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Original Article

Correlation of Wnt antagonist sFPR1, Slug and β-catenin with prognosis and metastasis in colorectal carcinoma

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Abstract: sFPR1 plays an important role in colorectal carcinoma (CRC) tumorigenesis, Slug is also considered to be related to the development of CRC. However, the relationship between them and the mechanism of their involvement in CRC metastasis remain unknown. In this study, immunohistochemistry (IHC) was used to detect the expression of sFPR1, β-catenin, and Slug in 145 samples of CRC and corresponding surrounding "normal" mucosa tissues. Furthermore, clinicopathological features such as age, sex and so on were also collected retrospectively. Western blot and Transwell were used to detect proteins expression and migration capacity. In present study, the expression of sFPR1, Slug and β-catenin proteins were significantly correlated with lymph node metastasis and tumor-node-metastasis (TNM) stage of patients with CRC. sFPR1 expression showed a negative correlation with Slug and β-catenin. Kaplan-Meier analysis indicated that the postoperative 5-year OS of patients was related to the expression of sFPR1 and Slug, multivariate Cox regression analysis revealed that sFPR1 expression was an independent prognostic factor for CRC patients. Moreover, we found that the expression of slug and β-catenin could be regulated by sFPR1 in SW480 cells, and migration capacity of SW480 cells was suppressed with sFPR1 restoration. In summary, our data suggest that sFRP1, Slug and β-catenin are related to metastasis and prognosis in CRC. sFPR1 could mediate CRC metastasis by regulating the expression of Slug and β-catenin. Combined detection of these factors may be of significant value in predicting the metastasis and prognosis in CRC patients.

Keywords: sFPR1, slug, β-catenin, metastasis, prognosis, CRC

Introduction

The morbidity of colorectal carcinoma (CRC) has increased in recent years. According to the survey by Chen et al. in 2015 [1], the morbidity of CRC ranked No. 4 and No. 5 in malignant tumor morbidity among females and males, respectively; its mortality ranked No. 5 among all the malignant tumors. Tumor metastasis is an important factor restricting patients' survival rates. The 5-year survival rates of patients with regional positive lymph nodes or distant metastasis are significantly lower than the survival rates of those without tumor metastasis [2]. Therefore, exploring the mechanism of CRC metastasis and searching for new targets are extremely important to increase the patients' survival rates and decrease tumor metastasis [3].

Recent studies reported that the abnormal activation of the Wnt signal transduction pathway exists in 90% of the CRC [4], and the canonical Wnt/β-catenin signaling pathway plays an extremely important role in the metastasis of CRC [5]. The Wnt/β-catenin signaling pathway influences the expression of downstream target genes by regulating the distribution of β-catenin in the cytoplasm and cell nucleus. The status is regulated by various extracellular secretory proteins. They mainly play the role of regulating the Wnt pathway by influencing the combination of Wnt ligand with a receptor protein on the cytomembrane [6]. Secreted frizzled-related proteins (sFRPs) are important antagonists in the Wnt pathway. sFPR family mainly comprises five proteins, sFPR1-5. The common characteristic (except SFRP3) is that they all have a cysteine-rich domain in N-terminal similar to the Fzd

Table 1. Patient characteristics

Patient characteristic	Frequency (n)	Percentage (%)
Gender		
Male	86	59.3
Female	59	40.7
Age		
< 60 years	57	39.3
≥ 60 years	88	60.7
Diameter of tumor		
< 5.0 cm	84	57.9
≥ 5.0 cm	61	42.1
Location		
Rectum	65	44.8
Colon	80	55.2
Differentiation		
Well	31	21.4
Moderated	77	53.1
Poor	37	25.5
Depth of invasion		
Under serous membrane	80	55.2
To serous membrane	65	44.8
Lymph node metastasis		
Negative	113	77.9
Positive	32	22.1
Distant metastasis		
Negative	126	86.9
Positive	19	13.1
TNM stage		
I+II	105	72.4
III+IV	40	27.6

receptor, and it can further compete with the Fzd receptor in binding with the Wnt ligand and inhibit the Wnt pathway [7]. sFPR1 was selected for the present study. The existing researches reported that as a tumor suppressor gene, sFPR1 is lowly expressed in various malignant tumors, such as lung cancer [8], nasopharynx cancer [9], cervical cancer [10], and so on.

Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells differentiate and transform into interstitial cells [11]. In the development of tumors, EMT is an important process for tumors to acquire the capabilities of invasion and metastasis [12]. Slug, also named as Snail2, is a member of the Snail family of zinc finger transcription factors important for EMT. The Snail family includes three members: Snail1 (Snail), Snail2 (Slug), and Snail3 (Smuc). All of them share the same SNAG structural domain in N-terminal, and the zinc finger domain in C-terminal is responsible for combining with E-boxes sequence and regulating the expression of target genes [13]. Slug is highly expressed in various malignant tumors and plays an extremely important role in tumor invasion and metastasis [14, 15].

