RESEARCH ARTICLE

Editorial Process: Submission:08/22/2025 Acceptance:05/01/2026 Published:05/18/2026

A Novel Circulating microRNA Signature Panel as a Prognostic Biomarker for in Patients with Colorectal Cancer

Noha G Bader El Din[1]*, Sally Farouk[1], Reem M El Shenawy[1], Rehab I Moustafa[1], Maha M. Elbrashy[2], Ahmed M Khairy[3], Marwa K Ibrahim[1]

Abstract

Background: Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide, necessitating the development of reliable prognostic biomarkers. Circulating microRNAs (miRs) have emerged as promising, non-invasive biomarkers. This study was designed as a comprehensive evaluation, conducted in two independent stages, to identify circulating miRs capable of providing an accurate prognosis of CRC. **Subjects:** The study included 225 subjects (150 Egyptian CRC patients and 75 healthy controls). We selected 25 miRs based on recent studies and our previous work and evaluated their expression in the Evaluation Group (65 subjects). The miRs showing significant differential expression were further confirmed in the Validation Group (160 subjects: 110 CRC patients and 50 controls). For all samples, miRNAs were extracted, and their concentration, integrity, and purity were measured using a NanoDrop, followed by cDNA synthesis and qRT-PCR analysis. **Results:** The serum levels of eight miRNAs (Let-7c, miR-21, miR-26a, miR-26b, miR-126, miR-146a, miR-223, and miR-374) were significantly higher in CRC patients and were able to discriminate between CRC patients and healthy controls. The combined analysis of all eight miRNAs showed higher specificity, with an AUC of 0.92 and a specificity of 99.73% for CRC patients. The combination of five miRNAs, which exhibited a greater fold change in CRC patients, achieved an AUC of 0.97 and a specificity of 99.6%. In contrast, the combination of miR-21, miR-26a, and miR-26b demonstrated the highest AUC value of 0.974, with a specificity of 99.27%. Computational analysis revealed that CCND1 and TP53 function dually as efficacy and prognostic biomarkers for CRC, and that these roles are regulated by let-7a-5p. Collectively, the data indicate that this novel miRNA signature panel improves prognostic accuracy compared to individual miRNA analysis and may support enhanced patient screening and personalized treatment strategies in CRC. **Conclusion:** We established a circulating miRs signature panel for the first time to accurately predict the prognosis of Egyptian CRC patients.

Keywords: miRNAs Signature- Expression- Prognosis- Colorectal Cancer

Asian Pac J Cancer Prev, **27 (5)**, 1735-1745

Introduction

Colorectal cancer (CRC) remains a significant public health challenge, accounting for substantial morbidity and mortality worldwide [1]. CRC is the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths in the Western world [2]. In Egypt, CRC is becoming an increasingly prevalent health issue. Historically, the incidence of CRC in Egypt was relatively low compared with that in Western countries. However, recent years have seen a rising trend, with CRC now considered one of the most common cancers in the Egyptian population [3]. The CRC ranks seventh among males and females with 5,940 new cases in 2022. The incidence rate is 6.4, the mortality rate is 3.4, and the 5-year prevalence per 100,000 is 4.5. The ASR incidence rate is 6.6 in males and 6.3 in females [4]. This increase is attributed to changes in dietary habits, environmental factors, lifestyle features, and genetic diversity.

Interestingly, the median age of CRC diagnosis in Egypt is younger compared to Western countries, suggesting potential genetic and environmental influences unique to the region. By 2030, the incidence rate of CRC in patients aged 20 to 34 is expected to increase by 90.0% and 124.2%, respectively [1]. The CRC in young adult patients differs from CRC in older adults in terms of clinicopathology and genetics. Moreover, many researches are needed to study the CRC pattern in young people [5].

Early CRC screening, innovative multimodal treatment approaches, and intensive knowledge of the molecular and genetic mechanisms promoting CRC development can improve CRC patients' clinical outcomes

*[1]Microbial Biotechnology Department, Biotechnology Research Institute, National Research Centre, Dokki, Cairo, Egypt. [2]Biochemistry Department, Biotechnology Research Institute, National Research Centre, Dokki, Cairo, Egypt. [3]Endemic Medicine Department, Faculty of Medicine, Cairo University, Giza, Egypt. *For Correspondence: nbabdereldin@yahoo.com*

[6, 7]. It has been documented that screening techniques like sigmoidoscopy, colonoscopy, CT colonography, stool DNA testing, and fecal immunochemical testing can lower the incidence and mortality of colorectal cancer [8]. Despite advancements in medical science, the prognosis for CRC patients remains bleak, especially in regions with limited access to advanced medical care and screening programs. It was reported that 30% of CRC patients were diagnosed at a late stage, had distant metastases with low 5-year survival rates and could experience death from their disease [9, 10]. Moreover, some CRC patients don't show any symptoms, and their recovery is usually impossible. In Egypt, this challenge is compounded by disparities in healthcare access and resources, emphasizing the need for innovative, accessible diagnostic tools and effective prognostic biomarkers [11].

Recent advances in molecular biology have underscored the potential of microRNAs (miRs) as non-invasive biomarkers for various cancers, including CRC [12]. The miRs can have diverse impacts on the translation of various proteins and affect their function. It is thought that the translation of approximately 50% of human genes is controlled by miRs [13, 14]. MiRs can identify a wide variety of mRNA transcripts and exert epigenetic control over fundamental cellular functions. In cancer progression, miRs may function as oncogenes or suppressors. Dysregulation of miRs in CRC patients has been associated with cancer initiation, development, metastases, and treatment response [15, 12, 16, 17].

Recently, several miRs have been identified as potentially promising prognostic, diagnostic biomarkers and can be used as therapeutic targets in CRC patients [18-20]. MiRs exhibit remarkable stability in different clinical samples, such as serum, plasma, urine, stool, exosomes, formalin-fixed paraffin-embedded tissues, and tissues [21]. MiR expression in serum is better than in tissues, as tumor cell heterogeneity yields variable results. Therefore, miR profiling in serum is a valuable, cost-effective, and practical tool for the diagnosis and prognosis of many types of cancer, including CRC. The expression and function of these miRs can vary significantly depending on diet habits, environmental changes, population demographics, and ethnic origin, highlighting the importance of context-specific biomarker development [22-26].

Notably, the knowledge about miRs dysregulation in Egyptian CRC patients is scarce and the studies are very limited [27-29]. Moreover, most of the previous studies were conducted on different populations other than the Egyptian. Therefore, this study investigates a novel circulating miRs signature panel comprising eight specific miRs as prognostic biomarkers for CRC. The findings highlight the potential of this miR panel to predict patient prognosis, thereby aiding in personalized treatment strategies. Computational analysis has been performed to investigate the interactions between the selected miRNAs and CRC-associated genes.

Materials and Methods

Clinical Samples

Serum samples were collected from 225 subjects enrolled in two groups (Evaluation and Validation). We selected, for the first time, 25 specific miRs from CRC studies across different populations and from our previous work. We assessed their expression in the evaluation group, which contained 65 subjects (40 CRC patients, 25 controls). Then, the miRs that showed significant differential expression were further validated in the validation group, which included 160 subjects (110 CRC, 50 controls).

All patients were investigated and done colonoscopy at the colonoscopy unit in the Endemic Medicine Department, Faculty of Medicine, Cairo University. The selection of CRC patients was based on inclusion/exclusion criteria. Patients who received treatment, had surgery, or had a clinical diagnosis of hereditary nonpolyposis or familial adenomatous polyposis were excluded from the study. Every patient underwent a diagnostic interview, including a detailed disease history and clinical examination. Furthermore, diagnostic colonoscopy and pathological examination were performed for all patients. The G-grade scale was used to evaluate the degree of CRC differentiation, and tumor size was also measured to determine the CRC clinical stage.

