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ABOUT COVER

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AIM AND SCOPE

World Journal of Gastrointestinal Oncology (*World J Gastrointest Oncol*, *WJGO*, online ISSN 1948-5204, DOI: 10.4251) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJGO covers topics concerning carcinogenesis, tumorigenesis, metastasis, diagnosis, prevention, prognosis, clinical manifestations, nutritional support, molecular mechanisms, and therapy of benign and malignant tumors of the digestive tract. The current columns of *WJGO* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of gastrointestinal oncology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJGO*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Gastrointestinal Oncology (*WJGO*) is now indexed in Science Citation Index Expanded (also known as SciSearch®), PubMed, and PubMed Central. The 2018 edition of Journal Citation Reports® cites the 2017 impact factor for WJGO as 3.140 (5-year impact factor: 3.228), ranking WJGO as 39 among 80 journals in gastroenterology and hepatology (quartile in category Q2), and 114 among 222 journals in oncology (quartile in category Q3).

EDITORS FOR THIS ISSUE

Retrospective Study

Prognostic value of vascular endothelial growth factor receptor 1 and class III β-tubulin in survival for non-metastatic rectal cancer

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Abstract

AIM

To assess the long-term prognostic value of vascular endothelial growth factor receptor 1 (VEGFR1) and class III β-tubulin (TUBB3) mRNA expression in non-metastatic rectal cancer.

METHODS

A total of 75 consecutive patients with non-metastatic rectal cancer from March 2004 to November 2008 were analyzed retrospectively at our institute. The mRNA expressions of VEGFR1 and TUBB3 were detected by multiplex branched DNA liquid-chip technology. The Cut-off Finder application was applied to determine cutoff point of mRNA expression. SPSS software version 22.0 was used for analysis.

RESULTS

The median follow-up was 102.7 mo (range, 6-153.6). The χ^2 and Fisher's exact tests showed that VEGFR1 expression was related to lymph node metastasis (P = 0.013), while no relationships between TUBB3 and clinicopathological features were observed. Univariate analysis showed that T stage, lymph node metastasis, tumor differentiation, VEGFR1 and TUBB3 mRNA expression were correlated to overall survival (OS) (P = 0.048, P = 0.003, P = 0.052, P = 0.003 and P = 0.015, respectively). Also, lymph node metastasis and VEGFR1 expression independently influenced OS by multivariate analysis (P = 0.027 and P = 0.033). VEGFR1 expression was positively correlated with TUBB3 (P = 0.024). The patients with low expression of both TUBB3 and VEGFR1 presented a better OS (P = 0.003). In addition, the receiver operating characteristic analysis suggested that the combination of lymph node metastasis and VEGFR1 had a more favorable prognostic value (P < 0.001).

CONCLUSION

VEGFR1 expression and lymph node metastasis independently and jointly affect survival. Moreover, low expression of VEGFR1 and TUBB3 presented a better OS in patients with non-metastatic rectal cancer, which might serve as a potential prognostic factor.

Key words: Rectal cancer; Class III β-tubulin; Vascular endothelial growth factor receptor 1; Overall survival

Core tip: Nowadays, personalized and precision medicine becomes vital in cancer treatment. Herein, we focus on the long-term prognostic value of vascular endothelial growth factor receptor 1 (VEGFR1) and class III β-tubulin (TUBB3) mRNA expression in non-metastatic rectal cancer. In the 75 consecutive patients enrolled, we found that VEGFR1 expression and lymph node metastasis were independent factors influencing overall survival, and the combination of them showed a favorable prognostic value. Also, VEGFR1 expression was significantly related to lymph node metastasis. In addition, VEGFR1 expression was positively correlated with TUBB3 expression.

INTRODUCTION

Rectal cancer is one of the most diagnosed malignancies among both males and females worldwide with worse outcomes than colon cancer[1,2]. Clinically, patients showed various outcomes to multimodality therapies. Nowadays, personalized and precision medicine has become essential in the treatment of rectal cancer. Recent studies conducted gene expression profiling to predict the response and long-term prognosis of malignancies[3,4]; however, no consensus was achieved on prognostic gene profiling for rectal cancer.

Vascular endothelial growth factor (VEGF) possesses a significant role in angiogenesis by binding to VEGFR1 and VEGFR2, which is required for cancer progression and metastasis[5,6]. A phase II trial indicated that VEGF could predict the pathological response to locally advanced rectal cancer patients treated with neoadjuvant cetuximab-based chemoradiation[7]. In addition, class III β-tubulin (TUBB3) has been reported to play a critical role in tumor development and malignant transformation as a β-tubulin isotype. The variable levels of expression of the gene have been reported in colon, lung, ovary, kidney, prostate, and throat cancer with solid tumors[8-10]. However, only a few studies focused on its role in rectal cancer.

