ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Plasma miR-378a as a novel biomarker for the early detection of colon cancer

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Abstract

Introduction: Prognosis and treatment outcomes of colon cancer (CC) patients are poor due to the limitations of the current early diagnostic techniques. Recently, innovations in liquid biopsy technology have inspired research into identifying early peripheral blood-based diagnostic markers for CC.

Objective: The objectives of the study are to evaluate the potential of plasma miR-378a as a diagnostic biomarker for early CC.

Methods: In this study, quantitative reverse transcription polymerase chain reaction was used to measure plasma miR-378a levels in CC patients, patients with colon polyps (CPs), and healthy controls (HCs). The study included 94 CC patients (56 males, 38 females; age range 35 – 82 years, mean age 57.7 ± 10.1 years) and 65 patients with CPs (40 males, 25 females; age range 42 – 91 years, mean age 63.0 ± 11.4 years), including 24 adenomatous polyps, 15 inflammatory polyps, 12 hamartomatous polyps, 14 hyperplastic polyps, and 45 HCs. The correlation between miR-378a and clinicopathology features was analyzed, and diagnostic value of miR-378a in each group was assessed using receiver operating characteristic (ROC) curve analysis.

Results: CC patients had significantly lower plasma miR-378a levels compared to patients with CPs ($p<0.0001$) and HCs ($p=0.0018$). miR-378a levels were significantly correlated with TNM stage, tumor size, and lymph node metastasis ($p<0.05$). ROC curve analysis illustrated strong diagnostic discriminative power of miR-378a for CC and CPs, with an area under the curve of 0.8202 (95% confidence interval: 0.7528 – 0.8876), sensitivity of 89.36%, and specificity of 66.15%.

Conclusion: These findings suggest that plasma miR-378a may serve as a potential biomarker for early diagnosis of CC.

Keywords: Mir-378a; Colon cancer; Early diagnosis; Marker; Plasma

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Citation: Guo L, Zhang Y, Liu J, *et al*. Plasma miR-378a as a novel biomarker for the early detection of colon cancer. *Eurasian J Med Oncol*. 2026;10(1):110-119. doi: 10.36922/EJMO025140088

Received: April 4, 2025

Revised: May 8, 2025

Accepted: May 22, 2025

Published online: July 1, 2025

1. Introduction

Colon cancer (CC) is the most common malignant tumor of the digestive system, with the 4th highest cancer morbidity and mortality rates in the world.[1] As the early symptoms

of CC are not distinct, most patients are already in the advanced stages of CC at the time of their diagnosis, which seriously affects subsequent treatment and prognosis.[2] Therefore, diagnosing cancer in the early stage has become the key to improving prognosis. At present, colonoscopy, endoscopy, and serum carcinoembryonic antigen (CEA) testing are the main methods for early clinical diagnosis of CC.[3-5] Colonoscopy is widely recognized as the reference standard for diagnosing colorectal cancer (CRC) due to its ability to detect and remove precancerous polyps. However, its invasive nature and the need for specialized equipment and trained personnel may limit its accessibility and application worldwide. While the accuracy of colonoscopy in diagnosing CRC is well-documented, it is important to consider alternative screening methods that may be less invasive and more widely applicable, especially in regions with limited resources.[6-8] Furthermore, owing to the upregulated CEA levels commonly seen in other malignancies, CEA is considered having limited sensitivity and specificity for diagnosis, which substantially hampers its use in the early diagnosis of CC.[5,9] In recent years, continuous research into diagnostic techniques has identified certain tumor markers that can be detected using signal amplification techniques from patients' plasma and other body fluids, which may serve as a basis for diagnosis.[10] Moreover, due to its advantages such as convenient sampling, suitability for large-scale screening, applicability in resource-limited regions, and minimal physiological injury, peripheral blood has become a focal point in identifying specific tumor markers, which has attracted significant research interest.[11,12]

