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Upregulation of miR-21 and miR-106b in Plasma and Tissues as a Possible Prognostic Marker in Aggressive Breast Cancer

Andia Seyedi Moghaddam[1], Mahdieh Salimi[2,*], Najmeh Ranji[1] and Hossein Mozdarani[3]

[1]Department of Biology, Faculty of Sciences, Rasht Branch, Islamic Azad University, Rasht, Iran
[2]Department of Medical Genetics, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
[3]Department of Medical Genetics, Tarbiat Modares University, Tehran, Iran

*** *Corresponding author:*** Mahdieh Salimi, Department of Medical Genetics, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran. Tell: +982144787382; Email: salimi@nigeb.ac.ir

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Abstract

Background: The miRNAs are referred to small non-coding RNAs (consisting of 18 to 25 nucleotides). Functional studies have shown their functions to be oncogenes or tumor suppressor genes in different types of cancers. The miR-106b and miR-21 have been identified to participate in the biological behaviors of cells.

Objectives: This study aimed to evaluate the tissue and plasma levels of miR-21 and miR-106b in patients with breast cancer who were diagnosed with ductal carcinoma.

Methods: In total, 40 cases of breast cancer patients 180 samples were examined in this project. Samples included ductal carcinoma breast tumors (n=40), normal breast tissues of the margin of the tumor (n=40) and 20 samples from unaffected mammary tissue of females undergoing reduction mammoplasty (control group), plasma samples of patients with breast cancer (n=40), and plasma of non-affected individuals (n=40). The expression levels of miR-106b and miR-21 were determined using SYBR Green real-time RT-PCR assay in breast tissues and plasma of cancerous patients in comparison to the controls.

Results: MiR-106b and miR-21 revealed much higher expression in tissues and plasma of patients with breast cancer in comparison to that in the group of control (P<0.001). High levels of mir-106b and miR-21 expression in plasma and tumor tissues were highly correlated with tumors in higher stages and lymph node involvement (P<0.0001).

Conclusion: Based on the obtained results, upregulation of miR-106b and miR-21 in the plasma of patients with breast cancer can act as a possible non-invasive biomarker for breast cancer prognosis. Further follow-up studies are required to confirm this.

Keywords: Biomarker, Cancer, miR-21, miR-106b, Prognosis

1. Background

Cancer is a disease that is related to genetics, which may be caused by inherited or acquired mutations (1). Breast cancer is a heterogeneous condition and the most current malignancy among females. It is the major cause of death by cancer in the world. Approximately, 5-10% of breast cancer cases are congenital with obvious molecular subtypes (1). Breast cancer could be categorized according to such factors as cellular receptors, tumor tissue origin, metastasis, tumor stage and grade, morphological features, and gene expression profile. Five main intrinsic subtypes of breast cancer based on cellular receptors include Luminal B, Luminal A, HER2+, basal-like, and normal-like breast cancer (2).

The most studied class classes of non-coding RNAs (ncRNAs) are microRNAs with about 18-25 nucleotides in length, which belong to the classification of small non-coding RNAs (ncRNAs). Various bioinformatics and cloning studies have shown that the human genome may contain more than 1,000 miRNAs and their expression is influenced by intracellular factors and various environmental variables (3) Similar to the genes, they can act as tumor suppressors and oncogenes (3,4). The miRNA genes exist throughout the genome and they constitute about 2–5% of all human genes and after transcription, they regulate approximately 10–30% of the surface protein-coding genes. By partial complementary sequence to the 3'UTR region of the mRNA, they contribute to a diversity of biological processes, including cell proliferation, apoptosis, and hematopoiesis, as well as tumorigenesis and drug resistance (3,4). Upregulation of miRNAs that act as oncogenes helps tumor development by inhibiting tumor suppressor genes (5,6). To date, bioinformatics studies have suggested that a group of miRNAs may serve as a diagnostic and prognostic marker in breast cancer (7).

