ISSN Print: 2664-6188
ISSN Online: 2664-6196
Impact Factor: RJIF 5.35
IJCBB 2025; 7(2): 26-31
www.biochemistryjournal.net
Received: 21-05-2025
Accepted: 25-06-2025

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Elevated expression of circulating miRNA-21 as a potential diagnostic biomarker for colorectal cancer in Iraqi patients

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DOI: https://www.doi.org/10.33545/26646188.2025.v7.i2a.102

Abstract
Colorectal cancer (CRC) ranks as the third most frequently diagnosed malignancy globally and is a primary contributor to cancer-related mortality. Notwithstanding advancements in treatment, late-stage diagnosis continues to be a significant obstacle to enhancing survival outcomes. MicroRNA-21 (miR-21), an oncogenic modulator of tumor-associated pathways, has surfaced as a potential biomarker for cancer diagnosis. This study sought to assess the diagnostic significance of circulating miR-21 in Iraqi individuals with colorectal cancer (CRC). A total of 30 colorectal cancer patients and 30 healthy controls were recruited, and serum samples were obtained for RNA extraction utilizing the TRIzol™ method. Quantitative real-time PCR (RT-qPCR) was conducted to evaluate miR-21 expression, normalized to U6 as an internal control. Results indicated a substantial elevation of circulating miR-21 in colorectal cancer patients, with a 3.4-fold increase relative to controls. Expression levels exhibited a correlation with illness stage, varying from 1.89-fold in stage I to 4.70-fold in stage IIIA. These data underscore circulating miR-21 as a prospective non-invasive biomarker for colorectal cancer diagnosis and disease progression. Integrating miR-21 into screening methodologies may augment early detection and enhance patient prognoses, especially in resource-constrained environments. Extensive, multicenter investigations are necessary to confirm its clinical efficacy.

Keywords: miRNA-21, biomarker, circulating miRNA, early detection, colorectal cancer, Iraqi patients

1. Introduction
In 2020, colorectal cancer was the third most frequently detected type of cancer and the second foremost reason for cancer-related mortality globally, with over 1.9 million new reported cases and 900,000 fatalities recorded [1]. Incidence and mortality rates, along with trends and future projections, exhibit considerable variation across diverse regions and nations [2, 3]. Significantly, there has been a transformation in the age distribution of cases, with an increasing prevalence of diagnoses among younger individuals, particularly in developed nations [4]. These disparities likely arise from differing exposures to risk factors, including lifestyle and environmental influences [5]. Mitigating modifiable risks such as alcohol consumption, tobacco use, obesity, and poor dietary habits, while enhancing protective factors like physical activity, aspirin usage, and balanced nutrition, is crucial for primary prevention. Moreover, screening initiatives aimed at detecting and excising precancerous colorectal lesions constitute a crucial aspect of secondary prevention and are broadly acknowledged as effective in cancer control strategies [6-7].
Although numerous studies have explored different facets of colorectal cancer, key challenges remain in pinpointing its primary risk factors and determining the most effective prevention methods for varied populations. A clear and current synthesis of this knowledge could greatly assist researchers, clinicians, and policymakers. This review delves into the most recent literature to offer updated insights into the epidemiology, risk factors, and prevention of colorectal cancer [8]. Additionally, discovering a more sensitive, easily identifiable, and representative biomarker is essential for enhancing early detection, tracking disease development, forecasting outcomes, and uncovering new treatment opportunities. Genetic mutations, such as those in KRAS, NRAS, and BRAF, impact how patients respond to treatments, especially targeted therapies like EGFR inhibitors [9].

MicroRNAs are short, non-coding RNA molecules, roughly 20 nucleotides long, which help regulate gene expression during and after transcription. They are involved in key biological processes, including cell differentiation, growth, and programmed cell death [10]. Growing evidence suggests that miRNAs hold promise as effective biomarkers for identifying and managing cancer.

2. Materials and Methods

2. 1 Sample collection

This research encompassed 30 patients with CRC and 30 healthy individuals serving as controls. Recruitment occurred from September 2024 to January 2025 at the Oncology Teaching Hospital in Medical City and Al-Yarmook Hospital in Baghdad. Participants' ages varied from 33 to 72 years. An aliquot of 5 mL of venous blood was extracted from both patients and healthy subjects. Patients were diagnosed with primary colorectal cancer via histological analysis. Blood samples were collected in gel tubes, incubated at ambient temperature for 30 min, and subsequently centrifuged for 10 min to isolate the serum.

