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CLINICAL STUDY

Differential expression of miRNAs related to autophagy pathway in tissue and serum samples of colorectal cancer patients

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ABSTRACT

BACKGROUND: This study was aimed to investigate the relationship of miR-17-5p, miR-30b, miR-30d, miR-216a and miR-216b associated with autophagy gene beclin 1, and beclin 1 gene with colorectal cancer (CRC).

MATERIALS AND METHODS: Forty-seven patients with CRC and 50 healthy individuals with no cancer history were included in this study. In the serum, tumor and non-tumoral tissue samples of the CRC patients, and in the serum samples of the healthy subjects, expression levels of miRNAs were detected by qRT-PCR. The beclin 1 gene expression levels were determined by qRT-PCR, and protein levels were determined by Western blot method in tumor and non-tumor tissue samples of the patients.

RESULTS: The miR-17-5p and miR-30d expressions were found to be higher in tumor tissue as compared to patient non-tumor tissues, while expressions of beclin-1, miR-30b and miR-216a were found to be lower. In addition, the beclin-1 protein levels were significantly decreased in the tumor tissue as compared to those in the patient non-tumor tissues. The miR-30d expression was significantly reduced in the serum of the patients when the serum samples of CRC patients and healthy controls were compared.

CONCLUSION: The beclin 1 gene may play a role as a tumor suppressor in CRC. Moreover, these miRNAs cannot be used as highly reliable biomarkers in serum for CRC diagnosis *(Tab. 2, Fig. 6, Ref. 46)*. Text in PDF *www.elis.sk*

KEY WORDS: colorectal cancer, autophagy, beclin-1, miRNA, biomarker.

Introduction

Colorectal cancer (CRC), constituting 10 % of all cancer cases, ranks third most common cancer type worldwide, but also is second in terms of mortality. In 2020, 1.9 million people were diagnosed with CRC, and 48 % of these patients died (1). Autophagy, defined as the type II programmed cell death mechanism, plays a critical role in cancer. Autophagy is a catabolic degradation process in which unnecessary or long-lived proteins and damaged organelles or pathogens are delivered to lysosomes, digested by lysosomal enzymes to generate vital substrates such as amino acids, glucose and fatty acids and energy sources like ATP, and ultimately recycled into the cytosol (2). The relation between autophagy and cancer is very complicated and controversial, since in cancer, this mechanism acts as a double-edged sword (3). Autophagy is generally considered as a death mechanism, however, the more common view is that the role of autophagy regarding cell death is primarily protective (4). Although autophagy acts as a tumor suppressor in the occurrence and development of cancer, it also helps tumor cells to develop tolerance to stress and resistance to adverse circumstances during cancer progression (5). Beclin-1 is an important autophagy-inducing gene involved in the organization of cell death and survival of various cell types (6). It has been named as the keeper gene with the duty of initiating or inhibiting autophagy and reported as the target of therapies all the time (7). Beclin-1, plays a key role in the fork of two critical pathways, autophagy and apoptosis, namely cellular survival and death, respectively (8).

Monoallelic deletions of the beclin 1 gene have been shown in ovarian, breast and prostate cancers (9). Moreover, recently, it has been shown in many cancer types that beclin 1 expression is regulated by microRNAs (miRNAs), therefore, miRNAs play very important roles in the regulation of autophagy (10). MiRNAs are small, single-chain regulatory RNA molecules about 18–24 nucleotides in length, located in intronic or exogenous regions or intergenic regions of protein-coding genes (11). They are encoded by highly conserved DNA regions, yet do not undergo transla-

