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Original Article

Correlation of Th17/Treg associated transcription factors with clinicopathological features of colorectal cancer and their prognostic significance

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Abstract: Objective: To analyze the correlation of Th17/Treg associated transcription factors (TFs) with clinicopathological features of colorectal cancer (CRC) and their prognostic significance. Methods: This research enrolled 56 CRC patients (experimental group, EG) and 50 healthy controls (control group, CG), who presented to Deqing People's Hospital between June 2017 and January 2019. The levels of Th17, Treg and their TFs [forkhead box protein P3 (Foxp3), retinoid acid receptor-related orphan receptor gamma t (RORγt)] and secreted inflammatory factors (IFs) [interleukin-17 (IL-17), interleukin-22 (IL-22)] were detected in the peripheral blood (PB) of both groups, and the TFs' phosphorylated protein expression was observed by Western blot. Further, the correlation of TFs with patients' pathological features was analyzed. Finally, a 3-year prognostic follow-up was performed on CRC patients. Receiver operating characteristic (ROC) determined the predictive value of Th17/Treg on the prognostic mortality of patients. Results: Peripheral blood Th17 and Treg showed higher levels in the EG than in the CG, demonstrating excellent diagnostic effects on CRC ($P<0.05$). The EG also exhibited reduced Foxp3 and p-Foxp3 protein expression, and elevated RORγt and p-RORγt levels compared with the CG (all $P<0.0001$). In addition, the EG exhibited statistically higher IL-17 and IL-22 levels than the CG (all $P<0.05$). Further, the analysis of pathological features revealed close correlations of Th17/Treg, RORγt and Foxp3 with tumor size, TNM staging, degree of differentiation, and lymph node metastasis (LNM) of CRC patients (all $P<0.001$). Finally, the prognostic follow-up results identified that TNM staging, degree of differentiation, LNM, RORγt, Th17 and Treg were independent risk factors for prognostic mortality of CRC patients, while Foxp3 was an independent protective factor (all $P<0.001$). Conclusion: Th17/Treg associated TFs are of great significance for the prognosis evaluation of CRC, the imbalance of which can cause aggravation of the inflammatory reaction and promote malignancy of CRC.

Keywords: Th17/Treg associated transcription factors, colorectal cancer, pathological features, prognosis

Introduction

Colorectal cancer (CRC) is the third most prevalent malignancy in the world, following breast cancer and lung cancer. According to the statistical survey of the World Health Organization, there were about 1.8 million new CRC cases worldwide in 2018, with 880,000 deaths [1, 2]. The morbidity and mortality of CRC are steadily increasing each year, with a notable rise among individuals under 40, indicating a shift towards a younger populations [3]. Currently, CRC treatment primarily involves surgery or (and) chemoradiotherapy. However, the treatment effect is far from satisfactory, these treatments often yield unsatisfactory results due to the typically advanced stage of CRC at diagnosis, resulting in high mortality rates [4]. Therefore, enhancing early diagnosis and developing more effective treatment strategies are critical goals in CRC research [5]. There is a clinical consensus that a thorough understanding of the pathogenesis of CRC and exploring its pathological change from a molecular perspective could significantly advance diagnosis and treatment [6, 7]. Timely and effective evaluation of the pathological development of CRC is crucial for improving the treatment efficiency [8]. Although many studies have proposed new directions for the future diagnosis and treatment of CRC, such as the

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detection and targeted therapy of small-molecule RNAs and mesenchymal stem cells-derived exosome drug delivery [9, 10], these methods face numerous challenges before they can be clinically applied, leaving current limitations in CRC diagnosis and treatment unresolved. Th17/Treg cells, as important cells regulating inflammatory and immune responses in the human body, play an extremely important role in many diseases [11, 12]. For CRC, the potential significance of Th17/Treg may also provide a novel research direction for future diagnostic and therapeutic strategies. Hence, this study holds significant clinical implications.

