Expression of *RUNX3* gene and miR-363 in colorectal cancer and the relationship with clinicopathological features

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Abstract. Expression of *RUNX3* gene and miR-363 in colorectal cancer was studied to explore its relationship with clinicopathological characteristics of colorectal cancer and to analyze the value of *RUNX3* combined with miR-363 in the diagnosis of colorectal cancer. In total, 85 patients diagnosed with colorectal cancer in the First People's Hospital of Xiaoshan Hangzhou from March 2014 to July 2016 were the experiment group. Seventy healthy individuals who underwent physical examination were the control group. RT-qPCR was used to detect the expression levels of *RUNX3* gene and miR-363 in peripheral blood of the two groups. The relationship between the expression of *RUNX3* and miR-363 with its clinicopathological characteristics was analyzed as well. The expression of *RUNX3* in the experiment group was significantly lower than that in the control group (P<0.05). The expression level of miR-363 was significantly lower than in the control group (P<0.05). However, there was a correlation with tumor size, degree of differentiation, lymph node metastasis, depth of invasion and clinical stages (P<0.05). *RUNX3* and miR-363 were significantly positively correlated with the degree of differentiation (r=0.7381, r=0.5375; P<0.05); *RUNX3* and miR-363 were significantly negatively correlated with clinical stages (r=-0.7167, -0.6700; P<0.05). The area under the ROC curve of the combined test was larger than the single test. The expression of *RUNX3* gene and miR-363 in peripheral blood of patients with colorectal cancer was lower than in the normal controls. The low expression of *RUNX3* and miR-363 was closely related to various biological behaviors of colorectal cancer. A potential reference is provided for the evaluation of patients with colorectal cancer and expected to have an important guiding effect in the treatment of colorectal cancer. Moreover, combined test of *RUNX3* and miR-363 has important significance in the diagnosis and treatment evaluation of colorectal cancer.

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Key words: *RUNX3*, miR-363, colorectal cancer, clinical stages, clinicopathological features, degree of differentiation

Introduction

Colorectal cancer is one of the most common malignant tumors in the gastrointestinal tract (1), including both colon and rectal cancer. The occurrence of malignant lesions is mainly caused by various carcinogenic factors such as the environment or heredity of the intestinal mucosal epithelium (2). The disease has the third highest occurrence rate in the male tumors and second highest in the females (3). The incidence rate ranks fourth and the mortality rate ranks fifth in the malignant tumors of China (4,5). The incidence of colorectal cancer has shown a significant upward trend due to factors such as environment, diet and social pressure (6). Most colorectal cancers are insidious, not easily identified in the early stage and have an extremely low diagnostic rate. The majority of patients are already in the advanced stage when they are diagnosed with colorectal cancer, and missed the best time for treatment. Colorectal cancer is most likely to metastasize to the liver, lung, bone and retroperitoneal lymph nodes. It is already in the advanced stage when distant metastasis occurs. Therefore, routine detections and early diagnosis are required for the treatment of colorectal cancer (7).

RUNX3 is a tumor suppressor gene that has important regulatory effect on the proliferation, growth and apoptosis of cells. The development, metastasis and prognosis of various malignant tumors are related to the expression of *RUNX3* and is expected to become an important indicator for prognosis and evaluation of tumor invasions (8,9). Evidence has shown that, *RUNX3* can be used as a basis for judging the degree of malignancy of colon cancer and an indicator for assisted diagnosis (10). MicroRNAs (miRNAs), as a class of endogenous non-coding small RNAs, generally approximately 22 nt in length, can be found in many organisms such as animals, plants and viruses (11). It has been reported that miRNA, as a protooncogene or tumor suppressor gene, may be involved in the development and progression of tumors (12,13). Related research by Hu *et al* (14) found that miR-363 can inhibit the proliferation and metastasis of tumors. Genes showed a low expression in colorectal cancer, due to its close association with the occurrence, development and metastasis of colorectal cancer, therefore, it may be a new target for the gene diagnosis and treatment in colorectal cancer. However, there are only a few studies on the association between the expression of miR-363 and clinicopathological characteristics in colorectal cancer.

Table I. Primer sequences.

Internal reference	Upstream primer	Downstream primer
RUNX3	5'-AGGCAATGACGAGAACTACTCC-3'	5'-CGAAGGTCGTTGAACCTGG-3'
GAPDH	5'-GCACCGTCAAGGCTGAGAAC-3'	5'-ATGGTGGTGAAGACGCCAGT-3'
miR-363	5'-ACACTCCAGCTGGGAATTGCACGGTATCCA-3'	5'-TGGTGTCGTGGAGTCG-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'

The expression of *RUNX3* and miR-363 in colorectal cancer were detected in the present study. The relationship between the expression levels of *RUNX3*, miR-363 and the clinicopathological characteristics were studied and the effect of *RUNX3* and miR-363 in the development, progression and prognosis of colorectal cancer were investigated. In addition, the value of *RUNX3*, and miR-363 single diagnosis and two combined diagnosis in colorectal cancer were compared to provide a potential theoretical basis to help early clinical diagnosis and treatment.

