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Original Article

# MIRNA-mediated regulation of the PI3K/AKT signaling pathway in colorectal cancer: A study based on data mining

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**Abstract**

**Aim:** In colorectal cancer (CRC), expression of genes involved in the PI3K/Akt signaling pathway varies significantly. Studies have shown that microRNAs (miRNA) have important roles in the development of CRC. Accordingly, the aim of this study was to determine miRNAs affecting the critical genes in the PI3K/Akt pathway by analysis of The Cancer Genome Atlas (TCGA) CRC data sets and to evaluate the clinical significance of these miRNAs.

**Material and Methods:** Initially, CRC mRNA, miRNA expression levels and patient data were obtained from TCGA database. The study included 220 CRC patients. MiRNAs that were negatively correlated with genes in the PI3K/Akt signaling pathway were selected, and their expression levels were compared with the clinical and demographic characteristics of CRC patients.

**Results:** miR-18a, miR-19b, miR-17, miR-106b, miR-130b and miR-135b were found to be negatively correlated with genes that play key roles in the PI3K/Akt pathway. Also, miR-18a, miR-19b, miR-17 and miR-135b were found to vary significantly according to the CRC subtype.

**Conclusion:** Consequently, the PI3K/Akt signaling pathway was found to be deregulated in CRC and the majority of genes involved in this signaling pathway were associated with miRNAs. Thus, PI3K/Akt miRNA axis might serve as a potentially distinctive diagnostic, prognostic and therapeutic avenue against CRC.

**Keywords:** Bioinformatics; colorectal cancer; miRNA; PI3K/AKT pathway; TCGA

## INTRODUCTION

Colorectal cancer is the third most common cancer in the world with high mortality. According to American cancer statistics, about 145,000 new cancer cases and 606880 cancer related deaths are projected to occur in 2019 (1). The survival rate in CRC is directly related to the TNM stage in the diagnosis. While 5-year survival rate reaches 90 % in early stages, this rate decreases to 10% in advanced stages (1). Although the molecular mechanism of CRC has been well studied, its development is still not fully decrypted yet. Thus, illuminating the molecular pathogenesis of CRC development is of great interest.

The PI3K/Akt signaling pathway is one of the most frequently activated signaling pathway in cancer. PI3K/Akt signaling leads to suppression of apoptosis, stimulation of cell growth and proliferation. Under normal circumstances, the activation of PI3K/Akt is tightly controlled. However, genetic abnormalities leading to PI3K/Akt hyper-activation cause disruption of this control mechanism and accelerate the cancer process (2). Activation of PI3K/Akt signaling pathway is controlled by four main sensors: receptor tyrosine kinases (RTKs), cytokine and G-protein coupled receptors and integrins. Upon appropriate binding, these sensors, together with their cofactors, activate downstream kinases in the phosphatidylinoside 3-kinase (PI3Ks) family. In the downstream part of PI3K, AKT is activated by PI3K. Activation of AKT stimulates the expression of a series of genes regulating cell survival, apoptosis and proliferation. The different expression profile of genes in this signaling pathway has been associated with the development of many cancers, including CRC (2,3). Particularly, blockers of the PI3K/Akt signal have been proposed as potential

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therapeutic agents in CRC. More than 60 % of the CRC cases were shown to have activating mutations in genes involved in PI3K/Akt signaling pathway and deactivating mutations in genes involved in the negative regulation of Akt such as phosphatase and tensin homolog (PTEN). In addition, downstream effectors of PI3K/Akt signaling pathway such as APC and β-catenin genes were shown to be frequently mutated in CRC (3).

As we all known, miRNAs are conserved post-transcriptional regulators of gene expression that are involved nearly all cellular processes such as cell growth, proliferation, differentiation and death as well as regulation of several physiological conditions at the organism level. Therefore, there is no doubt that deregulation of these tiny molecules is associated with many types of human disease, especially cancer (4).

Accordingly, here we aimed to determine the miRNAs affecting the critical genes involved in the PI3K/Akt pathway and to evaluate the clinical significance of these miRNAs by bioinformatics analysis of TCGA CRC data sets.

