

Association between the expression level of miRNA-374a and TGF- β 1 in patients with colorectal cancer

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Abstract. Colorectal cancer (CRC) poses a significant threat to global health with increasing incidence and mortality rates. Exploring different molecular mechanisms and aspects underlying the development and progression of CRC will promote the early diagnosis and lead to better outcomes. MicroRNAs (miRNAs/miRs) are one of the molecular aspects related to the development and diagnosis of CRC. The present study aimed to investigate the association between the expression levels of miRNA-374a and transforming growth factor (TGF)- β 1 in patients with CRC to explore the roles of these markers in carcinogenesis and their use as diagnostic biomarkers. For this purpose, 195 subjects were enrolled. The expression level of miRNA-374a was assessed in serum samples of 145 subjects (screening phase) in addition to 50 CRC tissue samples to validate the tumor-derived expression of miRNA-374a (confirmation phase). The analysis of miRNA-374a expression was performed by the extraction of miRNAs followed by reverse transcription-quantitative PCR. Additionally, the TGF- β 1 level was measured in all patients with CRC and control serum samples. ROC curve analysis was performed to determine the diagnostic potential of miRNA-374a and TGF- β 1. The patients with CRC exhibited significantly elevated levels of miRNA-374a compared with the healthy individuals, with a fold change of 12.92 ($P=0.0001$). Moreover, the expression of miRNA-374a varied, depending on the CRC grade; patients with grade I disease exhibited a 9.2-fold increase and those with grade II disease a 21.84-fold increase in miRNA-374a expression. The relative expression level of miRNA-374a in CRC tissues was significantly upregulated by 4.48-fold compared with the adjacent non-cancerous tissues ($P<0.001$). Furthermore, the TGF- β 1 expression level was significantly

elevated in patients with CRC compared with the controls ($P=0.0001$). The area under the curve was 0.9560 for CRC serum samples ($P<0.0001$), 0.8972 for CRC tissue ($P<0.0001$) and 0.8075 for TGF- β 1 expression levels ($P=0.001$). In addition, a positive correlation was found between the miRNA-374 expression level and TGF- β 1 serum levels ($\text{Rho}=0.8912$, $P<0.0001$). On the whole, the present study demonstrates that the elevated expression levels of miRNA-374 and TGF- β 1 in patients with CRC indicate their crucial roles in driving tumor progression and highlight their potential for use diagnostic biomarkers and therapeutic targets in CRC.

Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed types of cancer and is ranked a the third most common cancer worldwide. The age-standardized incidence rate (ASR) per 100,000 individuals is 11.4% for colon cancer and 7.6% for rectal cancer. CRC is the second leading cause of cancer-related mortality worldwide, with a mortality rate of 9.4%. Of note, ~1.93 million new cases of CRC (10% of new cancer cases) were diagnosed in 2020, with the ~935,000 related deaths attributed to the disease (1). This incidence is expected to reach 2.2 million new cases by the year 2030, with 1.1 million cancer-related deaths (2). Immense efforts are being made to decrease the high patterns of the incidence and mortality associated with CRC through cancer prevention, early diagnosis and appropriate treatment (2). Recently, it has been widely acknowledged that the molecular landscape of CRC varies according to the ethnic group and geographic location (3).

Egyptian patients with CRC have attracted attention among various populations due to distinct genetic and environmental factors that may influence disease progression and treatment outcomes (4). In Egypt, CRC was ranked as the fourth most commonly diagnosed type of cancer in females and the seventh in males in 2020. CRC represents 3.9% of new cases with a mortality rate of 3.3% and the 5-year prevalence in all ages has been shown to be 15.6% (5). The ASR per 100,000 subjects has been shown to be 6.3 in females and 6.6% in males (6). Thus, this significant burden of CRC, in terms of incidence and mortality, highlights the importance of investigating the various mechanisms that contribute to its development and underscores the importance of defining novel prognostic factors and therapeutic targets (7,8).

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CRC is a multifactorial disease influenced by lifestyle, environmental and genetic factors (9). CRC is a highly heterogeneous disease, with different causes and inter-individual variation. The majority of the colorectal malignant lesions are classified as adenocarcinomas. CRC occurs when the cellular and molecular signaling pathways are disrupted or dysregulated, which leads to the abnormal proliferation of colon or rectal cells. These abnormalities lead to cancer initiation, growth, progression and metastasis (10).

CRC progression and metastasis are great challenges and are responsible for treatment failure and the high mortality rates. In the early stages of cancer, cancer cells change from an epithelial phenotype to a mesenchymal phenotype and this process known as epithelial-mesenchymal transition (EMT), which results in the progression of the primary tumor, invasion and metastasis (11). Several signaling pathways, such as the Wnt/ β -catenin, transforming growth factor- β 1 (TGF- β 1), Notch, Hedgehog and NF- κ B play role in the development of EMT (12).

