ANTICANCER RESEARCH *36*: 5449-5454 (2016)
doi:10.21873/anticanres.11124

# *miR-21*, *miR-221* and *miR-150* Are Deregulated in Peripheral Blood of Patients with Colorectal Cancer

MIROSLAVA SARLINOVA[1], MIRKO HALASA[2,3], DUSAN MISTUNA[3,4], LUDOVIT MUSAK[5], ROBERT ILIEV[6], ONDREJ SLABY[6], JANA MAZUCHOVA[7], VANDA VALENTOVA[8], LUKAS PLANK[9] and ERIKA HALASOVA[1,8]

*[1]Department of Molecular Medicine, Martin Biomedical Centre, and Departments of [3]Surgery, [7]Dermatovenerology, [8]Medical Biology and [9]Pathological Anatomy, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovak Republic; Departments of [2]Surgery and [5]Occupational Medicine and Toxicology, and [4]Transplantation Centre, University Hospital in Martin, Martin, Slovak Republic; [6]Molecular Oncology II–Solid Cancer Research Group, Masaryk University Central European Institute of Technology, Brno, Czech Republic*

**Abstract.** *The Aim of this study was to evaluate the expression levels of miR-21, miR-221, miR-150, let-7a and miR-126a in peripheral blood of 71 patients with colorectal cancer and 80 matched healthy control individuals. We determined expression levels of these microRNAs in peripheral blood samples and used small nucleolar RNA (RNU48) as an internal control. Expression levels of miR-21 ($p<0.0001$) and miR-221 ($p<0.0001$) were significantly higher, whereas expression levels of miR-150 ($p=0.0054$) were significantly lower in the blood samples of patients with colorectal cancer in comparison to the control group. The combination of these three microRNAs enabled us to distinguish patients with colorectal cancer from healthy donors with a sensitivity of 80% and specificity of 74% ($p<0.0001$). We did not observe any correlation of the studied microRNAs with clinicopathological features of colorectal cancer, indicating that expression of these microRNAs is more likely related to the host response to the tumour than the tumour itself.*

Colorectal cancer (CRC) represents a significant global health problem. Despite prevention and screening, this disease has an increasing incidence and high mortality. It is the most frequently occurring malignancy in the world, accounting for more than one million cases and 500,000 deaths per year (1, 2).

The invasive nature of current diagnostic procedures for CRC and the relatively high percentage of false-positive results limit the application of modern detection tools. The discovery and clinical evaluation of new specific and robust non-invasive biomarkers for diagnosis of CRC at an early stage, as well as for better prognosis and prediction of response to therapy, are very challenging (3). It is necessary to introduce a new non-invasive, but sufficiently sensitive method for detecting this disease. Numerous studies indicate that microRNA (miRNAs) can play an important role as reliable biomarkers for cancer detection and prognostic prediction, and even as novel targets for cancer therapy (4, 5).

The study of miRNA molecules is rapidly expanding the field of molecular biology and knowledge of their regulatory competence is becoming one of the basic building blocks of tumour biology. miRNAs are small, single-stranded, non-coding RNA molecules with a length of 19-25 nucleotides that regulate gene expression, significantly contribute to the management of the proteosynthesis, regulate the activation of genes at the post-transcriptional level, and participate in the regulation of the cell cycle. Their expression is different in healthy and disease-affected individuals (6, 7).

The detection of miRNAs circulating in blood has enormous potential because miRNAs serve as non-invasive biomarkers, not only for the diagnosis and prognosis of the disease, but also as novel predictors of response and sensitivity to cancer treatment (8).

In this study, we delt with CRC and identified types of miRNA molecules that have diagnostic relevance for this type of cancer. miRNAs, as new markers, could contribute to the improvement of existing CRC classification and lead

*Correspondence to:* doc. RNDr. Erika Halasova, Ph.D., Department of Molecular Medicine, Comenius University in Bratislava, Jessenius Faculty of Medicine, Mala Hora 11161/4C, Martin, Slovak Republic. Tel: +4210432633439, e-mail: halasova@jfmed.uniba.sk

*Key Words:* Colorectal cancer, microRNAs, biomarker, miRNA expression.

to earlier detection and diagnosis. This could also enhance accurate prediction of the disease course.

The aim of the present study was to analyse the expression of selected microRNAs (*miR-21, miR-221, miR-150, let-7a* and *miR-126a*) and to determine whether the given values of analysed miRNAs could serve as relevant predictors for determination of disease.

