RESEARCH ARTICLE

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Expression and Prognostic Value of Serum *microRNA- 375* and *microRNA- 182* in Correlation with Tissue Immunehistochemical Expression of Related Proteins, Serving as Novel Biomarkers in Colorectal Cancer

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Abstract

Background: Colorectal cancer (CRC) commonly evolves through precursor adenomatous polyps; however, reliable non-invasive biomarkers capable of distinguishing benign from malignant colorectal lesions remain limited. Circulating microRNAs have emerged as promising molecular indicators of tumour initiation and progression. **Objectives:** This study aimed to evaluate the diagnostic, prognostic, and mechanistic relevance of circulating *miR-375* and *miR-182* in patients with colorectal polyps and colorectal cancer. In addition, their performance was compared with conventional tumour markers and immunohistochemical staining of the tissues with *caspase-3* was performed to assess apoptotic activity in malignant and adenomatous tissue. **Methods:** A total of 450 participants were enrolled, including 150 patients with CRC, 150 patients with colorectal polyps, and 150 healthy controls. Clinical, anthropometric, biochemical, histopathological, and immunohistochemical data were collected. Circulating *miRNA-375* and *miRNA-182* expression levels were quantified and correlated with clinicopathological parameters. Diagnostic accuracy was assessed using receiver operating characteristic (ROC) curve analysis, and logistic regression models were applied to identify independent predictors of CRC. Immunohistochemical staining for *caspase-3* was performed on tissue samples. **Results:** *miR-375* expression showed a progressive and significant downregulation from controls to polyps and CRC, whereas *miR-182* demonstrated marked upregulation across the same spectrum (both $p < 0.001$). *miR-375* and *miR-182* exhibited excellent diagnostic performance for CRC detection. Logistic regression analysis identified *miR-375* (OR = 0.003, p = 0.002) and *miR-182* (OR = 8.254, p = 0.001) as independent predictors of CRC. Caspase positivity independently predicted lymph node metastasis (OR = 12.038, p = 0.004). *miR-375* demonstrated significant inverse correlations with CEA and CA 19-9 levels. **Conclusions:** The reciprocal dysregulation of *miR-375* and *miR-182* reflects a molecular transition from benign colorectal polyps to invasive CRC. These circulating miRNAs exhibit superior diagnostic accuracy compared with conventional tumour markers and may represent valuable non-invasive tools for early detection and risk stratification of colorectal cancer.

Keywords: microRNA profiling- adenoma–carcinoma sequence- biomarker validation- apoptosis- lymph node metastasis

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Introduction

Colorectal cancer (CRC) is among the most prevalent malignancies globally and remains a leading cause of cancer related mortality, despite advances in surgical and medical management. Early detection significantly improves survival, as localized CRC exhibits markedly better prognosis than advanced disease. Standard screening modalities, including colonoscopy and faecal occult blood testing, are effective but limited by their invasive nature, variable sensitivity for early lesions, cost, and suboptimal patient compliance [1, 2]. Consequently, there is a persistent need for reliable, minimally invasive biomarkers capable of detecting both precancerous lesions and early-stage CRC, ideally with prognostic and predictive potential.

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression post-transcriptionally, modulating key cellular processes such as proliferation, apoptosis, angiogenesis, and differentiation. Dysregulation of miRNA networks is increasingly recognized as a hallmark of cancer, contributing to initiation, progression,

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metastasis, and therapy resistance [2, 3]. Circulating miRNAs are stable in serum, plasma, and other biofluids due to protection within extracellular vesicles or protein complexes, making them attractive candidates for liquid biopsy biomarkers [4]. Recent meta-analyses have confirmed the diagnostic potential of circulating miRNAs in CRC, showing high sensitivity and specificity, often surpassing traditional biomarkers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19 9) [4, 5]. Moreover, many miRNAs map directly to oncogenic pathways, including PI3K/AKT, Wnt/β-catenin, and epithelial-mesenchymal transition signalling, highlighting their potential as mechanistic indicators of disease progression [5, 6].

Among candidate miRNAs, miR 375 has been consistently reported as a tumour suppressor, with downregulation linked to CRC progression and poor prognosis, whereas miR 182 exhibits oncogenic properties, promoting proliferation, invasion, and metastasis [5-7]. Despite these promising findings, studies directly comparing the expression of specific circulating miRNAs across healthy controls, patients with colorectal polyps (premalignant lesions), and CRC within the same cohort remain limited. Furthermore, their clinical applicability for risk stratification, early diagnosis, and prognostic prediction has not been fully established.

caspase-3 (*CASP3*) is a major mediator of apoptosis activated during cellular exposure to cytotoxic drugs, radiotherapy, or immunotherapy. It is often used as a marker for efficacy of cancer therapy. However, recent reports indicate that *caspase-3* also has non-apoptotic roles such as promotion of tumor relapse and tumor angiogenesis. Therefore, the roles of *caspase-3* in tumor progression remain to be defined clearly [8].

Therefore, the present study aimed to evaluate the expression profiles of circulating miR 375 and miR 182 as well as caspase immunohistochemical expression across the spectrum of colorectal neoplasia, including healthy individuals, patients with colorectal polyps, and patients with CRC (Figure 1, 2). We also aimed to assess their diagnostic, clinical, and prognostic relevance, exploring correlations with tumour stage, histological grade, lymph node involvement, and standard laboratory biomarkers. By delineating these relationships, this study seeked to provide insights into the molecular mechanisms underlying colorectal tumorigenesis and evaluate the potential of these miRNAs as clinically actionable biomarkers for early detection and disease management.

Materials and Methods

Ethical approval

Prior to study initiation, ethical approval was obtained from the Institutional Review Board of the Theodor Bilharz Research Institute (FWA00010609); approval number PT :(775), in compliance with the principles of the Declaration of Helsinki (1964) and its subsequent revisions. Written informed consent was obtained from all participants prior enrollment. Recruitment was conducted between October 2023 and September 2024 through inpatient hepatology and gastroenterology units, as well as outpatient clinics of surgery department at the Theodor Bilharz Research Institute.