The healthy control subjects had normal colonoscopic and pathological examinations. None of the controls has any cancer or any other indication of colorectal neoplasia. The collected serum samples were stored at -80 C until use. The study was reviewed and approved by the National Research Centre's Ethics Committee reg. Number 13060119-1 in accordance with the Helsinki Declaration. All study subjects provide written informed consent before participating in the study and before their blood samples are collected.

Quantitative Real-Time PCR (q RT-PCR) reaction

The first step of the study was to extract total RNA, including miR, from 225 serum samples using the RNeasy Serum/Plasma Kit from Qiagen, Germany, in accordance with the manufacturer's instructions. The extracted RNA was measured and kept for further cDNA synthesis at -20°C.

For RT-PCR analysis, the RNA was initially reverse-transcribed to produce cDNA using the miScript Reverse Transcription Kit (Qiagen, Germany). In the reverse transcription reaction, 60 ng of total RNA was used as the starting material, and the reverse transcription was carried out under the manufacturer's conditions. Then, the reaction was incubated at 37°C for 60 minutes, and finally, the miScript Reverse Transcriptase mix was inactivated at 95°C for 5 minutes. Following that, the cDNA was kept at -20°C till the real-time PCR reaction was carried out.

Next, Quantitative RT-PCR (Qiagen, Germany) was used to profile 25 mature miR (Let-7c, -9, -21, -26a, -26b, -92a, -93, -126, -128, -146a, -184, -193a, -204, -205, 208a, -210, -221, -223, -296, -374a, -375, -423, -499a, -574, -885) in 24 CRC patients and 15 healthy controls (Evaluation Group). The final volume was 20

µl, comprising 2 µl of cDNA, 2 µl of universal primer, 2 µl of a specific miRNA primer, and 8 µl of miScript SYBR Green mix. The SNORD95 housekeeping gene was used to normalize relative miR expression levels. The RT-PCR reaction mix was first activated at 95°C for 15 minutes. Next, it underwent a three-step cycling process: denaturation at 94°C for 15 seconds, annealing at 55°C for 30 seconds, and extension at 70°C for 40 cycles (30 s). The reaction was performed on the Qiagen Rotor-Gene Real-Time PCR machine (Qiagen, Germany).

After that, the highly expressed 8 miRs (Let-7c,-21,-26a,-26b,-126,-146a,-223,-374a) were chosen to be further confirmed in the (Validation Group). The expression of the 8 miRs was evaluated in an additional 160 serum samples (110 CRC patients and 50 healthy controls) using qRT-PCR.

miRs Data Analysis

Each miR's relative expression was determined using the $2^{-\Delta\Delta CT}$ method, and fold-changes were used to represent the differences in miR expression. The t-test was used to obtain the p-value, which is considered significant if ≤.05 and highly significant if ≤.01. The correlation between miR expression levels and clinicopathological parameters in CRC was detected.

Statistical analysis

All statistical analyses were performed using Prism GraphPad version 9 (GraphPad Software, Inc., Boston). The data were statistically represented as mean and SD. The Mann-Whitney test was used to compare the studied groups. To test the diagnostic efficacy, the receiver operating characteristic (ROC) analysis was performed on each miRNA separately and combined to detect the best cut-off, sensitivity, and specificity values. P-values less than 0.05 were considered statistically significant with a confidence interval of 95%.

Computational analysis for the studied miRNAs with CRC

To examine the effect of significantly expressed miRNAs on colorectal cancer-interrelated pathways, in silico analyses were carried out. The prediction of target gene, biomarker identification, and subcellular localization annotation were carried out using QIAGEN's Ingenuity Pathway Analysis (IPA) software (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis). The basic algorithms applied in IPA were formerly done by Krämer et al.2013 [30].

Results

The subjects' demographic and clinical–histopathological Data

For the Evaluation Group, 65 subjects (40 CRC patients and 25 controls) were enrolled, and for the Validation Group, 160 subjects (110 CRC patients and 50 controls) were examined. The age of all subjects ranged from 25 to ≤65 years. The mean age of the subjects in the Evaluation Group was (55.21±11.28), while in the Validation Group it was (53.50±12.3). All clinical and pathological parameters of the screening and validation sets are shown in Table 1. None of the CRC patients has any other disease complications. In the Evaluation Group, 15 (37%) of CRC patients were Grade I and 25 (63%) were Grade II while in the Validation Group 35 (32%) of CRC patients were Grade I and 75(68%) were Grade II.

The qRT-PCR of the selected miRs

a) Screening Group

The dysregulation of 25 circulating miRs levels was investigated in the screening group (40 CRC patients, 25 controls) by Quantitative RT-PCR. The expression of the 25 miRs was shown in Table 2. The 40 CRC patients were 15 Grade I and 25 Grade II, and the miRs expression levels in different grades were evaluated and displayed in Table 3. Out of 25 expressed miRs, 8 miRs (Let-7c,-21,-26a,-26b,-126,-146a,-223,-374a) showed high differential regulation when compared to healthy controls with statistical significance difference P≤ .05.

b) Validation Group

To confirm the 8 miRs upregulation we evaluated their level in the Validation group (110 CRC, 50 controls). The SNORD 95 gene was used to normalize the miRs expression levels. The data analysis showed highly significant upregulation of the eight miRs. They can discriminate between CRC patients and healthy controls (P = .001), as shown in Figure 1. Also, the expression of the 8 miRs differed significantly between CRC grades I and II. These results highlight an association between the upregulation of the 8 miRs and the development of colorectal cancer.

ROC curve analysis of differentially expressed miRs

The ROC curve was used, and the AUC was calculated for each expressed miR to assess their efficacy as diagnostic biomarkers for CRC. The sensitivity, specificity, and AUC for the 8 miRs are listed in Table 4. The ROC curves for the individual and combined miRs demonstrated higher accuracy in distinguishing CRC patients from the control group. The combination of the eight miRs showed higher specificity, with an AUC of 0.92, sensitivity of 68.4, and specificity of 99.73 (P = 0.0001) in CRC patients. The combining of the 5 miRs (miR-21, miR-26a, 26b, 233, 374a), which showed higher fold change in CRC patients, had an AUC of 0.97 and specificity of 99.6, while the combining of miR-21, miR-26a, 26b showed the highest AUC significance of 0.974, and specificity 99.27 (P = 0.0001) as shown in Figure 2. The 5 miRs had higher fold regulation in both the Evaluation and Validation Group associated with high diagnostic accuracy and specificity. This signature panel provides a more comprehensive prognostic assessment than individual miRs, enhancing patient screening and informing personalized treatment strategies.

Exploring interactions and detecting biomarkers included in miRNA-intermediated regulation of colorectal cancer (CRC) pathways by in-silico analysis.