Herein, our study attempted to explore the potential prognostic value of VEGFR1 and TUBB3 for long-term survival in non-metastatic rectal cancer.

MATERIALS AND METHODS

Patients

Eighty cases of well-preserved formalin-fixed and paraffin embedded tumor tissue specimens that had undergone total mesorectal excision (TME) at the Fujian Cancer Hospital from March 2004 to November 2008 were retrospectively examined. Among these, two patients with previous malignancy and three with distant metastasis were excluded. Finally, 75 patients who fulfilled the following inclusion criteria were enrolled in the study: (1) Pathologically confirmed as primary rectal adenocarcinoma; (2) underwent TME; (3) no evidence of distant metastasis; (4) no previous or concurrent malignancy; and (5) complete follow-up information was obtained.

The variables such as gender, age, preoperative carcino-embryonic antigen (pre-CEA), pre-operative hemoglobin (pre-Hb), distance to the verge, T stage, lymph node metastasis, venous invasion, and tumor differentiation were considered. The T stage and lymph node metastasis were re-diagnosed based on the 8th Edition of the American Joint Committee on Cancer (AJCC)[11].

Treatments and follow-up

All patients underwent TME, including abdominoperineal resection and low anterior resection. Of these, eight cases received neoadjuvant chemoradiotherapy followed by TME. A total of 66 cases received 5-fluorouracil (5-FU)-based chemotherapy. The overall survival (OS)

Table 1 Patient characteristics	
Characteristics	**Data, *n* (%)**
Gender	
Female	36 (48)
Male	39 (52)
Age (yr)	
median (range)	52 (29-74)
≤ 60	58 (77.3)
> 60	17 (22.7)
Pre-CEA (ng/mL)	
≤ 5	36 (63.2)
> 5	21 (36.8)
Pre-Hb (g/L)	
≤ 120	26 (34.7)
> 120	49 (65.3)
Distance to verge (cm)	
≤ 5	46 (61.3)
> 5	29 (38.7)
T stage	
T1 + T2	13 (17.3)
T3 + T4	63 (82.6)
Lymph node metastasis	
Negative	22 (29.3)
Positive	53 (70.6)
Venous invasion	
Negative	68 (90.7)
Positive	7 (9.3)
Tumor differentiation	
Poorly differentiated	20 (26.7)
Moderately-well differentiated	55 (73.3)
Chemotherapy	
No	9 (12)
Yes	66 (88)
TUBB3 expression	
Low-expression	39 (52)
High-expression	36 (48)
VEGFR1 expression	
Low-expression	53 (70.7)
High-expression	22 (29.3)
TUBB3 and VEGFR1	
Both low expression	32 (42.6)
Others	43 (57.3)

Pre-CEA: Preoperative carcino-embryonic antigen; Pre-Hb: Preoperative hemoglobin.

was defined as the duration from the date of diagnosis to the last follow-up or the date of death due to any cause, which was obtained from the medical records and telephonic interviews.

Multiplex branched DNA liquidchip technology
The formalin-fixed and paraffin embedded (FFPE) tumor tissue specimens containing more than 70% of tumor cells were selected. The Multiplex branched DNA liquidchip (MBL) technology (Guangzhou SurExam Bio-Tech Co., Ltd., China) was implemented to determine the mRNA expression levels of VEGFR1 and TUBB3. The FFPE tissue samples were lysed in the presence of proteinase K, at 56℃ for 2 h. Then, the lysate was transferred to a 96-well plate containing the blocking reagent, capture beads with probes for VEGFR1 and TUBB3, and target gene-specific probe sets. The sandwich nucleic acid hybridization was carried out for 16 h. The unbound RNA was removed by three washes

with buffer under a vacuum system. The signal bound to the target mRNA was amplified with a streptavidin-conjugated phycoerythrin solution at 50℃ for 30 min. The fluorescence values of the samples were identified and analyzed using Luminex 200 system (Luminex, Austin, TX, United States), which were regarded as the RNA expression levels of each gene. The cutoff point of mRNA expression affecting the survival was determined by the Cutoff Finder application[12].

