using the Sage-Capture Chemiluminescent Imaging System (ChampChemi 610 Plus, China).

Methyl Thiazolyl Tetrazolium (MTT) assay

Cells were seeded in a 96-well plate at a density of 1,000-10,000 cells per well in 200 μL of culture medium containing 10% FBS. After incubation, 20 μL of MTT solution (5 mg/mL in PBS, pH 7.4) was added to each well, followed by an additional 4 h of incubation. Then, the supernatant was removed, and 150 μL of DMSO was added with gentle shaking for 10 minutes to fully dissolve the formazan crystals. Absorbance was measured at 490 nm using a microplate reader, and the results were recorded.

Colony forming assay

For the colony formation assay, cells were harvested and seeded into 6-well plates at a density of 5×10^5 cells per well. After a 24-hour treatment with CDCA, 10 μL of the cell suspension was plated in solid medium and cultured at 37°C with 5% CO_2 for 14 days. Colonies were observed under a microscope and quantified using ImageJ software.

Transwell

Cells were starved in serum-free medium for 24 hours before the transwell assay. The ECM gel was pre-cooled, diluted to 1 mg/mL with serum-free medium, and added to transwell chambers, followed by incubation at 37°C for 1-3 hours. After removing the unbound gel, the chambers were rehydrated with 100 μL of serum-free medium. Once no fluid passed through, cells were seeded at a 1:1 ratio with the sample. The chambers were incubated at 37°C in a 5% CO_2 atmosphere for 24-48 hours. After fixation with 4% paraformaldehyde, cells were stained with crystal violet, washed, and air-dried. Migrated cells were observed under a microscope and quantified using ImageJ software.

Statistical analysis

Continuous data were expressed as mean ± standard deviation (SD), whereas categorical variables were expressed as number (percentage). Comparisons between two groups were performed using an unpaired t-test with Welch's

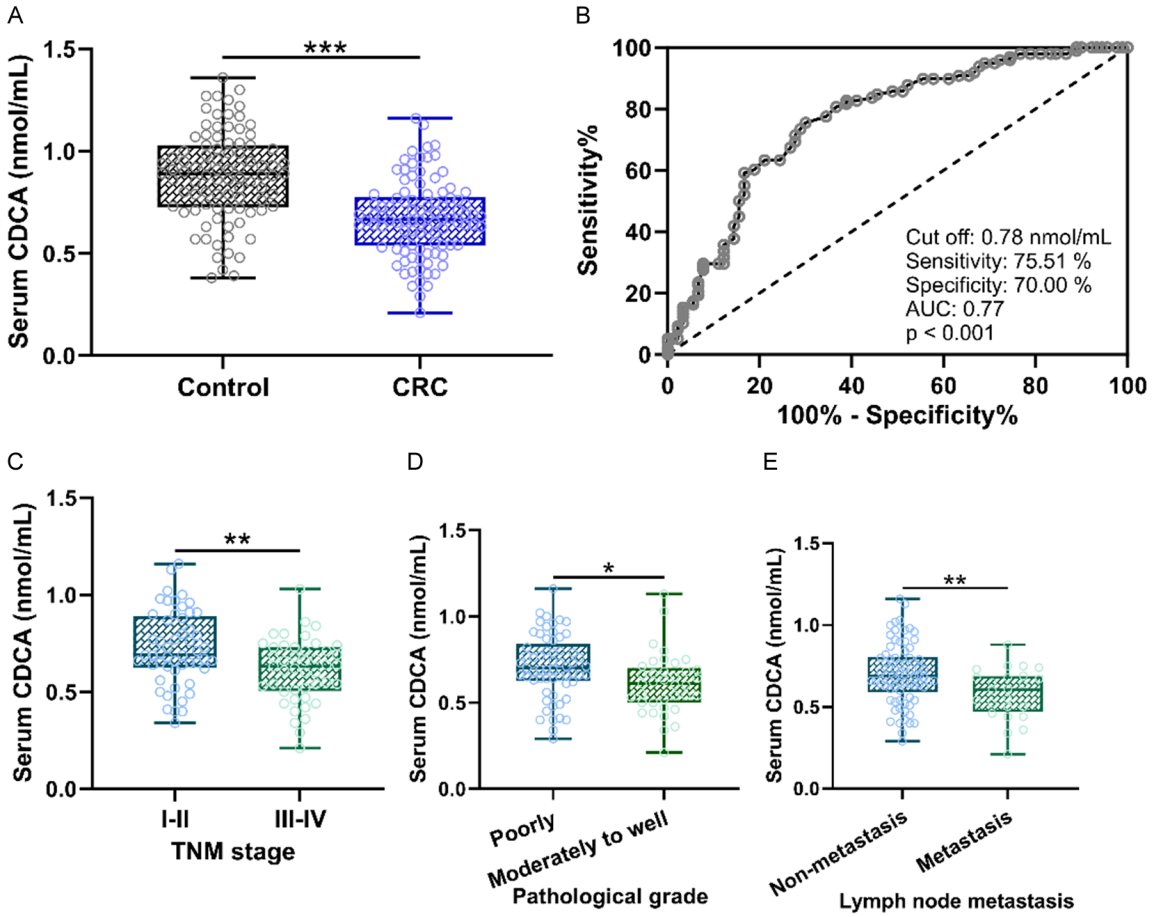

Figure 1. Serum chenodeoxycholic acid (CDCA) served as a biomarker for the diagnosis of colorectal cancer. (A) Comparison of serum CDCA between CRC patients (n = 98) and healthy controls (n = 90). (B) ROC analysis of the value of serum CDCA level in the diagnosis of CRC. (C-E) Comparisons of serum CDCA between TNM stages of I-II and III-IV (C), pathological grades of poorly differentiated and moderately to well differentiated (D), lymph node non-metastasis and metastasis (E) in CRC patients. Data were shown with box plot. *P < 0.05, **P < 0.01, ***P < 0.001 from Unpaired t test with Welch's correction.

correction, while comparisons among multiple groups were performed using the Brown-Forsythe ANOVA test, followed by Dunnett's T3 multiple-comparisons test. Categorical variables were compared using Fisher's exact test. Survival curves were generated using the Kaplan-Meier method, and differences in survival between groups were assessed using the log-rank test. For the MTT assays involving repeated measurements over time, repeated-measures ANOVA was used for overall between-group comparisons, followed by Bonferroni post hoc tests for pairwise comparisons at different time points within groups. Statistical analyses were performed using GraphPad Prism 9.0 software (GraphPad Software, CA, USA). **P < 0.01 indicates statistical significance compared to the control group, while #P < 0.05 and ##P < 0.01 denote significance compared to the CDCA treatment group.