To explore the possible functions of selected miRNAs, thousands of networks between miRNAs and their mRNA targets were computer-generated. As a result, 40 mRNAs

Table 1. The Clinicopathological Parameters of Evaluation and Validation Groups

	Evaluation Group (n=65)		Validation Group (n=165)	
	CRC Patients (n=40)	Healthy Control (n=25)	CRC Patients (n=110)	Healthy Control (n=50)
Age	55.21±11.28	44.60±12.96	53.50±12.3	43.88±11.56
Gender M/F	20 /20	15/10	58 /52	28/22
Vomiting	25 (62%)	3 (12%)	39 (35%)	13 (26%)
Abdominal Pain	23 (57%)	13 (52%)	65 (59%)	28 (58%)
Bleeding	20 (50%)	6 (24%)	66 (60%)	17 (34%)
Weight loss	28 (70%)	6 (24%)	18 (17%)	16 (34%)
Constipation	19 (47%)	6 (28%)	13 (11%)	19 (38%)
Diarrhea	11 (27%)	4 (16%)	27 (25%)	14 (28%)
Grade		----------		----------
G1	15 (37%)		35 (32%)	
G2	25 (63%)		75 (68%)	
Tumor Site		----------		----------
Colon	32 (80%)		66 (60%)	
Rectum	8 (20%)		44 (40%)	
Tumor size		----------		----------
< 5cm	14 (35%)		53 (48%)	
≥ 5cm	26 (65%)		57 (32%)	

Table 2. The Fold Regulation of 25 miRs in CRC Patients Compared to Healthy Controls

No.	MicroRNA	Fold Regulation in CRC Patients	P-value
1	let-7c-5p	4.8104	0.0864
2	miR-9-5p	2.7777	0.3905
3	miR-21-5p	5.0809	0.0018
4	miR-26a-5p	7.4234	0.0006
5	miR-26b-5p	8.5209	0.0177
6	miR-92a-3p	1.5627	0.9097
7	miR-93-5p	1.1215	0.3294
8	miR-126-3p	2.5522	0.0050
9	miR-128-3p	3.103	0.0773
10	miR-146a-5p	5.7038	0.0009
11	miR-184	1.8756	0.5255
12	miR-193a-5p	2.3401	0.0293
13	miR-204-5p	1.9566	0.7942
14	miR-205-5p	2.0904	0.8320
15	miR-208a-3p	3.5743	0.1015
16	miR-210-3p	-2.4267	0.7072
17	miR-221-3p	1.2691	0.7681
18	miR-223-3p	9.8976	0.0003
19	miR-296-5p	-1.3186	0.4962
20	miR-374a-5p	7.2925	0.0001
21	miR-375	-1.1582	0.9468
22	miR-423-5p	3.0101	0.4349
23	miR-499a-5p	2.9199	0.2989
24	miR-574-3p	-2.1964	0.0307
25	miR-885-5p	-1.2259	0.9962

were recognized to be regulated by 7 selected miRNAs (Figure 1). Despite miR-26b-5p and miR-26a-5p having diverse roles, they are labeled as miR-26a-5p. Similarly, let-7a-5p and let-7c-5p, which also have different functions, have the same designation in the IPA database.

Most miRNA-target genes are within transmembrane receptors (17 genes), then nuclear proteins (10 genes), with some cytoplasmic ones (7 genes), and extracellular space proteins (6 genes). Underlining the importance of interactions with extracellular cytokines such as WNT1, WNT3A, Il6, WNT7A, IFNG, and TNF. FZD3 transmembrane receptor is regulated by four miRNAs, underscoring its importance in CRC signaling pathways. Both let-7a-5p and miR-146a-5p target the same mRNAs, namely WNT1 and TLR4. Two miRNAs control STAT1, while STAT3 is regulated only by miR-21. Notably, only miR-26a can control the IFNG target gene.

The analysis showed that let-7a-5p could interact with 18 mRNA genes, while miR-146a-5p and miR-21-5p could target 9 different mRNA genes. MiR-26a-5p targets 7 genes; however, miR-223-5p targets only 4 mRNAs. Finally, miR374b-5p interacts with two mRNAs, and miR-126a-5p connects with only one mRNA target (Figure 4).

Figure 5 illustrates the potential of the studied miRNAs to interact with biomarker genes. Some of these interacting genes are compatible for use as biomarkers for CRC. The analysis revealed that Cyclin D1 (CCND1) and TP53 can serve as promising prognostic and efficacy biomarkers. While interleukin 6 (Il6) is the only disease diagnosis biomarker. Analysis has identified TP53 as a biomarker to evaluate response to therapy. While SMAD4 acts only as a prognostic biomarker for colorectal cancer.

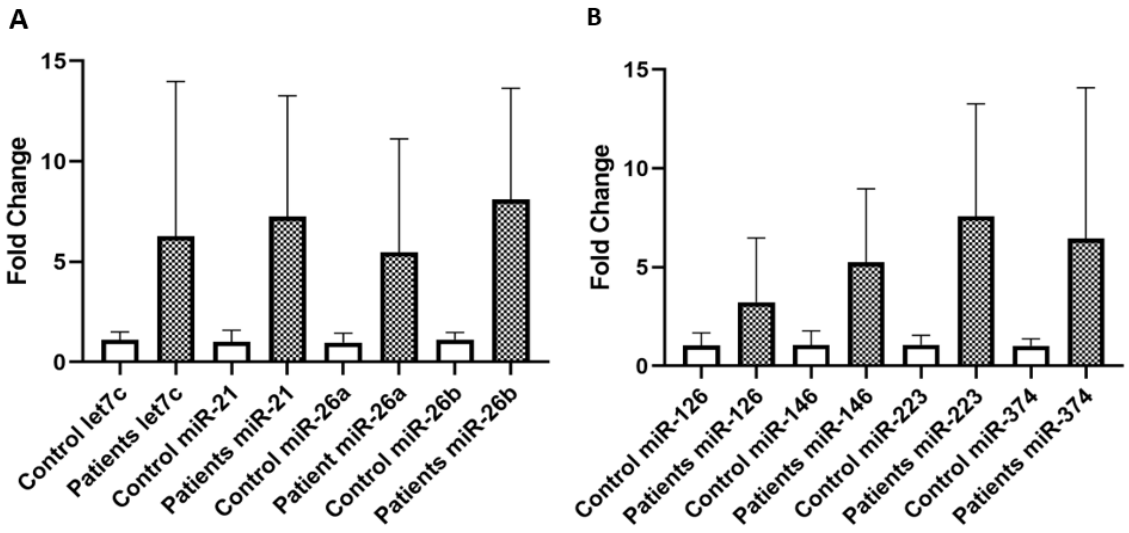

Figure 1. Diagrammatic Representation of the Expression Level of A) miRs (Let 7c, -21, -26a, -26b) B) miRs (-126, -146a, -223, 374a) in CRC patients versus the healthy controls. The dysregulation of miRs was evaluated by qRT-PCR. The data was normalized using the SNORD 95 expression and displayed as fold-change relative to the healthy controls.

Discussion

Different miRs can regulate multiple targets rather than just one gene, and they are crucial for gene regulation. Other cancer types have dysregulated miRs expression. Several studies have examined the roles of miRs in the initiation, proliferation, and migration of colorectal cancer. MiRs have been found to function as tumor suppressor genes, oncogenes, controllers of cell division, and enhancers of cancer development. MiRs are useful because of their exceptional stability and specificity across many bodily fluids.

CRC has been extensively studied in Western countries, whereas its etiology and the high prevalence of early-onset CRC in Egypt are less well understood, and fewer data are available. In the current study, we evaluated the expression of 25 miRs in Egyptian CRC patients. To our knowledge, this is the first study to assess this signature miRNA panel in Egyptian patients with CRC (Grades I and II). The results highlighted a novel circulating miR signature panel consisting of five miRs (miR-21, miR-26a, miR-26b, miR-223, and miR-374a). Furthermore, the upregulation of these miRs was confirmed in the validation group. The results showed a statistically significant difference in these miRs between CRC patients and healthy controls. Also, this miR upregulation was associated with clinical

