Expression of chicken ovalbumin upstream promoter-transcription factor II and liver X receptor as prognostic indicators for human colorectal cancer

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Abstract. Cholesterol increases the risk of colorectal cancer. Liver X receptor (LXR), retinoid X receptor (RXR)α and sterol regulatory element binding protein (SREBP)-1c are transcriptional regulators of lipid metabolism. Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) serves an essential role in angiogenesis and development, but its role in cancer is controversial. The expression of COUP-TFII, LXR, RXRα and SREBP-1c in colorectal cancer, as well as their association with clinicopathologic features, was assessed, and their utility as prognostic indicators in colorectal cancer evaluated. Colorectal cancer samples (n=707 patients) were analyzed for COUP-TII, LXR, RXRα and SREBP-1c expression by immunohistochemistry. Overall survival curves of patients with tumors expressing different levels of these proteins were produced and risk factors were assessed. Of the 707 patients, 32.7, 50.9, 56.4, and 41.7% were positive for COUP-TFII, LXR, RXRα, and SREBP-1c, respectively. The lack of COUP-TFII or LXR expression was associated with lower overall survival rates (P=0.0154 for COUP-TFII, and 0.0113 for LXR). Following adjustment for other clinical risk factors (age, sex, tumor size, grade, vascular invasion, and Tumor-Node-Metastasis stage), the lack of COUP-TFII or LXR expression was a negative independent prognostic factor for survival. The expression of COUP-TFII and LXR alone or in combination may be biomarkers to indicate a positive prognosis in patients with colorectal cancer.

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Key words: colorectal cancer, chicken ovalbumin upstream promoter-transcription factor II, liver X receptor, retinoid X receptor α, prognosis

Introduction

Colorectal cancer is one of the most common types of cancer worldwide (1). Despite advances in diagnostic and therapeutic strategies, clinical outcomes and prognoses for patients with colorectal cancer remain unsatisfactory (2). Therefore, the identification of molecular markers for the more aggressive colorectal tumor phenotypes is required, to allow patient treatment to be adjusted accordingly. However, predictive molecular indicators of regional disease invasion and metastasis are not well defined.

Nuclear receptors (NRs) are ligand-activated transcription factors that control the expression of genes involved in nearly all aspects of development, physiology, and disease (3). The majority of NRs are receptors for small lipophilic ligands (metabolites, hormones, drugs, and environmental compounds) that directly modulate their transcriptional activities (4). The NRs liver X receptor α (LXRα) and β are key regulators of lipid, cholesterol and carbohydrate metabolism and homeostasis (5). They function as transcription factors by heterodimerizing with retinoid X receptor (RXR) and increasing the expression of target genes that encode proteins implicated in lipid metabolism, particularly in cholesterol efflux and fatty acid synthesis (6). Cholesterol controls cell proliferation; disruptions in cholesterol metabolism are associated with the development of colon cancer (7). Previous studies have indicated that LXRs may couple cholesterol homeostasis to proliferation (8-14). Synthetic (compounds T0901317 and GW3965) and natural (22[R]-hydroxycholesterol and 24[S]-hydroxycholesterol) LXR ligands suppress the proliferation of a number of human cancer cell lines, including prostate, breast, colon, ovarian and leukemia cancer cells (8-14). Furthermore, downregulation of the S-phase-associated kinase protein-2 (Skp2) component of ubiquitin ligase, which regulates $p27^{Kip1}$ degradation (15) and the resulting $p27^{Kip1}$ protein stabilization and retinoblastoma protein dephosphorylation, may contribute to the inhibition of cell proliferation (16). In addition, LXRs inhibit the proliferation of human colorectal cancer cells and the growth of intestinal tumors in mice (7).

RXR is an NR family member that has been implicated in cancer chemoprevention (17,18). RXRα expression is decreased in mouse skin tumors (19), whereas RXRβ expression is increased in non-small cell lung tumors (20) compared with healthy tissue. However, the clinical significance of RXR in colorectal cancer remains unclear.

Sterol regulatory element-binding protein-1c (SREBP-1c) is a transcriptional intermediary for the insulin stimulation of fatty acid synthase (FAS) gene expression (21). Induction of FAS expression and the consequential enhanced fatty acid synthesis is required for neoplastic transformation and tumor progression (22). SREBP-1 may be implicated in tumorigenesis, as the high expression of SREBP-1 is reported to predict a poor prognosis in patients with pancreatic cancer (23).