The Wnt/β-catenin signaling pathway and EMT play extremely important roles in the development of CRC [16, 17]. sFPR1, β-catenin, and Slug were selected for this study, and all of them were closely related to the development of CRC [18-20]. However, the research on their correlation with CRC metastasis and prognosis is limited; especially the mechanism of sFPR1 regulating CRC metastasis is still not clear. The present study explored the following hypotheses: the aforementioned three factors are interrelated and also closely related to CRC metastasis and prognosis.

Materials and methods

Patients and tissue samples

A total of 145 CRC radical surgery samples were collected in the First Affiliated Hospital of Bengbu Medical College from January 2009 to June 2010. Each sample was collected from tumor tissues and corresponding surrounding "normal" mucosa tissues (≥ 3 cm away from the tumor margin and pathologically proved as normal intestinal mucosa tissue). All were sporadic cases without genetic CRC history. All of them had complete clinical pathological information, and none of them received radiotherapy or chemotherapy. The results of the postoperative histopathological test showed that all were CRC. The follow-up lasted for 5 years, and the postoperative survival time was 3-60 months with an average survival time of 38.2±19.7 months. Tumor differentiation grade was performed according to the standard of World Health Organization in 2015. Clinical stages were performed according to the standard of American Joint Committee on Cancer in 2009. A total of 86 males and 59 females were enrolled with age ranging from 22 to 78 years (average age 58.0±11.3 years). Other clinicopathogical characteristics are listed in **Table 1**. The study was

approved by the ethics committee of the First Affiliated Hospital of Bengbu Medical College and conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

Immunohistochemical analysis

All samples were fixed with 10% neutral formalin, embedded in paraffin, and sliced at 4 μm thickness. Then, 3% H_2O_2 was applied to inactivate endogenous superoxide dismutase for 10 min, and the samples were boiled in 1.0% citrate, pH 6.0, at high pressure for 2 min. Primary antibodies, including rabbit anti-human SFPR1 antibody (1:100, Abcam, UK) and mouse anti-human Slug antibody (1:100, Santa Cruz Biotechnology, USA), were added. Mouse anti-human β-catenin monoclonal antibody (1:200, Santa Cruz Biotechnology, USA) was added to incubate at 4°C overnight. Then, the samples were washed with phosphate-buffered saline (PBS) three times. The ready-to-use secondary antibody was added for further incubation for 30 min at 37°C and developed using 3,3'-diaminobenzidine (DAB). Hematoxylin was applied to re-stain the cell nucleus, and then the samples were sealed using a neutral gum. PBS replacing primary antibody was taken as the negative control, and the known corresponding protein-positive slice was taken as the positive control.

Evaluation of immunostaining

Each slice was observed by two experienced pathology doctors in a double-blind manner. Cells without color were marked as 0, cells stained pale yellow were marked as 1, cells stained pale brown were marked as 2, and cells stained dark brown were marked as 3. Ten fields (×400) were selected randomly, and every field had about 100 cells; 0% was marked as 0, < 25% was marked as 1, 25%-50% was marked as 2, > 50%-75% was marked as 3, and > 75% was marked as 4. After combination, ≤ 2 was negative and ≥ 3 was positive. sFPR1 protein-positive reaction was localized in the cytomembrane and cytoplasm [21]. Slug protein-positive reaction was localized in the cytoplasm [22].

The positive marker for β-catenin was the appearance of pale brown granules in cells, and the positive substances were localized in the cytomembrane, cytoplasm, and/or cell nucleus. The staining results were judged according to the criteria described in the study by Maruyama et al. [23]. More than 70% of the cells with positive staining in the cytomembrane were normal. More than 10% of the cells showing staining in the cytoplasm and/or cell nucleus indicated positive expression. Negative expression in the cytomembrane and positive expression in the cytoplasm and/or cell nucleus indicated abnormal expression.

Cell culture

Chemicals and reagents: RPMI 1640 culture medium and trypsin were purchased from Gibco (USA). Dimethyl sulfoxide (DMSO) and 5-aza-2'-deoxycytidine (5-aza-dC) were obtained from Sigma (USA). 5-aza-dC was dissolved in DMSO to make a stock solution (10 mmol/L) and stored at -20°C. Te DMSO concentration was kept below 0.1% in all experiments and did not exert any detectable effect on cell growth or cell death. The Transwell assay kit was purchased from Corning (USA). Goat anti-human sFPR1 antibody, rabbit anti-human Slug antibody, mouse anti-human β-catenin monoclonal antibody, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) monoclonal antibody were purchased from Santa Cruz (USA).