Results

Serum CDCA levels were decreased in colorectal cancer

To compare serum CDCA levels between CRC patients and healthy controls, serum samples from 90 healthy individuals and 98 CRC patients were analyzed. Serum CDCA levels were significantly lower in CRC patients than in healthy controls (**Figure 1A**). Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic potential of serum CDCA for CRC. Serum CDCA showed an area under the curve (AUC) of 0.77, with a

Table 1. Serum chenodeoxycholic acid (CDCA) level and clinicopathological factors in colorectal cancer patients (n = 98)

Variables	Serum CDCA level		*p* value
	Low (n = 49)	High (n = 49)	
Age (years)			
< 65	19 (38.8%)	26 (53.1%)	0.223
≥ 65	30 (61.2%)	23 (46.9%)	
Gender			
Male	27 (55.1%)	24 (49%)	0.686
Female	22 (44.9%)	25 (51%)	
Tumor location			
Colon	26 (53.1%)	28 (57.1%)	0.839
Rectum	23 (46.9%)	21 (42.9%)	
TNM stage			
I-II	19 (38.8%)	34 (69.4%)	0.004
III-IV	30 (61.2%)	15 (30.6%)	
Pathological grade			
Poorly differentiated	24 (49%)	37 (75.5%)	0.012
Moderately to well differentiated	25 (51%)	12 (24.5%)	
Lymph node metastasis			
Non-metastasis	32 (65.3%)	42 (85.7%)	0.033
Metastasis	17 (34.7%)	7 (14.3%)	

Fisher's exact test.

sensitivity of 75.51% and specificity of 70.00% at a cutoff value of 0.78 nmol/mL (**Figure 1B**), suggesting moderate diagnostic performance.

Additionally, subgroup analyses were performed to explore the relationship between serum CDCA levels and clinicopathological features of CRC. Serum CDCA levels were lower in patients with advanced TNM stage (III-IV) than in those with early-stage disease (I-II) (**Figure 1C**). Furthermore, CDCA levels were lower in patients with moderately to well-differentiated tumors compared to those with poorly differentiated tumors (**Figure 1D**). Moreover, serum CDCA levels were significantly reduced in patients with lymph node metastasis compared to those without metastasis (**Figure 1E**).

Together, these results suggested that serum CDCA may have value as a biomarker for CRC diagnosis, progression, and prognosis.

Association between serum CDCA levels and clinicopathological factors in colorectal cancer patients

To investigate the relationship between serum CDCA levels and clinicopathological factors in CRC patients, an analysis was conducted as shown in **Table 1**. Patients were divided into high and low serum CDCA groups based on the median CDCA level. The analysis revealed that low serum CDCA levels were significantly associated with advanced TNM stages (III-IV, P = 0.004), moderately to well-differentiated tumors (P = 0.012), and lymph node metastasis (P = 0.033). No significant differences were observed in age, gender, or tumor location between the groups. These findings suggested that reduced serum CDCA levels may be associated with tumor progression and metastatic potential.

The association of FXR mRNA levels with clinicopathological factors in colorectal cancer patients

To assess changes in FXR gene expression in CRC, qRT-PCR analysis was performed on paired tumor and adjacent normal tissues from CRC patients. Patients were classified into high and low FXR mRNA level groups according to the median expression level. As summarized in **Table 2**, low FXR mRNA expression was significantly associated with advanced TNM stage (P = 0.042), pathological grade (P = 0.003), and lymph node metastasis (P = 0.009), but not with age, gender, or tumor location. These findings suggest that reduced FXR mRNA expression in tumor tissues was associated with CRC progression, highlighting its potential role as a biomarker for CRC prognosis.

Decreased FXR mRNA was associated with tumor progression and metastasis

To evaluate the level of FXR mRNA in CRC tissues and its clinical significance, FXR mRNA levels were analyzed in paired tumor and adjacent normal tissues. The results revealed a significant downregulation of FXR mRNA in tumor tissues compared to adjacent normal tissues (**Figure 2A**, **2B**). Consistently, analysis of a public dataset showed that FXR expression tended to be lower in CRC tumor tissues than in non-tumor tissues (Figure S1A). Furthermore, patients with higher FXR expression showed a trend toward better overall survival (Figure

Table 2. FXR mRNA level and clinicopathological factors in colorectal cancer patients (n = 98)

Variables	FXR mRNA level		*p* value
	Low (n = 49)	High (n = 49)	
Age (years)			
< 65	21 (42.9%)	24 (49%)	0.685
≥ 65	28 (57.1%)	25 (51%)	
Gender			
Male	23 (46.9%)	28 (57.1%)	0.419
Female	26 (53.1%)	21 (42.9%)	
Tumor location			
Colon	29 (59.2%)	25 (51%)	0.543
Rectum	20 (40.8%)	24 (49%)	
TNM stage			
I-II	21 (42.9%)	32 (65.3%)	0.042
III-IV	28 (57.1%)	17 (34.7%)	
Pathological grade			
Poorly differentiated	23 (46.9%)	38 (77.6%)	0.003
Moderately to well differentiated	26 (53.1%)	11 (22.4%)	
Lymph node metastasis			
Non-metastasis	31 (63.3%)	43 (87.8%)	0.009
Metastasis	18 (36.7%)	6 (12.2%)	

Fisher's exact test.

S1B). ROC analysis showed that FXR mRNA expression had diagnostic value for CRC, with an AUC of 0.79, 70.41% sensitivity, and 74.49% specificity (**Figure 2C**, P < 0.001).