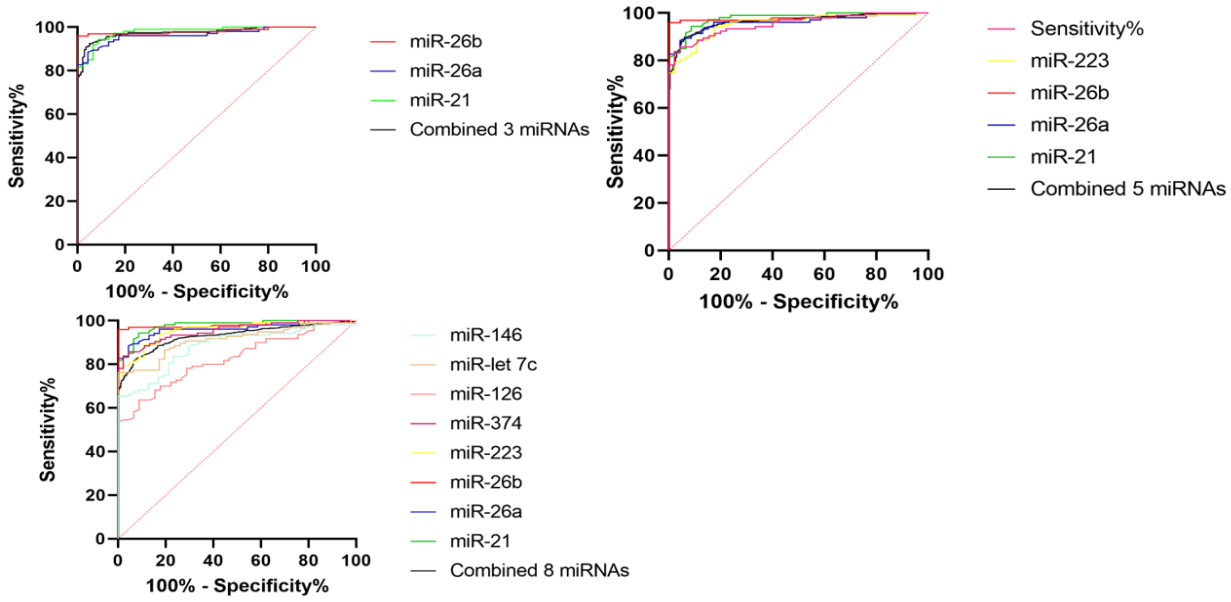

Figure 2. ROC Analysis of the Combining miRs in CRC Patients Compared to Healthy Controls. A) Combining of the three miRs (-21, -26a, -26b) AUC=0.9738 AUC=0.9738. AUC=0.9707 (c) Combining of the 8 miRNAs (Let 7c, 21, 26a, 26b, 126, 146a, 233, 374a) AUC=0.9299, p=0.0001.

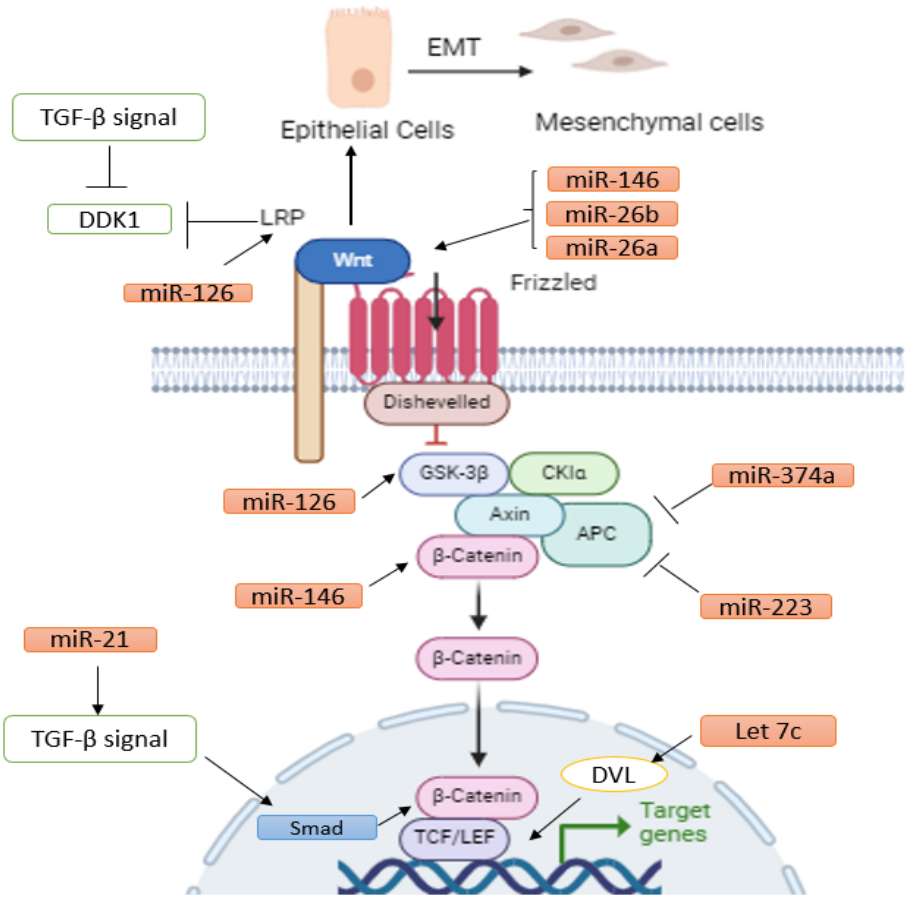

Figure 3. The Interaction between the Signature miRNAs Panel and Both TGF-β and Wnt Signaling Pathways. MiRs target different proteins and regulators that activate WNT pathway which is main stimulator of epithelial-mesenchymal transition that lead to carcinogenesis and colorectal cancer progression.

and pathological parameters in patients and has garnered attention for its prognostic potential in CRC.

These findings confirmed the previous studies on the function of these miRs in CRC development and progression. The substantial upregulation of miR-21 in CRC patients in our study confirms the findings of previous researches which highlighted the significance of miR-21 expression as a valuable and reliable biomarker in oncogenesis [31, 32]. In accordance with our results, [33, 34] reported that human and mouse CRC sera and tissues have higher levels of miR-21, which promote CRC metastasis. MiR-21 has long been recognized as an "oncomir" in several epithelial malignancies, such as esophageal, colorectal, and breast carcinomas. MiR-21 suppresses the programmed cell death 4 (PDCD4) protein, which stimulates cancer invasion [35]. Recently, the role of miR-21 in the development, proliferation, survival, and prognosis of CRC [36-40].

Considering miR-26a & 26b, our results confirmed the previous studies, which documented that the upregulation of miR-26a targets the retinoblastoma (Rb) tumor suppressor gene that enhances the E-cadherin–catenins and CRC [41, 42]. Additionally, align with our results Fan et al., 2018, revealed that upregulation of miR-26b in CRC patients with activated EMT and cancer stem cells. MiR-26b was reported to suppress WNT5A and PTEN expression, which encourages CRC metastasis. Recently, [43] showed that HCG11 stimulates miR-26 b, which promotes cellular malignancy and induces antiapoptotic activity in CRC cells. The study results provide a rationale for both miR 26a & 26b being very important for CRC oncogenesis and metastasis.

Our results regarding miR-223, which showed higher expression in CRC patients, demonstrated its crucial role in CRC development. These findings confirmed the previous studies on miR-223 conducted in different populations [18, 27, 28, 44] which highlighted its role in the promotion and progression of CRC. MiR-223 is an oncogenic miR that targets GTPase-activating protein 1 (RASA1) and suppresses the tumor suppressor F-box/WD repeat-containing protein 7 (FBXW7), which accelerates the development of CRC. Moreover, miR-223 stimulates the WNT/TGF-β pathway, which in turn promotes colorectal cell proliferation [45, 46]. This makes it unsurprising that higher miR-223 expression level has been previously linked to worse clinical and survival outcomes in CRC.