## MATERIAL and METHODS

### Data acquisition

To identify differentially expressed miRNAs in CRC, dbDEMC 2.0 (http://www.picb.ac.cn/dbDEMC/index.html) was screened with the "colon cancer", "colorectal cancer" and "miRNA" keywords. Subsequently, the same studies were eliminated and the upregulated and/or downregulated miRNAs in CRC were determined. Of these miRNAs, those with at least 2-fold changes (increasing or decreasing) were selected (4).

The data sets used for this study were obtained from the TCGA database (https://www.cbioportal.org/) in July 2019 (5). Flow of data acquisition was provided in Figure 1 in detail. In particular, CRC data sets were mined and miRNA and mRNA gene expression results were extracted from these experiments. All RNASeqV2 and clinical data were selected, and then the Build Archive function was performed. With ".txt", all quantitative files were selected and all files in the metadata and clinical departments were examined. Experiments where miRNA and mRNA expression levels were not found were excluded from this study. As a result, in the present study, 220 CRC patients were included. Demographic and clinical characteristics of CRC samples were collected. Samples with demographic and clinical data and expression level of genes were included in the study. Genes associated with the PI3K / Akt signaling pathway were selected using PI3K-Akt signaling pathway - Homo sapiens (human) (https://www.genome.jp/kegg-bin/show_pathway?hsa04151). Subsequently, genes that have critical functions in the PI3K/Akt signaling pathway and whose expression level was studied in TCGA CRC samples and miRNAs that showed significant negative correlation with these genes were determined. Of these miRNAs, those with available expression data and show significant alteration were selected. Accordingly, 16 genes associated with PI3K/Akt signaling pathway and 51 miRNAs with potential to regulate these genes were included in the study (Figure 1). Information about validated miRNAs of genes involved in PI3K/Akt signaling pathway was presented in Table 1.

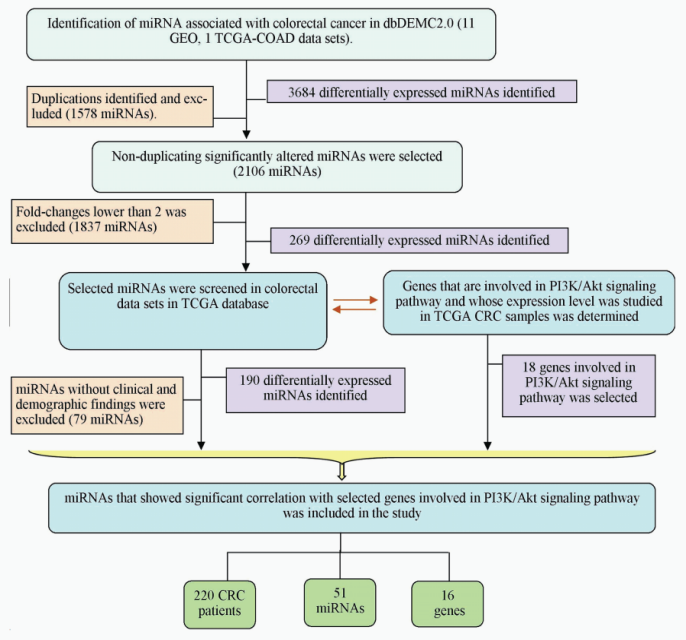


**Figure 1.** Flow chart of data acquisition. Data of CRC patients were collected using different databases. Duplicated studies and studies lacking clinical and expression data were excluded. Genes associated with the PI3K / Akt signaling pathway was selected from the KEGG database. Also, miRNAs that could potentially regulate these genes were selected. As a result, 220 CRC patients, 51 miRNAs and 16 genes were included in the study

### Statistical analysis

The demographic and clinical findings of the patients were selected from the CRC data sets in the TCGA database. Age and overall survival findings were presented as mean ± SD. Other findings were subdivided and frequencies were presented. The clinical characteristics of the patients and miRNA expression were compared according to the normality of the distribution. Student's t-test was used between the groups with normal distribution and non-parametric Mann-Withney U test was used between the groups without normal distribution. In comparison of multiple groups, One-Way-ANOVA test was used among the groups with normal distribution and non-parametric Kruskal Wallis test was used among the groups without normal distribution. For all findings, $p<0.05$ was considered statistically significant.