Furthermore, consistent with **Table 2**, FXR mRNA levels were lower in patients with advanced TNM stages (III-IV) than in those with early stages (I-II) (**Figure 2D**). Similarly, patients with moderately to well-differentiated tumors exhibited lower FXR levels than those with poorly-differentiated tumors (**Figure 2E**). Additionally, lower FXR levels were significantly associated with lymph node metastasis in comparison to non-metastatic cases (**Figure 2F**).

These findings indicated that FXR mRNA was downregulated in CRC tumor tissues and correlated with tumor progression and metastasis, supporting its potential value as a diagnostic and prognostic biomarker.

Correlation of serum CDCA and tumor FXR mRNA level

We next examined the correlation between serum CDCA levels and tumor FXR mRNA expression and evaluated their prognostic significance in CRC patients. A significant positive correlation was observed between serum CDCA levels and tumor FXR mRNA expression (r = 0.64, P < 0.001, **Figure 3A**). Patients were divided into low and high groups according to the median serum CDCA level or tumor FXR mRNA expression. Kaplan-Meier survival analysis revealed that patients with higher serum CDCA levels had significantly longer 5-year overall survival compared to those with lower levels (P = 0.022, **Figure 3B**). Similarly, patients with higher tumor FXR mRNA expression also had longer 5-year overall survival than those with lower expression (P = 0.008, **Figure 3C**).

Together, these findings suggested that higher serum CDCA levels and tumor FXR mRNA expression were associated with better overall survival in CRC patients.

CDCA and FXR inhibited the growth of CRC cells

The regulatory effects of CDCA and FXR on the proliferation of CRC cell lines were examined. A dose-dependent decrease in cell viability was observed in HCT116 and SW620 cells treated with increasing concentrations of CDCA (**Figure 4A** and **4B**). CDCA inhibited cell viability in a time-dependent manner, with the most pronounced effect observed at 0.4 mM. In contrast, FXR knockdown increased cell viability in both HCT116 and SW620 cells, especially at later time points (**Figure 4C** and **4D**). Similarly, CDCA treatment decreased HT-29 cell viability, and this inhibitory effect was attenuated by FXR knockdown (Figure S2A). This suggested that FXR knockdown promoted CRC cell growth. In addition, the FXR mRNA levels were increased after CDCA treatment, and this induction was attenuated by shFXR in both HCT116 and HT-29 cells (Figure S2B, S2C). These findings indicated that CRC cell proliferation was inhibited by CDCA treatment in a time- and dose-dependent manner, while FXR knockdown promoted cell growth. Together, these results suggested that the CDCA-FXR axis may be involved in regulating CRC cell proliferation.

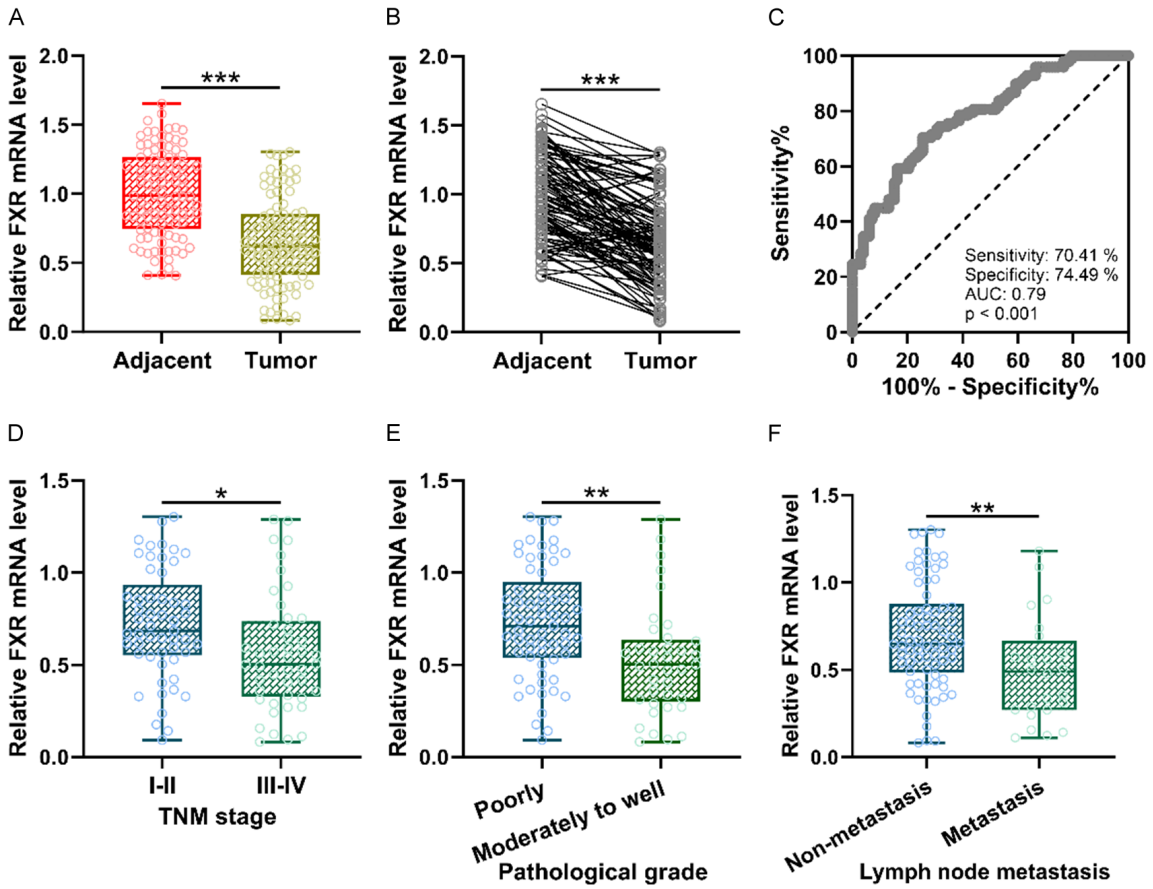

Figure 2. The expression of FXR in CRC tumor tissues. qRT-PCR was used to determine the expression of FXR mRNA between adjacent and tumor tissues of CRC patients (n = 98). Unpaired t-test with Welch's correction (A) and Paired t-test (B). (C) ROC analysis of the value of FXR mRNA level in the diagnosis of CRC. (D-F) Comparisons of FXR mRNA level in tumor tissues between TNM stages of I-II and III-IV (D), pathological grades of poorly differentiated and moderately to well differentiated (E), lymph node non-metastasis and metastasis (F) in CRC patients. Data were shown with box plot. *P < 0.05, **P < 0.01, ***P < 0.001 from Unpaired t test with Welch's correction.