Notably, only one study documented the upregulation of miR-374a expression level in CRC patients [47]. So, the higher fold change in miR-374a in our study highlights its uniqueness and potential role in carcinogenesis. MiR-374a has been identified as a modulator of cell proliferation and apoptosis. Its dysregulation in many cancers has been correlated with tumor aggressiveness, severe

Table 3. The Fold Regulation of 25 miRs in CRC Grade I and II Patients Compared to Healthy Controls

No.	MicoRNA	Fold Regulation in CRC Grade I	P-value	Fold Regulation in CRC Grade II	P-value
1	let-7c-5p	7.0524	0.0012	3.8238	0.0395
2	miR-9-5p	3.5881	0.2344	2.3823	0.6988
3	miR-21-5p	3.6097	0.0040	6.2376	0.0020
4	miR-26a-5p	5.3373	0.0045	9.0484	0.0003
5	miR-26b-5p	9.3459	0.0006	8.0612	0.0282
6	miR-92a-3p	1.7028	0.7863	1.4842	0.9617
7	miR-93-5p	-1.0863	0.5620	1.2625	0.4174
8	miR-126-3p	2.1103	0.0221	2.8606	0.0047
9	miR-128-3p	2.5813	0.1963	3.4653	0.0700
10	miR-146a-5p	4.8947	0.0103	6.2521	0.0005
11	miR-184	3.1025	0.9251	1.3867	0.4435
12	miR-193a-5p	2.4196	0.0407	2.2937	0.0474
13	miR-204-5p	2.171	0.9417	1.8383	0.6817
14	miR-205-5p	3.8861	0.9841	1.4409	0.7653
15	miR-208a-3p	4.8639	0.0709	2.9711	0.1798
16	miR-210-3p	-1.5717	0.6887	-3.1492	0.7543
17	miR-221-3p	-1.3018	0.6066	1.7151	0.4481
18	miR-223-3p	7.1795	0.0038	12.0003	0.0002
19	miR-296-5p	-2.0931	0.3917	1.0007	0.5568
20	miR-374a-5p	6.9158	0.0004	7.5283	0.0001
21	miR-375	-1.0571	0.9664	-1.2235	0.9094
22	miR-423-5p	2.5162	0.5172	3.3519	0.4904
23	miR-499a-5p	3.8957	0.2300	2.456	0.4961
24	miR-574-3p	-2.0752	0.2885	-2.2726	0.0433
25	miR-885-5p	-1.3315	0.7734	-1.1666	0.8039

Table 4. The ROC Analysis of the 8 Individual and Combined miRs in CRC Patients

	AUC	Cut off	Sensitivity%	95% CI	Specificity%	95% CI	P value
Let 7c	0.9113	> 2.314	76.29	66.93% to 83.65%	97.73	88.66% to 99.89%	<0.0001*
MiR-21	0.9786	> 3.734	83.18	74.97% to 89.09%	97.83	88.66% to 99.89%	<0.0001*
MiR-26a	0.9624	> 2.463	83.65	75.37% to 89.54%	97.83	88.66% to 99.89%	<0.0001*
MiR-26b	0.9813	> 2.375	95.96	90.07% to 98.42%	97.8	88.43% to 99.89%	<0.0001*
MiR-126	0.8246	> 2.990	54.55	45.24% to 63.54%	97.74	88.43% to 99.89%	<0.0001*
MiR-146	0.8808	> 3.283	66.35	56.83% to 74.70%	95.74	85.75% to 99.24%	<0.0001*
MiR-223	0.9547	> 3.959	79.44	70.83% to 86.01%	97.83	88.66% to 99.89%	<0.0001*
MiR-374	0.9534	> 2.370	83.81	75.59% to 89.64%	97.81	88.43% to 99.89%	<0.0001*
Combined the best 3 miRNAs	0.9738	> 3.721	78.06	73.13% to 82.31%	99.27	95.98% to 99.96%	<0.0001*
Combined the best 5 miRNAs	0.9707	> 3.918	77.41	70.81% to 79.25%	99.6	97.56% to 99.98%	<0.0001*
Combined the all 8 miRNAs	0.9299	> 3.904	68.43	65.19% to 71.49%	99.73	98.47% to 99.99%	<0.0001*

clinicopathological features, and poor patient survival [48, 49]. It targets genes involved in the Wnt/β-catenin signaling pathway [50, 51]. Furthermore, a positive correlation was observed between the expression level of miR-374a and serum TGF-β1 levels, suggesting the potential value of assessing miR-374a in future translational research studies. As part of our miRs panel, miR-374a contributes to the overall prognostic accuracy and is highly specific for Egyptian CRC patients. Computational investigation showed that miR-21-5p, miR-146a-5p, and let-7a-5p regulate key oncogenic genes, including STAT3, STAT1, and MYC, respectively. Moreover, let7a-5p influences

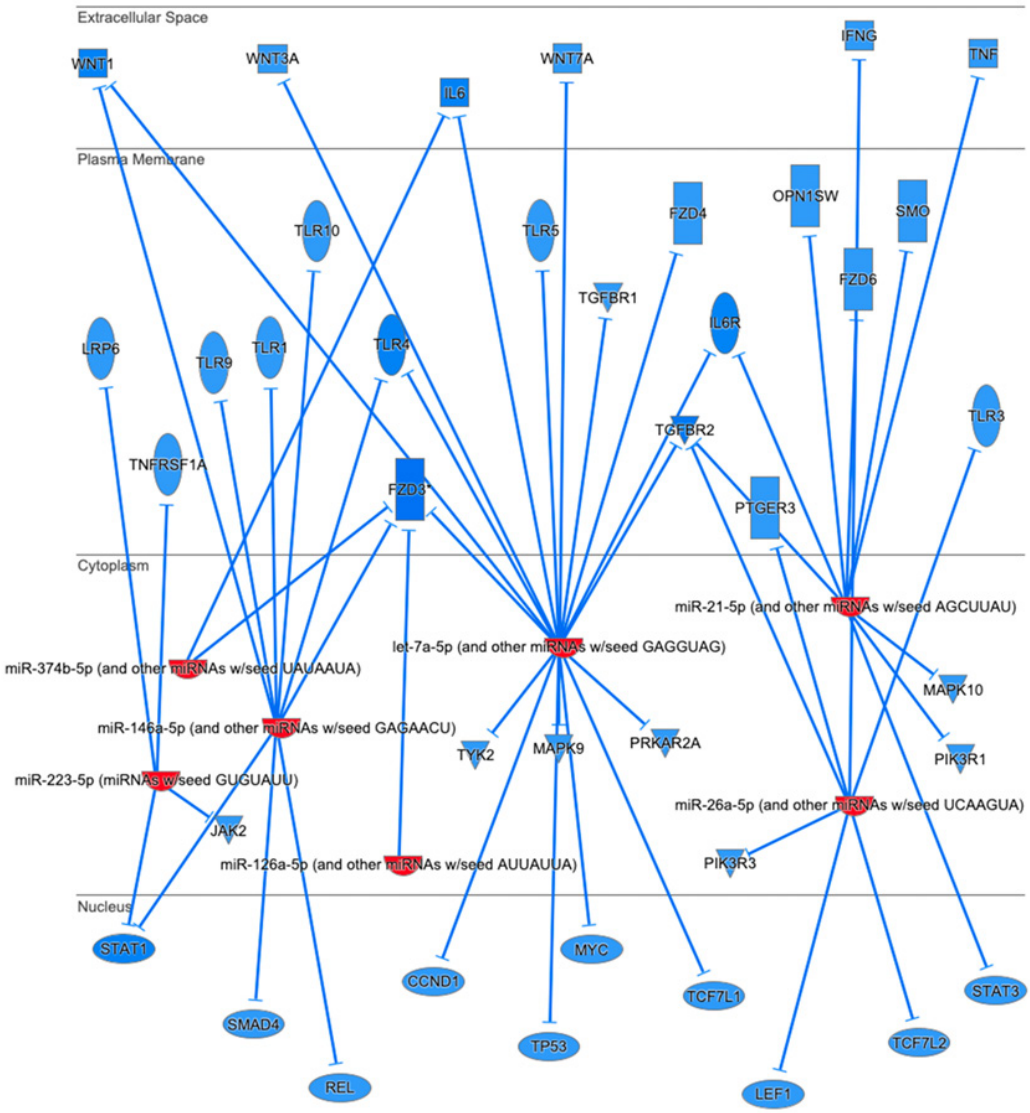

Figure 4. Computer-Generated Analysis of Predicted Target Genes Distributed within Subcellular Compartments in Colorectal Cancer by using Ingenuity Pathway Analysis (IPA). Different shapes are utilized to characterize molecular types: kinases are displayed as triangles, transcriptional regulators as ovals, cytokines as squares, G-protein-coupled receptors as rectangles, transmembrane receptors as stretched hexagons, and mature miRNAs as incomplete ovals. The color scheme emphasizes expression status, with red indicating elevated expression levels and blue denoting predicted components. Suppresive interactions are exemplified by blue lines, indicating predicted relationships.

the WNT signaling pathway through WNT1, WNT3A, and WNT7A, in addition to regulating TGF, TP53, and FZD3, thereby boosting tumorigenesis (Figures 4 and 5).