Patients and methods

General information. Eighty-five patients who were diagnosed with colorectal cancer in the First People's Hospital of Xiaoshan Hangzhou (Hangzhou, China) from March 2014 to July 2016 were collected as the experiment group which included 52 cases of males, 33 cases of females and the mean age was 59.51±8.98 years. There were 40 cases of colon and 45 cases of rectum. There were also 15 cases of highly differentiated, 52 cases of moderately differentiated and 18 cases of poorly differentiated. Clinical stages were: 19 cases in stage I, 29 cases in stage II, 22 cases in stage III and 15 cases in stage IV. Additionally, 70 healthy individuals who underwent physical examination during the same period were included as the control group, comprising 41 males, and 29 females, with a mean age of 58.68±8.81 years.

The inclusion criteria were: i) Patients with complete clinical and pathological data; ii) not treated with neoadjuvant chemotherapy, radiotherapy or immunotherapy; iii) all received tests such as blood tests, urine routine test, liver and kidney function test electrocardiogram and others; iv) patients were diagnosed with colorectal cancer in the postoperative pathology report.

The exclusion criteria were: i) Patients with autoimmune system defects; ii) non-primary tumor patients; iii) patients with liver dysfunction or other severe organ disease; iv) patients who are pregnant or during the period of lactation; v) patients have mental illness or have had a family history of mental illness.

This study was approved by the Ethics Committee of The First People's Hospital of Xiaoshan Hangzhou and the experimental content of the study was described in detail. The subjects agreed to participate and signed an informed consent.

Blood collection. Peripheral blood (2 ml) was collected from patients who were on empty stomach in the morning (experiment group), then loaded into an anticoagulation tube and sent to the laboratory. In the control group, 2 ml of fasting venous blood (peripheral blood) was taken in the morning on the day of the physical examination. After coagulation for 60 min (20-25˚C), centrifugation at 1,006.2 x g for 10 min at 4˚C, the supernatant was collected, avoiding repeated freezing and thawing.

Experimental instruments and reagents. TRIzol kit (Shanghai Shenggong Bio Co., Shanghai, China); DNase I (Shanghai Shenggong Bio Co., Shanghai, China); cDNA Reverse Transcription kit (Takara Biotechnology, Co., Ltd., Dalian, China); Ultraviolet spectrophotometer (Beijing Youpu General Technology Co., Ltd., Beijing, China), and real-time PCR kit (Beijing Aide Lai Biotechnology Co., Ltd., Beijing, China) were used in the study. The ABI 7500 real-time PCR detector was purchased from Applied Biosystems (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Experimental procedures of RT-qPCR. Total RNA in serum was extracted using TRIzol reagent according to the protocol. The template RNA with DNase I (RNA-free) was digested and the contamination of genomic DNA was eliminated. Ultraviolet spectrophotometer was used for the determination of purity and concentration, and 1.5% agarose gel electrophoresis was used for detection of RNA integrity. The RNA concentration was adjusted to 500 ng/μl. The transcription of RNA samples was reversed into cDNA by using reverse transcriptase and was conducted in strict accordance with the protocol. The SYBR RT-qPCR (Thermo Fisher Scientific, Inc.) system was 20 μl, 2X Ultra SYBR One-Step RT-qPCR buffer 10 μl, RNA template 2 μl, nuclease-free water 5.5 μl, 1 μl each of the upstream and downstream primers and upper enzyme mix 0.5 μl. The RT-qPCR reaction conditions were: Pre-denatured at 95˚C for 10 min, denatured at 95˚C for 15 sec, annealed and extended at 60˚C for 1 min, with 40 cycles. The primers in this experiment were designed by Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA, USA) primer design software, generated by Tianjin Saier Biotechnology Co., Ltd. (Tianjin, China). *GAPDH* was used as an internal reference for *RUNX3* and *U6* was used as an internal reference for miR-363. The specific primer sequences are shown in Table I. The above system configuration is strictly in accordance with the instructions. The results showed that fluorescence signal in the process of amplification of the cycle number Cq value started from the number of cycles corresponding to the inflection point from the background to the exponential growth phase. The relative expression level of the target gene *RUNX3* mRNA and miR-363 in blood was calculated by $2^{-\Delta Cq}$ (15).