Statistical analysis
The end point of our analysis was OS. The association of gene expression level and clinicopathological features was studied by the χ^2 and Fisher's exact tests. The association between the mRNA expressions of VEGFR1 and TUBB3 was studied by the Spearman correlation test. The Kaplan–Meier test was used to analyze the OS, and Cox regression model (LR forward) was employed for univariate and multivariate analysis. Receiver operating characteristic (ROC) analysis was employed for assessing the specificity as well as the sensitivity of predicting OS by specific parameters. The statistical significance of area under the ROC (area under curve, AUC) was calculated by Delong's test[13]. *P*-values < 0.05 were deemed significant. The statistical analysis was conducted by SPSS version 22.0 (IBM Corporation, Armonk, NY, United States). The statistical methods of our study were reviewed by Qian-yu Ni from The First Affiliated Hospital of Fujian Medical University.

RESULTS
Patient characteristics
A total of 75 patients were enrolled in the present study. The characteristics of non-metastatic patients are summarized in Table 1. Median follow-up time was 102.7 mo (range: 6.0-153.6). The cohort comprised of 39 (52%) male and 36 (48%) female cases with the median age 52 years (range, 29-74). Among these patients, 21 (36.8%) cases presented pre-CEA records that were higher than 5 ng/mL, while they could not be accessed for 18 cases. In the case of pre-Hb, 26 (34.7%) patients were ≤ 120 g/L and the remaining were > 120 g/L. In terms of the tumor location, 46 (61.3%) patients had low rectal cancer (0-5 cm distance to verge), while the other 29 (38.7%) patients were > 5 cm. In all, 22 (29.3%) with lymph node metastasis positive and 53 (70.6%) were negative. Twenty (26.7%) patients were identified as poorly differentiated and 55 (73.3%) as moderate-to-well differentiated. According to the Cutoff Finder software, 0.0575 and 0.2025 were considered as the optimal cutoff point for the VEGFR1 and TUBB3 expression value, respectively (Figure 1). In addition, 36 (48%) and 22 (29.3%) patients showed a high expression of VEGFR1 and TUBB3, respectively.

Associations between mRNA expression and clinicopathological features
The correlations between VEGFR1/TUBB3 mRNA expre-
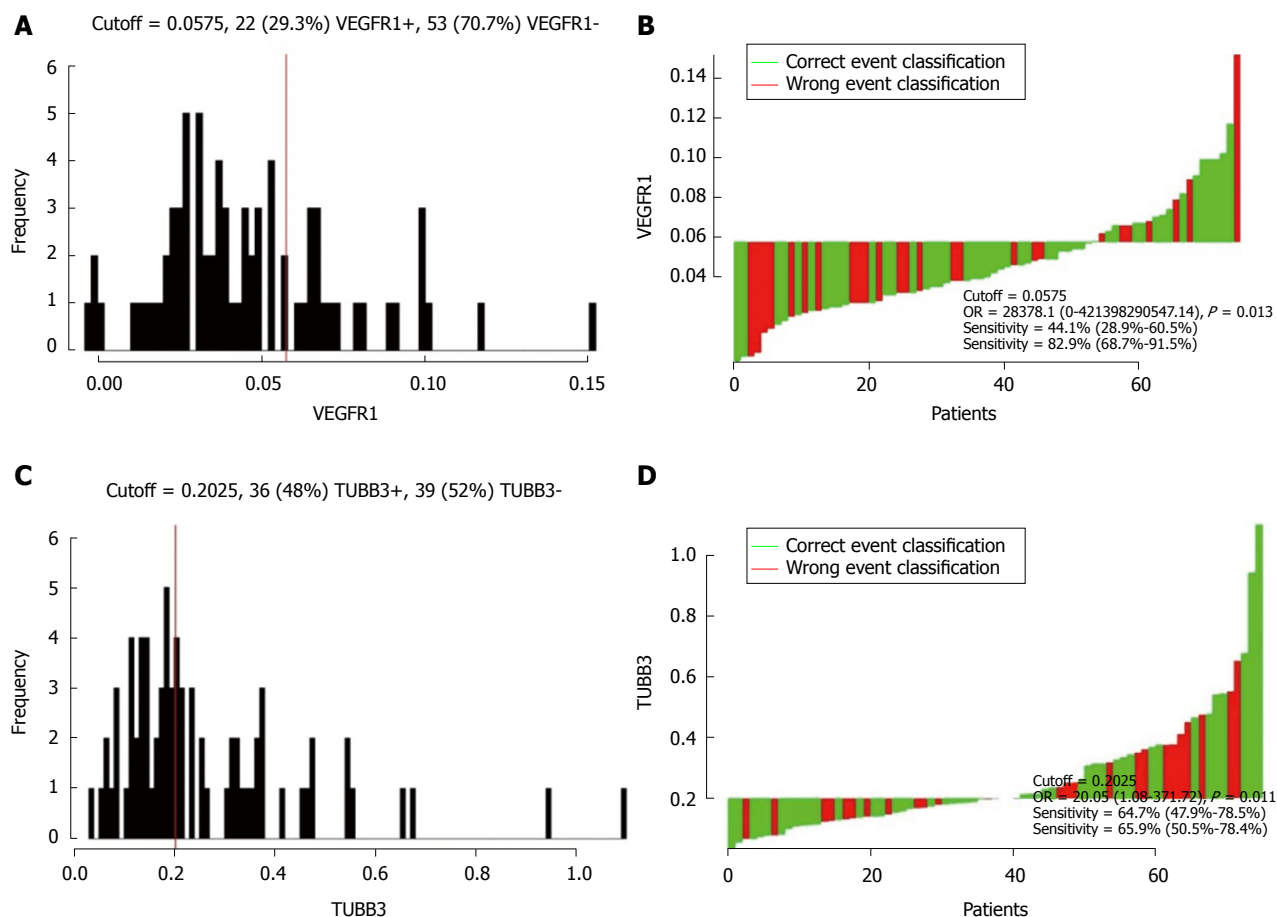
Figure 1 Distribution-based cutoff optimization of vascular endothelial growth factor receptor 1 and class Ⅲ β-tubulin expression value in 75 non-metastatic rectal cancer patients. A: Histograms of vascular endothelial growth factor receptor 1 (VEGFR1) expression value; B: Waterfall plot of optimal dichotomization for VEGFR1 expression value; C: Histograms of class Ⅲ β-tubulin expression value; D: Waterfall plot of optimal dichotomization for VEGFR1 expression value.

