Advances in Biological Research 7 (4): 144-119, 2013
ISSN 1992-0067

DOI: 10.5829/idosi.abr.2013.7.4.928

An Investigation on 10 Micro RNAs in Colorectal Cancer as Biomarkers to Predict Disease Progression

[1,3]*Ghasem Janbabai,* [1,2,3]*Touraj Farazmandfar and* [3]*Sahar Khosravi*

[1]Molecular and Cell Biology Research Center,
Mazandaran University of Medical Science, Sari, Iran
[2]Faculty of Advanced Medical Science Technologies,
Golestan University of Medical Sciences, Gorgan, Iran
[3]Cancer Research Center, Mazandaran University of Medical Science, Sari, Iran

Abstract: MicroRNAs constitute a class of small noncoding RNAs that function as post-transcriptional gene regulators. MicroRNAs have potential as diagnostic biomarkers and therapeutic targets in colorectal cancer. The purpose of present study was to define 10 major microRNAs and their correlation with disease progression in human colorectal cancer. MiRNAs of miR-21, miR-20a, miR-92a, miR-106a, miR-135b and miR-181b were up-regulate and miRNAs of miR-143, miR-145, miR-133b and let-7a-1 were down-regulated. The miR-21 and miR-135b be correlated with more advanced stages of the disease. We found differences in microRNAs expression between tumors and paired nontumorous tissues. Tumors with high expression of miR-21 and miR-135b were associated with more advanced stages. The miR-21 and miR-135b may be useful Biomarkers for early detection of colorectal cancers.

Key words: Colorectal cancer · MicroRNA expression · Biomarker · Staging

INTRODUCTION

MicroRNAs (miRNAs) are short 21–23 nucleotide RNA molecules which are crucial for post-transcriptional regulators of gene expression by complementary binding to the 3' untranslated regions of mRNAs; thereby causing mRNA degradation or translation inhibition [1-3]. Previous studies have shown that miRNAs participate in regulation of cellular development, differentiation, proliferation, apoptosis and affecting major biological systems such as maintenance of stem cell potency, immunity and cancer [4-11]. According to bio-informatics data Up to 30% of human genes are thought to be regulated by miRNAs and more than 50% of miRNA genes are located at specific chromosomal regions, including fragile sites and regions of deletion or amplification that are altered in human cancer [4, 12]. Depending on target genes they can either be considered as tumor suppressors or oncogenes [5, 12-15]. These short non-coding RNAs may be abnormally down-regulated or up-regulated in cancerous tissues [13, 16-18]. MiRNA expression was shown to be promising factors for cancer diagnosis and progression. Number of miRNAs were identified to be correlated with the clinical outcome of lung adenocarcinoma [19], chronic lymphocytic leukemia [20] and breast cancer [21]. Several articles were recently published about the importance of miRNAs in colorectal cancer [22-25]. However, whether a miRNA can predict the clinical outcomes of colorectal cancer has yet to be specified.

In this study, expression of 10 miRNAs; let-7a-1, miR-92a, miR-133b, miR-143, miR-145, miR-20a, miR-21, miR-106a, miR-135b, miR-181b were investigated and their correlation with disease progress is monitored.

MATERIALS AND METHODS

Formalin-fixed paraffin-embedded (FFPE) samples of cancerous tissue and adjacent noncancerous tissues were acquired from 87 patients who had undergone operations

Corresponding Author: Touraj farazmandfar, Molecular and Cell Biology Research Center,
Mazandaran University of Medical Science, Sari, Iran. Zip Code: 481751665.
Tel: +989111513013