## Materials and Methods

*Characteristics of the patients and controls*. The study was approved by the Ethics Committee (EK 1010/2012) of Jessenius Faculty of Medicine in Martin and all individuals signed informed consent before participation. The study consisted of 71 patients (aged 40-86 years) with diagnosed CRC and 80 controls (aged 44-86 years). The patient group consisted of 46 men (mean age±SD= 66.54±9.25 years) and 25 women (mean age±SD=66.36±10.66 years). The control group was formed by 58 men (mean age±SD= 59.36±11.05 years) and 22 women (mean age±SD=67.64±12.28 years). There was no significant difference between the age of patients and control s ($p$=0.0075).

*Methods*. Peripheral blood (2.5 ml) was collected before therapy using PAXgene Blood RNA Tubes (Qiagen, Hilden, Germany) for *in vitro* diagnostic purposes. After blood collection, the tubes were kept for 2 h at room temperature. The RNA concentration and purity were confirmed by the spectrophotometric ratio using absorbance measurements at wavelengths of 260 nm and 280 nm on a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, MA, USA). Isolated RNA was stored at −80˚C.

Reverse transcription (RT) was performed with 2 ng total RNA in a 10-μl reaction using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. RT primers were included in TagMan MicroRNA Assay Kit (Applied Biosystems). cDNA samples were stored at −20˚C until subsequent quantitative polymerase chain reaction (qPCR).

The 20-μl reaction mixture required for the qPCR included RT product, TaqMan Universal PCR Master Mix no UNG (Applied Biosystems), Nuclease-free water and probe mixture of the TaqMan MicroRNA Assay Protocol (Applied Biosystems). All reactions were performed in duplicate.

The qPCRs were performed using an IQ5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Relative quantification (RQ) was carried out using the delta delta CTcomparative method.

*Normalization and data analysis*. In the context of finding appropriate endogenous controls, 60 samples were tested. This group consisted of 30 patients and 30 controls. In order to select the appropriate endogenous control for data normalization, three internal reference genes (three small nucleolar RNAs: *RNU6B, RNU44*, and *RNU48*) were tested. Obtained data were evaluated using GenEx software (MultiD Analyses AB, Munich, Germany) consisting of geNorm and NormFinder algorithms.

Analysis and statistical evaluation of the results were performed with Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA). Differences between miRNA levels and clinico-pathological parameters were evaluated using two tests: for testing of two groups, the Mann–Whitney *U*-test was used, and for testing of three or more groups, the Kruskal–Wallis test was used. A value of $p<0.05$ was considered significant.

The rate of expression of statistically significant miRNAs in relation to CRC was evaluated by receiver operating characteristic (ROC) analysis.

## Results

The results of comparing endogenous controls suitable for data normalization are shown in Figure 1. *RNU48* gene was selected as a suitable reference for the normalization of miRNA expression data.

The expression levels of three analyzed miRNA were significantly different between patients and control group; miR-21 ($p<0.0001$), miR-221 ($p<0.0001$) were up-regulated, and miR-150 ($p=0.0145$) was found down-regulated in patients (Figure 2).

Statistically significant differences were not found in expression levels of *let-7a* ($p=0.1404$) and *miR-126a* ($p=0.2739$). However, a slightly increased level of *let-7a* and slightly reduced level of *miR-126a* in patients with CRC were observed.

By ROC analysis, it was confirmed that *miR-21* and *miR-221*, and a combination of *miR-21*, *miR-221* and *miR-150* was able to serve as a potential diagnostic biomarker of CRC (summarized in Table I, Figure 3), although no correlation between analysed miRNA and clinicopathological characteristics of patients was found. Results are presented as the median relative expression values, with ranges defined by 25th-75th percentiles, and *p*-values in Table II.

## Discussion

Although significant results have recently been achieved in the field of clinical diagnosis of CRC, and the mortality rate due to some malignant diseases in developed countries has decreased, the cancer incidence has remained the same. A significant factor which influences mortality rate is an early and accurate diagnosis (1). The first step in cancer treatment should be a proper characterization of an individual's tumour; this means analysis of tumour cells that are removed during surgery. In order to avoid the need for such strong intervention, new suitable non-invasive alternatives have been introduced, such as the fluid biopsy (circulating tumour cells) (9) and miRNA-based biomarkers (10). Both methods allow for early detection of various cancer types and their proper differential diagnostics.

miRNAs that circulate freely or exist in exosomal shells are less vulnerable to RNase-mediated degradation, and can be extracted from a wide variety of biological and clinical materials, collected in clinical settings (archival formalin-fixed paraffin-embedded) tissues and body fluids. Endogenous miRNAs in the blood tend to remain stable for

