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Expression and Prognostic Implication of the Nuclear Receptors Farnesoid X Receptor and Pregnane X Receptor in Human Cholangiocarcinoma

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Abstract

Background/Aim: Cumulative evidence reveals the contribution of nuclear receptors in the development and progression of cancers. The present study aimed to investigate the expression of two nuclear receptors, farnesoid X receptor (FXR) and pregnane X receptor (PXR), in cholangiocarcinoma (CCA) tissues and the correlation of their expression with clinicopathological features.

Materials and Methods: FXR and PXR expression was evaluated in 111 resected CCA patient samples using immunohistochemistry, and the association of its expression with clinicopathological parameters was analyzed. The effectiveness of the expression of these nuclear receptors in predicting patients' outcomes was also assessed.

Results: FXR and PXR positivity was noted in all examined samples, with their expression predominantly localized in the cytoplasm. Patients with intraductal papillary neoplasm of the bile duct had significantly lower FXR expression (p=0.042). Elevated FXR expression was also significantly associated with lymph node metastasis (p=0.006) and advanced tumor stage (p=0.007). The results of the log-rank test analysis showed that the survival time of the patients was not associated with the expression of these two nuclear receptors. However, the CCA patients presenting low FXR/PXR expression tended to have a longer overall survival (p=0.125).

Conclusion: FXR may contribute to the progression of CCA to more advanced stages. The prognostic significance of FXR and PXR expression appears relevant in CCA. These nuclear receptors may have a function in the prediction and treatment of this deadly disease.

Keywords: FXR, PXR, cholangiocarcinoma, nuclear receptor.

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Introduction

Cholangiocarcinoma (CCA) is an aggressive hepatobiliary malignant neoplasm of bile ducts occurring predominantly in the Southeast Asia countries, especially Thailand (1). The etiology of CCA in this region has a stronger link to liver fluke infection particularly *Opisthorchis viverrine* and *Clonorchis sinensis* (2). Primary sclerosing cholangitis and chronic hepatitis viral infection represent other risk factors of CCA in other regions (1). Although the diagnosis and management of CCA has been improved, research exploring molecular targets or biomarkers that are correlated with the disease progression and metastasis is still helpful to develop a strategy to enhance the treatment efficacy of this deadly disease.

Nuclear receptors are ligand-dependent transcription factors that are activated by various endogenous and xenobiotic substances. They act as a hub for orchestrating cellular complex regulatory networks to control downstream molecular events involved in many human biological processes including development, metabolism, reproduction, and immunity (3). Farnesoid X receptor (FXR), also known as NR1H4, is a member of the nuclear receptor superfamily. It is predominantly expressed in enterocytes, hepatocytes, and cholangiocytes (4). FXR plays a crucial role in maintaining bile acid homeostasis by acting as a bile acid sensor to regulate the expression of genes involved in the synthesis, uptake, transport, and metabolism of bile acids (4). FXR also regulates the metabolism of cholesterol, lipids, and glucose. Besides its role in metabolic regulation, FXR modulates the expression of genes involved in inflammation, fibrosis, cell differentiation, and proliferation (5, 6). Moreover, cumulative evidence also reveals the contribution of FXR in the development and progression of cancers (7, 8). However, the results concerning the role and the expression of FXR in cancers are controversial. It has been reported that FXR promoted the transforming growth factor-β-stimulated epithelial-mesenchymal transition (EMT) process in hepatocellular carcinoma (9). Suppression of FXR could inhibit glycochenodeoxycholate-induced EMT and metastasis of gallbladder cancer cells (10). In addition, over-expression of FXR in pancreatic and gallbladder cancer tissues has been shown to be associated with higher tumor grade, poor survival, and nodal metastasis (8, 11, 12). Contrastingly, the anticancer effects of FXR have been demonstrated in different studies. It possesses anticancer activity against hepatocellular carcinoma *via* inhibiting β-catenin activity (13). In CCA, a previous study found that FXR inhibits migration, invasion, and EMT of CCA cells *via* suppression of IL-6 expression (14). Moreover, the high expression of FXR in breast tumor tissues was related with prolonged survival and reduced metastasis (15). However, due to these contradictory findings, the clinical significance and functional roles of FXR in CCA remain challenging to elucidate.

In addition to FXR, pregnane X receptor (PXR), also known as NR1I2, is considered a nuclear receptor that plays a critical role in regulating cellular signaling cascades under physiological and pathological conditions such as inflammation and cancers (16). PXR is a member of transcription factors with high expression in the liver, small intestine, and colon. It is activated by a diverse array of endogenous compounds, such as steroid hormones, bile acids, and xenobiotics, including therapeutic drugs. PXR prevents the toxicities of exogenous substances to the body by functioning as a master regulator to manipulate the expression of detoxification enzymes (17). Apart from its role in endobiotic and xenobiotic metabolism, previous studies have reported that PXR exhibits an anti-inflammatory effect by interacting with NF-κB and AP-1 (18). In cancer, it has been recently reported that PXR dramatically suppresses the progression of liver cancer in a chemical-induced carcinogenesis mouse model (19). Although the detailed mechanism is not clear, transcriptome analyses suggest that PXR can suppress EMT associated with transformation and infiltration of malignant cells (19). Similarly, the ectopic expression of PXR in HepG2 cells suppressed metastasis behavior, suggesting that PXR may play an anticancer role in the liver (20). On the contrary, some studies have revealed a correlation between

activation of PXR and hyperproliferation of hepatocytes (21). Despite the well-known relationship between bile acids and nuclear receptors, pleiotropic effects of PXR in CCA have not been completely unraveled. A previous study has reported the over-expression of a PXR partner, RXRα, in human CCA tissues and CCA cell lines (22). The role of RXRα in CCA growth and survival has also been demonstrated in that study (22). However, the evaluation of the PXR expression in CCA specimens and its prognostic significance remains scarce.