Table 1: Primers information

miRNAs	Primer sequence (5'-3')	Gene ID
miR-20a	GTAGCACTAAAGTGCTTATA	157704454
miR-21	TGTCGGGTAGCTTATCAGAC	157713538
miR-92a	CTTTCTACACAGGTTGGGAT	157704454
miR-106a	CCTTGGCCATGTAAAAGTGC	224589822
miR-135b	CCTTGGCCATGTAAAAGTGC	224589822
miR-181b	GTAGCACTAAAGTGCTTATA	224589800
let-7a-1	TGGGATGAGGTAGTAGGTTG	224589821
miR-133b	CCTCAGAAGAAAGATGCCC	224589818
miR-143	GCGCAGCGCCCTGTCTCCCAG	224589817
miR-145	ACCTTGTCCTCACGGTCCAG	224589817

between 2005 and 2011 at Emam Hospital (Sari, Iran). Tumor staging was determined based on pathological records and the tumor node metastasis (TNM) classification [26]. Samples with familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer were left out of this study. This work was approved by the Clinical Research Ethics Committee in Mazandaran University of Medical Science.

Total RNA was isolated from micro dissected FFPE tissues using the miRNeasy FFPE Kit (Qiagen Hilden, Germany). Quantitative reverse transcriptase PCR (QRT-PCR) was performed in triplicate using normalization to U6 snRNA. First strand cDNA was synthesized using a miScript reverse transcription kit (Qiagen, Hilden, Germany). Primers for each miRNA designed by using GENE RUNNER software and be reviewed in NCBI and BLAST (Table 1). MiRNA were quantified by a miRscript SYBR Green PCR kit (Qiagen, Hilden, Germany) using 100 ng cDNA 0.5 μM miRNA-specific forward primers and the universal reverse primer per reaction. Real time PCR was performed at 40 cycles (95°C for 20 s, 58°C for 20 s, 72°C for 20 s) after an initial denaturation step (95°C for 10 min) in 7500 Real Time PCR system (Applied Biosystem, USA). Fold expression was calculated from threshold cycles (Ct) values by using the ΔΔCt method [27].

Statistical analyses were performed with SPSS for windows. The expression levels of miRNA in tumor and normal tissues were analyzed by the paired t□test. The p value less than 0.05 were used for statistical significance.

RESULTS

A maximum of 87 paired samples was analyzed by real-time RT-PCR assay to quantify the expression. miRNAs of miR-21, miR-20a, miR-92a, miR-106a, miR-135b and miR-181b were up-regulate and miRNAs of miR-143, miR-145, miR-133b and let-7a-1 were down-regulated. The mean expression levels of miR-21, miR-20a, miR-92a, miR-106a, miR-135b and miR-181b were higher in tumor than non-tumor samples (Fig. 1). The percentage of cases in which the expression levels of miR-21, miR-20a, miR-92a, miR-106a, miR-135b and miR-181b were higher in tumor than non-tumor samples, were 65.5, 59.8, 68.9, 67.8, 60.9 and 63.2%, respectively (see Table 2). In addition, the mean expression levels of miR-143, miR-145, miR-133b and let-7a-1 were lower in tumor than non-tumor samples (see Fig. 2). The percentage of cases in which the expression levels of miR-143, miR-145, miR-133b and let-7a-1 were lower in tumor than non-tumor samples, 59.8, 66.6, 60.9 and 70.1%, respectively (Table 2).

The only expression of miR-21 and miR-135b is correlated with more advanced stages of the disease. Stage I expressed lower levels of miR-21 and miR-135b than tumors and more advanced tumors expressed high levels of miR-21 and miR-135b using the quantitative RT-PCR data (test for trend, P<0.001) (Fig. 3).

Table 2: The expression level of miRNAs was studied on paired samples of colorectal cancer

miRNAs	Up-regulated/all cases (%)	Mean expression level in tumor (mean ± SD)	Mean expression level in non-tumor (mean ± SD)	Fold change	*P*□value
Up-regulated miRNAs					
miR-21	57/87 (65.5)	2.52±2.12	0.62±0.32	2.34	0.0011
miR-20a	52/87 (59.8)	2.12±1.56	0.53±0.22	2.78	0.0004
miR-92a	60/87 (68.9)	2.02±1.78	0.73±0.42	2.12	0.0022
miR-106a	59/87 (67.8)	2.61±1.89	1.03±0.62	2.45	0.0001
miR-135b	53/87 (60/9)	2.06±1.55	1.33±0.72	2.79	0.0002
miR-181b	55/87 (63.2)	2.14±1.28	1.83±1.12	2.86	0.0014
Down-regulated miRNAs					
miR-143	52/87 (59.8)	0.42±0.22	1.14±0.81	1.25	0.0010
miR-145	58/87 (66.6)	0.63±0.32	2.01±1.32	1.12	0.0017
miR-133b	53/87 (60.9)	0.52±0.36	1.94±1.23	1.31	0.0016
let-7a-1	61/87 (70.1)	0.66±0.31	2.03±0.68	1.18	0.0019