Observation indices. The clinical basic information between the groups was compared. The differences of *RUNX3* mRNA

Table II. Comparison of clinical general data between the groups (mean ± SD)/[n (%)].

Characteristics	Experiment group (n=85)	Control group (n=70)	χ^2/t	P-value
Sex			0.109	0.74
Male	52 (61.18)	41 (58.67)		
Female	33 (38.82)	29 (41.43)		
Age (years)	59.51±8.98	58.68±8.87	0.576	0.57
Body mass index (kg/m^2)	20.12±2.18	20.32±2.09	0.579	0.56
Smoking status			1.127	0.29
Smoking	45 (52.94)	43 (61.43)		
Non-smoking	40 (47.06)	27 (38.57)		
Alcoholic status			0.231	0.63
Alcoholic	47 (55.29)	36 (51.43)		
Non-alcoholic	38 (44.71)	34 (48.57)		
Diastolic blood pressure (mmHg)	76.23±12.13	77.36±12.45	0.570	0.57
Systolic blood pressure (mmHg)	113.34±18.24	117.39±19.35	2.321	0.98
WBC (x10^9/l)	5.89±3.65	6.21±3.51	0.553	0.58
HB (gm/dl)	11.87±1.91	12.59±2.16	1.865	0.06
PLT (x10^9/l)	153.67±21.81	156.17±22.71	0.697	0.49
RBC (10^{12}/l)	4.76±0.61	4.64±0.58	1.246	0.21

WBC, white blood cell; HB, hemoglobin; PLT, platelet; RBC, red blood cell.

and miR-363 expression levels between the experiment and control groups were observed. The correlation between the expression levels of *RUNX3* mRNA and miR-363 and clinical stage and differentiation was analyzed in accordance with the clinicopathological features of patients with colorectal cancer.

Statistical analysis. The statistical analysis of the experimental data was performed by SPSS 19.0 software system (IBM Corp., Armonk, NY, USA). The enumeration data are expressed as [n (%)]. Chi-square test was used for the comparison between groups and mean ± SD was used to represent the measurement data. Paired t-test was used for comparison between the groups. One-way analysis of variance (ANOVA) and LSD post hoc test were used for comparisons between the means of multiple groups. The correlation between the expression levels of *RUNX3* and miR-363 with the clinical stage and degree of differentiation was based on the Spearman correlation coefficient. The sensitivity and specificity of individual and combined tests were assessed using the receiver operating curve (ROC). The diagnostic value of *RUNX3* and miR-363 combined test of colorectal cancer was analyzed by binary logistic regression. P<0.05 was considered to indicate a statistically significant result.

Results

Comparison of general information. The clinical baseline information in terms of sex, age, body mass index, smoking status, alcoholic status, diastolic blood pressure, systolic blood pressure, white blood cell (WBC), hemoglobin (HB), red blood cell (RBC) count and platelet (PLT) count were collected from the experiment group and the control group. There was no difference between the groups (P>0.05) (Table II).

Table III. Comparison of the expression levels of *RUNX3* and miR-363 in the groups (mean ± SD).

Item	No. of cases	*RUNX3*	miR-363
The experiment group	85	0.93±0.38	0.47±0.21
The control group	70	2.18±0.87[a]	1.57±0.68[a]
t		11.94	19.77
P-value		<0.001	<0.001

[a]P<0.05 indicates data comparison with the experiment group.

Comparison of RUNX3 and miR-363 expression levels between the groups. RT-qPCR was used to detect the expression of *RUNX3* and miR-363. The expression of *RUNX3* in the experiment group was significantly lower than that in the control group, and the difference between the groups was statistically significant (P<0.05) (Table III). The expression level of miR-363 was also significantly lower than that in the control group (P<0.05) (Table III).

Association between the expression of RUNX3 and miR-363 with clinicopathological characteristics in patients with colorectal cancer. The clinical information of 85 patients with colon cancer was collected for comparison. Some relevant data showed that the expression level of *RUNX3* in the blood of patients with colorectal cancer was not significantly associated with sex, age and tumor location (P>0.05). However, it was

Table IV. Association between *RUNX3*, miR-363 and clinicopathological characteristics (mean ± SD).