In the present study, we aimed to investigate the clinical significance of FXR and PXR protein expression in CCA tissues using immunohistochemistry. The correlation between the expression levels and various clinicopathologic parameters was then analyzed. The potential of these nuclear receptors as prognostic predictors of CCA was also evaluated.

Materials and Methods

Human specimens. This study was retrospectively conducted on 111 specimens of paraffin-embedded liver tissues of patients with histologically proven CCA who underwent surgical resection. These tissues were obtained from the specimen bank of the Cholangiocarcinoma Research Institute, Khon Kaen University, Thailand. The study protocol was approved by the ethical committee for human research of Khon Kaen University (HE651204). The hospital medical charts and pathology records of patients were reviewed for sex, age, tumor location, tumor size, histological type and grade, tumor stage, the presence or absence of intraductal (ID) component, which refers to intraductal papillary neoplasm of the bile duct (IPNB), lymph-node involvement (LN), the presence or absence of lymphovascular invasion (LVSI), and the presence or absence of mucin. The staging of tumor was evaluated based on TNM classification.

Immunohistochemical staining and scoring. The experiment was performed using standard immunohistochemical assay. Briefly, 3 μm sections of formalin-fixed paraffin-embedded sections were mounted on glass slides. Thereafter, the specimens were deparaffinized in xylene and dehydrated in ethanol. Antigen retrieval was performed using microwave treatment for 30 min in 10 mM citrate buffer (pH 6.0). Endogenous peroxidase was inactivated by incubation with 3% hydrogen peroxide in water. After washing with Tris-buffered saline, the tissues were then incubated with 3% bovine serum albumin to block non-specific binding sites. After that, the sections were incubated with specific primary antibodies against FXR (dilution 1:100, #DF12511, Affinity Biosciences, Cincinnati, OH, USA) or PXR (dilution 1:200, #DF4827, Affinity Biosciences) at 4°C overnight. After complete incubation, the tissue sections were incubated with Dako EnVision HRP-labelled polymer anti-rabbit system (K4003; Dako, Kyoto, Japan) according to the manufacturer's instruction for 1 h at room temperature, and visualized with ImmPACT® DAB substrate Peroxidase (SK-4105, Vector Laboratories, Inc., Newark, CA, USA). Sections were then counterstained with hematoxylin and mounted in VectaMount® Permanent Mounting Medium (H-5000-60, Vector Laboratories, Inc.). Staining of FXR and PXR was semi-quantitatively scored based on the number of positive cells and stain intensity (H-scores).

Statistical analysis. Pearson' s chi square test was performed to analyze the relationship between FXR or PXR expression and clinicopathological parameters of CCA patients. Association between FXR and PXR was analyzed using Spearman's rho correlation test. The Kaplan–Meier method with a log-rank test was used to analyze the difference in survival rates between groups. A multivariate analysis by the Cox's proportional hazard regression model was performed to determine the correlation between clinicopathologic parameters and survival time of patients after tumor removal. The parameters found to be significant in univariate analysis and covariates considered as essential parameters for patients' outcome were included in the model. A *p*-value less than 0.05 was considered statistically significant.

Results

Clinicopathological and histopathological characteristics of the studied tumor tissues. The clinicopathological and histopathological features of the patients are summarized in Table I. The study comprised 43 female (38.73%) and 68 male (61.26%) patients. The age range was 43 to 83 years, with a mean age of 63 years. Forty-nine cases (44.14%) were less than 63 years old. In our series, 45 cases (40.54%) were classified as intrahepatic CCA, 64 cases (57.66%) as perihilar CCA, and two cases (1.80%) as distal CCA. Most tumors were grade G1 (83.78%) and well-differentiated (83.78%). Regarding the histological subtypes, most cases were periductal infiltration in intrahepatic mass-forming CCA (39.64%). Most of the tumors (49.55%) were at an advanced stage (stage IV). Mucin was observed in 91 cases (81.98%). LN and LVSI were present in 53 cases (47.75%) and 95 cases (85.59%), respectively.

Expression of FXR and PXR in CCA tissues. All 111 specimens showed positive staining for FXR expression in varying proportions and different intensities (Figure 1). The subcellular pattern of FXR distribution was cytoplasmic in 108 cases (97.3%) and both cytoplasmic and nuclear in three cases (2.7%) (Figure 1B and C). In this study, both cytoplasmic and nuclear staining were considered to semi-quantify FXR expression. The specimens were then divided into two subgroups; low and high expression, with 55 tissues (49.55%) exhibiting low expression and 56 samples (50.45%) showing high expression. FXR expression was also observed in normal bile duct cells adjacent to the tumor (Figure 1D).

