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Original Article

High expression of COUP-TF II cooperated with negative Smad4 expression predicts poor prognosis in patients with colorectal cancer

Chaoqun Wang[1,2*], Yun Zhou[3*], Ruoyun Ruan[1], Maojin Zheng[2], Wencan Han[2], Linchuan Liao[1]

[1]College of Basic and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China; [2]Department of Pathology, Xuzhou Medical College, Xuzhou 221004, Jiangsu, China; [3]Department of Radiation Oncology, Xuzhou City Central Hospital, Xuzhou 221004, Jiangsu, China. [*]Equal contributors.

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Abstract: Objective: In order to evaluate whether the role of chicken ovalbumin upstream promoter transcription factor II (COUP-TF II) could sever as a predictor to stratify risk of human colorectal cancer (CRC) patients, and to elucidate the preliminary molecular mechanisms of COUP-TF II involved in the development and advancement of CRC reflected by investigating the relationship of COUP-TF II with PTEN, Smad4. Methods: 112 cases tissue microarray and immunohistochemical SP method were used to detect the expression of COUP-TF II, PTEN and Smad4 in CRC tissues and adjacent non-tumorous tissues. The clinical relevance and prognosis of COUP-TF II, PTEN, Smad4 in CRC patients were analyzed. Furthermore, Cox proportional hazards model was performed to indicate the independent prognostic factors for CRC patients using various clinicopathological parameters and COUP-TF II, PTEN and Smad4. Results: COUP-TF II proteins were positively expressed in 65.2% of CRC tissues and 15.5% paired non-CRC tissues, respectively. The expression of COUP-TF II was significantly correlated with TNM stage and lymph node metastasis and a negative correlation with Smad4 expression. Patients bearing higher levels of COUP-TF II expression showed lower DFS and OS. Most importantly, Cox proportional hazards regression analyses showed COUP-TF II positive/Smad4 negative status (DFS, P=0.001; OS, P=0.005) were independent prognostic factors for CRC patients. Conclusion: Positive COUP-TF II expression levels has significant value in determining CRC stage and metastasis and cooperates with negative Smad4 expression contributing to assess prognosis in patients with colorectal cancer, suggesting Smad4 may be involved in the above regulation progress probably.

Keywords: Colorectal neoplasm, COUP-TF II, PTEN, smad4, tissue microarray, prognosis

Introduction

Almost 55% of Colorectal Cancer (CRC), the third most common cancer in men (746,000 cases, 10.0% of the total) and the second in women (614,000 cases, 9.2% of the total) worldwide, occur in more developed regions. Respectively, there is wide geographical variation in mortality across the world, with the highest estimated rates in both sexes in Central and Eastern Europe (20.3 per 100,000 for men, 11.7 per 100,000 for women), the lower in China (9.0 and 6.1, respectively) [1]. The main prognosis of CRC based on several histopathological and clinical criteria, as reflected by the AJCC/UICC TNM-classification-the gold standard for tumor evaluation and risk assessment, including the local invasion of the tumor, lymph node involvement, and distal metastasis [2, 3]. However, even with the use of the TNM-classification method, these prognostic factors do not fully predict individual clinical outcome [4]. Therefore, it is necessary to establish novel prognostic markers to aid the existing tumor classification systems in determining CRC prognostication accordingly. As a result, the need for an accurate diagnosis, prognosis, and efficient therapeutic approach has led to attempts to elaborate molecular classifications [5-8].

Chicken ovalbumin upstream promoter-transcription factors (COUP-TFs), orphan nuclear receptors, belong to the steroid/thyroid hormone receptor superfamily of nuclear receptor proteins and play essential roles in development, cellular homeostasis, and disease includ-

Table 1. Expression of COUP-TF II, PTEN, Smad4 in CRC tissues and peripheral normal mucosa tissues

	Cases (n)	COUP-TF II (+)		PTEN (-)		Smad4 (-)	
		Numble (%)	*P*-value	Numble (%)	*P*-value	Numble (%)	*P*-value
Cancerous tissues	112	73 (65.2)	<0.001	63 (56.3)	0.001	60 (53.6)	<0.001
Normal tissues	103	16 (15.5)		12 (11.7)		22 (21.4)	

COUP-TF II, chicken ovalbumin upstream promoter transcription factor II. *P*-value is based on Fisher's exact test.