The encoding gene of miR-21 is located on chromosome 17 (17q23.1) on the plus strand. MiR-21-5p is one of the well-known proto-oncogenes and numerous studies have confirmed its significant effect on the incidence and growth of tumors (8). Based on various studies, miR-21 upregulation can induce cell proliferation, cancer invasion and inhibit apoptosis in several varieties of cancer, including colorectal (CRC) (9), prostate (10), and ovarian cancers (11) via targeting tumor suppressor genes, such as *PTEN*, *PDCD4*, *RECK*, *FOXO1*, *RhoB* as well as *Cdc25a* (6,9,11).

MiR-106b is a member of the miR-106b-25 cluster located on chromosome 7 and its upregulation was

described in various cancer types (12) such as renal cell carcinoma/clear cell renal cell carcinoma (RCC/ccRCC) (13), laryngeal carcinoma (14), CRC (15), glioma (16), hepatocellular carcinoma (17) and cervical cancer (18), although its downregulation was reported in ovarian carcinoma (19) and gastric cancer (20). Thus, miR-106b functions as an oncogene (21). However, some studies have reported its tumor-suppressor function in some cancer types, including papillary thyroid cancer (PTC) (22). Also, it was reported that miR-106b can induce cell proliferation, migration, invasion, angiogenesis, as well as EMT-like changes (21).

The correlation between microRNA expression in tumors and plasma was confirmed in different types of cancer (23-25).

2. Objectives

This study compared the expression levels of miR-21 and miR-106b in breast tumor tissues and plasma samples with control groups. In addition, the association of expression levels of the microRNAs with clinicopathological features was analyzed.

3. Methods

3.1. Patients and samples

Inclusion criteria for breast cancer patients were diagnosis of ductal carcinoma breast cancer, not starting any treatments, and access to the patients' clinic and pathologic information. Also for the normal control group matching age and no history of cancer not in them nor their first-degree family were considered.

In total, 180 samples consist of 80 plasma samples (40 samples from breast cancer patients and 40 samples from unaffected matched individuals as normal control) and 100 breast tissue samples (40 samples from breast tumor tissues, 40 samples from normal adjacent tissues, and 20 samples from unaffected mammary tissue of women who underwent reduction mammoplasty as a control group) were collected from Asia Hospital, Tehran, Iran (2017-2018). This study has been approved by the Ethics Committee of the NIGEB according to the Helsinki Declaration (IR.NIGEB.1397.11.10.E). The informed written consent was obtained from all participants. The clinicopathologic features of patients were obtained after surgery and confirmed by a pathologist. Table 1 presents the demographic characteristics of the patients Whole blood samples were collected in sterile tubes with EDTA from patients before surgery, while fresh tissue samples were collected in cryotubes and transferred to the laboratory in liquid nitrogen. Plasma was separated from blood using 2 step centrifugation (first centrifuged at 3000 rpm at 4 °C for 10 min; then centrifuged at 12000 rpm at 4 °C for 10 min).

3.2. Total RNA extraction

The total RNA (including miRNA) was extracted from breast tumor tissue samples and plasma using Sigma-Aldrich total RNA purification kit (Germany) based on the instructions provided by the manufacturer. Absorbance measurements using a NanoDrop 2000 (Thermo Scientific, USA) were performed to confirm both RNA concentration and purity.

3.3. Reverse Transcription

BonmiR cDNA kits (Bon biotech, Iran) were used

Table 1. Characteristics of breast cancer patients and controls.