A positive signal for PXR expression was detected in all cases. Among 111 specimens studied, 79 cases (71.2%) exhibited diffuse cytoplasmic pattern (Figure 2B), while 32 cases (28.8%) displayed cytoplasmic granular patterns (Figure 2C). In this study, both staining patterns were considered to semi-quantify PXR expression. Based on the intensity and frequency of staining, 47 tissues (42.34%) showed low expression and 64 tissues (57.66%) exhibited

Table I. *Clinical and pathological characteristics of all patients.*

Clinical and pathological characteristics	N=111	%
Sex		
Male	68	61.3
Female	43	38.7
Age, mean (years)		
<63	49	44.1
≥63	62	55.9
Tumor location		
Intrahepatic	45	40.5
Perihilar	64	57.7
Distal	2	1.8
Histological grade		
Grade 1	93	83.8
Grade 2	16	14.4
Grade 3	2	1.8
Histological grade		
Well differentiation	93	83.8
Not well differentiation	18	16.2
Histological type		
Intraductal (ID)	4	3.6
Periductal-infiltrating (PI)	2	1.8
Mass-forming (MF)	23	20.7
ID + PI	1	0.9
ID + MF	24	21.6
PI + MF	44	39.6
ID + PI + MF	13	11.7
Intraductal papillary neoplasm of the bile duct		
No	69	62.2
Yes	42	37.8
Tumor size		
<4.5 cm	62	55.9
≥4.5 cm	49	44.1
Mucin		
Absence	20	18.0
Presence	91	82.0
Lymphovascular invasion		
No	16	14.4
Yes	95	85.6
Lymph node invasion		
No	58	52.3
Yes	53	47.7
Tumor stage		
Stage I	8	7.2
Stage II	13	11.7
Stage III	35	31.5
Stage IV	55	49.5
Presence of OV		
None	105	94.6
Gross	6	5.4

ID: Intraductal; PI: periductal-infiltrating; MF: mass-forming; OV: *Opisthorchis viverrine*.

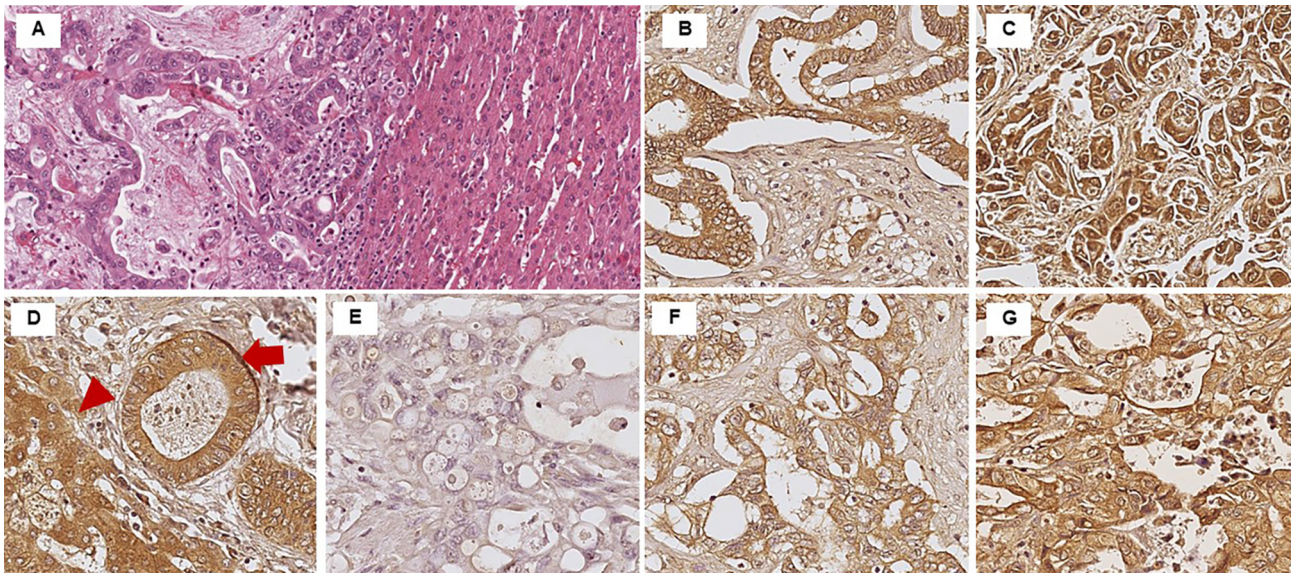

Figure 1. *Farnesoid X receptor (FXR) expression in cholangiocarcinoma. The tumor cells invade the hepatic parenchyma (A, H&E). Cytoplasmic expression (B) and cytoplasmic and nuclear expression (C). FXR expression in adjacent normal bile duct (arrow) and hepatocytes (arrowhead) (D). Intensity of FXR expression; score 1+ (E), 2+ (F), and 3+ (G). Original magnification 40×.*