The orphan NR chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) is involved in the regulation of gene expression (24,25), development, differentiation, and homeostasis (26); however, its role in cancer is debated as contradictory tumor-suppressive and oncogenic capacities have been reported (27,28). Increased expression of COUP-TFII was shown to enhance the invasiveness of human lung carcinoma cells (27). By contrast, a previous report demonstrated that overexpression of COUP-TFII in MDA-MB-435 breast cancer cells led to reduced growth and plating efficiency (28). The prognostic significance of high or low expression of COUP-TFII appears to vary; its expression may be a favorable (e.g., ovarian and colon cancer) or an unfavorable (e.g., breast and prostate cancer) prognostic factor in patients with different types of cancer, and its expression is tumor-specific (29). The underlying mechanisms that trigger altered expression of this gene in individual tumors remains poorly understood (30-32). According to a previous study, patients with COUP-TFII-positive tumors had a significantly higher 3-year overall survival (OS) rate compared with the COUP-TFII-negative group (33). However, the follow-up period was short and few patients with colorectal cancer were included in the study. Therefore, in the present study, the aim was to investigate the association between COUP-TFII expression and clinicopathological factors further and to confirm its prognostic significance in a larger number of patients with colorectal cancer. The association between LXR, RXRα, and SREBP-1c expression and clinicopathological factors was also assessed in the study participants.

Table I. Clinical characteristics and immunohistochemistry expressions of the study participants (n=707).

Variable	Patients, n (%)
Sex	
Male	403 (57.0)
Female	304 (43.0)
Age, years	
<65	392 (55.5)
≥65	315 (44.6)
Grade	
1	398 (56.3)
2	262 (37.1)
3+4	47 (6.7)
Tumor size, cm	
<5	247 (34.9)
≥5	460 (65.1)
Vascular invasion	
Negative	605 (85.6)
Positive	102 (14.4)
TNM stage	
0+I	95 (13.4)
II	295 (41.7)
III+IV	317 (44.8)
COUP-TEII expression	
Negative	476 (67.3)
Positive	231 (32.7)
LXR expression	
Negative	347 (49.1)
Positive	360 (50.9)
RXRα expression	
No data[a]	3 (0.4)
Negative	305 (43.1)
Positive	399 (56.4)
SREBP-1c expression	
Negative	412 (58.3)
Positive	295 (41.7)

[a]Three samples are missing in the RXRα immunohistochemical data. COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; LXR, liver X receptor; RXRα, retinoid X receptor α; SREBP-1c, sterol regulatory element binding protein-1c.

Materials and methods

Patients and tissue samples. Consecutive patients with colorectal cancer who were eligible and underwent surgery at Dong-A University Hospital between March 2002 and July 2011 (n=707) were enrolled in the study, including 403 males (age range, 29.0-87.0 years; mean age, 61.8 years) and 304 females (age range, 22.0-84.0 years; mean age, 62.1 years). Tissue samples from the patients were formalin-fixed and paraffin-embedded. Patients with familial adenomatous polyposis or inflammatory bowel disease or synchronous colorectal or extracolorectal cancer, and those lost to follow-up, were excluded. None of the patients had a family history of colorectal cancer, and none had received preoperative chemotherapy or radiotherapy. Information concerning age, sex, histological grade and Tumor-Node-Metastasis (TNM) stage (34) was retrieved by reviewing pathological and surgical reports. The present study was approved by the institutional review board of Dong-A University (Busan, Korea; approval no., 2-104709-AB-N-01-201504-BR-004-02).

Tissue microarrays and immunohistochemistry. Cores (1 mm) were removed from colorectal cancer samples that had previously been formalin-fixed and paraffin-embedded. For all arrays, three cores from different areas of the tumor were collected and placed in a new blank recipient paraffin block,