MicroRNAs (miRNAs/miRs) are key regulators of gene expression, exerting a significant impact on a variety of cellular functions, including differentiation, proliferation and death (13). miRNAs can function either as tumor suppressors or oncogenes, depending on the cellular context, environmental factors and ethnicity. Recently, miRNAs have emerged as promising novel biomarkers for CRC due to their roles in regulating gene expression involved in cancer progression. Specific miRNA profiles are associated with CRC diagnosis, prognosis, and treatment response, rendering them valuable tools for early detection and personalized therapy. The miRNA signatures offer a non-invasive diagnostic potential and provide insight into the molecular mechanisms driving CRC, paving the way for improved clinical outcomes through targeted therapeutic strategies (14). Several studies have proven that miRNA-374a plays a complex role in the etiology and spread of various types of cancer (15-18). Previous research has demonstrated that miRNA-374a may play a role in CRC tumorigenesis (19). It has been reported that miR-374a expression is decreased in CRC tissue compared with normal tissue (20). However, recent studies have revealed that miRNA-374a expression is increased in CRC and is associated with a poor prognosis of patients with CRC (18,19).

The higher expression level of miR-374a has been detected in primary tumors and has been shown to be associated with a poor survival rate. It has been demonstrated that miR-374a targets Wnt/ β -catenin signaling and suppresses a number of its negative regulators, which leads to the promotion of EMT and the development of cancer (12,21). Furthermore, the activation of Wnt/ β -catenin signaling has been reported to induce TGF- β 1 expression and enhance its downstream effects. The canonical Wnt/ β -catenin pathway and TGF- β 1 signaling mutually stimulate each other, promoting EMT and myofibroblast differentiation (22). It has been documented that transcription factors, such as Twist, Slug and Snail are activated during EMT and can bind directly to the TGF- β 1 gene promoter region and increase its transcription level (23).

TGF- β 1 is a potent inducer of EMT that leads to cancer progression (24,25). It plays a dual role in CRC, functioning as both a tumor suppressor and a promoter of tumor progression, depending on the cancer stage. In

the early stages, TGF- β 1 inhibits cell proliferation and promotes apoptosis, thereby suppressing tumor development. However, in advanced CRC, TGF- β 1 signaling can promote EMT, enhance tumor invasion, metastasis and contribute to immune evasion (26,27). Moreover, there is a complex regulatory network between miRNA-374a, EMT and TGF- β 1 and their dysregulation, which can lead to pathological consequences and promote cancer development, invasiveness and metastasis. Furthermore, studies have indicated that CRC progression influences the feedback regulation of TGF- β 1, resulting in elevated serum levels of the protein (26). Therefore, miRNA-374a and TGF- β 1 are considered critical targets for therapeutic intervention, as modulating their activity can inhibit cancer progression or prevent metastasis. To the best of our knowledge, no published studies to date have investigated the expression of miRNA-374a in Egyptian patients with cancer, to determine its exact role in CRC development, progression and metastasis. Thus, the present study aimed to assess the expression level of miRNA-374a in Egyptian patients with CRC and to explore its association with the TGF- β 1 expression level. The interplay between these markers is crucial for providing a better understanding of the molecular mechanisms underlying cancer progression, identifying prognostic biomarkers and for the development of targeted CRC therapies (27,28).

Patients and methods

Study subjects. The present study included 195 participants: A total of 150 patients with CRC and 45 healthy controls. The study was conducted in two phases: Screening and confirmation. The screening phase involved serum samples of 145 participants (100 patients with CRC and 45 controls) investigating miRNA-374a levels in serum. The confirmation phase involved 50 patients with CRC, examining both tumor tissue and adjacent normal tissue to validate the tumor-derived expression of miRNA-374a. All subjects underwent colonoscopy at the Endemic Medicine Department, Kaser El Aini Hospital, Cairo University, Cairo, Egypt, having biopsy and blood samples taken for examination from September, 2023 to January, 2024. All study procedures and protocols met the ethical standards of the Declaration of Helsinki 1975 revised in 2008, and were approved by the Medical Research Ethics Committee of the National Research Centre (registration no. 114125062023). Each subject signed an informed consent form prior to their participation in the study.

miRNA extraction and quantification. Tissue samples were preserved in RNAlater (Qiagen GmbH) following excision by colonoscopy needle aspiration immediately, and then stored in -80°C till usage. Prior to miRNA extraction, tissue samples were homogenized using TissueLyser (Qiagen GmbH). For serum samples, 3 ml whole blood was withdrawn from the patients with CRC and the controls, and serum was then separated from whole blood via centrifugation at $2,000 \times g$, 10 min, 25°C collected and stored at -80°C immediately until use. Any hemolyzed serum samples were excluded. Total cellular miRNA was extracted and purified from all tissue and serum samples using the RNeasy Mini kit following the manufacturer's instructions (Qiagen GmbH). The miRNA concentration

and purity were assessed with a NanoDrop spectrophotometer (UV-VIS-Spectrophotometer Q 5000). The isolated RNA was kept at -80°C until use in reverse transcription-quantitative PCR (RT-qPCR).