## RESULT

To identify miRNAs that are significantly involved in the formation and progression of CRC, miRNAs associated with CRC were evaluated in data sets obtained from different databases. Total number of 269 miRNAs was shown to be differently expressed in CRC as presented in Figure 2A and

2B. miRNAs that are significantly altered in CRC and have available associated clinical and demographic findings were selected for further analysis. MiRNAs with strong negative correlation with genes having crucial functional role in the PI3K/Akt signaling pathway were identified. As a result, total numbers of 51 miRNAs that have key roles in the PI3K/Akt signaling pathway were shown to be differentially expressed in CRC (Figure 2C and 2D).

In addition, the genes associated with the PI3K/Akt pathway and have available expression data were as follows: PIK3R1, PIK3R2, PIK3CA, KRAS, PTEN, PDK1, AKT1, AKT2, TSC1, TSC2, MTOR, BCL2, BCL2L2, BAD, CDKN1A, GSK3B, CCND1, CASP9 (Figure 2E and 2F). miRNAs that have marked negative correlation with these genes were presented Table 2. As a result of the detailed analyzes, miR-17, miR-18a, miR-19b, miR-106b, miR-130b and miR-135b were found to be negatively correlated with several genes in the PI3K/Akt pathway. In particular, genes that show strong negative correlation with miRNA are as follows: miR-17 with PIK3CA, KRAS, PTEN, BCL2, and BCL2L2; miR-18a, -19b and - 130b with PIK3CA, KRAS, and BCL2; miR-106b with PIK3CA, KRAS, PTEN, BCL2L2; miR135b with PTEN, BCL2, and BCL2L2.

Demographic and clinical data from TCGA CRC patient data sets were extracted and are shown in Table 3. Of the patients included in the study, 123 were males (age (year) mean±SD: 66.73±12.82) and 97 (age (year) mean±SD: 63.49±13.60) were females. In addition, the frequencies of clinical data such as stage, cancer type, primary tumor site, perineural invasion status, overall status and overall survival are shown according to gender. Stage IIA was found to be the most commonly diagnosed stage in women and men. In addition, colon adenocarcinoma was found to be higher than mucinous adenocarcinoma in both sexes. Tumor location was mostly seen in sigmoid colon in both sexes and cecum was the second most common tumor localization site. Overall survival was higher in women (35.47 ± 36.39 months) than in men (30.20 ± 27.59 months).

Moreover, MiR-17, miR-18a, miR-19b, miR-106b, miR-130b and miR-135b miRNAs that are associated with genes in the PI3K/Akt pathway were evaluated in terms of clinical and demographic characteristics (Table 4). MiR-19b was shown to be markedly associated with the age of CRC patients. Besides, miR-18a (p=0.0474), miR-19b (p=0.0081), miR-17 (p=0.0036) and miR-135b (p=0.049) showed statistically significant changes between colon adenocarcinoma and mucinous adenocarcinoma.