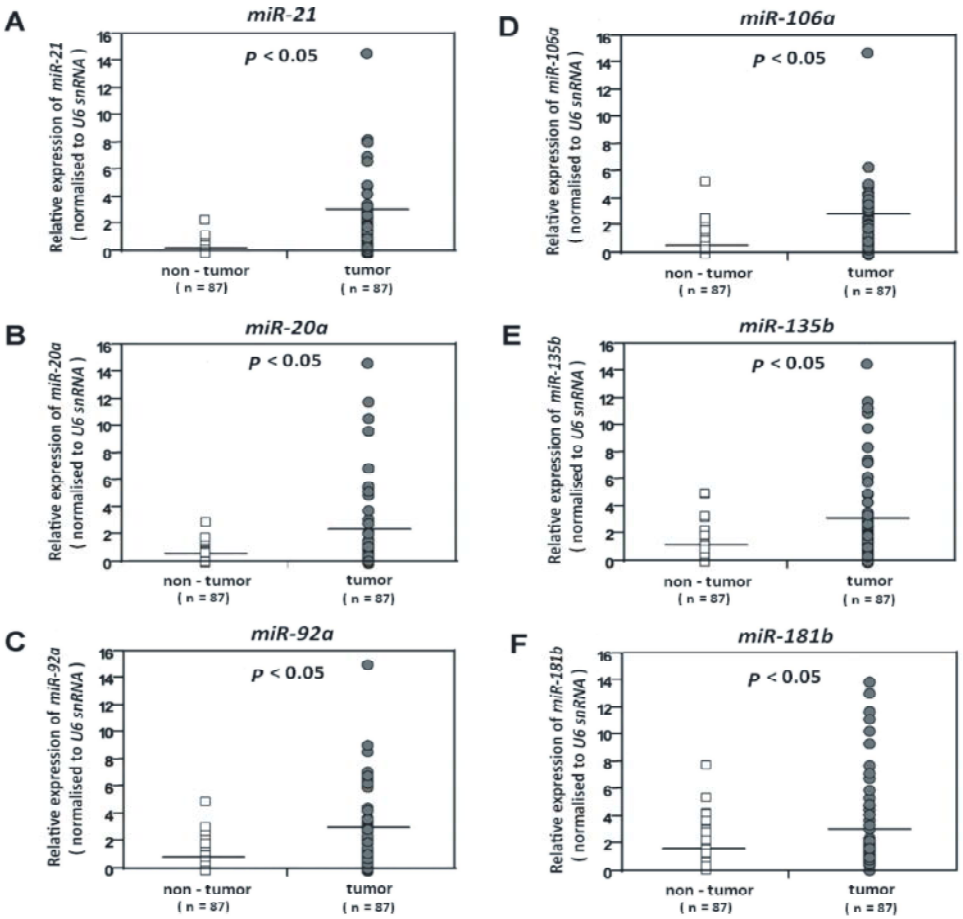

Fig. 1: Real-time RT-PCR analysis of 6 up-regulated miRNAs in tumor and non-tumor samples from colorectal cancer cases