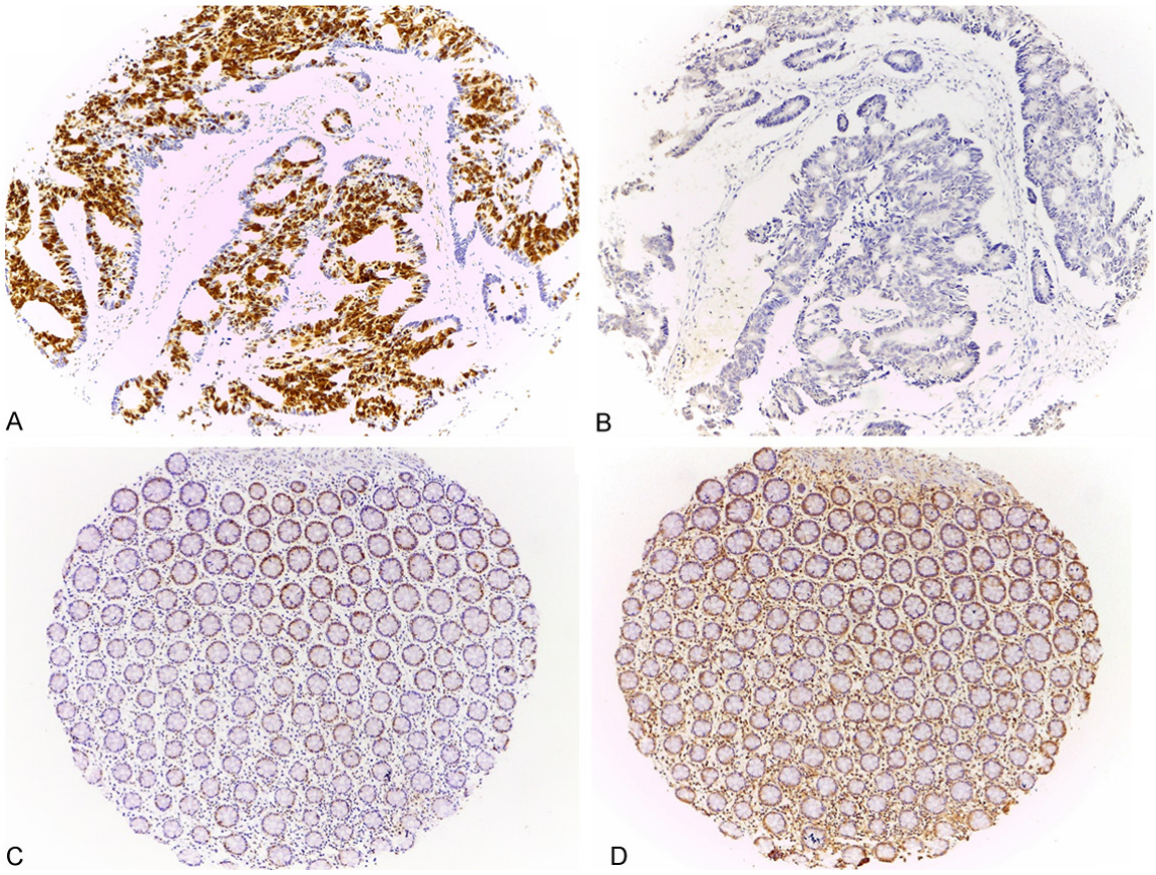

Figure 1. Representative illustrations of COUP-TF II, Smad4 immunohistochemistry in colorectal cancer tissue and adjacent normal epithelium. A. Immunohistochemistry for COUPTF II protein in colorectal cancer tissue (×100, H-score=9). B. The same core with A, immunohistochemistry for Smad4 protein in colorectal cancer tissue microarray (×100, H-score=0). C. Immunohistochemistry for COUPTFII protein in normal colorectal epithelium (×100, H-score=1). D. The same core with A, immunohistochemistry for Smad4 protein in normal colorectal epithelium (×100, H-score=4).