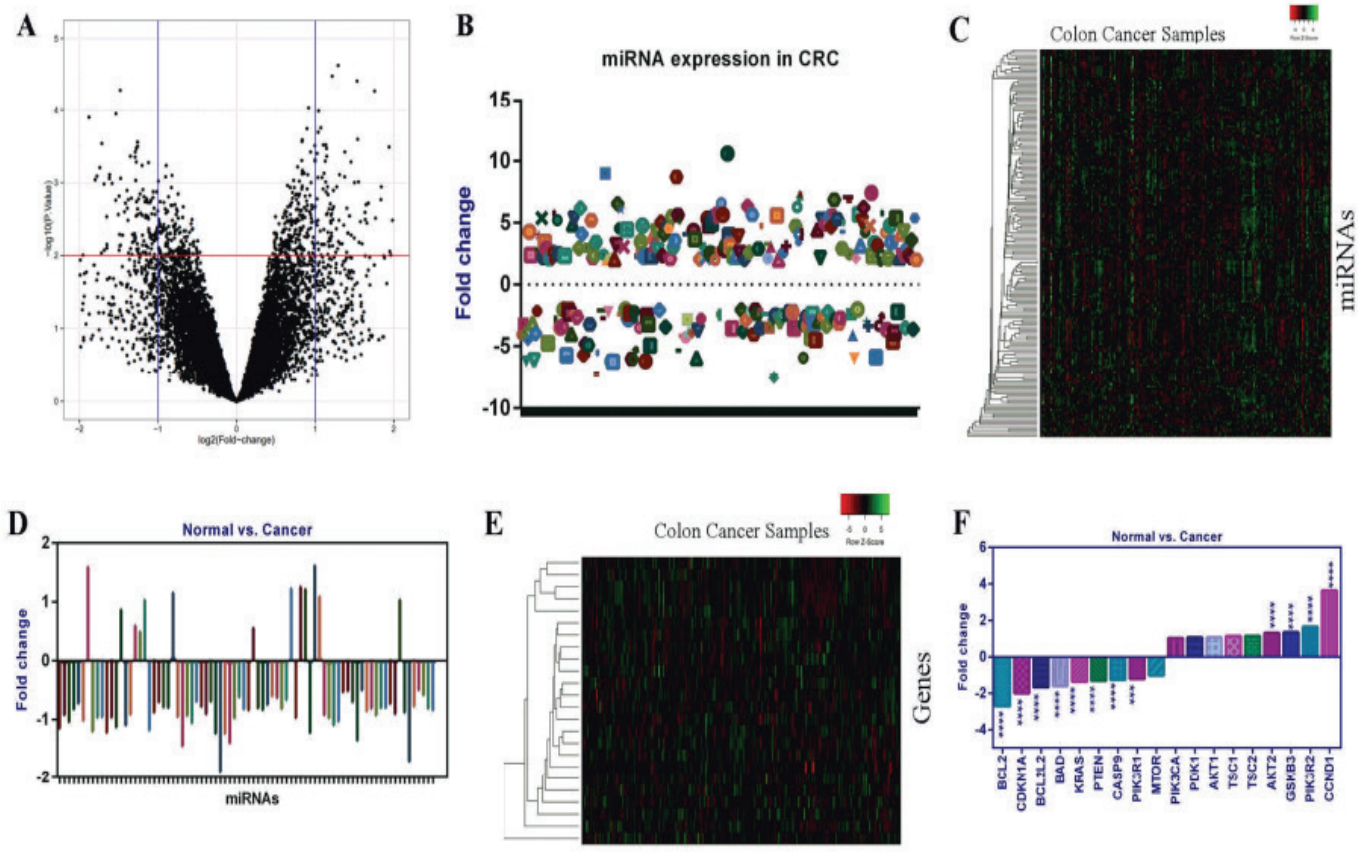


**Figure 2.** Expression levels of miRNAs and genes associated with PI3K/Akt signaling pathway in CRC (TCGA data set) (A) volcano plot showing expression levels of miRNAs (B) Expression levels of miRNAs that significantly altered in CRC (C) Heat-map graph showing miRNA expression levels (D) expressions of miRNAs associated with genes in the PI3K/Akt signaling pathway (E) Heat-map graph showing expression of critical genes in PI3K/Akt signaling pathway in CRC (F) Expression profile of critical genes in the PI3K/Akt signaling pathway in CRC

**Table 1. Demonstration of miRNAs associated with genes in the PI3K/Akt signaling pathway with validated miRNA-target gene relationship**