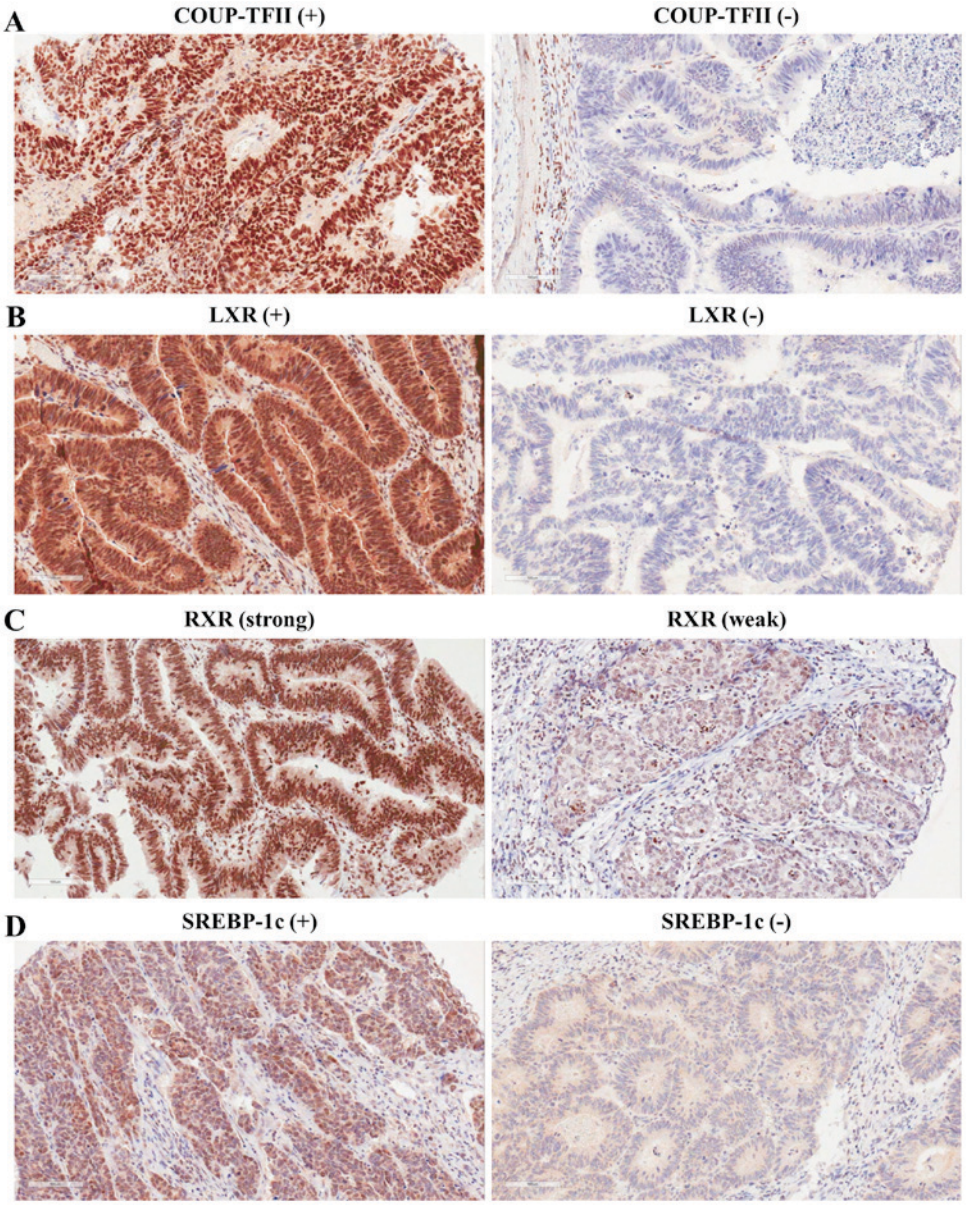

Figure 1. Representative images of immunohistochemical staining for COUP-TFII, LXR, RXRα, and SREBP-1c in colorectal cancer tissue. (A) Left, COUP-TFII-positive colorectal cancer tissue. Right, COUP-TFII-negative colorectal cancer tissue. (B) Left, LXR-positive colorectal cancer tissue. Right, LXR-negative colorectal cancer tissue. (C) Left, strong RXRα immunoreactivity detected in well-differentiated colorectal cancer tissues. Right, weak RXRα immunoreactivity detected in poorly differentiated colorectal cancer tissues. (D) Left, SREBP-1c-positive colorectal cancer tissue. Right, SREBP-1c-negative colorectal cancer tissue. Magnification, x200. COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; LXR, liver X receptor; RXRα, retinoid X receptor α; SREBP-1c, sterol regulatory element binding protein-1c.

according to a previously described method (35). Sections were deparaffinized using a series of xylene baths; rehydration was performed using a series of graded alcohol solutions. Sections (4-μm thick) were used for immunohistochemical staining. To enhance immunoreactivity, microwave antigen retrieval was performed at 750 W for 30 min in Tris EDTA (pH 9.0). Subsequent to blocking endogenous peroxidase activity with 5% hydrogen peroxidase for 10 min, incubation with the primary antibody was performed for 1 h at room temperature. The primary antibodies used in immunostaining included a mouse monoclonal antibody directed against COUP-TFII (clone H7147; catalog no., PP-H7147-00; 1:100; Perseus Proteomics Inc., Tokyo, Japan), a rabbit polyclonal antibody directed against LXRα/β (clone S-20; catalog no., sc-1000; 1:400; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), a mouse monoclonal antibody directed against RXRα (clone F-1; catalog no., sc-46659; 1:50; Santa Cruz Biotechnology, Inc.), and a rabbit polyclonal antibody directed against SREBP-1 (clone H-160; catalog no., sc-8984; 1:100; Santa Cruz Biotechnology, Inc.). An Envision™Chem™ Detection kit (DakoCytomation, Carpinteria, CA, USA) was used for the secondary antibody at room temperature for 30 min. After washing the tissue samples in TBS for 10 min, 3,3'-diaminobenzidine was used as a chromogen, and then Mayer's hematoxylin counterstain was applied for 1 min at room temperature. Archival, 10% formalin-fixed (for 18-48 h at room temperature), paraffin-embedded human normal kidney, thyroid, skin and testis tissues (obtained from tissue archives

Table II. Differential distribution of LXR, RXRα, and SREBP-1c according to COUP-TFII expression.