CDCA inhibited the proliferation and migration of CRC cells in an FXR-dependent manner

To investigate whether FXR mediates the antitumor effects of CDCA, SW620 cells were treated with 0.4 mM CDCA after FXR knockdown by shFXR. CDCA treatment significantly inhibited the colony-forming ability of SW620 cells compared to the control group (**Figure 5A**). However, this inhibitory effect was attenuated by FXR knockdown, suggesting that CDCA suppressed CRC cell proliferation at least partly through FXR activation (**Figure 5A**).

Similarly, CDCA significantly reduced the migration activity of SW620 cells (**Figure 5B**). In contrast, the inhibitory effect on migration was markedly attenuated after FXR was knocked down (**Figure 5B**).

These findings suggested that CDCA inhibited CRC cell proliferation and migration at least partly through an FXR-dependent mechanism.

CDCA activated FXR and inhibited Wnt/β-catenin signaling in CRC

To assess the effects of CDCA on FXR activation in CRC cells, the mRNA level of FXR was measured in SW620 cells after treatment with 0.4 mM CDCA. The results showed that CDCA significantly upregulated FXR mRNA expression (**Figure 6A**), and this effect was markedly attenuated by FXR silencing. Similarly, CDCA treatment also increased FXR protein levels, which were attenuated by shFXR (**Figure 6B** and **6C**).

These results indicated that CDCA upregulated FXR at both the mRNA and protein levels.

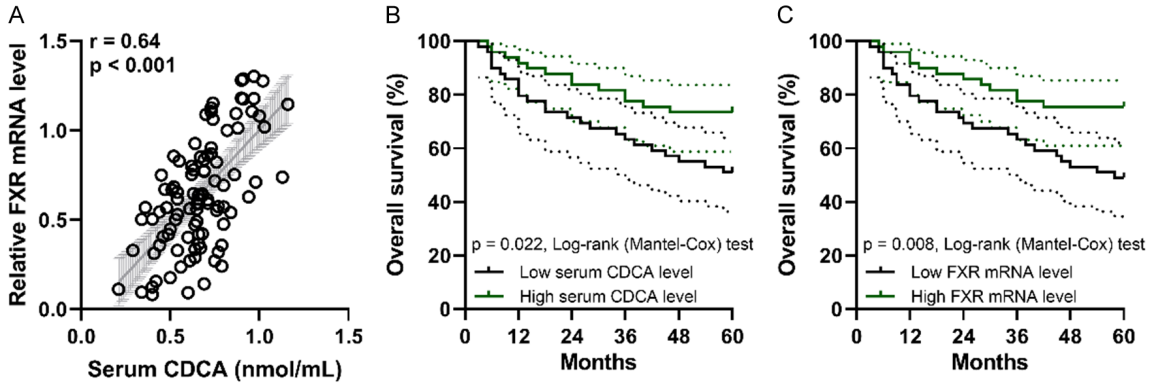

Figure 3. Correlation of serum CDCA and tumor FXR mRNA level in CRC patients and their prognostic values. (A) Pearson correlation analysis of serum CDCA and tumor FXR mRNA level in CRC patients. According to the median of serum CDCA and tumor FXR mRNA level, CRC patients were divided into low serum CDCA and high serum CDCA groups (B), low FXR mRNA level, and high FXR mRNA level (C). The overall survival curves during the 5 years of follow-up were plotted respectively.