ssion and clinicopathological features were analyzed (Table 2). A majority of the patients displayed positive lymph node metastasis in the high-expression group of VEGFR1 ($P = 0.013$). However, no significant difference was found between the expression level of TUBB3 expression and clinicopathological features (gender, age, pre-CEA, pre-Hb, distance to the verge, T stage, lymph node metastasis and venous invasion, all $P > 0.05$).

Impact of VEGFR1 and TUBB3 on OS

The Cox regression analysis of OS influencing factors was shown in Table 3. Univariate analysis showed that T stage, lymph node metastasis, tumor differentiation, and VEGFR1 and TUBB3 expression were significantly related to OS ($P = 0.048$, $P = 0.003$, $P = 0.052$, $P = 0.003$ and $P = 0.015$, respectively) (Figures 2, 3 A and B). Moreover, Kaplan-Meier analysis showed that the rates of 1-, 3-, and 5-year OS in the TUBB3 low- and high-expression groups were 94.9% *vs* 94.4%, 76.9% *vs* 52.8%, and 71.8% *vs* 47.2%, respectively ($P = 0.017$). The rates of OS in the VEGFR1 low- and high-expression groups were 98.1% *vs* 86.4%, 77.4% *vs* 36.4%, and 69.8% *vs* 36.4%, respectively ($P = 0.003$).

Moreover, lymph node metastasis (HR = 3.042, 95%CI: 1.137-8.142, $P = 0.027$) and VEGFR1 (HR = 2.151, 95%CI: 1.062-4.355, $P = 0.033$) were independent factors influencing OS, as evaluated by the multivariate Cox regression model.

Prognostic value of different combinations on survival

VEGFR1 and TUBB3 expression were positively correlated ($P = 0.006$, $r = 0.315$) by the Spearman's correlation test. Both low expression of VEGFR1 and TUBB3 were observed in 32 (42.6%) cases. Moreover, the Kaplan-Meier analysis showed that the 1-, 3-, and 5-year OS of both low-expression patients *vs* others were 96.9% *vs* 93.0%, 84.4% *vs* 53.5%, and 78.1% *vs* 46.5%, respectively ($P = 0.003$, Figure 3C). Meanwhile, Kaplan-Meier analysis showed that the rates of 1-, 3-, and 5-year OS in positive lymph node metastasis patients with high expression of VEGFR1 *vs* others were 90.0% *vs* 98.2%, 35.0% *vs* 78.2%, and 30.0% *vs* 70.9%, respectively ($P < 0.001$) (Figure 3D).