RT-qPCR. The differential expression of miRNA-374a was detected using RT-qPCR. Firstly, 60 ng pure miRNA (extracted as described above) were reverse transcribed into cDNA at 37°C for 60 min using the miScript II RT Kit (Qiagen GmbH). qPCR was then performed using the miScript miRNA PCR master mix (Qiagen GmbH). Briefly, the reaction contained cDNA template, 2x QuantiTect SYBR-Green Master Mix, specific miRNA-374a primer, 10X miScript Universal Primer and RNase-free water. The SNORD-95 housekeeping gene was used as an internal control to determine miRNA relative expression (the assay was ready-made by Qiagen; the primers sequences were secured and owned by Qiagen). The reaction was commenced with an initial incubation at 95°C for 15 min, followed by 40 cycles of amplification consisting of denaturation at 94°C for 15 sec, annealing at 55°C for 30 sec, and extension at 70°C for 30 sec. The reaction was performed on the Rotor-Gene PCR cycler (Qiagen GmbH).

The following sequence was for miRNA-374a: 5'-UUAUAAUACAACCUGAUAGUG-3'. All PCR reactions were performed in triplicate and the data are presented as the mean $\pm$ SD. The gene expression profiles were normalized to SNORD-95 and calculated using the formula $2^{-\Delta\Delta C_q}$ (29). The variations in gene expression were assessed as fold change and compared to the mean of the healthy controls. miRNA expression was considered upregulated when the fold change was >2 , while it was considered downregulated when the fold change was <0.5 .

Measurement of TGF- β 1 levels in serum. The expression level of TGF- β 1 in the serum samples of both the healthy controls and patients with CRC were assessed using the DRG Human TGF- β 1 (EIA-1864) ELISA kit (DRG Diagnostics GmbH) according to the manufacturer's recommendations and guidelines.

Prediction of miRNA target genes: miRDB (<https://mirdb.org/>) was used for miRNA target prediction and functional annotations. A bioinformatics tool, predict gene targets in miRDB which was developed by analyzing thousands of miRNA-target interactions from high-throughput sequencing experiments.

Statistical analysis. All data were analyzed using Prism Graph Pad version 9 software (Dotmatics). Data are presented as the mean $\pm$ SD. The normality test revealed that the studied groups were normally distributed. Comparisons between groups were performed using an unpaired t-test. One-way ANOVA test was used for comparisons between multiple groups followed by Tukey's multiple comparison tests. The receiver operating characteristic (ROC) curve with the area under curve (AUC) analysis were performed to detect the optimal cut-off value for both serum miR-374a expression levels, in tissues of patients with CRC and TGF- β 1 levels compared with control sera, adjacent non-cancerous tissues and control sera, respectively. Correlation analysis was performed using Spearman's

correlation analysis. P-values <0.05 were considered to indicate statistically significant differences with a confidence interval of 95%.

Results

Characteristics of the study population. A total of 195 individuals were recruited in the present study; these included 150 patients with CRC and 45 healthy controls. The sex distribution was equal in both study groups. The age of CRC patients and control subjects ranged between 35 and 62 years. The 45 healthy subjects constituted of 20 females and 25 males with a mean age of 44.38 ± 11.47 years. The 150 patients with CRC included 66 females and 84 males with a mean age of 53.57 ± 12.46 years. The majority of patients with CRC who participated in the study suffered from weight loss, bleeding and abdominal pain. In the patients with CRC, grade II and large-size tumors >5 cm in size were dominant. Furthermore, the majority of the tumors found in the patients with CRC were localized in the colon, sigmoid and rectum. The study population characteristics and clinical data of the screening phase (100 patients with CRC) and the confirmation phase (50 patients with CRC) are summarized in Table I.