Pro-inflammatory T helper type 17 (Th17) cells and regulatory T cells (Treg) paly crucial roles in regulating inflammatory and immune responses of the human body. The Th17/Treg equilibrium not only indicates the normal functioning of human immune system and the inhibition of inflammatory response, but also reflects the stable state of organ function to a certain extent [13, 14]. Current evidence indicates that Th17/Treg can activate the release of inflammatory factors (IFs) in the human body through their specific transcription factors (TFs), a pathological process that is of great significance for various tumors such as lung carcinoma, endometrial cancer and osteosarcoma [15-17]. Th17 is a CD4+ T cell subset distinct from Th1 and Th2 subsets, the biological function of which is related to the expression of cytokines such as IL-17 (also known as IL-17A), IL-17F, IL-21, and IL-22 [18]. The differentiation of Th17 cells from naïve CD4+ T cells is regulated by cytokines (including IL-6 and TGF-β), signal transducers (including STAT-3 and Smad), and transcription factors retinoic acid receptor-related orphan receptors (including RORγt and RORα) [19, 20]. Treg is another functionally and structurally distinct subset of CD4+ T cells, which expresses the specific transcription factor Foxp3 [21]. Th17 and Treg have opposing functions, with Th17 representing a pro-inflammatory subset, while Treg has an antagonistic effect; however, their developmental pathways are reciprocally interconnected [22]. Furthermore, Velikova et al. reported obvious imbalance of Th17/Treg in CRC, and upregulation of Th17/Treg-related genes [transcription factor forkhead box protein P3 (Foxp3), interleukin-10 (IL-10) and interleukin-23 (IL-23)] as crucial for CRC development [23]. However, an in-depth analysis of this relationship is still lacking.

To address this gap, we enrolled CRC patients to examine the levels of Th17 and Treg in peripheral blood mononuclear cells (PBMCs) by flow cytometry. We believe that Th17/Treg associated TFs may also be of great significance to the progression assessment of CRC, which may provide new directions and ideas for future diagnosis and treatment of CRC. Consequently, this study conducted a preliminary analysis of the relationship between Th17/Treg cell TFs and CRC, aiming at providing new references for CRC management.

Materials and methods

Patient data

In this retrospective study, the blood sample of 56 CRC patients (experimental group, EG) and 50 healthy controls (control group, CG) who presented to Deqing People's Hospital between June 2017 and January 2019 were collected. The study was approved by Ethics Committee of Deqing People's Hospital, Huzhou, China. Each specimen was collected after the patient's permission, and all patients signed an informed consent release.

Inclusion criteria

Experimental Group (EG): 1) Adults (age >18 years old); 2) Patients who underwent open or laparoscopic radical colorectal resection, following surgical principles such as total rectal mesenteric resection [24] or total colonic mesenteric resection [25]; 3) A diagnosis of CRC by pathological biopsy; 4) Adenocarcinoma indicated by histopathological examination; 5) Long-term regular outpatient and telephone follow-up visits; 6) Complete clinical data and follow up data. Control Group (CG): 1) Adults (age >18 years old); 2) Normal physical examination results; 3) No major medical history.

Exclusion criteria

1) Patients whose post-operative pathology did not confirm CRC; 2) Absence of tumor tissue in the pathology specimen; 3) Incomplete clinical data and follow up data; 4) Presence of other cardio-cerebrovascular diseases, autoimmune defects, or mental illness; 5) Deaths due to serious surgical complications within a short period of time after the operation; 6) Pregnant and lactating patients. The flow diagram detail-