Human CRC SW480 cell line was purchased from the cell bank of the Chinese Academy of Sciences and stored by Laboratory of Pharmacology, Bengbu Medical College. The cells were grown adhering to the wall in RPMI 1640 culture medium containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and streptomycin at 37°C and in the presence of 5% CO_2 in an incubator with saturated humidity. The cell passage was performed every 2-3 days and digested with 0.25% trypsin.

Treatment with 5-aza-dC to process the SW480 cell line

SW480 cells in the logarithmic phase were collected, digested with 0.25% trypsin, and made into a cell suspension. The cells were seeded at 6×10^4/mL in a six-well plate (2 mL/well). After incubation for 24 h, 5-aza-dC at a final concentration of 5 μmol/L was added to SW480 cells [24, 25]. The group with 0.1% DMSO replacing an equal volume of 5-aza-dC was taken as control. The medium was replaced every 24 h by the medium with the same drug concentration,

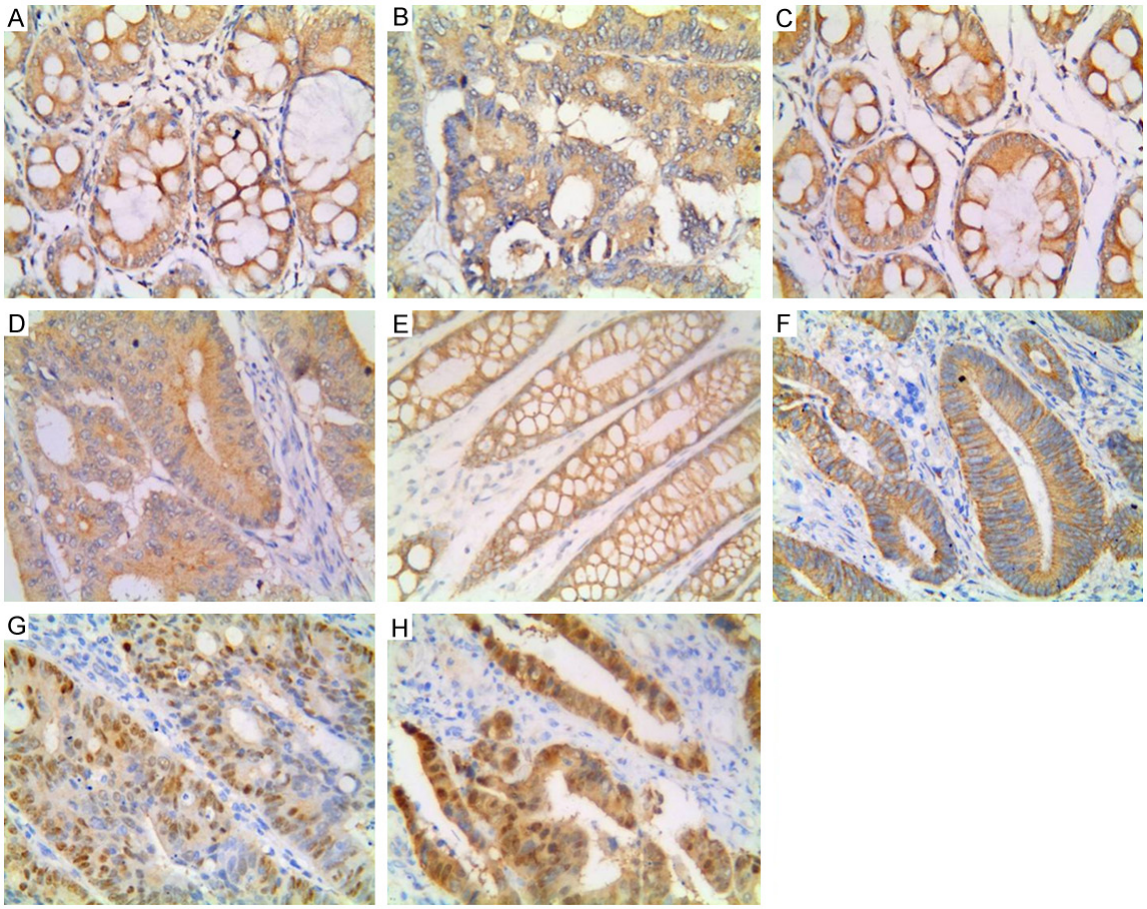

Figure 1. Expression of the proteins in colorectal carcinoma (×400 magnification). A. Positive sFPR1 expression in the cytoplasm of "normal" mucosa cells. B. Positive sFPR1 expression in the cytoplasm of cancer cells. C. Positive Slug expression in the cytoplasm of "normal" mucosa cells. D. Positive Slug expression in the cytoplasm of cancer cells. E. Positive β-catenin expression in the membrane of "normal" mucosacells. F. Positive β-catenin expression in the membrane of cancer cells. G. Positive β-catenin expression in the nucleus of cancer cells. H. Positive β-catenin expression in the nucleus and cytoplasm of cancer cells.

and the cells in the two groups were collected for further experiments.