Expression of miRNA-374a in different CRC locations, stages and grades. Using RT-qPCR, 100 CRC and 45 healthy control serum samples were examined for the expression of miRNA-374a. It was found that the miRNA-374a expression levels were significantly higher in the patients with CRC than in the healthy controls (fold change, 12.92; $P=0.0001$). In addition, miRNA-374a was differentially expressed in different CRC stages and grades. The fold change in grade I was 9.2, while that in grade II was 21.84 ± 17.55 . It was found that miRNA-374a expression could distinguish between patients with CRC grade I or II disease ($P=0.0001$), and its expression was affected by tumor location, such as colon, sigmoid and rectum ($P=0.0003$). There was a significant difference in miRNA-374 expression between the colon and rectum ($P=0.0017$), and between the colon and sigmoid ($P=0.0005$). In addition, the findings demonstrated a substantial difference in miRNA-374a expression between CRC stage I (tumor size <5 cm) and stage II (tumor size ≥ 5 cm) ($P=0.0001$), as shown in Fig. 1. These findings indicate that miRNA-374a expression is significantly elevated in patients with CRC compared with healthy controls, and varies across different stages and grades of CRC. It can be used to distinguish between various CRC subtypes and tumor sizes.

Validation of the tumor-derived expression of miRNA 374a. The relative miRNA-374a expression level in CRC tissues (50 pairs of tumor tissues and adjacent normal tissues) was assessed. The expression of miRNA-374a was significantly increased in cancerous tissues (fold change, 4.280; $P<0.001$) compared with the adjacent noncancerous tissues, as shown in Fig. 2. Furthermore, significant positive correlations were found between the relative expression levels of miRNA-374a in serum samples and the expression level in CRC tissues (Fig. 3). Moreover, the results revealed that miRNA-374a expression differed significantly between patients with CRC grade I and grade II ($P=0.001$) Fig. 1B. These results highlight

Table I. The characteristics and clinical data of the study population.

Characteristic	Control, n=45	Patients with CRC, n=50 (tissues)	Patients with CRC, n=100 (serum)
Age, years			
<40	16 (36%)	13 (26%)	21 (21%)
$\geq$ 40	29 (64%)	37 (74%)	79 (79%)
Sex, male/female	25 (55%)/20 (45%)	29 (58%)/21 (42%)	55 (56%)/45 (44%)
Diabetes mellitus (yes)	14 (31%)	16 (32%)	25 (25%)
HTN (yes)	16 (35%)	21 (42%)	19 (19%)
Smoking (yes)	17 (37%)	19 (38%)	35 (35%)
Constipation (yes)	14 (31%)	31 (62%)	50 (50%)
Diarrhea (yes)	12 (26%)	27 (54%)	24 (24%)
Bleeding	17 (37%)	35 (70 %)	58 (58%)
Abdominal pain	24 (53%)	34 (68%)	58 (58%)
Weight loss	13 (29%)	37 (74%)	74 (74%)
Vomiting	12 (26%)	19 (38%)	38 (38%)
Grade			
G1	-	20 (40%)	36% (36%)
G2	-	30 (60%)	64% (64%)
Tumor site			
Colon vs. sigmoid	-	25 (50%)	50 (50%)
Sigmoid vs. rectum	-	13 (26%)	25 (25%)
Rectum vs. colon	-	12 (24%)	25 (25%)
Tumor size			
<5 cm	-	18 (36%)	41 (41%)
$\geq$ 5 cm	-	32 (64%)	59 (59%)

the significant upregulation of miRNA-374a in CRC tissues compared with adjacent normal tissues and indicate a strong correlation between miRNA-374a expression in serum and tumor tissues, with distinct expression differences between CRC grade I and grade II. Moreover, the results revealed a strong positive correlation of miR-374 expression in CRC tissues and their corresponding serum samples ($Rho=0.9727$, $P<0.0001$), as shown in Fig. 3.

TGF- β 1 protein expression levels in CRC serum samples. The serum TGF- β 1 protein level was evaluated in patients with CRC and healthy controls. As shown in Fig. 4, there was a significant increase in the TGF- β 1 expression level in patients with CRC compared with the healthy controls ($P=0.0001$). The average concentration in patients with CRC was 462.4 ± 99.47 pg/ml, while that in the healthy controls was 340.6 ± 559 pg/ml. Notably, the concentration of TGF- β 1 was higher in patients with CRC with grade II disease (503.2 ± 97.99 pg/ml) than in those with grade I disease (389.2 ± 46.16 pg/ml). These findings indicate that the serum TGF- β 1 levels are significantly elevated in patients with CRC compared with healthy controls, with higher levels observed in CRC patients with grade II compared with grade I disease.