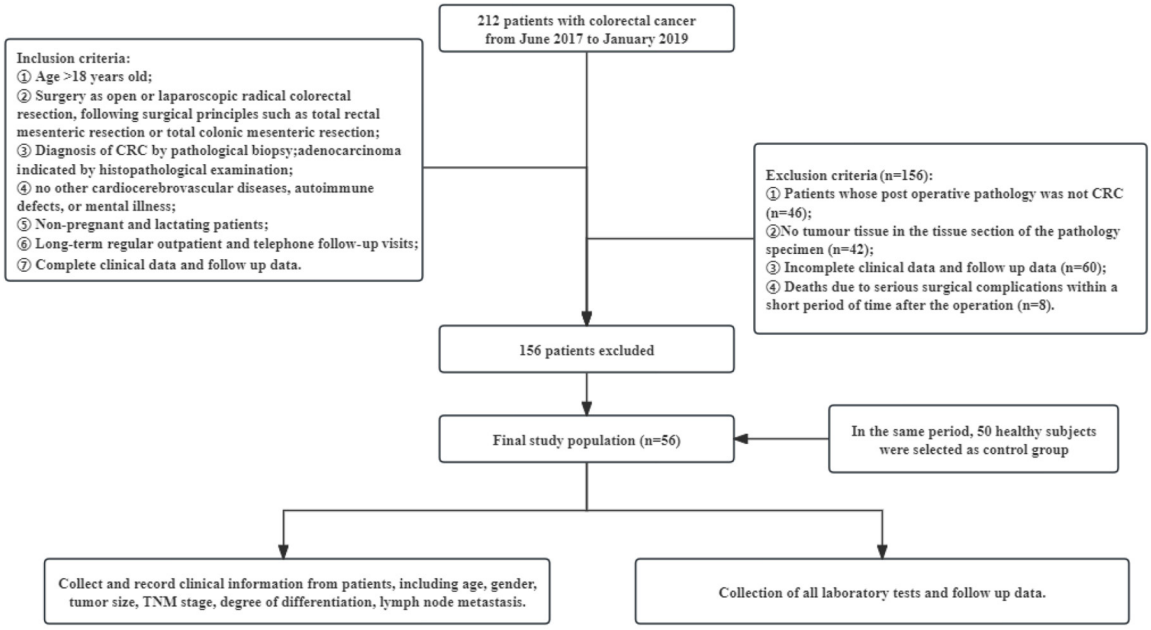

Figure 1. Flow diagram detailing the selection of patients included in this study.

Table 1. Primer sequences for reverse transcription-quantitative PCR

	Primer sequence (5'-3')
RORγt	Forward: ACAGAGACACCACCGAACATC
	Reverse: ATGCCAGATGACTTGTCCCC
Foxp3	Forward: AGAAGCAGCGGACACTCAA
	Reverse: CACTTGTGCAGACTCAGGTTGT
β-actin	Forward: GCCACTGCCGCATCCTCTTC
	Reverse: AGCCTCAGGGCATCGGAACC

ing the selection of patients included in this study is shown in **Figure 1**.

Data collection

Clinical information collected from patients included age, gender, tumor size, TNM stage, degree of differentiation, lymph node metastasis. All laboratory tests results were also collected.

Methods

Fasting venous blood was sampled in the early morning and was divided into two portions, for determination of peripheral blood (PB) Th17/Treg cell ratio and quantification of Th17/Treg Associated TFs and IFs. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples (10-15 mL) by adding 1.077 g/ml Ficoll (Lymphosep; Biosera, East Sussex, UK) and centrifuging at 450 g for 25 min followed by washing twice with RPMI-1640 medium (Sigma, Germany). Th17 and Treg cells were detected using flow cytometry. The expression of Th17/Treg associated TFs, retinoid acid receptor-related orphan receptor gamma t (RORγt), forkhead box protein P3 (Foxp3), was measured by quantitative real-time reverse-transcription PCR (qRT-PCR). Downstream IFs interleukin-17 (IL-17) and interleukin-22 (IL-22) levels were evaluated by enzyme linked immunosorbent assay (ELISA) after serum collection via routine centrifugation. The operation process strictly followed the instructions of kits all supplied by Beijing Solarbio Science & Technology.

Real-time PCR

Total RNA was extracted from PBMCs by using the Trizol reagent (Invitrogen, USA) according to the manufacturer's guidelines. The reverse transcription of total RNA for cDNA synthesis was performed with a cDNA Reverse Transcription Kit (Applied Biosystems, USA). The qRT-PCR was performed on an ABI 7900HT system (Applied Biosystems, USA) and SYBR Green Master Mix (Applied Biosystems, USA). Primers were synthesized by Sangon Biotech (Shanghai, China). β-actin was used as an internal control. The primer sequences are shown in **Table 1**.

Table 2. Comparison of baseline data between the two groups

	Control group (n=50)	Experimental group (n=56)	t/χ^2	P
Age	62.40±6.66	61.16±6.74	0.951	0.344
Gender (Male/Female)	20/30	24/32	0.089	0.766
Smoking (Yes/No)	21/29	19/37	0.732	0.392
Drinking (Yes/No)	17/33	16/40	0.363	0.547
Family history of CC (Yes/No)	3/47	5/51	0.325	0.569
Previous intestinal inflammation (Yes/No)	11/39	14/42	0.132	0.717
Degree of education			0.352	0.553
Primary and junior high schools	16	21		
High school and above	34	35		