| Gene | miRNA | Cancer/Tissue Type | Validation method | Ref |
|---|---|---|---|---|
| **PIK3R2** | miR-30a | Non-small Cell Lung Cancer | qPCR, Western Blot, mimic transfection | (2) |
| | miR-141 | Colorectal cancer | qPCR | (6) |
| | miR-130b | Gastric cancer | qPCR, Immunohistochemistry | (7) |
| **IK3CA** | miR-222 | Colorectal cancer | qPCR | (6) |
| | miR-19b | Breast cancer | Luciferase reporter gene assay, qPCR | (8) |
| | miR-17 | Lymphoma | qPCR, Immunohistochemistry | (9) |
| | miR-18a | Ovarian cancer | Luciferase reporter gene assay, qPCR | (10) |
| | miR-19a/b | Colorectal cancer | Dual-luciferase reporter assay, qPCR | (11) |
| **KRAS** | miR-224 | Colorectal cancer | qPCR, mimic transfection | (12) |
| | miR-301a | Lung and Colorectal cancer | qPCR, Western Blot | (13) |
| | miR-17 | Pancreatic cancer | qPCR, Immunohistochemistry | (14) |
| | miR-106b | Esophageal squamous cell carcinoma | qPCR, Western Blot, Luciferase reporter gene assay | (15) |
| | miR-17 | Colorectal cancer | Microarray, qPCR, Western Blot, Luciferase reporter gene assay | (3) |
| | miR-188 | Gastric cancer | Microarray, qPCR, Western Blot, Luciferase reporter gene assay | (16) |
| **PTEN** | miR-15b | Nasopharyngeal carcinoma | qPCR | (17) |
| | miR-135b | Colorectal cancer | qPCR | (18) |
| | miR-29b | Breast cancer | qPCR, Western Blot, mimic transfection | (19) |
| | miR-301a | Cervical cancer; Pancreatic cancer | 3'-UTR luciferase assay, qPCR, Western Blot; qPCR, Western Blot, Luciferase reporter gene assay | (20) |
| **AKT2** | miR-182 | Neuroblastoma | qPCR, Western Blot | (21) |
| | miR-182 | Renal cancer | qPCR, mimic transfection | (22) |
| **MTOR** | miR-335 | Nasopharyngeal carcinoma | qPCR | (17) |
| | miR-135b | Rectal cancer | Microarray, RT-PCR | (23) |
| | miR-150 | Myoblasts | CLIP-seq | (24) |
| **GSK3B** | miR-155 | Murine transplantation model | Microarray, qPCR, Western Blot, Luciferase reporter gene assay | (25) |
| | miR-21 | Podocytes | qPCR, Western Blot | (26) |
| | miR-31 | Corneal epithelium | qPCR, Western Blot | (27) |
| | miR-15a | Osteosarcoma | qPCR, Western Blot, Luciferase reporter gene assay | (28) |
| | miR-15b | B-cell malignancies | qPCR, Western Blot, Luciferase reporter gene assay | (29) |
| **CCDN1** | miR-155 | Gastric cancer | Target protector (TP) assay, qPCR, Western Blot, Luciferase reporter gene assay | (30) |
| | miR-186 | Lung Adenocarcinoma | qPCR, Western Blot | (31) |
| | miR-148a | Hepatocellular carcinoma | qPCR, Immunohistochemistry | (32) |
| **CDKN1A** | miR-125b | Prostate cancer | qPCR, Western Blot | (33) |
| | Let-7c | HIV-1 infection | qPCR, Luciferase reporter gene assay | (34) |
| **BAD** | miR-100 | Pancreatic cancer | qPCR, Western Blot | (35) |
| | miR-224 | Oocytes, cumulus cells, and blastocysts | qPCR | (36) |
| **BCL2** | miR-19b | Cardiomyocyte | qPCR, mimic transfections | (37) |
| | miR-222 | Bladder cancer | qPCR, Immunohistochemistry | (38) |
| | miR-17 | Acute lymphoblastic leukemia | qPCR, Western Blot | (39) |
| **BCL2L2** | miR-16 | Oral squamous cell carcinoma | qPCR, Luciferase reporter gene assay | (40) |

ALT: Alanine aminotransferase; hs-CRP. High sensitivity C-reactive protein; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; BP. Blood pressure; HbA1c: Glycated hemoglobin; HOMA-IR: Homeostasis model assessment insulin resistance; OGTT: Oral glucose tolerance test; FPG: Fasting plasma glucose; BMI: Body mass index. Data was presented as mean ± standard deviation

**Table 2. miRNAs that show significant negative correlations with important genes in the PI3K/Akt signaling pathway in TCGA CRC patient data sets**

