Overexpression of FZD7 is associated with poor survival in patients with colon cancer

Chengwei Ye[a,1], Meifang Xu[b,1], Mengxin Lin[c,1], Yiyi Zhang[a], Xin Zheng[b], Yanwu Sun[a], Yu Deng[a], Jie Pan[d], Zongbin Xu[a,*], Xingrong Lu[a,*], Pan Chi[a,*]

[a] Department of Colorectal Surgery, Fujian Medical University Union Hospital, Fuzhou, China
[b] Department of Pathology, Fujian Medical University Union Hospital, Fuzhou, China
[c] Department of Oncology, Fujian Medical University Union Hospital, Fuzhou, China
[d] Department of Emergency surgery, Fujian Medical University Union Hospital, Fuzhou, China

ABSTRACT

Background: Overexpression of Frizzled-7 (FZD7) has been associated with tumor invasion and distant metastases, but little is known about the relationship between FZD7 expression and prognosis in colon cancer.
Patients and methods: A total of 114 patients with colon cancer between June 2010 and December 2010 were enrolled in this study. The expression of FZD7 in cancerous and adjacent non-cancerous tissues was determined by immunohistochemistry, and the association between FZD7 expression and patient's clinicopathological characteristics was explored. The correlation between FZD7 expression and prognosis of colon cancer patients was analyzed using the Oncomine database and R2.
Results: FZD7 expression levels were significantly higher in colon cancer tissues compared with adjacent non-cancerous tissues ($P < 0.001$). High expression of FZD7 was significantly associated with metastatic or recurrent disease in colon cancer ($P = 0.010$). Kaplan-Meier survival analysis demonstrated that colon cancer patients with high expression of FZD7 had a significantly poorer OS ($P = 0.013$) and DFS ($P = 0.010$). Cox regression demonstrated that the expression of FZD7 was an independent prognostic factor for DFS (HR = 6.647, $P = 0.023$). A meta-analysis from the Oncomine database demonstrated that FZD7 mRNA levels were significantly higher in colorectal cancer tissues than in normal colorectal tissues, and FZD7 high expression was associated with a significantly poorer event and relapse-free survival time by analyzing the data from the R2: Genomics Analysis and Visualization Platform.
Conclusions: Overexpression of FZD7 was associated with poor survival in patients with colon cancer. Our data suggest that FZD7 expression could be an effective prognostic biomarker for colon cancer.

1. Introduction

Colon cancer is a commonly occurring cancer and remains the third cancer-related death worldwide [1]. Although radical surgery and adjuvant chemotherapy have improved colon cancer survival, tumor relapse remains the main cause of poor prognosis [2]. However, the complexities of tumor biology make it difficult to accurately predict the prognosis of colon cancer patients [3]. Therefore, there is an urgent need to identify risk factors and biomarkers in order to offer individualized therapeutic intervention strategies [4,5].

Frizzled-7 (FZD7), a member of the Frizzled receptor family, is a downstream target of the Wnt/β-catenin signaling pathway [6]. It can

activate the canonical and/or the non-canonical Wnt signaling pathways by binding with Wnt ligands [7]. Recent studies have demonstrated that high expression of FZD7 was an independent prognostic biomarker in various types of cancers, such as hepatocellular carcinoma [8], breast cancer [9], and gastric cancer [10]. Accumulating evidence indicates that canonical Wnt/β-catenin signaling pathway can regulate transcription factors and cytokines, modulate epithelial-mesenchymal transition (EMT), and thereby promoting colon cancer cell adhesion, migration, and invasion [7,11]. However, little was known about the relationship between FZD7 expression and clinical features of colon cancer.

In this study, we examined the expression of FZD7 in cancerous and

adjacent non-cancerous colon tissues by immunohistochemistry. We then evaluated the correlations between FZD7 expression and clinicopathological characteristics of colon cancer. Additionally, we determined the prognostic significance of FZD7 expression in predicting overall survival (OS) and disease-free survival (DFS). Finally, our findings were confirmed by bioinformatic analysis.