MicroRNAs (miRNAs) are small, non-coding RNA molecules with a length of approximately 18 – 22 nucleotides each that play important roles in human hematopoietic processes, growth and development, inflammation occurrence and progression, cell proliferation and migration, as well as tumor progression and prognosis.[13] Research has shown that miRNAs have specific expression profiles in cancer cells and tumor tissues and can enter the blood and body fluid through the rupture of tumor cells or tumor-associated vesicles. Thus, miRNAs may have potential as tumor markers.[14] In addition, miRNAs have good stability in plasma,[15] offering another advantage as markers for the early diagnosis of tumors. Studies have reported that miRNAs play an important role in regulating the development and progression of CC and have good prognostic value.[16-18] However, few studies have reported the use of miRNAs in the early diagnosis of CC.

A growing body of research has reported altered plasma expression of specific miRNAs in the early stages of CC. For example, a recent review has summarized these findings, emphasizing the potential of miRNAs as non-invasive biomarkers for early detection. Among these, miR-378a has been identified as having a notably altered expression in the early phases of CC, which may offer unique insights into the molecular mechanisms of the disease. As a biomarker, miR-378a detection could effectively complement existing screening protocols, particularly during initial risk assessment. This approach could help clinicians in identifying patients who require further invasive colonoscopy examinations, ultimately leading to more efficient allocation of medical resources.

This study focuses on miR-378a for several key reasons. First, miR-378a has been observed to exhibit significant expression changes in the early stages of CC, implying its potential involvement in tumor initiation and progression, rather than functioning solely as a secondary phenomenon in advanced tumors. Second, despite its distinct expression profile and known regulatory roles in cancer biology, research on miR-378a, especially in the context of CC, remains limited. This gap in the literature presents an opportunity for further investigation. Finally, the inherent stability and specificity of miRNAs in plasma make miR-378a particularly suitable as a circulating marker. Its ability to remain stable during blood sample processing and storage enhances the reliability of diagnostic assay. The underexplored status of miR-378a highlights its potential as a novel, non-invasive diagnostic marker for CC. Evidence indicates a relationship between miR-378a and the mechanisms of CC progression. Studies have shown that miR-378a is involved in the biological processes of proliferation, cell cycle, and apoptosis in CRC.[19] It has been hypothesized that miR-378a may affect the abnormal cell proliferation by regulating cell cycle-related genes or inhibiting apoptotic pathways. Furthermore, miR-378a may influence the migration and invasiveness of CC cells by regulating epithelial-mesenchymal transition-related genes, which may explain its correlation with clinicopathologic features such as TNM stage and lymph node metastasis. This research aims to fill this gap by analyzing miR-378a expression levels in plasma samples from CC patients, correlating these levels with clinicopathological features, and assessing its diagnostic potential for early-stage CC using receiver operating characteristic (ROC) curve analysis (Figure 1).

In contrast to previous studies, this study included a cohort containing three well-defined groups – patients with CC, patients with colon polyp (CP), and healthy control (HC) – and specifically analyzed patients with intestinal polyps as an independent group. This design allowed a comprehensive assessment of miR-378a dynamics in both intestinal polyps and CC, rather than comparison to CC and HC. By comparing the expression differences between the polyp and CC groups, the value of miR-378a

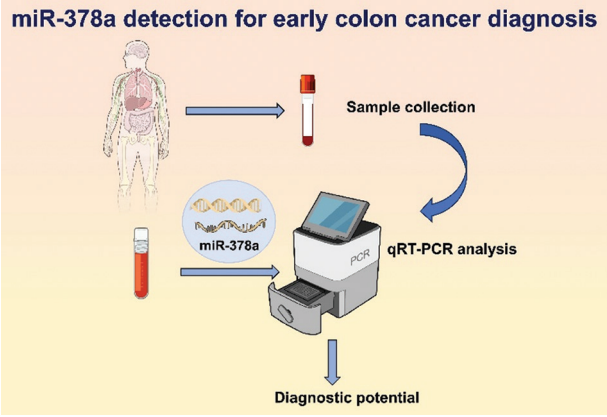

Figure 1. Study design for evaluating miR-378a as a novel early diagnostic biomarker across the colon disease spectrum

in predicting the cancerous potential of polyps can be assessed, which is important for guiding clinical diagnostic strategies.