Diagnostic potential of the expression of miRNA-374a and TGF β 1 in patients with CRC. ROC curve analysis

was performed to determine the diagnostic potential of miRNA-374a and TGF- β 1 in patients with CRC, as shown in Fig. 5. The AUC, the sensitivity and specificity of differentially expressed miRNA-374a and TGF- β 1 in CRC serum samples were evaluated, as presented in Table II and Fig. 5. The AUC of miRNA-374a in CRC serum samples was 0.9560 ($P<0.0001$), with sensitivity of 84.4% and a specificity of 97.5% in the prognosis of CRC; the AUC of miRNA-374a in CRC tissue was 0.8972 ($P<0.0001$). On the other hand, the AUC for the TGF- β 1 expression level in CRC prognosis was 0.8075 ($P=0.001$). Furthermore, a positive correlation was found between the miRNA-374 expression level and TGF- β 1 serum levels ($Rho=0.8912$; 95% CI, 0.8389 to 0.9272, $P<0.0001$) as shown in Fig. 6. These results suggest that miRNA 374a and TGF- β 1 have potent diagnostic potential for CRC, with a significant positive correlation existing between their expression levels in serum.

Discussion

CRC is a heterogeneous disease characterized by various molecular changes that contribute to its etiology and progression. Among these changes, the dysregulation of miRNA expression and the TGF- β 1 signaling pathway play a vital role in the cancer development and progression (13,30). Recently, the dysregulation of miRNA-374a has gained increasing attention, as it has been implicated in the development of several

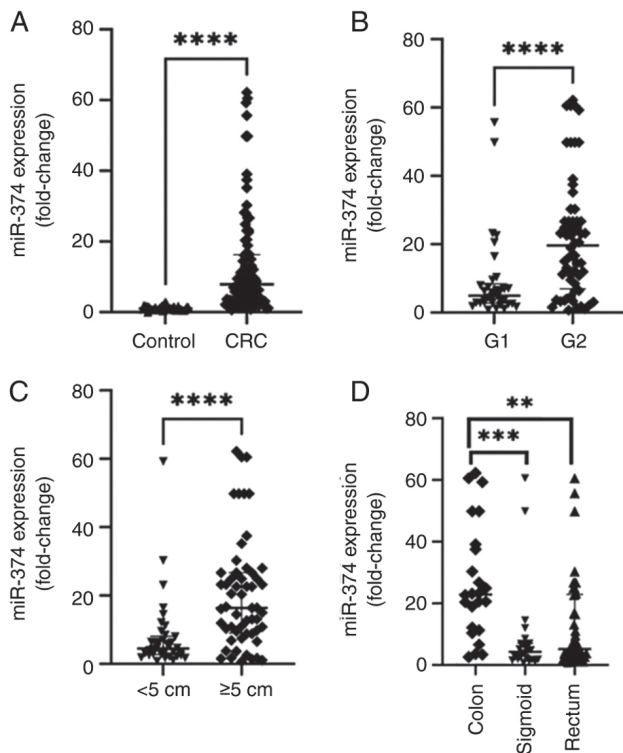


Figure 1. (A) The miRNA-374a expression level was significantly higher in patients with CRC than in the healthy controls (**** $P=0.0001$). (B) miRNA-374a expression can distinguish between patients with CRC grade I or II (**** $P=0.0001$). (C) Substantial difference in miRNA-374a expression was observed between CRC stage I (tumor size <5 cm) or stage II (tumor size ≥ 5 cm) (**** $P=0.0001$). (D) miRNA-374 expression can be affected by tumor location, such as colon, sigmoid and rectum ($P=0.0003$); miRNA-374 expression differed significantly between colon and rectal cancer (* $P=0.0017$), and between colon and sigmoid cancer (** $P=0.0005$). CRC, colorectal cancer.

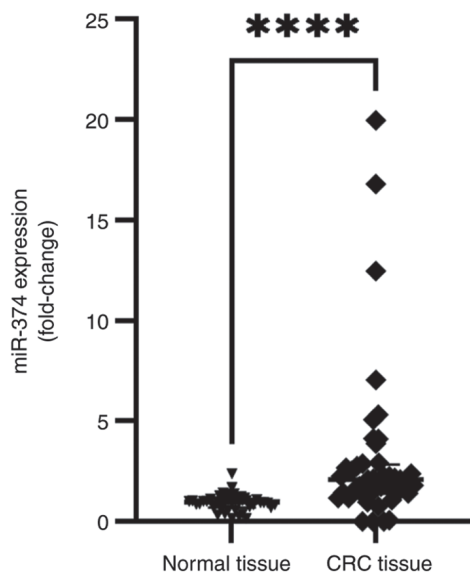


Figure 2. miRNA-374 was expression significantly increased in cancerous tissues compared with the adjacent non-cancerous tissues (fold change, 4.280; **** $P<0.001$).