Finally, we combined the two independent prognostic factors, lymph node metastasis and VEGFR1 expression, to construct a prognostic model and supplemented the VEGFR1 expression to the lymph node metastasis

Table 2 Correlation between vascular endothelial growth factor receptor 1 and class III β-tubulin expression with clinicopathological features

Parameter	TUBB3			VEGFR1		
	Low (*n*)	High (*n*)	*P*	Low (*n*)	High (*n*)	*P*
Gender			0.426			0.081
Female	17	19		22	14	
Male	22	17		31	8	
Age (yr)			0.31			1
≤ 60	32	26		41	17	
> 60	7	10		12	5	
Pre-CEA			0.203			0.244
≤ 5	20	16		26	10	
> 5	8	13		12	9	
Pre-Hb			0.801			0.206
≤ 120	13	13		16	10	
> 120	26	23		37	12	
Distance to verge (cm)			0.608			0.792
≤ 5	25	21		32	14	
> 5	14	15		21	8	
T stage			0.883			0.744
T1 + T2	7	6		10	3	
T3 + T4	32	30		43	19	
Lymph node metastasis			0.071			0.013
Negative	15	7		20	2	
Positive	24	29		33	20	
Tumor thrombus			0.25			1
Negative	37	31		48	20	
Positive	2	5		5	2	
Tumor differentiation			0.754			0.939
Poorly	11	9		14	6	
Moderately-well	28	27		39	16	
Chemotherapy			0.156			0.051
No	7	2		9	0	
Yes	32	34		44	22	
VEGFR1			0.024			
Low	32	21				
High	7	15				

Pre-CEA: Preoperative carcino-embryonic antigen; Pre-Hb: Preoperative-hemoglobin.

by ROC analysis to assess the improvement of the model for OS. The lymph node metastasis (AUC: 0.688, 95%CI: 0.567–0.808, $P = 0.005$) showed a better prognostic value than VEGFR1 expression (AUC: 0.635, 95%CI: 0.507–0.764, $P = 0.045$). Furthermore, a better prognostic value was shown when combining the lymph node metastasis and VEGFR1 expression (AUC: 0.748, 95%CI: 0.637–0.859, $P < 0.001$) (Figure 4).

DISCUSSION

Firstly, we evaluated the long-term prognostic value of VEGFR1 and TUBB3 expression after the diagnosis of non-metastatic rectal cancer with a median follow-up of 102 mo. Here, we found that VEGFR1 and TUBB3 expression affected OS in non-metastatic rectal cancer by univariate analysis. Moreover, a favorable OS in both low expression of VEGFR1 and TUBB3 was noted as compared to others. In addition, the association between VEGFR1 expression and lymph node metastasis was also assessed. The combination of lymph node metastasis and VEGFR1 expression might also provide a promising tool for the prognosis of non-metastatic rectal cancer.

Reportedly, VEGFR correlates with poor prognosis, metastasis, and recurrence in various tumor types, including breast and lung cancers[14,15]. Moreover, previous studies demonstrated that VEGF plays a crucial role as a potent angiogenic factor in both experimental and human studies with respect to colorectal cancer progression and metastasis[16-18]. The co-expression of VEGF and VEGFR1/2 in the nucleus stimulates the proliferation and migration of endothelial cells, thereby providing nutrition for growing tumors and establishing a continuity between tumor cells and host vasculature[19].

VEGFR1 is primarily localized in the nucleus of endothelial cells; As the predominant receptor of the tumor microenvironment, it is essential for the survival of endothelial cells[20]. Tsai *et al*[21] reported that the overexpression of VEGF is a significant positive predictor for early postoperative relapse in stage Ⅰ-Ⅲ colorectal cancer patients, leading to poor OS ($P = 0.002$). Similarly, Nriagu *et al*[22] reported that the overexpression of VEGF mRNA was an independent factor affecting OS as assessed by multivariate analysis (HR = 1.94, $P = 0.005$). Herein, we found that the low expression of VEGFR1 might positively affect OS with a 5-year OS of 69.8% for low

Table 3 Cox regression analysis for overall survival

Variables	Univariate				Multivariate		
	HR	95%CI	*P*		HR	95%CI	*P*
Gender							
Female/male	1.018	0.519-1.997	0.958				
Age							
≤ 60/> 60	1.175	0.548-2.518	0.679				
Pre-CEA							
≤ 5/> 5	1.067	0.496-2.298	0.868				
Pre-Hb							
≤ 120/> 20	0.651	0.328-1.290	0.219				
Distance to verge (cm)							
≤ 5/> 5	1.265	0.642-2.491	0.497				
T stage							
T1 + T2/T3 + T4	4.221	1.011-17.632	0.048		4.05	0.968-116.93	0.055
Lymph node metastasis							
Negative/positive	6.247	1.905-20.491	0.003		3.042	1.137-8.142	0.027
Tumor thrombus							
Negative/positive	1.303	0.458-3.705	0.62				
Tumor differentiation							
Poorly/moderately-well	0.503	0.251-1.006	0.052		-	-	0.18
Chemotherapy							
No/yes	1.407	0.430-4.605	0.572				
TUBB3 expression							
Low/high	2.407	1.188-4.877	0.015		-	-	0.1
VEGFR1 expression							
Low/high	2.817	1.424-5.570	0.003		2.151	1.062-4.355	0.033

