INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies in the world, with high incidence and mortality. Abnormally expressed microRNAs (miRNAs) are considered novel biomarkers in cancer diagnosis. The aim of this study was to investigate the diagnostic value of miR-92a-1 in patients with CRC. Serum samples were collected from 148 patients pathologically diagnosed with CRC and 68 gender- and age-matched healthy volunteers. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure serum miR-92a-1 level. Relationship between miR-92a-1 and clinicopathological features of CRC cases was analysed via chi-square test. Receiver operating characteristic (ROC) curve was plotted to estimate the diagnostic value of miR-92a-1 in CRC. Serum miR-92a-1 was significantly up-regulated in CRC patients compared with healthy individuals (P < .001). Moreover, miR-92a-1 expression was correlated with TNM stage (P = .02), histological stage (P = .003), lymph node metastasis (P = .003) and distant metastasis (P < .001). ROC analysis showed that the area under the ROC curve (AUC) was 0.914, suggesting high diagnostic accuracy of miR-92a-1 in ROC. The optimal cut-off value was 1.485, with a sensitivity of 81.8% and a specificity of 95.6%. MiR-92a-1 is increased in CRC patients and correlated with aggressive clinical characteristics. Serum miR-92a-1 may be a potential diagnostic biomarker for CRC.

KEYWORDS
Colorectal cancer, Diagnosis, MiR-92a-1

1 | INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies in the world, with high incidence and mortality. In China, the morbidity and mortality of CRC show rising trends, due to changes in lifestyle and diets. Although treatment methods for CRC have been improved, the survival rates of CRC patients are still not ideal. Tumour stage at diagnosis is a major factor influencing therapeutic effects. Reportedly, CRC patients diagnosed at early stage were more likely to be cured, while those in advanced stage frequently faced dismal prognosis. Thus, finding novel and effective biomarkers for early diagnosis of CRC is a valuable way to improve clinical outcome of the patients.

Growing evidences have indicated that the expression patterns of microRNAs (miRNAs) possess close association with tumour development and progression, which might hold the potential to serve as biomarkers for diverse cancers, including CRC. MiRNAs are a class of small non-coding RNAs and play important roles in regulating
gene expression.\textsuperscript{11} MiRNAs can bind to the 3' untranslated region (3' UTR) of their target mRNAs, thus preventing gene translation.\textsuperscript{12} Aberrant expression of miRNAs can contribute to various human diseases, like malignancy.\textsuperscript{13} In previous tumour investigations, numerous miRNAs were reported to act as oncogenes or tumour suppressors in the progression of CRC. For example, microRNA-429 (miR-429), miR-1260b and miR-10b played oncogenic roles in CRC development.\textsuperscript{14-16} While miR-526-3p, miR-185 and miR-452 could inhibit aggressive progression of the malignancy.\textsuperscript{17-19} Abnormally expressed miRNAs may provide a novel way for CRC diagnosis.

MicroRNA-92a-1 (miR-92a-1) is a member of miR-17-92 cluster. MiR-17-92 cluster includes four families: miR-17, miR-18, miR-19 and miR-92a.\textsuperscript{20} MiR-92a family includes four members: miR-25, miR-92a-1, miR-92a-2 and miR-363. The members of miR-92a family play significant roles in the development and progression of CRC, and might serve as biomarkers for the cancer. A study carried out by Li et al demonstrated that the expression of miR-25 was up-regulated in CRC tissues and that its overexpression predicted poor prognosis for the patients.\textsuperscript{2} In the study of Xu et al, the expression profile of miR-363 was confirmed to be a novel diagnostic biomarker for CRC.\textsuperscript{21} MiR-92a has ever been reported to be involved in the development and metastasis of CRC and serve as an effective biomarker for the cancer diagnosis and prognosis.\textsuperscript{22-24} Therefore, we speculated that the expression of miR-92a-1 might also act as an indicator in early detection of CRC.

In this study, we sought to investigate the expression level of miR-92a-1 in CRC patients, and its association with clinical characteristics. In addition, we explored the diagnostic value of miR-92a-1 in CRC.