2. Materials and methods

2.1. Patients

This was a retrospective study at a single institution. A total of 114 patients with colon cancer receiving surgical resection from June to December 2010 at Fujian Medical University Union Hospital (Fujian, China) were enrolled in this study. The inclusion criteria were as follows: [1] histologically proven adenocarcinomas [2], tumors located in the colon, including ascending, transverse, descending, and sigmoid colon, and [3] an Eastern Cooperative Oncology Group (ECOG) performance status score less than 2. Patients were excluded from the study if they were [1]: age less than 18 years [2], severe complications [3], familial adenomatous polyposis (FAP) or Lynch syndrome, and [4] multiple primary tumors. Informed consent was obtained from all participants included in the study. The study procedure was approved by the Institutional Review Board of Fujian Medical University Union Hospital.

2.2. Data collection

Tissue samples, including cancerous and adjacent non-cancerous colon tissues, were collected during operation, and the surgical specimens were subjected to routine pathological examination. Clinical information including gender, age, body mass index (BMI), preoperative carcinoembryonic antigen (CEA) level, preoperative carbohydrate antigen 199 (CA199) level, tumor location, gross type, histopathology, differentiation, and pathological TNM stage were collected from the patient's medical records. Survival data were collected from postoperative follow-up.

2.3. Immunohistochemistry

Immunohistochemistry was performed using the streptavidin-biotin complex method. The primary antibody was anti-FZD7 (Bioss Antibodies, Beijing, China), and negative control of phosphate-buffered saline was used. The staining intensity scores were as follows: 0 (no staining), 1 (light yellow staining), 2 (brown staining), and 3 (deep brown staining). The percentage of positive cells was scored as follows: 0 (< 5% stained cells), 1 (5%–25% stained cells), 2 (25%–50% stained cells), 3 (50%–75% stained cells), and 4 (> 75% stained cells). The final score of FZD7 was obtained by multiplying these two scores: 0 for negative (−), 1–3 for weakly positive (+), 4–8 for positive (+ +), and 9–12 for strongly positive (+ + +). Representative figures of FZD7expression were shown in Fig. 1. If the final score of FZD7 expression in cancerous tissue was higher than that in non-cancerous tissues, the patient was classified as the high expression group. Otherwise, the patient was classified as the low-expression group.

2.4. Follow-up

Postoperative follow-up was performed every 3 months for the first 3 years, then every 6 months for the next 2 years, and annually thereafter. During each visit, a physical examination, serum CEA test, chest X-ray, or CT, and abdominopelvic MRI or CT scans were performed. A colonoscopy was performed annually after surgery. Positron emission tomography (PET) was performed when needed. OS was defined as the time from the date of the primary treatment to the date of death from any cause or until the date of the last follow-up. DFS was defined as the time from the date of the primary treatment to the date of diagnosis of tumor relapse or the date of the last follow-up. Postoperative follow-up lasted until death or the cut-off date of September 30, 2018.

2.5. Bioinformatic analysis of FZD7

To further verify the FZD7 expression in a large number of colon cancer tissues, the Oncomine database [12] (A cancer microarray database and integrated data-mining platform aimed at facilitating discovery from genome-wide expression analyses; https://www.oncomine.org) was utilized to perform the validation of FZD7 expression. Additionally, we analyzed the prognostic value of FZD7 for colorectal cancer (CRC) patients using the R2: Genomics Analysis and Visualization Platform [13] (a biologist-friendly web-based genomics analysis and visualization application, which contained several gene microarrays or RNA-Seq data, http://r2.amc.nl) to explore the potential prognostic value of FZD7.

2.6. Statistical analysis

Statistical analyses were performed using SPSS version 24.0 (SPSS Inc., Chicago). Categorical variables were expressed as numbers with percentages) and compared using the Chi-square test or Fisher's exact test when appropriate. Normally distributed data were described by means ± standard deviations and analyzed using Student's t-tests. Significant variables in the univariate analysis were entered into a multivariate Cox regression model. Cox's proportional hazard model analysis was performed to identify risk independent factors for OS and DFS. The Kaplan-Meier method was used to assess survival outcomes. The log-rank test was used to compare survival between groups. $P < 0.05$ was defined as statistically significant.