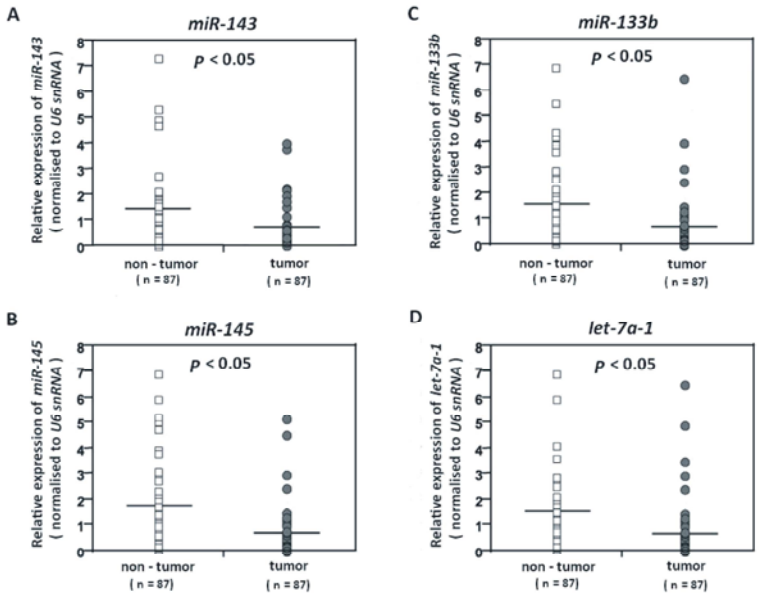

Fig. 2: Real-time RT-PCR analysis of 4 down-regulated miRNAs in tumor and non-tumor samples from colorectal cancer cases

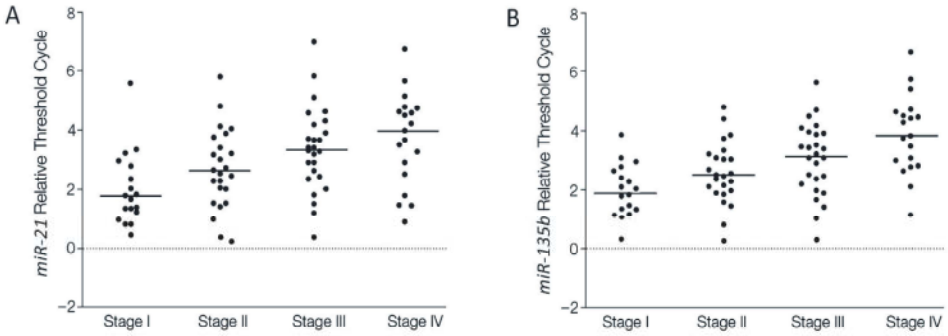

Fig. 3: Dot plots represent miR-21 and miR-135b relative Ct values from qRT-PCR for tumor expression levels. Each has been normalized to paired nontumorous tissue, respectively. Tissue types have been ordered from stage I through IV tumors. Horizontal bars indicate median expression value. The Cuzick nonparametric test for trend was used to evaluate trends (p for trend <0.001).

DISCUSSION

There is a lot of information that shows the miRNAs are involved in carcinogenesis [13-15]. It is important to recognize colorectal cancer-related miRNAs to enhance understanding of colorectal cancer biology. In this study, according to reported in several recent studies [28, 29], the expression levels of miR-21, miR-20a, miR-92a, miR-106a, miR-135b and miR-181b is higher in tumor than non-tumor samples and the expression levels of miR-143, miR-145, miR-133b and let-7a-1 were lower in tumor than non-tumor samples. In this study, base on Schetter *et al.* [24] studied the expression of miR-21 be correlated with more advanced stages of the disease and tumor development and It is elevated expression levels with disease progression. Over-expression of miR-21 in most solid tumors and its activity as an antiapoptotic factor in human glioblastoma cells has previously been studied [11, 21]. The miR-21 studies in human cell lines have shown it can target the tumor suppressor genes, phosphatase and tensin homolog (PTEN) [30] and tropomyosin 1 (TPM1) [31]. It also became clear that the miR-135b expression is elevated with disease progression. The miR-135b is a regulator of APC and has a strong effect on Wnt pathway activity [32]. Moreover, this miR-135b is highly expressed in colorectal adenomas and carcinomas, simultaneously with low levels of APC, indicating that up-regulation of this miRNA might be involved in colorectal cancer pathogenesis [33]. All of these data suggested that the expression of miRNAs changes during tumorigenesis. If increased miR-21 and miR-135b expression promotes colorectal cancer progression, inhibiting the activity of these two miRNAs may help prevent tumor progression at high risk populations of colorectal cancer. In addition, these associations indicating that miR-21 and miR-135b expression may be a useful prognostic biomarker and may help predict the benefits of therapy. However Further studies are needed to confirm the relationship between miR-21 and miR-135b and the progression of colorectal cancer to determine the potential of these two miRNAs as either a biomarker or therapeutic target.