types of cancer (31). Furthermore, TGF- β 1 is recognized for its pleiotropic effects on the initiation of CRC (32). The present

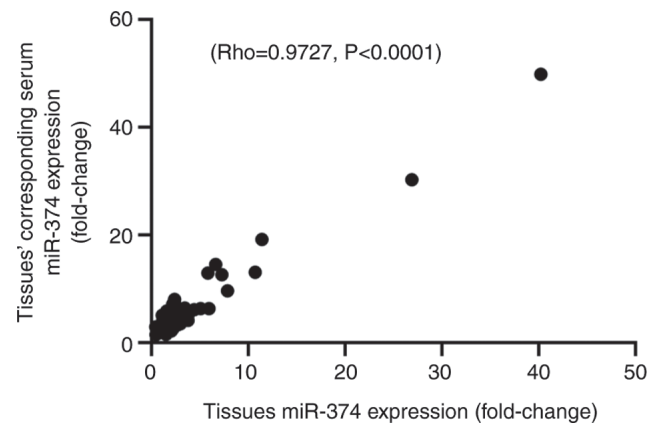


Figure 3. Correlation between miRNA-374a expression levels in colorectal cancer tissues and its expression in the corresponding serum samples for better clarity and understanding.

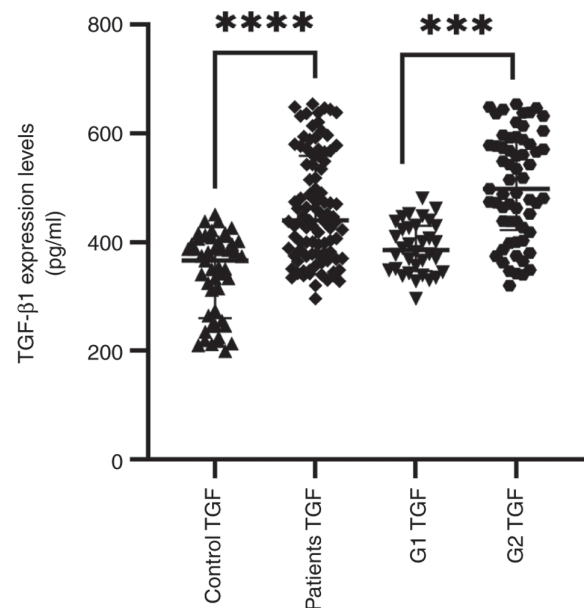


Figure 4. A significant increase was found in the TGF- β 1 expression level in patients with CRC compared with the healthy controls (**** $P=0.0001$). Notably, the concentration of TGF- β 1 was higher in patients with CRC with grade 2 (G2) than in those with grade 1 (G1) disease. CRC, colorectal cancer (*** $P<0.0001$).

study evaluated the expression of miRNA-374a and TGF- β 1 in 150 patients with CRC and 45 controls to explore the interplay between them in CRC development, and highlight their potential use as biomarkers for diagnosing and predicting cancer outcomes, as well as for potential therapeutic targets. In the first part of the present study, the expression level of miRNA-374a was evaluated in the serum samples of patients with CRC (fold change, 12.92; $P=0.0001$) in comparison to healthy controls. Consistent with the CRC serum results, the tissue level of miRNA-374a was significantly higher in cancerous tissues compared with adjacent non-cancerous tissues (fold change, 4.280; $P<0.001$). These findings are in accordance with those in the study by Bayatiani *et al* (19), who further explored the increased miRNA-374a expression levels in CRC tissues, demonstrating that it targets adenomatous polyposis coli

Table II. Diagnostic efficacy of miRNA 374 and TGF- β 1 expression.

Parameter	AUC	Cut-off value	Sensitivity	95% CI	Specificity	95% CI	P-value
Circulating miR-347 expression	0.9560	>2.576	84.38%	75.81 to 90.30	97.50%	87.12 to 99.87%	<0.0001 ^a
Tissue miR-374 expression	0.8972	>2.085	65.22	50.77 to 77.32	95.45	84.87 to 99.19	<0.0001 ^a
TGF- β 1	0.8075	>422.5	60.00%	49.95 to 69.28	91.84%	80.81 to 96.78	<0.0001 ^a

^aIndicates statistically significant difference (P<0.05). AUC, area under the curve; CI, confidence interval.