Characteristic	No. of cases	*RUNX3*	F/t	P-value	miR-363	F/t	P-value
Sex			0.699	0.49		1.379	0.17
Male	52	0.98±0.37			0.64±0.21		
Female	33	0.92±0.41			0.58±0.17		
Age (years)			0.729	0.47		1.111	0.27
<50	39	0.95±0.39			0.57±0.19		
≥50	46	1.02±0.48			0.62±0.22		
Tumor locations			1.776	0.08		1.837	0.07
Colon	40	0.93±0.35			0.54±0.17		
Rectum	45	1.08±0.42			0.61±0.18		
Tumor sizes (cm)			2.346	0.02		0.708	0.48
<5	57	1.13±0.54			0.51±0.19		
≥5	28	0.86±0.40[c]			0.48±0.17		
Differentiation			17.91	<0.001		13.55	<0.001
Highly differentiated	15	1.43±0.49			0.63±0.23		
Medium differentiated	52	1.03±0.31[a]			0.47±0.17[a]		
Poorly differentiated	18	0.75±0.16[a,b]			0.32±0.10[a,b]		
Lymph node metastasis or non-metastasis			3.809	<0.001		4.340	<0.001
Metastasis	49	0.79±0.38			0.43±0.14		
Non-metastasis	36	1.20±0.61[c]			0.59±0.20[c]		
Infiltration depth			4.849	<0.001		4.807	<0.001
T1+T2	28	1.18±0.43			0.60±0.17		
T3+T4	57	0.81±0.27[c]			0.44±0.13[c]		
Clinical stages			8.896	<0.001		9.498	<0.001
I + II	48	1.48±0.63			0.69±0.21		
III + IV	37	0.53±0.13[c]			0.30±0.15[c]		

[a]P<0.05 indicates comparison with high differentiation; [b]P<0.05 indicates comparison with medium differentiation; [c]P<0.05 indicates comparisons between two groups in the same type.

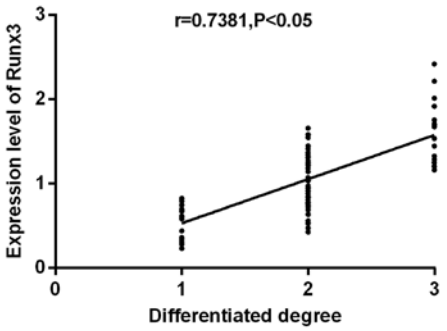

Figure 1. Correlation between the expression level of *RUNX3* mRNA and the degree of differentiation. The expression level of *RUNX3* mRNA was positively correlated with the degree of differentiation (r=0.7381; P<0.05). The higher the degree of differentiation, the higher the expression level of *RUNX3* mRNA. In the abscissa: 1 poor differentiation, 2 medium differentiation and 3 high differentiation.

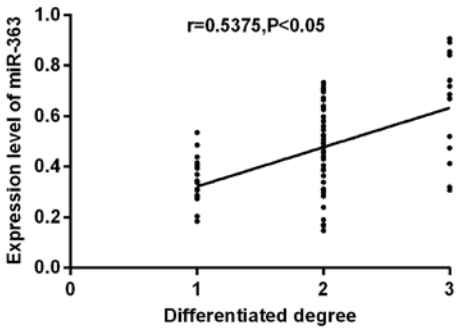

Figure 2. Correlation between the expression level of miR-363 and the degree of differentiation. miR-363 was significantly positively correlated with the degree of differentiation (r=0.5375; P<0.05). The higher the degree of differentiation, the higher the expression level of miR-363. In the abscissa: 1 poor differentiation, 2 medium differentiation and 3 high differentiation.

associated with tumor size, degree of differentiation, lymph node metastasis, depth of invasion and clinical stages (P<0.05). The expression level of miR-363 was not associated with sex, age tumor location and tumor size (P>0.05). It was correlated with the degree of differentiation, lymph node metastasis, depth of invasion and clinical stages (P<0.05) (Table IV).

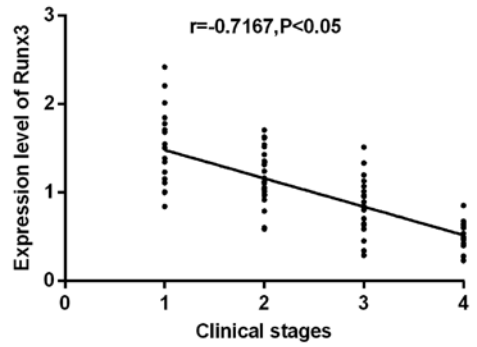

Figure 3. Correlation between the expression level of *RUNX3* mRNA and clinical stages. *RUNX3* mRNA was significantly negatively correlated with clinical stage (r=-0.7167; P<0.05), as the clinical stage increases, the expression level of RUNX3 mRNA gradually decreases. In the abscissa: 1 stage I, 2 stage II, 3 stage III and 4 stage IV.