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tion. MiRNAs negatively regulate the expression of the respective genes on a transcriptional or posttranscriptional level either by direct degradation or by inhibiting translation via binding to the 3'-UTR region, coding regions or 5'-UTR region of mRNAs (12). The downregulation of beclin 1 was shown in many types of cancer, including gastric cancer and colon cancer. Furthermore, a worse prognosis in patients expressing low beclin 1 levels has been demonstrated in many types of cancer (9). This indicates that beclin 1 may have a potential tumor suppressor role (13). It has been demonstrated that miRNAs are associated with genes that regulate the induction, vesicle nucleation, vesicle elongation, maturation and autophagosome formation stages of autophagy (14). According to our reviews of literature, it was determined that miR-17-5p (15), miR-30b (16), miR-30d (17), miR-216a (18) and miR-216b (19) target the beclin 1 mRNA. Additionally, numerous studies have suggested that miRNAs can act as biomarkers in the cancer diagnosis. The expressions of miR-17-5p, miR-30b, miR-30d, miR-216a and miR-216b have been suggested as potential serum biomarkers for the diagnosis of CRC. However, some miRNAs have been reported to show different expression levels in the tissue and serum of cancer patients (20).

Beclin 1 plays a regulatory role in the balance of autophagy between tumorigenesis and cell survival and tumor suppression and cell death in both tumor and non-tumor tissues of CRC patients (21, 22). In this study, it was aimed to reveal how beclin 1 plays a part in CRC by investigating the gene and protein levels of beclin -1. Moreover, investigated were the differences between the expression levels of beclin 1-related miRNAs, namely miR-17-5p, miR-30b, miR-30d, miR-216a and miR-216b in tumor tissues and those in non-tumor tissue samples of CRC patients. Furthermore, since miRNAs are suggested as promising biomarkers in cancer diagnosis, the expression levels of beclin 1-related miRNAs, namely miR-17-5p, 30b, 30d, 216a, and 216b in the serum samples of CRC patients were compared to those in healthy controls in order to ascertain the reliability of the use of these miRNAs as biomarkers in CRC diagnosis.

Clinical significance

– Autophagy may be a mechanism that leads to cell death; beclin 1 may be a tumor suppressor gene in CRC.
– miR-17-5p and miR-30d may target beclin 1 acting as oncomiRs in CRC.
– The expression levels of miR-17-5p, miR-30b, miR-30d, miR-216a and miR-216b were lower in the serum of CRC patients than in their tissues.
– miR-17-5p, miR-30b, miR-30d, miR-216a and miR-216b are not reliable biomarkers for the diagnosis of CRC from serum samples.

Material and methods

Ethics statement and collection of blood and tissue samples

This study was approved by the Istanbul University, Istanbul Faculty of Medicine, Clinical Research Ethics Committee (#2015/1248). The informed consent form was collected from all volunteers included in the study. The tumor tissues and non-tumor tissues of CRC patients were obtained to detect beclin 1 and miR-17-5p, 30b, 30d, 216a, and 216b expression levels. The blood samples were obtained from CRC patients and healthy individuals to determine serum expression levels of miRNAs. From 47 patients diagnosed with CRC, peripheral blood samples were drawn before operation, and tumor tissue and non-tumor tissue (adjacent normal tissues) were collected during surgery performed at the Department of General Surgery, Istanbul Faculty of Medicine, Istanbul University. Blood samples were also collected from the healthy control group constituted of 50 subjects with no personal or family cancer history.

Total RNA and miRNA extraction

Total RNA (Qiagen, RNeasy Plus Mini Kit, USA) and miRNA (Qiagen, miRNeasy Mini Kit, USA) from tumor tissue and adjacent non-tumor tissue samples of CRC patients were isolated using commercial kits according to the manufacturer's directions. MiRNA (Qiagen, miRNeasy Serum/Plasma Kit, USA) from the serum samples of CRC patients and healthy controls were isolated using commercial kits according to the manufacturer's directions.

The cDNA synthesis from total RNA and miRNA samples

For the Beclin-1 gene, the cDNA was synthesized from total RNA samples using the AccuScript High Fidelity1st Strand cDNA Synthesis Kit (Agilent Technologies, USA), according to the manufacturer's directions. For miRNAs, cDNA was synthesized from total miRNA samples using the miRNA 1st Strand cDNA Synthesis Kit (Agilent Technologies, USA) according to the manufacturer's directions.