Western blot

The bicinchoninic acid kit was applied to detect protein concentration and further calibrate the protein level. Then, the protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis, and then the membrane was transferred onto polyvinylidene difluoride (PVDF) membrane. The membrane was blocked using 5% skimmed milk for 1 h. Primary antibody sFPR1 (1:1000), Slug (1:1000), and β-catenin (1:1000) were added at 4°C overnight, followed by horseradish peroxidase-labeled rabbit anti-goat secondary antibody (1:10000), and shacked for 2 h. The Bio-Rad imaging system was used for imaging and acquisition. The Digital scanning imaging system was used to analyze the intensity of bands.

Transwell migration assay

The collected cells were resuspended a medium containing 0.05%-0.2% FBS without serum, and the density was adjusted to 5×10^5/mL. The suspension (200 μL) was seeded in the upper chamber, and the medium containing 5% FBS (500 μL) was added into the lower chamber. After incubation for 24 h, the cells in the matrix gel and upper chamber were wiped away carefully with a swab. The membrane was air-dried under room temperature. Then, 4% paraformaldehyde was added onto the lower cell surface to fix for 15 min. Ten fields in each well were selected and observed using an inverted microscope (×200). The photos were taken to calculate the average number of penetrating cells.

Table 2. The relationship between expression of sFPR1, β-catenin, Slug and clinicopathogical characteristics of (CRC)

Variables	SFPR1 expression		P	β-catenin expression		P	Slug expression		P
	Negative	Positive		Negative	Positive		Negative	Positive	
Sex			0.533			0.733			0.930
Male	57	29		34	52		56	30	
Female	42	17		25	34		38	21	
Age			0.260			0.680			0.986
< 60 years	42	15		22	35		37	20	
≥ 60 years	57	31		37	51		57	31	
Diameter of tumor			0.395			0.951			0.608
< 5.0 cm	55	29		34	50		53	31	
≥ 5.0 cm	44	17		25	36		41	20	
Location			0.621			0.623			0.691
Rectum	43	22		25	40		41	24	
Colon	56	24		34	46		53	27	
Differentiation			0.369			0.267			0.468
Well	18	13		16	15		23	8	
Moderate	54	23		27	50		48	29	
Poor	37	10		16	21		23	14	
Depth of invasion			0.018			0.064			0.691
Under serous membrane	48	32		38	42		53	27	
To serous membrane	51	14		21	44		41	24	
Lymph node metastasis			0.002			0.014			0.005
Negative	70	43		52	61		80	33	
Positive	29	3		7	25		4	28	
Distant metastasis			0.008			0.062			0.026
Negative	81	45		55	71		86	40	
Positive	18	1		4	15		8	11	
TNM stage			0.002			0.002			0.007
I+II	64	41		51	54		75	30	
III+IV	35	5		8	32		19	21	

Statistics

SPSS version 20.0 software (SPSS, IL, USA) was used to perform the statistical analysis. The χ^2 and Fisher exact tests were used to analyze the correlation between protein expression rate and clinicopathogical characteristics. Spearmen correlation was used to analyze the correlation among proteins. Multivariate logistic regression analysis was used to clarify the relative factors for lymph node metastasis. The Kaplan-Meier method was used for the univariate survival analysis, and the log-rank test was used for the comparison among survival curves. The Cox's regression model was used for the multivariate survival analysis, and concomitant variables included age, gender, tumor diameter, location, differentiation degree, invasion depth, lymph node metastasis, distant metastasis, TNM stage, and expression of sFPR1, Slug, and β-catenin. The analysis used 95% confidence interval. For the cytological experiment, the data were expressed as mean ± standard deviation. The comparisons between groups were analyzed by one-way analysis of variance and *t* test. A *P* value less than 0.05 was termed as statistical significance.