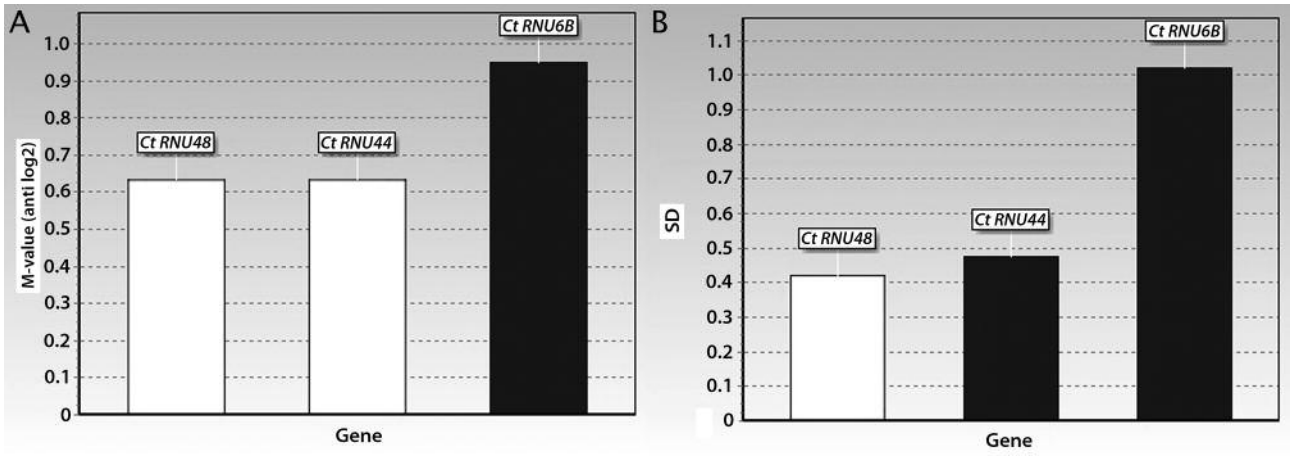


Figure 1. *Selected suitable endogenous control using algorithm geNorm (A) and NormFinder (B).*

a long time, and can withstand several repeated freeze-thaw cycles. Growing evidence indicates that cancer cells secrete miRNAs into the systemic circulation (11, 12). This fact is one of the main reasons for the study of miRNAs as biomarkers in the field of cancer research (11).

One of the major concerns of using miRNA expression analysis in body fluids, such as blood, is an inadequate selection of internal controls for the normalization of miRNA expression (13). Consequently, there is no established housekeeping miRNA gene for normalization of the expression of circulating miRNAs in body fluids (14, 15). MicroRNA with stable expression for specific experimental conditions is most often used as a suitable endogenous control for data normalisation of miRNA expression. Reference genes of small nuclear RNAs, for example, *RNU6B* (16, 17, 18), *RNU48* (19) or a *5S* rRNA (20) are frequently used for the analysis of tissue miRNA. Mitchell *et al*. (21) reported that synthetic versions of miRNA from other organisms (*Caenorhabditis elegans*) can be beneficially used for the normalization of data for circulating miRNA and identification of an appropriate endogenous control is required prior to each experiment. Based on literature data (17, 18, 22, 23), we analysed a combination of three reference genes *RNU48, RNU6B, RNU44*, and the gene RNU48 was found to be an appropriate endogenous control.

Current research suggests that there is a relationship between the levels of miRNA in peripheral blood and in the tumour tissue (24, 25).

In our study, the expression of selected miRNAs was determined in samples of whole peripheral blood from patients with CRC and healthy controls.

One of the most studied miRNAs is *miR-21*. Its deregulation and increased expression was demonstrated in a wide variety of tumours, such as breast, pancreas, lung, liver, chronic lymphocytic leukemia, glioblastoma, and CRC (26, 27). It was found that miR-21 participates in the multistep process of colorectal tumorigenesis by regulation of the mitogen-activated protein kinase pathway, as well as in association with wingless-type protein/β-catenin signaling by targeting phosphatase and tensin homolog (*PTEN*), programmed cell death (*PDCD*), and dickkopf WNT signaling pathway inhibitor 2 (*DKK2*) genes (11). Kannan *et al*. dealt with free circulating miR-21 in the plasma and their study demonstrated that plasma miR-21 could be used to identify patients with CRC and differentiate them from healthy volunteers with high sensitivity (90%) and specificity (90%) (28).