Although previous studies have reported changes in miR-378a expression in CC, few studies have specifically focused on its value for application as an early diagnostic marker, especially its potential to distinguish malignant tumors from benign polyps. In this study, the relationship between miR-378a and clinicopathological features such as TNM stage, tumor size, and lymph node metastasis was systematically analyzed, revealing an expression pattern associated with disease progression that has not been fully explored in previous studies.

In this study, peripheral blood samples were collected from patients with CC and CP and compared with samples from healthy individuals undergoing health checkups during the same period. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to quantify miR-378a expression in plasma across the three distinct groups. Subsequently, the correlation between miR-378a levels and clinicopathological characteristics in CC patients was analyzed, and the diagnostic value of miR-378a for early-stage CC was further evaluated using ROC curve analysis.

2. Materials and methods

2.1. Study subjects

Plasma samples were collected from patients with CC and those with CP who were treated and pathologically diagnosed at the Affiliated Hospital of Guangdong Medical University between September 2018 and January 2020. Enrollment details are provided in Table 1. Of these patients, 65 had CP, comprised of 40 males and 25 females, aged 42 – 91 years, with a mean age of 63.0 ± 11.4 years. These included 24 cases of adenomatous polyps, 15 inflammatory polyps, 12 hamartomatous polyps, and 14 hyperplastic polyps. A total of 94 patients were diagnosed with CC, comprised of 56 males and 38 females, aged 35 – 82 years, with a mean age of 57.7 ± 10.1 years. These patients were clinically staged according to the TNM staging system established by the American Joint Committee on Cancer, comprising 17 cases at Stage I, 20 at Stage II, 33 at Stage III, and 24 at Stage IV. In addition, plasma samples were collected from 45 healthy individuals who underwent health checkups during the same period. There were no significant differences in sex or age among the three groups (p>0.05). This study was approved by the Ethics Committee of the Affiliated Hospital of Guangdong Medical University. None of the subjects had been treated with surgery, radiotherapy, or chemotherapy before blood collection, and those with previous underlying diseases or primary tumors at other sites were excluded. Informed consent was obtained from all patients and their families.

Table 1. Information of the enrolled subjects

Variable	Colon polyp patients (n=65)	Colon cancer patients (n=94)	Healthy controls (n=45)
Age			
⩽60 years	26	50	16
>60 years	39	44	29
Sex			
Male	40	56	29
Female	25	38	16
Pathological nature of polyps			
Adenomatous polyps	24		
Inflammatory polyps	15		
Hamartomatous polyps	12		
Hyperplastic polyps	14		
TNM staging			
Stage I		17	
Stage II		20	
Stage III		33	
Stage IV		24	

2.2. Plasma collection

Approximately 5 mL of venous blood was collected from each participant in the morning on an empty stomach. The blood samples were drawn into an ethylenediaminetetraacetic acid-containing anticoagulant blood collection tube, gently mixed, and then transported to the laboratory under low-temperature conditions. Blood

samples were then centrifuged at 2,800 g for 10 min at 4°C. The upper plasma layer (supernatant) was transferred into RNase-free cryogenic vials and stored in a freezer at −80°C for later use.

2.3. Reagents

The miRNeasy Serum/Plasma Kit (Cat: 217184; Qiagen, Germany) was used for RNA extraction, and the Mir-X miRNA qRT-PCR TB Green® Kit (Cat: 638316; TAKARA, Japan) was used for qRT-PCR analysis.

