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Original research

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Profile Expression and Validation of Plasma miR-92a-3p in Patients with Colorectal Cancer as a Candidate Diagnostic Biomarker: A Study in the Indonesian Population

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Abstract

Mutations, epigenetic alterations, signalling pathway dysregulation, and gut microbiome cause colorectal cancer. Epigenetic modifications affect gene expression patterns without changing the

Information Classification: General

DNA base sequence. Research on cancer and normal tissues demonstrates changes in miRNA expression during carcinogenesis.

This study aims to obtain the signature profile of miRNA in colon cancer patients, as well as its biological functions through bioinformatic analysis, and to validate miR-92a-3p in the plasma of colorectal cancer patients to obtain diagnostic biomarkers.

Plasma miRs were isolated from patients with colorectal cancer and non colorectal cancer. The miRNA profiles were initially evaluated using NanoString analysis, after which selected candidate miRNAs were validated by qRT-PCR.

Differential expression was analyzed and diagnostic performance was assessed using ROC curve analysis. The results indicate that in patients with colorectal cancer, there is upregulation of miR-101-3p, miR-129-5p, miR-146-3p, and miR-198, miR-384, miR-4448, miR-92a-3p as well as downregulation of miR-1228-3p, miR-223-3p, miR-3690, miR-377-3p, miR-379-5p, miR-423-5p, miR-513c-3p, miR-548y, and miR-561-3p compared to non colorectal cancer. In silico analysis showed that these regulated miR play role in colorectal and cancer pathways. Validation of miR-92a-3p expression showed a 1.29 fold upregulation ($p < 0,001$) in colorectal cancer patients compared to non colorectal cancer with an AUC value of 0.83, indicating that this miRNA has good potential as a diagnostic biomarker that requires further validation.

Keywords: miRNA, colorectal cancer, biomarker, miR-92a-3p profiling miRs, validation

Introduction

Colorectal cancer (CRC) is the third most common malignancy in the world after lung and breast cancer. According to the Global Burden Cancer (GLOBOCAN) data from WHO in 2022, the incidence of lung cancer was 2.48 million cases, breast cancer 2.30 million cases, and colorectal cancer 1.92 million cases.[1] With an estimated 1.2 million new cases and more than 600 thousand deaths each year, CRC is the second leading cause of cancer-related deaths worldwide.[2]

The fecal occult blood test has been implemented as one of the screening modalities for early-stage cancer detection. The fecal occult blood test is currently the most widely available non-invasive screening tool, but it has limitations in sensitivity and requires strict dietary restrictions.[3]

Colonoscopy, as the gold standard for early CRC detection, has limitations due to its invasive nature and high cost.[4] Therefore, a new non-invasive examination approach is urgently needed to improve early-stage CRC detection.

Colorectal cancer is caused by various factors such as genetic mutations, epigenetic changes, dysregulation of signaling pathways, and gut microbiota.[5] Epigenetic changes are alterations in gene expression patterns that occur not due to changes in the DNA base sequence. Research comparing miRNA expression profiles in cancer and normal tissues shows differences in miRNA expression during carcinogenesis.[6] Changes in miRNA expression can drive the development of carcinogenesis. Evidence from studies conducted in other populations cannot necessarily be extrapolated to Indonesia, despite the extensive investigation of circulating miRNAs as potential diagnostic biomarkers for lung cancer. Differences in miRNA expression may arise from variation in genetic background, dietary and other lifestyle-related factors, environmental exposure, as well as characteristics unique to particular populations.[25]

Upregulation and downregulation of miRNA expression are observed in KKR conditions 9. Some miRNAs exhibit tissue or developmental stage-specific expression patterns and play a potential role in maintaining tissue identity and function. The pathogenesis of colorectal cancer (CRC) primarily occurs via the chromosomal instability pathway, characterized by the accumulation of specific, non-random increases in DNA copy numbers.[12]

The miR-17-92 cluster comprises miRNA-92a together with five additional mature miRNAs, namely miR-17-5p, miR-18a, miR-19a, miR-19b-1, and miR-20a. Particular interest has been directed toward the miR-17-92 cluster because of its involvement in colorectal cancer. In CRC, expression of the miR-17-92 cluster is elevated and may contribute to tumor progression through the promotion of angiogenesis.[7] The identification of miRNAs and their disease-associated expression patterns has provided new opportunities for the development of cancer diagnostic approaches.[8] Zhou et al. previously reported that excessive expression of miRNA-92a correlates with tumor size, lymph node status, and the TNM classification stage, as well as poor CRC prognosis.[9] Therefore, miRNA-92a is believed to play a role in the mechanism of cancer cell formation.

Understanding miRNA expression levels in cancer patients can provide insights into affected molecular pathways and help identify new therapeutic targets. The high stability of miRNAs in biological fluids, their ease of extraction and measurement, and their promising sensitivity and specificity make them potential biomarkers for colorectal cancer. Identification and measurement of miRNA levels can be used to aid in the early detection of cancer.[10] For this reason, based on this, this study aims to analyze how strongly the plasma miRNA-92-a-3p biomarker candidate has consistent expression and to analyze the target genes involved in the mechanisms of cancer cell formation.

Materials and methods

Research sample collection

The research protocol received ethical approval from the Research Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran, under approval number 626/UN6.KEP/EC/2025. Enrollment in the study was undertaken only after written informed consent had been obtained from each participant. The procedures undertaken in this study adhered to the applicable regulatory and ethical requirements, including the principles outlined in the Declaration of Helsinki.

This research is an observational analytical study with a cross-sectional design. This study uses a consecutive sampling method. The target population of this study is adult patients diagnosed with colorectal cancer who undergo colonoscopy. The accessible population is adult patients suspected of having colorectal cancer from May 2025 to May 2026. This study uses blood plasma examination materials. The plasma examination materials come from patients who visit the Digestive Surgery Polyclinic, have been diagnosed by clinicians as having colorectal cancer or not having colorectal cancer, and are willing to participate in the study by signing an informed consent after being given an explanation. Inclusion criteria for subjects include patients with a histopathologically confirmed diagnosis of colorectal cancer (CRC), patients who have not yet received CRC-related therapy, aged ≥18 years, willing to provide a blood sample (indicated by agreeing to and signing the informed consent), and available clinical data including tumor size (T), lymph node metastasis (N), distant metastasis (M), and stage. Then the exclusion criteria are patients with other malignancies

such as leukemia and lung cancer, and samples in a lysis condition. The plasma examination materials come from venous blood samples of patients diagnosed with KKR and also non-KKR. This study uses clinical data from patients, including demographic data and TNM staging data of KKR patients.

Specimen collection and examination procedure

Specimen collection

Blood samples were collected intravenously of 5 ml using aseptic technique, placed into EDTA vacutainer and centrifuged at 4000 rpm for 10 minutes. The supernatant plasma was transferred to two labeled sterile Eppendorf tubes. Plasma samples were stored at -80°C until the RNA extraction process and followed by the miRNA-92a-3p testing.

miRNA profiling was conducted based on two group categories, namely the colorectal cancer group and the non-colorectal cancer group. The obtained plasma is extracted to obtain total RNA, then the total RNA containing miRNA from the extraction is analyzed using the nCounter NanoString platform.

miRNA isolation from blood plasma samples

Total RNA from blood plasma in both the CRC group and the non-CRC group will be isolated using the miRNeasy Serum/Plasma Kit – microRNA Isolation (Qiagen #217184).

miRNA profiling analysis

Out of 12 RNA samples, they were analyzed using the nanoString instrument. RNA expression was characterized using NanoString technology, which enables direct measurement of individual RNA molecules. Using the nCounter miRNA Expression Panel, expression profiles of as many as 827 human miRNAs can be assessed simultaneously. The data obtained will be processed with nSolver Analysis Software 4.0 and mirnet. Raw measurements generated by the nCounter NanoString platform are provided as RCC (Reporter Code Count) files., which contain raw data on the number of readings for each miRNA, positive control, and negative control. Other supporting files include

RLF (Reporter Library Files) and Raw Count.csv. The analysis of miRNA expression was conducted using R Studio software, with a differential expression analysis approach. Clustering of miRNA expression was performed based on categories of upregulation and downregulation using the partitioning around medoids (PAM) method in the fpc R library to generate a heatmap of exosomal miRNA expression patterns. miRNAs with significant expression changes ($p<0.05$) were further analyzed to determine gene targets using the multimiR R library, which integrates several databases such as MIRTarbase, miRDB, TargetScan, and Diana MicroT. The results are presented as raw data for each miRNA along with several negative and positive controls. This data was then imported into the NSolver 4.0 software. Within nSolver, miRNA measurements are evaluated relative to the overall dataset mean, allowing the miRNAs showing the greatest positive or negative deviations to be identified. Detection of each miRNA in an individual sample was established by comparing its signal with the internal negative-control measurement from the same sample. The amount corresponding to the detected miRNA is then normalized using a set of housekeeping genes, where the geometric mean of the housekeeping genes is calculated. The software then estimates the arithmetic mean of the geometric means for all sample lanes. A normalization coefficient for each sample lane was obtained from the ratio between the overall arithmetic mean and the geometric mean calculated for that lane. and multiplying each amount by this factor to provide the normalized value. These normalized amounts are then compared between samples to obtain the fold change of miRNA. The relative fold change values are calculated for each miRNA. The heatmap depicts miRNA expression changes on a log2 scale after normalization, with values centered according to the corresponding pathway mean.