Results

Expression of sFPR1, β-catenin, and Slug in CRC and surrounding "normal" mucosa tissues

The positive expression rates of sFPR1 were 31.72% (46/145) and 66.9% (97/145), respectively, in CRC and surrounding "normal" muco-

Table 3. Correlation between expression of sFPR1, Slug, β-catenin in CRC

Variables	β-catenin		r	p	Slug		r	P
	Negative	Positive			Negative	Positive		
sFPR1			-0.250	0.002			-0.254	0.002
Negative	32	67			56	43		
Positive	27	19			38	8		
β-catenin							0.287	<0.001
Negative					48	11		
Positive					46	40		

sa tissues, with statistical significance (P < 0.05) (**Figure 1A** and **1B**). The positive expression rates of Slug protein in CRC and surrounding "normal mucosa" tissues were 35.17% (51/145) and 7.59% (11/145), respectively, with statistical significance (**Figure 1C** and **1D**). β-catenin was totally expressed on the cytomembrane in the normal tissues (**Figure 1E**), and only 2.07% (3/145) was abnormally expressed in the cytoplasm. The abnormal expression rate of β-catenin in CRC tissues was 59.31% (86/145) (**Figure 1F-H**).

Correlations between the expression of sFPR1, β-catenin, and Slug and clinicopathological characteristics *for CRC*

The expression of sFPR1, β-catenin, and Slug had no correlation with gender, age, tumor sites, diameter, and differentiation degree (P < 0.05). The expression of sFPR1, β-catenin, and Slug protein were significantly correlated with lymph node metastasis and TNM stage of patients with CRC (P < 0.05). The expression of sFPR1 and Slug proteins was significantly correlated with distant metastasis in patients with CRC (P < 0.05) (**Table 2**).

Correlation analysis of the expression of sFPR1, β-catenin, and slug in CRC

Spearman bivariate analysis indicated that the expression of sFPR1 in CRC tissues was negatively correlated with the expression of β-catenin and Slug (r = -0.250, P = 0.002; r = -0.252, P = 0.002); the expression of β-catenin showed a positive correlation with the expression of Slug protein (r = 0.287, P < 0.01) (**Table 3**).

Correlation analysis for lymph node metastasis

Multinomial logistic regression suggested that the depth of invasion and the expression of sFPR1 and Slug proteins were the key factors for lymph node metastasis (P < 0.05) (**Table 4**).

Univariate analysis for survival rate of patients with CRC

The Kaplan-Meier analysis indicated that the postoperative 5-year OS of patients was related to the expression of sFPR1, β-catenin, and Slug; lymph node metastasis; distant metastasis; and TNM stage (P < 0.05). Among them, the survival rate in the group with the positive expression of sFPR1 was significantly higher than that in the group with the negative expression of sFPR1 (log-rank = 17.415, P < 0.001). The survival rates in the groups with the positive expression of β-catenin and Slug were lower than those in the groups with the negative expression of β-catenin and Slug (log-rank = 21.387, P < 0.001; log-rank = 10.415, P < 0.001). It has also been found that on combining the positive expression of sFPR1 with the negative expression of β-catenin and Slug, the OS was significantly higher than that on combining the negative expression of sFPR1 with the negative expression of β-catenin and Slug (log-rank = 34.157, P < 0.001) (**Figure 2**; **Table 5**).

Multivariate analysis for survival rate of patients with CRC

Cox's regression analysis indicated that distant metastasis and the expression of sFPR1 and β-catenin were independent ifactors affecting patients' 5-year OS, as shown in the **Table 6**.

Detection of the expression of sFPR1, β-catenin, and Slug in SW480 cells before and after treatment with 5-aza-dC

The expression of sFPR1 protein increased and the expression of β-catenin and Slug proteins decreased in the experimental group compared with the control group (P < 0.05) (**Figure 3**).

Detection of changes in migration of SW480 cells before and after treatment with 5-aza-dC

The results of the Transwell assay showed that the migration and invasion of SW480 cells

Table 4. Multivariate analysis of factors affecting lymph node metastasis

Variables	Categories	Multivariate analysis		
		HR	95% CI	P
Depth of invasion	Under serous membrane/To serous membrane	2.626	1.104-6.246	0.029
sFPR1	Negative/positive	0.249	0.069-0.905	0.035
Slug	Negative/positive	2.545	1.079-6.002	0.033