Quantitative real-time PCR

Quantitative real-time PCR analyses were carried out using Agilent Mx3005P (Agilent Technologies, USA) and gene expression analyses of beclin 1 and GAPDH using RT^2 QPCR Primer Assay (Qiagen, USA) and Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent Technologies, USA). Final volumes of the reactions were set as 20 µl using 10 µl 2X SYBR Green QPCR Master Mix, 0.5 µl Forward Primer (10 µM), 0.5 µl Reverse Primer (10 µM), 7 µl RNase-free water and 2 µl cDNA. PCR conditions were established according to the manufacturer's suggestions as 5 minutes at 95 °C and 40 cycles of 10 seconds at 95 °C, 30 seconds at 61 °C and 2 minutes at 72 °C. Melting-curve analyses were performed following each QPCR application. All samples were studied twice. The relative gene expression of beclin 1 was normalized to GAPDH (reference gene) and calculated using the $2^{-\Delta\Delta}$ Ct method. For this, firstly, the difference between the Ct values of beclin 1 and those of GAPDH (reference gene) genes were calculated to find the $^{\Delta}$Ct value of the beclin 1 gene in tumor tissue and adjacent non-tumor tissue samples. Then, the value of $^{\Delta\Delta}$Ct , i.e., of the difference between the $^{\Delta}$Ct value of beclin 1 in the tumor and that in adjacent non-tumor tissue samples was calculated. The fold change between the expression of the beclin 1 gene in the tumor and that in adjacent non-tumor tissue samples was then equal to $2^{-\Delta\Delta}$Ct.

The miScript Primer Assay (Qiagen, USA) and miRNA QPCR Master Mix (EvaGreen) (Agilent Tecnologies, USA) were used

for the expression analyses of miR-17-5p, 30b, 30d, 216a, 216b, and RNU6 (reference gene). Final volumes of the reactions were set as 25 µl using 2.5 µl 10X Core PCR Buffer, 2.25 µl $MgCl_2$ (50 mM), 1 µl dNTP mix (20 mM), 1.25 µl 20X EvaGreen dye, 1 µl Universal Reverse Primer (3,125 mM), 1 µl Forward Primer (3,125 mM), 0.5 µl Enzyme (High-Specificity PCR enzyme blend), 13.5 µl RNase-free water and 2 µl cDNA. PCR conditions were established according to the manufacturer's suggestions as 10 minutes at 95 °C and 40 cycles of 10 seconds at 95 °C, 15 seconds at 63 °C for miR-17-5p, miR-30d and miR-216a, and 15 seconds at 60 °C for miR-30b and miR-216b, and 20 seconds at 72 °C. Melting-curve analyses were performed following each QPCR application. All samples were studied twice. The relative expression levels of miRNAs were normalized to RNU6 (reference gene) and calculated by the $2^{-\Delta\Delta}$ Ct method. To compare the expression level of miRNAs in tissue samples, firstly, the difference between Ct values of miRNAs and and those of RNU6 (reference gene) were calculated to find the $^{\Delta}$Ct values of the miRNAs in tumor and adjacent non-tumor tissue samples. Then, $^{\Delta\Delta}$Ct, i.e., the difference between the $^{\Delta}$Ct values of miRNAs in the tumor and those in non-tumor tissue samples were calculated. The fold change between the expressions of the miRNAs in the tumor tissue and those in adjacent non-tumor tissue samples were then equal to $2^{-\Delta\Delta}$Ct. To compare the expression levels of miRNAs in serum samples, firstly, the differences between the Ct values of miRNAs and those of RNU6 (reference gene) were calculated to find the $^{\Delta}$Ct values of the miRNAs in the serum samples of CRC patients and healthy controls. Then, $^{\Delta\Delta}$Ct, i.e., the difference between the $^{\Delta}$Ct values of miRNAs in the serum samples of CRC patients and those in healthy control were calculated. The fold changes in expressions of the miRNAs between serum samples CRC patients and healthy controls was then equal to $2^{-\Delta\Delta}$Ct.