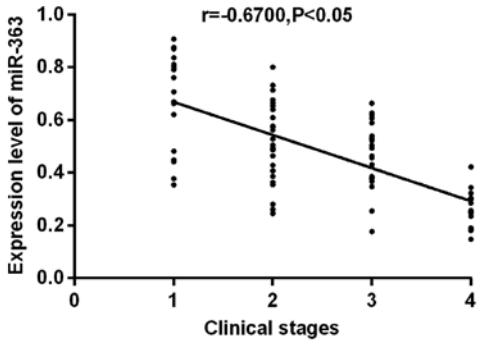

Figure 4. Correlation between the expression level of miR-363 and clinical stage. There was a significant negative correlation between miR-363 and clinical stage (r=-0.6700; P<0.05). As clinical stages continue to increase, the expression level of miR-363 gradually decreases. In the abscissa: 1 stage I, 2 stage II, 3 stage III and 4 stage IV.

Correlation between the expression levels of RUNX3 and miR-363 with clinical stages and differentiation. The expression of *RUNX3* and miR-363 was detected by RT-qPCR. Correlation between the expression levels of *RUNX3* and miR-363 with the degree of differentiation were analyzed (Figs. 1 and 2). *RUNX3* and miR-363 were significantly positively correlated with the degree of differentiation (r=0.7381, r=0.5375; P<0.05). The higher the degree of differentiation, the higher the expression level of *RUNX3* and miR-363. The correlation between the expression levels of *RUNX3* and miR-363 with clinical stages were analyzed (Figs. 3 and 4). *RUNX3* and miR-363 were significantly negatively correlated with clinical stages (r=-0.7167, -0.6700; P<0.05). As clinical staging continues to increase, the expression levels of *RUNX3* and miR-363 gradually decrease.

Comparison of the value of RUNX3 and miR-363 single test and two combined tests for the diagnosis of colorectal cancer. The comparison of the diagnostic value in this part was taken from the participation in the discussion between the experiment group and the normal control group. Then the sensitivity, specificity and Youden indices in the single and combined tests were compared. Sensitivity levels from high to low from the combined test was (88.57%), miR-363 (85.71%) and *RUNX3* (78.57%). The specificity level from high to low in the combined test was (96.47%), miR-363 (95.29%) and *RUNX3* (80.0%). The results suggest that *RUNX3*+miR-363 combined test has the highest sensitivity and specificity levels. Among them, Youden had the largest indices in the combined test. For the screening of colorectal cancer, the larger the Youden index, the better the detection and the higher the authenticity (Table V).

Table V. Comparison of the value of *RUNX3* and miR-363 single test and two combined tests for colorectal cancer diagnosis.

Test	Sensitivity	Specificity	Youden indices
RUNX3	78.57%	80.00%	0.59
miR-363	85.71%	95.29%	0.81
RUNX3+miR-363	88.57%	96.47%	0.85

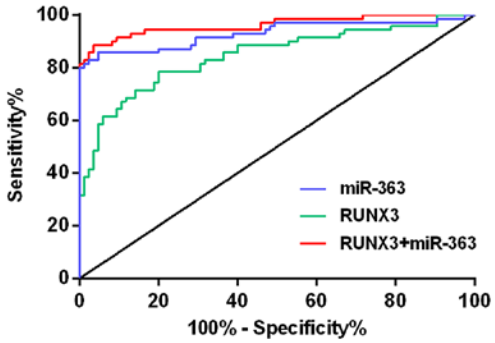

Figure 5. ROC curve of *RUNX3* and miR-363 single test and combined diagnosis of colorectal cancer. The combined test of *RUNX3* and miR-363 in peripheral blood has the highest sensitivity and specificity level, the area under the ROC curve (AUC) in the combined test is larger than the single test and has a higher diagnostic value. In the ROC curve, the combined test is closer to the upper left corner than the single test, and has a higher detection accuracy than the single test.

Evaluation of RUNX3 and miR-363 single test and combined test in the diagnosis of colorectal cancer. ROC curves were plotted based on their sensitivity and specificity levels for both single and combined tests. In the area under the ROC curve (AUC) the larger the AUC, the greater the diagnostic value. Combination diagnosis of AUC is greater than single diagnosis. The optimal thresholds for *RUNX3* and miR-363 are 1.367 and 0.752, respectively, the diagnostic efficiency is the highest at this time-point (Table VI and Fig. 5).

Discussion

Colorectal cancer has become the third most common cancer in the world. It is the fourth malignant tumor that causes human death (16). It has a high morbidity and mortality rates and there is a significant heterogeneity among individual patients (17). The main prevention and treatment of colorectal cancer is to control its incidence and reduce its mortality rates. Therefore, it is very necessary to find a sensitive and specific index to judge the risk of occurrence, development, metastasis and recurrence of colorectal cancer patients, thus an individualized treatment

Table VI. Evaluation of *RUNX3* and miR-363 single test and two combined tests for the diagnosis of colorectal cancer.