ing cancer where over-or under expression of COUP-TFs has prognostic significance for patient survival [9-12]. For example, high amounts of COUP-TF II expression, reported in Prostate tumor samples [11], breast cancer [13], colon cancer [14] and ovarian cancer [15], exhibit tumor-specific pro-oncogenic or tumor suppressor-like activity.

Recent studies have demonstrated that COUP-TF II expression or activity is correlated with tumour recurrence and inversely associated with transforming growth factor (TGF-β) signaling [11, 16, 17]. Moreover, overexpression of COUP-TF II cooperates with PTEN loss driving the indolent tumor to become a metastatic-prone prostate cancer. Mechanistic investigations further reveal that COUPTFII-mediated inhibition of TGF-β signaling is crucial for the progression of PTEN-mutant Prostate cancer [11]. But it remains poorly understood that the biological and clinical significance of COUP-TF II in colorectal tumorigenesis and prognosis. In this study, we investigated the expression of

Table 2. Correlation between patient characteristics and COUP-TF II, PTEN, Smad4, COUP-TF II (+)/ Smad4 (-) expression

Clinicopathological factors	Cases (n)	COUP-TF II (+)		PTEN (-)		Smad4 (-)		COUP-TF II (+)/ Smad4 (-)	
		Numble (%)	*P*-value	Numble (%)	*P*-value	Numble (%)	*P*-value	Numble (%)	*P*-value
Gender									
Male	62	43 (69.4)	0.325	36 (58.1)	0.705	32 (51.6)	0.705	25 (40.3)	1.000
Female	50	30 (60)		27 (54)		28 (56)		20 (40)	
Age (year)									
<56	54	34 (63)	0.694	29 (53.7)	0.704	26 (48.1)	0.343	18 (33.3)	0.180
≥56	58	39 (67.2)		34 (58.6)		34 (58.6)		27 (46.6)	
Size (cm)									
<5	56	32 (51.7)	0.112	32 (57.1)	1.000	23 (41.1)	0.013	18 (32.1)	0.123
≥5	56	41 (73.2)		31 (55.4)		37 (66.1)		27 (48.2)	
Depth of invasion									
Uninvolved Serosa	31	17 (54.8)	0.186	17 (54.8)	1.000	9 (29)	0.002	6 (194)	0.006
Serosal invasion	81	56 (69.1)		46 (56.8)		51 (63)		39 (48.1)	
Differentiation									
Well + Moderate	99	64 (64.6)	1.000	52 (52.5)	0.037	49 (49.5)	0.019	36 (36.4)	0.034
Poorly	13	9 (69.2)		11 (84.6)		11 (84.6)		9 (69.2)	
Lymph node status									
-	62	34 (54.8)	0.016	29 (46.8)	0.035	24 (38.7)	0.001	14 (22.6)	<0.001
+	50	39 (78)		34 (68)		36 (72)		31 (62)	
TNM stage									
I+II	59	32 (54.2)	0.017	27 (45.8)	0.023	21 (35.6)	<0.001	12 (20.3)	<0.001
III+IV	53	41 (77.4)		36 (67.9)		39 (73.6)		33 (62.3)	

COUP-TF II, chicken ovalbumin upstream promoter transcription factor II. *P*-value is based on Fisher's exact test.