Western blot

To minimize protein degradation in the tissue samples, they were first treated with a protease inhibitor cocktail and phosphatase inhibitor. Before Western blotting, the samples were equalized to 50 µg following quantitation with fluorometric Qubit (Invitrogen, USA). Then, the samples were separated with an SDS-PAGE electrophoresis according to their molecular weights and transferred to a nitrocellulose membrane with a semi-dry transfer system. The membrane was blocked by applying 5 % Tween 20 containing skimmed milk powder in a shaking incubator for 2 hours. After that, it was incubated with monoclonal beclin 1 antibody diluted to the concentration of 1:1,000 overnight at +4 °C and following washing it was treated with horseradish peroxidase (HRP) tagged secondary antibody for an hour. The membrane was viewed using a Wealtec Keta Imaging System with a CCD camera. The densitometric measurements were carried out with the Image J program. The GAPDH protein was utilized as a control, confirming the equal amount of protein loading for each sample. The required normalizations were performed after calculating GAPDH differences in the samples.

Statistical analysis

While evaluating the findings, the SPSS 22.0 program was utilized for statistical analysis and p values below 0.05 were considered significant. The normal distribution of data was evaluated with Kolmogorov–Smirnov and Shapiro Wilk tests. In addition, the Kruskal–Wallis test was performed to compare three independent groups; the adjusted computed p values were then used for *post hoc* test. The Mann–Whitney U test was utilized to compare two independent groups. Receiver operating characteristic (ROC) curve was performed to detect the sensitivity and specificity values of miR-17-5p, 30b, 30d, 216a, and 216b that may be used to define CRC. In case of AUC < 0.6, it was considered insignificant; additionally, a potential discriminator was assumed in the range of 0.7–0.89, although AUC > 0.9 was considered to be a significant discriminator.

Results

The 47 patients diagnosed with CRC admitted to the Department of General Surgery, Istanbul Faculty of Medicine with mean age of 57.31 ± 21.56 and 50 healthy individuals with no personal or family cancer history with mean age of 55.89 ± 11.23 were included in this study. In the patient group, 20 subjects were men and 27 were women, and in the healthy control group, 33 were men and 17 were women. Demographic parameters of the CRC patients and healthy controls are given in Table 1. Also, clinicopathological features of the CRC patients are given in Table 2. There was

Tab. 1. Demographic parameters of the CRC patients and healthy control.

Demographic parameters	Patient (n:47)	Control (n:50)
Age (year)	57.30±21.56	56.30±19.56
Gender (F/M)	20/27	17/33

Tab. 2. Clinicopathological features of the CRC patients (n = 47).

Features	n (%)
Tumor Stage	
T1	3 (6.38)
T2	3 (6.38)
T3	20 (42.55)
T4	21 (44.68)
Lymph node involvement	
N0	17 (36.17)
N1	17 (36.17)
N2	13 (27.65)
Metastasis	
Yes	26 (55.31)
No	21 (44.68)
Perineural invasion	
Yes	22 (46.8)
No	25 (53.19)
Differentiation	
Poorly	14 (29.78)
Well	12 (25.53)
Moderately	21 (44.68)
Tumor localization	
Sigmoid colon	20 (42.55)
Rectum	9 (19.14)
Right colon	11 (23.40)
Left colon	1 (2.12)
Rectosigmoid	5 (10.63)
Splenic flexure	1 (2.12)

no difference among the groups in terms of both demographic parameters and clinicopathological features.

The expression levels of beclin 1, miR-17-5p, 30b, 30d, 216a, and 216b were compared between tumor and adjacent non-tumor tissue samples collected from the CRC patients. When comparing the expression levels of beclin 1, miR-30b and miR-216a in the tumor tissues and non-tumor tissues, those in the non-tumor tissues were lower 5.64-fold (95% CI: 3.82–6.54; $p < 0.001$), 2.94-fold (95% CI: 2.21–4.82; $p < 0.001$), and 4.32-fold (95% CI: 2.79–5.82; $p < 0.001$), respectively (Fig. 1). However, when comparing the expression levels of miR-17-5p, miR-30d, and miR-216b in non-tumor and tumor tissues, those in the tumor samples were higher 2.78-fold (95% CI: 1.32–4.26; $p < 0.001$), 4.04-fold (95% CI: 2.72–5.37; $p < 0.001$) and 1.68-fold, respectively (Fig. 1). Additionally, the ROC curve proposed an important diagnostic capability for miR-30b and miR-216a in CRC patients. The results demonstrated that the area under the ROC curve (AUC) was 0.911 (99% CI: 0.857–0.966; $p < 0.001$) and 0.837 (99% CI: 0.757–0.916; $p < 0.001$) for the tumor tissue versus non-tumor tissue, respectively (Fig. 2).