Our analysis showed that there was a statistically significant increase in the expression of *miR-21* in peripheral blood of patients with CRC, compared to a control group. This corresponds to the data found in the literature. We also found that increased expression of *miR-21* differentiated patients with CRC from the control group with 71.8% sensitivity and 67.5% specificity.

Pu *et al*. analyzed the *miR-221* levels in 103 patients with CRC and 37 healthy controls (29). The plasma *miR-221* expression was increased and differentiated patients from the controls with 86% sensitivity and 41% specificity. The results showed that plasma *miR-221* correlated with the expression of p53 in formalin-fixed paraffin-embedded tissue. An increased level of miRNA in this patient group was also confirmed in serum, together with *miR-134, miR-146a, miR-222* and *miR-23*. Tsunoda *et al*. in their study described that kirsten rat sarcoma viral oncogene homolog (KRAS) can increase the expression of oncogenic cluster *miR-221/222* and thereby contributes to overall disease progression in patients with CRC (30).

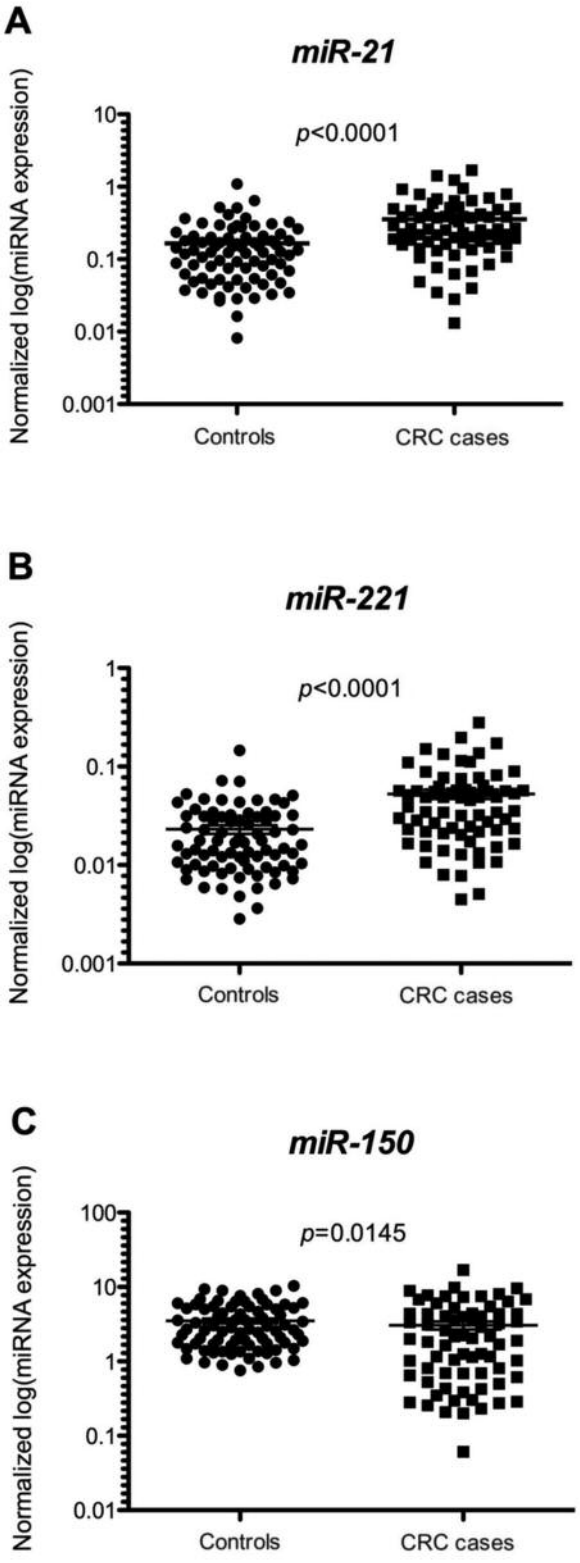


Figure 2. *Different expressions of miR-21 (A), miR-221 (B) and miR-150 (C) in samples from patients with colorectal cancer (CRC). and controls. miR-21 and miR-221 showed significantly higher expression in patients, whereas miR-150 was significantly lower in patients.*