To understand the relationship between the different miRs in the signature panel, we used www.mirdb.org and www.targetscan.org. We found that our miRNAs affect the TGF-β1 and WNT signaling pathways, which regulate EMT, a process crucial for cancer progression, as shown in Figure 3. In the WNT pathway, Let-7c targets DVL3 directly, whereas β-catenin targets it indirectly. Nevertheless, miR-21 regulates Wnt expression through the FZD8-TGFβ complex [52]. It also stimulates TGF-β1, which leads to EMT. Notably, both miRNAs 26a and 26b upregulated matrix metalloproteinase-9 (MMP9), facilitating extracellular matrix remodeling and cancer cell invasion [42, 53]. Also, they target WNT5A, a non-canonical WNT ligand that can promote EMT and develop cancer [54]. Interestingly, miRs 126 interact with low-density lipoprotein receptor-related protein 6 (LRP6) and Glycogen synthase kinase-3 beta (GSK-3β) [55] while miR-146a induced β-catenin/Wnt Signaling [56] On the other hand, miRs-223 and -374a downregulate the adenomatous polyposis coli (APC) and affect TGFB1 which enhances WNT signaling, stabilizing β-catenin and promoting transcription of WNT target genes [51, 57]. Finally, the ROC curve confirmed that the unique five-miR (21, 26a, 26b, 223, 374a) signature can distinguish CRC patients from healthy controls with a strong diagnostic accuracy of 0.97 and specificity of 99.6, while the three miRs (21, 26a, 26b) had an AUC of 0.973 and specificity

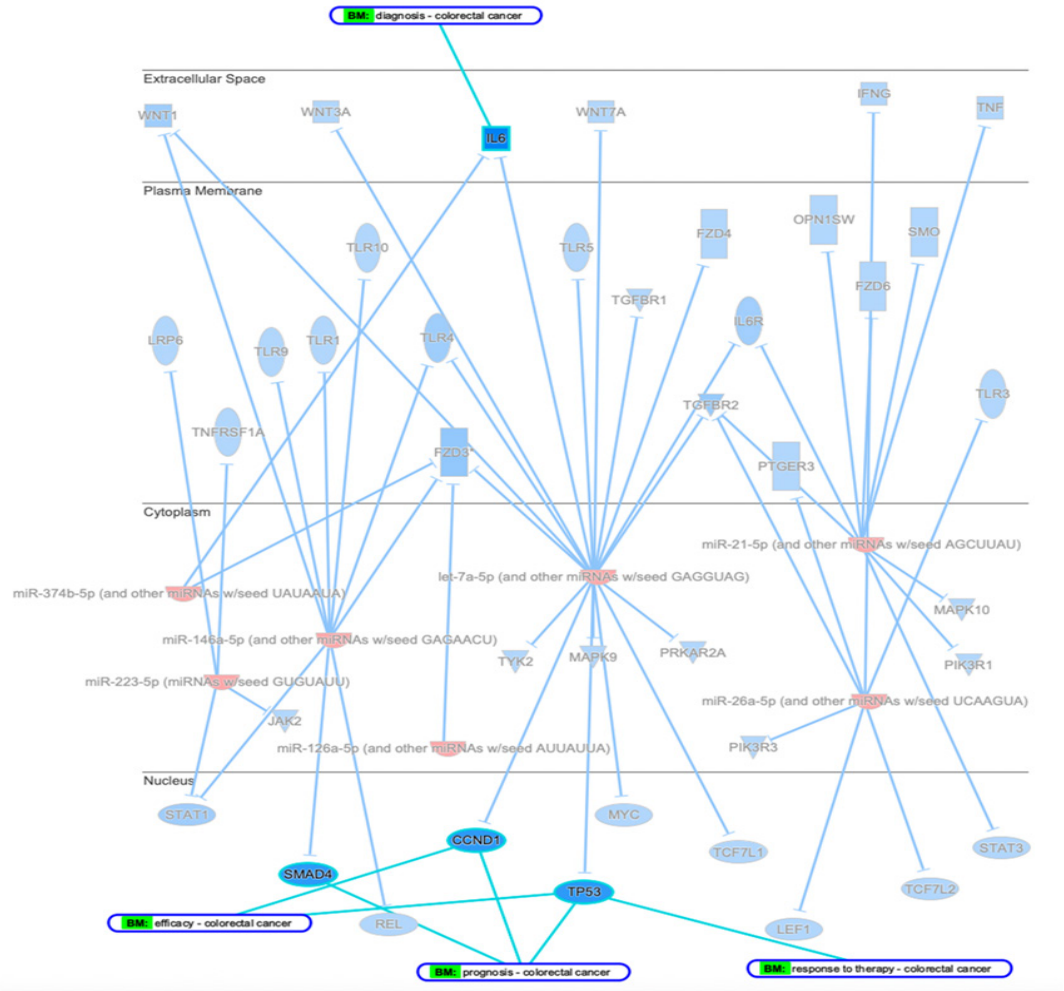

Figure 5. In- silico Analysis of Biomarkers in Colorectal Cancer. The figure was done using IPA (Ingenuity Pathway Analysis).

of 99.27. These results highlight the potential of our five miRs panel as a prognostic and diagnostic biomarker for Egyptian patients with CRC. However, further translational research is needed to improve the current diagnosis and treatment for CRC patients.

In conclusion, the high diagnostic accuracy of our 5 miR panel underscores its potential for non-invasive CRC screening, which could complement existing screening methods such as colonoscopy and fecal occult blood tests. The integration of miR-based biomarkers into clinical practice could revolutionize the management of CRC, particularly in resource-limited settings. The comprehensive miRs signature panel could hold promise for enhancing the accuracy of CRC prognosis, guiding personalized treatment strategies, and ultimately improving patient outcomes worldwide. However, future studies should focus on validating these findings in larger, independent cohorts and exploring the mechanistic roles of these miRs in CRC biology.

Author Contribution Statement

NB designed, prepared, performed experimental work, and wrote the manuscript. SF performed in preparing the manuscript draft, miRNAs extraction, cDNA reaction, and qRT-PCR, Statistical analysis, Figure preparation. RE, RM, ME and MI performed sample extraction, cDNA reaction, qRT-PCR, and data interpretations. ME performed computational analysis. AK samples collection and provided data sheets, All Authors reviewed the manuscript.

Acknowledgements

Ethics approval and consent to participate

This study was approved by the Medical Research Ethics Committee of the National Research Centre reg no. 13060119-1. Each subject signed an informed consent form before participating in this study. All the study procedures and protocols met the ethical standards of the 1964 Declaration of Helsinki (2008 revision).

Availability of data and materials

All data used and analyzed in this study were available upon reasonable request from the corresponding author.

Funding

NRC project fund No.13060119

Declaration of interest

All authors declare there is no conflict of interest.