When the beclin 1 protein values in tumor and adjacent non-tumor tissue samples of CRC patients were compared, it was determined that beclin 1 protein values in tumor tissues (0.32 ± 0.18) were statistically lower than in adjacent non-tumor tissues (0.,65 $\pm$ 0.34) ($p < 0.001$) (Fig. 3).

When the expression levels of miR-17-5p, 30b, 30d, 216a, and 216b between the serum samples of the CRC patients and the healthy controls were examined, the expression of miR-30d was found to be 5.64-fold lower in the patient group (95% CI: 1.32–3.52; $p < 0.001$) (Fig. 4).

The expression levels of miRNAs in the tumor tissue and serum samples of the CRC patients were evaluated. When comparing the miR-17-5p, miR-30b, miR-30d, miR-216a, and miR-216b expression levels in the tissue samples and serum, those in the serum were lower 9.96-fold (95% CI: 5.08–7.92; $p < 0.001$), 8.38-fold (95% CI: 7.41–9.81; $p < 0.001$), 6.79-fold (95% CI: 7.96–5.35; $p < 0.001$), 7.96-fold (95% CI: 6.12–9.21; $p < 0.001$), and 4.32-fold (95% CI: 2.56–5.68; $p < 0.001$), respectively (Fig. 5).

No significant discriminator was found in the comparison of ROC curve analysis of serum samples from CRC patients and healthy controls, as it was considered insignificant when AUC < 0.6 (Fig. 6).

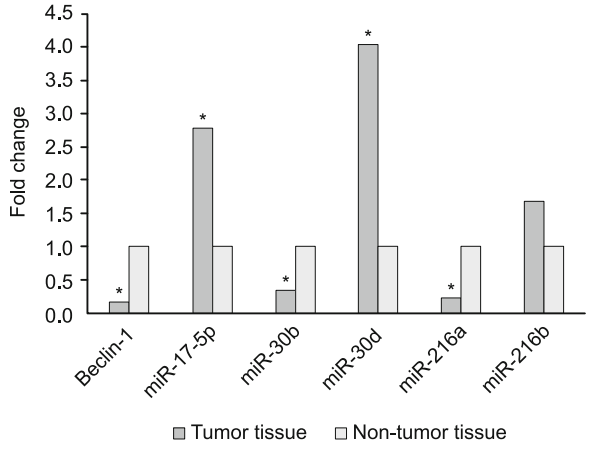

Fig. 1. Beclin-1 gene and miRNAs expression fold changes in the tumor tissues as compared to non-tumoral tissue samples of the CRC patients (significance levels: *p < 0.001).

Discussion

Despite the recent development of promising diagnostic and therapeutic modalities, the mortality associated with CRC is still very high worldwide (23). Therefore, new molecular mechanisms such as autophagy, which can play a role in the CRC pathogenesis, and new regulatory molecules called miRNAs have attracted