3. Results

3.1. FZD7 expression in colon cancer tissues

A total of 114 patients (76 males and 38 females) were included in this study. The clinicopathological characteristics of colon cancer patients are summarized in Table 1. In order to explore the expression and significance of FZD7 in colon cancer tissues, we determined the expression of FZD7 protein in 114 cancerous and matched adjacent non-cancerous colon tissues by immunohistochemistry. Immunohistochemical staining demonstrated that the expression of FZD7 in the colon cancer tissues was higher than that in adjacent non-cancerous tissues (Fig. 2, $P < 0.001$). According to the FZD7 expression between cancerous and adjacent non-cancerous tissues, 46 patients (40.4%) were classified as the FZD7 low-expression group and 68 patients (59.6%) as the FZD7 high expression group.

3.2. Association between FZD7 expression and clinicopathological characteristics in colon cancer patients

We further explored the association between expression of FZD7 and clinicopathological characteristics in colon cancer patients, as demonstrated in Table 2. Low expression of FZD7 was more often observed in ascending colon cancer patients (45.7%), while high expression was more often seen in sigmoid colon cancer (66.2%, $P = 0.020$). High expression of FZD7 was significantly associated with metastatic or recurrent disease in colon cancer ($P = 0.010$). Additionally, the expression of FZD7 was not associated with gender, age, BMI, preoperative CEA level, preoperative CA199 level, tumor location, gross type, histopathology, histologic differentiation, and the pathological stage (all $P > 0.05$).
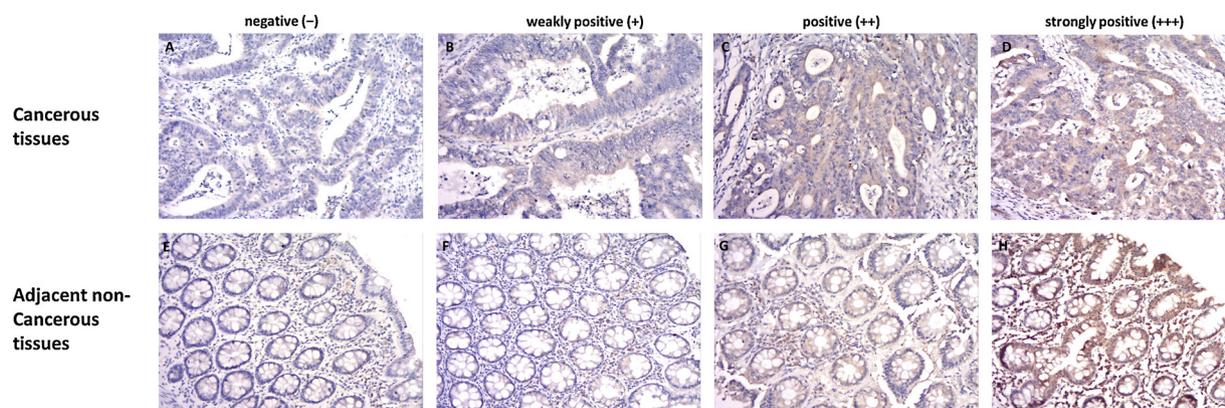
Fig. 1. Representative figures of FZD7 expression cancerous (A–D) and adjacent cancerous (E–H) colon tissues.

Table 1
Baseline characteristics of patients with colon cancer.

Characteristics	N (114)	%
Gender		
Male	76	66.7
Female	38	33.3
Age (mean ± SD, years)	62.6 ± 13.4	
BMI (mean ± SD, kg/m^2)	21.9 ± 2.8	
Pretreatment CEA level (mean ± SD, ng/ml)	17.7 ± 40.0	
Pretreatment CA199 level (mean ± SD, U/ml)	44.3 ± 145.4	
Tumor location (%)		
Ascending colon	34	29.8
Transverse colon	4	3.5
Descending colon	11	9.6
Sigmoid colon	65	57.0
Gross type (%)		
Expanding	45	39.5
Ulcering	64	56.1
Infiltrating	5	4.4
Histopathology (%)		
Adenocarcinoma	101	88.6
Mucinous or signet ring adenocarcinoma	13	11.4
Tumor differentiation (%)		
Well and moderately differentiated	87	76.3
Poorly differentiated and others	27	23.7
Pathological T stage (%)		
T1	7	6.1
T2	9	7.9
T3	67	58.8
T4	31	27.2
Pathological N stage (%)		
N0	60	52.6
N1	37	32.5
N2	17	14.9
Pathological M stage (%)		
M0	101	8.6
M1	13	11.4
Metastasis or recurrence		
Yes	29	25.4
No	85	74.6
Expression of FZD7		
Low	46	40.4
High	68	59.6