| Gene | miRNA | Correlation | Gene | miRNA | Correlation | Gene | miRNA | Correlation |
|---|---|---|---|---|---|---|---|---|
| | let-7f | -.182** | | miR-150 | -.293** | | miR-106b | -.144* |
| | let-7a | -.185** | | miR-146b | -.268** | | miR-17 | -.211** |
| PIK3R1 | miR-185 | -.232** | | miR-155 | -.233** | | miR-188 | -.268** |
| | miR-22 | -.183** | GSK3B | miR-223 | -.371** | PTEN | miR-15b | -.214** |
| | miR-146b | -.184** | | miR-21 | -.276** | | miR-135b | -.209** |
| | miR-30a | -.214** | | miR-22 | -.296** | | miR-29b | -.242** |
| | miR-22 | -.222** | | miR-31 | -.320** | | miR-301a | -.209** |
| PIK3R2 | miR-10b | -.258** | | miR-15a | -.400** | | miR-17 | -.231** |
| | let-7c | -.214** | | miR-15b | -.350** | | miR-10a | -.244** |
| | miR-18a | -.386** | | miR-146b | -.347** | | miR-16 | -.262** |
| | miR-141 | -.365** | | miR-155 | -.163* | | miR-135b | -.297** |
| | miR-106b | -.403** | CCDN1 | miR-200a | -.337** | BCL2L2 | miR-222 | -.242** |
| PIK3CA | miR-130b | -.434** | | miR-200b | -.285** | | miR-221 | -.228** |
| | miR-222 | -.364** | | miR-186 | -.318** | | miR-106b | -.225** |
| | miR-19b | -.355** | | miR-148a | -.358** | | miR-26b | -.231** |
| | miR-17 | -.300** | | miR-16 | -.384** | | miR-224 | -.318** |
| | miR-18a | -.227** | | miR-217 | -.207** | | miR-18a | -.244** |
| | miR-19a | -.248** | | miR-218 | -.276** | | miR-19b | -.295** |
| | miR-19b | -.253** | | miR-100 | -.266** | BCL2 | miR-135b | -.439** |
| | miR-196 | -.223** | CDKN1A | miR-125b | -.295** | | miR-222 | -.323** |
| | miR-224 | -.227** | | miR-181d | -.285** | | miR-17 | -.295** |
| KRAS | miR-106b | -.232** | | miR-328 | -.247** | | miR-130b | -.155* |
| | miR-130b | -.210** | | let-7c | -.291** | | miR-100 | -.154* |
| | miR-301a | -.273** | | miR-1266 | -.170* | | miR-1 | -.160* |
| | miR-324 | -.209** | | miR-331 | -.163* | BAD | miR-129 | -.173** |
| | miR-17 | -.199** | TSC1 | miR-223 | -.164* | | miR-19a | -.209** |
| | miR-135b | -.258** | | miR-134 | -.188** | | miR-139 | -.157* |
| MTOR | miR-335 | -.302** | | miR-1266 | -.290** | | miR-182 | -.173** |
| | miR-182 | -.229** | TSC2 | miR-31 | -.235** | AKT2 | miR-31 | -.181** |
| CASP9 | miR-100 | -.192** | | | | | | |