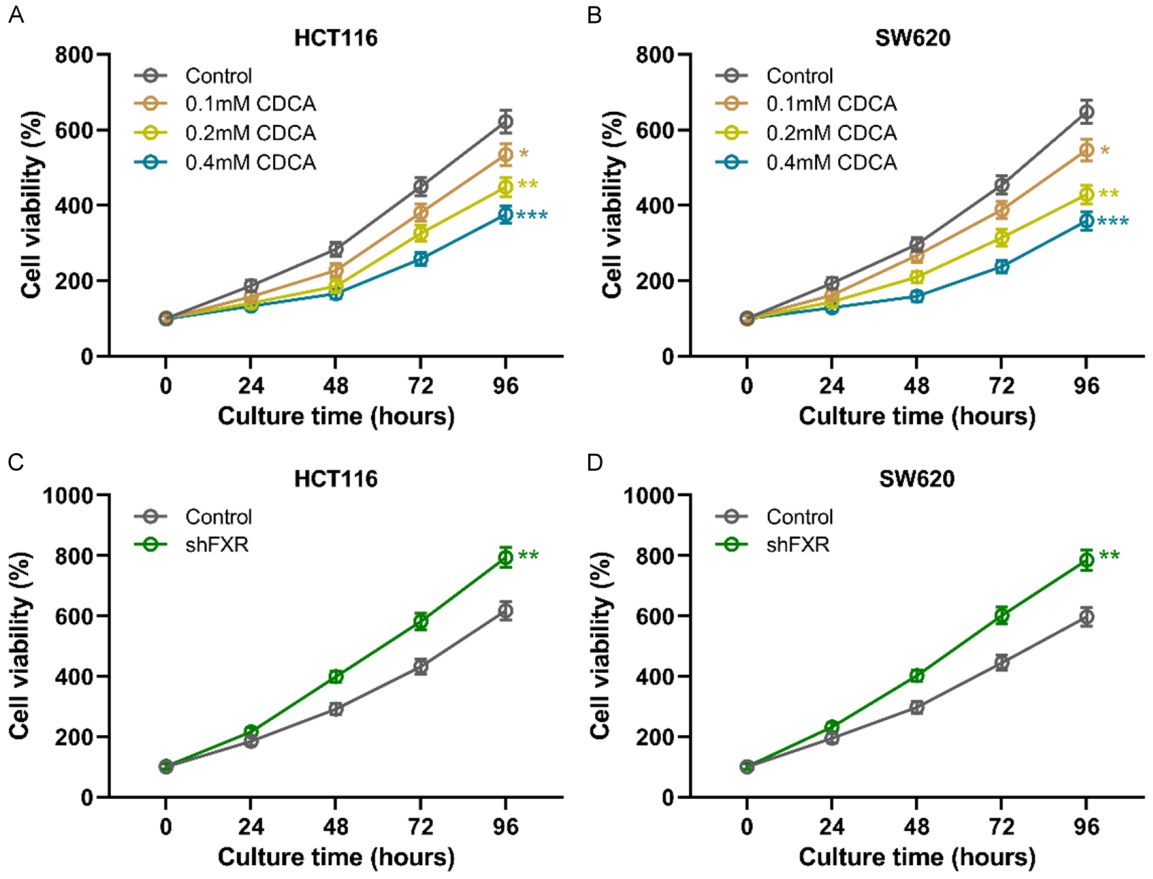

Figure 4. CDCA and FXR inhibited the growth of CRC cells. HCT116 and SW620 cells were stimulated with different concentrations of CDCA, and the cell viability at different times was measured by MTT (A and B). HCT116 and SW620 cells were transfected with shFXR, and the cell viability at different times was measured by MTT (C and D). Data were shown with mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 compared to control. Repeated-measures ANOVA was used for comparisons among multiple groups with time as a within-subject factor, followed by post hoc tests.

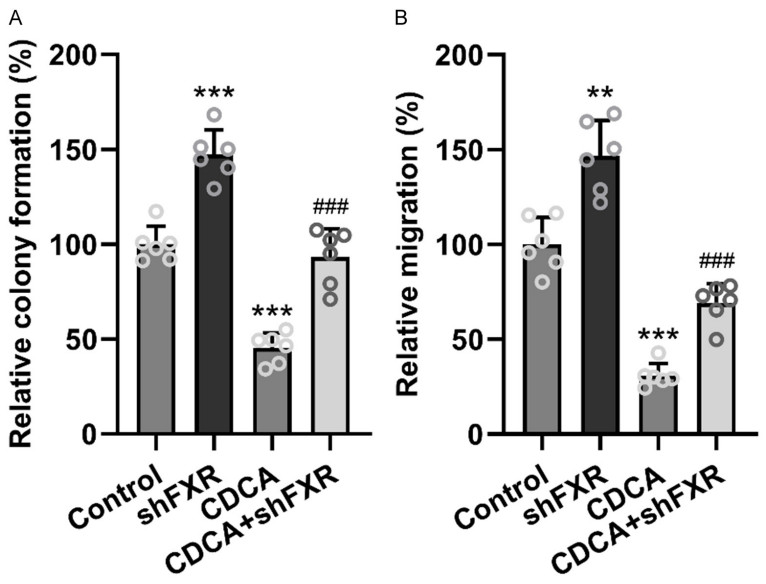

Figure 5. CDCA inhibited the proliferation and migration of CRC cells. SW620 cells were stimulated with 0.4 mM CDCA. SW620 cells were transfected with shFXR for 48 hours and then stimulated with 0.4 mM CDCA. A. The colony formation assay was conducted after 10 days of culture and the relative colony formation was compared. B. The migration assay was conducted after two days of culture by transwell assay and the relative migration was compared. Data were shown with mean ± SD. ***P < 0.001 compared to control, ##P < 0.01, ###P < 0.001 compared to CDCA. Brown-Forsythe ANOVA test.

Furthermore, CDCA treatment also reduced the protein expression of β-catenin and Wnt3a, and this effect was partially reversed by FXR knockdown (**Figure 6D-F**). To further evaluate downstream Wnt/β-catenin signaling, we examined the protein levels of c-Myc and Cyclin D1 in SW620 cells. CDCA treatment significantly decreased c-Myc and Cyclin D1 protein levels compared with the control group, whereas FXR knockdown attenuated the inhibitory effects of CDCA on these proteins (Figure S3).

Together, these findings suggested that CDCA activated FXR at both the mRNA and protein levels, thereby inhibiting Wnt/β-catenin signaling.

Discussion

The global incidence and mortality rates of colorectal cancer are increasing, with its development attributed to both genetic and environmental factors. Among environmental factors, Western dietary patterns characterized by high fat and low fiber intake have been closely linked to altered bile acid metabolism. Increased intestinal exposure to dysregulated bile acids may promote colorectal tumorigenesis through oxidative stress, DNA damage, inflammatory responses, and pro-proliferative signaling [17, 18]. Our study shows that serum CDCA levels are significantly lower in CRC patients than in healthy controls, supporting its potential value as a diagnostic and prognostic biomarker.

In recent years, dysregulation of bile acid metabolism has gained increasing attention in CRC research. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) based serum bile acid profiling has also been reported as a potential approach for CRC screening [19]. Recent evidence further supports the association between altered bile acid profiles and CRC risk. In particular, updated clinical evidence indicates that fecal bile acid profiles are associated with CRC incidence and risk stratification, while experimental evidence suggests that CDCA-related microbial metabolism may influence susceptibility to high-fat diet-induced CRC [20, 21]. In line with these findings, our study showed a significant decrease in serum CDCA levels in colon cancer patients, further supporting the potential value of CDCA as a candidate biomarker for CRC.