	Patient N (%)	Control N (%)
number	40	40
Age (years)		
Mean	45.9±11.6	48.5± 16.4
Range	27-84	25-82
Stage at diagnosis		
Stage I	3 (7.5)	
Stage II	17 (42.5%)	
Stage III	14 (35%)	
Stage IV	6 (15%)	
Lymph node status		
N0	18 (45%)	
N+	22(55%)	
Distance metastasis		
yes	5 [1 bone, 4 lung] (12.5%)	
No	35 (87.5%)	
Hormone receptor status (IHC)		
ER positive	24 (60%)	
ER negative	16 (40%)	
PR positive	21 (52.5%)	
PR negative	19 (47.5%)	
HER-2 status (IHC)		
+++	12 (30%)	
Negative	24 (60%)	
Triple-negative breast cancer	4 (10%)	

for microRNAs reverse transcription. All the steps were performed based on the instructions provided by the manufacturer. The SNORD47 was applied as the internal control.

3.4. miRNA Expression Level Measurement by Real-time RT-PCR

Following the RT step, miRNAs expression levels were measured through SYBR Green real-time RT-PCR. The primer set for expression analysis of microRNAs was designed and synthesized by Stem Cell Technology Company (Tehran, Iran). The ordered set consisted of miR-21 and miR-106b specific forward primer, universal reverse primer, and internal control forward primer (SNORD47). The thermal cycling conditions involved an initial denaturation stage at 95 °C for 2 min as well as 40 cycles at 95 °C for 5 s and 60 °C for 30 s. The amplification of a linear standard curve (from 0.24 to 1,000 ng) of total cDNA measured by ultraviolet spectrophotometer was adopted to determine amplification efficiency for each pair of primer. It was demonstrated that standard curves possess satisfactory linearity and amplification (100%). The expression level was calculated by the $2^{-\Delta\Delta CT}$ method. It is worth mentioning that the data were shown as the fold change in gene expression normalized to an endogenous reference gene and relative to the controls. The 2-fold and higher, between 0.5- and 2-fold, and 0.5-fold and lower expression of RNA were considered as up-regulation, normal regulation, and down-regulation.

3.5. Statistical Analysis

Data were evaluated using GraphPad Prism 8.0.2 (California Corporation, USA). Numerical data were analyzed using the Mann-Whitney U test as well as the Kruskal-Wallis test. Correlation and consistency were analyzed using Spearman correlation analysis. Quantitative data were shown as the mean±SD. A p-value less than 0.05 was considered statistically significant.

4. Results

4.1. Expression of miR-21 and miR-106b in plasma samples and tumor in patients with breast cancer in compared to controls

The findings showed that the expression level of miR-21 in the tumor (4.52±2.42) and plasma (1.99±1.05) samples of breast cancer was higher, compared to the control group (P<0.001) and this difference was significant. The expression level of miR-106b in the breast cancer tumor (3.07±1.1) and plasma (1.85±0.74) was also higher compared to the controls (P<0.001), and the alterations were statistically significant (Figure 1). As shown in Figure 2, the data revealed a positive association between plasma and tumor samples in terms of miR-21 and miR-106b expression (Figure 2).

4.2. The relationship between the expression of miR-21 and miR-106 and clinicopathological parameters of patients with breast cancer

Based on the obtained results, a significant association was observed between the expression levels of miR-21 and miR-106b and lymph node metastasis and higher tumor stages (all P<0.0001; Table 2). However, other clinical factors such as receptor status (HER2, ER, and PR) were not significantly associated with the expression levels of miR-21 and miR-106b (all P>0.05; Table 2). The receiver operating characteristics (ROC) curve analysis based on miR-21 and miR-106b expression in plasma samples and tumors of those with breast cancer focusing on lymph node involvement situations confirmed the association of miR-21 and miR-106b expression and node involvement (Figure 3).