Validation of miRNA-92a-3p expression using qRT-PCR method

Total RNA was extracted from patient plasma samples using the miRNeasy Serum/Plasma Kit (Qiagen #217184) according to the manufacturer's instructions. RNA extraction protocol diagram (Appendix 4) The RNA concentration was measured first before cDNA synthesis. cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystem #4368814) with specific RT primers for the target miRNA-92a-3p (RT-primer: hsa-miR-92a, Applied Biosystem) and miRNA U6 snRNA (RT-primer: U6 snRNA, Applied Biosystem). Total volume 15µL

consisting of 1.5 uL Reverse Transcription Buffer (10X), 1 uL MultiScribe™ Reverse Transcriptase, 0.15 uL 100mM dNTPs (with dTTP), 0.19 uL RNase Inhibitor, 20 U/µL, 5 µL total RNA (2 ng/uL), 3 µL RT target primer [miRNA 92a] or endogenous control [U6 snRNA], 4.16 uL Nuclease-free water. The PCR program used during the reverse transcription process: 16ºC for 30 minutes, 42ºC for 30 minutes, 85ºC for 5 minutes.

The measurement of miRNA-92a-3p expression was performed using the TaqMan® microRNA Assay miRNA-92a (ThermoFischer Scientific). Meanwhile, U6 snRNA expression was examined using the TaqMan® microRNA Assay U6 snRNA (ThermoFischer Scientific). The examination was conducted in triplicate. Each reaction consists of 10 uL TaqMan® Fast Advanced Master Mix (2×), 1 uL TaqMan® microRNA Assay (20×) [miRNA-92a-3p or U6 snRNA], 1.33 uL cDNA Sample [miRNA-92a-3p or U6 snRNA], and 7.67 uL RT-PCR Grade water. The PCR examination was conducted using the Realtime PCR Cfx 96 Opus Dx (Biorad) device. The PCR program used is 50ºC for 2 minutes, 95ºC for 20 seconds, followed by 40 cycles (95ºC for 3 seconds, 60ºC for 30 seconds). Fluorescent reading was performed at a temperature stage of 60ºC for 30 seconds. Gene expression analysis was performed using the Livak formula with the calculation of the $2^{\Delta\Delta C}$ value. The relative expression of miRNA-92a-3p was calculated using the $2^{-\Delta\Delta CT}$ equation where ΔΔCT = (average Ct miRNA-92a-3p – average Ct U6 snRNA) patient sample - (average Ct miRNA-92a-3p – average Ct U6 snRNA) control sample.[11] Statistical analysis will be conducted using t-test and variance analysis to determine the significance value.

Bioinformatics analysis

A combined functional analysis was undertaken to characterize the potential contribution of miRNA-92a-3p to the development and progression of CRC. Potential targets of miRNA-92a-3p were identified by integrating experimentally supported target information available in TarBase, miRTarBase, and DIANA microT. The use of multiple databases aims to enhance the reliability of the prediction results, as each database has different curation methods and analytical approaches. TarBase and miRTarBase contain miRNA targets supported by experimental evidence, while DIANA microT provides target predictions based on bioinformatics approaches.[12,13] miRTarBase,

which is a database of miRNA-target interactions that are comprehensively annotated and experimentally validated in the field of miRNA research.[14] The complete set of miRNA-92a-3p target genes was subsequently analyzed using the online Database for Annotation, Visualization and Integrated Discovery (DAVID).. DAVID was employed to characterize the functional properties of the target genes through Gene Ontology (GO) classification and to identify relevant biological pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG).[15,16] p-value < 0.05 and a minimum of 2 genes were chosen as the threshold.

The relationships among miRNA-92a target genes were further examined using protein–protein interaction information retrieved from the Search Tool for the Retrieval of Interacting Genes (STRING) database.[17] The PPI network was prepared based on PPI data with a combined score > 0.4 and visualized using the advanced tool Cytoscape. Key nodes within the network were characterized using three complementary topological parameters, namely degree, closeness, and betweenness centrality., based on the CytoNCA plug-in in the prepared network. Additionally, module analysis was applied to filter important modules active in the network using the Molecular Complex Detection (MCODE) plug-in from Cytoscape.[18] The final results included functional enrichment with interactions between nodes/proteins and genes involved in the identified modules. P values < 0.05 are considered to represent statistical significance.

MicroRNA (miRNA)-92a-3p may also be involved in the onset and progression of CRC., as miRNA-92a-3p can predict the initiation and progression of CRC. Functional enrichment analysis of the miRNA-92a-3p target genes was performed using GO and KEGG pathway analyses. The GO analysis classified the enriched terms into three domains: biological process (BP), cellular component (CC), and molecular function (MF).

Data analysis

Data were processed using SPSS version 26.0 with Windows as the operating system. The p-value considered significant is <0.05. Before conducting statistical tests on ratio data, a normality test is performed using the Shapiro-Wilk test. Statistical analysis for comparisons of more than two groups was conducted using a parametric test, namely ANOVA (analysis of variance). That is, the

comparison of each dependent variable in the normal and control groups, the characteristics of the research subjects are presented descriptively. Categorical data is presented as a percentage, while numerical data is presented as the mean and standard deviation for normally distributed data or the median or range for non-normally distributed data. The comparison between the normal group and the KKR group on each day was conducted using an independent t-test for statistical analysis. If there are groups with non-normally distributed normality test results, the testing is conducted using the Mann-Whitney test.

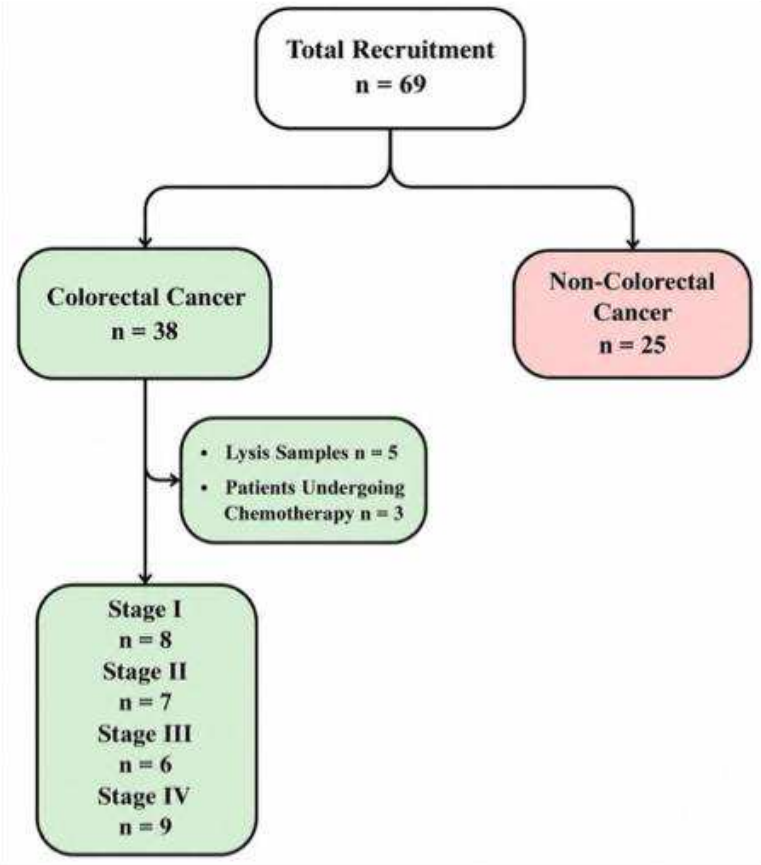


Figure 1 Flowchart of research subject recruitment and selection

Results

Overview of the study sample

This prospective observational study was conducted on 55 subjects as shown in Figure 1. The case

group consisted of 30 patients with colorectal cancer (CRC) who were treated at the Digestive Surgery Clinic. The characteristics of the study subjects can be seen in Table 1.

Table 1 Characteristics of Research Subjects

Variable	**CRC (n= 30)**	**Non-CRC (n= 25)**	**p-Value**
Gender (%)			0.522[a]
Male	17 (56.70)	13 (52.00)	
Female	13 (43.30)	12 (48.00)	
Age (years)			0.298[c]
Median (IQR)	56 (51 - 65)	55 (38 - 65)	
Min-Max	43 - 83	20 - 81	
Colonoscopy (%)			
Normal	0	0	
Inflamed	0	8	
Tumor	30	0	
Cholelithiasis	0	0	
Others	0	11	
Histopathologic examination results (%)			
Poorly differentiated adenocarcinoma	24 (73.00)	NA	
Well differentiated adenocarcinoma	5 (16.67)	NA	
Signet ring carcinoma	1 (3.33)	NA	

Tumor location (%)			
Anus	1 (3.33)	NA	
Transversum colon	2 (6.67)	NA	
Left colon	1 (3.33)	NA	
Sigmoid	2 (6.67)	NA	
Rectum	24 (80.00)	NA	
Stage (%)			
I	8 (26.67)	NA	
II	7 (23.33)	NA	
III	6 (20.00)	NA	
IV	9 (30.00)	NA	

Notes: Analysis using [a]*Chi Square*, [b]*Fisher Exact*.