2.4. Plasma RNA extraction

This procedure was conducted in strict accordance with the instructions of the miRNeasy Serum/Plasma Kit (Qiagen, Germany). After the plasma was thawed, it was centrifuged at 12,000 rpm for 10 min at 4°C. A total of 200 µL of supernatant were pipetted into a new Eppendorf centrifuge (EP) tube, and 1 mL of QIAzol Lysis Reagent was added. The mixture was then vortexed and incubated for 5 min at room temperature. After 200 µL chloroform was added, the sample was shaken vigorously for 15 s and then centrifuged at 12,000 rpm for 15 min at 4°C, and the supernatant was transferred into a new EP tube. Anhydrous ethanol with a volume of 1.5 times that of the supernatant was added and pipetted evenly into the mixture. The sample was then transferred to an RNA separation column and centrifuged at room temperature to allow RNA binding to the membrane. After the filtrate was discarded, RNA washing buffer solution I, RNA washing buffer solution II, and 80% ethanol were added into the separation column in sequence. The sample was then passed through the column by centrifugation at room temperature. The filtrate was discarded, and the sample was dried at room temperature for 15 min. The RNA separation column was then attached to a 1.5 mL RNase-free EP tube, and 15 µL of RNase-free H_2O was added to elute the RNA.

2.5. Synthesis of first-strand complementary DNA (cDNA) and real-time fluorescence quantitative polymerase chain reaction (qPCR)

First-strand cDNA was synthesized using reverse transcription in strict accordance with the instructions of the Mir-X miRNA qRT-PCR TB Green® Kit (TAKARA, Japan). The 20 µL reverse transcription reaction system consisted of 10 µL of mRQ Buffer (×2), 7.5 µL of RNA template, and 3.5 µL of mRQ Enzyme. The reaction conditions were as follows: Incubation at 37°C for 1 h, followed by enzyme inactivation at 85°C for 5 min. The resulting cDNA product was diluted 1:5 with RNase-free H_2O and used as the template for qPCR. The expression of miR-378a was quantified using the TB green dye method. The specific forward primer for miR-378a was designed by Primer 5.0 software and synthesized by TAKARA after BLAST comparison. The forward primer sequence was 5'-ACTGGACTTGGAGTCAGAAGG-3'. The reverse primer of miR-378a and both primers for the internal reference (U6 small nuclear RNA) were supplied in the kit. The reaction conditions were as follows: Initial denaturation at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s and annealing/extension at 60°C for 30 s. Reaction tubes without a template were used as the no-template negative controls. Three replicate wells were set for each sample. After amplification, cycle threshold (Ct) values were calculated, and U6 expression was used as the internal reference for normalization. The relative expression level of miR-378a was determined using the $2^{-\Delta\Delta Ct}$ method and expressed as $\log_{10}(2^{-\Delta\Delta Ct})$.

2.6. Statistical analysis

The relative expression level of miR-378a was expressed as $\log_{10}(2^{-\Delta\Delta Ct})$. Statistical analyses were conducted using the Statistical Package for the Social Sciences 22.0 (IBM Corporation, USA) and GraphPad Prism 9.0 (GraphPad Software, USA). The median and interquartile range was used to describe the levels of miR-378a in all participants. All data were tested for normality. For normally distributed data, independent samples *t*-test was used for comparisons between the two groups. For non-normally distributed data, a nonparametric test was used for comparisons between the two groups. One-way analysis of variance, Chi-square test, and Fisher's exact test were used to analyze the demographic information of the subjects. A $p<0.05$ indicated that the difference was statistically significant. ROC curve analysis was used to determine the diagnostic cut-off value of miR-378a, and corresponding diagnostic indexes such as sensitivity, specificity, and area under the curve (AUC) were calculated to evaluate the application of miR-378a in early diagnosis of CC, with a significance level set at $\alpha = 0.05$.

3. Results

3.1. Expression levels of miR-378a in CC, CPs, and HCs

Figure 2 is a scatter plot showing the miR-378a levels in samples from the three groups. The miR-378a levels in the plasma of the CC group were significantly lower than those in the CP and HC groups ($p<0.0001$, $p=0.0018$). In addition, the miR-378a levels in the CP group were significantly lower than those in the HC group ($p<0.0001$).