BMI: body mass index; CEA: carcinoembryonic antigen; CA199: carbohydrate antigen 19-9; FZD7: frizzled 7; SD: standard deviation.

3.3. High expression of FZD7 in colon cancer tissues is associated with poor survival

Kaplan-Meier survival analysis demonstrated that colon cancer patients with high expression of FZD7 had a significantly poorer OS (P = 0.013) and DFS (P = 0.010) rate, as shown in Fig. 3. The 5-year OS and DFS rates among colon cancer patients with high FZD7 expression were 64.7% and 69.5%, significantly lower than 82.6% and
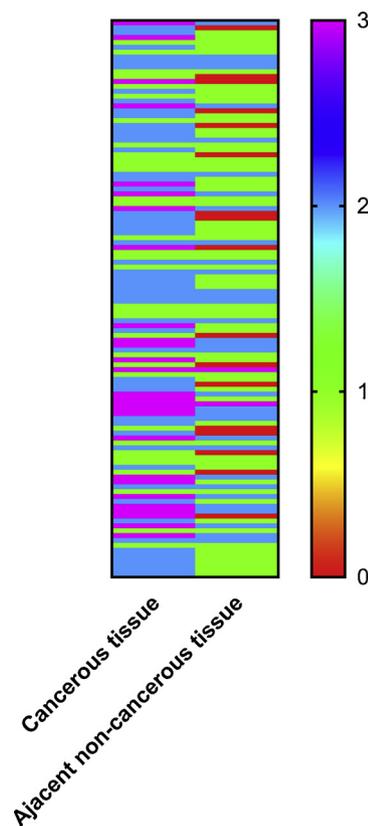

Fig. 2. The immunohistochemical score of FZD7 in cancerous and adjacent cancerous tissue.

90.5% among patients with low FZD7 expression. The median OS time was 70.9 and 84.8 months (P = 0.013), respectively, in high and low FZD7 expression group. While the DFS time was 72.5 and 89.6 months (P = 0.010), respectively.

High FZD7 expression is a prognostic predictor in patients with colon cancer

In the next step, univariate and multivariate analysis was performed to identify independent predictive factors for DFS and OS in patients with colon cancer by using a Cox proportional-hazard model (Table 3). On the univariate analysis, BMI (P = 0.049), preoperative CA19-9 level (P = 0.002), and preoperative CEA level (P = 0.013) were independent prognostic factors for DFS but not OS. The pathological N stage (DFS: HR = 5.234, P < 0.001; OS: HR = 2.883, P < 0.001) and the expression of FZD7 (DFS: HR = 5.632, P = 0.022; OS: HR = 2.459,

Table 2
Association between FZD7 expression and clinicopathological characteristics in colon patients.