2.2 Protocol of miRNA extraction from the serum blood samples

The extraction of total RNA, which included microRNA, was performed on the serum samples following the standard procedure outlined in the TRIzol™ Reagent manual.

2.3 RNA Quantitation by Qubit 4.0

The Qubit® RNA HS Assay Kits quantified total RNA, yielding accurate measurements of RNA concentrations across a wide range (4.7-46.1 ng/µl). No substantial disparities in RNA concentrations were observed between tumor and control samples. Likewise, RNA purity was uniform across the identical sample groups.

2.4 Reverse transcription for complementary DNA (cDNA) synthesis

A mixture of 4 µl of RNA and 1 µl of stem-loop reverse transcription primers targeting miR-21 was prepared, and expression analysis was quantified. This was normalized with the U6 housekeeping gene in the normal sample.

2.5 Primers

The primer three designs online and the NCBI-GenBank database were used to design the U6 primer. Macrogen Company provided all these primers that are given in the table below:

Table 1: Information on the reference gene used for primers

Gene	Sequence
U6 F	GAGAAGATTAGCATGGCCCCT
Primer R	ATATGGAACGCTTCACGAATTTGC

The miR-21 primers were custom-designed for this study based on the Sanger Center miR database registry, delivered as given in the table below:

Table 2: Information of the reference gene used for primers

Gene	Sequence
Has-miR-21	GTTGGCTCTGGTGCAGGGTCCGAGG
RT Primer	TATTCGCACCAGAGCCAACTCAACA
Has-miR-21 F	GTTTGGTAGCTTATCAGACTGA
Primer R	GTGCAGGGTCCAGGT

2.6 Quantitative Real-Time polymerase chain Reaction (RT-qPCR)

Table 3 provides the composition of the PCR master mix, and Table 4 specifies the RT-PCR program and thermal cycling conditions used for miR-21 amplification. While Figures 1 and 2 show the Amplification plots for MIR-21 expression obtained by RT PCR and the miR-21 expression melting curve, respectively.

Table 3: Components of qRT-PCR for miRNA-21

Master mix components	Volume Reaction (µl)
SYBR Green master mix	5
Forward primer	0.5
Reverse primer	0.5
Nuclease-free water (N.F.W.)	3
cDNA	1
Total volume	10
Aliquot per single rxn	9 µl of master mix per tube, and add 1 µl of template

Table 4: RT-PCR program

Cycle Step	Temperature (°C)	Time (sec)	Cycles
Initial Denaturation	95	8	1
Denaturation Extension	95 55	15 30 (+plate read)	50
Extension	72	20 (min)	1