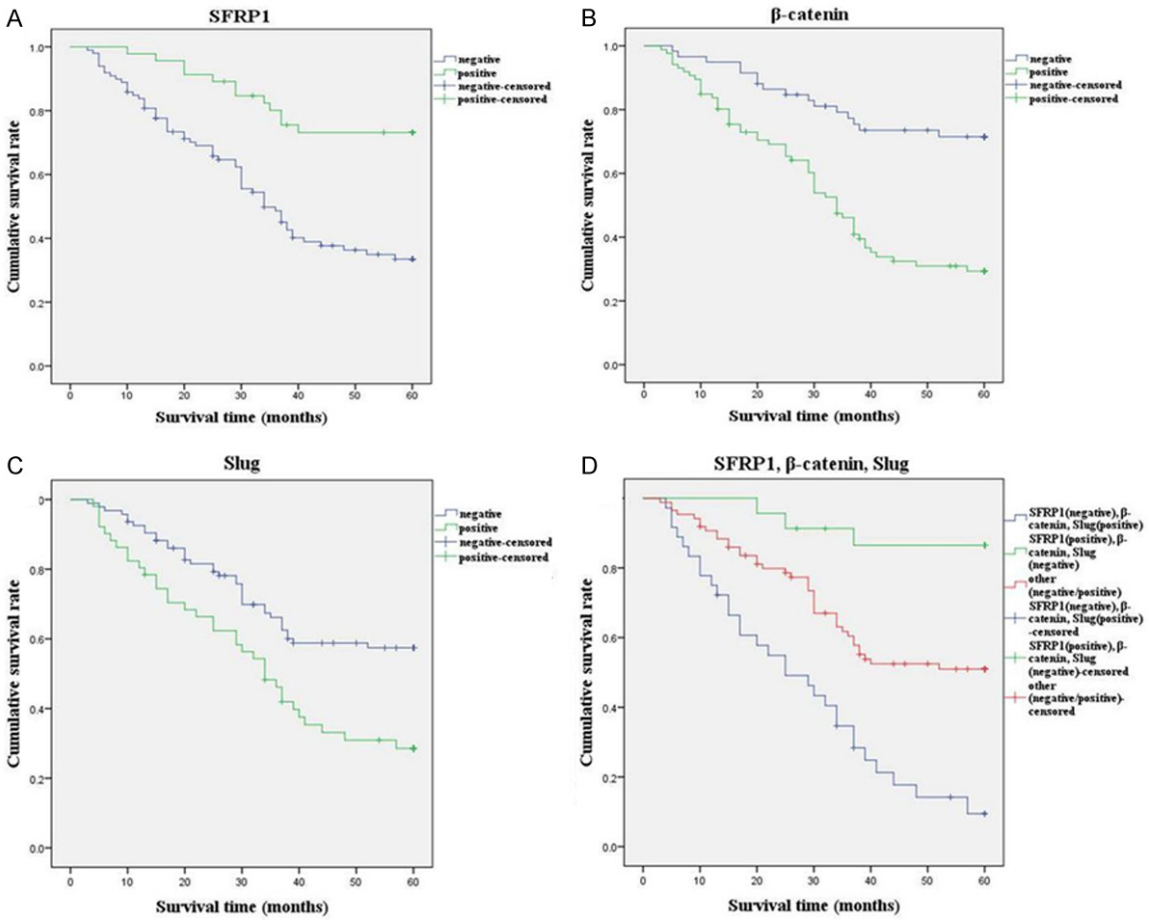

Figure 2. Kaplan-Meier analysis of the survival rate of patients with colorectal carcinoma. (A) Overall survival of all patients in relation to sFPR1 expression (log-rank = 17.415, $P < 0.001$). (B) Overall survival of all patients in relation to β-catenin expression (log-rank = 21.387, $P < 0.001$). (C) Overall survival of all patients in relation to Slug expression (log-rank = 10.415, $P = 0.001$). In (A-C) analyses, the green line represents positive expression of proteins and the blue line represents negative expression of proteins. (D) Overall survival of all patients in relation to the combination of sFPR1, β-catenin and Slug expression (log-rank = 34.157, $P < 0.001$). The green line represents positive expression of sFRP1 and negative expression of Slug, β-catenin and the blue line represents negative expression of sFRP1 and positive expression of Slug, β-catenin. The red line represents other positive or negative expression of the proteins. In all analyses, †represents censored observation.

weakened in the treatment group compared with the control group ($P < 0.05$) (**Figure 4**).

Discussion

Tumor metastasis plays an important role in decreasing the survival rate of patients with CRC. sFPR1 is an antagonist in the Wnt/β-catenin signaling pathway and also a tumor suppressor gene, playing a vital role in the occurrence and development of CRC. However, the mechanism underlying its involvement in CRC metastasis is not clear. Slug is an important transcription factor for EMT. It also plays