Pre-CEA: Preoperative carcino-embryonic antigen; Pre-Hb: Preoperative hemoglobin.
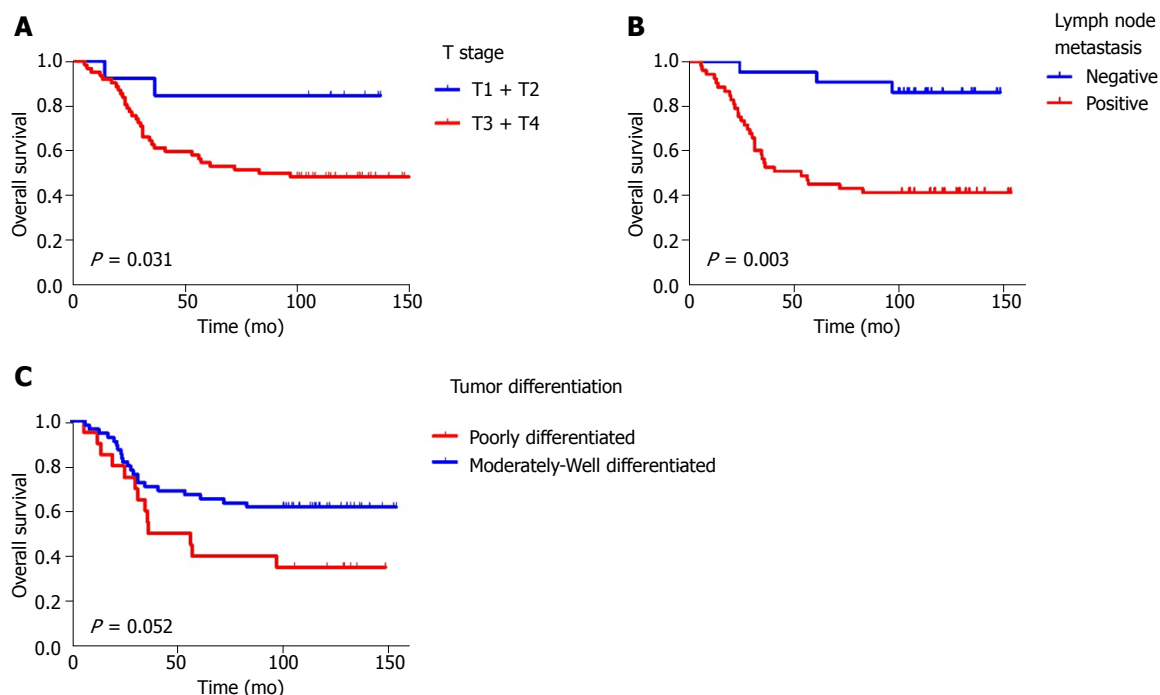
Figure 2 Kaplan-Meier survival curves of overall survival. A: T stage (T1 + T2 *vs* T3 + T4, *P* = 0.031); B: Lymph node metastasis (negative *vs* positive, *P* = 0.003); C: Tumor differentiation (poorly differentiated *vs* moderately-well differentiated, *P* = 0.052).

vs 36.4% for the high-expression group (HR = 2.151, *P* = 0.033). These results indicated that VEGFR1 functions as a positive regulator of angiogenesis[23], which might lead to poor survival in cancer patients.