3.2. Correlation between plasma miR-378a levels and clinicopathological features of CC

The 94 patients with CC were divided into two groups based on plasma miR-378a expression: 47 cases with low expression and 47 with high expression. The relationship

between miR-378a levels and clinicopathological features (age, sex, tumor size, TNM stage, tumor type, and lymph node metastasis) was analyzed. Plasma miR-378a levels were significantly correlated with TNM stage, tumor size, and lymph node metastasis ($p<0.05$) but not with age, sex, or tumor type ($p>0.05$) (Table 2). The results showed that plasma miR-378a levels are inversely proportional to TNM stage in the CC group. miR-378a expression levels did not show significant associations with sex, age, and tumor subtype, implying that miR-378a expression is independent of these factors and may be applicable as a biomarker in patient groups with diverse demographic characteristics, thereby enhancing its versatility as a clinical diagnostic tool.

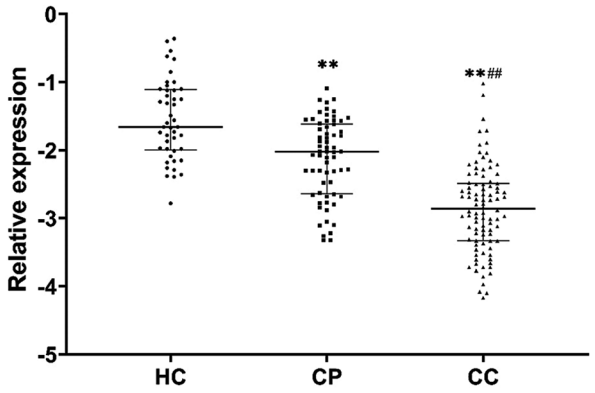

Figure 2. Expression levels of miR-378a in patients with CC, colon polyps, and HCs. $^{**}p<0.01$, $^{*}p<0.05$ compared with the HC group; $^{\#\#}p<0.01$, $^{\#}p<0.05$ compared with the CP group
Abbreviations: CC: Colon cancer; CP: Colon polyp; HC: Healthy control.

Table 2. Comparison of clinicopathological features between the high and low miR-378a expression groups in colon cancer patients

Pathological features	*n*	miR-378a			
		Low expression	High expression	Chi-square	*p*-value
Sex					
Male	56	29	27	0.1767	0.674
Female	38	18	20		
Age (years)					
⩽60	50	27	23	0.6836	0.408
>60	44	20	24		
Tumor size					
⩽4	51	18	33	9.644	<0.01
>4	43	29	14		
TNM staging					
Stage I – II	37	4	33	34.94	<0.01
Stage III – IV	57	43	14		
Lymph node metastasis					
Yes	34	27	7	18.43	<0.01
No	60	20	40		
Tumor subtypes					
Ulcerative	41	21	20	0.4326	0.835
Non-ulcerative	53	26	27		

3.3. Diagnostic value of miR-378a in CC

The diagnostic efficacy of miR-378a in CC was rigorously assessed using ROC curve analysis, a standard approach for evaluating the performance of a binary classifier. The AUC, 95% confidence interval (CI), cut-off point, sensitivity, and specificity were determined for each group and are detailed in Table 3 and illustrated in Figure 3.

For the HC group, the AUC for miR-378a in diagnosing CC was 0.9356 (95% CI: 0.8946 – 0.9748), indicating a high discriminatory ability. This high AUC value, close to 1, suggests miR-378a effectively differentiates CC patients from healthy individuals. Sensitivity was 77.66%, and specificity was 99.78% (Figure 3A), which underscores the potential of miR-378a as a reliable biomarker for early CC detection in the general population.

In the CP group, the AUC was 0.8202 (95% CI: 0.7528 – 0.8876), with sensitivity of 89.36% and specificity of 66.15% (Figure 3B). The decrease in AUC and specificity suggests that while miR-378a is sensitive in identifying CC in this group, false positives are more likely compared to the HC group.