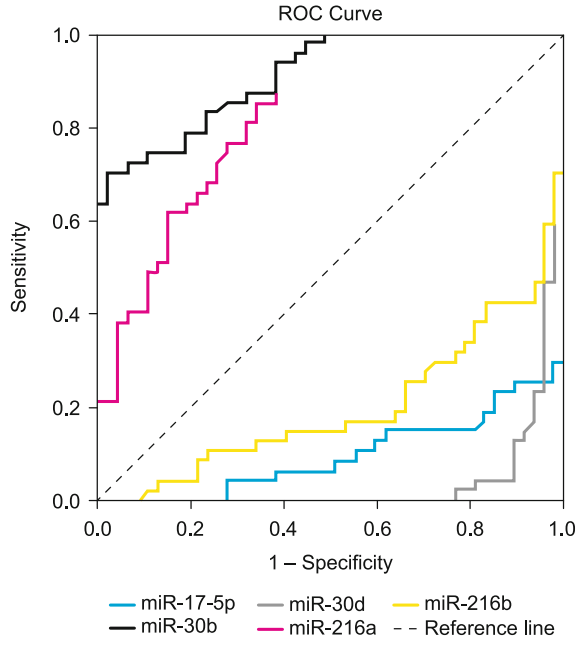

Fig. 2. ROC curve analysis of miRNAs for tumor tissue versus non-tumor tissue of CRC patient.

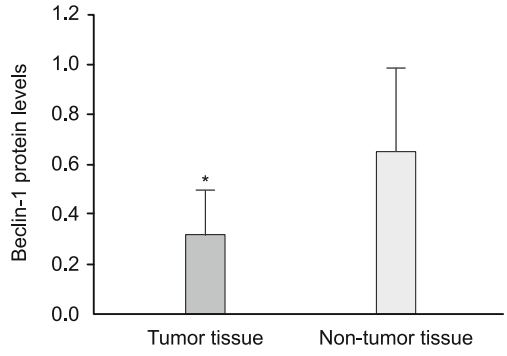

Fig. 3. Beclin-1 protein levels in the tumor tissue and non-tumor tissue samples of the CRC patients (Data are presenting mean (± SD) values, non-tumor tissue (0.65 ± 0.34) and tumor tissue (0.32 ± 0.18; significance levels: *p < 0.001).

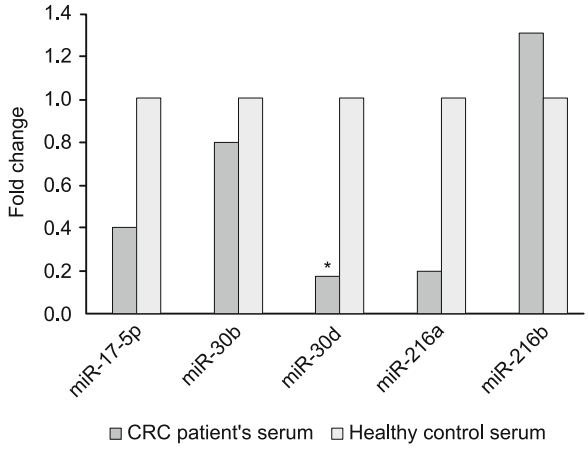

Fig. 4. miRNA expression fold changes in the serum samples of the CRC patients as compared to the healthy controls (significance levels: *p < 0.001).

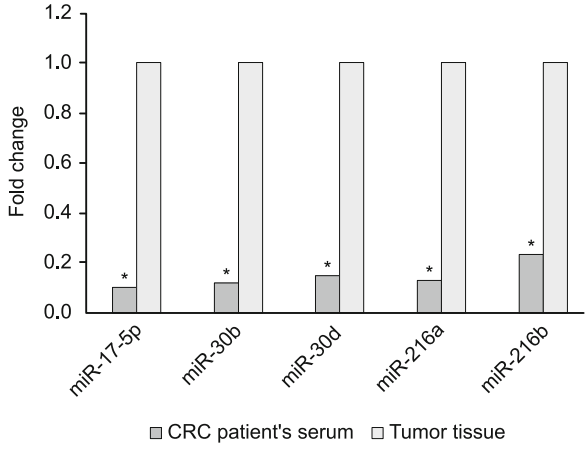

Fig. 5. miRNA expression fold changes in the tumor tissue samples of CRC patients as compared to serum samples of the CRC patients (significance levels: *p < 0.001).