Abbreviations: NA, not available

In this study, the number of male patients was higher than that of female patients, with a percentage of 56.7% compared to 43.3%. The age of the patients in this study had a median of 56 years with a range of 51-65 years. Based on the histopathological results, the majority of subjects in this study had the following histopathological examination results: 73% poorly differentiated adenocarcinoma, 16.67% well differentiated adenocarcinoma, and 3.33% with signet ring carcinoma. In this study, the most common tumor location was in the rectum at 80%, followed by the sigmoid at 6.67%, the transverse colon at 6.67%, the descending colon at 3.33%, and the left colon at 3.33%. The majority of patients in this study were in stage IV (30%), followed by stage I (26.67%), stage II (23.33%), and stage III (20%).

Analysis of miRNA Profiling in CRC Group

In Figure 2, the expression of miRNA shows both increases and decreases in each research subject, whether in colorectal cancer patients or non-colorectal cancer patients. The red color in Figure 2 indicates an increase in miRNA expression, while the blue color indicates a decrease in expression. Additionally, the intensity of the color shows the difference in expression, with brighter and more intense colors indicating a higher expression of miRNA.

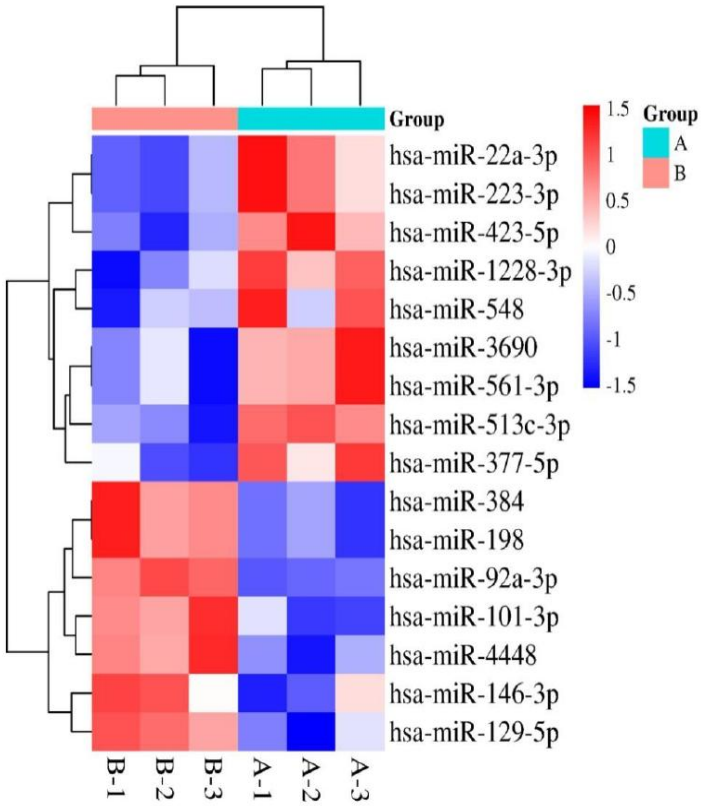


Figure 2. Screening differentially expressed miRNAs using a Nanostring. Candidate differentially expressed in colorectal cancer and non colorectal cancer are shown in a heat map

Based on the results of miRNA expression profiling, in the KKR condition, seven types of onco-miRs were found, which is fewer than the nine types of tumor suppressor miRNAs. Sixteen miRNAs in Table 2 show diverse expression patterns, and some miRNAs have different mechanistic bases in various literatures, although the direction of regulation is not always consistent across populations or specific types of cancer. miRNA-146-3p, which showed the highest fold change in

this study of 5.00 with a p-value of 0.001. The specific role of miRNA-146b-3p in colorectal cancer is currently still relatively new and limited to one study by Wang et al., so the role of miRNA-146b-3p in CRC has not been extensively explored in other research. The literature on the biological functions and gene targets of miRNA-146b-3p in colorectal cancer specifically is still very limited.

Table 2 The value of expression change for each type of miRNA

miRNA	**Fold Change**	**p-Value**
hsa-miR-101-3p	1.97	0.001
hsa-miR-1228-3p	-1.82	0.02
hsa-miR-129-5p	2.29	0.04
hsa-miR-146-3p	5	0.03
hsa-miR-198	2.25	0.05
hsa-miR-223-3p	-2.13	0.002
hsa-miR-3690	-1.56	0.01
hsa-miR-377-3p	-1.63	0.04
hsa-miR-379-5p	-1.63	0.002
hsa-miR-384	2,11	0.05
hsa -miR-4448	1.44	0.03
hsa-miR-423-5p	-1.76	0.05
hsa -miR-513c-3p	-1.63	0.002
hsa-miR-548y	-2.1	0.001
hsa-miR-561-3p	-1.55	0.01
hsa -miRNA-92a-3p	1.55	0.05

From Table 3, the miRNA with the highest degree and betweenness values is miR-16-5p with a degree value of 802 and betweenness of 590198.7, as well as miR-7b-5p with a degree value of 798 and betweenness of 620605.5. However, in Table 2, no statistically significant fold change was observed. The miRNA candidates to be validated in colorectal cancer patients are derived from onkomiR. This is based on the consideration that the expression of onkomiR miRNAs will always be measurable and validated using qRT-PCR in colorectal cancer patient samples. Based on the profiling results, miRNA-92a-3p was selected as a candidate for the validation stage because it showed a significant increase in expression compared to the non-cancer group. In addition to showing consistent expression changes, miRNA-92a-3p has also been widely reported to be associated with the development of various malignancies, including colorectal cancer, thus having the potential to serve as a diagnostic or prognostic biomarker.

Table 3 miRNA profiling using Mirnet

miRNA	**Degree**	**Betwenness**
hsa-miR-16-5p	802	590198.7
hsa-miR-7b-5p	798	620605.5
hsa-miR-92a-3p	594	334000.4
hsa-miR-93-5p	594	268663.3
hsa-miR-15a-5p	484	176267.3

Analysis of miRNA-92a-3p Expression in CRC Compared to Non-CRC

Before conducting the difference test for miRNA-92a-3p, a normality test was performed first. The results of the normality test showed that the miRNA-92a-3p data did not have a normal distribution ($p<0.05$), so the difference test used a non-parametric analysis with the Mann Whitney test. Table 4 shows a significant difference in miRNA-92a-3p expression between the KKR and non-KKR groups with a p-value of 0.002 (1.81±0.94 and 0.95±0.45).

Table 4 miRNA profiling using Mirnet

Group	n	miRNA-92a-3p expression	p
CRC	30	1.81±0.94	0.002
non-CRC	25	0.95±0/45	

The distribution of MiRNA-92a-3p expression results in the colorectal cancer group compared to the non-colorectal cancer group can be seen in Figure 3. The vertical axis shows the expression value of miRNA-92a-3p, while the horizontal axis shows the two compared groups, namely CRC and non-CRC. The middle line of each box represents the median value, the upper and lower bounds of the box indicate the interquartile range (Q1-Q3), while the vertical lines (whiskers) depict the distribution of values outside that range. The points outside the whiskers in the CRC group are extreme values (outliers) that reflect the biological variation of miRNA-92a expression among individuals in that group.

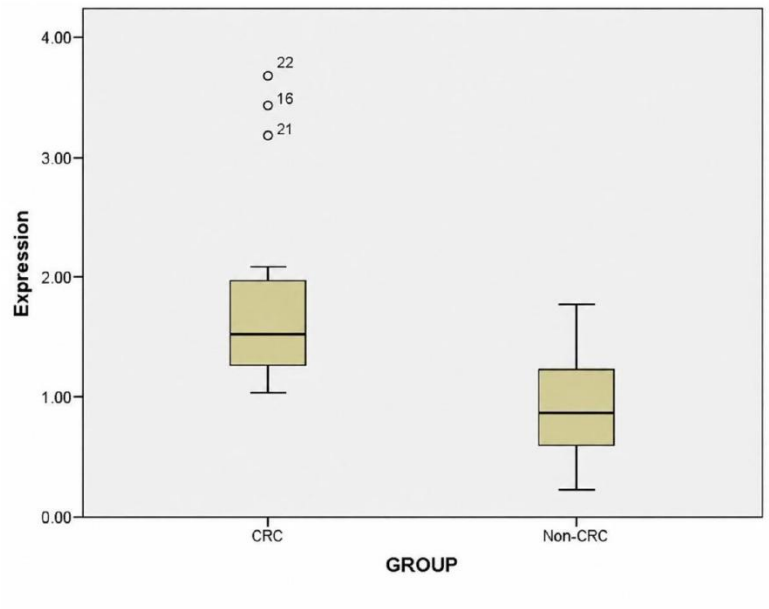


Figure 3 illustrates the mean distribution of miRNA-92a-3p expression differences between the colorectal cancer and non-colorectal cancer groups.

Analysis of the Correlation between miRNA-92a-3p Expression and the Severity Level of CRC

The results of the Kruskal-Wallis test indicate that there is a significant difference in the expression levels of miRNA-92a-3p among stages of colorectal cancer ($p = 0.033$) (Figure 4). Further analysis showed that significant differences were found between stage I and stage III ($p = 0.034$) as well as between stage I and stage IV ($p = 0.013$). Meanwhile, comparisons between stage I and II ($p = 0.598$), stage II and III ($p = 0.133$), and stage II and IV ($p = 0.162$) did not show statistically significant differences.