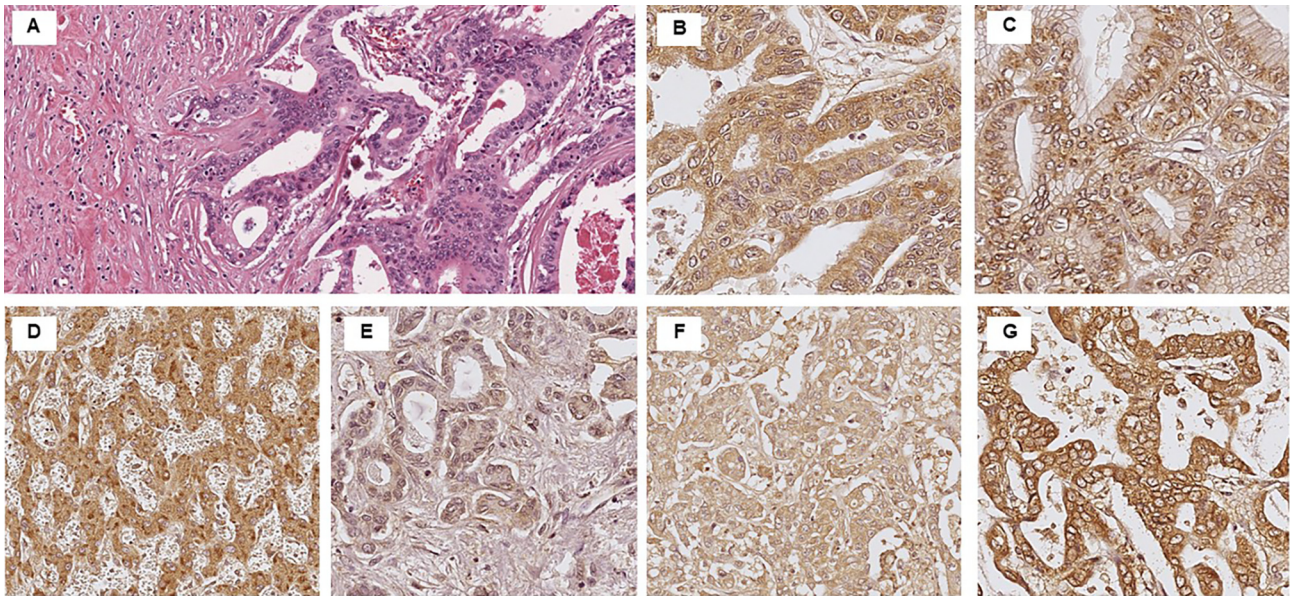

Figure 2. *Pregnane X receptor (PXR) expression in cholangiocarcinoma. The tumor cells invade the bile duct wall (A, H&E). Diffuse cytoplasmic expression (B) and cytoplasmic granular expression (C). PXR expression in adjacent normal hepatocytes (D). Intensity of PXR expression; score 1+ (E), 2+ (F), and 3+ (G). Original magnification 40×.*

high expression. A significant correlation between FXR and PXR expression was found in the specimens studied using Sperman correlation analysis (Figure 3).

Correlation between FXR and PXR expression and clinicopathological characteristics. The relationship between the expression of FXR and PXR in CCA tissues and the known

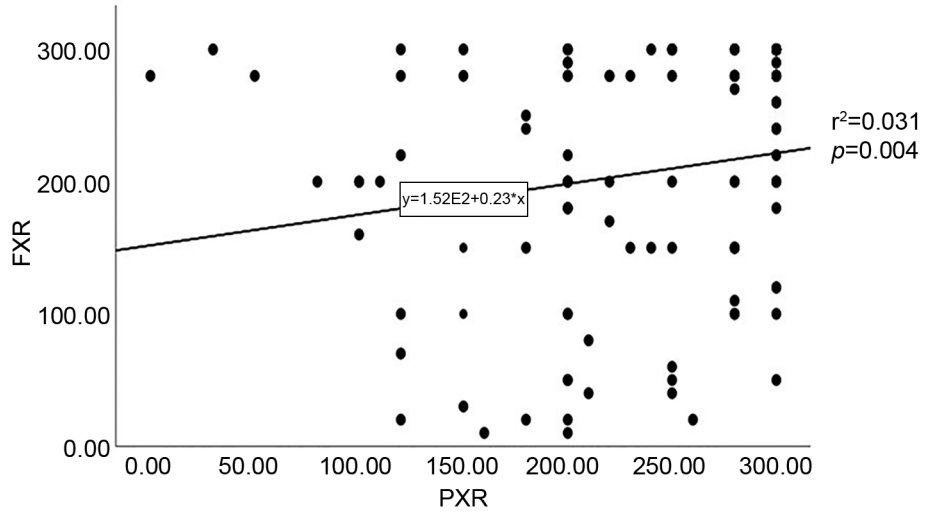

Figure 3. *Scatter plot graph showing the correlation between the expression of farnesoid X receptor (FXR) and pregnane X receptor (PXR). Significant positive correlation was found between the immunohistochemical score of FXR and PXR (p=0.004).*

clinicopathological features of the patients was assessed using a univariate analysis. The results showed that FXR expression was not associated with sex, age, tumor location, tumor size, histological type and grade, and the presence or absence of LVSI and mucin. However, there was a statistically significant association between FXR expression and the presence or absence of intraductal papillary neoplasm of the bile duct (IPBN) in CCA tissues (p=0.042), lymph node metastasis (p=0.006), and tumor stage (p=0.007) (Table II and Figure 4). For PXR expression, no significant associations were found between its expression and all evaluated parameters (Table II).