	LXR expression, n (%)		RXRα expression[b], n (%)		SREBP-1c expression, n (%)	
COUP-TFII expression	Negative	Positive	Negative	Positive	Negative	Positive
Negative	279 (58.6)	197 (41.4)	224 (47.1)	251 (52.7)	296 (62.2)	180 (37.8)
Positive	68 (29.4)	163 (70.7)	81 (35.1)	148 (64.1)	116 (50.2)	115 (49.8)
P-value[a]	<0.0001		0.0035		0.0027	

[a]Calculated by χ^2 test. [b]Three samples are missing in the RXRα immunohistochemical data. COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; LXR, liver X receptor; RXRα, retinoid X receptor α; SREBP-1c, sterol regulatory element binding protein-1c.

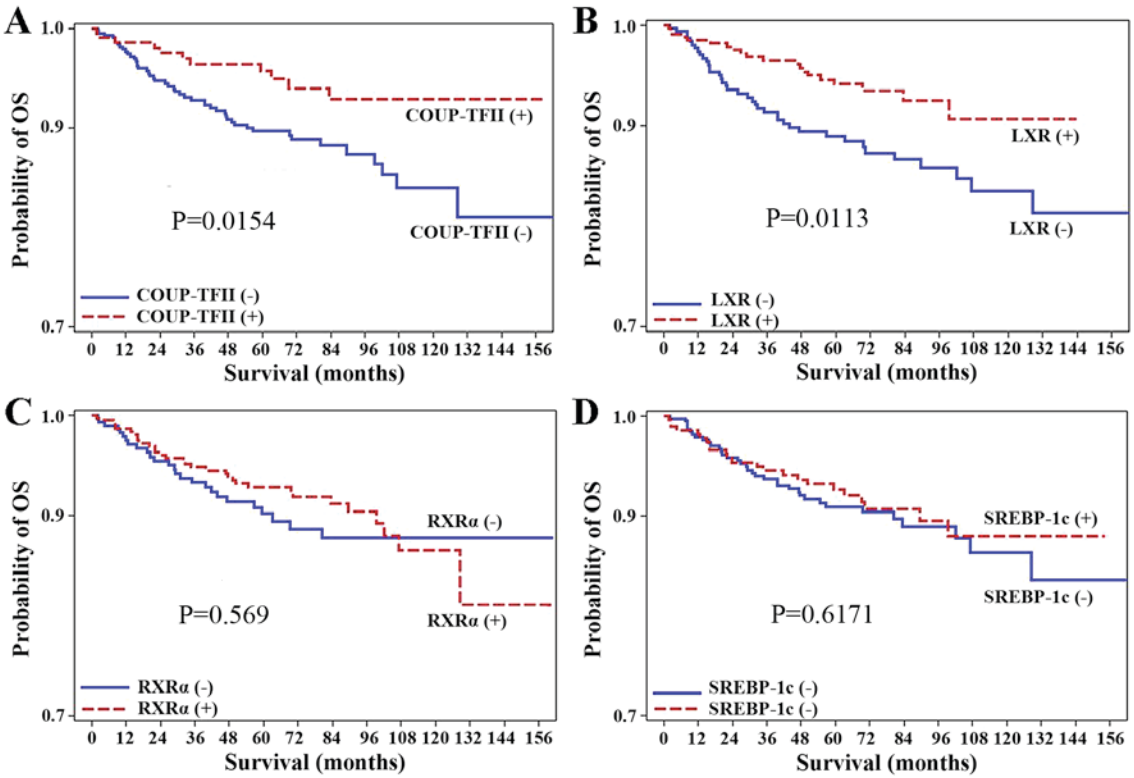

Figure 2. Kaplan-Meier OS curves for 707 patients with colorectal cancer, according to expression levels of COUP-TFII, LXR, RXRα, and SREBP-1c. (A) Patients stratified according to COUP-TFII expression. (B) Patients stratified according to LXR expression. (C) Patients stratified according to RXRα expression. (D) Patients stratified according to SREBP-1c expression. OS rates are indicated in each panel. OS, overall survival; COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; LXR, liver X receptor; RXRα, retinoid X receptor α; SREBP-1c, sterol regulatory element binding protein-1c.

at Dong-A University Hospital) were used as positive controls for COUP-TFII, LXRα/β, RXRα, and SREBP-1c, according to the antibody manufacturer's protocol. A negative control was obtained by substituting the primary antibody with buffer.

Immunohistochemical assessment. The percentage and intensity of immunoreactive tumor cells in each core were recorded, and the final value of the positive tumor cells was determined as the mean of the immunoreactivity of the three cores. The presence of tumor tissue in ≥2 interpretable cores was required for the inclusion of a case in statistical analyses. All slides were independently evaluated by two independent experienced pathologists (MSR and MGP) who were blinded to clinicopathological data. There were only minor discrepancies in the evaluation; slides with discrepancies between evaluations were reevaluated under a multi-head microscope until a consensus evaluation was obtained. The percentage of positive tumor cells and the staining intensity (weak or strong) were assessed. Staining intensity was scored visually and stratified as follows: Negative, weak (if the staining appeared as a blush), or strong (if it was markedly positive at 20x magnification).