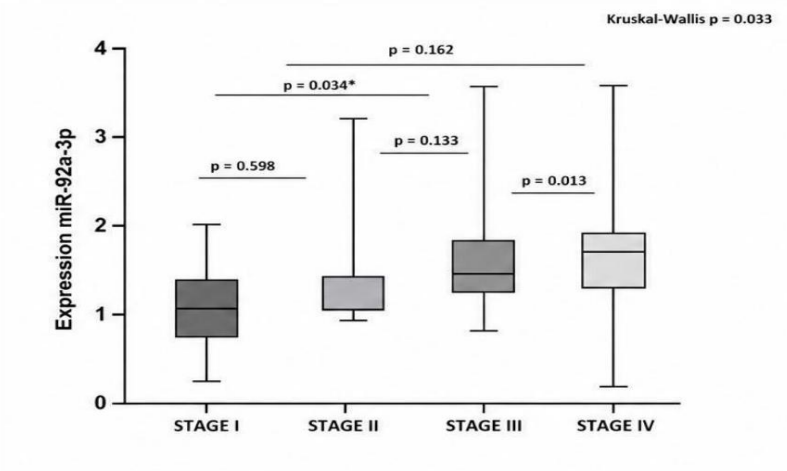


Figure 4 Mean miRNA-92a-3p Levels at Each Stage of CRC

The mean expression values of miRNA-92a-3p in stages I, II, III, and IV were 21.59, 24.43, 33.90, and 35.57, respectively. The analysis results show a correlation value of r = 0.033, indicating a statistically significant relationship between miRNA-92a-3p expression and colorectal cancer stages (p-value < 0.05) (Table 5).

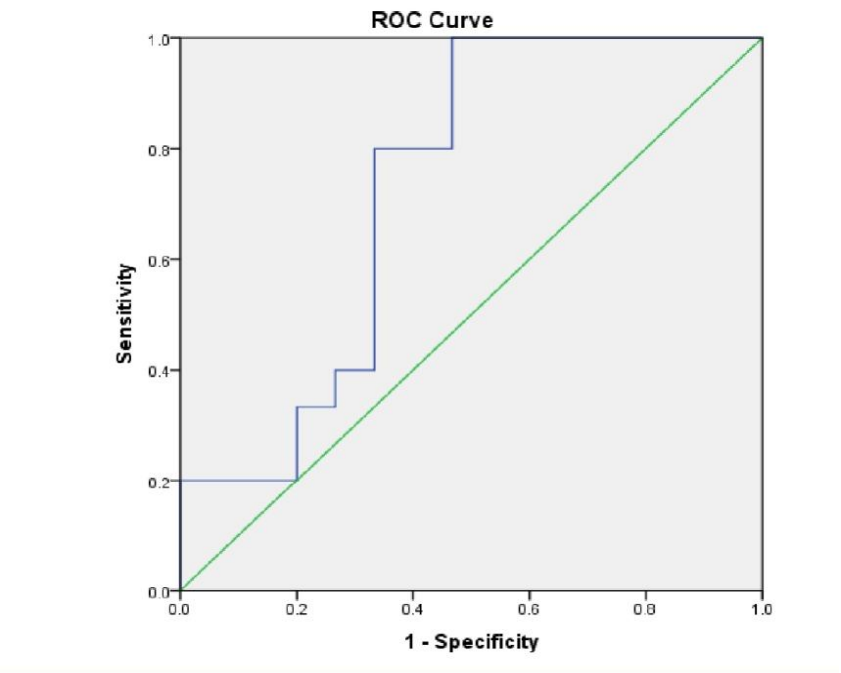


Figure 5 Receiver operating characteristic (ROC) curve for miRNA-92a-3p expression in differentiating between CRC and non-CRC

Table 5 Correlation of miRNA-92a-3p levels with CRC stage

Stage	Mean value of miRNA-92a-3p	Correlation coefficient
I	21.59	0.003
II	24.43	
III	33.90	
IV	35.57	

Analysis of miRNA-92a-3p Expression for CRC Screening

The cut-off expression value of ≥1.29 resulted in an AUC of miRNA-92a-3p of 0.83 (95% confidence interval (CI): 0.68–0.97, P < 0.001), with a specificity of 80% and a sensitivity of 73.3%, as shown

in Figure 5. ROC analysis shows that hsa-miR-320a has an AUC value of 0.718. The interpretation of that value is moderate[19], so miRNA-92a-3p can be considered as a diagnostic biomarker. miRNA-92a-3p has a moderate strength to differentiate colorectal cancer patients from non-colorectal cancer patients.

Bioinformatics Analysis of miRNA-92a-3p in CRC

There are 78 intersections between miRNA-92a-3p and colorectal cancer, namely: ACAP2, AGO2, ARID1A, ATM, AURKA, AXIN1, BMPR1A, BRAF, CASP9, CCND1, CCND2, CD276, CDC27, CDH1, CHEK1, CRTC3, CTSB, DCC, DNMT1, E2F1, EGFR, EP300, EPHB2, EZH2, FASLG, FBXW7, GSK3B, GSTP1, HDAC1, HIF1A, HMGB1, HOXA5, IDH1, IGF1R, IRS2, ITGA5, KAT2B, KLF4, MAP2K4, MAP2K6, MCL1, MDM2, MIER3, MKI67, MYC, MYO1B, NFKB1, NOTCH1, NRAS, PDGFRA, PIK3CA, PIK3CB, PIK3R1, PKM, POLE, PROX1, PTEN, PTPRJ, RBFOX1, RECQL, REV3L, SATB2, SDC2, SIRT1, SLC7A11, SMAD2, SMAD4, SMAD7, SMARCA5, SP1, STAT3, TCF4, TET2, TGFBR1, TGFBR2, TOP1, UCHL1, WNT5A (Figure 6). The targets of hsa-miRNA-92a-3p are closely related to cancer (Figure 6).

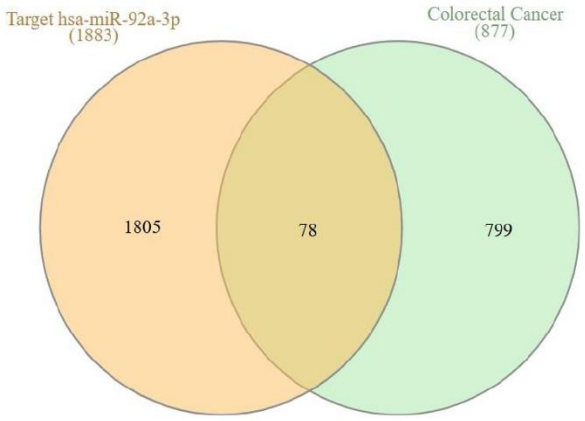


Figure 6 Venn Diagram of the Association between miRNA-92a-3p and Colorectal Cancer

Based on their primary role in cancer, there are several groups of genes associated with cancer, including: Oncogenes: BRAF, EGFR, MYC, NRAS, PIK3CA, PIK3CB, MDM2, CCND1, CCND2, AURKA, GSK3B, DNMT1. Tumor Suppressor Genes: PTEN, ARID1A, ATM, CDH1, FBXW7, MAP2K4, SMAD2, SMAD4, TET2, CHEK1.

The resulting gene set was subsequently subjected to pathway enrichment analysis through ShinyGO and KEGG to determine the biological processes and pathways represented by the identified genes.The analysis results show that the target gene of miRNA-92a-3p is significantly ($p < 0.05$) enriched in various pathways, with the highest enrichment value of 20 displayed in Figure 7. Several pathways that show the highest fold enrichment values include endometrial cancer, chronic myeloid leukemia, colorectal cancer, pancreatic cancer, and prostate cancer.

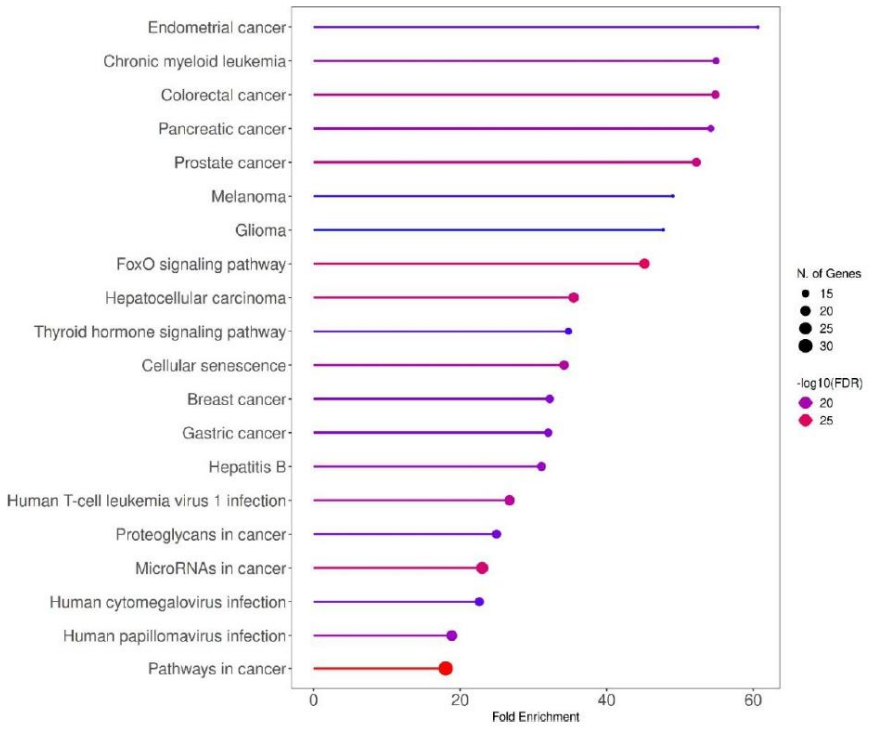


Figure 7 Top 20 target intersections for miRNA-92a-3p associated with colorectal cancer.