Table 5. Results of univariate analyses of (OS) time

Variables	N	Mean OS (months)	P	Log-Rank
sFPR1 expression			<0.001	17.415
Negative	99	32.9±19.2		
Positive	46	49.6±15.7		
β-catenin expression			<0.001	21.387
Negative	59	46.9±17.7		
Positive	86	32.2±18.9		
Slug expression			0.001	10.415
Negative	94	40.8±19.3		
Positive	51	33.3±19.7		
Sex			0.090	2.868
Male	86	40.9±19.2		
Female	59	34.3±20.0		
Age			0.780	0.078
< 60 years	57	37.5±19.3		
≥ 60 years	88	38.7±20.1		
Diameter of tumor			0.606	0.265
< 5.0 cm	84	38.1±19.6		
≥ 5.0 cm	61	38.4±20.1		
Location			0.906	0.014
Rectum	65	37.2±20.0		
Colon	80	39.0±19.6		
Differentiation			0.165	3.606
Well	31	35.6±20.1		
Moderate	77	38.7±20.1		
Poor	37	36.8±19.0		
Depth of invasion			0.539	0.377
Under serous membrane	80	39.6±18.4		
To serous membrane	65	36.4±21.3		
Lymph node metastasis			<0.001	17.811
Negative	113	41.4±18.7		
Positive	32	26.8±19.2		
Distant metastasis			<0.001	103.527
Negative	126	42.3±17.6		
Positive	19	11.1±5.6		
TNM stage			<0.001	31.591
I+II	105	43.6±17.5		
III+IV	40	24.0±18.3		

Table 6. Results of multivariate analyses of (OS) time

Variables	Categories	Multivariate analysis		
		HR	95% CI	P
Distant metastasis	Negative/positive	12.582	5.005-31.631	<0.001
sFPR1	Negative/positive	0.433	0.219-0.855	0.016
β-catenin	Negative/positive	2.584	1.394-4.791	0.003

an important role in CRC metastasis. Thus, a correlation might exist between the expression of sFPR1 and Slug and CRC. This study detected the expression of sFPR1, β-catenin, and Slug in CRC. They were found to be closely related to CRC metastasis and to each other. The exploration of the interaction mechanism may provide insights for the prevention and treatment of CRC.

In present study, the expression of sFPR1 in CRC tissues was found to be significantly lower than that in the surrounding "normal" mucosa tissues ($P < 0.05$) [18]. Furthermore, the expression of sFPR1 was found to have a negative correlation with CRC and tumor stage [21]. The aforementioned study indicated that the supermethylation of sFPR1 gene promoter might be the main mechanism for the decrease in expression in CRC [26]. Silva et al. [27] also suggested that the supermethylation of sFPR1 was closely related to the abnormal activation of the Wnt pathway in CRC. The Western blot analysis in the present study indicated that the expression of sFPR1 protein increased after treatment with 5-aza-dC [24, 25, 28]. 5-aza-dC is the first commercial DNA methyltransferase inhibitor approved by Food and Drug Administration (FDA) to treat malignant tumors [29]. Meanwhile, the Transwell assay demonstrated that cell migration decreased. In addition, the survival analysis of 145 patients indicated that the 5-year OS of the positive expression of sFPR1 was significantly higher that of the negative expression [21], and an independent factor influencing the prognosis of CRC. These findings suggested that sFPR1

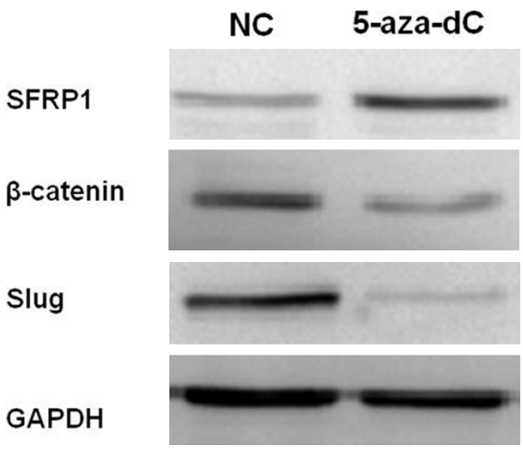

Figure 3. The expression of sFPR1, β-catenin and Slug was determined by Western blotting in the SW480 cell line treated with 5-aza-dc.

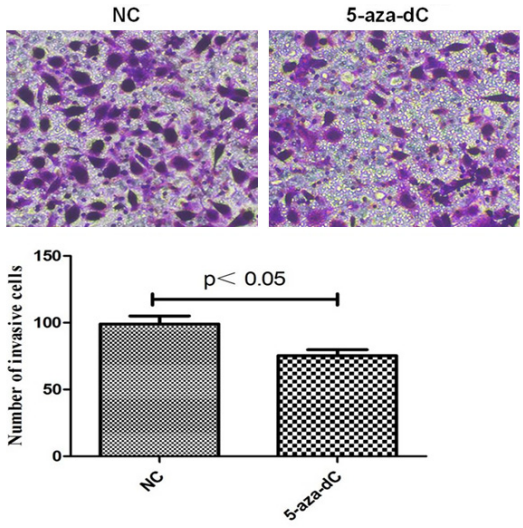

Figure 4. The migration capacity of SW480 cells treated with 5-aza-dC was detected by transwell assay. P < 0.05.

might play an important role in CRC development and prognosis [30].