For COUP-TFII, immunoreactivity was defined as cells showing nuclear staining in the tumor tissue with minimal background staining. Tumors with strong staining intensity in >10% of tumor cells were recorded as having positive immunoreactivity for COUP-TFII. For LXRα/β, immunoreactivity was defined as cells exhibiting nuclear staining with/without

Table III. Univariate analysis of the associations between clinical characteristics and COUP-TFII, LXR, RXRα, and SREBP-1c expression.

	COUP-TFII		LXR		RXRα		SREBP-1c	
Variable	Positive, n (%)	P-value	Positive, n (%)	P-value	Positive, n (%)	P-value	Positive, n (%)	P-value
Sex		0.3883		0.7376		0.7935		0.4272
Male	137 (34.0)		203 (50.4)		225 (56.3)		163 (40.5)	
Female	94 (30.9)		157 (51.6)		174 (57.2)		132 (43.4)	
Age, years		0.8618		0.0790		0.8739		0.4837
<65	127 (32.4)		188 (48.0)		220 (56.4)		159 (40.6)	
≥65	104 (33.0)		172 (54.6)		179 (57.0)		136 (43.2)	
Grade		0.2914		0.2749		0.0160		0.7015
1	134 (33.7)		196 (49.3)		240 (60.5)		171 (43.0)	
2	78 (29.8)		143 (54.6)		141 (53.8)		104 (39.7)	
3+4	19 (40.4)		21 (44.7)		18 (40.0)		20 (42.6)	
Tumor size, cm		0.6494		0.6620		0.2653		0.0537
<5	153 (33.3)		237 (51.5)		266 (58.2)		204 (44.4)	
≥5	78 (31.6)		123 (49.8)		133 (53.9)		91 (36.8)	
Vascular invasion		0.0184		0.0334		0.6958		0.7546
Negative	208 (34.4%)		42 (41.2)		343 (57.0)		44 (43.1)	
Positive	23 (22.6%)		318 (52.6)		56 (54.9)		251 (41.5)	
TNM stage		0.0215		0.3616		0.5857		0.6859
0+I	38 (40.0)		51 (53.7)		51 (53.7)		36 (37.9)	
II	106 (35.9)		157 (53.2)		173 (58.8)		123 (41.7)	
III+IV	87 (27.4)		152 (48.0)		175 (55.6)		136 (42.9)	

COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; LXR, liver X receptor; RXRα, retinoid X receptor α; SREBP-1c, sterol regulatory element binding protein-1c.

cytoplasmic staining patterns in the tumor tissue with minimal background staining; tumors with strong staining intensity in >10% of the tumor cells were recorded as having positive immunoreactivity for LXRα/β. For RXRα, immunoreactivity was defined as cells exhibiting nuclear staining in the tumor tissue with minimal background staining. Cases were divided into those with weak or strong RXRα expression according to staining intensity, since immunoreactivity was typically evenly distributed within a tumor sample, but varied in intensity. For SREBP-1c, immunoreactivity was defined as cells exhibiting nuclear staining with/without cytoplasmic staining patterns in the tumor tissue with minimal background staining; tumors with a strong staining intensity in >10% of tumor cells were recorded as having positive immunoreactivity for SREBP-1c.

Statistical analysis. The χ^2 test was used to analyze differences in clinical characteristics and immunohistochemically-assessed expression levels. Survival curves were calculated by the Kaplan-Meier method, and comparisons of survival curves were made with the log-rank test. Multiple analyses were performed with the Cox proportional hazards model to assess the association of COUP-TFII, LXR, RXRα, and SREBP-1c expression with the OS rate. P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed with SAS 9.4 software (SAS Institute, Cary, NC, USA).

Results

Expression of COUP-TFII, LXR, RXRα, and SREBP-1c in human colorectal cancer tissues. Our previous study revealed expression of COUP-TFII in 55/95 (57.89%) colorectal carcinoma tissue specimens (33). To confirm the expression pattern of COUP-TFII in a larger number of patients with human colorectal carcinoma, immunohistochemistry was performed with an antibody against COUP-TFII. Positive COUP-TFII expression was observed in 231/707 (32.7%) colorectal carcinoma tissue specimens. Immunostaining occurred predominantly in the nuclei of tumor cells (Fig. 1). The expression levels of LXR, RXRα, and SREBP-1c were also assessed in human colorectal tumors by immunohistochemistry. Positive expression of LXR and SREBP-1c was observed in 360 (50.9%) and 295 (41.7%) of the 707 colorectal carcinoma tissue specimens, respectively (Fig. 1; Table I). Positive expression of RXRα was observed in 399/704 (56.4%) colorectal carcinoma tissue specimens (Fig. 1 and Table I). Core tissue was lost during the preparation of three colorectal carcinoma tissue specimens.