A total of 78 target intersections between hsa-miR-92-3p and colorectal cancer were then analyzed using network pharmacology with STRING DB and Cytoscape. STRING DB was used to observe

protein-protein interactions (PPI), while Cytoscape was used to view the network topology score. Greater centrality was represented by circles with larger dimensions and darker intensity (Figure 8). To examine the possible contribution of miRNA-92a-3p targets to CRC biology, the identified genes were mapped onto the colorectal cancer pathway available in KEGG. The analysis results showed that several miRNA-92a-3p target genes are distributed across various components of the colorectal cancer pathway, marked in red on the pathway map as seen in Figure 9. The redder the color on the pathway map and the larger the diameter of the circle, the greater the role of miRNA-92a-3p in that pathway. The KEGG “Pathways in Cancer” category was subsequently examined to determine how the miRNA-92a-3p target genes are distributed among pathways implicated in carcinogenesis. The mapping results showed that several miRNA-92a-3p target genes are involved in various components of the carcinogenesis pathway and are marked in red on the pathway map, as seen in Figure 10.

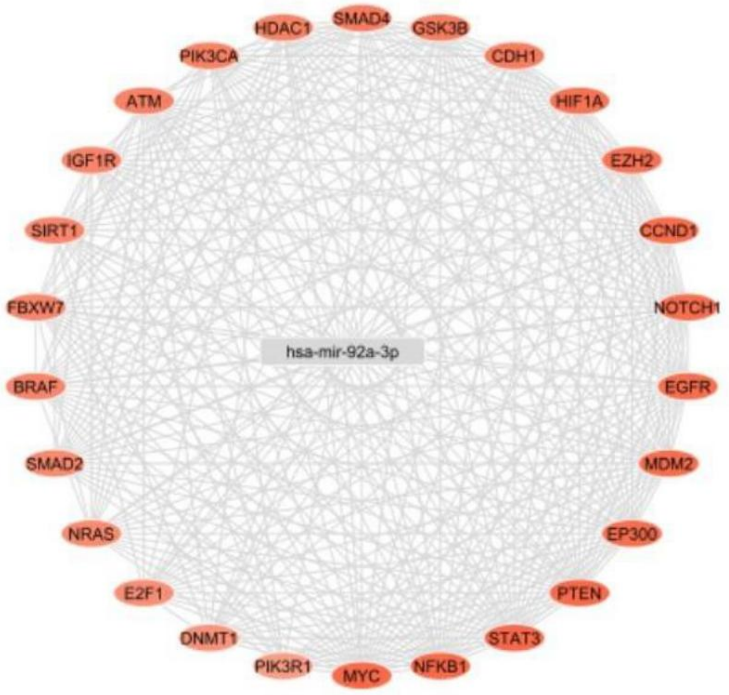
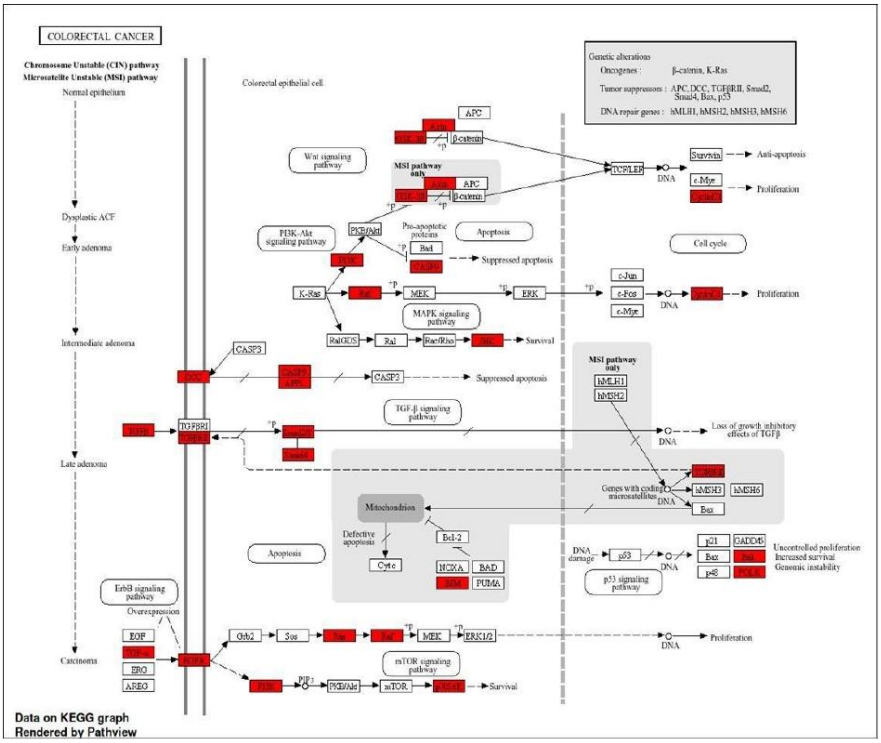


Figure 8 Top protein-protein interaction targets of hsa-miR-92-3p based on MCODE clustering.



385

386 **Figure 9** Involvement of the Target Gene hsa-miRNA-92a-3p in Colorectal Cancer
387 Pathway[16,20,21]

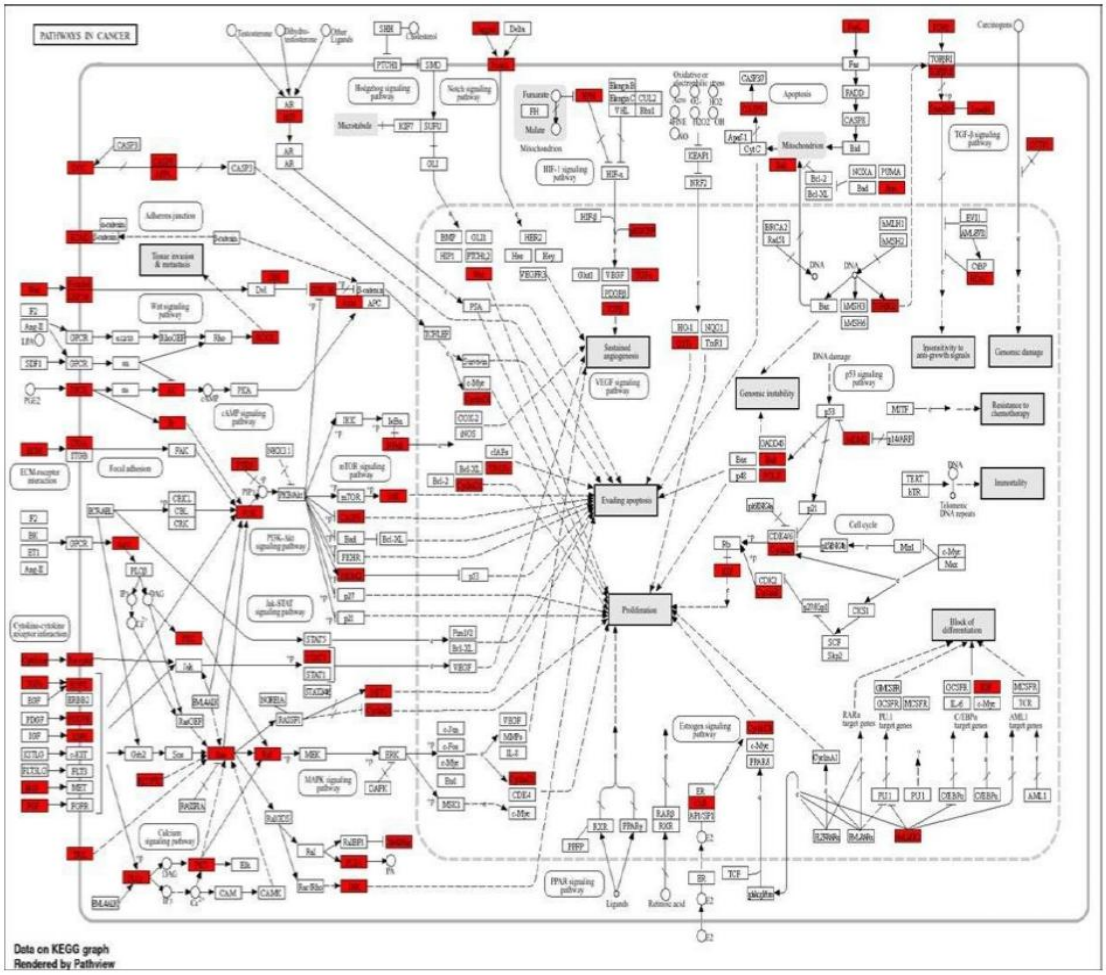


Figure 10 miRNA-92a-3p-Associated Carcinogenesis Pathway Network [16,20,21]

Analysis of Kaplan Meier Test Prediction

Publicly available datasets were analyzed using Kaplan–Meier methods to examine whether expression of miRNA-92a-3p target genes was related to clinical outcomes among patients with colorectal cancer (Figure 11). Survival analysis identified significant associations involving NFKB1, PTEN, STAT3, and MDM2, whereas no statistically significant association was observed for MYC.