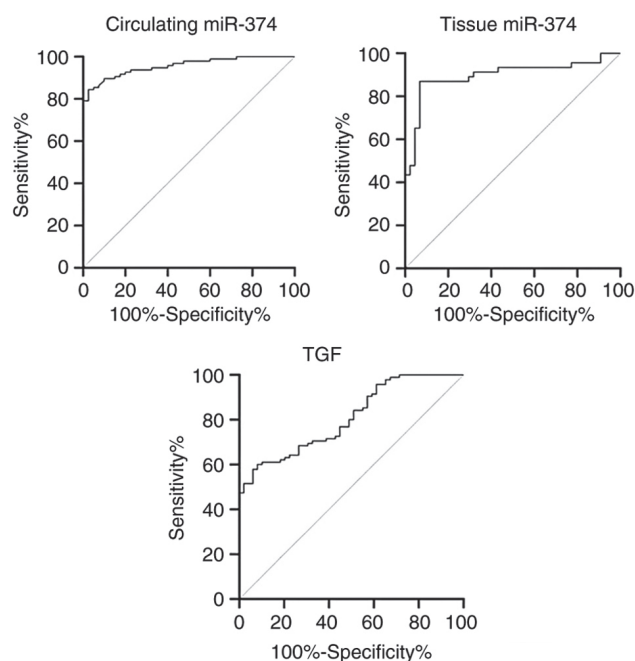


Figure 5. ROC curve analysis was performed to determine the diagnostic potential of miRNA-374a in serum, tissues and TGF- β 1 in patients with CRC. The AUC of miRNA-374a in CRC serum samples was 0.9560 (P<0.0001) with a sensitivity of 84.4% and a specificity of 97.5% in the prognosis of CRC, while the AUC for CRC tissue was 0.8972 (P<0.0001). On the other hand, the AUC for the TGF- β 1 expression level in CRC prognosis was 0.8075 (P=0.001). These data are presented in detail in Table II. CRC, colorectal cancer; ROC, receiver operating characteristic; AUC, area under the curve.

(APC) and glycogen synthase kinase-3 β , leading to the release of β -catenin, the activation of the Wnt pathway and subsequent cancer development.

Another study documented the elevated expression of miRNA-374a in the serum of patients with gastric cancer, exhibiting superior diagnostic value compared with the conventional diagnostic markers, alpha-fetoprotein and carcinoembryonic antigen (17). In addition, Kim *et al* (15) demonstrated that the high expression of miR-374a was associated with lung adenocarcinoma, pleural invasion and a decreased disease-free survival. Moreover, the analysis of miRNA-374a expression levels in tissues confirmed the tissue-derived nature of miRNA-374a and its upregulation in association with CRC carcinogenesis. These results are in line with previous findings that validated the role of other miRNAs in CRC (33,34).

Several studies on different cancer cell lines have reported miRNA-374a as an oncogenic miRNA. In osteosarcoma

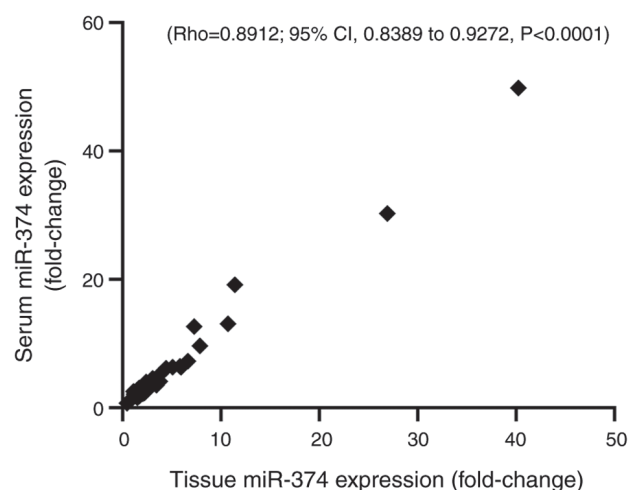


Figure 6. A strong positive correlation was found between the miRNA-374 expression level and TGF- β 1 serum levels (Rho=0.8912; 95% CI, 0.8389 to 0.9272, P<0.0001).

cell lines, high miRNA-374a levels have been shown to be associated with increased cell propagation and colony formation (35). Furthermore, the downregulation of miRNA-374a in breast cancer cell lines, suppresses cell progression, migration and invasion (36). Additionally, functional studies conducted in *in vivo* and *in vitro* models have demonstrated that elevated miRNA-374a levels promote cancer growth in triple-negative breast cancer (37).

Conversely, Chen *et al* (38) demonstrated that the high expression of miR-374a in colon cell lines suppressed cancer progression by inhibiting and inactivating the PI3K/AKT pathway (38); miR-374a has also been shown to suppress cancer cell proliferation and metastasis *in vitro* (39). The discrepancies in these results highlight the dual nature of miR-374a in cancer development, where its effects can vary depending on the primary target pathway involved in a particular type of cancer.