Correlation between FXR and PXR expression and cumulative survival rates of CCA patients. To implicate the clinical significance of FXR and PXR in CCA patients, the correlation between the expression of these nuclear receptors and patient survival time was evaluated. The Kaplan–Meier analysis indicated no correlation between overall survival and the expression levels of FXR or PXR when analyzed separately (Figure 5A and B). The respective mean survival times in days were 930.24±78.54 (95%CI=776.29-1,084.19 days) for FXR low expression and 967.32±76.23 (95%CI=817.90-1,116.74 days) for high FXR expression. CCA patients with low PXR expression demonstrated a mean survival time of 1,004.66±80.28 (95%CI=847.31-1,162.00 days) compared to 920.28±76.40 (95%CI=770.52-1,070.03 days) for those with high PXR expression. The log-rank test analysis was also conducted on CCA patients with varying degrees of concomitant FXR and PXR expression. The results showed that the survival time of the patients was not associated with the expression of these two nuclear receptors. However, patients with low expression of both FXR and PXR tended to have a longer survival time (Figure 5C and D).

A multivariate Cox proportional hazard-regression analysis was conducted to determine the impact of clinicopathological characteristics on the overall survival of patients. The statistically significant variables from the univariate analysis (p<0.05), namely sex, histological grade, the presence or absence of LVSI and LN, and tumor stage, were included in the multiple regression. Although FXR and PXR expression levels were not statistically significant in the univariate analysis, they were included in the multivariable analysis due to their clinical relevance. As shown in Table III, sex and histological grade had a significant prognostic impact on CCA patients when either FXR, PXR, both FXR and PXR, or varying degrees of FXR

Table II. *Relationship between Farnesoid X receptor (FXR) and Pregnane X receptor (PXR) expression and clinicopathological parameters of cholangiocarcinoma patients.*

Parameter		FXR		*p*-Value	PXR		*p*-Value
		Low (%)	High (%)		Low (%)	High (%)	
Sex	Male	34 (50.0)	34 (50.0)	0.905	27 (39.7)	41 (60.3)	0.480
	Female	21 (48.8)	22 (51.2)		20 (46.5)	23 (53.5)	
Age	<63	28 (57.1)	21 (42.9)	0.155	20 (40.8)	29 (59.2)	0.772
	≥63	27 (43.5)	35 (56.5)		27 (43.5)	35 (56.5)	
Tumor location	Intrahepatic	25 (55.6)	20 (44.4)	0.163	20 (44.4)	25 (55.6)	0.284
	Perihilar	28 (43.8)	36 (56.3)		25 (39.1)	39 (60.9)	
	Distal	2 (100)	0 (0)		2 (100)	0 (0)	
Histological grade	Grade 1	46 (49.5)	47 (50.5)	0.479	37 (39.8)	56 (60.2)	0.450
	Grade 2	7 (43.8)	9 (56.3)		9 (56.3)	7 (43.8)	
	Grade 3	2 (100)	0 (0)		1 (50.0)	1 (50.0)	
Histological grade	Well differentiation	46 (49.5)	47 (50.5)	0.967	37 (39.8)	56 (60.2)	0.215
	Not well differentiation	9 (50.0)	9 (50.0)		10 (55.6)	8 (44.4)	
Histological type	ID	3 (75.0)	1 (25.0)	0.471	1 (25.0)	3 (75.0)	0.874
	PI	1 (50.0)	1 (50.0)		1 (50.0)	1 (50.0)	
	MF	9 (39.1)	14 (60.9)		10 (43.5)	13 (56.5)	
	ID + PI	1 (100)	0 (0)		1 (100)	0 (0)	
	ID + MF	15 (62.5)	9 (37.5)		9 (37.5)	15 (62.5)	
	PI + MF	19 (43.2)	25 (56.8)		18 (40.9)	26 (59.1)	
	ID + PI + MF	7 (53.8)	6 (46.2)		7 (53.8)	6 (46.2)	
IPNB	No	29 (42.0)	40 (58.0)	0.042*	29 (42.0)	40 (58.0)	0.932
	Yes	26 (61.9)	16 (38.1)		18 (42.9)	24 (57.1)	
Tumor size	<4.5 cm	30 (48.1)	32 (51.6)	0.783	25 (40.3)	37 (59.7)	0.628
	≥4.5 cm	25 (51.0)	24 (49.0)		22 (44.9)	27 (55.1)	
Mucin	Absence	11 (50.0)	9 (45.0)	0.590	12 (60.0)	8 (40.0)	0.078
	Presence	44 (48.4)	47 (51.6)		35 (38.5)	56 (61.5)	
LVSI	Absence	11 (68.8)	5 (31.3)	0.097	6 (37.5)	10 (62.5)	0.672
	Presence	44 (48.4)	47 (51.6)		41 (43.2)	54 (56.8)	
LN	Absence	36 (62.1)	22 (37.9)	0.006*	25 (43.5)	33 (56.9)	0.865
	Presence	19 (35.8)	34 (64.2)		22 (41.5)	31 (58.5)	
Tumor stage	Stage I-II	16 (76.2)	5 (23.8)	0.007*	8 (38.1)	13 (61.9)	0.662
	Stage III-IV	39 (43.3)	51 (56.7)		39 (43.3)	51 (56.7)	
Presence of OV	None	51 (48.6)	54 (51.4)	0.438	44 (41.9)	61 (58.1)	0.697
	Gross	4 (66.7)	2 (33.3)		3 (50.0)	3 (50.0)	

Data are presented as number (percentage). *Significance defined by $p<0.05$. ID: Intraductal; PI: periductal-infiltrating; MF: mass-forming; IPBN: intraductal papillary neoplasm of the bile duct; LVSI: lymphovascular invasion; LN: lymph node invasion; OV: *Opisthorchis viverrine*.

and PXR expression were included in the analysis model (Table III). Female sex and well-differentiated histological grade in CCA were associated with improved survival rates in CCA patients.