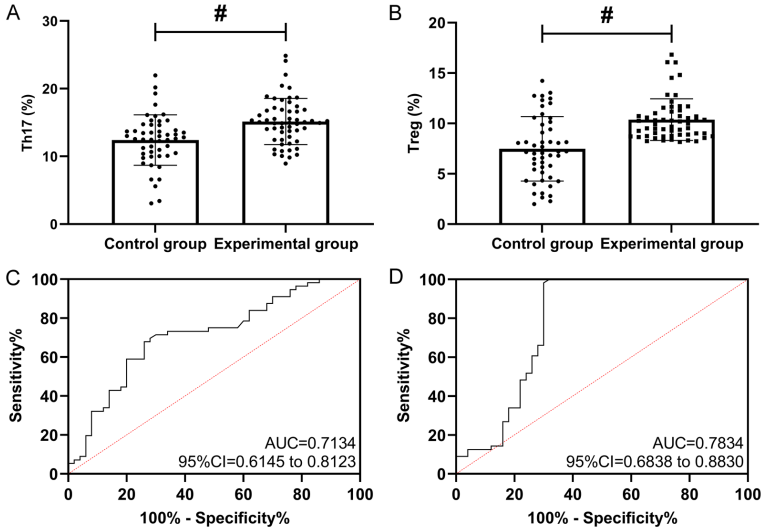

Figure 2. Comparison of Th17/Treg cell levels. A. Comparison of Th17 contents between the experimental group and the control group. B. Comparison of Treg contents between the experimental group and the control group. C. ROC curve of Th17 in diagnosing CRC. D. ROC curve of Treg in diagnosing CRC. #*P*<0.05. CRC, colorectal cancer.

Western blot

Western blots were performed to measure RORγt, Foxp3 and other phosphorylated proteins in serum to verify the expression of Th17/Treg associated TFs. Total proteins, extracted by adding protein lysate into the serum, were transferred to a polyvinylidene fluoride (PVDF) membrane after verifying the purity. After blocking with 4% skim milk, the primary antibody protein was added to incubate overnight at 4°C. The primary antibodies were as follows: RORγt (ab207082, 1:2000, Abcam), Foxp3 (ab20034, 1:1000, Abcam), β-actin (ab8226, 1 μg/mL, Abcam). After being rinsed with tris-buffered saline with 0.1% tween ® 20 detergent (TBST) on the second day, the second antibody (1:2000) was added, and the bands' gray values were analyzed by Image J after protein development.

Prognostic follow-up

A 3-year follow-up was performed on all CRC patients in the form of regular hospital reexamination or telephone enquiries, with no more than 3 months between follow-up visits. Patients' prognosis and survival were recorded, and the survival curve was drawn.

Endpoints

Primary outcomes: (1) Correlations of Th17/Treg cells and their TFs with the pathological features of CRC were discussed; (2) Related factors influencing CRC patients' outcomes and survival were analyzed.

Secondary outcomes: Differences in Th17/Treg cells and levels of their TFs between EG and CG.

Statistics and methods

SPSS 22.0 was used for statistical processing of the data collected. Quantitative data were denoted by ($\bar{x}\pm s$), and the inter- and multi-group comparisons were performed by the independent samples *t* test and the one-way ANOVA plus LSD post-hoc testing, respectively. Categorical data, denoted by (%), were ana-