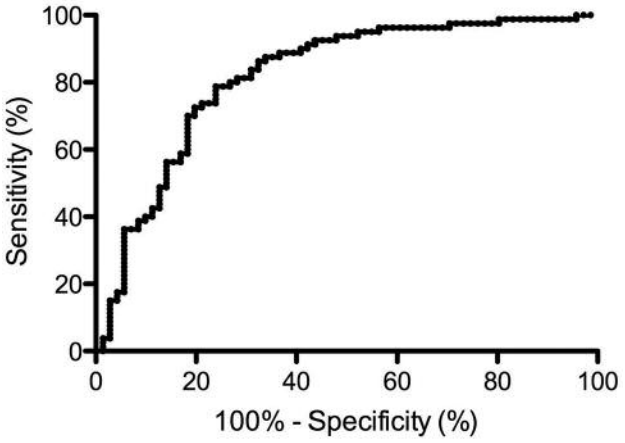


Figure 3. *Combined receiver operating characteristic curve for miR-21, miR-221 and miR-150.*

Table I. *Results of receiver operating characteristic (ROC) analysis for miR-21, miR-221, miR-150 and their combination.*

| | *miR-21* | *miR-221* | *miR-150* | Combination |
|---|---|---|---|---|
| Sensitivity | 71.83% | 71.83% | 57.75% | 80% |
| Specificity | 67.50% | 68.75% | 56.25% | 74% |
| AUC | 0.7401 | 0.7535 | 0.6315 | 0.8180 |
| *p*-Value | <0.0001 | <0.0001 | 0.0145 | <0.0001 |

AUC: Area under the ROC curve.

The current findings suggest that *miR-150* may be a key regulator of tumorigenesis and progression of CRC. Based on *in vitro* as well as *in vivo* studies, Feng *et al.* reported that *miR-150* acts as a tumour suppressor (31). *miR-150* is involved in the mechanism of the CRC at the molecular level by inhibiting the target gene proto-oncogene sequences (*c-MYB*). Kent *et al.* analysing HT29 cell line of colorectal adenocarcinoma demonstrated that *miR-150* down-regulates expression of c-MYB and b-cell leukemia/lymphoma 2 (*BCL2*) genes (32).

Our analysis showed a decrease in *miR-150* level in the peripheral blood of patients compared to controls. This finding is in accordance with previously published data dealing with miR-150 targets and suggests that this miRNA could play a key role in both inhibition (if normally expressed) and progression (if its expression is decreased) of CRC tumorigenesis.

The more we understand the etiological and biological nature of CRC, the better equipped we will be for designing effective preventive, diagnostic and therapeutic tools to help reduce the burden of this disease.

Table II. *Peripheral blood miRNAs differentially expressed between patients with colorectal cancer (CRC) and healthy donors and correlation of miRNAs with clinicopathological features of CRC.*