Discussion

Nuclear receptors play important roles in oncogenic transformation by modifying various genes and molecular pathways that drive the malignant behavior of normal cells (8, 16). In this study, the expression of two nuclear receptors, FXR and PXR, was determined through immunohistochemical examination. Our results showed that high levels of FXR expression correlate with disease progression and advanced stages of CCA. In addition, patients with low expression of both FXR and PXR tended to have longer survival times. These data suggest that FXR and PXR may play roles in the prediction and treatment of CCA.

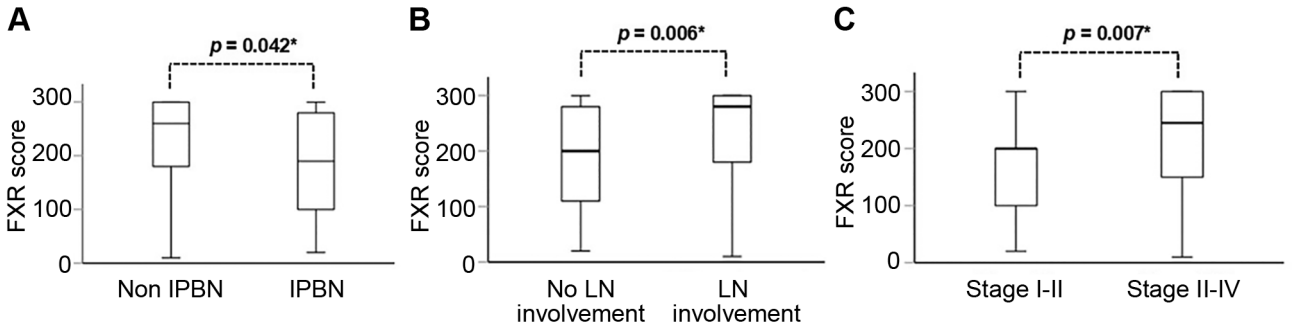

Figure 4. *The association of farnesoid X receptor (FXR) expression with clinicopathological parameters. (A) Mean FXR expression in CCA tissues with the absence of intraductal papillary neoplasm of the bile duct (IPBN) (non-IPBN group) was significantly higher than in CCA tissues with the presence of IPBN (IPBN group) (*p<0.05). (B) There is a significant difference in FXR expression among cholangiocarcinoma (CCA) patients with lymph node involvement compared to those without (*p<0.05). (C) FXR expression in CCA patient tissues at stage I-II was lower than those of stage III-IV patients (*p<0.05).*

FXR is a ligand-modulated nuclear receptor that is normally translocated from the cytoplasm to the nucleus upon activation, where it functions as a transcription factor to regulate the expression of various genes (23). It is the main nuclear receptor responsible for maintaining bile acid homeostasis. Besides, FXR is one of the nuclear receptors associated with various cancers (7, 8). In this study, FXR positivity (immunohistochemistry score >0) was noted in all 111 specimens investigated. The FXR expression was also observed in normal bile ducts adjacent to the tumor. It is important to highlight that among the tested tissues, only some tumor specimens contained normal bile ducts and the expression of FXR in bile duct tissues of healthy subjects was not determined in our study. Therefore, further research is needed to evaluate whether FXR expression differs between CCA tissues and normal bile duct tissues. Based on our finding, we observed that FXR was predominantly localized in the cytoplasm in CCA samples. A similar expression pattern was observed in the invasive breast carcinoma cases (15). Contrary to our results, previous studies reported that FXR immunoreactivity was primarily detected in cellular nuclei in hepatocellular carcinoma (24) and esophageal adenocarcinoma tissues (25). These data indicate that the subcellular distribution of FXR varies among different cancers. The retention of FXR in the cytoplasm in CCA may be explained by the significantly elevated levels of taurine- and glycine-conjugated bile acids in the patients (26), as these conjugated bile acids are weak FXR activators (27). Moreover, alterations in the translocation process of FXR to the nucleus, the excess production of FXR, or the absence of FXR ligands in CCA patients might contribute to the high FXR levels in the cytoplasm during malignancy. The mechanism underlying this phenomenon remains to be further studied.

In the present study, no significant correlation was found between FXR expression and the location, histological type, or grade of CCA. In contrast, FXR over-expression was shown to correlate with high histological grades of esophageal cancer (28). However, a significant relationship was observed between high FXR expression in CCA tissues and tumor metastasis, specifically with LN and advanced tumor stage. This suggested that FXR expression might be associated with the progression of CCA. However, this observation is only correlative; therefore, further investigation into the underlying mechanisms by which FXR regulates CCA progression is needed. Previous studies also demonstrated that FXR over-expression contributed to lymph node metastasis in esophageal adenocarcinoma (28) and pancreatic cancer (8). In the current study, FXR expression was not found to be a reliable prognostic marker for CCA. Patients with low FXR expression showed no difference in overall survival compared to those with high expression. Nevertheless, it