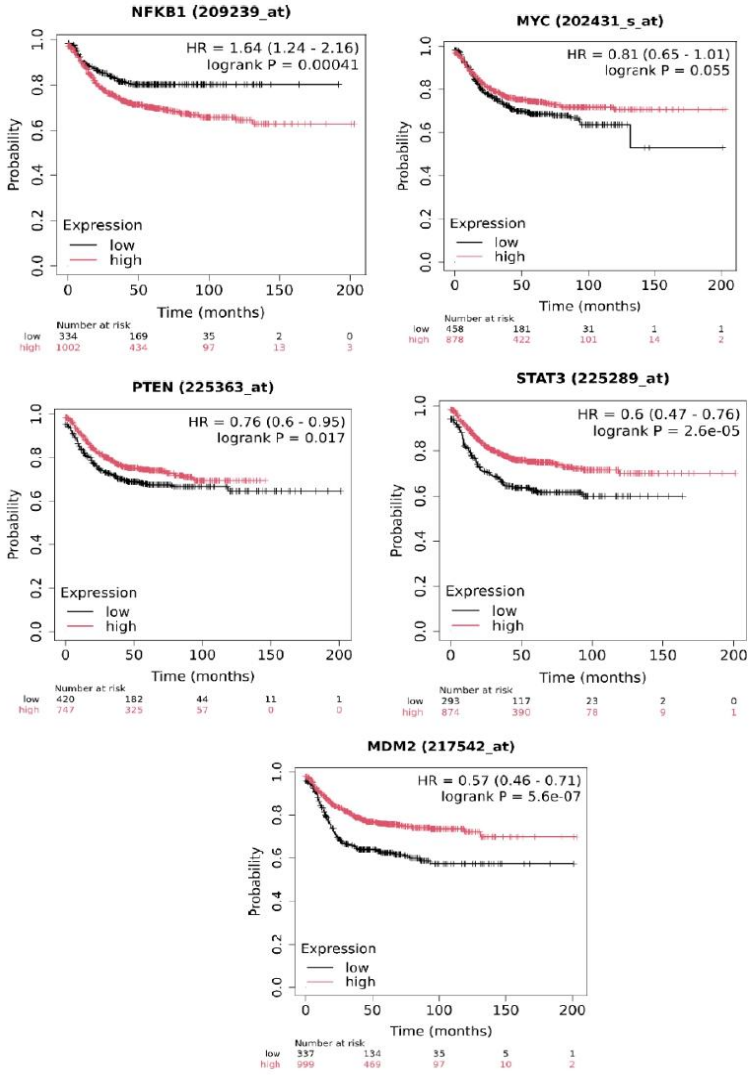


Figure 11 Kaplan–Meier curves based on target gene expression levels in colorectal cancer patients.

High expression of NFKB1 is associated with a worse prognosis compared to low expression, with an HR value of 1.64 (95% CI: 1.24–2.16; p = 0.00041). Conversely, high expression of PTEN is linked to a better prognosis (HR = 0.76; 95% CI: 0.60–0.95; p = 0.017). Similar results were found for STAT3 (HR = 0.60; 95% CI: 0.47–0.76; p = 2.6×10^{-5}) and MDM2 (HR = 0.57; 95% CI: 0.46–0.71; p = 5.6×10^{-7}), where groups with high expression showed a better survival probability compared to groups with low expression. Meanwhile, MYC does not show a significant relationship

with patient survival (HR = 0.81; 95% CI: 0.65–1.01; p = 0.055), although there is a tendency for increased survival probability in the high expression group. Overall, the in-silico Kaplan–Meier analysis shows that the four target genes of miRNA-92a-3p, namely NFKB1, PTEN, STAT3, and MDM2, have a significant relationship with the survival of colorectal cancer patients, while MYC does not show a meaningful relationship.

Discussion

The age characteristics of the colorectal cancer population showed a median age of 60 years and a range of 52-67 years. The number of male subjects was higher than that of female subjects. The age range of subjects in this study is consistent with the research conducted by Roshandel et al. and Matsuda et al., who found an increase in CRC cases starting from the age group of 40 years in both men and women. According to Roshandel et al., age increase is a major risk factor for CRC incidence, where individuals over 50 years old are specifically in the high-risk category.[22,23] In a review by Roshandel et al., it was also explained that the risk of CRC is higher in men with an age-standardized incidence of 23.4 per 100,000 compared to women with 16.2 per 100,000 incidence, with a sex ratio of 1.4.[23]

Most subjects in this study had low-grade adenocarcinoma histopathological examination results, with 73% having low-grade adenocarcinoma, 16.67% having low-grade adenocarcinoma, and 3.33% with signet ring carcinoma results. This is similar to the results in the study by Jurescu et al., which found that most of their research subjects (83.3%) had low-grade adenocarcinoma results. There were 7 cases or 0.50% of subjects with signet ring carcinoma histopathological results.[24] Then, in this study, the most common tumor location was in the rectum, with a frequency of 37%, followed by the sigmoid at 33.3%, which is consistent with research from several major cities in Indonesia and reports from Greystoke et al.[17,25,26]

Based on the stage of the subjects in this study, most were in stage IV (30%), followed by stage I (27%), stage II (23%), and stage III (20%). The results of this study are similar to those of Zaki et al. The majority of the research subjects were in the late stages (III and IV) with a percentage of 90.7%.[27] This is slightly different from the results obtained by Golder et al., where out of 1,146 CRC

patients, 40% were in stage III, followed by stage II with 38%, stage I with 14%, and stage IV with 8% of the subjects.[28]

The analysis results in this study show significant differences in the expression levels of miRNA-92a-3p across stages of colorectal cancer. However, differences were only found between stage I and III and stage I and IV. These findings are consistent with Yamada et al., who also found an increased regulation of miRNA-92a-3p in smaller cancers and cancers classified as clinical stages I to IV with p-values of 0.017 and 0.022, respectively. According to Yamada et al., the expression of miRNA-92a-3p is related to advanced clinical stages and the depth of cancer invasion.[29] Different results were reported by Zaki et al., who found that although there was an increase in miRNA-92a-3p expression from early to late stages, no significant difference in miRNA-92a-3p expression was found with a p-value of 0.303. However, it was also explained that the expression of miRNA-92a-3p positively correlates only with advanced stage colorectal cancer.[27]

miRNA profiling in CRC group

Analysis of the expression data revealed a heterogeneous miRNA profile, with some miRNAs showing increased expression while others demonstrated reduced expression based on their calculated fold changes. Based on the fold change values, the miRNAs that exhibit increased expression include miRNA-146-3p, miRNA-129-5p, miRNA-198, miRNA-384, and miRNA-92a-3p. Conversely, several miRNAs such as miRNA-223-3p, miRNA-548y, miRNA-513c-3p, and miRNA-579-5p show decreased expression in the cancer group. The observed pattern suggests that colorectal carcinogenesis is accompanied by alterations in multiple miRNA regulators, which may collectively influence numerous downstream target genes.[30,31]

The results of the fold change analysis showed that there were three types of miRNAs with the lowest expression decrease, namely miRNA-223-3p by -2.13 times, miRNA-548y by -2.10 times, and miRNA-1228-3p by -1.82 times lower compared to the non-colorectal cancer group. This expression pattern raises the possibility that these three miRNAs may have tumor-suppressive functions in colorectal cancer. In The biological characteristics previously attributed to tumor-suppressive miRNAs are generally consistent with the findings observed among the downregulated

miRNAs in this study. Previous evidence has described miR-377-3p as a potential tumor suppressor in CRC, with its activity linked to suppression of Wnt/β-catenin signaling through regulation of XIAP and ZEB2.[32]

Based on the profiling results, miRNA-92a-3p was selected for further analysis. MiRNA-92a-3p was subsequently selected for further investigation because its expression was significantly higher in patients with CRC than in the non-CRC group, as well as various previous reports indicating the involvement of miRNA-92a-3p in the development of colorectal cancer. MiRNA-92a-3p is a member of the miRNA-17-92 cluster, which is known to have oncogenic activity and plays a role in regulating cell proliferation, angiogenesis, migration, and tumor cell survival.[33,34] Therefore, the validation of miRNA-92a-3p expression was conducted to confirm the initial profiling results.

Analysis of miRNA-92a-3p Expression in CRC Compared to Non-CRC

The difference in miRNA-92a-3p expression between the CRC group and the non-CRC group showed a p-value of 0.002, indicating a significant difference in miRNA-92a-3p expression between the CRC group and the non-CRC group. Our findings are consistent with those reported by Fathi et al., who observed elevated miRNA-92a-3p expression among individuals with CRC relative to healthy controls, with values of 12.46±9.22 compared to 0.61±0.37 and a p-value of <0.001.[35] Zaki et al. also showed a significant increase in the median plasma levels of miRNA-92a-3p in CRC patients (31.1; 1.2-9753.8) compared to controls (0.5; 0-2.3) with a p-value of <0.001. Previous research also supports miRNA-92a-3p as an oncomiR in the carcinogenesis process of colorectal cancer and has the ability to support tumor proliferation, migration, and invasion.[27]

The fold change value in this study was 1.55 at the profiling stage, with a validation average expression of 1.81 in the cancer group, falling within the range reported in studies conducted in Asia, which reported an average increase of 1.5–3 times compared to the control.[31] The variation in reported expression levels may partly reflect methodological and population-related differences, including specimen type, normalization strategy, patient characteristics, and geographic background.