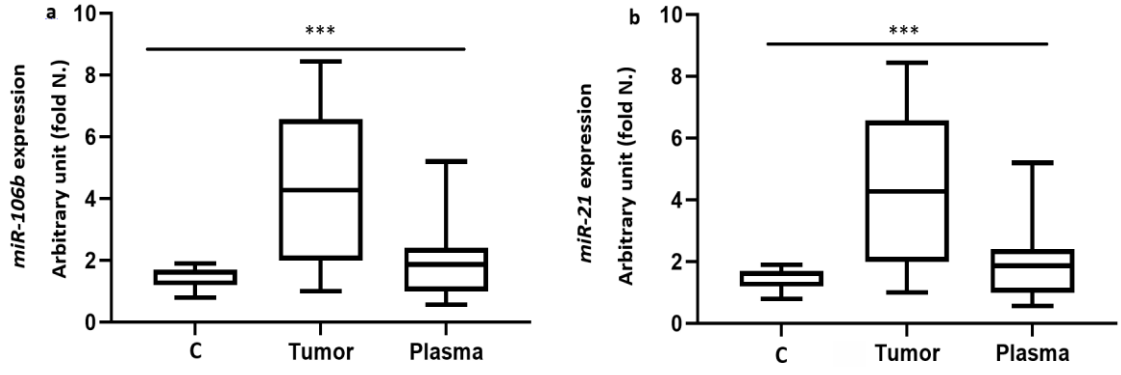

Figure 1. Expression of miR-106b (a) and miR-21(b) in the tumor and plasma samples of those with breast cancer in comparison to the normal control group. Results are presented as a fold number increase versus control. The values were normalized to *SNORD47* RNA previously

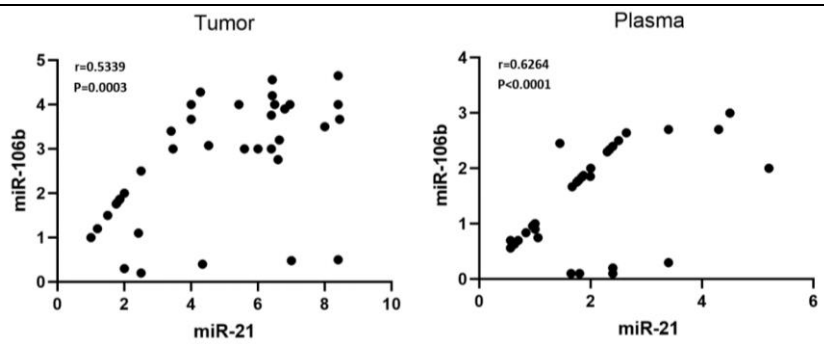

Figure 2. Association between miR-106b and miR-21 in breast cancer tumor and plasma samples. There is a positive correlation between the relative expression based on the results obtained by Spearman's correlation analysis, levels of miR-106b and miR-21 in both plasma samples and tumors of females with breast cancer (r=0.5339, P=0.0003, and r=0.6264, P<0.0001). miR, micro ribonucleic acid.

Table 2. Expression of miR-21 and miR-106-b in different clinicopathological features of BC patients in the research group composed of tumor and plasma samples (mean ± standard deviation).

Parameter	Average expression level of miR-21 in breast tumor tissues	P-value	Average expression level of miR-21 in plasma samples	P-value	Average expression level of miR-106b in breast tumor tissues	P-value	Average expression level of miR-106 in plasma samples	P-value
ER$^+$	5.16 ±2.39	0.16	2.24±1.1	0.19	2.88 ±1.26	0.3858	1.63 ±0.72	0.5579
ER$^-$	4.03±2.18		1.08±0.90		2.50±1.3		1.66±0.97	
PR$^+$	4.9±2.2	0.47	2.11±0.84	0.72	2.93 ±1.27	0.4571	1.67 ±0.72	0.6463
PR$^-$	4.39±2.55		1.98±1.26		2.57 ±1.34		1.65±0.94	
HER2$^+$	4.56± 2.52	0.72	4.56±1.16	0.62	2.42 ±1.37	0.3361	1.63 ±1.02	0.8830
HER2$^-$	4.43±2.18		1.92±1.02		2.86 ±1.25		1.67 ±0.74	
Triple negative	4.23±2.57	0.8324	2.28±1.39	0.34	2.12 ±1.43	0.2695	1.38±0.82	0.4458
Non-triple negative	4.63±2.39		2±0.98		2.75 ±1.32		1.59 ±0.86	
Lymph node +	6.03±1.89	***< 0.0001	2.60±0.95	***<0.0001	3.67 ±0.63	***<0.0001	2.15 ±0.4	***<0.0001
Lymph node	2.67±1.58		1.25±0.59		1.35 ±0.8		0.79 ±0.61	
Stage 1	1.23±0.2		0.9±0.14		0.4 ±0.08		0.16 ±0.09	
Stage 2	2.84±1.51	***< 0.0001	1.32±0.57	***<0.0001	1.78 ±0.83	***<0.0001	1.16 ±0.75	***<0.0001
Stage 3	5.8±1.6		2.55±0.97		3.63 ±0.57		1.98 ±0.46	
Stage 4	7.43±0.96		2.95±0.77		4.20 ±0.31		2.51 ±0.24	