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

**Table 3. Demographic and clinical characteristics of patients**

| | | Total | | Gender | | | |
|---|---|---|---|---|---|---|---|
| | | Patients n=220 | Frequency (%) | Male (n=123) | Frequency (%) | Female (n=97) | Frequency (%) |
| **Age (Mean±SD) (year)** | | **65.30±13.24** | | **66.73±12.82** | | **63.49±13.60** | |
| **Stage** | Stage I | 30 | 13.64 | 17 | 13.82 | 13 | 13.40 |
| | Stage IA | 1 | 0.45 | 1 | 0.81 | 0 | 0.00 |
| | Stage II | 11 | 5.00 | 7 | 5.69 | 4 | 4.12 |
| | Stage IIA | 73 | 33.18 | 43 | 34.96 | 30 | 30.93 |
| | Stage IIB | 3 | 1.36 | 1 | 0.81 | 2 | 2.06 |
| | Stage IIC | 1 | 0.45 | 1 | 0.81 | 0 | 0.00 |
| | Stage III | 6 | 2.73 | 3 | 2.44 | 3 | 3.09 |
| | Stage IIIA | 3 | 1.36 | 2 | 1.63 | 1 | 1.03 |
| | Stage IIIB | 36 | 16.36 | 18 | 14.63 | 18 | 18.56 |
| | Stage IIIC | 20 | 9.09 | 12 | 9.76 | 8 | 8.25 |
| | Stage IV | 15 | 6.82 | 7 | 5.69 | 8 | 8.25 |
| | Stage IVA | 10 | 4.55 | 4 | 3.25 | 6 | 6.19 |
| | Stage IVB | 1 | 0.45 | 1 | 0.81 | 0 | 0.00 |
| | NA | 10 | 4.55 | 6 | 4.88 | 4 | 4.12 |
| **Cancer subtype** | Colon Adenocarcinoma | 188 | 85.45 | 105 | 85.37 | 83 | 85.57 |
| | Colorectal Adenocarcinoma | 1 | 0.45 | 0 | 0.00 | 1 | 1.03 |
| | Mucinous Adenocarcinoma | 31 | 14.09 | 18 | 14.63 | 13 | 13.40 |
| **Primary Tumor Site** | Ascending Colon | 43 | 19.55 | 25 | 20.33 | 18 | 18.56 |
| | Descending Colon | 10 | 4.55 | 7 | 5.69 | 3 | 3.09 |
| | Sigmoid Colon | 66 | 30.00 | 31 | 25.20 | 35 | 36.08 |
| | Transverse Colon | 14 | 6.36 | 7 | 5.69 | 7 | 7.22 |
| | Hepatic Flexure | 13 | 5.91 | 8 | 6.50 | 5 | 5.15 |
| | Splenic Flexure | 4 | 1.82 | 3 | 2.44 | 1 | 1.03 |
| | Cecum | 56 | 25.45 | 33 | 26.83 | 23 | 23.71 |
| | Rectosigmoid Junction | 1 | 0.45 | 0 | 0.00 | 1 | 1.03 |
| | NA | 13 | 5.91 | 9 | 7.32 | 4 | 4.12 |
| **Perineural invasion** | Yes | 39 | 17.73 | 23 | 18.70 | 16 | 16.49 |
| | No | 98 | 44.55 | 43 | 34.96 | 55 | 56.70 |
| | NA | 83 | 37.73 | 57 | 46.34 | 26 | 26.80 |
| **Overall status** | Living | 170 | 77.27 | 91 | 73.98 | 79 | 81.44 |
| | Deceased | 50 | 22.73 | 32 | 26.02 | 18 | 18.56 |
| **Overall survival (months)** | | 32.52±31.80 | | 30.20±27.59 | | 35.47±36.39 | |