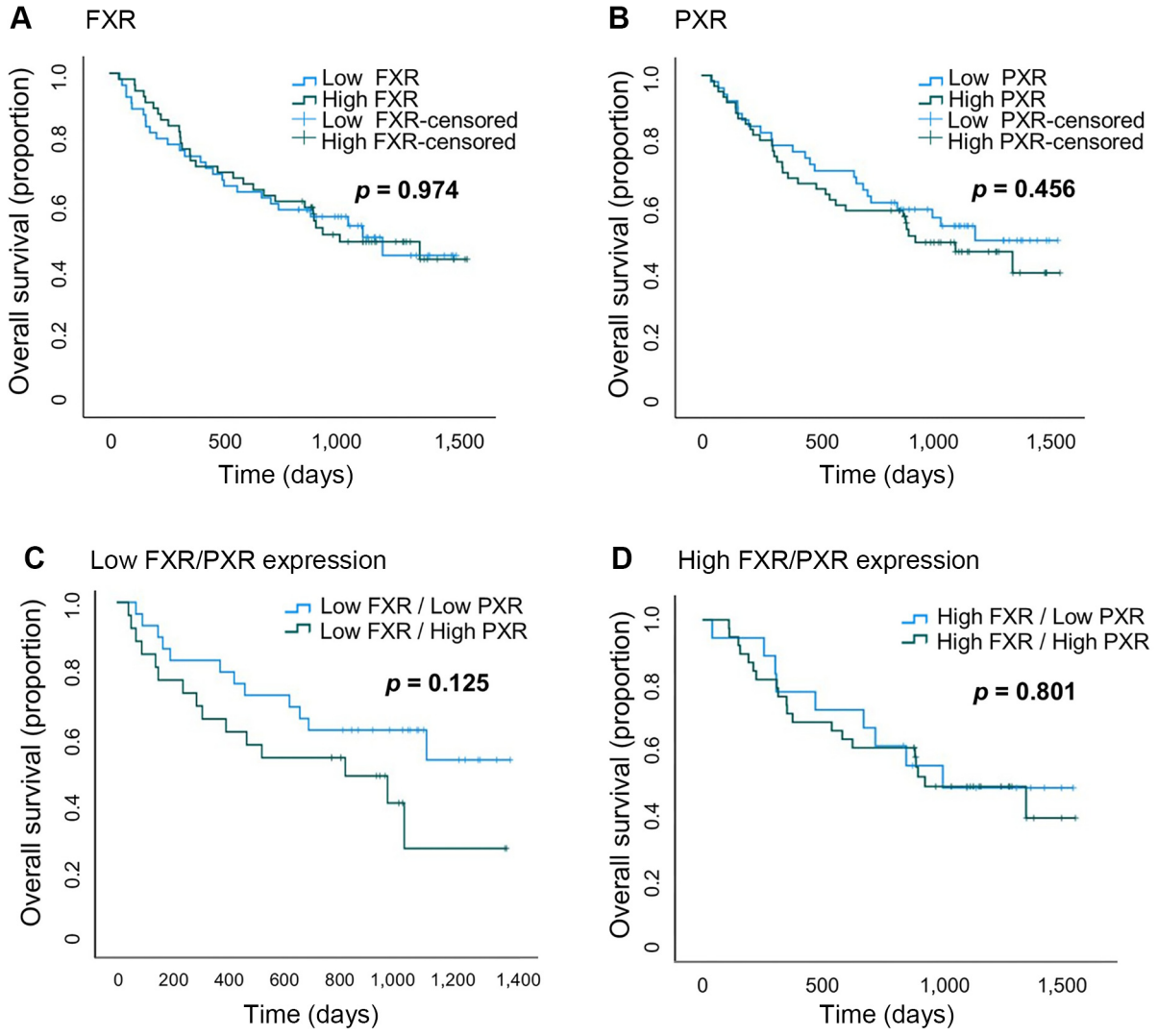

Figure 5. *Correlation between farnesoid X receptor (FXR) and pregnane X receptor (PXR) expression and cumulative survival rate (Kaplan–Meier method). (A) Survival curves of low and high FXR expression groups. (B) Survival curves of low and high PXR expression groups. (C) Survival curves of low FXR/low PXR expression and low FXR/high PXR expression group. (D) Survival curves of high FXR/low PXR expression and high FXR/high PXR expression groups.*

should be noted that the immunoreactivity signal in both cytoplasmic and nuclear staining was taken into account to semi-quantify FXR expression. Subgroup analyses of the correlation between clinical parameters, survival time, and immunohistochemical localization of FXR in nuclei were not conducted in this study due to the limited number of cases with nuclear pattern staining. Previous studies have reported controversial results regarding FXR levels and the survival time of cancer patients. The association between high FXR expression and better patient outcomes has been found in intrahepatic CCA specimens (14), invasive breast carcinoma (15) and colon carcinoma patients (29). On the other hand, the expression of this nuclear receptor has been identified as a prognostic indicator of poor survival in pancreatic cancer patients (12). These data suggest a dynamic regulation of FXR in cancers, indicating that its

Table III. *Univariate and multivariate analysis of factors influencing overall survival in cholangiocarcinoma patients.*