Variables	FZD7 expression		P-value
	Low (N = 46)	High (N = 68)	
Gender (%)			0.070
Male	26(56.5)	50(73.8)	
Female	20(43.5)	18(26.2)	
Age (mean ± SD, years)	60.6 ± 15.2	64.0 ± 12.0	0.186
BMI (mean ± SD, kg/m²)	21.5 ± 3.0	22.2 ± 2.6	0.255
Pretreatment CEA (mean ± SD, ng/ml)	13.7 ± 23.1	20.4 ± 48.2	0.054
Pretreatment CA199 (mean ± SD, U/ml)	20.0 ± 21.6	60.7 ± 186.3	0.144
Tumor location (%)			**0.020**
Ascending colon	21(45.7)	13(19.1)	
Transverse colon	2(4.3)	2(2.9)	
Descending colon	3(6.5)	8(11.8)	
Sigmoid colon	20(43.5)	45(66.2)	
Gross type (%)			0.160
Expanding	22(47.8)	23(33.8)	
Ulcering	21(45.7)	43(63.2)	
Infiltrating	3(6.5)	2(2.9)	
Histopathology (%)			0.766
Adenocarcinoma	40(87.0)	61(89.7)	
Mucinous or signet ring adenocarcinoma	6(13.0)	7(10.3)	
Tumor differentiation (%)			0.183
Well moderately differentiated	14(30.4)	13(19.1)	
Poorly differentiated and others	32(69.6)	55(80.9)	
Pathological T stage (%)			0.120
T1	5(4.9)	2(2.9)	
T2	4(8.7)	5(7.4)	
T3	29(63.0)	38(55.9)	
T4	8(17.4)	23(33.8)	
Pathological N stage (%)			0.073
N0	30(65.2)	30(44.1)	
N1	10(21.7)	27(39.7)	
N2	6(13)	11(16.2)	
Pathological M stage (%)			0.276
M0	42(91.3)	57(86.8)	
M1	4(8.7)	11(16.2)	
Metastasis or recurrence (%)			**0.010**
Yes	5(10.9)	21(30.9)	
No	41(89.1)	47(69.1)	

FZD7: frizzled 7; BMI: body mass index; CEA: carcinoembryonic antigen; CA199: carbohydrate antigen 19-9; SD: standard deviation.

P = 0.017) were found to be prognostic factors for both DFS and OS. On multivariate analyses, pathological N stage remained to an independent prognostic factor for DFS (P < 0.001) and OS (P < 0.001), and the expression of FZD7 was an independent prognostic factor for DFS (HR = 6.647, P = 0.023).

3.4. Validation of FZD7 expression in Oncomine and R2

To further evaluate the relationship between survival and FZD7 expression in colon cancer, we analyzed data from Oncomine and R2. Meta-analysis of 10 GEO-sourced data sets mined from the Oncomine database demonstrated that FZD7 mRNA levels were significantly higher in colorectal cancer tissues than in normal colorectal tissues (P < 0.001; Fig. 4A). The R2: Genomics Analysis and Visualization Platform was used to generate Kaplan-Meier overall survival curves using data sets "Tumor Colon-Smith-232-MAS5.0-u133p2", "Tumor Colon (Core Exon)-Sveen-333-rma-sketch-huex10p", "Tumor Colon Sieber-290-MAS5.0-u133p2", and "Tumor Colon CIT (Combat)-Marisa-566- rma-u133p2". FZD7 high expression was associated with a significantly poorer event and relapse-free survival time (all P < 0.05; Fig. 4B, C, D, E). Moreover, we found that in "Mixed Colon Adenocarcinoma-TCGA-174 custom-agg4502a073" data set (P = 0.118), the high expression of FZD7 was associated with poorer overall survival, but without statistical significance (Fig. 4F).

4. Discussion

Accumulating evidence has indicated that FZD7 plays an important role in tumor invasion and metastasis. However, little is known about the relationship between FZD7 expression and prognosis in colon cancer. In the present study, we used immunohistochemistry and bioinformatic approaches to investigate the relationship between FZD7 expression and the clinicopathological features of colon cancer patients. Our results indicated that FZD7 expression was higher in colon cancer tissues than in adjacent non-cancerous colon tissues. High expression of FZD7 was significantly associated with metastatic or recurrent disease. Additionally, high expression of FZD7 was an independent prognostic factor for DFS. We further used bioinformatic databases to confirm the relationship between higher FZD7 expression and poor prognosis, and the results were in good accordance with our analysis.