References

1. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. CA Cancer J Clin. 2020;70(3):145-64. https://doi.org/10.3322/caac.21601.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. https://doi.org/10.3322/caac.21660.
3. Soliman AS, Bondy ML, El-Badawy SA, Mokhtar N, Eissa S, Bayoumy S, et al. Contrasting molecular pathology of colorectal carcinoma in egyptian and western patients. Br J Cancer. 2001;85(7):1037-46. https://doi.org/10.1054/bjoc.2001.1838.
4. Ayed AW, Saber RA, Ali Sholkami NH. Clinico–epidemiological study on colorectal cancer through el-minia governorate during the years 2015–2019. Minia J Med Res. 2023;34(4):35-44.
5. Mauri G, Sartore-Bianchi A, Russo AG, Marsoni S, Bardelli A, Siena S. Early-onset colorectal cancer in young individuals. Mol Oncol. 2019;13(2):109-31. https://doi.org/10.1002/1878-0261.12417.
6. Ryan E, Sheahan K, Creavin B, Mohan HM, Winter DC. The current value of determining the mismatch repair status of colorectal cancer: A rationale for routine testing. Crit Rev Oncol Hematol. 2017;116:38-57. https://doi.org/10.1016/j.critrevonc.2017.05.006.
7. Ladabaum U, Dominitz JA, Kahi C, Schoen RE. Strategies for colorectal cancer screening. Gastroenterology. 2020;158(2):418-32. https://doi.org/10.1053/j.gastro.2019.06.043.
8. Ladabaum U, Mannalithara A, Mitani A, Desai M. Clinical and economic impact of tailoring screening to predicted colorectal cancer risk: A decision analytic modeling study. Cancer Epidemiol Biomarkers Prev. 2020;29(2):318-28. https://doi.org/10.1158/1055-9965.Epi-19-0949.
9. Brouwer NPM, Bos A, Lemmens V, Tanis PJ, Hugen N, Nagtegaal ID, et al. An overview of 25 years of incidence, treatment and outcome of colorectal cancer patients. Int J Cancer. 2018;143(11):2758-66. https://doi.org/10.1002/ijc.31785.
10. Aguiar Junior S, Oliveira MM, Silva D, Mello CAL, Calsavara VF, Curado MP. Survival of patients with colorectal cancer in a cancer center. Arq Gastroenterol. 2020;57(2):172-7. https://doi.org/10.1590/s0004-2803.202000000-32.
11. Brand Bateman L, Khamess S, Abdelmoneim SE, Arafat W, Fouad MN, Khamis Y, et al. Designing an effective colorectal cancer screening program in egypt: A qualitative study of perceptions of egyptian primary care physicians and specialists. Oncologist. 2020;25(10):e1525-e31. https://doi.org/10.1634/theoncologist.2019-0687.
12. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microrna expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A. 2006;103(7):2257-61. https://doi.org/10.1073/pnas.0510565103.
13. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mrnas are conserved targets of micrornas. Genome Res. 2009;19(1):92-105. https://doi.org/10.1101/gr.082701.108.
14. Tétreault N, De Guire V. Mirnas: Their discovery, biogenesis and mechanism of action. Clin Biochem. 2013;46(10-11):842-5. https://doi.org/10.1016/j.clinbiochem.2013.02.009.
15. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. Microrna expression profiles classify human cancers. Nature. 2005;435(7043):834-8. https://doi.org/10.1038/nature03702.
16. Tan Z, Liu Q, Jiang R, Lv L, Shoto SS, Maillet I, et al. Interleukin-33 drives hepatic fibrosis through activation of hepatic stellate cells. Cell Mol Immunol. 2018;15(4):388-98. https://doi.org/10.1038/cmi.2016.63.
17. El-Awady MK, Tabll AA, Atef K, Yousef SS, Omran MH, El-Abd Y, et al. Antibody to e1 peptide of hepatitis c virus genotype 4 inhibits virus binding and entry to hepg2 cells in vitro. World J Gastroenterol. 2006;12(16):2530-5. https://doi.org/10.3748/wjg.v12.i16.2530.
18. Moridikia A, Mirzaei H, Sahebkar A, Salimian J. Micrornas: Potential candidates for diagnosis and treatment of colorectal cancer. J Cell Physiol. 2018;233(2):901-13. https://doi.org/10.1002/jcp.25801.
19. Ahadi A. The significance of microrna deregulation in colorectal cancer development and the clinical uses as a diagnostic and prognostic biomarker and therapeutic agent. Noncoding RNA Res. 2020;5(3):125-34. https://doi.org/10.1016/j.ncrna.2020.08.003.
20. Chen B, Deng YN, Wang X, Xia Z, He Y, Zhang P, et al. Mir-26a enhances colorectal cancer cell growth by targeting rreb1 deacetylation to activate akt-mediated glycolysis. Cancer Lett. 2021;521:1-13. https://doi.org/10.1016/j.canlet.2021.08.017.
21. Howe K. Extraction of mirnas from formalin-fixed paraffin-embedded (ffpe) tissues. Methods Mol Biol. 2017;1509:17-24. https://doi.org/10.1007/978-1-4939-6524-3_3.
22. Fadaka AO, Ojo BA, Adewale OB, Esho T, Pretorius A. Effect of dietary components on mirna and colorectal carcinogenesis. Cancer Cell Int. 2018;18:130. https://doi.org/10.1186/s12935-018-0631-y.
23. Guz M, Jeleniewicz W, Malm A, Korona-Glowniak I. A crosstalk between diet, microbiome and microrna in epigenetic regulation of colorectal cancer. Nutrients. 2021;13(7). https://doi.org/10.3390/nu13072428.
24. Coleman D, Kuwada S. Mirna as a biomarker for the early detection of colorectal cancer. Genes (Basel). 2024;15(3). https://doi.org/10.3390/genes15030338.
25. Dawood RM, El-Meguid MA, Ibrahim MK, Bader El Din NG, Barakat A, El-Wakeel K, et al. Dysregulation of fibrosis related genes in hcv induced liver disease. Gene. 2018;664:58-69. https://doi.org/10.1016/j.gene.2018.04.032.
26. Bader El Din NG, Farouk S, El-Shenawy R, Ibrahim MK, Dawood RM, Elhady MM, et al. Tumor necrosis factor-α -g308a polymorphism is associated with liver pathological changes in hepatitis c virus patients. World J Gastroenterol. 2016;22(34):7767-77. https://doi.org/10.3748/wjg.v22.i34.7767.
27. Bader El Din NG, Ibrahim MK, El-Shenawy R, Salum GM, Farouk S, Zayed N, et al. Micrornas expression profiling in egyptian colorectal cancer patients. IUBMB Life. 2020;72(2):275-84. https://doi.org/10.1002/iub.2164.
28. Bader El Din NG, Farouk S, Abdel-Salam LO, Khairy A. The potential value of mirna-223 as a diagnostic biomarker for egyptian colorectal patients. Eur J Gastroenterol Hepatol. 2021;33(1):25-31. https://doi.org/10.1097/meg.0000000000001961.
29. Farouk S, Khairy A, Salem AM, Soliman AF, Bader El Din NG. Differential expression of mir-21, mir-23a, and mir-27a, and their diagnostic significance in egyptian colorectal cancer patients. Genet Test Mol Biomarkers. 2020;24(12):825-34. https://doi.org/10.1089/gtmb.2020.0184.
30. Krämer A, Green J, Pollard J, Jr., Tugendreich S. Causal