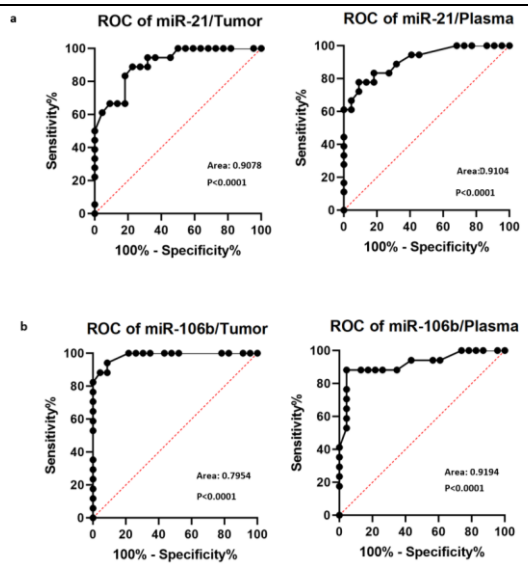

Figure 3. Receiver-operating characteristics plots from the comparison of miR-21 (a) and miR-106b (b) expression in LN+ versus LN – breast cancer in tumor and plasma samples.

5. Discussion

Breast cancer is a heterogeneous disease on the molecular level and is the most prevalent malignancy among females (26). It has been one of the major death causes among female malignancies (approximately 1 in 10) (27). The major reason for the death from breast cancer is late diagnosis. The

miRNAs are a group of small RNAs with various roles in regulating different biological processes through the inhibition of translation; therefore, their expression may be changed in body fluids such as blood, plasma, urine, etc. in a variety of cancers. Numerous miRNAs have been demonstrated to be potential biomarkers that serve to diagnose cancer and other diseases (28,29). In the present study, the data revealed up-regulation of mir-21 and miR-106b in tumors and plasma of females with breast cancer.

It has been revealed that miR-106b is overexpressed in different varieties of cancer, including gastric, RCC, glioma and hepatocellular carcinoma (12), CRC, cervical cancer, laryngeal carcinoma, and pituitary adenoma (29,30). Based on the results of a study conducted by Zhao et al., the upregulation of miR-106b in CRC serum samples from patients has been remarkably associated with distant and lymph node metastasis (15). It was reported that the overexpression of miRNA-106b in glioma tumor samples was significantly associated with tumor grade (16). Yu et al. determined that miR106b was highly expressed in HCC tumors in comparison to adjacent non-neoplastic liver tissues, which was considerably associated with tumor grade (31). Based on the findings in this study, a significant elevation was observed in the miR-106b expression in the plasma samples as well as tumor tissues of patients with breast cancer compared to controls, while a positive association was observed between the upregulation of miR-106b-5p and lymph node involvement and the higher tumor stages. However, no significant correlation was found between cellular receptor status and up-regulation of miR-106b-5p in breast cancer.