The presence of these outliers needs to be interpreted critically: the relatively high standard deviation in the cancer group (SD = 0.94) reflects the biological heterogeneity of colorectal cancer, where the level of oncomiR activation can vary depending on the stage, histological subtype, genetic background of the patient, and the stability of miRNA in the specimen. This phenomenon is commonly reported in miRNA biomarker research on colorectal cancer, especially in studies with a limited number of samples and a mix of various stages.[31] This heterogeneity may also explain why single biomarkers like miRNA-92a-3p are not yet sufficient as the sole diagnostic marker, prompting some researchers to propose the combination of miRNA panels to enhance detection accuracy.

Correlation Analysis of miRNA-92a-3p Expression with CRC Stage

Unlike gender and age, analysis based on disease stage shows a statistically significant relationship. Patients with stage III and IV had a higher average expression of miRNA-92a-3p compared to stage I–II ($p = 0.001$). The positive correlation between miRNA-92a-3p expression and disease stage has significant biological implications. The increase in miRNA-92a-3p with the progression of the stage indicates that this miRNA is not merely involved in tumor initiation but actively contributes to the processes of invasion and metastasis. Mechanistically, this can be explained through the ability of miR-92a to suppress PTEN, thereby activating the PI3K-AKT pathway which plays a role in cell motility and metastasis formation, as well as suppressing the RECK gene which functions as an inhibitor of matrix metalloprotease.[18] Similar findings were reported by Zhou et al. from China, who found a significant correlation between the overexpression of miRNA-92a-3p in tumor tissue and lymph node metastasis and advanced TNM stage in East Asian colorectal cancer patients.[22]

These findings are consistent with Zhou et al.'s results, which reported a significant correlation between the expression of miRNA-92a-3p in tumor tissue and advanced TNM stage in the East Asian population, and further strengthen the evidence that miRNA-92a-3p plays a role not only in the initiation but also in the progression of colorectal tumors.[22] However, not all studies on Asian populations show a consistent pattern. Ng et al. and Huang et al., who studied miRNA-92a-3p in

519 the plasma of CRC patients in Hong Kong and China, reported that although plasma miRNA-92a-
520 3p levels could distinguish CRC from healthy controls with good sensitivity and specificity, their
521 levels did not correlate with TNM stage.[10,11] This difference in results is likely due to the sample
522 source being plasma in both studies, as in this research, considering that miRNA levels in
523 circulation are influenced by various additional factors such as release from non-tumor cells, renal
524 clearance, and preanalytical variability, which can obscure their relationship with the degree of local
525 tumor severity.
526
527 ***Analysis of miRNA-92a-3p Expression for Colorectal Cancer Screening***
528 The results of the analysis in this study show that the cut-off expression value of ≥1.29 resulted in
529 an AUC of miRNA-92a-3p of 0.83 (95% confidence interval (CI): 0.68–0.97, P < 0.001), the
530 corresponding sensitivity and specificity were 73.3% and 80%, respectively. A slightly higher result
531 was obtained in the study by Zaki et al. with a cut-off value of 1.43, an AUC of 0.994, a sensitivity
532 of 98.1%, and a specificity of 93.9%.[27] In the study by Fathi et al., miRNA-92a-3p showed an AUC
533 of 0.938 (95% CI = 0.880-0.995), indicating good diagnostic capability, with high sensitivity (88%),
534 high specificity (90%), high PPV of 90%, and high NPV of 88%. Based on the analysis using the
535 Youden Index, Fathi et al. obtained an optimal cut-off value of 6.01 with a sensitivity of 73.60% and
536 a sensitivity of 73.60%.[35]
537
538 ***Bioinformatics Analysis of miRNA-92a-3p in CRC***
539 MiRNA-92a-3p has received substantial attention in CRC research, partly because it is encoded
540 within the polycistronic miR-17-92 cluster, located on chromosome 13q13, a This cluster has been
541 implicated in cancer biology through effects on tumor-cell proliferation, inhibition of apoptosis, and
542 promotion of CRC progression.[36] By acting as an oncomiR in CRC, miRNA-92a-3p induces cell
543 proliferation and differentiation thru the activation of oncogenic signaling pathways.[35]
544 Comparison of the two gene sets identified 755 genes shared between the predicted miRNA-92a-
545 3p targets and genes related to colorectal cancer. The overlap suggests that miRNA-92a-3p may
546 affect multiple biological processes relevant to CRC development through regulation of its target

genes. These findings also show that miRNA-92a-3p does not work thru a single target, but rather thru a broad network of gene regulation. These characteristics align with the nature of miRNA, which can regulate multiple targets simultaneously, resulting in a complex biological impact on the process of carcinogenesis.[30]

Due to the large number of overlapping genes obtained, functional analysis is required to understand the biological processes collectively influenced by these genes. Therefore, pathway enrichment analysis was done with the ShinyGO platform, which is integrated with the KEGG (Kyoto Encyclopedia of Genes and Genomes) database. This analysis aims to identify biological pathways that are significantly enriched by the target genes of miRNA-92a-3p. The analysis results show 20 main pathways with a p-value < 0.05. In the bubble plot visualization, the amount of genes involved in the pathway is represented by the size of the circles, while the color gradient reflects the level of enrichment significance based on the −log10(FDR) value.

In addition to the pathways in cancer conditions, the PI3K-Akt signaling pathway also shows a very high level of significance. Among the enriched pathways, PI3K–AKT represents a major signaling axis involved in the control of cellular growth, proliferation, metabolism, and survival. Dysregulated activation of PI3K–AKT signaling has been implicated in the pathogenesis of multiple malignancies, including CRC.[37] Enrichment results also indicate the involvement of the FoxO signaling pathway. The FoxO pathway acts as an important regulator in controlling the cell cycle, apoptosis, DNA repair, and response to oxidative stress.[38] The relationship between the FoxO pathway and miRNA-92a-3p is indirect but mutually reinforcing. When AKT is activated due to the suppression of PTEN by miRNA-92a-3p, AKT phosphorylates FoxO proteins, causing FoxO to translocate out of the nucleus, which inhibits the transcription of pro-apoptotic genes such as BIM and FASL. As a result, apoptosis decreases, allowing cancer cells to survive.

The Hippo signaling pathway is also included in the group of pathways that experience significant enrichment. The Hippo pathway has a primary function in controlling organ size, cell proliferation, and tissue regeneration. When FoxO signaling is disrupted, transcriptional programs favoring cell growth may become activated while apoptotic mechanisms are suppressed.. In various types of cancer, including colorectal cancer, dysregulation of the Hippo signaling pathway has been

reported to contribute to increased tumor cell proliferation and invasion capabilities.[39] The involvement of this pathway indicates that the target of miRNA-92a-3p may also influence the balance between cell proliferation and death.

In addition to pathways directly related to proliferation and apoptosis, the analysis also showed significant enrichment in Proteoglycans in Cancer and Focal Adhesion. Proteoglycans play a role in the interaction between cancer cells and the tumor microenvironment, including the regulation of adhesion, migration, and intercellular communication. Meanwhile, focal adhesion is an important component that connects the cell cytoskeleton with the extracellular matrix. The activation of these two pathways is often associated with increased invasion and metastasis capabilities of cancer cells.[40] Interestingly, the Colorectal Cancer pathway ranked third highest with a fold enrichment value of 8.10. These findings indicate a strong correlation between the target genes of miRNA-92a-3p and the molecular mechanisms underlying colorectal cancer.

Further analysis of specific components in the Colorectal Cancer pathway (hsa05210) shows that several target genes of miRNA-92a-3p occupy central positions in the colon carcinogenesis cascade. In the MAPK signaling and transduction pathway, the BRAF and NRAS genes are found to function as key components in regulating cell proliferation and growth.[41] In the apoptosis pathway, the BCL2L11 (Bim), BAK1, and CASP9 genes are identified. Among these genes, CASP9 serves as the main mediator of programd cell death thru the intrinsic apoptosis pathway.[42] Additionally, the DCC gene and components of the TGF-β pathway such as SMAD2, SMAD4, and TGFBR2 are also identified. Within the TGF-β signaling system, SMAD4 and TGFBR2 are important mediators involved in controlling epithelial cell growth and maintaining tumor-suppressive activity in the colon.[43]

The miRNA-92a-3p target genes mapped to the colorectal cancer pathway include genes involved in the Wnt signaling pathway (AXIN and GSK-3β), TGF-β signaling (TGFβ, TGFBRII, SMAD2, SMAD3, and SMAD4), PI3K-Akt signaling (PI3K, PDK1, and p70S6K), MAPK signaling (RAS, RAF, JNK), cell cycle regulation (Cyclin D1), and apoptosis (CASP9, BIM, BAK, and DCC). Previous research has additionally indicated that members of the miR-17-92 cluster can modulate important components of TGF-β signaling, including SMAD2, SMAD4, and TGFBR2. in neuroblastoma. In