Nonetheless, the activation of the Wnt-pathway by miRNA 374a expression, promotes TGF- β 1 expression. Both TGF- β 1 and the Wnt pathway induce EMT, triggering a wide variety of cellular responses and promoting cancer development. Barnard *et al* (40) reported a gradient increase in the TGF- β 1 level within the colonic epithelium, emphasizing its crucial role in CRC carcinogenesis. Moreover, multiple studies have proven that TGF- β 1 is associated with tumor development and progression (28,41). These previous reports are in line with the findings of the present study, which demonstrated a

significantly higher TGF- β 1 level among patients with CRC than in healthy individuals ($P=0.0001$).

Concurrently, Itatani *et al* (42) reported that TGF- β 1 expression was increased in CRC tissues and was positively related to tumor size, invasion depth and metastasis. In addition, these results are consistent with those in the previous study by Hao *et al* (43), who documented that TGF- β 1 functions as a tumor promoter by stimulating EMT and enhancing cancer development. This leads to tumor angiogenesis activation, metastasis, immune response evasion and chemotherapeutic resistance. Moreover, the results of the present study revealed a positive correlation between the expression level of miRNA-374a and the TGF- β 1 serum level ($Rho=0.8912$, $P<0.0001$). These results are in accordance with the findings in the study by Bayatiani *et al* (19) and Farouk *et al* (33), which revealed that miRNA-374a targets compounds affecting the Wnt/ β -catenin signaling, which in turn increases the TGF- β 1 activity, and promotes the EMT, invasion and metastasis of CRC cells. The reciprocal regulation of TGF- β 1 and miRNA-374a highlights their collaborative function in the pathophysiology of CRC (19,33). Notably, using the miRDB.org software for target prediction confirmed the present findings. This identified the tumor suppressor gene, APC, as a target of miRNA-374a (data not shown). This interaction stimulates the Wnt pathway, promoting cell proliferation and carcinogenesis. The activation of both the Wnt and TGF- β pathways induces EMT, ultimately leading to increased cell proliferation and metastasis.

According to the findings of ROC analysis, both miRNA-374a and TGF- β 1 can effectively discriminate between the healthy controls and patients with CRC, with high accuracy and specificity. The AUC for miRNA-374a in CRC serum samples was 0.9560 ($P<0.0001$), with a sensitivity of 84.4% and a specificity of 97.5% in the prognosis of CRC, while the AUC for CRC tissue was 0.8972 ($P<0.0001$). On the other hand, the AUC for the TGF- β 1 expression level in CRC prognosis was 0.8075 ($P=0.001$). These results are consistent with those of previous studies and corroborate the clinical significance of miRNA-374a and TGF- β 1 in cancer. For instance, Kim *et al* (15) and O'Brien *et al* (18) reported that high serum levels of miRNA-374a were associated with a poor response to chemotherapy and an increased risk of recurrence in patients with cancer, highlighting its potential as a predictive biomarker for treatment response and prognosis. Similarly, Chen *et al* (44) and Liu *et al* (45) demonstrated that elevated serum levels of TGF- β 1 were predictive of aggressive tumor behavior and poor outcomes in patients with CRC, implicating its prognostic value in clinical practice.

In conclusion, the present study demonstrates the elevated expression levels of miRNA-374 and TGF- β 1 in patients with CRC, which indicates their crucial roles in driving tumor progression and metastasis. In the future, the authors aim to perform further studies to confirm the synergistic effects of both miRNA-374 and TGF- β 1 on promoting EMT, invasion and metastasis underscore their potential as promising diagnostic and prognostic biomarkers and therapeutic targets in CRC. However, additional experiments are required to elucidate the role of miRNA-374a in CRC and its interaction with TGF- β 1, such as the analysis of gene expression profiles, immunoprecipitation experiments, *in vitro* assays using cell

lines and *in vivo* models. These investigations of the molecular mechanisms underlying the interplay between miRNA-374a and TGF- β 1 signaling pathways are required in order to exploit their therapeutic potential and improve the clinical outcomes of patients with CRC.

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Availability of data and materials

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

Authors' contributions

NGBED was involved in the design of the study, in study supervision, and in the writing and preparation of the draft of the manuscript. NGBED, RES, RIM and SF performed sample extraction, cDNA reaction, RT-qPCR and ELISA. AK was involved in sample collection and clinical data sheets, and SF was involved in preparing the draft of the manuscript, in statistical analysis and in the preparation of the figures. All authors have read and approved the final manuscript. NGBED and SF confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Medical Research Ethics Committee of the National Research Centre (registration no. 114125062023). Each subject signed an informed consent form before participating in the study. All the study procedures and protocols met the ethical standards of the Declaration of Helsinki 1964 (2008 revision).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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