Based on previous studies, miR-21 functions as a proto-oncogene, which is upregulated in several solid tumors, such as breast, pancreatic, prostate, and colorectal cancers, and is associated with migration, proliferation, and inhibition of apoptosis via targeting different tumor suppressor genes, including *PTEN*, *PDCD4*, *SPRY*, *RECK*, etc. (32). Si et al. studied the expression of 10 miRs (miR-665, miR-125b, miR-17, miR-106b, miR-21, miR-625, miR-558, miR-185 miR-92a, and miR-93) on serum samples and tissue of patients with breast cancer and compared it with the clinicopathological characteristics. They reported that the level of miR-21 has been remarkably higher in serum samples and tissue of patients with breast cancer compared to the controls. In addition, increased levels of miR-21 have been positively correlated with lymph node status and tumor size ($p<0.001$) (33). Papadaki et al. reported a relationship between the expression levels of miR-21 in metastatic breast cancer plasma as well as disease characteristics and hormone receptor status (34). Zhu et al. assessed the levels of expression of miR-21 in CRC. Based on their results, miR-21-5p was highly overexpressed in CRC tissues and serum exosomes compared with negative controls. Consequently, miR-21 can be considered a prognostic biomarker for the early diagnosis of various types of cancer (30). Our data showed that miR21 expression was highly upregulated in the tumor tissues and plasma samples of those with breast cancer. Moreover, a positive association was found between the upregulation of miR-21-5p and lymph node involvement and tumor stage. Therefore, it seems that upregulation of mir21 can be related to tumor stage and lymph node involvement in Iranian patients. Nevertheless, no association was found between receptor status and upregulation of miR-21-5p in breast cancer.

6. Conclusion

Based on the obtained results in this study, levels of miR-21 and miR-106b expression are remarkably higher in breast tumor tissue and plasma of patients with breast cancer in comparison to controls. Moreover, upregulation of these miRNAs was related to clinical stage and lymph node metastasis of those with breast cancer, while there were was no correlation with cellular receptor status statuses. Based on the obtained results, upregulation of miR-21 and miR-106b in plasma of females with breast cancer may be used as a possible non-invasive biomarker for breast cancer prognosis. Further follow-up studies are required to confirm this.

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Footnotes

Conflicts of Interest: The authors assert that they have no potential conflicts of interest regarding the publication of this study.
Funding: None
Ethical approval: In this study procedures involving human participants were conducted based on the 1964 Helsinki declaration and further amendments.
Informed consent: Informed consent was taken from each participant at the beginning of the study.

References

1. Savad S, Mehdipour P, Miryounesi M, Shirkoohi R, Fereidooni F, Mansouri F, et al. Expression analysis of MiR-21, MiR-205, and MiR-342 in breast cancer in Iran. *Asian Pac J Cancer Prev*. 2012;**13**(3):873-7. doi: 10.7314/apjcp.2012.13.3.873. [PubMed: 22631664].
2. Gray JM, Rasanayagam S, Engel C, Rizzo J. State of the evidence 2017: an update on the connection between breast cancer and the environment. *Environ Health*. 2017;**16**(1):94. doi: 10.1186/s12940-017-0287-4. [PubMed: 28865460].