| | | | Median expression (25-75th percentile) | | | | |
|---|---|---|---|---|---|---|---|
| | | n | *miR-21* | *miR-221* | *miR-150* | *let-7* | *miR-126* |
| Cohort | Patients | 71 | 0.25 (0.16-0.47) | 0.04 (0.02-0.06) | 1.98 (0.64-4.35) | 0.85 (0.39-1.51) | 0.17 (0.09-0.37) |
| | Healthy donors | 80 | 0.13 (0.06-0.21) | 0.02 (0.01-0.03) | 2.82 (1.89-5.46) | 0.62 (0.37-1.01) | 0.21 (0.11-0.37) |
| Fold change* | | | 2.0279 | 2.3675 | 0.7027 | 1.382 | 0.8299 |
| *p*-Value | | | **<0.0001** | **<0.0001** | **0.0054** | 0.1404 | 0.2739 |
| Clinical stage | I | 20 | 0.26 (0.19-0.5) | 0.03 (0.02-0.06) | 1.46 (0.62-5.86) | 0.69 (0.28-2.06) | 0.21 (0.07-0.38) |
| | II | 9 | 0.44 (0.18-0.89) | 0.06 (0.04-0.12) | 0.78 (0.32-2.12) | 1.28 (0.3-1.65) | 0.17 (0.09-0.28) |
| | III | 22 | 0.35 (0.19-0.48) | 0.05 (0.03-0.09) | 3.1 (0.98-4.59) | 0.89 (0.42-1.41) | 0.17 (0.08-0.42) |
| | IV | 18 | 0.17 (0.11-0.37) | 0.03 (0.02-0.06) | 1.63 (0.69-4.32) | 0.67 (0.39-0.98) | 0.15 (0.09-0.41) |
| *p*-Value | | | 0.1372 | 0.0599 | 0.3314 | 0.6627 | 0.9822 |
| T-Stage | T1 | 7 | 0.29 (0.18-0.49) | 0.05 (0.03-0.08) | 1.67 (0.81-3.09) | 1.37 (0.54-2.89) | 0.17 (0.06-0.24) |
| | T2 | 14 | 0.22 (0.14-0.54) | 0.02 (0.01-0.04) | 1.57 (0.53-6.2) | 0.41 (0.21-1.44) | 0.25 (0.07-0.39) |
| | T3 | 42 | 0.26 (0.14-0.49) | 0.05 (0.02-0.06) | 2.09 (0.48-4.32) | 0.81 (0.42-1.51) | 0.15 (0.09-0.31) |
| | T4 | 6 | 0.23 (0.16-0.38) | 0.03 (0.01-0.13) | 2.69 (1.04-6.54) | 0.55 (0.13-1.19) | 0.36 (0.14-0.78) |
| *p*-Value | | | 0.8195 | 0.0727 | 0.8988 | 0.1784 | 0.3173 |
| N-Stage | N0 | 31 | 0.26 (0.18-0.51) | 0.04 (0.02-0.06) | 1.63 (0.43-5.44) | 0.98 (0.36-1.65) | 0.18 (0.08-0.36) |
| | N1 | 29 | 0.25 (0.12-0.45) | 0.05 (0.02-0.06) | 3.34 (0.75-4.32) | 0.67 (0.42-1.13) | 0.14 (0.09-0.34) |
| | N2 | 9 | 0.24 (0.17-0.41) | 0.03 (0.02-0.07) | 1.43 (0.29-6.29) | 0.69 (0.32-1.37) | 0.22 (0.11-0.59) |
| *p*-Value | | | 0.7236 | 0.879 | 0.4699 | 0.5885 | 0.4736 |
| M-Stage | M0 | 51 | 0.29 (0.18-0.50) | 0.04 (0.02-0.07) | 2.15 (0.61-4.39) | 0.89 (0.36-1.64) | 0.17 (0.08-0.37) |
| | M1 | 18 | 0.17 (0.11-0.37) | 0.03 (0.02-0.06) | 1.25 (0.52-3.94) | 0.65 (0.35-0.98) | 0.14 (0.09-0.31) |
| *p*-Value | | | 0.0596 | 0.2693 | 0.6504 | 0.1831 | 0.8097 |
| Grade | Well-diff | 19 | 0.23 (0.17-0.36) | 0.04 (0.02-0.06) | 2.07 (0.41-3.77) | 0.69 (0.39-1.17) | 0.18 (0.08-0.35) |
| | Moderately diff | 38 | 0.25 (0.14-0.58) | 0.03 (0.02-0.07) | 1.63 (0.69-4.36) | 0.75 (0.29-1.77) | 0.14 (0.08-0.39) |
| | Poorly diff | 12 | 0.37 (0.14-0.57) | 0.004 (0.002-0.006) | 3.04 (0.57-7.59) | 0.66 (0.39-1.36) | 0.18 (0.12-0.38) |
| *p*-Value | | | 0.4504 | 0.9984 | 0.5193 | 0.7651 | 0.8324 |
| Localization | Proximal | 24 | 0.26 (0.16-0.41) | 0.05 (0.02-0.06) | 2 (0.53-4.28) | 0.48 (0.35-1.42) | 0.2 (0.1-0.4) |
| | Distal | 47 | 0.25 (0.16-0.52) | 0.03 (0.02-0.06) | 1.89 (0.69-4.35) | 0.89 (0.41-1.53) | 0.2 (0.1-1) |
| *p*-Value | | | 0.7199 | 0.7938 | 0.9952 | 0.3524 | 0.2875 |

diff: Differentiated. *Relative to controls. Significant *p*-values shown in bold.

Defining the specific miRNA profiles of a patient's blood in the future could contribute not only to early diagnosis of the disease but could also enable determination of predictive factors that would lead to the most appropriate personalized and targeted treatment of patients with CRC.

## Conflicts of Interest

The Authors confirm that there are no conflicts of interest in regard to this study.

## Acknowledgements

This work was supported by the Ministry of Health of the Slovak Republic under the contract 2012/25-UKMA-2, by the project Competence Center for Research and Development in the Field of Diagnostics and Therapy of Oncological Diseases, ITMS: 26220220153 and by the project "Biomedical Center Martin" ITMS code: 26220220187, the project is co-financed from EU sources.

## References

1 Siegel R, Naishadham D and Jemal A: Cancer statistics. CA Cancer J Clin *62(1)*: 10-29, 2012.

2 Luo X, Burwinkel B, Tao S and Brenner H: MicroRNA signatures: novel biomarker for colorectal cancer? Cancer Epidemiol Biomarkers Prev *20(7)*: 1272-1286, 2011.