When considering the non-cancer groups (CP and HC), the AUC was 0.8674 (95% CI: 0.8179 – 0.9169), with sensitivity of 87.23% and specificity of 75.45% (Figure 3C). This indicates robust diagnostic performance of miR-378a across both the CP and HC groups, with a balanced sensitivity and specificity.

In addition, the AUC for miR-378a in distinguishing CC from HC was 0.9356, showing the best performance in this scenario, while the AUC for distinguishing CC from polyps was 0.8202, showing relatively weaker performance. The diagnostic performance of miR-378a as a CC biomarker varied in different control settings, and this variability enhances its clinical applicability, allowing it to be used in accordance with different clinical contexts (general screening, follow-up in high-risk groups) to adjust the application strategy. Specificity and sensitivity of using miR-378a as a biomarker for detecting CC show differences when different controls are used. In the CC versus CP group, the sensitivity and specificity were 89.36% and 66.15%, respectively; in the CC versus HC group, sensitivity and specificity were 77.66% and 99.78%, respectively; and in the CC versus combined CP and HC group, sensitivity and specificity were 87.23% and

Table 3. Diagnostic value of plasma miR-378a for colon cancer

Diagnostic group	Truncated value	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)
Colon cancer versus colon polyp group	<−2.13	0.8936 (0.813 – 0.9748)	0.6615 (0.5335 – 0.7743)	0.8202 (0.7528 – 0.8876)
Colon cancer versus healthy group	<−2.425	0.7766 (0.679 – 0.8561)	0.9978 (0.8823 – 0.9994)	0.9356 (0.8964 – 0.9748)
Colon cancer versus colon polyp+healthy groups	<−2.195	0.8723 (0.7876 – 0.9323)	0.7545 (0.6633 – 0.8316)	0.8674 (0.8179 – 0.9169)

Abbreviations: AUC: Area under the curve; CI: Confidence interval.

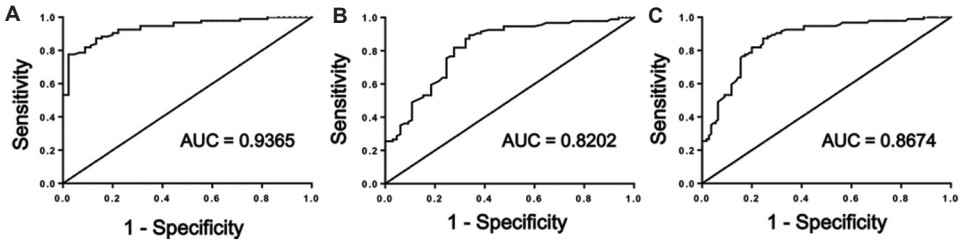

Figure 3. Diagnostic value of miR-378a in colon cancer. (A) ROC curve of miR-378a for diagnosing colon cancer in the healthy control group; (B) ROC curve of miR-378a for diagnosing colon cancer in the colon polyp group; (C) ROC curve of miR-378a for diagnosing colon cancer in the non-cancer groups (colon polyp + healthy control).
Abbreviations: AUC: Area under the curve; ROC: Receiver operating characteristic.

75.45%, respectively. These differences may be attributable to the involvement of miR-378a in the early stages of carcinogenesis, with expression changes starting at the polyp formation stage and becoming more pronounced in cancer. This explains for the better performance of miR-378a in distinguishing healthy tissue from malignant tissue. The expression of miR-378a may therefore show a gradient from healthy tissue to polyps to cancer.

In summary, miR-378a showed high diagnostic efficacy in all groups, especially in distinguishing CC from healthy individuals, as evidenced by high AUC and specificity values. These results indicate that miR-378a holds significant potential as a biomarker for the early diagnosis of CC.