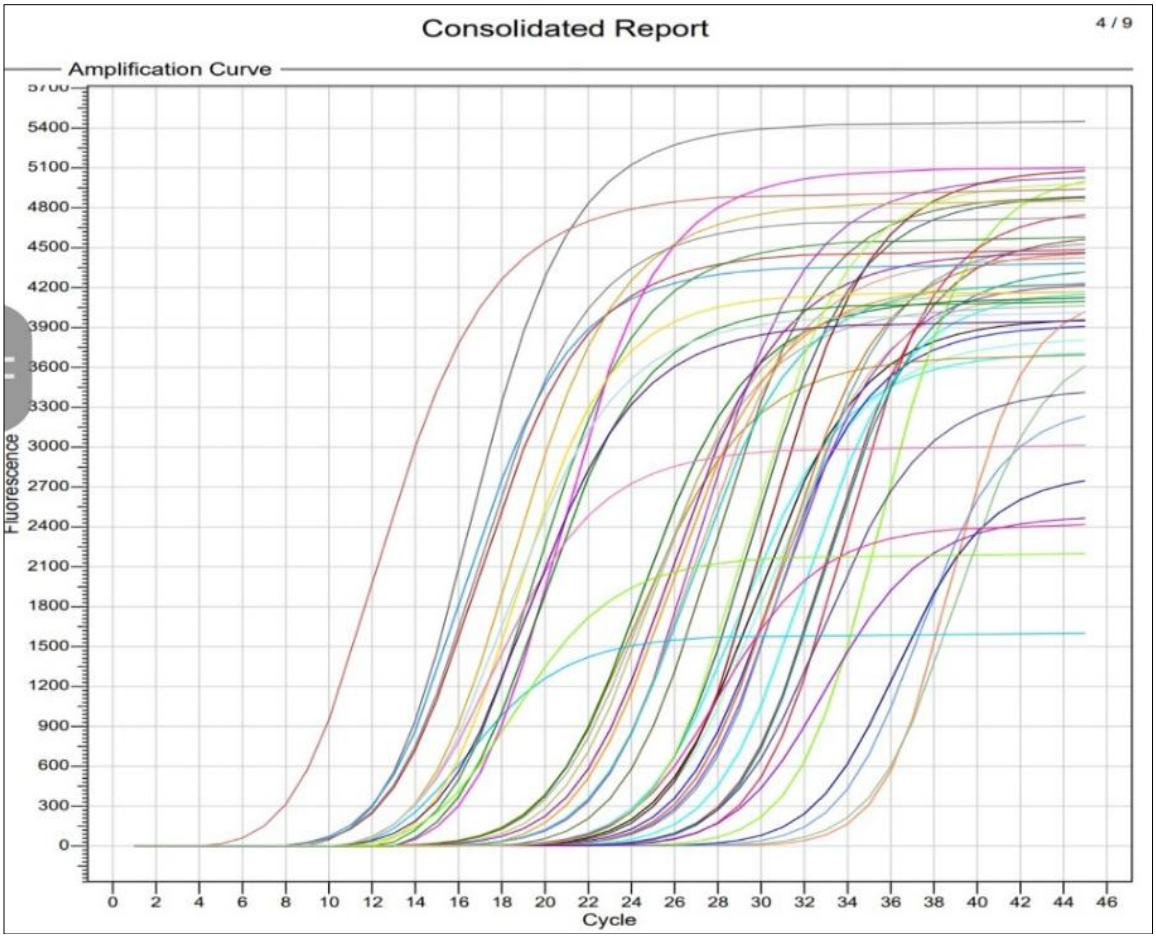

Fig 1: MIR-21 expression amplification plots collected using RT PCR.