## 2 | METHODS AND MATERIALS

### 2.1 | Patients and sample collection

The present study was a retrospective investigation. A total of 148 CRC patients were recruited from Cangzhou Central Hospital. Inclusion criteria for CRC patients were as follows: (a) pathophysiological confirmed; (b) not receiving any anti-tumour treatments previously; (c) possessing available clinical records. Meeting any one of the following conditions, patients would be excluded: (a) without pathological diagnosis; (b) dying within one month after diagnosis; (c) showing abnormal liver/kidney function and routine blood test results or other associated or co-existing diseases; (d) with other primary malignancies. In addition, 68 gender- and age-matched healthy volunteers were recruited as healthy controls in this study, who experienced physical examination in the physical examination centre of the hospital. None of the healthy individuals had the history of malignancies or abnormalities in liver/kidney function.

Blood samples were collected from CRC patients and healthy volunteers in the morning after fasting for 8-10h. The blood samples were centrifuged to isolate serum samples and stored at −80°C for further studies. Meanwhile, we recorded clinicopathological characteristics of the CRC patients, including age, gender, tumour size, tumour location, TNM stage, histological stage, lymph node metastasis and distant metastasis (Table 1). This study was approved by the ethics committee of the hospital. All participants provided written informed consents for this research.

### 2.2 | RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNAs were extracted from serum samples adopting TRizol reagent kit (catalogue number: 15596026, Invitrogen) as per the manufacturer’s protocols. The ratio of OD260/OD280 (1.9-2.0) was used to evaluate the purity of isolated RNAs. Total RNA was used for reverse transcription reaction employing TaqMan miRNA reverse transcription kit (catalogue number: 4366596, Applied Biosystems). Obtained cDNA was stored at −20°C until use.

Then, PCR was carried out in ABI 7300 Real-Time PCR System (Applied Biosystems) with SYBR Green PCR Master Mix (Applied Biosystems). Cycle threshold (CT) was defined as the cycle number of fluorescence signal reaching to threshold. We set threshold level at 0.1-0.3, and CT values of miRNA samples were automatically calculated based on miRNA abundance.\textsuperscript{25} U6 gene served as endogenous control to normalize relative expression level of miR-92a-1. All data were calculated with the method of 2\textsuperscript{−ΔΔCt}. Primer sequences were designed based on published data, as follows: miR-92a-1, forward-5'-ACACAGGTGGGATCGGTTG-3', and reverse-5'-CAAACTCAACAGGCAGCAGCAGGA-3'; U6, forward-5'-CTCGGCTCGGCAGCACA-3', and reverse-5'-AACGCTTACAGAATTTCAG-3'.

### 2.3 | Statistical analysis

Continuous data were present as mean ± SD and compared adopting Student’s t test. Chi-square test was used to examine the correlation of miR-92a-1 with clinicopathological features of CRC patients. Receiver operating characteristic (ROC) analysis was used to evaluate the diagnostic value of miR-92a-1 in CRC. All data analyses were performed in SPSS 21.0 statistical software. P-value less than .05 was considered as significant level.

## 3 | RESULTS

### 3.1 | Expression level of miR-92a-1

According to qRT-PCR, the expression of miR-92a-1 was significantly increased in CRC patients, compared with healthy controls (Figure 1).

### 3.2 | Association of miR-92a-1 with clinicopathological characteristics of CRC

In the current study, CRC patients were divided into high (n = 86) and low (n = 62) expression groups, according to their median expression...
| Features                  | Total N = 148 | MiR-92a-1 expression | P-values |
|---------------------------|---------------|----------------------|----------|
|                           | Low (n = 62)  | High (n = 86)        |          |
| Age (years)               |               |                      | .764     |
| ≤60                       | 57            | 23                   |          |
| >60                       | 91            | 39                   |          |
| Gender                    |               |                      | .721     |
| Male                      | 67            | 27                   |          |
| Female                    | 81            | 35                   |          |
| Tumour size (cm)          |               |                      | .471     |
| ≤5                        | 76            | 34                   |          |
| >5                        | 72            | 28                   |          |
| Location                  |               |                      | .785     |
| Colon                     | 64            | 26                   |          |
| Rectum                    | 84            | 36                   |          |
| TNM stage                 |               |                      | .020     |
| I–II                      | 74            | 38                   |          |
| III–IV                    | 74            | 24                   |          |
| Histological stage        |               |                      | .003     |
| Well; Moderate            | 74            | 40                   |          |
| Poor                      | 74            | 22                   |          |
| LN metastasis             |               |                      | .003     |
| Yes                       | 72            | 39                   |          |
| No                        | 76            | 23                   |          |
| Distant metastasis        |               |                      | <.001    |
| Yes                       | 78            | 22                   |          |
| No                        | 70            | 40                   |          |

**FIGURE 1** Serum level of miR-92a-1 in CRC patients and healthy individuals. The expression of miR-92a-1 was significantly up-regulated in patients with CRC compared with the healthy individuals. ***: suggested P < .001

relationship between miR-92a-1 and age, gender, or tumour size or location (all P > .05, Table 1).