To evaluate the associations between COUP-TFII expression and LXR, RXRα, and SREBP-1c expression in colorectal cancer, the χ^2 test was used. In 70.7% (163/231) of patient samples in which COUP-TFII was expressed, LXR was also expressed (P<0.0001). In 64.1% (148/229) of patient samples in which COUP-TFII was expressed, RXRα was also expressed (P=0.0035). In 49.8% (115/231) of patient samples in which COUP-TFII was expressed, SREBP-1c was also expressed (P=0.0027; Table II). These data suggest that COUP-TFII expression is positively associated with LXR, RXRα and SREBP-1c expression.

Table IV. Crude HRs for COUP-TFII, LXR, RXRα, and SREBP-1c expression.

Expression status	HR	95% CI	P-value
COUP-TFII			
Negative	2.20	1.14, 4.24	0.0182
Positive	ref.		
LXR			
Negative	1.99	1.16, 3.42	0.0130
Positive	ref.		
RXRα			
Negative	1.16	0.69, 1.95	0.5693
Positive	ref.		
SREBP-1c			
Negative	1.14	0.68, 1.93	0.6174
Positive	ref.		
COUP-TFII + LXR			
C(-)L(-)	2.43	1.17, 5.04	0.0171
C(-)L(+)	1.05	0.43, 2.53	0.9201
C(+)L(-)	0.50	0.11, 2.33	0.3800
C(+)L(+)	ref.		

COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; LXR, liver X receptor; RXRα, retinoid X receptor α; SREBP-1c, sterol regulatory element binding protein-1c; HR, hazard ratio; CI: confidence interval; ref., hazard ratio reference value of 1; C, COUP-TFII; R, RXRα; L, LXR.

Associations between the expression of COUP-TFII, LXR, RXRα and SREBP-1c, and clinicopathological features. Following the analysis of COUP-TFII, LXR, RXRα and SREBP-1c staining in tumors, the χ^2 test was used to evaluate the association between COUP-TFII, LXR, RXRα and SREBP-1c expression, and the clinicopathological features of the study population. As shown in Table III, there was a significant association of vascular invasion (P=0.0184) and the TNM stage (P=0.0215) with COUP-TFII expression, and samples that exhibited vascular invasion and higher TNM stages tended to be COUP-TFII-negative. No significant association was identified between COUP-TFII expression and the patient's age or sex, or the tumor size or grade (Table III). There was a significant negative association between LXR expression and vascular invasion (P=0.0334). No significant association was found between LXR expression and the patient's age or sex, the tumor size or grade, or the TNM stage (Table III). There was a significant association between tumor grade (P=0.0160) and RXRα expression, with high grades tending to be RXRα-negative. No significant association was identified between RXRα expression and age, sex, TNM stage, tumor size or vascular invasion (Table III). No significant association was identified between SREBP-1c expression and age at the time of surgery, sex, size, grade, TNM stage or vascular invasion (Table III).

Association of COUP-TFII and LXR expression with good prognosis in colorectal cancer patients. To assess whether

Table V. HRs for COUP-TFII, LXR, or RXRα expression and clinical characteristics.

										Multiple models					
	Crude model			COUP-TFII			LXR			RXRα			SREBP-1c		
Characteristic	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
Expression															
Negative				2.16	1.11-4.23	0.0243	1.83	1.06-3.17	0.0306	1.16	0.69-1.95	0.5822	1.20	0.71-2.03	0.5076
Sex															
Male	1.80	1.03-3.13	0.0381	1.88	1.08-3.29	0.0266	1.80	1.03-3.13	0.0399	1.76	1.00-3.07	0.0490	1.81	1.03-3.15	0.0378
Age, years															
<65	1.48	0.85-2.58	0.1641	1.39	0.80-2.43	0.2425	1.33	0.76-2.33	0.3130	1.37	0.78-2.40	0.2678	1.39	0.80-2.43	0.2441
Grade															
3+4	1.69	0.66-4.44	0.2746	1.89	0.72-4.95	0.1954	1.57	0.61-4.07	0.3521	1.30	0.46-3.73	0.6213	1.56	0.60-4.03	0.3601
2	1.19	0.69-2.04	0.5370	0.95	0.55-1.65	0.8519	0.98	0.56-1.71	0.9370	0.92	0.53-1.62	0.7826	0.93	0.54-1.62	0.8014
Tumor size															
≥5 cm	1.30	0.75-2.26	0.3591	1.19	0.67-2.12	0.5459	1.19	0.67-2.11	0.5454	1.16	0.66-2.07	0.6057	1.19	0.67-2.12	0.5480
Vascular invasion															
Positive	2.11	1.16-3.84	0.0151	1.53	0.82-2.86	0.1839	1.56	0.84-2.90	0.1636	1.73	0.93-3.21	0.0844	1.68	0.91-3.11	0.0983
TNM stage															
III+IV	4.29	1.32-13.95	0.0154	3.41	1.02-11.46	0.0473	3.55	1.06-11.91	0.0406	3.52	1.04-11.88	0.0425	3.56	1.06-11.96	0.0405
II	2.25	0.67-7.58	0.1900	1.93	0.55-6.71	0.3017	1.96	0.57-6.81	0.2872	1.99	0.57-6.96	0.2792	1.93	0.55-6.74	0.3014

COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; LXR, liver X receptor; RXRα, retinoid X receptor α; SREBP-1c, sterol regulatory element binding protein-1c; HR, hazard ratio; CI, confidence interval; ref., hazard ratio reference value of 1; TNM, Tumor-Node-Metastasis.