Detection method	Optimal critical value	AUC	P-value	95% Confidence interval	
				Upper limit	Lower limit
RUNX3	1.367	0.845	<0.001	0.780	0.909
miR-363	0.752	0.930	<0.001	0.883	0.976
RUNX3+miR-363	-	0.961	<0.001	0.930	0.992

plan has to be developed (18). Currently, pathogenesis of colorectal cancer is not yet fully understood. There are some reports showing that its carcinogenesis may originate from intestinal mucosal epithelial cells, a series of genetic alterations are involved in the sequence changes of 'abdominal epithelial dysplasia - adenoma - carcinoma' (19).

RUNX3, as a tumor suppressor gene, a member of the *RUNX3* family of transcription factors, it has an important effect in cell differentiation and apoptosis, and cell cycle regulation (20). An important reason for the downregulation of *RUNX3* expression was due to methylation of CpG islands in the *RUNX3* promoter region. Its anticancer mechanism is mainly related to the TGF-β pathway which induces growth inhibition and apoptosis (21,22). However, the TGF-β/Smad signaling pathway is involved in the development and progression of colorectal cancer (23). Studies have shown that (24) *RUNX3* gene may be a key target for TGF-β signaling pathways, participated in the negative regulation of epithelial cells by the TGF-β pathway, promoter region methylation and heterozygosity loss was the main mechanism. miR-363 is a novel small molecule RNA that has an important effect in the occurrence and development of some tumors. It was first discovered that the expression of miR-363 was in the head and neck squamous cell carcinoma of lymph node metastasis, and miR-363 has a low expression in tissues. Furthermore, it also showed a low expression level in some highly invasive tumor cell lines. Further studies have found that miR-363 affects the invasion and metastasis of tumor cells mainly through targeted inhibition of podoplanin (PDPN) (25,26).

In the present study, by comparing the information of the general clinical baseline, it was found that there was no difference between the experiment group and the control group. The interference from other factors on the experimental results were excluded, to ensure reliable results in this research. The data showed that the expression level of *RUNX3* in the blood of the experiment group was lower than the control group. Ku *et al* (10) used RT-qPCR to detect cancer cell lines in colon cancer patients. It was found that approximately half of the colon cancer cell lines had no expression or decreased expression of *RUNX3*. The expression level of miR-363 in the blood of the experiment group was also significantly lower than the control group (P<0.05). Xu *et al* (27) detected the expression of miR-363 in colorectal cancer tissues by RT-qPCR. Compared with normal colorectal epithelial cell lines, miR-363 expression was significantly downregulated in six colorectal cancer cell lines. The differences were statistically significant (P<0.05) and consistent with the results of this research. The study used peripheral blood, which is easier to obtain than tissue. Through the research of the relationship between the expression of *RUNX3* and miR-363 with clinicopathological features in patients with colorectal cancer, the results have shown that *RUNX3* was associated with tumor size, degree of differentiation, lymph node metastasis, depth of invasion and clinical stages (P<0.05), which is consistent with the results of Watanabe *et al* (28). However, the results of Mu *et al* (29) found that *RUNX3* expression was not associated with tumor size, differentiation and histological types. There are some differences between this research and the results of Watanabe *et al*, so further research is required. We should be aware of that with the development of colorectal cancer, the expression level of *RUNX3* gradually decreases. In the development of colorectal cancer, the loss of *RUNX3* expression presents a cumulative process. Therefore, *RUNX3* can be used as a new marker to judge the clinical stages of colorectal cancer. Moreover, Soong *et al* (20) found that the positive expression of *RUNX3* in the nucleus of colon cancer tissues is related to the stage of disease. miR-363 is associated with differentiation, lymph node metastasis, depth of invasion and clinical stages (P<0.05). Also studies have shown, the comparison of miR-363 among different pathological differentiation, TNM stages and lymph node metastasis in patients with colorectal cancer, the difference was statistically significant and consistent with our research (14). It has been shown that the clinical stage and differentiation of cervical cancer are related to miR-363 and that there is scarce researches on colorectal cancer (30). Therefore, miR-363 requires a deeper study on colorectal cancer. The results of this study showed that *RUNX3* and miR-363 are significantly positively correlated with the degree of differentiation. Both genes have an important effect in the occurrence and development of colorectal cancer. However, *RUNX3* and miR-363 showed a significant negative correlation with clinical stage. As the disease worsens, the expression levels of *RUNX3* and miR-363 are downregulated. This indicates that *RUNX3* and miR-363 can be used to determine the severity of patients' condition. Based on relevant reports, there is scarce research on the diagnosis of colorectal cancer with *RUNX3* and miR-363 and no research was found on the two combined diagnosis. The present study compared the diagnostic significance of *RUNX3* and miR-363 single and combined tests in colorectal cancer, it was found that the sensitivity and specificity levels of the combined diagnosis of *RUNX3* and miR-363 were higher than the single test. Also the combined test has the largest Youden indices, for the screening of colorectal cancer, the larger the Youden index, the better the detection effect and the higher the authenticity. By plotting the ROC curve, the AUC shows

that the combined diagnosis is larger than the single diagnosis, and the larger the AUC, the greater the diagnostic value. The above two points indicate that the value of the combined test of *RUNX3* and miR-363 is higher than the single test.