### 3.3 Diagnostic value of miR-92a-1 in CRC

To explore diagnostic value of miR-92a-1 in CRC, ROC analysis was performed and showed an area under the ROC curve (AUC) of 0.914, suggesting its high diagnostic accuracy in CRC. The cut-off value of miR-92a-1 for CRC diagnosis was 1.485, with a sensitivity of 81.8% and a specificity of 95.6% (Figure 2).

### 4 DISCUSSION

CRC is a prevalent malignant tumour in the world. Despite significant improvements in surgical, neoadjuvant chemotherapy and radiotherapy, the survival rates of patients with CRC are still dismal. Early diagnosis is essential for survival in CRC patients. Until now, the gold standard for CRC diagnosis is colonoscopy, but its invasive nature limits its wide application in clinical practices. Commonly used serum biomarkers for CRC diagnosis include CEA and CA19–9. However, both of them only show low accuracy in CRC diagnosis.
Consequently, novel biomarkers with high sensitivity and specificity are urgently required for CRC diagnosis.

MiRNAs, short non-coding RNAs, play important roles in physiological and pathological conditions. Their expression profiles show close association with tumour development, progression and treatment response, suggesting their indicative functions in human malignancy. In addition, the expressions of miRNAs are stable and can be easily detected in archived tissue specimens and body fluids. According to existing documents, miRNAs may provide promising approaches for cancer diagnosis. In previous studies, several miRNAs have been confirmed to play predictive roles in the processes of CRC. Wang et al reported that circulating miR-210 level was significantly different between CRC patients and healthy individuals, which showed diagnostic and prognostic capability for the cancer. MiR-223, as another example, was reportedly up-regulated in CRC tissues, and its elevated expression was correlated with positive metastasis and poor prognosis in the patients. In a word, abnormally expressed miRNAs are involved in the aetiology of CRC and might serve as indicators for the cancer.

As a member of miR-17-92 cluster, miR-92a was correlated with the progression of CRC. Therefore, we speculated that miR-92a-1, the precursor of miR-92a, might play a crucial role in the progression of CRC. In the current study, we investigated serum level of miR-92a-1 in CRC patients and healthy individuals via qRT-PCR. The results suggested that serum miR-92a-1 was up-regulated in CRC patients compared to healthy individuals. Moreover, increased expression of miR-92a-1 was closely linked to advanced TNM stage, poor histological stage and positive lymph node metastasis and distant metastasis, which suggested that miR-92a-1 might be involved in CRC development and progression. Alterations in miR-92a-1 expression among CRC patients were normal, in comparison with healthy controls, and in further study, we will check the role of miR-92a-1 in the progression of CRC. Experimental data revealed that miR-92a-1, an oncogene, could promote aggressive progression of CRC. However, specific mechanisms for miR-92a-1 affecting CRC were not investigated in the present study. Further analyses are still required.

The members of miR-92a family play significant roles in the development and progression of CRC and might serve as biomarkers for the cancer. However, diagnostic performance of miR-92a-1 in CRC remained unclear. To examine diagnostic value of miR-92a-1, ROC curve was constructed in the current study. The results demonstrated that miR-92a-1 could distinguish CRC patients from healthy individuals with high sensitivity and specificity. MiR-92a-1 might be a potential diagnostic biomarker for CRC. It was worth noting that the sample size in the current study was relatively small, and the application value of miR-92a-1 in CRC diagnosis requires further identifications. Future studies should be performed to verify our findings and investigate relevant mechanism both in vivo and in vitro.

In summary, miR-92a-1 is up-regulated in CRC patients and correlated with malignant tumour development and progression. Serum miR-92a-1 may be a novel diagnostic biomarker for CRC.

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CONFLICT OF INTEREST
None.

AUTHOR CONTRIBUTIONS
YS conceived and designed the experiments; ZL conceived and performed the experiments; and YS prepared figures. YS and ZL wrote the main manuscript text. All authors reviewed the manuscript. With the approval of Cangzhou Central Hospital Ethics Committee, written informed consent was obtained from every patient.

DATA AVAILABILITY STATEMENT
All data generated or analysed during this study are included in this article.

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