4. Discussion

Research has shown that treatment outcomes and prognosis of CC are related to the stage of cancer progression at the time of diagnosis.[20] Those CC patients who were diagnosed at advanced stage or with a distant metastasis experience significantly poorer treatment responses and prognosis than those diagnosed at an early stage, which had sharply shortened survival periods.[21,22] However, as the early symptoms of CC are not distinct, effective early diagnostic methods are lacking. As a result, most patients are diagnosed at intermediate or advanced stages, which greatly reduce their quality of life and treatment outcomes.[23] Therefore, early diagnosis of CC is a key to improving patient quality of life. However, current early clinical diagnostic methods for CC have limitations and are not widely implemented in developing countries. In recent years, greater attention has been given to identifying early markers of CC that can be measured non-invasively.[24] As the most important sample type of a liquid biopsy, peripheral blood contains a variety of biological macromolecules, among which free RNA carries tumor genetic information and may be used as a potential diagnostic marker for tumor.[25,26]

The development and progression of CC is a slow process, and adenomatous polyps and polyp syndromes are closely correlated with its pathogenesis.[27,28] Numerous studies suggest that nearly all cases of colon adenocarcinoma evolve from adenomas through the adenoma-carcinoma sequence. The risk of adenomatous polyps progressing to CC has been reported to be 5 times higher than in patients without adenomatous polyps, and the risk in patients with multiple adenomas is approximately twice as high as in patients with a single adenoma.[29] Although the malignant transformation of intestinal polyps is a complex biological phenomenon involving multiple factors and genes, early diagnosis and treatment of precancerous adenomatous intestinal polyps can significantly reduce the incidence and mortality of CC. Therefore, plasma samples from CC patients, patients with CP, and HC were collected to evaluate the diagnostic efficacy of a potential marker among the groups, aiming to determine the application of miR-378a in the early diagnosis of CC.

In various types of tumor, miRNAs can promote or inhibit tumor development by regulating the expression of downstream target oncogenes or tumor suppressor genes.[30] Numerous studies have shown that miRNAs are abnormally expressed in CC tissues.[31,32] Furthermore, differences have been observed in miRNA levels between the serum of CC patients and HC, which were also correlated with

clinicopathological features and patient prognosis.[33] Wang and Gu[34] found that serum miR-29a levels in CC patients were significantly higher than those in the HC group and were positively correlated with TNM staging. They also reported that its sensitivity and specificity in differentiating between metastatic and non-metastatic CC were higher than 75%, indicating it was significantly superior to serum CEA.[34] A prognostic analysis by Yan *et al.*[35] found that serum levels of secreted miR-6803-5p in CC patients were significantly higher than those in HC, with higher expression in patients with advanced TNM stage, lymph node metastasis, and liver metastasis, suggesting a worse prognosis. A clinical study by Yuan *et al.*[36] involving a large sample of CC patients shown that the preoperative serum levels of miR-200b, miR-203, miR-29a, and miR-31 were correlated with the risk of postoperative recurrence. Furthermore, serum miR-31 and miR-29a could independently serve as markers for recurrence detection. In addition, Yin *et al.*[37] found that serum miR-126, miR-14, and miR-21 as potential biomarkers for liver metastasis of CC. These studies indicate that miRNAs not only have significant differential expression in CC tissues but can also remain stable in the bloodstream, suggesting that clinical characteristics of CC can be detected in the miRNA levels of peripheral blood.[16]