FZD7 can bind with Wnt ligands and transduces canonical and non-canonical Wnt/β-catenin signaling pathways and thus promotes tumor invasion and metastasis [11]. Accumulating evidence has indicated
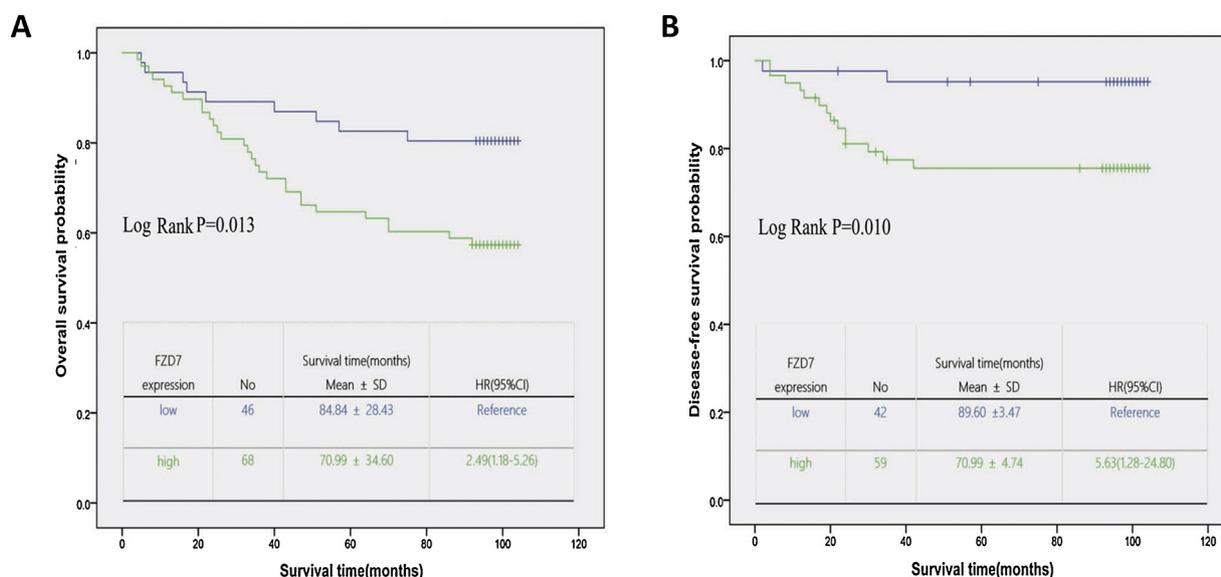
Fig. 3. Kaplan-Meier curves for colon cancer patients stratified by the expression level of FZD7. A. overall survival; B. disease-free survival.

Table 3
Univariate and multivariate analyses of the risk factors of OS and DFS in patients with colon cancer.

	OS (n = 114)				DFS (n = 101)			
Variables	Univariate analysis		Multivariable analysis		Univariate analysis		Multivariable analysis	
	HR(95% CI)	p value	HR(95% CI)	p value	HR(95% CI)	p value	HR(95% CI)	p value
Gender (male vs female)	0.550(0.260-1.161)	0.117			0.553(0.178-1.715)	0.305		
Age	1.020(0.994-1.048)	0.136			1.023(0.982-1.066)	0.278		
BMI	1.064(0.955-1.187)	0.261			1.182(1.001-1.397)	0.049	1.018(0.819-1.264)	0.874
Pretreatment CEA	1.005(1.000-1.010)	0.054			1.020(1.007-1.034)	0.002	1.017(0.998-11.035)	0.077
Pretreatment CA199	1.001(1.000-1.003)	0.127			1.002(1.000-1.004)	0.013	1.001(0.999-1.003)	0.468
Tumor location		0.194				0.993		
Ascending colon	Reference	0.117			Reference	0.791		
Transverse colon	0.530(0.240-1.171)	0.460			0.863(0.289-2.574)	0.986		
Descending colon	1.721(0.408-7.257)	0.119			0.000(0.000-0.000)	0.945		
Sigmoid colon	0.385(0.091-1.621)				1.055(0.228-4.883)			
Gross type		0.129				0.339		
Expanding	Reference	0.964			Reference	0.939		
Ulcering	1.048(0.134-8.190)	0.451			0.534(0.011-6.809)	0.933		
Infiltrating	2.154(0.293-15.854)				1.249(0.126-15.88)			
Histopathology(Mucinous or signet ring adenocarcinoma vs Adenocarcinoma)	1.745(0.784-3.886)	0.173			2.047(0.583-7.186)	0.263		
Tumor Differentiation (poorly vs moderately and well differentiated)	1.934(0.732-5.110)	0.183			1.743(0.605-5.016)	0.303		
Pathological T stage	2.676(1.555-4.606)	< **0.001**	1.880(1.025-3.451)		1.677(0.583-7.186)	0.173		
Pathological N stage	2.883(1.911-4.349)	< **0.001**	2.106(1.318-3.366)	**0.002**	5.234(2.605-10.515)	< **0.001**	6.328(1.298-15.575)	< **0.001**
Pathological M stage	4.812(2.373-9.758)	< **0.001**	3.336(1.531-7.268)	**0.002**				
FZD7 expression	2.459(1.177-5.263)	**0.017**	1.606(0.730-3.532)	0.239	5.632(1.279-24.802)	**0.022**	6.647(1.298-34.025)	**0.023**