Study population

The study population comprised a total of 450 participants, stratified into three well-defined groups. The colorectal cancer (CRC) group included 150 patients with histopathologically-confirmed colorectal adenocarcinoma. Inclusion criteria for this group were newly diagnosed, treatment-naïve CRC cases, confirmed by colonoscopic biopsy or surgical resection of colorectal masses followed by histopathological examination. Patients with a prior history of malignancy, previous chemotherapy or radiotherapy, inflammatory bowel disease, hereditary colorectal cancer syndromes, or concurrent severe systemic or autoimmune disorders were excluded.

The colorectal polyp group consisted of 150 patients with endoscopically detected and histologically verified colorectal polyps. Eligible participants had benign adenomatous or hyperplastic polyps without evidence of invasive malignancy. Exclusion criteria included a history of colorectal cancer, inflammatory bowel disease, hereditary polyposis syndromes, previous colorectal surgery, or any prior oncological treatment.

The control group comprised 150 apparently healthy, age- and sex-matched individuals with no personal history of colorectal neoplasia, inflammatory bowel disease, irritable bowel syndrome, chronic diarrhoea, or constipation. Controls were also required to have no history of malignancy, chronic inflammatory or metabolic disorders, or ongoing medication known to influence inflammatory or metabolic status. Collectively, these stringent inclusion and exclusion criteria were applied to ensure homogeneity within groups and to minimize confounding factors that could influence circulating biomarker expression.

Clinical, Anthropometric, and Biochemical Assessment

All participants underwent a standardized clinical evaluation, including detailed medical history, smoking status, and family history of colorectal cancer. Anthropometric measurements were obtained using uniform protocols: body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2) and serves as a global indicator of overall adiposity; elevated BMI has been consistently associated with increased CRC risk in large prospective cohorts [9]. Waist circumference (WC) was measured at the midpoint between the lowest rib and the iliac crest and reflects central (abdominal) adiposity, which is more strongly linked to CRC risk than BMI alone due to its association with metabolic dysfunction and chronic inflammation [10, 11]. Waist-to-hip ratio (WHR) was calculated as the ratio of waist to hip circumference and quantifies body fat distribution; higher WHR has been independently associated with greater CRC risk, particularly in both sexes [12, 13]. Fasting venous blood samples were collected for biochemical analyses, including total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), liver and kidney function tests in the form of serum alanine aminotransferase (ALT)

activity, aspartate aminotransaminase (AST) activity, total protein, albumin, urea and creatinine. They were assayed by the Beckman Coulter AU 480 autoanalyzer .

For colorectal cancer patients, clinicopathological data such as tumour site, histological grade, stage, and lymph node involvement were obtained from medical records. These clinical and biochemical parameters were correlated with circulating studied miRNA expression to investigate associations with tumour presence and progression.

miRNA Extraction and Quantification

Total RNA was isolated from serum samples using the miRNeasy Serum/Plasma Kit (Qiagen , Cat. No.: 217184, supplied by Clinilab), following the manufacturer's instructions, and stored at −70 °C in RNase-free 1.5 mL vials. Two µL of C. elegans miR-39 miRNA mimic (Cat. No.: MS00019789) was spiked into each RNA sample to serve as an exogenous reference gene. RNA purity and concentration were assessed by spectrophotometry at 260/280 nm using a NanoDrop device. Reverse transcription of miRNAs was performed using the miScript II RT Kit (Qiagen, Cat. No.: 218160, supplied by Clinilab), which employs oligo-dT primers with a 3′ degenerate anchor and a 5′ universal tag sequence to enable amplification of mature miRNAs. The final cDNA volume was 20 µL, prepared according to the manufacturer's instructions using miScript HiSpec Buffer, and the reactions were carried out on a Biometra Thermal Cycler. The resulting cDNA was diluted to 200 µL in RNase-free water and stored at −20 °C until further analysis. Quantitative real-time PCR (qPCR) was performed using the miScript SYBR® Green PCR Kit (Qiagen, Hilden, Germany) with predesigned miScript Primer Assays for hsa-*miR-375* (Qiagen, Cat. No.: MS00007328, miRBase Accession: MI0000801) and hsa-*miR-182* (Qiagen, Cat. No.: MS00003705, miRBase Accession: MI0000280), (Table 1) The qPCR reactions were run on a QuantStudio™ 5 Real-Time PCR System with a melting curve analysis performed at the end of each run. Expression levels of miRNAs were normalized to the exogenous C. elegans miR-39 reference and calculated using the comparative ΔΔCT method set [14, 15]. Relative expression [fold change] for each candidate miRNA within each group was then calculated using the equation: 2 -ΔΔCT [15]. The ΔCT for each miRNA in each sample [cases] was calculated as follows: ΔCT sample = CT miR (375 or 182) - CT Cel mir-39. Then, ΔΔCT was calculated: ΔΔCT = ΔCT patients - ΔCT controls. This approach allowed precise quantification of *miR-375* and *miR-182* expression in CRC, polyp, and control serum samples for downstream diagnostic and prognostic analyses. Relative miRNA expression levels were calculated using the 2^{-} ΔΔCt method. Fold regulation was determined relative to healthy controls after normalization to the endogenous reference gene [15]. To ensure the reliability and accuracy of miRNA quantification, serum samples with a cycle threshold (CT) value greater than 40 in the qPCR assay were excluded from further analysis. Such high CT values were considered indicative of undetectable or extremely low target expression, likely reflecting technical limitations rather than true biological absence. Exclusion of these samples minimized potential bias and ensured that downstream statistical analyses, including relative quantification and comparative ΔΔCT calculations, were performed only on samples with robust and reproducible amplification signals, in accordance with established qPCR quality control standards.

Immunohistochemical Assessment of caspase-3

Tissue biopsies from the colorectal masses were obtained by colonoscopy from the studied patients. Tissue specimens were fixed and preserved in 10% neutral buffered formalin, processed and embedded in paraffin blocks. Tissue sections of 4 microns thickness were stained with Hematoxyllin and Eosin for histopathological examination and definitive pathological diagnosis, and then immunostained for *caspase-3*.