A previous study evaluated VEGF expression in 117 colorectal adenocarcinoma patients, and confirmed that lymph node metastasis (positive *vs* negative, *P* < 0.001) and TNM stage (stage Ⅲ *vs* Ⅰ/Ⅱ, *P* < 0.001) were related to increased VEGF expression. Moreover, the mean number of metastatic nodes was significantly associated with VEGF expression (1.06 ± 2.84 for low expression *vs* 2.45 ± 4.03 for high expression, *P* =
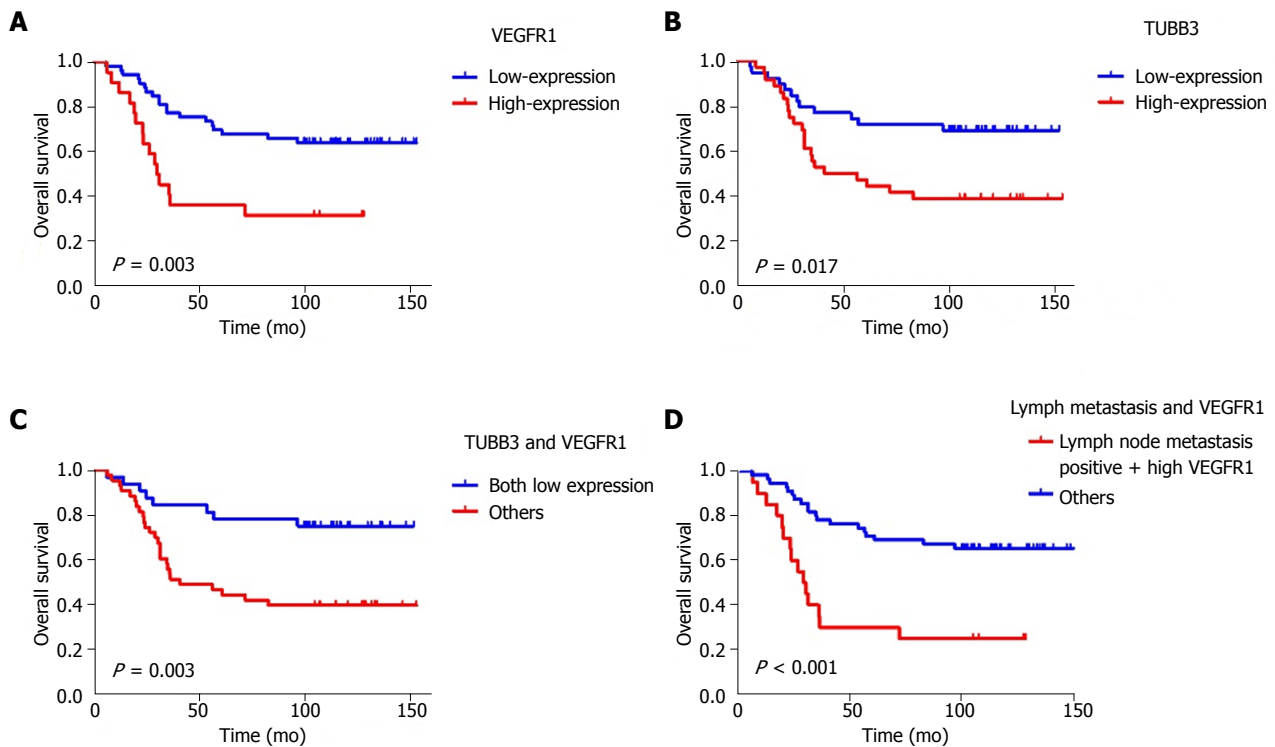
Figure 3 Kaplan-Meier survival curves of Overall Survival. A: Vascular endothelial growth factor receptor 1 (VEGFR1) expression (low *vs* high, *P* = 0.003); B: Class Ⅲ β-tubulin (TUBB3) expression (low *vs* high, *P* = 0.017); C: TUBB3 and VEGFR1 (both low expression *vs* others, *P* = 0.003); D: TNM stage and VEGFR1 (stage Ⅲ + high VEGFR1 expression vs others, *P* < 0.001).

0.031)[24]. Similarly, our study implied that VEGFR1 expression was related to lymph node metastases (*P* = 0.013). However, whether the function of VEGF/VEGFR1 affects lymph node metastasis is yet unclear. Nagy *et al*[25] hypothesized that tumor cells in the circulation directly reached the regional lymph nodes through the supply vessels or blood vessel-lymph vessel junctions.

A retrospective study reported that VEGF expression could identify an unfavorable subgroup of patients with stage Ⅱ colon cancer for optimal treatment strategy (the recurrence rate was 50% for VEGF-positive *vs* 11.7% for VEGF-negative, *P* = 0.001)[26]. As shown by ROC curves in our analysis, though low sensitivity of VEGFR1 (44.1%), the specificity was high with 82.9%, which exerted a similar effect on prognosis as lymph node metastasis. Moreover, the sensitivity increased when combined with lymph node status, and a superior prognostic value was noted for the combination. Further identification of a group of lymph node metastasis-positive with high VEGFR1 expression allows for selective treatment with adjuvant chemotherapy using antiangiogenic therapy, including VEGFR1 antisense and monoclonal antibodies, as well as postoperative follow-up.

Several clinical studies demonstrated that the increased expression of TUBB3 in various human malignancies was related to low response rate and poor survival in patients treated with taxane-based chemotherapies[27-30]. However, studies focusing on the relationship between TUBB3 and non-metastatic rectal cancer are limited. The current study showed that the low expression of TUBB3 had better OS in non-metastatic rectal cancer patients as assessed by univariate analysis (5-year OS, 71.8% *vs* 47.2%), although no significant difference was observed by multivariate analysis.