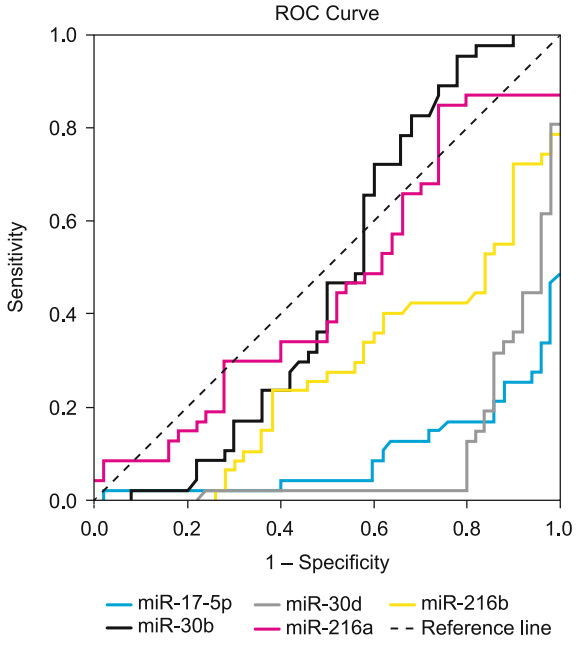

Fig. 6. ROC curve analysis of miRNAs for serum of CRC patients versus serum healthy controls.

attention (11). Some miRNAs may act as regulatory role in the development and progression of CRC pathogenesis by targeting mRNAs encoded by oncogenes or tumor suppressor genes (24).

As previously mentioned, the role of autophagy in cancers development and progression is still very complex. However, beclin 1, the key role player in the initiation of autophagy, has been generally defined as a tumor suppressor gene in cancer studies (9). In cell culture studies, it has been stated that decreased beclin 1 levels and autophagy inhibition upregulate CRC. Moreover, activating autophagy or upregulating beclin 1 might suppress the development and progression of colon cancer and be an effective treatment for CRCs (25, 26). In a study conducted in 2020, marked downregulation of beclin 1 was demonstrated in tumor tissue as compared to non-tumor tissue in CRCs, and a decrease in expression of beclin 1 was associated with a poor prognosis in CRC patients. Moreover, it was reported that the downregulation of beclin 1 distinctly increased CRC cells´ migration and aggression (21). Conformably, when we examined the difference between the expressions of beclin 1 in the tumor and those in the surrounding non-tumor tissues, we determined that both mRNA and protein levels of the beclin 1 gene were decreased in the tumor tissues. Therefore, we concluded that beclin 1 may have the function of a tumor suppressor gene in CRC and that autophagy may be a pathway leading to death of CRC cells.

The miR-17-5p expression levels were found to be high in CRC (27–29), and these high expression levels were associated with pathologic stages and grades in CRC patients (30, 31). Similar to the findings of previous studies, in our study, the expression level of miR-17-5p was found to be higher in tumor tissues than in non-tumor tissues in CRC patients. This indicated that miR-17-5p may be acting as an oncomiR in CRC. In line with our previous finding is the possibility that miR-17-5p can inhibit beclin 1, a tumor suppressor, and therefore, contribute to CRC development. However, when the differences between miR-17-5p expressions in CRC patient and those in healthy control serum samples were evaluated, only a slight change (0.4-fold) was observed. Therefore, we cannot suggest miR-17-5p as a sensitive diagnostic biomarker that could be detected in serum for CRC. Gremek et al. have reported that there was no overlap between the expression levels of miRNAs expressed from tissue and serum, suggesting that miRNAs in serum may not be properly released from CRC cells (20).

Various studies have showed a decrease in miR-30b expression in the tumor tissues of the CRC patients (32–34), even more in patients with liver metastasis (33). Furthermore, *in vitro* studies have demonstrated that miR-30b reduced the migration, invasion, and tumor growth of the CRC cells (33, 35). In agreement with previous CRC study, our study found miR-30b expression levels to be lower