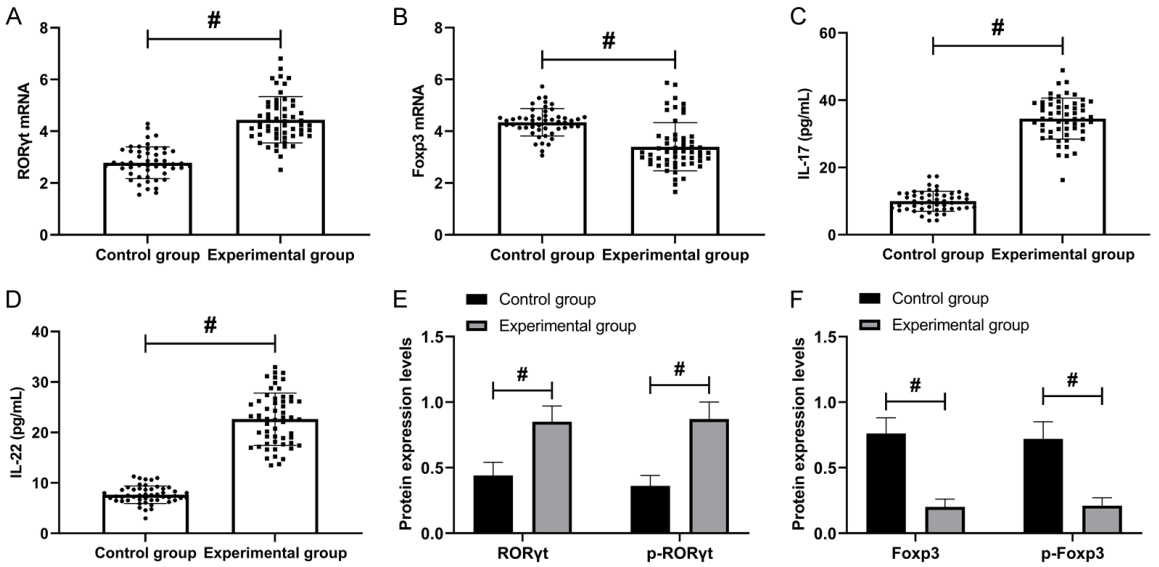

Figure 3. Comparison of Th17/Treg associated TFs. A. Comparison of RORγt mRNA level between the experimental group and the control group. B. Comparison of Foxp3 mRNA level between the experimental group and the control group. C. Comparison of IL-17 level between the experimental group and the control group. D. Comparison of IL-22 level between the experimental group and the control group. E. The protein expression levels of RORγt and p-RORγt. F. The protein expression levels of Foxp3 and p-Fox. $^{\#}P<0.05$.

lyzed between groups using the Chi-square test. Receiver operating characteristic (ROC) curve analysis was employed to identify the diagnostic value of relevant factors. The calculation and comparison of survival rate employed the Kaplan-Meier method and the Log-rank test, respectively. A *p*-value of <0.05 was chosen as the threshold of statistical significance.

Results

Baseline data comparison

A comparison of baseline data (age, gender, etc.) between the EG and the CG is presented in **Table 2**. No significant differences were found between the two groups in age (P=0.344), gender (P=0.766), smoking history (P=0.392), drinking history (P=0.547), family history of CC (P=0.567) and previous intestinal inflammation (0.717), confirming that the two groups were comparable.

Comparison of Th17/Treg cell levels

The Th17 cell level in the EG was (15.15±3.41)%, which was statistically higher than that of the CG (*P*<0.0001). The Treg level in the EG was (10.37±2.07)%, also higher compared with the CG (*P*<0.0001). According to ROC analysis, when blood Th17>13.95%, the sensitivity and specificity for diagnosing CRC were 67.86% and 74.00%, respectively. However, the sensitivity of Treg for diagnosing CRC was 98.21% and the specificity was 70.00% when blood Treg>8.19%. In addition, the AUC of Th17 in diagnosing CRC was 0.7134 while the AUC of Teg in diagnosing CRC was 0.7834 (**Figure 2**).

Comparison of Th17/Treg associated TFs

Subsequently, the detection of Th17/Treg associated TFs showed that RORγt mRNA in the EG was significantly higher than that in the CG, while Foxp3 mRNA was markedly lower (*P*<0.001). In addition, the EG exhibited higher IL-17 and IL-22 levels than the CG (*P*<0.001). Finally, the Western blot results also determined higher RORγt and p-RORγ protein expression while lower Foxp3 and p-Foxp3 in the EG compared with the CG (*P*<0.001), which was consistent with the above results (**Figure 3**).

Correlations of Th17/Treg associated TFs with pathological characteristics of CRC

The analysis showed that Th17, Treg, RORγt and Foxp3 levels did not show significant differences in CRC patients across different ages (P=0.745, P=0.707, P=0.563, P=0.282), gender (P=0.772, P=0.832, P=0.763, P=0.577),

Table 3. Correlation of Th17/Treg with pathological features of CRC

	n	Th17	*t*/*P*	Treg	*t*/*P*
Age			0.326/0.745		0.378/0.707
<61	27	14.99±3.05		10.26±1.99	
≥61	29	15.29±3.76		10.47±2.16	
Gender			0.291/0.772		0.213/0.832
Male	24	14.99±3.15		10.44±2.11	
Female	32	15.26±3.64		10.32±2.07	
Smoking			0.465/0.644		0.700/0.487
Yes	19	15.44±3.77		10.64±1.87	
No	37	14.99±3.25		10.23±2.17	
Drinking			0.661/0.512		0.275/0.784
Yes	16	15.62±3.03		10.25±1.66	
No	40	14.95±3.57		10.42±2.23	
Family history of CC			0.347/0.730		0.462/0.646
Yes	5	15.66±2.74		9.96±1.82	
No	51	15.10±3.49		10.41±2.10	
Previous intestinal inflammation			0.443/0.660		0.359/0.721
Yes	14	15.50±3.37		10.54±1.80	
No	42	15.03±3.46		10.31±2.16	
Tumor size (cm^2)			2.839/0.006		3.315/0.002
<5	41	14.18±3.27		9.86±1.20	
≥5	15	16.97±3.22		11.76±3.13	
TNM staging			4.492/0.001		4.986/0.001
I-II	41	14.08±2.76		9.67±1.09	
III-IV	15	18.06±3.39		12.27±2.85	
Degree of differentiation			3.586/0.001		2.266/0.028
Medium to high differentiation	44	14.37±3.06		10.05±1.87	
Low differentiation	12	17.98±3.21		11.52±2.41	
LNM			3.766/0.0004		3.592/0.001
No	44	14.34±2.95		9.90±1.47	
Yes	12	18.10±3.48		12.09±2.96	