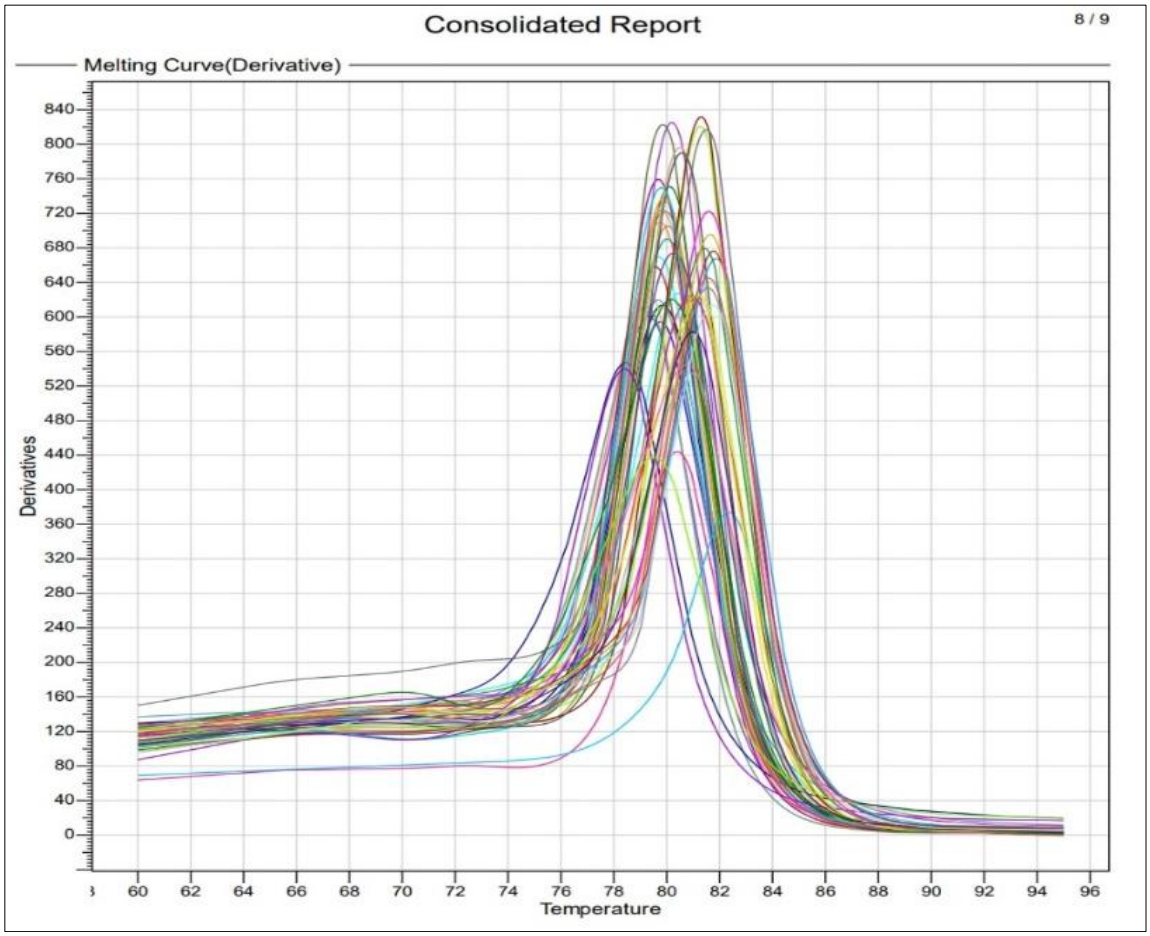

Fig 2: The miR-21 expression Melting Curve

Alterations in mature RNA expression were measured using the relative CT method, denoted by the formula ($2^{-\Delta\Delta Ct}$). This method, established by Livak and Schmittgen in 2001, is a reliable and widely utilized technique for assessing relative gene expression alterations. It elucidates the regulatory mechanisms underlying variations in miRNA expression.

3. Results

3.1 Results of clinical characteristics of the studied samples

The ages of participants in both the CRC group and the healthy controls ranged from 33 to 72 years. The Body Mass Index (BMI), derived from weight and height, served as an index of body size, with the majority of adults classified as overweight or obese. Genetic predisposition, particularly the presence of relatives with colorectal cancer, significantly influences disease risk. Table 5 indicates that a significant proportion of CRC patients possessed a familial history of the ailment. Smoking significantly increases the risk of CRC, and the risk rises with duration and intensity of smoking.

Table 5: Age, family history, smoking, and BMI in subjects

Age (years)	CRC patients	Control
33-44	6	9
44-55	10	10
55-72	14	11
Family history		
Yes	20	0
No	10	30
Smoking		
Yes	25	10
No	5	20
BMI		
25.0-29.9	13	11
30.0-39.9	17	19

3.2 Distribution of CRC patients according to stages of disease

Table 6 illustrates the CRC patient group; the results reveal that patients with stage IIA CRC represent the highest number.

Table 6. Distribution of patients according to stages

Stages	CRC
I	2
IIA	9
IIB	9
IIIA	10

3.3 Molecular analyses of the circulating miRNA-21 expression level

Using relative quantification based on CT values, the RT-qPCR analysis revealed that miR-21 expression was significantly elevated in all patients, showing a 3.402-fold increase relative to healthy controls, whose expression level was normalized to 1.00. Table 7 shows a comparison of miRNA-21 expression between the two studied groups.

Table 7: Comparison of miRNA-21 expression among study groups

Descriptive statistics	CRC patients	Healthy control
Median Fold of change	3.402	1

The relationship between miRNA-21 expression and CRC stages is shown in Table 8. These molecules demonstrate unique expression signatures in tumor cells and tissues and remain detectable in bodily fluids due to their strong chemical stability.

Table 8: Fold change in miRNA-21 expression in the CRC patient group

Stage	Fold
I	1.890
IIA	3.077
IIB	3.303
IIIA	4.699

4. Discussion

Various factors can enhance the possibility of colorectal cancer. These encompass age, where the possibility of developing colorectal cancer escalates with increasing age, particularly after 50 years old. Having a first-degree relative (parent, sibling, or child) with colorectal cancer or polyps significantly elevates an individual's likelihood of developing the condition [11]. Hereditary Cancer Syndrome, certain genetic mutations passed down through families, can significantly raise the risk of colorectal cancer. The most well-known hereditary conditions associated with this cancer are Lynch syndrome and FAP.