BMI: body mass index; CEA: carcinoembryonic antigen; CA199: carbohydrate antigen 19-9; HR: hazard ratio; CI: confidence interval.

FZD7 was a prognostic biomarker and a potential therapeutic target in various types of tumors [8–10,14]. Additionally, it has been reported that FZD7 can facilitate colorectal cancer progression and metastasis [15,16]. Ueno et al. [17] have reported the high FZD7 expression in colon cancerous tissues, and revealed that FZD7 may be important in the proliferation, invasion, and metastasis of colon cancer cells. In our study, we demonstrated that FZD7 expression was higher in colon cancer tissues than in adjacent non-cancerous colon tissues, which was consistent with previous results.

In our study, a total of 26 patients experienced tumor relapse, and high expression of FZD7 was significantly associated with metastatic or recurrent disease (p = 0.010). Tumor invasion and metastasis are believed to associate with mesenchymal-epithelial transition (EMT) [18]. One of the possible explanations may be the function of FZD7 in induction of EMT in Wnt/β-catenin signaling pathway [19]. Taken together, these results indicated that FZD7 might be involved in colon cancer invasiveness and metastasis.

Having shown the association between FZD7 overexpression and colon cancer invasiveness and metastasis, we further explore the prgnostic value of FZD7 expression in colon cancer. Kaplan-Meier survival analysis demonstrated that high expression of FZD7 was associated with a significantly poorer OS (P = 0.013) and DFS (P = 0.010). Cox regression demonstrated that the expression of FZD7 was an independent prognostic factor for DFS (HR 6.647, P = 0.023). The prognostic significance of FZD7 in colon cacner was consistent with previsous findings [8–10,14].

We further evaluated the relationship between survival and FZD7 expression in colon cancer by informatics analysis. A meta-analysis from the Oncomine database demonstrated that FZD7 mRNA levels were significantly lower in colorectal cancer tissues than in normal colorectal tissues, and FZD7 high expression was associated with a significantly poorer event and relapse-free survival time. Together, these results from bioinformatics analysis were in good accordance with our analysis.

There are several limitations to our study. First, the present study was a retrospective analysis based on a single institution and may be subjected to potential selection bias. Second, the sample size was relatively small, and subgroup analysis was not performed. Third, although the bioinformatics analysis was in good accordance with our results, these findings need to be confirmed with a larger number of patients. Given these limitations, our study adds to the understanding of the relationship between FZD7 expression and prognosis in colon cancer.

In conclusion, FZD7 expression levels were significantly higher in colon cancer tissues compared with adjacent non-cancerous tissues. Over-expression of FZD7 was associated with poor survival in patients with colon cancer. Our data suggest that FZD2 expression could be an effective prognostic biomarker for colon cancer.

Compliance with ethics guidelines

All authors declare that they have no conflict of interest.