Immunohistochemical analysis for *caspase-3* was conducted on formalin-fixed, paraffin-embedded colorectal tissue sections of 4 µm thickness. Sections were initially pretreated with proteinase K (Agilent Dako, CA, USA) to facilitate antigen retrieval, followed by washing with phosphate-buffered saline (PBS) for 5 minutes. Tissue slices were then incubated for 60 minutes at 37 °C with a primary antibody targeting *caspase-3* (Santa Cruz Biotechnology, USA). After washing, a secondary antibody (AgilentDako, CA, USA) was applied for an additional 60 minutes. The immunoreactive signal was visualized using 3,3'-diaminobenzidine (DAB) chromogen (Agilent Dako, CA, USA), producing brown cytoplasmic staining. Quantification was performed by assessing the percentage of *caspase-3*–positive cells in 10 consecutive high-power fields (×400) per specimen, providing a semi-quantitative measure of apoptotic activity in colorectal cancer tissues (Figure 3).

Statistical analysis

Data were coded and analyzed using IBM SPSS Statistics version 28 (IBM Corp., Armonk, NY, USA). Quantitative variables were summarized as mean ± standard deviation, median, minimum, and maximum, while categorical variables were presented as frequencies and percentages. Group comparisons for normally distributed quantitative variables were performed using unpaired t-tests or one-way analysis of variance (ANOVA) with post hoc multiple comparisons. For non-normally distributed quantitative variables, the Kruskal-Wallis test or Mann-Whitney U test was applied. Categorical data were compared using the Chi-square (χ^2) test, with Fisher's exact test used when expected frequencies were below five. Correlations between quantitative variables were assessed using Spearman's correlation coefficient. Receiver operating characteristic (ROC) curves were constructed to determine the optimal cutoff values of significant parameters for detecting CRC or polyp cases. Logistic regression analysis was conducted to identify independent predictors of advanced stage disease or lymph node positivity in CRC patients. A p-value <0.05 was considered statistically significant [16-19]. A priori sample size calculation was performed using G*Power software (version 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Germany). Based on an anticipated medium effect size

(d = 0.5), alpha error probability of 0.05, and statistical power of 80%, the minimum required sample size was calculated to be 128 participants per group for independent comparisons. To increase statistical robustness and account for potential data loss, 150 participants were included in each group.

Results

Baseline Demographic and Clinical Characteristics

A total of 450 participants were included: 150 healthy controls, 150 patients with colorectal polyps, and 150 patients with colorectal cancer (CRC). The three groups were comparable with respect to age and sex distribution, with no statistically significant differences observed (Table 2).

Smoking prevalence increased progressively across the groups, being absent in controls, present in 24.0% of polyp patients, and markedly higher in CRC patients (54.0%; $p < 0.001$). A positive family history of colorectal cancer in first-degree relatives was significantly more frequent among CRC patients (68.0%) compared with polyp patients (36.0%; $p = 0.001$). Central obesity was significantly more prevalent in CRC than polyp patients (88.0% vs. 72.0%; $p = 0.046$). Clinical symptoms, including chronic diarrhoea and history of mucus or bloody stool, were more frequently reported in polyp and CRC groups, although the difference between these two groups did not reach statistical significance. A history of inflammatory bowel disease was significantly associated with CRC (68.0%) compared with polyp patients (22.0%; $p < 0.001$).

Endoscopic, Histopathological, and Tumour Characteristics

Among patients with colorectal polyps, adenomatous polyps were equally distributed between high-grade and low-grade dysplasia. Endoscopic findings revealed either rectal adenomatous polyps (46.0%) or multiple colonic polyps (54.0%). CRC cases predominantly presented as rectal or sigmoid lesions, with moderately differentiated adenocarcinoma accounting for 80.0% of tumours. Most CRC patients had T3 tumours (60.0%), while lymph node involvement (N2) was observed in 40.0% of cases. Caspase immunohistochemical expression showed a striking contrast between groups, being uniformly positive in polyp tissues (100%), while 78.0% of CRC tissues were caspase-negative ($p < 0.001$), indicating altered apoptotic signalling with malignant transformation (Table 3).

Anthropometric and Metabolic Parameters

Anthropometric measurements demonstrated a stepwise increase in waist circumference, waist-to-hip ratio, and BMI from polyp patients to CRC patients, with highly significant intergroup differences ($p < 0.001$ for all). CRC patients exhibited the highest BMI and central adiposity indices. Biochemical analysis revealed progressive metabolic derangements across the disease spectrum. CRC patients showed significantly lower haemoglobin and platelet counts, alongside marked elevations in liver enzymes (ALT, AST, ALP), bilirubin levels, fasting glucose, total cholesterol, triglycerides, and LDL cholesterol ($p < 0.001$ for all comparisons). Serum albumin and total protein levels were significantly reduced in CRC patients, reflecting systemic inflammation and nutritional compromise (Table 4). Post hoc pairwise analysis confirmed that most laboratory abnormalities were significantly more pronounced in CRC compared with both controls and polyp patients.

Tumour Markers and Circulating miRNA Expression

Serum CEA and CA 19-9 levels were significantly elevated in CRC patients compared with polyp patients ($p < 0.001$), with wide variability reflecting tumour burden heterogeneity. Circulating *miR-375* expression showed a marked and progressive downregulation from controls to polyps and CRC, whereas *miR-182* demonstrated a strong stepwise upregulation, reaching its highest levels in CRC patients (both $p < 0.001$). Post hoc analysis confirmed statistically significant differences between all group pairs for both miRNAs, confirming that circulating *miR-375* expression showed a marked and significant

Table 1. miRNA details, miRBase IDs, and Qiagen primer information for *miR-375* and *miR-182*

miRNA	miRBase Accession	Qiagen Primer Assay Cat. No.	Sequence (5′→3′)	Notes
hsa-miR-375	MI0000801	MS00007328	UUUUGUUCGUUCGGCUCGCGUGA	Mature miRNA, serum quantification
hsa-miR-182	MI0000280	MS00003705	UUUGGCAAUGGUAGAACUCACACU	Mature miRNA, serum quantification
cel-miR-39	MI00000039	MS00019789	UCACCGGGUGUAAAUCAGCUUG	Exogenous reference for normalization