CONCLUSION

We found differences in microRNAs expression between tumors and paired nontumorous tissue. Tumors with high expression of miR-21 and miR-135b were associated with more advanced stages. The miR-21 and miR-135b may be useful Biomarkers for early detection of colorectal cancers and survival prognosis including response to therapy.

REFERENCES

1. Lagos-Quintana, M., R. Rauhut, W. Lendeckel and T. Tuschl, 2001. Identification of novel genes coding for small expressed RNAs. Science Signaling, 294(5543): 853-858.
2. Lee, Y., K. Jeon, J.T. Lee, S. Kim and V.N. Kim, 2002. MicroRNA maturation: stepwise processing and subcellular localization. The EMBO Journal, 21(17): 4663-4670.
3. Pillai, R.S., 2005.MicroRNA function: multiple mechanisms for a tiny RNA? Rna, 11(12): 1753-1761.
4. Sassen, S., E.A. Miska and C. Caldas, 2008. MicroRNA: implications for cancer. Virchows Archiv, 452(1): 1-10.

5. Calin, G.A., C.D. Dumitru, M. Shimizu and M. Bichi, 2002. June 4, 2013 Zupo, S. Noch, E. Aldler, H. Rattan, S. Keating, M. and Rai, K. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences, 99(24): 15524-15529.
6. Brennecke, J., D.R. Hipfner, A. Stark, R.B. Russell and S.M. Cohen, 2003. bantam Encodes a Developmentally Regulated microRNA that Controls Cell Proliferation and Regulates the Proapoptotic Gene *hid* in Drosophila. Cell, 113(1): 25-36.
7. Xu, P., S.Y. Vernooy, M. Guo and B.A. Hay, 2003. The *Drosophila* MicroRNA Mir-14 Suppresses Cell Death and Is Required for Normal Fat Metabolism. Current Biology, 13(9): 790-795.
8. Chen, C.Z., L. Li, H.F. Lodish and D.P. Bartel, 2004. MicroRNAs modulate hematopoietic lineage differentiation. Science, 303(5654): 83-86.
9. Calin, G.A. and C.M. Croce, 2006. MicroRNA signatures in human cancers. Nature Reviews Cancer, 6(11): 857-866.
10. Calin, G.A., C. Sevignani, C.D. Dumitru, T. Hyslop, E. Noch, S. Yendamuri, M. Shimizu, S. Rattan, F. Bullrich and M. Negrini, 2004. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proceedings of the National Academy of Sciences of the United States of America, 101(9): 2999-3004.
11. Chan, J.A., A.M. Krichevsky and K.S. Kosik, 2005. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Research, 65(14): 6029-6033.
12. Cho, W., 2007. OncomiRs: the discovery and progress of microRNAs in cancers. Molecular cancer, 6(1): 60.
13. Hammond, S.M., 2006. MicroRNAs as oncogenes. Current opinion in genetics & Development, 16(1): 4-9.
14. Kumar, M.S., J. Lu, K.L. Mercer, T.R. Golub and T. Jacks, 2007. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nature Genetics, 39(5): 673-677.
15. Gartel, A.L. and E.S. Kandel, 2008. miRNAs: Little known mediators of oncogenesis. in Seminars in Cancer Biology. 2008: Elsevier.
16. Slaby, O., M. Svoboda, P. Fabian, T. Smerdova, D. Knoflickova, M. Bednarikova, R. Nenutil and R. Vyzula, 2008.Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. Oncology, 72(5-6): 397-402.
17. Akao, Y., Y. Nakagawa and T. Naoe, 2007. MicroRNA-143 and-145 in colon cancer. DNA and Cell Biology, 26(5): 311-320.
18. Schepeler, T., S.T. Reinert, M.S. Ostenfeld, L.L. Christensen, A.N. Silahtaroglu, L. Dyrskjot, C. Wiuf, F.J. Sorensen, M. Kruhoffer and S. Laurberg, 2008. Diagnostic and prognostic microRNAs in stage II colon cancer. Cancer Research, 68(15): 6416-6424.
19. Yanaihara, N., N. Caplen, E. Bowman, M. Seike, K. Kumamoto, M. Yi, R.M. Stephens, A. Okamoto, J. Yokota and T. Tanaka, 2006. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell, 9(3): 189-198.
20. Calin, G.A., M. Ferracin, A. Cimmino, G. Di Leva, M. Shimizu, S.E. Wojcik, M.V. Iorio, R. Visone, N.I. Sever and M. Fabbri, 2005. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. New England Journal of Medicine, 353(17): 1793-1801.
21. Iorio, M.V., M. Ferracin, C.G. Liu, A. Veronese, R. Spizzo, S. Sabbioni, E. Magri, M. Pedriali, M. Fabbri and M. Campiglio, 2005. MicroRNA gene expression deregulation in human breast cancer. Cancer Research, 65(16): 7065-7070.
22. Michael, M.Z., S.M. O'Connor, N.G. Van Holst Pellekaan, G.P. Young and R.J. James, 2003. Reduced Accumulation of Specific MicroRNAs in Colorectal Neoplasia11Note: Susan M. O'Connor and Nicholas G. van Holst Pellekaan contributed equally to this work. Molecular Cancer Research, 1(12): 882-891.
23. Bandrés, E., E. Cubedo, X. Agirre, R. Malumbres, R. Zárate, N. Ramirez, A. Abajo, A. Navarro, I. Moreno, M. Monzó and J. García-Foncillas, 2006. Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. Molecular Cancer, 5: 29.
24. Schetter, A.J., S.Y. Leung, J.J. Sohn, K.A. Zanetti, E.D. Bowman, N. Yanaihara, S.T. Yuen, T.L. Chan, D.L. Kwong and G.K. Au, 2008. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA: the journal of the American Medical Association, 299(4): 425-436.