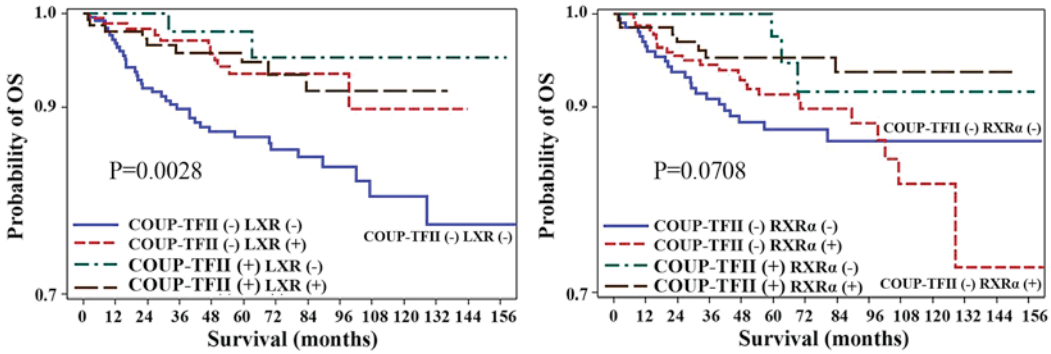

Figure 3. Association between combinations of COUP-TFII and LXR expression and survival of patients with colorectal cancer. Kaplan-Meier OS curves for 707 colorectal cancer patients, according to the expression levels of COUP-TFII and LXR in combination. OS rates are indicated in each panel. OS, overall survival; COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; LXR, liver X receptor.

Table VI. Adjusted HRs for combinations of COUP-TFII and LXR expression.

	COUP-TFII + LXR		
Characteristic	HR	95% CI	P-value
C(-)L(-)	2.33	1.11-4.89	0.0256
C(-)L(+)	1.15	0.47-2.80	0.7597
C(+)L(-)	0.54	0.12-2.50	0.4287
C(+)L(+)	ref.		
Sex			
Male	1.86	1.06-3.26	0.0299
Age, years			
<65	1.36	0.78-2.37	0.2828
Grade			
3+4	1.84	0.70-4.82	0.2128
2	0.97	0.56-1.69	0.9240
Tumor size, cm			
≥5	1.20	0.68-2.13	0.5227
Vascular invasion			
Positive	1.38	0.74-2.60	0.3144
TNM stage			
III+IV	3.28	0.98-11.02	0.0546
II	1.85	0.53-6.41	0.3338

COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; LXR, liver X receptor; RXRα, retinoid X receptor α; SREBP-1c, sterol regulatory element binding protein-1c; C, COUP-TFII; L, LXR; (-), negative expression; (+), positive expression; HR, hazard ratio; CI, confidence interval; ref., hazard ratio reference value of 1.

COUP-TFII expression is a significant prognostic factor for the survival of patients with surgically resected colorectal carcinoma, a log-rank test was used with Kaplan-Meier survival curves. The median follow-up duration was 63.39 months. Of the 707 patients analyzed, the patients positive for COUP-TFII expression (231 patients) had a significantly higher OS rate than those negative for COUP-TFII expression (P=0.0154; Fig. 2). Similarly, the positive expression of LXR was associated with better OS rate (P=0.0113; Fig. 2). However, the positive expression of RXRα and SREBP-1c were not associated with the OS rate (P=0.569, P=0.6171, respectively). Additionally, Cox proportional hazards regression analysis revealed that the negative expression of LXR [hazard ratio (HR), 1.99; 95% confidence interval (CI), 1.16-3.42; P=0.0130] or COUP-TFII (HR, 2.20; 95% CI, 1.14-4.24; P=0.0182) were associated with significantly worse prognoses than the positive expression of LXR or COUP-TFII (Tables IV and V).

Prognostic significance of combinations of COUP-TFII and LXR expression. The aforementioned results indicated that COUP-TFII or LXR expression may be positive prognostic factors for patients with colorectal cancer. Therefore, the OS rate for patients with LXR- and COUP-TFII-positive immunostaining was compared with that of patients with LXR- and COUP-TFII-negative immunostaining. Patients with LXR- and COUP-TFII-positive immunostaining had a significantly higher OS rate than those with LXR- and COUP-TFII-negative immunostaining (P=0.0028; Fig. 3). Additionally, Cox proportional hazards regression analysis revealed that the negative expression of LXR and COUP-TFII was associated with a significantly worse prognosis (HR, 2.43; 95% CI, 1.17-5.04; P=0.0171) compared with the positive expression of LXR and COUP-TFII (Tables IV and VI).