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

| Table 4. Comparison of miRNA levels according to clinical and demographic characteristics | | | | | | |
|---|---|---|---|---|---|---|
| **Demographic and clinical characteristics** | **miR-18a** | **miR-19b** | **miR-17** | **miR-135b** | **miR-106b** | **miR-130b** |
| **Age (≤50 vs. >50)** | 0.6028 | 0.0406* | 0.0532 | 0.0584 | 0.286 | 0.855 |
| **Gender (female vs. male)** | 0.828 | 0.143 | 0.299 | 0.861 | 0.298 | 0.342 |
| **Stage (within all groups)** | 0.506 | 0.877 | 0.948 | 0.423 | 0.2631 | 0.4176 |
| **Cancer subtype (Colon Adenocarcinoma vs. Mucinous Adenocarcinoma)** | 0.0474* | 0.0081** | 0.0036** | 0.049* | 0.2251 | 0.1401 |
| **Primary Tumor Site (within all groups)** | 0.388 | 0.138 | 0.301 | 0.797 | 0.538 | 0.472 |

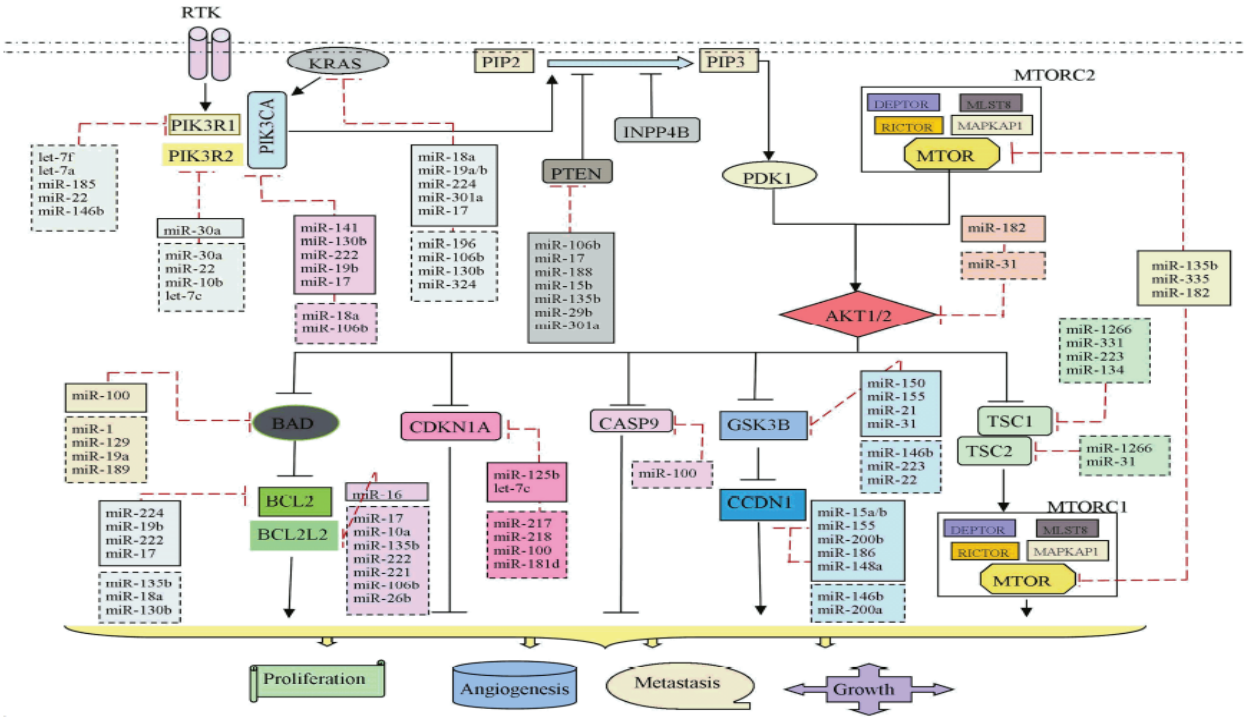


**Figure 3.** Figure 3. Prototypical representation of PI3K/Akt signaling pathway. In TCGA CRC patient data sets, miRNAs that are involved in the regulation of critical genes in PI3K/Akt signaling pathway were presented. miRNAs previously verified in other studies with the gene of interest were represented in boxes without dashed lines. MiRNAs identified as a result of this study were represented in boxes with dashed lines

## DISCUSSION

The PI3K/Akt signaling pathway is frequently activated in cancer. In the activation of this pathway, while the expression level of some genes increases, some of them decrease (2). It has also been shown that not only protein-encoding genes but non-coding RNAs such as miRNAs that are involved in PI3K/Akt signaling pathway are differently expressed (8). Thus, many genes involved in this signaling pathway, either coding for proteins or non-coding RNAs have become therapeutic targets. Therefore, a better understanding of the PI3K/Akt signaling pathway will allow the development of new diagnostic and therapeutic strategies, especially for CRC. In the present study, for a first time, we investigated the association between genes involved in PI3K/Akt signaling pathway and their regulatory miRNA genes in CRC.

As a result, genes in the PI3K/Akt signaling pathway and their corresponding miRNAs showing negative correlation with these genes have been shown to be differentially expressed in CRC. In this study, miR-17, miR-18a, miR-19b, miR-106b, miR-130b and miR135b miRNAs were shown to affect many genes in the PI3K/Akt signaling pathway. In addition, a miRNA was found to be negatively correlated with more than one protein coding gene involved in the PI3K/Akt signaling pathway. The negative correlation of a miRNA with multiple genes does not mean that it directly targets the gene of interest. However, it is likely that corresponding miRNAs are involved in the regulation of gene of interest. Therefore, future more comprehensive studies are needed to clarify the interactions between miRNAs and predicted target genes using in vivo and in vitro experimental methods to further support the findings

of this study.

Additionally, miRNAs associated with the PI3K/Akt signaling pathway were compared with the clinical and demographic characteristics of CRC patients, and miR-17, miR18a, miR-19 and miR-106b were shown to be markedly associated with the subtypes of CRC, suggesting that these miRNAs can be used as a biomarker in determining the subtype of CRC. However, more comprehensive investigations are required to validate these findings and adapt them to be used in the clinic.

## CONCLUSION

Consequently, the PI3K/Akt signaling pathway was found to be deregulated in CRC and the majority of genes involved in this signaling pathway were associated with miRNAs. However, findings obtained in the present work should also be confirmed and supported by in vivo/in vitro laboratory studies.

*Competing interests: All of the authors of this manuscript declared that there is no conflict of interest.*
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*Ethical approval: This study was approved by the Institutional Ethics Committee and conducted in compliance with the ethical principles according to the Declaration of Helsinki.*

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