Table 2. Demographic and Clinical Characteristics of theStudied Groups

Variable	Controls (n=150)	Polyps (n=150)	CRC (n=150)	P value
Age (years), mean ± SD	59.14 ± 4.56	58.64 ± 6.63	59.34 ± 5.31	0.811
Male sex, n (%)	108 (72.0)	102(68.0)	102 (68.0)	0.882
Smoking, n (%)	0 (0.0)	36 (24.0)	81 (54.0)	<0.001
Family history, n (%)	0 (0.0)	54 (36.0)	102 (68.0)	0.001
Central obesity, n (%)	0 (0.0)	108 (72.0)	132 (88.0)	0.046
Chronic diarrhoea, n (%)	0 (0.0)	99 (66.0)	72 (48.0)	0.069
History of IBD, n (%)	0 (0.0)	33 (22.0)	102 (68.0)	<0.001

Table 3. Endoscopic, Pathological, and Staging Characteristics of CRC and Polyp Patients

Parameter	Polyps (n=150)	CRC (n=150)
Adenoma, low-grade dysplasia	75 (50.0)	–
Adenoma, high-grade dysplasia	75 (50.0)	–
Rectal adenomatous polyp	69 (46.0)	–
Multiple colonic polyps	81 (54.0)	–
Moderately differentiated carcinoma	–	120 (80.0)
Well differentiated carcinoma	–	30 (20.0)
T3–T4 tumors	–	120 (80.0)
Lymph node positive (N2)	–	60 (40.0)

Data expressed as number and percentage.

Table 4. Anthropometric and Laboratory Parameters Across the Study Groups.

Parameter	Controls	Polyps	CRC	P value
Hemoglobin (g/dL)	13.78 ± 1.12	12.05 ± 1.70	10.68 ± 1.54	<0.001
Platelets (×10³/µL)	277.8 ± 73.5	191.3 ± 72.2	118.6 ± 72.5	<0.001
Albumin (g/dL)	4.17 ± 0.37	3.78 ± 0.67	2.85 ± 0.46	<0.001
ALT (U/L)	15.0 ± 7.1	52.9 ± 61.9	70.6 ± 56.2	<0.001
ALP (U/L)	62.7 ± 20.5	64.4 ± 50.1	167.4 ± 95.1	<0.001
Glucose (mg/dL)	79.7 ± 10.0	122.0 ± 59.9	222.9 ± 92.4	<0.001

Data expressed as mean ± 2 SD.

downregulation in CRC as compared to both polyps' group and healthy controls. On the contrary, *miR-182* demonstrated a strong significant upregulation in CRC patients as compared to polyps and controls (Table 5).

Association of miRNAs with Tumour Characteristics in CRC

Within the CRC group, *miR-375* expression was significantly lower in moderately differentiated tumours compared with well-differentiated tumours (p = 0.049). No significant associations were observed between *miR-375* or *miR-182* and tumour stage, lymph node status, or caspase expression intensity. Similarly, CEA and CA 19-9 levels did not differ significantly according to tumour stage, grade, or lymph node involvement. However, caspase positivity was significantly associated with tumour stage and lymph node metastasis (p = 0.046, 0.004 respectively), indicating its potential relevance in tumour aggressiveness (Supplementary Table 1, Figure 4).

Correlation Analysis

Correlation analysis stratified by study groups, distinct patterns were observed between circulating miRNA levels and clinic-metabolic parameters. In the control group, neither *miR-375* nor *miR-182* showed significant correlations with age or lipid profile parameters, although *miR-182* demonstrated a moderate positive association with triglyceride levels (r = 0.388, p = 0.005). Among patients with colorectal polyps, no statistically significant correlations were detected between either miRNA and anthropometric or lipid variables, with only borderline associations noted between *miR-375* and HDL-cholesterol (r = 0.271, p = 0.057) and between *miR-182* and triglycerides (r = −0.265, p = 0.063). In contrast, the CRC group exhibited more pronounced and biologically relevant associations. *miR-182* levels correlated positively with measures of central adiposity, including waist circumference (r = 0.364, p = 0.009) and waist-to-hip ratio (r = 0.330, p = 0.019), as well as with total cholesterol (r = 0.331, p = 0.019) and LDL-cholesterol (r = 0.327, p = 0.020), while showing an inverse correlation with HDL-cholesterol (r = −0.282, p = 0.048). Conversely, *miR-375* displayed consistent negative trends with anthropometric indices in CRC patients, although these associations did not reach statistical significance. Collectively, these findings suggest a disease-specific relationship between *miR-182* expression and metabolic dysregulation in

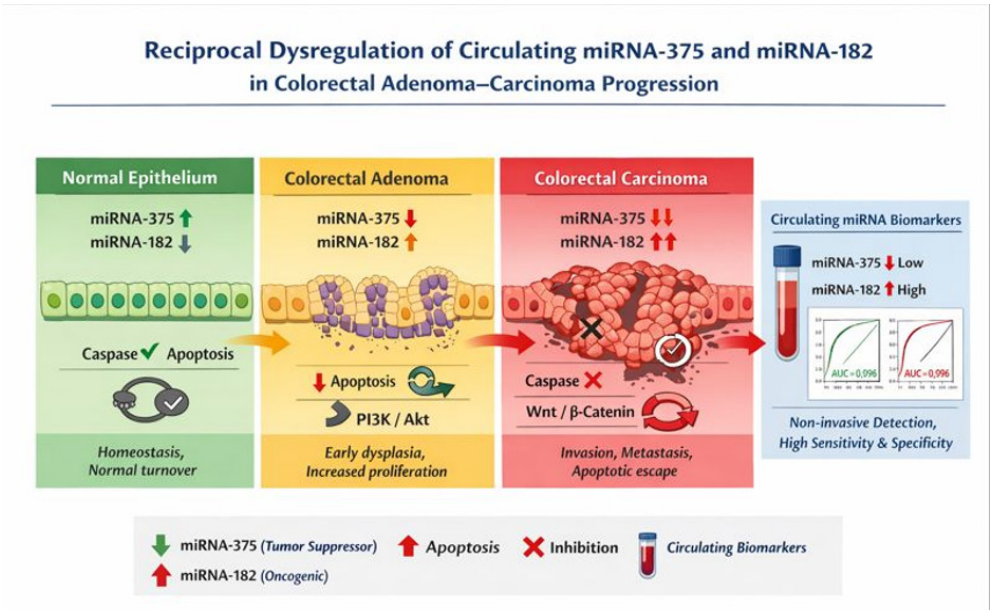

Figure 1. Graphical Abstract

Table 5. Tumour Markers and Circulating miRNA Expression Levels

Marker	Controls (n= 150)	Polyps (n= 150)	CRC (n= 150)	p value
CEA (ng/mL)	–	7.11 ± 16.25	160.0 ± 163.7	<0.001
CA 19-9 (U/mL)	–	26.0 ± 18.7	179.0 ± 212.8	<0.001
miRNA-375 (Fold regulation)	1.27 ± 0.41	0.68 ± 0.43	0.07 ± 0.12	<0.001
miRNA-182 (Fold regulation)	0.94 ± 0.49	2.38 ± 0.68	9.49 ± 8.84	<0.001