COUP-TF II by 112 patients sample analysis and compared this value with that of the corresponding adjacent non-tumorous mucosa tissues, and looked for correlations between the expression of the proteins and clinicopathological factors and prognosis in colorectal cancer patients by tissue microarray and immunohistochemical SP methods. As the follow-up work of further explicating COUP-TF II regulatory mechanism in CRC, we selected PTEN-a crucial tumor suppressor often mutated in CRC and Smad4-the core members of TGF-β pathway, to elucidate the preliminary molecular mechanisms of COUP-TF II involved in the development and advancement of CRC

Materials and methods

Patients and tissue samples

Samples were procured from 112 consecutive colorectal cancer patients who were eligible and received surgery at Xuzhou medical college affiliated hospital from 2007 to 2009 and received no preoperative adjuvant therapy such as radiotherapy or chemotherapy, 103 cases of adjacent non-tumorous mucosa tissues, distance from the tumor tissue than 5 cm, as control group. Samples consisted of 62 male cases and 50 female cases, aged 34 to 76, with the median patient age of 56 years old. 59 patients were classified as Stage I and II, according to the 2009 AJCC colorectal cancer TNM staging, and 53 as Stage III and IV. Other patient characteristics concerning gender, size, differentiation, nodal involvement described in the **Table 1** in detail, was retrieved by reviewing the pathology and surgical reports. Tissue samples from patients were formalin-fixed and paraffin-embedded. Ethical approvals for recruitment were obtained from the Ethical Committee of Xuzhou medical college affiliated hospital.

Construction of tissue microarray and immunohistochemistry

The sites of the representative cancerous sites and corresponding normal tissues were

Table 3. Spearman correlations of COUP-TF II expression to PTEN, Smad4 immunoreactivity

COUP-TF II	PTEN		Spearman Rho	*P*-value	Smad4		Spearman Rho	*P*-value
	-	+			-	+		
-	20	19	-0.073	0.443	15	24	-0.221	0.019
+	43	30			45	28		
Total	63	49			60	52		

COUP-TF II, chicken ovalbumin upstream promoter-transcription factor II.

Table 4. Five-year DFS and OS according to prognostic factors (univariate analysis)

Clinicopathological factors		Cases (n)	5-year DFS		5-year OS	
			Numble (%)	*P*-value	Numble (%)	*P*-value
Gender						
Male		62	38 (61.3)	0.667	37 (59.7)	0.872
Female		50	33 (66)		31 (62)	
Age (year)						
<56		54	37 (68.5)	0.283	37 (68.5)	0.113
≥56		58	34 (58.6)		31 (53.4)	
Size (cm)						
<5		56	42 (75)	0.010	41 (73.2)	0.006
≥5		56	29 (51.8)		27 (48.2)	
Depth of invasion						
Uninvolved Serosa		31	24 (77.4)	0.061	24 (77.4)	0.022
Serosal invasion		81	47 (58)		44 (54.3)	
Differentiation						
Well + Moderate		99	66 (66.7)	0.049	64 (64.6)	0.017
Poorly		13	5 (38.5)		4 (30.8)	
Lymph node status						
-		62	46 (74.2)	0.006	47 (75.8)	<0.001
+		50	25 (50)		21 (42)	
TNM stage						
I+II		59	46 (78)	<0.001	47 (79.7)	<0.001
III+IV		53	25 (47.2)		21 (39.6)	
COUP-TF II						
-		39	31 (79.5)	0.010	31 (79.5)	0.002
+		73	40 (54.8)		37 (50.7)	
PTEN						
-		63	36 (57.1)	0.149	36 (57.1)	0.383
+		49	35 (71.4)		32 (65.3)	
Smad4						
-		60	29 (48.3)	0.001	27 (45)	<0.001
+		52	42 (80.8)		41 (78.8)	
COUP-TF II	Smad4					
+	-	45	18 (40)	<0.001	16 (35.6)	<0.001
Others		67	53 (79.1)		52 (77.6)	

DFS, disease-free survival; OS, overall survival; COUP-TF II, chicken ovalbumin upstream promotertranscription factor II. *P*-value is based on a univariate two-sided log-rank test.