FXR, a key nuclear receptor in bile acid metabolism, plays a tumor-suppressive role in CRC. Our findings demonstrated that reduced FXR mRNA level in CRC tumor tissues correlated with advanced TNM stages, poor tumor differentiation, and lymph node metastasis, suggesting that lower FXR expression was associated with CRC progression. This is consistent with the known role of CDCA in colon cancer progression. Further analysis revealed significant downregulation of FXR mRNA in CRC tumors, reinforcing its association with tumor aggressiveness. Moreover, analysis of public CRC datasets showed a similar trend toward lower FXR expression in tumor tissues than in non-tumor tissues, and patients with higher FXR expression tended to have better overall

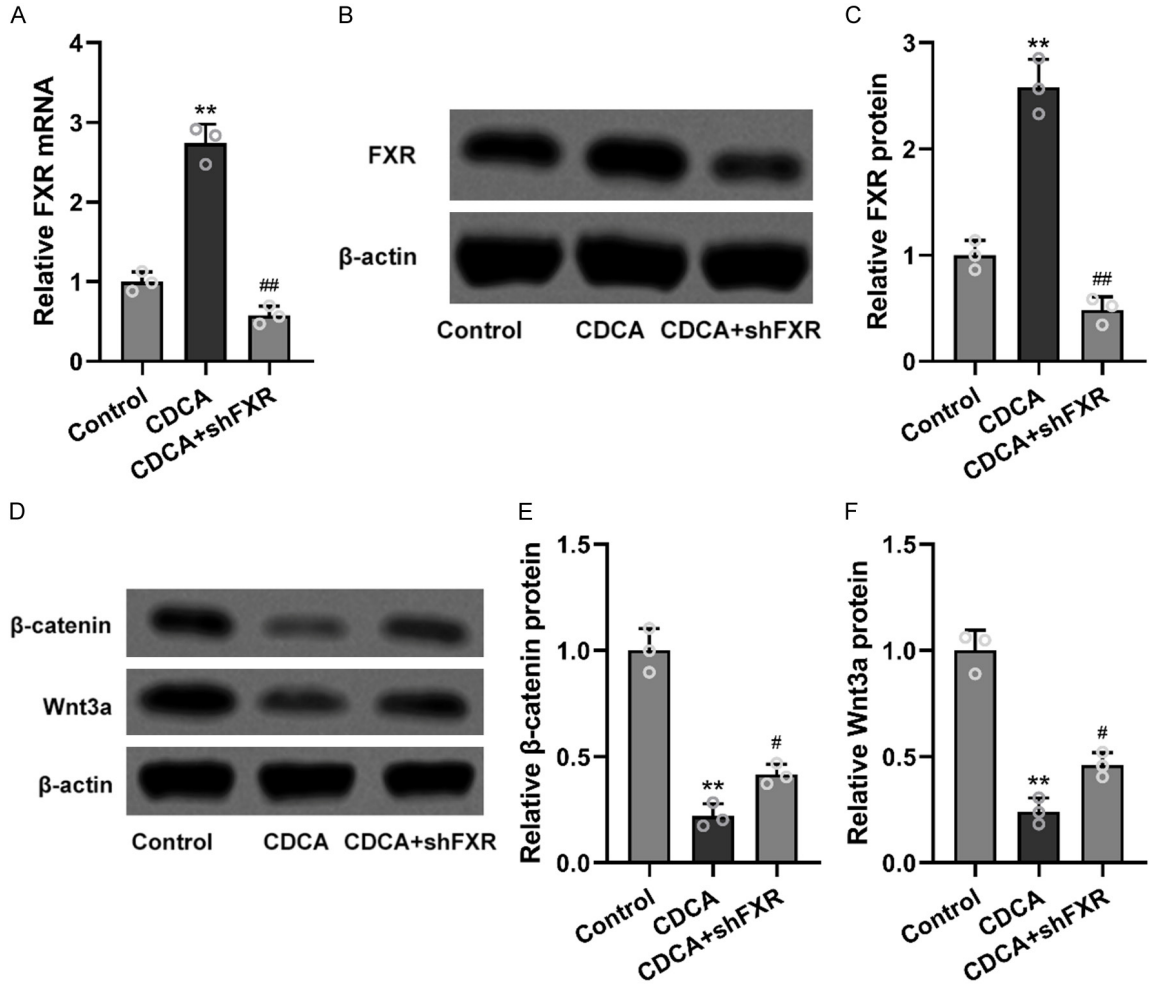

Figure 6. CDCA activated FXR and inhibited Wnt/β-catenin signaling in CRC. SW620 cells were stimulated with 0.4 mM CDCA for 48 hours. SW620 cells were transfected with shFXR for 48 hours and stimulated with 0.4 mM CDCA for 48 hours. qRT-PCR was used to determine the expression of FXR mRNA (A), and Western blotting was used to measure the protein expression of FXR (B and C). Western blotting was used to measure the protein expression of β-catenin and Wnt3a (D-F). Data were shown with mean ± SD. **P < 0.01 compared to control, #P < 0.05, ##P < 0.01 compared to CDCA. Brown-Forsythe ANOVA test.

survival. ROC analysis indicated FXR's potential as a diagnostic biomarker for CRC, with an AUC of 0.79, sensitivity of 70.41%, and specificity of 74.49%, supporting its role in CRC diagnosis and prognosis. In addition, we observed a significant positive correlation between serum CDCA levels and tumor FXR mRNA level ($r = 0.64$, $P < 0.001$). Kaplan-Meier survival analysis further showed that higher serum CDCA levels and elevated FXR expression were closely associated with longer 5-year overall survival. These results suggest that serum CDCA and tumor FXR mRNA may serve as prognostic biomarkers for CRC, reflecting their potential to guide CRC diagnosis, prognosis, and clinical management. However, the molecular mechanisms underlying the tumor-suppressive effects of CDCA and FXR remain unclear and require further investigation.