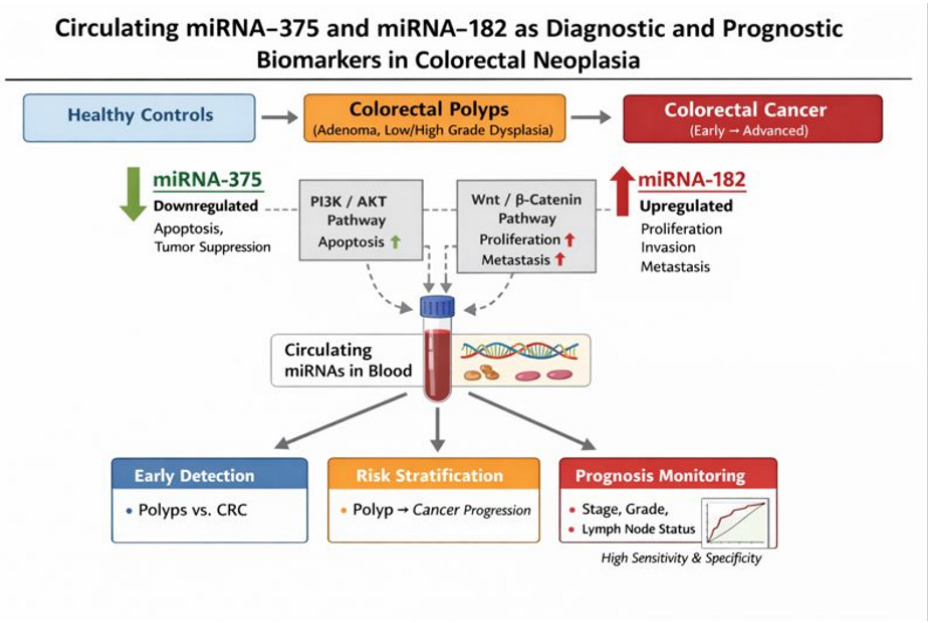

Figure 2. Graphical Summary Figure Representing the Study's Concepts

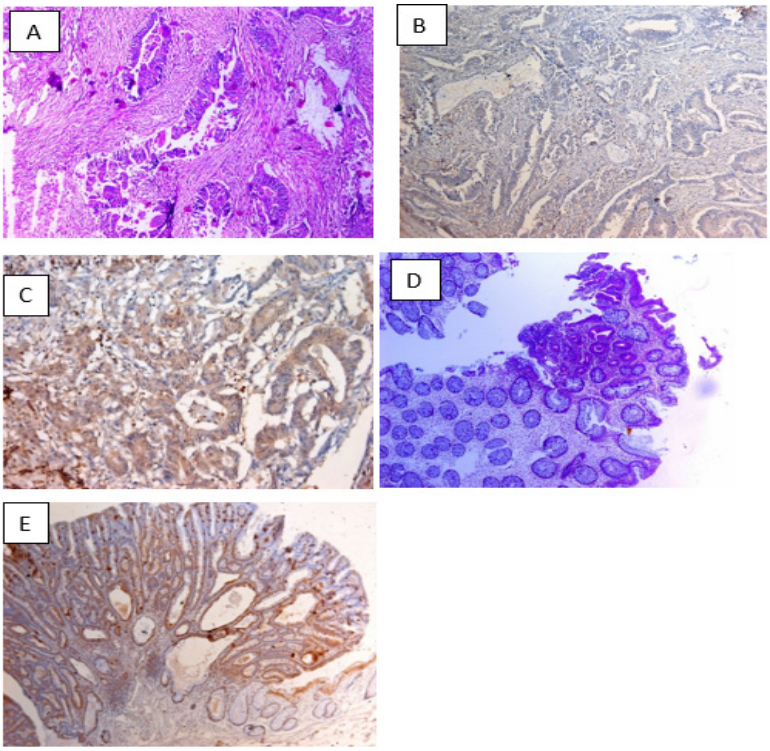

Figure 3. *Caspase-3* immunostaining in colorectal tissues. (A) Moderately Differentiated Adenocarcinoma of colon: Distorted, irregular and cribriform glands lined by pleomorphic cells with hyperchromatic nuclei. (H&E X100). (B) Negative Caspase 3 IHC staining at colonic Adenocarcinoma (X100).(C) Positive Caspase 3 IHC staining at colonic Adenocarcinoma (X100, 200 respectively) (Mild positivity).(D) Tubular Adenoma shows Irregular glands lined by hyperchromatic cells with nuclear stratification (H&Ex50).(E) IHC Caspase 3 staining show moderate positive staining (x50). (B) *Caspase-3* negative adenocarcinoma; (C) *Caspase-3* positive adenomatous polyp; (D) *Caspase-3* negative adenomatous polyp. Positive cytoplasmic staining is indicated by arrows. Images at 400× magnification.