In this study, the expression levels of *RUNX3* and miR-363 in the blood of patients were studied from various aspects, which provides a reference for clinical research. However, there is a lack of follow-up in patients after surgery, so the lifestyle and prognosis of patients need to be explored in future studies. Also the exact mechanism of action of *RUNX3* and miR-363 in the development of colorectal cancer remains to be further studied. Searches have shown that there only exist a few relevant documents on the combined tests of *RUNX3* and miR-363, so this aspect requires strengthening in subsequent research.

In summary, the expression of *RUNX3* gene and miR-363 in serum of patients with colorectal cancer is lower than normal people, the low expression of *RUNX3* and miR-363 is closely related to various biological behavior of colorectal cancer and shows potential as a reference for the evaluation of patients with colorectal cancer, and to provide a guidance for the treatment of colorectal cancer. Combined test of *RUNX3* and miR-363 has an important significance in the diagnosis and treatment evaluation of colorectal cancer.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

GL conceived and designed the study, collected the patients' data, analyzed and interpreted the patient data regarding the colorectal cancer, wrote the manuscript, and read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the First People's Hospital of Xiaoshan Hangzhou (Hangzhou, China). Signed informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The author declares no competing interests.

References

1. Wong MC, Lam AT, Li DK, Lau JT, Griffiths SM and Sung JJ: Factors associated with practice of colorectal cancer screening among primary care physicians in a Chinese population: A cross-sectional study. Cancer Epidemiol 33: 201-206, 2009.
2. Matsuoka K, Watanabe N and Nakamura K: *O*-glycosylation of a precursor to a sweet potato vacuolar protein, sporamin, expressed in tobacco cells. Plant J 8: 877-889, 1995.
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
4. Zheng R, Zeng H, Zhang S, Chen T and Chen W: National estimates of cancer prevalence in China, 2011. Cancer Lett 370: 33-38, 2016.
5. Wang XQ, Tang ZX, Yu D, Cui SJ, Jiang YH, Zhang Q, Wang J, Yang PY and Liu F: Epithelial but not stromal expression of collagen alpha-1(III) is a diagnostic and prognostic indicator of colorectal carcinoma. Oncotarget 7: 8823-8838, 2016.
6. Zhang ZJ, Zheng ZJ, Kan H, Song Y, Cui W, Zhao G and Kip KE: Reduced risk of colorectal cancer with metformin therapy in patients with type 2 diabetes: A meta-analysis. Diabetes Care 34: 2323-2328, 2011.
7. Zhong LL, Chen HY, Cho WC, Meng XM and Tong Y: The efficacy of Chinese herbal medicine as an adjunctive therapy for colorectal cancer: A systematic review and meta-analysis. Complement Ther Med 20: 240-252, 2012.
8. Kim HJ, Park J, Lee SK, Kim KR, Park KK and Chung WY: Loss of RUNX3 expression promotes cancer-associated bone destruction by regulating CCL5, CCL19 and CXCL11 in non-small cell lung cancer. J Pathol 237: 520-531, 2015.
9. Estécio MR, Maddipoti S, Bueso-Ramos C, DiNardo CD, Yang H, Wei Y, Kondo K, Fang Z, Stevenson W, Chang KS, *et al*: RUNX3 promoter hypermethylation is frequent in leukaemia cell lines and associated with acute myeloid leukaemia inv(16) subtype. Br J Haematol 169: 344-351, 2015.
10. Ku JL, Kang SB, Shin YK, Kang HC, Hong SH, Kim IJ, Shin JH, Han IO and Park JG: Promoter hypermethylation downregulates RUNX3 gene expression in colorectal cancer cell lines. Oncogene 23: 6736-6742, 2004.
11. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, *et al*: Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA 101: 2999-3004, 2004.
12. Ambros V: The functions of animal microRNAs. Nature 431: 350-355, 2004.
13. Zhang B, Pan X, Cobb GP and Anderson TA: microRNAs as oncogenes and tumor suppressors. Dev Biol 302: 1-12, 2007.
14. Hu F, Min J, Cao X, Liu L, Ge Z, Hu J and Li X: MiR-363-3p inhibits the epithelial-to-mesenchymal transition and suppresses metastasis in colorectal cancer by targeting Sox4. Biochem Biophys Res Commun 474: 35-42, 2016.
15. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
16. Favoriti P, Carbone G, Greco M, Pirozzi F, Pirozzi RE and Corcione F: Worldwide burden of colorectal cancer: A review. Updates Surg 68: 7-11, 2016.
17. Cohen SJ, Cohen RB and Meropol NJ: Targeting signal transduction pathways in colorectal cancer - more than skin deep. J Clin Oncol 23: 5374-5385, 2005.
18. Seymour MT, Maughan TS, Ledermann JA, Topham C, James R, Gwyther SJ, Smith DB, Shepherd S, Maraveyas A, Ferry DR, *et al*; FOCUS Trial Investigators; National Cancer Research Institute Colorectal Clinical Studies Group: Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): A randomised controlled trial. Lancet 370: 143-152, 2007.
19. Croitoru ME, Cleary SP, Di Nicola N, Manno M, Selander T, Aronson M, Redston M, Cotterchio M, Knight J, Gryfe R, *et al*: Association between biallelic and monoallelic germline MYH gene mutations and colorectal cancer risk. J Natl Cancer Inst 96: 1631-1634, 2004.
20. Soong R, Shah N, Peh BK, Chong PY, Ng SS, Zeps N, Joseph D, Salto-Tellez M, Iacopetta B and Ito Y: The expression of RUNX3 in colorectal cancer is associated with disease stage and patient outcome. Br J Cancer 100: 676-679, 2009.