3 Ferro A, Peleteiro B, Malvezzi M, Bosetti C, Bertuccio P, Levi F, Negri E, La Vecchia C and Lunet N: Worldwide trends in gastric cancer mortality (1980-2011), with predictions to 2015, and incidence by subtype. Eur J Cancer *50(7)*: 1330-1344, 2014.

4 Mishra PJ: MicroRNAs as promising biomarkers in cancer diagnostics. Biomark Res *2(19)*: DOI: 10.1186/2050-7771-2-19, 2014.

5 Sempere LF: Recent advances in miRNA-based diagnostic applications. Expert Rev Mol Diagn *12(6)*: 557-559, 2012.

6 Slaby O, Svoboda M, Besse A, Bienertova-Vasku, J, Cerna K, Dolezalova D, Dostalova-Merkerova M, Ehrmann J, Faltejskova P, Hajek R, Hajkova J, Juracek J, Krejcik Z, Kubiczkova L, Kulda V, Lakomy R, Luzna P, Machova-Polakova K, Mayer J,

Mayerova V, Mraz M, Musilova K, Pesta M, Poprach A, Pospisilova S, Redova M, Sana J, Sevcikova S, Sterba J, Votavova H and Vyzula R: MicroRNA in Oncology. First Edition, Galen, Praha, 2012.

7 Thomas J, Ohtsuka M, Pichler M and Ling H: MicroRNAs: Clinical relevance in colorectal cancer. Int J Mol Sci *16(12)*: 28063-28076, 2015.

8 Pichler M and Calin GA: MicroRNAs in cancer: From developmental genes in worms to their clinical application in patients. Br J Cancer *113(4)*: 569-573, 2015.

9 Mostert B, Sieuwerts AM, Martens JW and Sleijfer S: Diagnostic applications of cell-free and circulating tumor cell-associated miRNAs in cancer patients. Expert Rev Mol Diagn *11(3)*: 259-275, 2011.

10 Brase JC, Wuttig D, Kuner R and Sültmann H: Serum microRNAs as non-invasive biomarkers for cancer. Mol Cancer *9(306)*: DOI: 10.1186/1476-4598-9-306, 2010.

11 Okugawa Y, Toiyama Y and Goel A: An update on microRNAs as colorectal cancer biomarkers: where are we and what's next? Expert Rev Mol Diagn *14(8)*: 999-1021, 2014.

12 Turchinovich A, Weiz L, Langheinz A and Burwinkel B: Characterization of extracellular circulating microRNA. Nucleic Acids Res *39(16)*: 7223-7233, 2011.

13 Baraniskin A, Nöpel-Dünnebacke S, Ahrens M, Jensen SG, Zöllner H, Maghnouj A, Wos A, Mayerle J, Munding J, Kost D, Reinacher-Schick A, Liffers S, Schroers R, Chromik AM, Meyer HE, Uhl W, Klein-Scory S, Weiss FU, Stephan C, Schwarte-Waldhoff I, Lerch MM, Tannapfel A, Schmiegel W, Andersen CL and Hahn SA: Circulating U2 small nuclear RNA fragments as a novel diagnostic biomarker for pancreatic and colorectal adenocarcinoma. Int J Cancer *132(2)*: E48-E57, 2013.

14 Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK and Calin GA: MicroRNAs in body fluids--the mix of hormones and biomarkers. Nat Rev Clin Oncol *8(8)*: 467-477, 2011.

15 Zen K and Zhang CY: Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. Med Res Rev *32(2)*: 326-348, 2012.

16 Svoboda M, Izakovicova-Holla L, Sefr R, Vrtkova I, Kocakova I, Tichy B and Dvorak J: Micro-RNAs miR125b and miR137 are frequently upregulated in response to capecitabine chemoradiotherapy of rectal cancer. Int J Oncol *33(3)*: 541-547, 2008.

17 Kulda V, Pesta M, Topolcan O, Liska V, Treska V, Sutnar A, Rupert K, Ludvikova M, Babuska V, Holubec L Jr. and Cerny R: Relevance of miR-21 and miR-143 expression in tissue samples of colorectal carcinoma and its liver metastases. Cancer Genet Cytogenet *200(2)*: 154-160, 2010.

18 Cheng H, Zhang L, Cogdell DE, Zheng H, Schetter AJ, Nykter M, Harris CC, Chen K, Hamilton SR and Zhang W: Circulating plasma MiR-141 is a novel biomarker for metastatic colon cancer and predicts poor prognosis. PLoS One *6(3)*: e17745, 2011.