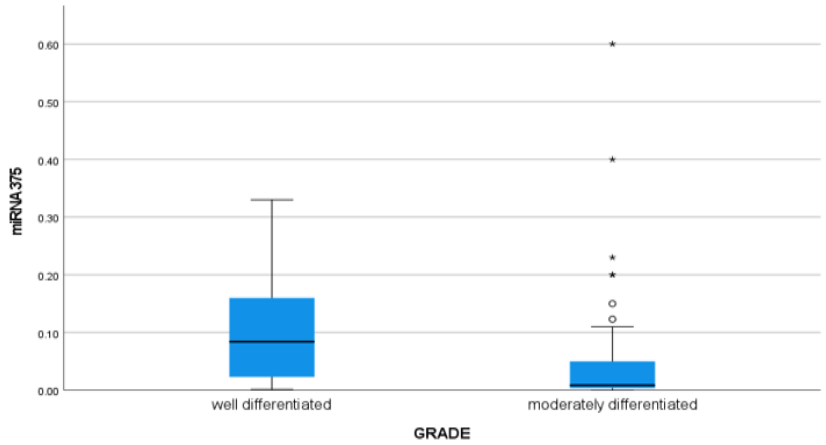

Figure 4. Association of miR-375 with Tumour Grade in CRC

colorectal cancer, which was not evident in controls or polyp patients (Supplementary Table 1, Figure 5-Supplementary Figures 1-8).

In CRC patients, *miR-375* levels did not differ significantly between males and females. In contrast, *miR-182* expression was significantly higher in males than in females, indicating a sex-related variation that may have biological or clinical relevance in colorectal cancer (Supplementary Table 2).

Diagnostic Performance of miRNAs and Tumour Markers

ROC curve analysis demonstrated that circulating *miR-375* and *miR-182* exhibit excellent diagnostic performance in colorectal cancer (CRC) (Supplementary Figures 9-14).. *miR-375* distinguished CRC from healthy controls with an AUC of 0.998 (95% CI: 0.992–1.000) at a cut-off ≤0.74, achieving 100% sensitivity and 98% specificity (PPV 96.1%, NPV 97.9%, p < 0.001). *miR-182* showed similar accuracy for CRC versus controls (AUC 0.996, 95% CI: 0.988–0.999, cut-off >2.1, sensitivity 98%, specificity 98%, PPV 94.1%, NPV 95.9%, p < 0.001). For differentiating CRC from polyps, *miR-182* achieved an AUC of 0.927 (95% CI: 0.880–0.974, cut-off >0.995, sensitivity 88%, specificity 90%), whereas *miR-375* yielded an AUC of 0.907 (95% CI: 0.851–0.963, cut-off <0.0545, sensitivity 70%, specificity 98%). *miR-182* also discriminated polyps from controls with high accuracy (AUC 0.970, 95% CI: 0.937–1.002, cut-off >1.525, sensitivity 94%, specificity 92%). Among conventional tumor markers, CEA showed superior performance for CRC versus polyps (AUC 0.986, 95% CI: 0.965–1.007, cut-off >14 ng/mL, sensitivity 98%, specificity 94%), while CA 19-9 achieved an AUC of 0.976 (95% CI: 0.951–1.002, cut-off >40.5 U/mL, sensitivity 92%, specificity 92%) (all p < 0.001). These findings highlight the potential of circulating *miR-375*, *miR-182*, CEA, and CA 19-9 as reliable biomarkers for CRC detection and differentiation from colorectal polyps (Supplementary Table 3-4).

Regression Analysis

Logistic Regression Analysis for Lymph Node Positivity

Multivariate logistic regression was performed to identify independent predictors of lymph node (LN) positivity in colorectal cancer patients. *caspase-3* positivity emerged as a significant independent predictor of LN metastasis (B = 2.488, OR = 12.038, 95% CI: 2.163–66.984, p = 0.004). In contrast, neither *miR-375* (B = -1.444, OR = 0.236, 95% CI: 0.001–51.413, p = 0.599) nor *miR-182* (B = 0.009, OR = 1.009, 95% CI: 0.939–1.085, p = 0.801) showed significant predictive value.

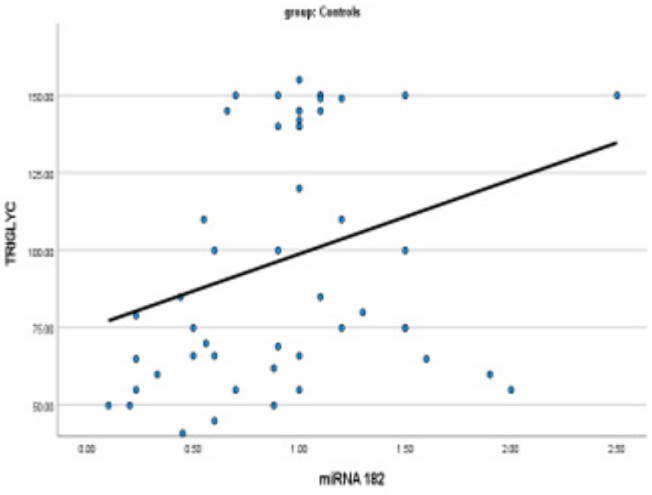

Figure 5. Correlation of miR-182 with Serum Triglycerides in Control Group

These findings suggest that while circulating miRNAs may have diagnostic utility, *caspase-3* expression in tumor tissue is a more robust marker for predicting LN metastasis in colorectal cancer (Supplementary Table 6, Supplementay Figure 15). Multivariate logistic regression was performed to assess the predictive value of circulating *miR-375* and *miR-182* for colorectal cancer. *miR-375* exhibited a strong inverse association with CRC (OR = 0.003, 95% CI: 0.000–0.107, p = 0.002), while *miR-182* showed a significant positive association (OR = 8.254, 95% CI: 2.325–29.302, p = 0.001). The regression model was statistically significant, with the following equation: Logit(P) = −5.124 − 5.974 × *miR-375* + 2.111 × *miR-182*. These findings indicate that low *miR-375* and high *miR-182* expression are independent predictors of colorectal cancer in the studied cohort (Supplementary Table 7, Supplementary Figure 16).

Discussion

Colorectal cancer (CRC) evolves through a multistep adenoma–carcinoma sequence, characterized by the accumulation of genetic and epigenetic alterations. Early detection of premalignant lesions remains critical to improve prognosis. The present study demonstrates a distinct molecular and clinicopathological continuum from colorectal polyps to invasive colorectal cancer, characterized by progressive metabolic dysregulation, suppression of apoptotic signaling, and marked alterations in circulating microRNA expression.