reviewed and mapped, each cancerous zone and normal peripheral zone selected three sites. The tissue microarrays were built using a tissue microarray instrument (MINICORE, Alphelys Instruments). A 1.0-mm diameter cylinder embedded into a new paraffin block, and serially sectioned into 4-μm slices. Immunohistochemical staining was performed using the Rabbit Polyclonal antibody for COUP-TF II (ab50487) and PTEN-(ab31392), the Rabbit Monoclonal antibody for Smad4 (ab40759), the HRP-conjugated secondary detection antibody and DAB, All of the above reagents purchased from Abcam company (United Kingdom). The avidin-biotinperoxidase complex method is carried out according to the instructions with PBS instead of the primary antibody as a negative control, definitely positive biopsy of colorectal cancer as a positive control. Two specialists who were blinded to patient outcome evaluated the staining independently. In each case, the importance of the staining was evaluated by counting the frequency of labeled cells in five high-power fields containing each roughly 100 cells. For the intensity, the grading scale ranged from 0 (negative), 1 (weak), 2 (moderate), to 3 (strong). Labeling frequency was scored as 0 (0%), 1 (1%-25%), 2 (26%-50%), 3 (51%-75%), and 4 (76%-100%) according to the percentage of positively stained cells. The final staining score was calculated by multiplying the staining intensity score by the staining extent score and the specimens were divided into the following two groups: negative ≤3 and positive >3.

Statistical analysis

Statistical differences were performed using SPSS 19.0 (IBM, SPSS Statistics). Multiple comparisons of COUP-TF II among each

Table 5. Univariate and multivariate analysis of DFS and OS in 112 CRC patients examined

Variables	5-Year disease-free survival (DFS)				5-Year overall survival (OS)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	P-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)
Gender (female/male)	0.67	0.874 (0.469-1.626)		-	0.837	0.952 (0.524-1.730)		-
Age (≥56 years/<56 years)	0.289	1.399 (0.752-2.605)		-	0.119	1.623 (0.884-2.980)		-
Tumor size (≥5 cm/<5 cm)	0.013	2.272 (1.190-4.336)	0.04	1.981 (1.033-3.798)	0.008	2.348 (1.256-4.390)	0.019	2.160 (1.138-4.102)
Serosal invasion (+/-)	0.07	2.124 (0.941-4.792)		-	0.028	2.481 (1.105-5.573)		-
Differentiation (poor/moderate, well)	0.056	2.127 (0.981-4.609)		-	0.022	2.363 (1.135-4.923)		-
Lymph node status (+/-)	0.008	2.343 (1.249-4.396)		-	0.001	2.996 (1.604-5.598)	0.014	0.208 (0.060-0.725)
TNM stage (III+IV/I+II)	0.001	3.086 (1.595-5.973)		-	<0.001	3.977 (2.044-7.740)	<0.001	11.366 (2.930-44.090)
COUP-TF II (+/-)	0.013	2.652 (1.224-5.750)		-	0.004	3.083 (1.428-6.653)		-
PTEN (+/-)	0.156	0.627 (0.329-1.195)		-	0.388	0.765 (0.417-1.405)		-
Smad4 (+/-)	0.001	0.311 (0.152-0.635)		-	0.001	0.308 (0.156-0.611)		-
COUP-TF II (+) Smad4 (-)/others	<0.001	3.826 (1.998-7.326)	<0.001	3.552 (1.848-6.827)	<0.001	4.071 (2.171-7.633)	0.005	2.632 (1.342-5.160)

Data considered significant ($P<0.05$) in the univariate analysis are shown in bold, and were examined in the multivariate analysis (Method: forward LR). CI, confidence interval; COUP-TF II, chicken ovalbumin upstream promoter transcription factor II; HR, hazard ratio.