in tumor tissues as compared to adjacent non-tumor tissues. Therefore, we agree that miR-30b may be a tumor suppressor miRNA in CRC. In the literature, there are scarcely any studies investigating the effect of miR-30b on autophagy, yet there are studies investigating miR-30b on apoptosis, another cellular death pathway. Furthermore, in the CRC cell line, overexpression of miR-30b specifically was reported to arrest cells in G1 phase and promote apoptosis (34). In addition, there was no difference in the expression level of miR-30b between the serum samples of the CRC patients and healthy controls. Normally, one would expect a tumor suppressor miRNA to be higher in the healthy control, however, this was not the case in our study and therefore, it is not possible to benefit from the examination of miR-30b in the serum for the diagnosis of CRC. In addition, miR-30b levels were decreased in the tumor tissues and serum samples of the CRC patients (36). In conclusion, the inconsistency between our findings and those published in previous studies may be due to the differences between expression patterns of miRNAs in different cancer types and ethnicities.

Previously, it has been reported that miR-30d expression was downregulated in colon cancer cell line (37), and CRC tissue samples (38). Zhang et al. have showed that miR-30d binding to 3'-UTR of beclin 1, and upregulation of miR-30d downregulated the autophagy in colon cancer cells (17). Moreover, Tanoğlu et al, have demonstrated in their expression profiling study that miR-30d level was decreased between the tumor-tissues of Stage-II CRC patients and surrounding normal-control tissues in the Turkish population (39). The number of studies on miR-30d is very limited to come to a definite conclusion. Furthermore, the findings of a study examining the role of miR-30d in Turkish patients with Stage-II CRC were in contrary to our results. According to our findings, the miR-30d expression level was increased in the CRC tumor tissues, however, it was reported decreased in an expression profiling study conducted on the Turkish population (39). Thus, it is difficult to say whether miR-30d acts as an oncomiR or a tumor suppressor in CRC. However, unlike that study, in our study, the patients with CRC were not separated according to their stages and there were patients from all four stages. Moreover, we also found that miR-30d, which was found to be increased in the tumor tissues of CRC patients, was decreased in the CRC patient serum samples as compared to the healthy control serum samples. The same result was obtained both times while the analyses were performed under similar conditions. Based on these results, it is necessary to increase the number and elaborate the miRNA optimization and normalization studies from the serum. This discrepancy suggests that circulating miRNAs do not always have to be secreted directly from tumor tissues.

In a recent study, the miR-216a expression levels have been demonstrated to be decreased in CRC (40). Also, the overexpression of miR-216a was shown to suppress invasion and metastasis of CRC cells, both *in vitro* and *in vivo* (41). Similar to the findings of previous studies, miR-216a levels were also decreased in tumor tissues of CRC patients in our study. Therefore, it can be said that miR-216a may be a tumor suppressor miRNA. There is only one study that has examined the effect of miR-216a on beclin 1, yet its effect on autophagy in cancer has not been examined in detail, so it is not possible to comment the effect of miR-216a on autophagy in CRC. Additionally, the miR-216a levels were not statistically different between the CRC patient and healthy control serum samples. Thus far, we have not encountered a study on miR-216a expression levels in the serum of patients with cancer. When more studies are conducted evaluating miR-216a in the serum of the cancer patients, it can be determined whether miR-216a is suitable for the diagnosis of CRC from serum samples.

So far, miR-216b has been investigated in CRC and has generally been considered a tumor suppressor (42, 43). For instance, miR-216b has been reported to be downregulated in CRC tissues and cell lines (44–46). In our study, the miR-216b expression level was found to be increased in the tumor tissues of the CRC patients, but it was not statistically significant. Furthermore, in our study, there was no difference between the patient and healthy control serum samples in terms of miR-216b expressions. However, miR-216b was reported to be downregulated in the serum of CRC patients (43). Therefore, it cannot be concluded whether it acts as an oncomiR or a tumor suppressor in CRC. Further studies may show its value as a diagnostic biomarker.

Consequently, it was determined that the key autophagy protein beclin 1 could function as a tumor suppressor in CRC, and miR-17-5p and miR-30d could act as oncomiR. Moreover, it is concluded that miR-17-5p, miR-30b, miR-30d, miR-216a and miR-216b levels are not reliable biomarkers for the diagnosis of CRC from serum samples, since there was no statistical difference between CRC patient serum and healthy control serum.

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