analysis approaches in ingenuity pathway analysis. Bioinformatics. 2014;30(4):523-30. https://doi.org/10.1093/bioinformatics/btt703.
31. Slaby O, Svoboda M, Fabian P, Smerdova T, Knoflickova D, Bednarikova M, et al. Altered expression of mir-21, mir-31, mir-143 and mir-145 is related to clinicopathologic features of colorectal cancer. Oncology. 2007;72(5-6):397-402. https://doi.org/10.1159/000113489.
32. Nielsen BS, Jørgensen S, Fog JU, Søkilde R, Christensen IJ, Hansen U, et al. High levels of microrna-21 in the stroma of colorectal cancers predict short disease-free survival in stage ii colon cancer patients. Clin Exp Metastasis. 2011;28(1):27-38. https://doi.org/10.1007/s10585-010-9355-7.
33. Bakirtzi K, Hatziapostolou M, Karagiannides I, Polytarchou C, Jaeger S, Iliopoulos D, et al. Neurotensin signaling activates microrna-21 and -155 and akt, promotes tumor growth in mice, and is increased in human colon tumors. Gastroenterology. 2011;141(5):1749-61.e1. https://doi.org/10.1053/j.gastro.2011.07.038.
34. Kulda V, Pesta M, Topolcan O, Liska V, Treska V, Sutnar A, et al. Relevance of mir-21 and mir-143 expression in tissue samples of colorectal carcinoma and its liver metastases. Cancer Genet Cytogenet. 2010;200(2):154-60. https://doi.org/10.1016/j.cancergencyto.2010.04.015.
35. Fassan M, Pizzi M, Giacomelli L, Mescoli C, Ludwig K, Pucciarelli S, et al. Pdcd4 nuclear loss inversely correlates with mir-21 levels in colon carcinogenesis. Virchows Arch. 2011;458(4):413-9. https://doi.org/10.1007/s00428-011-1046-5.
36. Schee K, Boye K, Abrahamsen TW, Fodstad Ø, Flatmark K. Clinical relevance of microrna mir-21, mir-31, mir-92a, mir-101, mir-106a and mir-145 in colorectal cancer. BMC Cancer. 2012;12:505. https://doi.org/10.1186/1471-2407-12-505.
37. Peng Q, Zhang X, Min M, Zou L, Shen P, Zhu Y. The clinical role of microrna-21 as a promising biomarker in the diagnosis and prognosis of colorectal cancer: A systematic review and meta-analysis. Oncotarget. 2017;8(27):44893-909. https://doi.org/10.18632/oncotarget.16488.
38. Davey M, Feeney G, Annuk H, Paganga M, Holian E, Lowery A, et al. Identification of a five-mirna expression assay to aid colorectal cancer diagnosis. Gastrointest Disord. 2022;4:190-204. https://doi.org/10.3390/gidisord4030018.
39. el-Awady MK, el-Din NG, el-Garf WT, Youssef SS, Omran MH, el-Abd J, et al. Antisense oligonucleotide inhibition of hepatitis c virus genotype 4 replication in hepg2 cells. Cancer Cell Int. 2006;6:18. https://doi.org/10.1186/1475-2867-6-18.
40. Salum GM, Bader El Din NG, Ibrahim MK, Anany MA, Dawood RM, Khairy A, et al. Vascular endothelial growth factor expression in hepatitis c virus-induced liver fibrosis: A potential biomarker. J Interferon Cytokine Res. 2017;37(7):310-6. https://doi.org/10.1089/jir.2016.0127.
41. Coronel-Hernández J, López-Urrutia E, Contreras-Romero C, Delgado-Waldo I, Figueroa-González G, Campos-Parra AD, et al. Cell migration and proliferation are regulated by mir-26a in colorectal cancer via the pten-akt axis. Cancer Cell Int. 2019;19:80. https://doi.org/10.1186/s12935-019-0802-5.
42. Farouk S, El-Shenawy R, Khairy AM, Bader El-Din NG. Overexpression of mirna 26a and 26b with mmp-9 are valuable diagnostic biomarkers for colorectal cancer patients. Biomarkers in medicine. 2023;17(3):159-69. https://doi.org/10.2217/bmm-2022-0861.
43. Guo P, Liu S, Sun X, Xu L. Association of tgf-ß1 polymorphisms and chronic hepatitis c infection: A meta-analysis. BMC Infect Dis. 2019;19(1):758. https://doi.org/10.1186/s12879-019-4390-8.
44. Zhang Z, Kim K, Li X, Moreno M, Sharp T, Goodheart MJ, et al. Microrna-26b represses colon cancer cell proliferation by inhibiting lymphoid enhancer factor 1 expression. Molecular cancer therapeutics. 2014;13(7):1942-51. https://doi.org/10.1158/1535-7163.Mct-13-1000.
45. Sun D, Wang C, Long S, Ma Y, Guo Y, Huang Z, et al. C/ebp-β-activated microrna-223 promotes tumour growth through targeting rasa1 in human colorectal cancer. Br J Cancer. 2015;112(9):1491-500. https://doi.org/10.1038/bjc.2015.107.
46. Yeh CH, Bellon M, Nicot C. Fbxw7: A critical tumor suppressor of human cancers. Mol Cancer. 2018;17(1):115. https://doi.org/10.1186/s12943-018-0857-2.
47. Bayatiani MR, Ahmadi A, Aghabozorgi R, Seif F. Concomitant up-regulation of hsa- mir-374 and down-regulation of its targets, gsk-3β and apc, in tissue samples of colorectal cancer. Rep Biochem Mol Biol. 2021;9(4):408-16. https://doi.org/10.52547/rbmb.9.4.408.
48. Kim Y, Sim J, Kim H, Bang SS, Jee S, Park S, et al. Microrna-374a expression as a prognostic biomarker in lung adenocarcinoma. J Pathol Transl Med. 2019;53(6):354-60. https://doi.org/10.4132/jptm.2019.10.01.
49. Ji R, Zhang X, Gu H, Ma J, Wen X, Zhou J, et al. Mir-374a-5p: A new target for diagnosis and drug resistance therapy in gastric cancer. Mol Ther Nucleic Acids. 2019;18:320-31. https://doi.org/10.1016/j.omtn.2019.07.025.
50. Cai J, Guan H, Fang L, Yang Y, Zhu X, Yuan J, et al. Microrna-374a activates wnt/β-catenin signaling to promote breast cancer metastasis. J Clin Invest. 2013;123(2):566-79. https://doi.org/10.1172/jci65871.
51. Lei Y, Chen L, Zhang G, Shan A, Ye C, Liang B, et al. Micrornas target the wnt/β-catenin signaling pathway to regulate epithelial-mesenchymal transition in cancer (review). Oncol Rep. 2020;44(4):1299-313. https://doi.org/10.3892/or.2020.7703.
52. Arisan ED, Rencuzogullari O, Freitas IL, Radzali S, Keskin B, Kothari A, et al. Upregulated wnt-11 and mir-21 expression trigger epithelial mesenchymal transition in aggressive prostate cancer cells. Biology (Basel). 2020;9(3). https://doi.org/10.3390/biology9030052.
53. Zucker S, Cao J, Chen WT. Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. Oncogene. 2000;19(56):6642-50. https://doi.org/10.1038/sj.onc.1204097.
54. Mikels AJ, Nusse R. Purified wnt5a protein activates or inhibits beta-catenin-tcf signaling depending on receptor context. PLoS biol. 2006;4(4):e115. https://doi.org/10.1371/journal.pbio.0040115.
55. Guo X, Ji Q, Wu M, Ma W. Naringin attenuates acute myocardial ischemia-reperfusion injury via mir- 126/gsk-3β/β-catenin signaling pathway. Acta cirurgica brasileira. 2022;37(1):e370102. https://doi.org/10.1590/acb370102.
56. Ghuwalewala S, Ghatak D, Das S, Roy S, Das P, Butti R, et al. Mirna-146a/akt/β-catenin activation regulates cancer stem cell phenotype in oral squamous cell carcinoma by targeting cd24. Front Oncol. 2021;11:651692. https://doi.org/10.3389/fonc.2021.651692.
57. Xu J, Lamouille S, Derynck R. Tgf-beta-induced epithelial to mesenchymal transition. Cell Res. 2009;19(2):156-72. https://doi.org/10.1038/cr.2009.5.