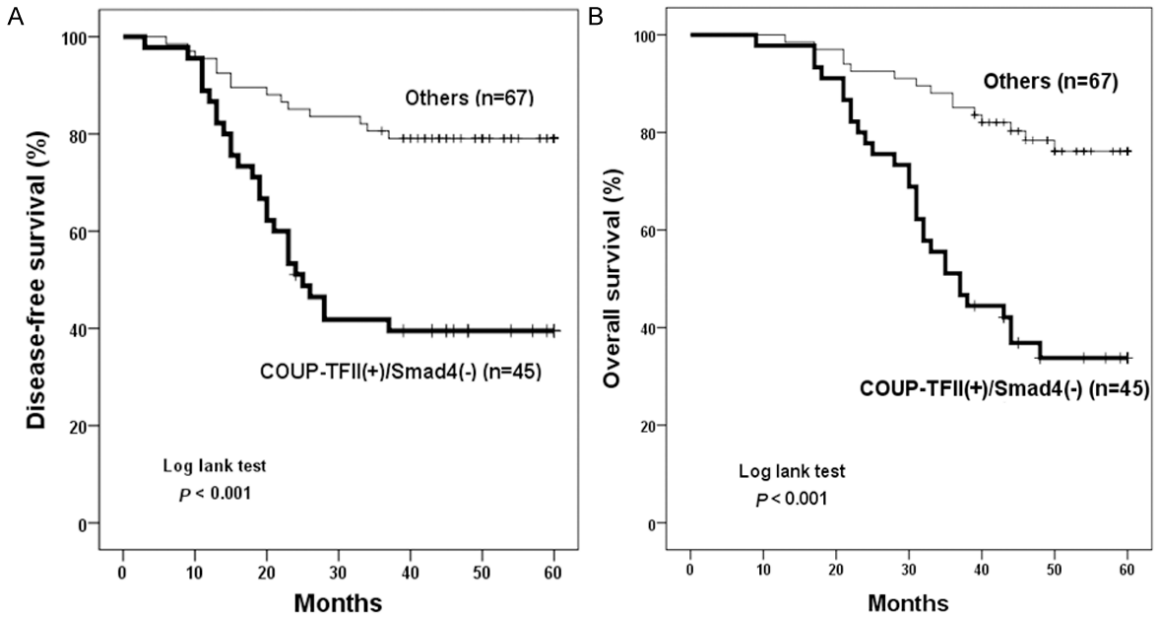

Figure 2. Kaplan-Meier survival curves for (A) Disease-free and (B) overall survival of 112 patients with colorectal carcinoma according to the expression status of COUP-TF II (+)/Smad4 (-). COUP-TF II (+)/Smad4 (-) expression status was significantly associated with an increased risk of adverse clinical outcome.

group of different clinicopathological parameters were performed with Fisher's exact test. The relationships between COUP-TF II immunoreactivity and the expression of PTEN, Smad4 were compared using Spearman correlation coefficients. Overall survival (OS) and Disease-free survival (DFS) curves were generated according to the Kaplan-Meier method for different factors and the statistical significance was calculated using the log-rank test. The Cox proportional hazards regression model and the hazard ratio were used for analysis the predictive value of COUP-TF II expression univariately with other clinical and pathological variables and 95% confidence intervals were computed. The multivariate Cox proportional hazards model was used to estimate an integrated risk score in univariate analysis. A value of $P<0.05$ was considered statistically significant.

Results

Expression of COUP-TF II, PTEN, Smad4 in human colorectal cancer tissues

Immunoreactivities for the COUP-TF II positive expression were defined by the presence of brown granules, located in nucleus. The cytoplasm and/or nucleus appearing brown granules were defined as positive expression of Smad4 and PTEN (**Figure 1**). In the present study, as summarized in **Table 1**, approximately 73 (65.2%) of 112 CRC tissue specimens exhibited intermediate to intense COUP-TF II staining, whereas only 16 (15.5%) positive COUP-TF II staining of normal colonic mucosa or scattered positive staining cells in adjacent tissues was detected, indicating the significant statistical difference in comparison ($P<0.05$). The overall increased negative expression of PTEN 56.3% (63/112), Smad4 53.6% (60/112) was indicated in colorectal cancer tissues in comparison to normal intestinal mucosa 11.7% (12/103), 21.4% (22/112) respectively ($P<0.05$), and supported the conclusion that PTEN and Smad4, as a tumor suppressor, is mutated in a large number of cancers at high frequency.