Research suggests that around 20% of colorectal cancer patients diagnosed before the age of 50 may carry one of these syndromes. For such individuals, genetic counseling and testing are highly advised [12]. Obesity people with a BMI higher than the normal range are at a certain risk for several diseases, including colorectal cancer. Maintaining a BMI between 19 and 24 is considered healthy and can help reduce this risk. Tobacco Consumption, smoking, or utilizing tobacco increases the risk of colorectal cancer. Diet, A high intake of processed and red meats, coupled with a low consumption of fruits, vegetables, and fiber, may elevate the risk of colorectal cancer. Inflammatory Bowel Disease (IBD) patients, such as Crohn's disease or ulcerative colitis, are at a very high hazard of developing colorectal cancer [13]. A personal history of colorectal cancer or the presence of polyps also raises the chance of future occurrences. The risk is further influenced by characteristics such as the number, size, and type of polyps found [14].

Excessive alcohol use is associated with an increased risk of colorectal cancer. Reducing alcohol consumption is an effective strategy to lower this risk. Also, a lack of regular physical activity can elevate the risk of developing colorectal cancer. Engaging in consistent exercise is a vital preventive measure. Five-year survival rates for colorectal cancer represent the proportion of patients who survive five years post-diagnosis. These rates are contingent upon the extent of cancer dissemination.

The details of the stages are illustrated below:

Localized (Stages 0-II): The neoplasm is fixed to the colon or rectum. The 5-year survival rate is approximately 90-91%. Diagnosis rate: Approximately 35% of cases are identified at this stage.

Regional (Stage III): The malignancy has metastasized to adjacent lymph nodes but not to distant organs. The 5-year survival rate is approximately 73-74%. Diagnosis rate: 36% of instances. Distant (Stage IV): The cancer has disseminated to remote organs such as the lungs or liver.

The 5-year survival rate is 15-16%, and the diagnosis rate is 23% of instances.

Unstaged Cases: The survival rates for these cases are less predictable, averaging approximately 49% [15].

The miR-21 overexpression has been identified as a central player in the progression of colorectal cancer, and most importantly, in terms of promoting metastasis. Recent research shows that miR-21 enhances the progression of colorectal cancer mainly by enhancing its metastatic capability. This underscores the complex interplay between the genetic and epigenetic factors in cancer and hence positions miR-21 as a key promoter of metastasis [16]. A good comprehension of the molecular mechanisms underlying miR-21 will also enhance the creation of targeted therapeutic approaches and methods for enhanced colorectal cancer control. MiR-21 is an oncomir that regulates the expression of many cancer-related genes like PTEN, TPM1, and PDCD4, and has been consistently reported to be overexpressed in numerous human cancers [17, 18].

In colorectal cancer, elevated tumor tissue expression levels of miR-21 have been identified as an independent prognostic and predictive biomarker [19, 20]. This necessitates the need to investigate the diagnostic utility of circulating miR-21 in CRC. While a number of studies have implicated the diagnostic promise of miR-21 in blood samples of CRC patients, the findings are not consistent, and the sample sizes in such studies are generally small [21, 22]. To resolve these inconsistencies, a meta-analysis was conducted to examine the diagnostic value of circulating miR-21 in colorectal cancer with the purpose of seeking the optimal biomarker for CRC diagnosis.

5. Conclusion

This study shows that CRC patients overexpress circulating miRNA-21 compared to healthy controls, which might present its use as a non-invasive biomarker for early detection. MiRNA-21's elevated levels in CRC patients suggest its role in tumor progression and metastasis, supporting previous findings that it regulates oncogenic pathways. These findings demonstrate miRNA-21's clinical potential for CRC diagnosis, particularly in resource-constrained countries like Iraq. More large, multi-center studies are needed to confirm its diagnostic accuracy and explore its prognostic and therapeutic potential. Adding miRNA-21 to screening protocols could improve CRC patient outcomes by improving early detection.

6. Acknowledgment

The author likes to thank Al-Nahrain University for partially supporting this work.

7. Funding

No funds were received.

8. Conflict of Interest

The author declares that there is no known conflict of interest associated with this work.