Central obesity was significantly more prevalent in CRC patients than in those with colorectal polyps, supporting growing evidence that visceral adiposity is a critical driver of colorectal carcinogenesis. Large prospective and case–control studies have demonstrated that central obesity measures, particularly waist circumference and waist-to-hip ratio, are more strongly associated with CRC risk than body mass index, likely through mechanisms involving insulin resistance, chronic low-grade inflammation, and altered adipokine signaling [9]. The progressive biochemical abnormalities observed in CRC patients including anemia, thrombocytopenia, elevated liver enzymes, hyperglycemia, and atherogenic dyslipidemia reflect the systemic metabolic and inflammatory burden accompanying advanced disease. Hypoalbuminemia and reduced total protein levels further indicate cancer-associated inflammation, hepatic dysfunction, and nutritional depletion, all of which have been linked to poor prognosis and aggressive tumor behavior in CRC [10]. Collectively, these findings support a metabolic–inflammatory continuum in which central obesity and metabolic derangement contribute to colorectal tumor progression from benign polyps to invasive malignancy.

A key finding of this study the is the reciprocal dysregulation of circulating *miRNA-375* and *miRNA-182* across healthy controls, patients with colorectal polyps, and CRC patients, highlighting their potential as minimally invasive biomarkers for early detection, disease monitoring, and prognostication. *miRNA-375* demonstrated a progressive decrease from controls to polyps and CRC, consistent with its role as a tumour suppressor [20-22]. Mechanistically, *miRNA-375* inhibits oncogenic pathways including PI3K/Akt and YAP1, promotes apoptosis and maintains epithelial homeostasis [21]. Its early downregulation in polyps may facilitate dysplastic transformation, while further suppression in CRC may permit malignant progression, invasion and metastasis.

Conversely, *miRNA-182* showed a significant and stepwise increase from normal controls to CRC, corroborating its role as an oncogenic mRNA (oncocomiR). *miRNA-182* promotes tumorigenesis via Wnt/β-catenin activation, cell-cycle progression, and apoptotic inhibition, consistent with our observation of reduced caspase activity in CRC tissues [22-24]. The reciprocal expression of these miRNAs suggests a coordinated shift favouring oncogenic dominance and apoptotic escape during colorectal tumorigenesis. The lower expression of *miRNA-375* in moderately differentiated compared with well-differentiated colorectal tumors supports its tumor-suppressive role in preserving epithelial differentiation and apoptotic control [21]. The lack of significant associations between circulating *miRNA-375* or *miRNA-182* levels and tumor stage or nodal status suggests that circulating miRNAs predominantly reflect early molecular alterations rather than tumor burden, as previously reported [4]. Similarly, conventional tumour markers such as CEA and CA 19-9 showed limited prognostic discrimination. In contrast, *caspase-3* positivity was significantly associated with advanced stage and lymph node metastasis, underscoring the importance of apoptotic dysregulation in tumor aggressiveness [7]. Mechanistically, suppression of *miRNA-375* and upregulation of *miRNA-182* may converge on caspase-dependent pathways, facilitating apoptotic escape and metastatic progression. *miRNA-182* expression in CRC patients showed inverse association with HDL cholesterol which further supports a connection between *miRNA-182* and pro-tumorigenic lipid metabolism. In contrast, the non-significant inverse trends observed for *miRNA-375* align with its tumor-suppressive role and suggest that its downregulation in CRC may represent a generalized loss of metabolic and apoptotic control rather than a direct reflection of adiposity measures [9, 13]. Collectively, these findings indicate that *miRNA-182* may function as a molecular interface linking metabolic derangement to colorectal cancer progression.

The sex-specific elevation of miRNA-182, but not *miRNA-375*, in CRC suggests hormonal and metabolic modulation of oncogenic miRNA signalling. *miRNA-182* has been shown to be influenced by sex hormone–responsive pathways and to interact with PI3K/AKT and Wnt/β-catenin signalling, which may contribute to its higher expression in males and to sex disparities in CRC incidence and outcomes [9-10]. Male-predominant metabolic and inflammatory profiles may further amplify miRNA-182–driven tumorigenic effects [22]. In contrast, the absence of sex-related differences in *miRNA-375* is consistent with its role as a tumour suppressor primarily regulated by tumour-intrinsic mechanisms, including control of apoptosis and epithelial differentiation, rather

than systemic hormonal factors [21]. These findings highlight *miRNA-182* as a potential sex-informed biomarker in CRC.