Relationship between the expression of COUP-TF II, PTEN, Smad4 and clinicopathological parameters of colorectal cancer tissues

Further correlation studies between COUP-TF II, PTEN, Smad4 expression and clinicopathologic features were performed, the outcome indicated in **Table 2**, in which COUP-TF II expression in tumour cells significantly associated with pathological predictors of CRC TNM stage and lymph node metastasis ($P<0.05$), the similar

correlation also involved in negative PTEN, Smad4. Diversely, negative PTEN expression was also associated with differentiation. In addition to this, the correlations between negative Smad4 expression and tumor size, Serosal invasion were also uncovered (P<0.05). No significant correlation was found between COUP-TF II expression and the gender, age, differentiation, over 5 cm-size tumor and serosa-involved. Remarkably, among 112 CRC cases, 45 CRC patients bearing COUP-TF II (+)/Smad4 (-) status which has a significant relationship with TNM stage, lymph node metastasis, differentiation, infiltration (P<0.05).

Correlation between the expression of COUP-TF II and PTEN, Smad4

Based on Spearman rank correlation test, an inverse relationship between COUP-TF II and Smad4 expression was noted in 112 cases of colorectal cancer tissues: 45 COUP-TF II-positive/Smad4-negative tumors and 24 COUP-TF II-negative/Smad4-positive tumors (rs=-0.221, P=0.019) (**Table 3**). There was no statistically significant relationship between COUP-TF II tumor expression and PTEN-negative tumors (P=0.443).

Clinicopathological parameters and COUP-TF II, Smad4, PTEN expression and outcome in colorectal cancer patients.

The log-rank test with Kaplan-Meier estimates demonstrated that high COUP-TF II status in CRC cases was significantly associated with poor survival or adverse clinical outcome (log-rank test: DFS, P=0.010; OS, P=0.002). The results of univariate analysis (**Table 4**) demonstrated that lymph node status (DFS, P=0.006; OS, P<0.001), histological grade (DFS, P<0.001; OS, P<0.001), tumor size (DFS, P=0.010; OS, P=0.006), differentiation (DFS, P=0.049; OS, P=0.017), Smad4 status (DFS, P=0.001; OS, P<0.001), COUP-TF II positive/Smad4 negative status (DFS, P<0.001; OS, P<0.001) and serosa-invasion (OS, P=0.022) were all significant prognostic factors for DFS and OS in the 112 patients examined. These results also indicated that patients no bearing higher levels of COUP-TF II exhibited favourable prognosis and five survival rates after proper routine clinical treatment, suggesting a positive role for COUP-TF II in driving CRC tumorigenesis. A subsequent multivariate analysis (**Table 5**, method: forward LR), however, revealed that lymph node status (OS, P=0.014), histological grade (OS, P<0.001), over 5 cm-size tumors (DFS, P=0.04; OS, P=0.019) and COUP-TF II positive/Smad4 negative status (DFS, P<0.001; OS, P=0.005) were independent prognostic factors for DFS and OS in these patients. The DFS and OS curves of COUP-TF II positive/Smad4 negative status in these analyses are shown in **Figure 2**.

Discussion

As far as the present state of study is concerned, COUP-TF II serves as a particular COUP-TF II family member, and plays essential roles in development, cellular homeostasis, and disease including cancer where aberrant expression of COUP-TF II associate with pathological predictors of tumorigenesis, progression and metastasis and the risk of patient survival [18-21]. COUP-TF II aberrant expression was frequently reported in different types of human tumors. Prostate tumor samples exhibited approximately 60% of all tumors exhibited intermediate to intense staining, whereas only 5% of the nontumor tissue stained positive for the receptor. More importantly, the expression of COUP-TF II correlates with increased risk of tumor recurrence and decreased survival in prostate cancer patients [11]. However, the prognostic significance of COUP-TF II varies between studies for breast cancer. Nagasaki S, et al revealed higher expression of COUP-TF II positively correlates with a poor clinical stage, lymph node status, histological grade and estrogen receptor alpha status, served as a negative prognostic factor for overall patient survival [12]. In contrast to this finding, Litchfield, et al reported that COUP-TF II expression is negatively associated with clinical outcome and disease progression [13]. Ovarian cancers with the higher COUP-TF II expression in the epithelium are associated with a trend toward greater likelihood of disease recurrence [15]. In pancreatic adenocarcinoma, COUP-TF II positivity expression is displayed in 61/89 tumor samples (Compared to low levels are detected in normal pancreas), correlates with the presence of lymph node, distant metastasis as well as the clinical stage, served as an indicator of worse prognosis in pancreatic cancer [22]. The present research uncovered that the overall increased positive expression of COUP-TF II in colorectal cancer tissues signifi-