In FXR-knockout mice, cholestyramine normalized bile acid levels but did not reduce tumorigenesis, suggesting that elevated bile acids alone do not drive tumor development in the absence of FXR [22]. Our study found that CDCA treatment significantly inhibited colony formation and migration activity of SW620 cells, suggesting its anti-proliferative and anti-migratory effects. Consistently, CDCA also reduced cell viability, and this inhibitory effect was attenuated by FXR knockdown in HT-29 cells. Additionally, CDCA increased FXR mRNA

expression in HCT116 and HT-29 cells, whereas shFXR reduced this induction. Together with the results from SW620 cells, these findings support the reproducibility of the CDCA-FXR axis across different CRC cell models. The attenuation of CDCA-induced inhibition after FXR knockdown further indicates that the anti-tumor effects of CDCA are largely dependent on FXR activity. These findings suggest that FXR is important for CDCA-mediated inhibition of CRC cell proliferation and migration.

Previous studies have shown that FXR knockout increased intestinal tumor incidence, tumor burden, and lymph node involvement. Moreover, β-catenin levels were significantly upregulated, accompanied by increased expression of its downstream targets, including c-Myc and Cyclin D1 [22, 23]. Zhang et al. found that TMAO activated Wnt/β-catenin signaling by inhibiting the FXR-FGF15 pathway [24], suggesting a regulatory link between FXR and Wnt/β-catenin signaling. Our study revealed that CDCA treatment suppressed the key components of the Wnt/β-catenin signaling pathway, including β-catenin and Wnt3a. This effect was partially reversed by FXR knockdown, suggesting that CDCA suppresses Wnt/β-catenin signaling at least partially through FXR activation. Furthermore, the additional western blot analyses showed that CDCA significantly decreased the protein levels of the canonical Wnt/β-catenin target proteins c-Myc and Cyclin D1 in SW620 cells. FXR knockdown attenuated the inhibitory effects of CDCA on these downstream proteins, further supporting that CDCA suppresses Wnt/β-catenin downstream signaling at least partly through FXR activation. These results support an important role for FXR activation in CDCA-mediated regulation of the Wnt/β-catenin pathway.

Moreover, FXR plays a crucial role in modulating intestinal mucosal immune responses. FXR knockout mice show increased immune cell infiltration within the intestinal epithelium and elevated TNF-α production [23]. Recent evidence indicates that FXR activation can ameliorate intestinal inflammation and inhibit colitis-associated tumor growth by regulating gut macrophage recruitment, polarization, and crosstalk with Th17 cells [25]. Therefore, it is possible that FXR could inhibit colon cancer progression by regulating gut macrophage recruitment.

Several limitations should be acknowledged in this study. First, the potential of CDCA as a diagnostic and prognostic biomarker must be validated in larger, independent cohorts to establish its clinical relevance. Additionally, although CRC cell proliferation was inhibited by CDCA treatment in vitro, the incomplete reversal of this effect after FXR knockdown suggests that other FXR-independent mechanisms may also contribute to the anti-tumor effects of CDCA. Further studies are needed to clarify the specific pathways through which FXR modulates the tumor-suppressive action of CDCA. Moreover, this study lacks in vivo validation using tumor-bearing animal models. Future studies are warranted to further confirm the anti-tumor effects of CDCA and its FXR-dependent mechanisms in vivo. Finally, the broader role of FXR in CRC progression, including its interactions with the immune response and tumor microenvironment, has yet to be fully explored and warrants further research.

Conclusions

This study showed that serum CDCA levels were significantly reduced in CRC patients and were associated with advanced tumor stages and poorer prognosis. Additionally, FXR mRNA expression was also decreased in CRC tissues and closely associated with tumor aggressiveness and metastasis. Functionally, CDCA treatment inhibited CRC cell proliferation and migration in an FXR-dependent manner, partially through the suppression of β-catenin and Wnt3a protein expression. Collectively, these findings suggest that serum CDCA levels may serve as potential biomarkers for CRC diagnosis and prognosis, while the CDCA-FXR axis may represent a therapeutic target.

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All patients signed the informed consent.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yuzheng Xue, Department of Gastroenterology, Affiliated Hospital of Jiangnan University, No. 1000 Hefeng Road, Wuxi 214122, Jiangsu, China. Tel: +86-15301516562; E-mail: 9862018034@jiangnan.edu.cn; Dr. Tong Wang, Department of General Surgery, Wuxi People's Hospital Affiliated to Nanjing Medical University, No. 299 Qingyang Road, Wuxi 214023, Jiangsu, China. Tel: +86-13013675222; E-mail: aanti@njmu.edu.cn

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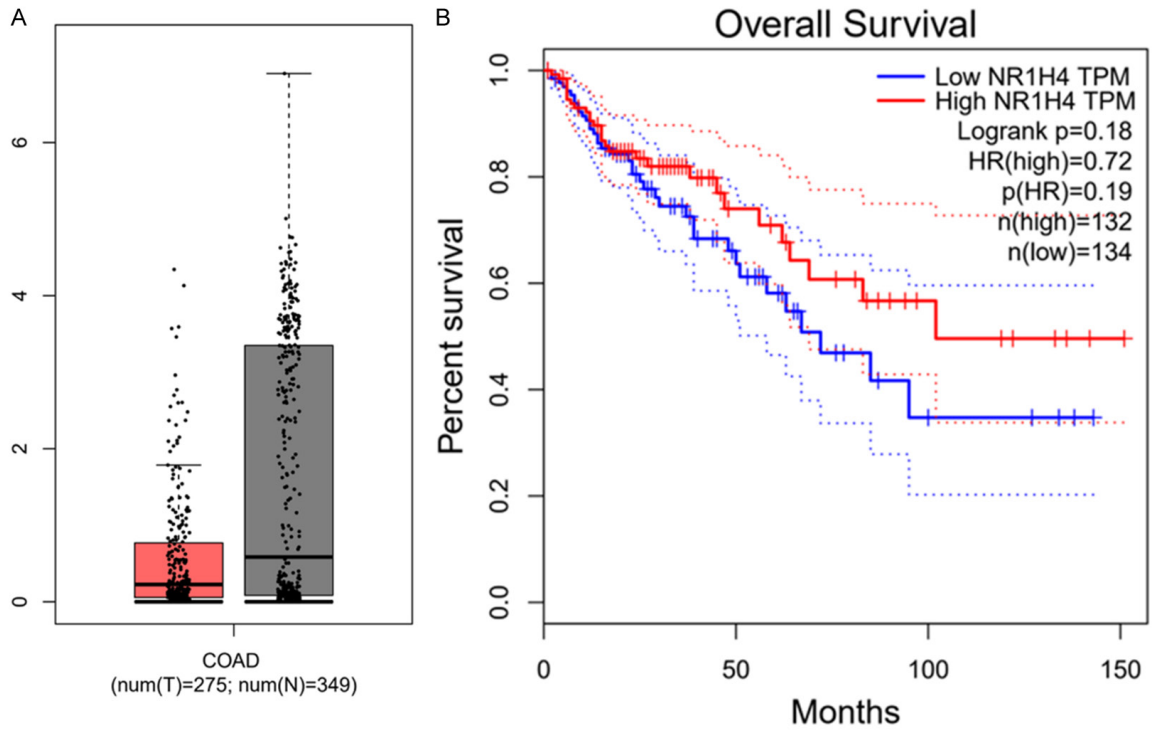