Our ROC curve analysis revealed that circulating *miRNA-375* and *miRNA-182* outperformed conventional tumor markers CEA and CA 19-9 in discriminating colorectal cancer from healthy controls. Specifically, *miRNA-375* achieved near-perfect diagnostic accuracy (AUC = 0.998), while *miRNA-182* also demonstrated excellent discriminative capability (AUC = 0.996). These findings corroborate and extend previous reports, emphasizing the potential of these circulating miRNAs as minimally invasive biomarkers for early CRC detection. Notably, their robust performance at the adenoma stage underscores their clinical advantage over conventional markers, which often exhibit limited sensitivity for premalignant lesions [25, 26]. Clinically, the ability to detect high-risk polyps through blood-based miRNA profiling could prioritize patients for colonoscopy, improve screening efficiency, and reduce the burden of late-stage diagnosis. Furthermore, longitudinal monitoring of miRNA levels may allow assessment of recurrence risk or treatment response, offering personalized surveillance strategies. Within the CRC cohort, lower *miRNA-375* correlated inversely with CEA and CA 19-9 levels, suggesting an association with tumour burden. Elevated miRNA-182, although not consistently associated with stage or grade, aligns with prior studies linking its overexpression to aggressive features and metastasis [27, 28]. Notably, logistic regression indicated that *miRNA-182* and caspase intensity predicted lymph node positivity, highlighting potential prognostic value for disease spread [29, 30]. These findings suggest that combining miRNA profiling with conventional tumour markers may enhance risk stratification and guide therapeutic decisions. Moreover, given their mechanistic involvement in key oncogenic pathways, miRNAs may serve as therapeutic targets, with potential to restore apoptotic activity or inhibit proliferation. Our findings are consistent with several case–control studies demonstrating downregulation of *miRNA-375* and upregulation of *miRNA-182* in CRC and adenomas. Liu et al.. [30] reported elevated miRNA-182 in CRC patients with strong diagnostic performance (AUC ~0.92). Mahmoud et al. [5] confirmed similar trends in Egyptian patients, demonstrating high sensitivity and specificity for CRC detection using circulating miR 182 and miR 223. Meta-analytic evidence further supports the enhanced accuracy of multi miRNA panels over individual markers [31]. Gatttuso et al. [32] made bioinformatic analyses for four miRNA signatures both in serum and malignant tissues and found that, miR 375 3p may possess prognostic value due to their regulatory roles in critical molecular pathways and target genes involved in colorectal cancer initiation and progression which come in accordance with our results. Although our study identified a clear pattern of progressive downregulation of miRNA 375 and upregulation of miRNA 182 across controls, polyps, and CRC, indicating their diagnostic relevance, not all studies have reported uniform expression trends for these miRNAs across all sample types and populations. Additionally, matched tissue plasma comparisons have demonstrated discordant expression for several candidate miRNAs, indicating that plasma miRNA profiles do not universally reflect tumor tissue expression and may be influenced by technical and biological factors such as RNA isolation method and sample handling [33]. These discrepancies could be attributed to differences in sample type, RNA isolation methods, population heterogeneity, or tumour subtype. Notably, the concordance between circulating and tissue miRNA expression remains an area of ongoing investigation. Standardization of pre-analytical and analytical methods is therefore critical before clinical translation [1]. The reciprocal expression pattern observed here supports the development of multi-miRNA panels rather than reliance on single markers. In our study, multivariable logistic regression analysis demonstrated that *miR-182* emerged as a significant positive predictor of CRC, whereas *miR-375* showed a strong inverse association with disease presence. The observed upregulation of *miR-182* in CRC patients is consistent with its established oncogenic role. *miRNA-182* has been reported to promote tumor proliferation, invasion, and metastatic potential through modulation of key signaling pathways, including PI3K/AKT and Wnt/β-catenin signaling, as well as suppression of tumor suppressor genes [27]. Its positive correlations with central obesity indices and atherogenic lipid parameters further suggest a link between metabolic dysregulation and oncogenic miRNA activation in colorectal carcinogenesis. The markedly elevated odds ratio associated with *miR-182* in our model underscores its robustness as a molecular indicator of malignant transformation within colorectal tissue. Conversely, *miR-375* exhibited a significant protective association, with reduced expression strongly linked to CRC occurrence in our study. *miR-375* is recognized as a tumor suppressor miRNA involved in regulating apoptosis, epithelial differentiation, and cellular metabolism [20], which aligns with its negative regression coefficient observed in the current analysis. Furthermore, in lymph node prediction analysis, *caspase-3* positivity emerged as an independent predictor of nodal involvement, highlighting the role of apoptosis-related pathways in tumor progression and metastatic spread. Ahmed et al. [34] found that Caspase 3 and other apoptotic markers have been shown to correlate with clinicopathological features and prognosis in colorectal cancer which come in accordance with our results. The striking difference in caspase expression patterns between polyp and CRC tissues provides additional mechanistic insight. Uniform caspase positivity in polyps contrasted sharply with its loss in most CRC cases, indicating disruption of apoptotic execution pathways as lesions progress toward malignancy. Importantly, caspase positivity independently predicted lymph node metastasis, highlighting its potential role as a marker of tumor aggressiveness rather than tumor burden [35]. Several recent studies support the concept that multi miRNA panels outperform single markers in discriminating CRC from benign lesions and healthy controls, reflecting the multifactorial molecular landscape of colorectal tumorigenesis [32]. The derived logistic regression model in our study demonstrates that circulating miRNA levels are strong and independent predictors

of CRC in our cohort. Specifically, reduced *miR-375* expression and elevated *miR-182* levels exert opposing influences on disease probability, with *miR-375* acting as a protective factor and *miR-182* promoting tumorigenesis. The model equation, Logit(P) = −5.124 − 5.974 × *miR-375* + 2.111 × *miR-182*, quantitatively reflects this reciprocal regulation, consistent with mechanistic studies showing *miR-375* suppresses oncogenic pathways such as PI3K/AKT and YAP1, while *miR-182* enhances Wnt/β-catenin signaling and cell proliferation. Our findings support the clinical utility of combined circulating miRNA profiling as a non-invasive, molecularly-informed diagnostic and prognostic tool for CRC. They offer promising avenues for early detection, risk stratification and precision oncology interventions.

Study strengths and limitations

This study has several key strengths. By integrating clinical, biochemical, histopathological, and molecular data, it provides a comprehensive perspective on colorectal adenoma–carcinoma progression. The focus on circulating *miR-375* and *miR-182* offers novel mechanistic insights and identifies non-invasive biomarkers with high diagnostic and prognostic potential. Inclusion of well-characterized control, polyp, and CRC groups, alongside detailed post hoc and correlation analyses, strengthens the validity of observed associations and links miRNA dysregulation to tumor stage, grade, lymph node involvement, and caspase activity. ROC analyses further highlight their clinical applicability for early detection and risk stratification. Despite robust findings, several limitations warrant consideration. First, the study design limits causal inference. Second, although sample size was sufficient for diagnostic analyses, external validation in independent, multi-center cohorts is necessary. Third, longitudinal assessment of miRNA dynamics during adenoma progression and post-treatment surveillance was not performed. Finally, the influence of potential confounders such as diet, microbiota composition, and medication history were not fully controlled. Future prospective studies addressing these factors are essential for clinical translation.

In conclusion, this study demonstrates that circulating *miR-375* and *miR-182* exhibit reciprocal dysregulation along the adenoma carcinoma sequence, reflecting key molecular events in colorectal tumorigenesis. Their combined diagnostic and prognostic utility surpass conventional biomarkers, while integration with apoptotic markers such as *caspase-3* further enhances predictive performance. These findings support the development of multi-miRNA panels as minimally invasive tools for early detection, risk stratification, and prognostic assessment in CRC, offering a foundation for precision medicine strategies targeting the molecular drivers of colorectal cancer.

Author Contribution Statement

All authors contributed equally in this study.

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