cantly is associated with pathological predictors of TNM stage and lymph node metastasis, suggesting a positive role for COUP-TF II in driving CRC cancerogenesis and metastasis. High COUP-TF II status, low Smad4 status, more importantly, both positive COUP-TF II status and negative Smad4 status was all significantly associated with poor survival or adverse clinical outcome in the 112 patients examined.

A subsequent multivariate analysis revealed that COUP-TF II positive/Smad4 negative status were independent prognostic factors for DFS and OS in these patients. This statement is not consistent with what Shin SW, et al have found [14]. However, the prognostic significance of COUP-TF II varies between studies and cancer types, and the precise roles of COUP-TF II in cancer progression and metastasis are still elusive. These differences reported in literature may be due to the intrinsic differences among various cancer types and COUP-TF II may exert its functions in a context dependent manner.

Elucidating the molecular mechanisms that COUP-TF II tightly correlated with tumour recurrence and progression is requisite for tumor diagnosis, treatment and prognosis assessments. It has been reported that COUP-TF II was identified as TGF-β/SMAD co-regulators and the presence or absence of COUP-TF II distinguished between up- and down-regulated TGF-β/SMAD targets [16]. In another study, COUP-TF II serves as a key regulator to inhibit TGF-β signaling by interacting with Smad4 to drive full malignant progression of PTEN-null prostate tumorigenesis in which COUP-TF II can antagonizes a growth barrier formed by PTEN-null-induced upregulate of TGF-β signaling [11]. One of the mechanisms for COUP-TF II inhibitory effects on TGF-β signaling is that COUP-TF II sequesters Smad4 binding to target genes [23, 24]. The functional counteraction between COUP-TF II and Smad4 is reinforced by an inverse relationship between COUP-TF II and Smad4 expression indicated in this study. Remarkably, COUP-TF II (+)/Smad4 (-) status in 45 CRC patients has a close relationship with TNM stage, lymph node metastasis, differentiation, infiltration and a worse prognosis. Our findings also provide evidence that sole Smad4 negative progress is associated with TNM stage, differentiation, the development of metastases and a poor prognosis in multiple subsets of patients with CRC. Although, weak PTEN expression in CRC tissues was significantly associated with lymph node metastasis, grade of differentiation, and TNM stage, but no statistically significant relationship with COUP-TF II expression.

In summary, our results highlights the importance value of high COUP-TF II expression in determining CRC stage and metastasis and stratifying the risk of prognosis for colorectal cancer patients, suggesting a causal role of COUP-TF II in disease progression. Further mechanistic investigation indicated that COUP-TF II expression inversely correlates with Smad4 in human colorectal tumors, supported by COUP-TF II positive/Smad4 negative status having a crucial role in tumor genesis, progression and predicting the DFS and OS of patients. Except for the pathological roles of COUP-TF II, the detailed mechanism for COUP-TF II action remains to be further elucidated. Although endogenous ligands for these receptors have not been identified, COUP-TF II, as a member of the nuclear receptor superfamily whose activity can be regulated by small molecules, may also be targeted by tissue-selective COUP-TF II modulators that can be developed as anticancer agents for treating cancer patients. Thus, this result provides us a rational basis for future targeting of COUP-TF II for cancer intervention.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Linchuan Liao, College of Basic and Forensic Medicine of Sichuan University, Chengdu 610041, Sichuan, China. Tel: +86-13808084803; Fax: +86-13808084803; E-mail: linchuanliao@163.com

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