EMT is an important process that influences tumor metastasis by regulating the expression of transcription factors, such as Snail, Slug, or Twist [12]. It was indicated that EMT also played an important role during the development of CRC [17]. In present study, the expression of Slug in CRC tissues was found to be significantly higher than that in the surrounding "normal" mucosa tissues ($P < 0.05$). Meanwhile, the expression of Slug was also indicated to exhibit a positive correlation with CRC lymph node metastasis, distant metastasis, and tumor stage, which was in line with the findings of Shioiri et al. [22], who demonstrated the expression of Slug in CRC tissues of 138 cases. Qian et al. [31] reported that tumor cell invasion and proliferation weakened after silencing Slug expression, which further confirmed the present conclusion. Moreover, the multivariate logistic regression analysis in this study suggested that Slug was the relevant risk factor for CRC lymph node metastasis [19]. Furthermore, the positive expression of Slug was found to have a negative correlation with 5-year OS [19, 22]. It suggested that Slug was closely related to the development of CRC.

It is believed that the occurrence and development of CRC are closely related to the abnormal activation of the Wnt/β-catenin signaling pathway. β-catenin is in the center of the pathway, and its localization in the cells decides the status of the whole pathway [20]. A previous study and this study, found that β-catenin protein was abnormally expressed in the cytoplasm and cell nucleus of CRC tissues compared with the surrounding "normal" mucosa tissues [32, 33]. Furthermore, the abnormal expression of β-catenin was found to be positively related to CRC stage and lymph node metastasis [34]. Overall, we conclude that β-catenin expression is significantly correlated with CRC metastasis.

The present study showed that sFPR1, Slug, and β-catenin were all closely related to the development of CRC. Meanwhile, the Spearman analysis found that the expression of sFPR1 had a negative correlation with the abnormal expression of β-catenin, which was in line with the mechanism of sFPR1 antagonizing the Wnt/β-catenin signaling pathway [18]. The multivariate logistic analysis in this study showed that sFPR1 was the relevant risk factor for lymph node metastasis, and had a significant correlation with tumor invasion depth and distant metastasis. The Transwell assay showed that the metastatic capacity of cells weakened after recovering the expression of sFPR1. Jiang et al. [35] believed that sFPR1 might inhibit the metastasis of hepatocellular carcinoma into lungs by decreasing the expression of β-catenin. Ren et al. [9] also found that the low expression of sFPR1 decreased the invasion capacity of nasopharynx cancer cells via the Wnt/β-catenin signaling pathway. Hence, it was suggested that sFPR1 might be involved in CRC metastasis via the Wnt/β-catenin signaling pathway. Moreover, IHC results indicated that the expres-

sion of sFPR1 was negatively correlated with the expression of Slug. Jin et al. [8] demonstrated that SFPR1 inhibited the EMT process in non-small cell lung cancer A549 cell line, and the metastasis of tumor were also weakened after recovering the expression of sFPR1. The expression of sFPR1 was also found to have a negative correlation with the expression of Slug, after treatment with 5-aza-dC compared with the control group. This indicated that sFPR1 might reduce the expression of Slug in CRC.

The Wnt/β-catenin signaling pathway regulates the EMT process and further influences the metastatic capacity of malignant tumors [36-38]. Wu et al. [39] found that the Wnt/β-catenin signaling pathway was involved in the EMT process mediated by Slug in breast cancer. The present study also found that the expression of β-catenin had a positive correlation with the expression of Slug. In addition, Chung et al. [10] found that sFPR1 regulated the expression of Slug via the Wnt/β-catenin signaling pathway and further influenced the EMT process in cervical cancer. Furthermore, the combination analysis of the three factors showed that on combining the positive expression of sFPR1 with the negative expression of β-catenin and Slug, the OS was significantly higher than that on combining the negative expression of sFPR1 with the positive expression of β-catenin and Slug. Therefore, the analysis of the combination of the three factors suggested that the high expression of sFPR1 could decrease the expression of β-catenin and Slug, reduce CRC metastasis, and further increase the survival rates of patients with CRC.

In conclusion, sFPR1, β-catenin, and Slug may an important role in metastasis and prognosis of CRC. Moreover, a correlation between these markers was also identified, the combined detection is important in judging patients' metastasis and prognosis. sFPR1 might regulate the expression of Slug and β-catenin influence metastasis of CRC. The limitation of our study is that there is no mRNA analysis from molecular level, only the analysis of the relevant protein expression. Future studies should explore more factors in the Wnt/β-catenin signaling pathway and EMT process, use gene transfer technology to regulate the expression of sFPR1.

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Disclosure of conflict of interest

None.

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