21. Tan SH, Ida H, Lau QC, Goh BC, Chieng WS, Loh M and Ito Y: Detection of promoter hypermethylation in serum samples of cancer patients by methylation-specific polymerase chain reaction for tumour suppressor genes including RUNX3. Oncol Rep 18: 1225-1230, 2007.
22. Smith E, De Young NJ, Pavey SJ, Hayward NK, Nancarrow DJ, Whiteman DC, Smithers BM, Ruszkiewicz AR, Clouston AD, Gotley DC, *et al*: Similarity of aberrant DNA methylation in Barrett's esophagus and esophageal adenocarcinoma. Mol Cancer 7: 75, 2008.
23. Nishio M, Sakakura C, Nagata T, Komiyama S, Miyashita A, Hamada T, Kuryu Y, Ikoma H, Kubota T, Kimura A, *et al*: RUNX3 promoter methylation in colorectal cancer: Its relationship with microsatellite instability and its suitability as a novel serum tumor marker. Anticancer Res 30: 2673-2682, 2010.
24. Kang KA, Kim KC, Bae SC and Hyun JW: Oxidative stress induces proliferation of colorectal cancer cells by inhibiting RUNX3 and activating the Akt signaling pathway. Int J Oncol 43: 1511-1516, 2013.
25. Sun Q, Zhang J, Cao W, Wang X, Xu Q, Yan M, Wu X and Chen W: Dysregulated miR-363 affects head and neck cancer invasion and metastasis by targeting podoplanin. Int J Biochem Cell Biol 45: 513-520, 2013.
26. Luo X, Burwinkel B, Tao S and Brenner H: MicroRNA signatures: Novel biomarker for colorectal cancer? Cancer Epidemiol Biomarkers Prev 20: 1272-1286, 2011.
27. Xu X, Wu X, Wu S, Jiang Q, Liu H, Chen R and Sun Y: Study on miR-490-5p and miR-363 as novel biomarkers for the diagnosis of colorectal cancer. Zhonghua Wei Chang Wai Ke Za Zhi 17: 45-50, 2014 (In Chinese).
28. Watanabe T, Kobunai T, Ikeuchi H, Yamamoto Y, Matsuda K, Ishihara S, Nozawa K, Iinuma H, Kanazawa T, Tanaka T, *et al*: RUNX3 copy number predicts the development of UC-associated colorectal cancer. Int J Oncol 38: 201-207, 2011.
29. Mu WP, Wang J, Niu Q, Shi N and Lian HF: Clinical significance and association of RUNX3 hypermethylation frequency with colorectal cancer: A meta-analysis. OncoTargets Ther 7: 1237-1245, 2014.
30. Tsuji S, Kawasaki Y, Furukawa S, Taniue K, Hayashi T, Okuno M, Hiyoshi M, Kitayama J and Akiyama T: The miR-363-GATA6-Lgr5 pathway is critical for colorectal tumourigenesis. Nat Commun 5: 3150, 2014.