TNM, Tumor-node-metastasis; LNM, lymph node metastasis; CRC, colorectal cancer.

smoking history (P=0.644, P=0.487, P=0.506, P=0.822), drinking history (P=0.512, P=0.784, P=0.389, P=0.802). However, they exhibited statistical difference in patients with different tumor size (P=0.006, P=0.002, P=0.001, P=0.001), TNM staging (P=0.001, P=0.001, P=0.001, P=0.001), degree of differentiation (P=0.001, P=0.028, P=0.001, P=0.001), and lymph node metastasis (All P=0.001), indicating a close association between these Th17/Treg associated TFs and the above pathological characteristics of CRC patients (**Tables 3**, **4**).

Relationship between Th17/Treg cells and the prognosis of CRC

We successfully tracked 54 cases of CRC over three years, with 11 death events. Comparing the Th17/Treg cell levels between the dead patients and the surviving patients, we found higher Th17 and Treg levels in the death group than in the surviving group (*P*<0.001). Similarly, ROC analysis revealed a sensitivity of 90.91% and a specificity of 69.77% of Th17 for diagnosing the 3-year mortality of CRC patients when Th17>15.28%. However, when Treg>10.08%, the sensitivity and specificity for diagnosing prognostic mortality were 81.82% and 60.47%, respectively. Meanwhile, the AUC for Th17 in predicting 3-year mortality in CRC patients was 0.8425 while AUC for Treg was 0.7241 (**Figure 4**).

In addition, according to the cut-offs of Th17 (15.06) and Treg (10.42), we have divided the

Table 4. Correlation of Th17/Treg associated transcription factors with pathological features of CRC

	n	RORγt mRNA	*t/P*	Foxp3 mRNA	*t/P*
Age			0.583/0.563		1.086/0.282
<61	27	4.37±0.84		3.26±0.75	
≥61	29	4.51±0.95		3.53±1.07	
Gender			0.303/0.763		0.561/0.577
Male	24	4.47±1.03		3.34±0.98	
Female	32	4.40±0.70		3.48±0.88	
Smoking			0.669/0.506		0.226/0.822
Yes	19	4.56±0.90		3.36±0.88	
No	37	4.39±0.90		3.42±0.97	
Drinking			0.868/0.389		0.252/0.802
Yes	16	4.61±0.91		3.45±0.85	
No	40	4.38±0.89		3.38±0.97	
Family history of CC			0.190/0.850		0.091/0.928
Yes	5	4.37±0.52		3.36±0.41	
No	51	4.45±0.92		3.40±0.97	
Previous intestinal inflammation			0.361/0.720		0.942/0.351
Yes	14	4.37±0.79		3.60±1.21	
No	42	4.47±0.93		3.33±0.82	
Tumor size (cm^2)			3.392/0.001		3.923/0.001
<5	41	4.22±0.81		3.14±0.68	
≥5	15	5.06±0.85		4.12±1.15	
TNM staging			5.961/0.001		5.892/0.001
I-II	41	4.11±0.64		3.05±0.56	
III-IV	15	5.37±0.85		4.35±1.08	
Degree of differentiation			3.411/0.001		3.836/0.001
Medium to high differentiation	44	4.25±0.80		3.18±0.76	
Low differentiation	12	5.16±0.89		4.22±1.07	
LNM			4.436/0.001		4.749/0.001
No	44	4.21±0.76		3.14±0.67	
Yes	12	5.32±0.80		4.36±1.14	

TNM, Tumor-node-metastasis; LNM, lymph node metastasis; CRC, colorectal cancer.

patients into low and high-level groups. KM analysis showed that high levels of Th17 and Treg were related to poor prognosis (**Figure 5**).