Variables	Median overall survival (Months)	Univariate analysis			Multivariate analysis			Multivariate analysis			Multivariate analysis			Multivariate analysis		
		HR	95%CI	*p*-Value	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Sex																
Male	28.73	1			1			1			1			1		
Female	36.34	0.557	0.315-0.986	0.044*	0.488	0.273-0.874	0.016*	0.493	0.275-0.885	0.018*	0.494	0.275-0.887	0.018*	0.497	0.276-0.897	0.020*
Age																
<63	34.07	1														
≥63	29.42	1.405	0.821-2.406	0.215												
Tumor location																
Intrahepatic	30.15	1														
Perihilar	31.96	0.961	0.562-1.644	0.885												
Distal	NA	0	0 (0-inf)	0.973												
Histological grade																
Well differentiation	34.02	1			1			1			1			1		
Not well differentiation	17.93	2.902	1.600 -5.265	<0.001*	2.529	1.339-4.778	0.004*	2.610	1.372-4.965	0.003*	2.613	1.378-4.954	0.003*	2.641	1.388-5.027	0.003*
Histological type																
Non-ID	37.05	1														
ID	36.20	0.624	0.355-1.094	0.100												
Tumor size																
<4.5 cm	34.07	1														
≥4.5 cm	28.42	1.493	0.833-2.523	0.135												
Mucin																
Absence	30.42	1														
Presence	28.42	0.939	0.473-1.864	0.857												
LVSI																
No	44.28	1			1			1			1			1		
Yes	29.27	5.996	1.461-24.616	0.013*	3.576	0.721-17.747	0.119	4.030	0.825-19.691	0.085	3.738	0.759-18.405	0.105	3.802	0.776-18.620	0.099
LN																
No	35.86	1			1			1			1			1		
Yes	26.19	2.121	1.230-3.656	0.007*	1.531	0.816-2.872	0.185	1.347	0.726-2.497	0.345	1.507	0.802-2.831	0.203	1.505	0.801-2.828	0.204
Tumor staging																
Stage I-II	40.87	1			1			1			1			1		
Stage III-IV	29.17	3.037	1.225-7.707	0.017*	1.278	0.414-3.947	0.670	1.138	0.376-3.442	0.819	1.257	0.410-3.852	0.689	1.296	0.424-3.962	0.649
FXR																
Low	40.87	1			1	1					1	1				
High	29.17	0.991	0.587-1.675	0.974	0.692	0.395-1.212	0.198				0.659	0.374-1.161	0.149			
PXR																
Low	32.94	1						1	1		1	1				
High	30.17	1.223	0.720-2.076	0.457				1.508	0.873-2.602	0.140	1.352	0.787-2.321	0.275			
FXR/PXR																
Low/Low	33.83	1												1		
Low/High	25.58	1.773	0.828-3.795	0.140										1.667	0.777-3.579	0.190
High/Low	32.08	1.224	0.515-2.907	0.647										0.667	0.273-1.630	0.375
High/High	31.04	1.349	0.659-2.759	0.413										1.037	0.495-2.147	0.923

CI: Confidence interval; HR: hazard ratio; ID: intraductal; LVSI: lymphovascular invasion; LN: lymph node invasion; FXR: farnesoid X receptor; PXR: pregnane X receptor. *$p<0.05$, Cox proportional hazards-regression test.

functions in the development and progression of different cancers should be separately evaluated according to the type of cancer.

Accumulative data reveal the pivotal role of PXR in cancer development and progression (16). Upon ligand binding, PXR forms a heterodimer with RXR, binds to a responsive element, and then activates the transcription of target genes (16). A previous study reported RXRα over-expression in human CCA tissues (22). However, the expression of PXR in CCA specimens and its prognostic significance remains unknown. The present study has, for the first time, explored PXR expression and its correlation with clinicopathological parameters in human CCA specimens. We noted PXR positivity in all 111 specimens investigated. However, its expression in bile duct tissues of healthy subjects was not assessed in our study. Previous studies have reported differential expression of PXR in various cancers. Its over-expression was reported in esophageal (30), breast (31), and bone cancers (32), while down-regulation of PXR was observed in colon (33), prostate (34), and cervical cancers (35). In this study, the majority of PXR expression in the examined CCA tissues exhibited cytoplasmic staining. In contrast, PXR displayed a nuclear staining pattern in esophageal adenocarcinoma (30) and pancreatic adenocarcinoma specimens (36). The high level of PXR in the cytoplasm of CCA tissues may indicate a change in the translocation process from the cytoplasm to the nucleus or an enhanced production of this nuclear receptor during CCA progression. The molecular mechanisms underlying the differential expression patterns of PXR and its role in various cancers need to be further explored. Regarding the clinical significance of PXR in CCA, there was no association between its expression and any of the examined clinicopathological parameters or patients' survival. In contrast to our results, a previous study demonstrated that elevated PXR expression was associated with histological grade, tumor size, and tumor stage in pancreatic adenocarcinoma tissues (36). Notably, patients with low expression of both FXR and PXR tended to have longer survival times. Further study with larger sample sizes are needed to confirm our observations.

Conclusion

In conclusion, our findings provide evidence that FXR is associated with favorable clinicopathologic parameters. The prognostic significance of FXR and PXR expression appears relevant in CCA. However, further studies with larger sample sizes are necessary to support this finding. Considering the critical roles of FXR and PXR in regulating cellular signaling pathways observed in several cancers, a better understanding of their putative roles in CCA should also be additionally explored.

Conflicts of Interest

The Authors declare no competing interests in relation to this study.

Authors' Contributions

PS: Methodology, investigation, data curation, formal analysis, writing – original draft. KY: Supervision, validation, writing – review & editing draft. RS: Supervision, validation, writing – review & editing draft. SK: Methodology, data curation, formal analysis. AP: Methodology, data curation, formal analysis. LS: Methodology, investigation, data curation, formal analysis, funding acquisition, writing & review & editing manuscript, conceptualization.

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Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

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