19 Faltejskova P, Svoboda M, Srutova K, Mlcochova J, Besse A, Nekvindova J, Radova L, Fabian P, Slaba K, Kiss I, Vyzula R and Slaby O: Identification and functional screening of microRNAs highly deregulated in colorectal cancer. J Cell Mol Med *16(11)*: 2655-2666, 2012.

20 Wang CJ, Zhou ZG, Wang L, Yang L, Zhou B, Gu J, Chen HY and Sun XF: Clinicopathological significance of microRNA-31, -143 and -145 expression in colorectal cancer. Dis Markers *26(1)*: 27-34, 2009.

21 Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB and Tewari M: Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA *105(30)*: 10513-10518, 2008.

22 Gee HE, Buffa FM, Camps C, Ramachandran A, Leek R, Taylor M, Patil M, Sheldon H, Betts G, Homer J, West C, Ragoussis J and Harris AL: The small-nucleolar RNAs commonly used for microRNA normalisation correlate with tumour pathology and prognosis. Br J Cancer *104(7)*: 1168-1177, 2011.

23 Matouskova P, Bartikova H, Bousova I, Hanusova V, Szotakova B and Skalova L: Reference Genes for Real-Time PCR Quantification of Messenger RNAs and MicroRNAs in Mouse Model of Obesity. PLoS One *9(1)*: e86033, 2014.

24 Zomer A, Vendrig T, Hopmans ES, van Eijndhoven M, Middeldorp JM and Pegtel DM: Exosomes: Fit to deliver small RNA. Commun Integr Biol *3(5)*: 447-450, 2010.

25 Montes-Moreno S, Martinez N, Sanchez-Espiridión B, Diaz Uriarte R, Rodriguez ME, Saez A, Montalbán C, Gomez G, Pisano DG, García JF, Conde E, Gonzalez-Barca E, Lopez A, Mollejo M, Grande C, Martinez MA, Dunphy C, Hsi ED, Rocque GB, Chang J, Go RS, Visco C, Xu-Monette Z, Young KH and Piris MA: miRNA expression in diffuse large B-cell lymphoma treated with chemoimmunotherapy. Blood *118(4)*: 1034-1040, 2011.

26 Yan LX, Wu QN, Zhang Y, Li YY, Liao DZ, Hou JH, Fu J, Zeng MS, Yun JP, Wu QL, Zeng YX and Shao JY: Knockdown of *miR-21* in human breast cancer cell lines inhibits proliferation, *in vitro* migration and *in vivo* tumor growth. Breast Cancer Res *13(1)*: DOI: 10.1186/bcr2803, 2011.

27 Mazeh H, Mizrahi I, Ilyayev N, Halle D, Brücher B, Bilchik A, Protic M, Daumer M, Stojadinovic A, Itzhak A and Nissan A: The diagnostic and prognostic role of microRNA in colorectal cancer - a comprehensive review. J Cancer *4(3)*: 281-295, 2013.

28 Kanaan Z, Rai SN, Eichenberger MR, Roberts H, Keskey B, Pan J and Galandiuk S: Plasma miR-21: a potential diagnostic marker of colorectal cancer. Ann Surg *256(3)*: 544-551, 2012.

29 Pu XX, Huang GL, Guo HQ, Guo CC, Li H, Ye S, Ling S, Jiang L, Tian Y and Lin TY: Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. J Gastroenterol Hepatol *25(10)*: 1674-1680, 2010.

30 Tsunoda T, Takashima Y, Yoshida Y, Doi K, Tanaka Y, Fujimoto T, Machida T, Ota T, Koyanagi M, Kuroki M, Sasazuki T and Shirasawa S: Oncogenic KRAS regulates miR-200c and miR-221/222 in a 3D-specific manner in colorectal cancer cells. Anticancer Res *31(7)*: 2453-2459, 2011.

31 Feng J, Yang Y, Zhang P, Wang F, Ma Y, Qin H and Wang Y: miR-150 functions as a tumour suppressor in human colorectal cancer by targeting c-Myb. J Cell Mol Med *18(10)*: 2125-2134, 2014.

32 Kent OA, McCall MN, Cornish TC and Halushka MK: Lessons from miR-143/145: the importance of cell-type localization of miRNAs. Nucleic Acids Res *42(12)*: 7528-7538, 2014.

*Received August 9, 2016*
*Revised September 12, 2016*
*Accepted September 21, 2016*