References

1. Ervik M, Lam F, Laversanne M, Ferlay J, Bray F. Global Cancer Observatory: Cancer Over Time. Lyon: International Agency for Research on Cancer; 2023. Available from: https://gco.iarc.fr/overtime
2. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. Global Cancer Observatory: Cancer Today. Lyon: International Agency for Research on Cancer; 2023. Available from: https://gco.iarc.fr/today
3. Ferlay J, Laversanne M, Ervik M, Lam F, Colombet M, Mery L, et al. Global Cancer Observatory: Cancer Tomorrow. Lyon: International Agency for Research on Cancer; 2023. Available from: https://gco.iarc.fr/tomorrow
4. Eng C, Hochster H. Early-onset colorectal cancer: the mystery remains. J Natl Cancer Inst. 2021;113(12):1608-1610. doi:10.1093/jnci/djab127
5. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. CA Cancer J Clin. 2023;73(3):233-254. doi:10.3322/caac.21772
6. Rex DK, Boland CR, Dominitz JA, Giardiello FM, Johnson DA, Kaltenbach T, et al. Colorectal cancer screening: recommendations for physicians and patients from the U.S. Multi-Society Task Force on Colorectal Cancer. Gastrointest Endosc. 2017;86(1):18-33. doi:10.1016/j.gie.2017.04.003
7. Qaseem A, Crandall CJ, Mustafa RA, Hicks LA, Wilt TJ, Forciea MA, et al. Screening for colorectal cancer in asymptomatic average-risk adults: a guidance statement from the American College of Physicians. Ann Intern Med. 2019;171(9):643-654. doi:10.7326/M19-0642
8. Maida M, Macaluso FS, Ianiro G, Mangiola F, Sinagra E, Hold G, et al. Screening of colorectal cancer: present and future. Expert Rev Anticancer Ther. 2017;17(12):1131-1146. doi:10.1080/14737140.2017.1392243
9. Murphy N, Moreno V, Hughes DJ, Vodicka L, Vodicka P, Aglago EK, et al. Lifestyle and dietary environmental factors in colorectal cancer susceptibility. Mol Aspects Med. 2019;69:9-23. doi:10.1016/j.mam.2019.06.005
10. Meng F, Henson R, Wehbe-Janek H, et al. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology. 2007;133(2):647-658. doi:10.1053/j.gastro.2007.05.022
11. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, et al. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. Ann Oncol. 2011;22(9):1958-1972. doi:10.1093/annonc/mdq653
12. Cai S, Li Y, Ding Y, Chen K, Jin M. Alcohol drinking and the risk of colorectal cancer death: a meta-analysis. Eur J Cancer Prev. 2014;23(6):532-539. doi:10.1097/CEJ.0000000000000076
13. Deng Y, Huang J, Wong MCS. Associations of alcohol and coffee with colorectal cancer risk in East Asian populations: a Mendelian randomization study. Eur J Nutr. 2023;62(2):749-756. doi:10.1007/s00394-022-03076-7
14. Zhou X, Wang L, Xiao J, Sun J, Yu L, Zhang H, et al. Alcohol consumption, DNA methylation and colorectal cancer risk: results from pooled cohort studies and Mendelian randomization analysis. Int J Cancer. 2022;151(1):83-94. doi:10.1002/ijc.33945
15. Thanikachalam K, Khan G. Colorectal cancer and nutrition. Nutrients. 2019;11(1):164. doi:10.3390/nu11010164

16. Hosonaga M, Saya H, Arima Y. Molecular and cellular mechanisms underlying brain metastasis of breast cancer. Cancer Metastasis Rev. 2020;39(3):711-720. doi:10.1007/s10555-020-09881-y
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^(-ΔΔCT) method. Methods. 2001;25(4):402-408. doi:10.1006/meth.2001.1262
18. Asangani IA, Rasheed SA, Nikolova DA, et al. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene. 2008;27(15):2128-2136. doi:10.1038/sj.onc.1210856
19. Schetter AJ, Leung SY, Sohn JJ, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA. 2008;299(4):425-436. doi:10.1001/jama.299.4.425
20. Nielsen BS, Jørgensen S, Fog JU, 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. doi:10.1007/s10585-010-9355-7
21. Xu F, Xu L, Wang M, et al. The accuracy of circulating microRNA-21 in the diagnosis of colorectal cancer: a systematic review and meta-analysis. Colorectal Dis. 2015;17(5):O100-O107. doi:10.1111/codi.12936
22. Yu W, Wang Z, Shen L, et al. Circulating microRNA-21 as a potential diagnostic marker for colorectal cancer: a meta-analysis. Mol Clin Oncol. 2016;4(2):237-244. doi:10.3892/mco.2015.700