Univariate analysis of the prognosis of CRC

Then, we analyzed the relevant factors influencing the prognosis of CRC. The results showed no differences in age (P=0.156), gender (P=0.830), and tumor size (P=0.063) between the death and the surviving groups. However, the dead patients had significantly higher RORγt (P<0.001) and lower Foxp3 levels (P<0.001) than the survivals, with more cases of III-IV TNM (P<0.001), poor differentiation and LNM (P=0.002), as shown in **Table 5**.

Discussion

In this study, we observed elevated levels of Th17/Treg cells in CRC patients, alongside their strong diagnostic effectiveness for CRC. The expression levels of Th17/Treg cells were consistent with the findings of Wang et al. [26], indicating obvious Th17/Treg cell imbalance in CRC patients. Meanwhile, their excellent diagnostic effectiveness for CRC also suggested that Th17/Treg cells can be used as one of the auxiliary diagnostic indicators of CRC in the future. While conventional tumor markers such as carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125) are widely acknowledged to be closely related to tumor diseases with

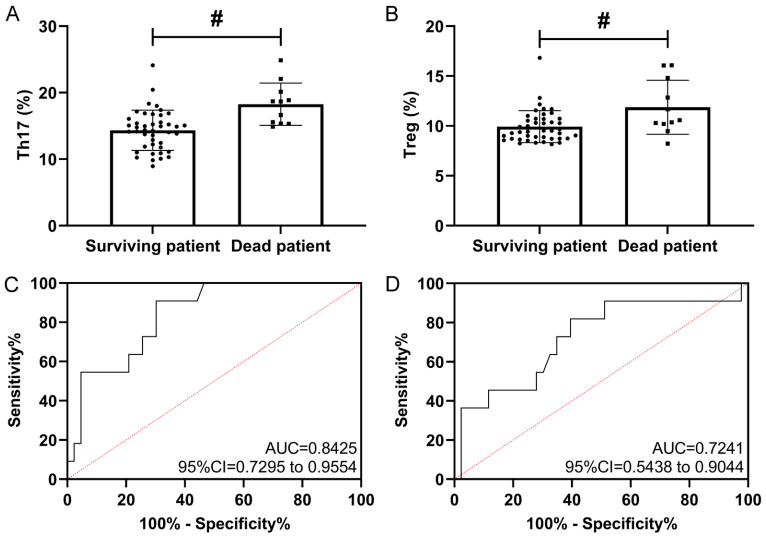

Figure 4. Association of Th17/Treg cells with the prognosis of CC. A. Comparison of Th17 between dead and survived patients. B. Comparison of Treg between dead and survived patients. C. ROC curve of Th17 for predicting 3-year mortality in CRC patients. D. ROC curve of Treg for predicting 3-year mortality in CRC patients. #*P*<0.05. CRC, colorectal cancer.

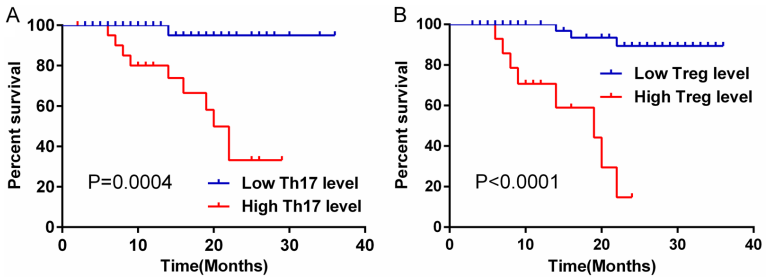

Figure 5. Comparison of survival outcome between patients with different levels of Th17 and Treg. A. Kaplan-Meier plots of overall survival of patients stratified by Th17 level. B. Kaplan-Meier plots of overall survival of patients stratified by Treg level.

high sensitivity [27], their low specificity limits their effectiveness in distinguishing tumors. Moreover, a study by Buzas et al. found these markers to be abnormally elevated in several inflammatory diseases [28], further diminishing their capacity for early tumor diagnosis. In contrast, our analysis of the diagnostic efficiency of Th17/Treg cells preliminarily demonstrates their potential to aid in CRC diagnosis. However, since the patients included in this study were at various stages of CRC, further research is needed to confirm the ability of Th17/Treg in the differential diagnosis of early CRC. Besides, we found that the TFs RORγt of Th17/Treg cells increased in CRC, while Foxp3 decreased, further demonstrating the close relationship between Th17/Treg and CRC. Studies have confirmed that the differentiation of Th17/Treg cells is mainly controlled by their TFs, and the absence of TFs can alter Th17/Treg activity [29]. In the early stage of abnormal immune response changes, primary CD4+ T cells can differentiate Foxp3 into RORγt, promote the differentiation of Th17/Treg cells, and activate the release of downstream IFs IL-17 and IL-22. This is supported by the detection results of IL-17 and IL-22 in this study. Similarly, the altered phosphorylated protein expression of Foxp3 and RORγt in CRC patients also confirmed the abnormal expression of Th17/Treg TFs, resulting in the imbalance of Th17/Treg.