603 the context of epithelial–mesenchymal interactions, previous research demonstrated that stromal
604 deletion of TGFBR2 in colorectal cancer can propamote tumor initiation and progression in vivo.
605 Consistent with these findings, Nishida et al. reported marked suppression of TGFBR2, SMAD2,
606 and SMAD3 in the tumor stroma, with these genes identified as potential targets of the miR-17-92a
607 and miR-106b-25 clusters.[44]
608
609 ***Role of miRNA-92a-3p Target Genes in Colorectal Cancer and Cancer Pathways***
610 In this study, the expression of miRNA-92a-3p was found to be significantly increased in the
611 colorectal cancer group compared to the non-cancer group, 1.81 ± 0.94 compared to 0.95 ± 0.45,
612 $p = 0.002$. This increase in expression has broad biological consequences, as miRNA-92a-3p has
613 been identified to target several key genes in the colorectal cancer pathway (hsa05210) and the
614 cancer pathway (hsa05200). To understand the mechanistic implications of these findings, the roles
615 of each target gene and their regulatory directions due to the increased expression of miRNA-92a-
616 3p are outlined below.
617 Glycogen Synthase Kinase 3 Beta (GSK3β) functions as a key element of the β-catenin destruction
618 complex, together with APC and Axin. Under physiological conditions, the complex promotes β-
619 catenin phosphorylation, thereby facilitating its subsequent degradation through the proteasome.
620 Thru this mechanism, GSK3β functions as a gatekeeper of the Wnt pathway by preventing the
621 accumulation of free β-catenin within the cell. When GSK3β is active and the destruction complex
622 functions normally, the expression of proliferative genes induced by β-catenin, such as MYC and
623 Cyclin D1, remains controlled.[45] In this study, the increased expression of miRNA-92a-3p found in
624 the colorectal cancer group is suspected to contribute to the decreased expression of GSK3β. The
625 decrease in GSK3β causes the destruction complex to not function optimally, leading to the
626 accumulation of free β-catenin in the nucleus, activating the transcription of Wnt target genes that
627 drive tumor proliferation and progression.
628 KRAS, BRAF, and NRAS are the main components of the MAPK pathway that play a role in
629 transducing mitogenic signals from cell surface receptors to the nucleus to induce the expression
630 of proliferation genes. Under normal conditions, the activation of these three genes is transient and

631 depends on growth factor stimulation. Somatic mutations in KRAS are found in approximately 40–
632 45% of colorectal cancers, while BRAF and NRAS are found in 5–15% and 3–5% of cases,
633 respectively, and all three lead to constitutive activation of the MAPK pathway.[41] Although KRAS,
634 BRAF, and NRAS generally act as oncogenes thru mutation mechanisms, the identification of these
635 genes as targets of miRNA-92a-3p indicates an additional layer of regulation involving miRNA
636 expression.
637 *PIK3CA and PIK3CB encode the catalytic subunits p110α and p110β of PI3K, while PIK3R1*
638 *encodes the regulatory subunit p85α that regulates PI3K activity. All three were identified as target*
639 *genes of miRNA-92a-3p in the colorectal cancer pathway. On the other hand, PTEN identified in*
640 *the cancer pathway is a major tumor suppressor that directly counteracts PI3K activity by*
641 *dephosphorylating PIP3 to PIP2, thereby preventing the recruitment and phosphorylation of AKT*.[37]
642 In this study, the increase in miRNA-92a-3p expression found to be 1.81 ± 0.94 in the cancer group
643 with a p-value of 0.002 is suspected to contribute to the decrease in PTEN expression.
644 TGFBR2 is a type II TGF-β receptor that receives antiproliferative signals and transduces them into
645 the cell thru the phosphorylation of SMAD2. The phosphorylated SMAD2 gene subsequently binds
646 with SMAD4 to form a transcription complex that activates the expression of cell cycle inhibitor
647 genes and pro-apoptotic genes. Thus, the TGF-β–SMAD pathway is one of the most important
648 tumor suppressor mechanisms in the colon epithelium, and the loss of any component of this
649 pathway is sufficient to disrupt the entire antiproliferative signal.[43] Loss of TGFBR2 function is found
650 in more than 30% of colorectal cancers with microsatellite instability, while deletion or mutation of
651 SMAD4 is found in 10–20% of cases and is associated with poor prognosis and increased risk of
652 metastasis.
653 CASP9, BCL2L11 (BIM), and BAK1 are core components of the intrinsic apoptosis pathway
654 mediated by mitochondria. The BCL2L11 gene functions as a BH3 cellular stress sensor that
655 activates BAK1 to permeabilize the outer mitochondrial membrane, triggering the release of
656 cytochrome c, and activating CASP9 as the initiator of the programd cell death cascade.[42] These
657 three genes functionally act as brakes against the proliferation of cells experiencing DNA damage
658 or oncogenic stress. In this study, the increased expression of miRNA-92a-3p, confirmed thru

qPCR testing (p = 0.002), is suspected to cause a simultaneous decrease in the expression of BCL2L11, BAK1, and CASP9.

Deleted in Colorectal Carcinoma (DCC) is a classic tumor suppressor gene located on chromosome 18q21. The DCC protein product functions as a dependence receptor for netrin-1. When the netrin-1 ligand is unavailable, DCC induces cell apoptosis as a mechanism to safeguard tissue integrity. Additionally, DCC plays a role in regulating cell adhesion and preventing cell invasion into surrounding tissues. Loss of heterozygosity (LOH) at 18q, which includes the DCC and SMAD4 loci, is found in more than 60% of advanced colorectal cancer and is directly associated with an increased risk of liver metastasis. In this study, the higher expression of miRNA-92a-3p in stages III–IV compared to stages I–II with a p-value of 0.002 aligns with the hypothesis that the suppression of DCC by miRNA-92a-3p contributes to increased invasiveness and metastatic potential in advanced stages.

Based on the overall analysis of the target genes identified in the Colorectal Cancer Pathway and the Cancer Pathway, the increased expression of miRNA-92a-3p found in this study, with an average expression of 1.81 ± 0.94 in the cancer group compared to 0.95 ± 0.45 in the non-cancer group (p = 0.002), is estimated to simultaneously suppress various essential tumor suppressor genes, including PTEN, GSK3β, SMAD4, TGFBR2, BCL2L11, BAK1, CASP9, and DCC. Thus, the increased expression of miRNA-92a-3p found in this study is consistent with its role as an oncomiR that simultaneously activates proliferation pathways, inhibits apoptosis, and promotes colorectal cancer progression. These findings reinforce the potential of miRNA-92a-3p as a diagnostic biomarker and an RNA-based therapeutic target worthy of further development in colorectal cancer.[9,31,33]

Results of in silico Kaplan-Meier prediction analysis

Based on the results of the Kaplan-Meier test, three groups of genes were found to influence the predictors of KKR severity. In the good prognosis group, patients with high gene expression levels had better survival rates than those with low expression levels. The genes included in this category are MDM2 & STAT3. These two genes show the largest curve ranges with very small p-values

(10^{-7} and 10^{-5}), indicating that this difference is not coincidental but has a very high statistical power to be used as prognostic biomarkers. Next, the PTEN gene is one of the tumor suppressor genes, and in line with its general role as a tumor suppressor, high levels of PTEN expression indicate better survival. The p-value of 0.017 is below the significance threshold of 0.05, so this difference is considered significant. Ren et al. in their study explain that PTEN-deficient KKR cells exhibit an increased CIN phenotype and are highly sensitive to STAT3 inhibition.[46] This is different from Karjalainen et al. who did not find a significant prognostic value of PTEN expression in KKR. In the analysis of KKR-specific mortality, the multivariable hazard ratios associated with PTEN loss were evaluated across the two cohorts. The corresponding HRs for full PTEN expression were 1.19 (95% CI 0.88–1.61) in cohort 1 and 0.85 (95% CI 0.55–1.31) in cohort 2. Across both cohorts, PTEN loss showed an association with BRAF mutation status and MMR deficiency. However, no independent association was observed between PTEN loss and either the composition of tumor-infiltrating immune cells or the expression levels of PD-L1, PD-1, IDO, and ARG.[47]

In the non-significant group (MYC), the MYC gene is one of the oncogene groups. Although the high and low expressions are quite widely separated, they have a 95% CI value (0.65 - 1.01) with a range crossing the number 1.0, and a Logrank with a p-value of 0.055 (> 0.05), the difference in survival ability between high and low MYC expression in this data is not considered statistically significant. Meanwhile, in a study by Saed et al., it is explained that MYC plays a role in promoting the survival of KKR cells, proliferation, and immune evasion with increased proteostress. Inhibiting the oncogenic program driven by MYC causes hyperactivation of the integrated stress response (ISR) and immunogenic cell death (ICD).[48]

In the poor prognosis group, the NFKB1 gene was the only data with a low expression range above the high expression range, with an HR value of 1.64 (>1), indicating that patients with high NFKB1 expression have a 1.64 times higher risk of death compared to patients with low expression. The p-value = 0.00041 indicates that this result is highly statistically valid. NFKB1 acts as a bad prognostic marker in cases of CRC. Increased NFKB1 in response to the EGFR oncogene can be detrimental to the tumor population by eliminating the oncogene and allowing the tumor to survive[49]

Conclusion

This study shows that miRNA-92a-3p is one of the microRNAs identified thru profiling analysis and has higher expression levels in colorectal cancer patients compared to the non-cancer group. These findings suggest that miRNA-92a-3p has the potential to be used as a candidate biomarker to differentiate colorectal cancer patients from non-colorectal cancer individuals.

Ethics approval and consent to participate

Ethical approval for the research protocol was granted by the Research Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran, under approval number 626/UN6.KEP/EC/2025.

Consent for publication

Not applicable

Availability of data and materials

The research data are maintained in an institutional repository and can be made available upon reasonable request to the corresponding author.

Competing interests

The author(s) report no conflicts of interest in this work.

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Authors' contributions

Conceptualization, R.H.; writing-original draft preparation, R.H.; writing-review and editing, R.H., M.B.B., R.R., N.S., S.H.A and I.P.

visualization, S.H.A., N.S.; supervision, M.B.B., I.P. and R.R. All authors have read and agreed to the published version of the manuscript.

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