miR-378 is a huge family system containing more than 10 molecular subtypes.[38] miR-378a, is located at chromosome 5p13, plays different roles in different cancer types. Previous studies have shown that miR-378a has low expression in gastric cancer[39] and liver cancer,[40] where it acts as a tumor suppressor by inducing apoptosis in tumor cells. Conversely, miR-378a is highly expressed in non-small cell lung cancer,[41] leukemia,[42] and renal cell carcinoma,[43] where it acts as an oncogene. Previous research has indicated that miR-378a is downregulated in CC, and its overexpression can inhibit the malignant biological function of CC cells[44] and increase their sensitivity to therapeutic drugs.[45] In this study, plasma miR-378a levels were quantified in CC patients, patients with CP, and HC using qRT-PCR. The results indicated that miR-378a expression was the highest in the HC group ($p<0.05$) and lowest in the CC group ($p<0.05$). The above results are consistent with previously reported miR-378a expression patterns in CC tissue.[46] Based on these results, it was hypothesized that miR-378a may be correlated with the clinical features, as well as the development and progression of CC. Correlation analysis revealed that miR-378a expression levels were significantly associated with TNM stage, tumor size, and lymph node metastasis. Lower miR-378a expression levels were observed in more advanced TNM stages, whereas no significant correlation was found with sex, age, or tumor type. These results suggest that plasma miR-378a levels are correlated with the development and progression of CC. Furthermore, ROC curve analysis demonstrated that the AUC values for miR-378a in diagnosing CC in among all three groups were higher than 0.8. Especially for diagnosing CC in the CRC group, miR-378a exhibited a sensitivity of 89.36% and a specificity of 66.15%, demonstrating strong diagnostic efficacy and supporting its potential as a biomarker for the early diagnosis of CC.

The expression level of miR-378a in the plasma of CC patients was found to be significantly different from that of HC and was correlated with clinical features of CC such as TNM stage, tumor size, and lymph node metastasis. However, several limitations in this study may affect the generalizability and reproducibility of the results.

First, the findings have not yet been verified in independent cohorts. This limits the generality of the findings and may affect reproducibility. To address this issue, future studies should validate miR-378a expression levels in different patient populations to ensure its stability and reliability as a biomarker. Although this study demonstrated the significant potential of miR-378a as a single marker, integrated modeling could address the limitations of single-marker approaches. The investigation of miR-378a provides an excellent basis for developing such an integrated approach. Future studies should systematically evaluate various biomarker combinations to identify the optimal combination that complements miR-378a, potentially transforming strategies for CC early detection.

Second, the cohort size in this study was relatively small, which may have limited the representativeness and generalizability of the results. To overcome this limitation, future studies should be conducted in larger patient populations to improve the statistical power and strengthen the generalization of the findings.

In addition, although the findings suggest that miR-378a is associated with clinical features of CC, this study did not account for all potential factors that may influence miR-378a expression, such as a patient's genetic background, lifestyle, and environmental factors. Future studies should consider these factors to provide a more comprehensive understanding of the role of miR-378a in CC development.

Despite these limitations, this study provides preliminary evidence supporting miR-378a as a potential biomarker for the early diagnosis of CC. These results provide new directions for future research and provide a new perspective for early diagnosis and treatment of CC. With further research and validation, miR-378a may become an important tool for early diagnosis and monitoring treatment response in CC.

In conclusion, miR-378a may have clinical applications as a potential biomarker for the early diagnosis of CC. However, its specific application still requires confirmation through extensive clinical studies.

5. Conclusion

Early diagnosis is critical for successful CC treatment. The lack of readily apparent early symptoms, coupled with the strong link between stage and prognosis, necessitates improved diagnostic tools and strategies to enhance patient outcomes.

Acknowledgments

None.

Funding

This project was supported by the Natural Science Foundation of Guangdong Province (grant number: 2018A0303130252) and the Discipline Construction Project of Guangdong Medical University (grant number: 4SG21011G).

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Hua Ye
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Investigation: Long Guo, Yan Zhang, Jun Liu
Methodology: Hua Ye
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Writing – review & editing: Xiaodan Li, Yanwen Liang, Wenjing Cheng

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Clinical Research of the Affiliated Hospital of Guangdong Medical College (approval no. PJ2016030KY and 3 March 2016). Written informed consent was obtained from all participants, including patients and healthy volunteers, before enrollment in the study. Specifically, healthy volunteers provided written informed consent after having been made aware of the purpose, procedures, potential risks, and benefits of this study.

Consent for publication

Verbal consent was obtained from all providers for the samples used in our study.

Availability of data

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

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