Figure S1. The expression of FXR in CRC tumor tissues (A) and the overall survival curves of CRC patients according to the median of FXR expression (B). Data were from http://gepia.cancer-pku.cn/index.html.

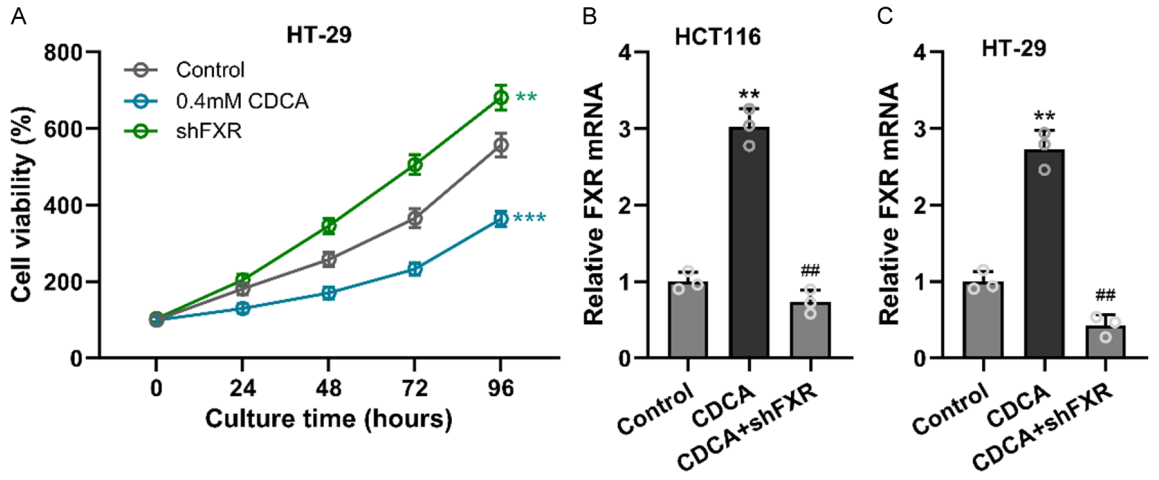

Figure S2. CDCA and FXR inhibited the growth of HT-29 cells. A. HT-29 cells were stimulated with 0.4 mM CDCA or transfected with shFXR and the cell viability at different time were measured by MTT. B, C. HCT116 and HT-29 cells were transfected with shFXR for 48 hours and stimulated with 0.4 mM CDCA for 48 hours. qRT-PCR was used to determine the expressions of FXR mRNA. Data were shown with mean ± SD. **P < 0.01, ***P < 0.001 compared to control, ##P < 0.01 compared to CDCA. Brown-Forsythe ANOVA test.

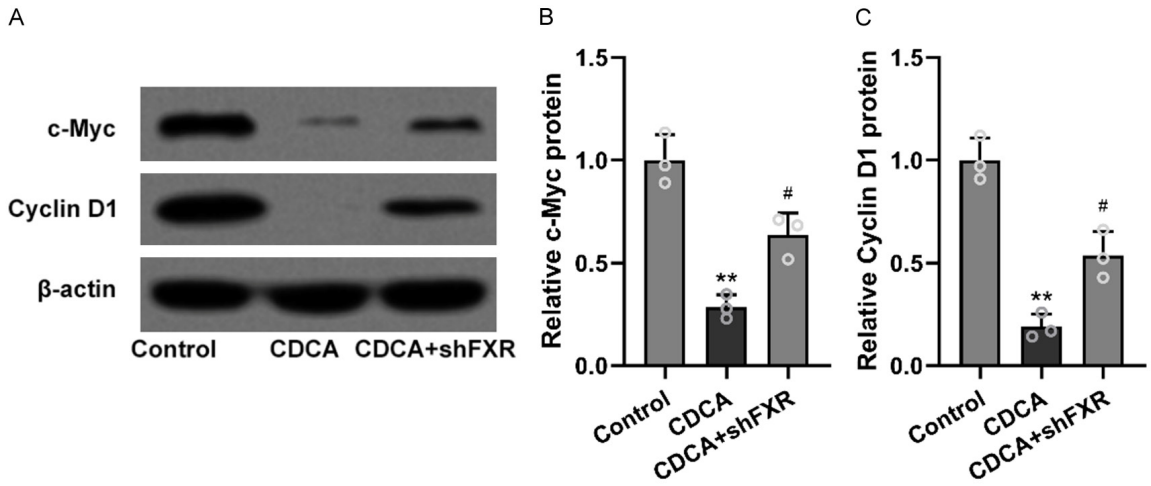

Figure S3. SW620 cells were stimulated with 0.4 mM CDCA for 48 hours. SW620 cells were transfected with shFXR for 48 hours and stimulated with 0.4 mM CDCA for 48 hours. Western blotting was used to measure the protein expressions of c-Myc and Cyclin D1 (A) and the comparisons among three groups (B and C). Data were shown with mean ± SD. **P < 0.01 compared to control, #P < 0.05 compared to CDCA. Brown-Forsythe ANOVA test.