Furthermore, Makarchenko *et al*[31] and Widow *et al*[32] reported that VEGFR1 regulated the chemo-resistant genes such as TUBB3, which might result in the poor prognosis of lung and gastroesophageal cancers. The current study established a positive correlation between VEGFR1 and TUBB3 (r = 0.315, *P* = 0.006), and a favorable OS was observed in both low expression groups (*P* = 0.003). Paradiso *et al*[33] had investigated the combination of TUBB3 and VEGFR1 in advanced breast cancer. Hypoxia in the tumor microenvironment promotes angiogenesis, and VEGFR1 is known to be related to angiogenesis[23]. TUBB3 was found to be involved in an adaptive response to low oxygen levels and poor nutrient supply in solid tumors[34,35]. Therefore, we speculate that the underlying mechanism of the two correlations might be related to anoxic environments.

Notably, this study was limited to a small-sample retrospective analysis. Thus, additional mRNA expression data might help to establish a superior predictor. Finally, prospective data and large sample size are essential for further substantiation of the results.

We confirmed that the increased expression of VEGFR1 and TUBB3 might be negatively correlated with long-term prognosis of non-metastatic rectal cancer.

ROC curves for OS of non-metastatic rectal cancer
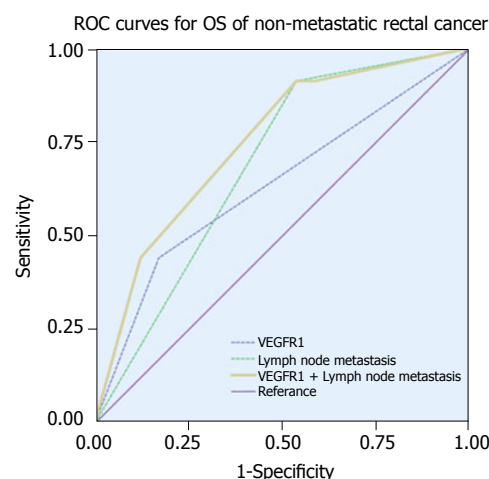
	AUC	95%CI	P
VEGFR1	0.635	0.507-0.764	0.045
Lymph node metastasis	0.688	0.567-0.808	0.005
VEGFR1 + lymph node metastasis	0.748	0.637-0.859	< 0.001

Figure 4 Receiver operating characteristic analyses in non-metastatic rectal cancer patients. *P*-values show the area under the receiver operating characteristic (ROC) curves in the three models. ROC analyses of the prediction of overall survival by vascular endothelial growth factor receptor 1 (VEGFR1) expression model, lymph node metastasis, and the combined VEGFR1 expression-lymph node metastasis model.

Furthermore, VEGFR1 expression and lymph node metastasis affected the survival independently as well as synergistically. These results might provide additional prognostic information compared to the conventional tumor histopathological factors.

ARTICLE HIGHLIGHTS

Research background
Rectal cancer is one of the most common form of cancer in both men and women. Gene expression profiling for predicting the response and long-term prognosis of malignancies has been reported in recent decades. Vascular endothelial growth factor (VEGF) and class Ⅲ β-tubulin (TUBB3) have been reported to play a vital role in cancer progression. However, few studies focused on their role in rectal cancer.

Research motivation
We try to explore the potential prognostic value of VEGFR1 and TUBB3 for long-term survival in non-metastatic rectal cancer.

Research objectives
A total of 75 patients diagnosed with primary rectal adenocarcinoma without metastases were retrospectively analyzed.

Research methods
Multiplex branched DNA liquidchip technology was applied to detected mRNA expressions of VEGFR1 and TUBB3. The cutoff point of mRNA expression was determined by Cutoff Founder.

Research results
VEGFR1 expression was positively correlated to TUBB3. Patients with both low expression of TUBB3 and VEGFR1 presented a better overall survival (OS). In addition, VEGFR1 and lymph node metastasis had potential as prognostic factors for OS in non-metastatic rectal cancer patients, and the combination of

them showed a favorable prognostic value.

Research conclusions
We confirmed that the increased expression of VEGFR1 and TUBB3 might be negatively correlated with long-term prognosis of non-metastatic rectal cancer. Furthermore, VEGFR1 expression and lymph node metastasis affected the survival independently, as well as synergistically. These results might provide additional prognostic information compared to the conventional tumor histopathological factors.

Research perspectives
VEGFR1 has the potential to contribute to decision making regarding individual treatment in rectal cancer. A larger sample size and additional mRNA expression data are warranted to establish a superior prognosis model.

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