Discussion

The identification of biomarkers for predicting the prognosis of colorectal cancer will aid the adjustment of therapeutic strategies to individual patients. Cholesterol is a known risk factor for patients with colorectal cancer. There are several transcription factors involved in cholesterol homeostasis, including LXR, RXRα and SREBP-1c. The role served by LXR in carcinogenesis was investigated in several tumor types in previous studies (8-16). The tumor-protective actions of LXR were revealed in a previous study, which revealed that the ligand-induced activation of LXR or transfection with LXRα blocked entry into G1 phase, increased caspase-dependent apoptosis and slowed the growth of xenograft tumors in mice (7). Gene expression analysis revealed that the activation of LXRα affected lipid metabolic networks and increased

cholesterol efflux in the intestine (7). However, to the best of our knowledge, the clinical significance of LXR expression in colorectal cancer has not been previously investigated.

In the present study, LXR expression was observed in 50.9% of colorectal cancer patients and was associated with favorable clinical outcomes, such as improved OS rates and lack of vascular invasion. However, it was not possible to discriminate between the expression of LXRα or β in the present study, as an anti-LXRα/β antibody was used. A future study will determine which type of LXR is more predictive of the prognosis in colorectal cancer. To the best of our knowledge, the present study is the first to demonstrate that LXR can be a positive prognostic factor for colorectal cancer, although there are several reports demonstrating that ligands of LXR inhibit cell proliferation in a number of cancer cell lines (8-14).

RXR has been implicated in cancer chemoprevention (17,18). However, the clinical significance of RXRα in colorectal cancer remains unknown. RXRα expression was observed in 56.4% of colorectal cancer patients in the present study and it was inversely associated with tumor grade. Further studies using RNA interference, or transfection with RXRα and LXR, are required to reveal the association between OS rates and LXR expression in patients with colorectal cancer.

No associations were found between expression of SREBP-1c and clinicopathological characteristics; this result is different from another study, in which the high expression of SREBP-1 predicted a poor prognosis for patients with pancreatic cancer (23). The different roles of SREBP-1c in cancer may depend on the tumor type.

In the present study, COUP-TFII expression was associated with an improved OS rate in a large cohort of patients with colorectal cancer with a long follow-up period. Our previous study revealed that COUP-TFII expression was not associated with lymph node metastasis or vascular invasion; this result may have been due to the relatively small number of patients with colorectal cancer who were included in the study (33). COUP-TFII expression was significantly negatively associated with vascular invasion and TNM stage; these results are similar to those of another study, which identified that high COUP-TFII transcript levels were associated with increased survival time, and that its expression inhibits the transforming growth factor-β (TGF-β)-dependent epithelial-mesenchymal transition (EMT) in breast cancer (36). In this study, the expression of TGF-β in patients with colorectal cancer was not examined. However, we hypothesize that there will be the downregulation of TGFβ in COUP-TFII-positive tumors. Our future study may investigate the expression of TGF-β and genes involved in EMT in patients with colorectal cancer.

Several studies have demonstrated that COUP-TFII is involved in cancer progression and metastasis (27,37,38). A recent study described the positive regulation of Snail1 by COUP-TFII, with the consequent downregulation of E-cadherin in colon cancer cell lines (37). The discrepancies between the results of the present study and those of Bao *et al* (37) may be due to other proteins associated with COUP-TFII in different cell lines, the sample size, and the genetic background of patients with colorectal cancer who were included in the studies. Although extensive studies have been performed recently (36-38), uncertainties remain concerning the role of COUP-TFII in cancer. Further studies using COUP-TFII-knockdown or overexpression are required to identify why the expression of COUP-TFII is negatively associated with TNM stage or vascular invasion in colorectal cancer.

Patients with LXR- and COUP-TFII-positive immunostaining had significantly better OS rates than those with LXR- and COUP-TFII-negative immunostaining. These data suggest that immunostaining for LXR and COUP-TFII in colorectal cancer samples at diagnosis may aid the prediction of the prognosis of patients with colorectal cancer.

The present study has limitations that should be noted. First, mortality during the study may have been too low to yield statistically significant data about whether TNM stage and vascular invasion were prognostic factors for patients in the study. Second, it was not demonstrated which type of LXR is a more important prognostic factor for colorectal cancer. Third, the molecular mechanisms responsible for the observed improvement in OS in patients with LXR-, or COUP-TFII-positive tumors have not been identified.

In summary, the present study demonstrated that LXR and COUP-TFII expression may be positive prognostic markers for patients with colorectal cancer. The results of the current study also suggest that the combined immunohistochemical examination of LXR and COUP-TFII expression in diagnostic samples of colorectal cancer may aid prognostic prediction. Future prospective and mechanistic studies evaluating the molecular interactions of LXR and COUP-TFII are required to confirm the findings of the present study.

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