25. Hermanek, P. and R. Hutter, 1997. TNM atlas: illustrated guide to the TNM/pTNM classification of malignant tumours. 1997: Springer Verlag.
26. Sobin, L.H. and I.D. Fleming, 1997. TNM classification of malignant tumors, Cancer, 80(9): 1803-1804.
27. Schmittgen, T.D. and K.J. Livak, 2008. Analyzing real-time PCR data by the comparative CT method. Nature Protocols, 3(6): 1101-1108.
28. Yang, L., N. Belaguli and D.H. Berger, 2009. MicroRNA and colorectal cancer. World Journal of Surgery, 33(4): 638-646.
29. Aslam, M.I., M. Patel, B. Singh, J.S. Jameson and J.H. Pringle, 2012. MicroRNA manipulation in colorectal cancer cells: from laboratory to clinical application. Journal of Translational Medicine, 10(1): 128.
30. Meng, F., R. Henson, M. Lang, H. Wehbe, S. Maheshwari, J.T. Mendell, J. Jiang, T.D. Schmittgen and T. Patel, 2006. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. Gastroenterology, 130(7): 2113.
31. Zhu, S., M.L. Si, H. Wu and Y.Y. Mo, 2007. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). Journal of Biological Chemistry, 282(19): 14328-14336.
32. Silver, S.J., J.W. Hagen, K. Okamura, N. Perrimon and E.C. Lai, 2007. Functional screening identifies miR-315 as a potent activator of Wingless signaling. Proceedings of the National Academy of Sciences, 104(46): 18151-18156.
33. Nagel, R., C. Le Sage, B.A. Diosdado, M. Van der Waal, J.A.O. Vrielink, A. Bolijn, G.A. Meijer and R. Agami, 2008. Regulation of the adenomatous polyposis coli gene by the miR-135 family in colorectal cancer. Cancer Research, 68(14): 5795-5802.