Authors' contributions

Chengwei Ye, Xingrong Lu, Zongbin Xu and Pan Chi designed the study, performed the evaluation of the IHC staining, participated in data analysis and drafted the manuscript. Yiyi Zhang, Mengxin Lin, and Yu Deng participated in data analysis. Xin Zheng, Jie Pan and Meifang Xu provided tissue specimens. All authors reviewed the manuscript for important intellectual content. All authors read and approved the final manuscript.
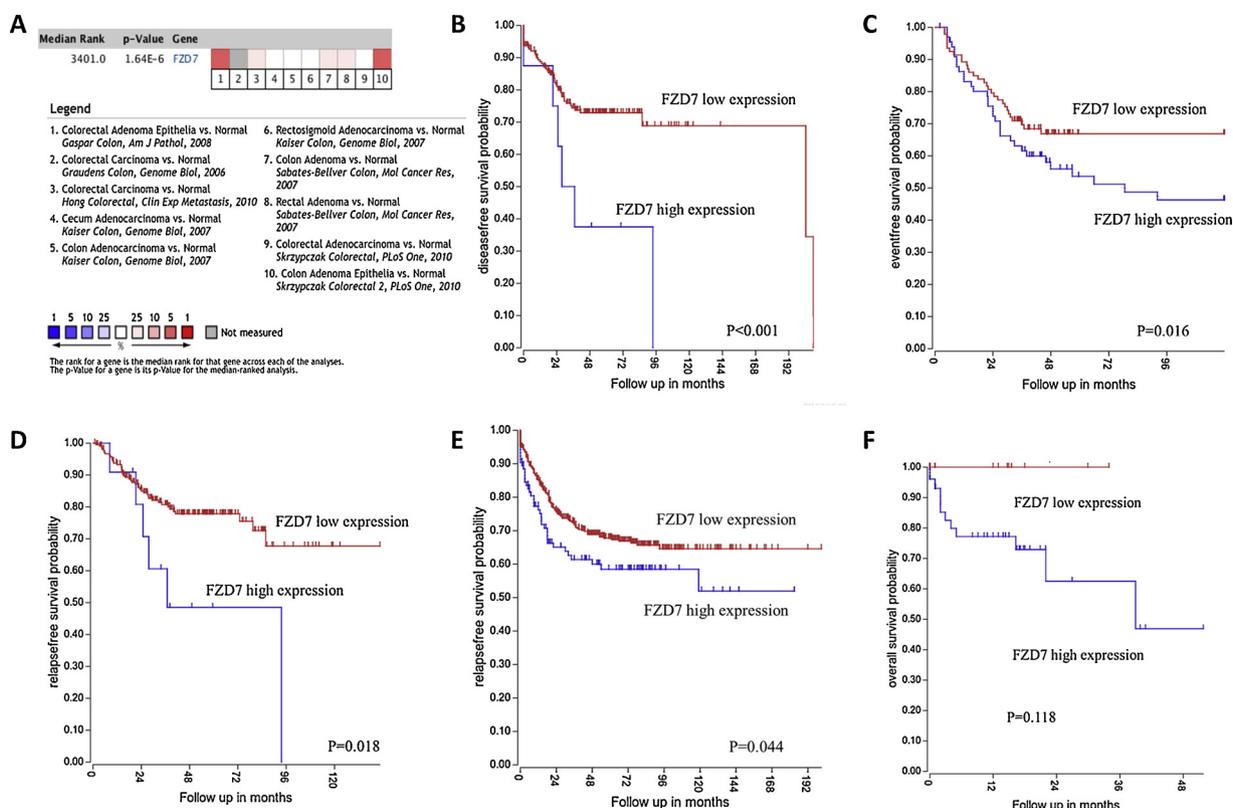
Fig. 4. Validation of FZD7.

(A) Meta-analysis of 10 GEO-sourced data sets mined from the Oncomine database showed that FZD7 mRNA levels were significantly lower in CRC tissues than in normal colon tissues (P < 0.001).

(B–E) High expression of FZD7 was associated with a significantly poorer event and relapse-free survival time. (all P < 0.05).

(F) High expression of FZD7 was associated with a poorer event and relapse-free survival, but without statistical significance(P = 0.118).

Ethics approval and consent to participate

Ethics approval and consent to participate was given by all patients in writing (ethics approval was given by the Institutional Review Board of Fujian Medical University Union Hospital).

Patient consent for publication

Not applicable.

Consent for publication

Written informed consent for publication of their clinical details was obtained from the patients.

Competing interests

The authors declare that they have no competing interests.

Conflicts of interests

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152478.

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