In our analysis of the correlation between Th17/Treg and the pathological features of CRC, we also found that Th17/Treg and their TFs were closely related to tumor size, TNM staging, degree of differentiation, and LNM of CRC. This indicates that Th17/Treg are closely related to the pathological process of CRC. Previous studies have mentioned that imbalance of Th17/Treg can mediate the involvement of neutrophile granulocytes in pro-inflammatory response, thereby compromising the defense and repair ability of mucosal tissues on the surface of organs and tissues [30]. Therefore, we speculate that in CRC, the aggravation of pathological changes in CRC may also lead to greater bodily damage in patients, reflected by the obvious imbalance of Th17/Treg cells. A similar conclusion was drawn in the research by Wang et al. [31], which supports our hypothesis. Finally, in the prognostic analysis of CRC patients, we found that the Th17/Treg levels of the deceased patients were elevated. Additionally, Th17/Treg cells demonstrated an ideal diagnostic effect on the prognostic

Table 5. Univariate analysis of the factors affecting the prognosis of CRC patients

	Surviving patients (n=43)	Dead patients (n=11)	χ^2 or t/P
Age			2.011/0.156
<61	22 (51.16)	3 (27.27)	
≥61	21 (48.84)	8 (72.73)	
Gender			0.046/0.830
Male	18 (41.86)	5 (45.46)	
Female	25 (58.14)	6 (54.55)	
Smoking			0.379/0.538
Yes	16 (37.21)	3 (27.27)	
No	27 (62.79)	8 (72.73)	
Drinking			2.404/0.121
Yes	14 (32.56)	1 (9.09)	
No	29 (67.44)	10 (90.91)	
Family history of CC			1.410/0.235
Yes	5 (11.63)	0 (0.0)	
No	38 (88.37)	11 (100.0)	
Previous intestinal inflammation			0.262/0.609
Yes	11 (25.58)	2 (18.18)	
No	32 (74.42)	9 (81.82)	
Tumor size (cm^2)			3.455/0.063
<5	35 (81.40)	6 (54.55)	
≥5	8 (18.60)	5 (45.45)	
TNM staging			10.230/0.001
I-II	36 (83.72)	4 (36.36)	
III-IV	7 (16.28)	7 (63.64)	
Degree of differentiation			13.710/<0.001
Medium to high differentiation	38 (88.37)	4 (36.36)	
Low differentiation	5 (11.63)	7 (63.64)	
LNM			9.946/0.002
No	5 (11.63)	6 (54.55)	
Yes	38 (88.37)	5 (45.46)	
RORγt mRNA	4.21±0.78	5.59±0.77	5.249/<0.001
Foxp3 mRNA	3.10±0.65	4.45±1.01	5.450/<0.001

TNM, Tumor-node-metastasis; LNM, lymph node metastasis; CRC, colorectal cancer.

mortality of CRC, suggesting their potential application in future diagnosis and treatment of CRC. Consistently, a study also found that Th17/Treg can be used as a prognostic marker of severe pancreatitis [32], further verifying the close relationship between Th17/Treg and tumor diseases. This suggests that Th17/Treg could play an auxiliary evaluation role in various tumors in the future. As for TNM staging, differentiation degree, and other factors, they are directly related to the pathological process of the tumor and have been extensively verified and analyzed in the past.

Of course, we need to include more case data to further improve the representativeness and comprehensiveness of our results. Additionally, in vitro experiments are needed to confirm the specific mechanism of Th17/Treg cells and their TFs in CRC, so as to provide more accurate reference for clinical practice.

In summary, Th17/Treg associated TFs are closely related to the occurrence and development of CRC, and the imbalance of their levels can aggravate the inflammatory reaction and promote malignancy of the disease, which is of

great significance for the diagnosis, prognosis evaluation and treatment of CRC patients.

Disclosure of conflict of interest

None.

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