3. Paranjape T, Slack FJ, Weidhaas JB. MicroRNAs: tools for cancer diagnostics. *Gut*. 2009;**58**(11):1546-54. doi: 10.1136/gut.2009.179531. [PubMed: 19834118].
4. Li J, Zhang Z, Chen F, Hu T, Peng W, Gu Q, et al. The diverse oncogenic and tumor suppressor roles of microRNA-105 in cancer. *Front Oncol*. 2019;**9**:518. doi: 10.3389/fonc.2019.00518. [PubMed: 31281797].
5. Palmero EI, de Campos SG, Campos M, de Souza NC, Guerreiro ID, Carvalho AL, et al. Mechanisms and role of microRNA deregulation in cancer onset and progression. *Genet Mol Biol*. 2011;**34**(3):363-70. doi: 10.1590/S1415-47572011000300001. [PubMed: 21931505].
6. Wang W, Luo YP. MicroRNAs in breast cancer: oncogene and tumor suppressors with clinical potential. *J Zhejiang Univ Sci B*. 2015;**16**(1):18-31. doi: 10.1631/jzus.B1400184. [PubMed: 25559952].
7. Adhami M, Haghdoost AA, Sadeghi B, Malekpour Afshar R. Candidate miRNAs in human breast cancer biomarkers: a systematic review. *Breast Cancer*. 2018;**25**(2):198-205. doi: 10.1007/s12282-017-0814-8. [PubMed: 29101635].
8. Karimi Kurdistani Z, Saberi S, Tsai KW, Mohammadi M. MicroRNA-21: mechanisms of oncogenesis and its application in diagnosis and prognosis of gastric cancer. *Arch Iran Med.* 2015;**18**(8):524-36. [PubMed: 26265521].
9. Wu Y, Song Y, Xiong Y, Wang X, Xu K, Han B, et al. MicroRNA-21 (Mir-21) promotes cell growth and invasion by repressing tumor suppressor PTEN in colorectal cancer. *Cell Physiol Biochem*. 2017;**43**(3):945-58. doi: 10.1159/000481648. [PubMed: 28957811].
10. Leite KR, Reis ST, Viana N, Morais DR, Moura CM, Silva IA, et al. Controlling RECK miR21 promotes tumor cell invasion and is related to biochemical recurrence in prostate cancer. *J Cancer*. 2015;**6**(3):292-301. doi: 10.7150/jca.11038. [PubMed: 25663948].
11. Lou Y, Yang X, Wang F, Cui Z, Huang Y. MicroRNA-21 promotes the cell proliferation, invasion and migration abilities in ovarian epithelial carcinomas through inhibiting the expression of PTEN protein. *Int J Mol Med*. 2010;**26**(6):819-27. doi: 10.3892/ijmm_00000530. [PubMed: 21042775].
12. Yang C, Dou R, Yin T, Ding J. MiRNA-106b-5p in human cancers: diverse functions and promising biomarker. *Biomed Pharmacother*. 2020;**127**:110211. doi: 10.1016/j.biopha.2020.110211. [PubMed: 32422566].
13. Liu K, Pan X, Peng X, Zhang C, Li H, Guan X, et al. Associations of high expression of miR-106b-5p detected from FFPE sample with poor prognosis of RCC patients. *Pathol Res Pract*. 2019;**215**(6):152391. doi: 10.1016/j.prp.2019.03.019. [PubMed: 31076282].
14. Cai K, Wang Y, Bao X. MiR-106b promotes cell proliferation via targeting RB in laryngeal carcinoma. *J Exp Clin Cancer Res*. 2011;**30**(1):73. doi: 10.1186/1756-9966-30-73. [PubMed: 21819631].
15. Zhao Y, Ren Q, Zhu K. Serum miR-106b upregulation predicts poor prognosis in patients with colorectal cancer. *Int J Clin Exp Pathol*. 2018;**11**(8):4197-204. [PubMed: 31949814].
16. Liu F, Gong J, Huang W, Wang Z, Wang M, Yang J, et al. MicroRNA-106b-5p boosts glioma tumorigensis by targeting multiple tumor suppressor genes. *Oncogene*. 2014;**33**(40):4813-22. doi: 10.1038/onc.2013.428. [PubMed: 24166509].
17. Yau WL, Lam CS, Ng L, Chow AK, Chan ST, Chan JY, et al. Over-expression of miR-106b promotes cell migration and metastasis in hepatocellular carcinoma by activating epithelial-mesenchymal transition process. *PLoS One*. 2013;**8**(3):e57882. doi: 10.1371/journal.pone.0057882. [PubMed: 23483935].
18. Cheng Y, Guo Y, Zhang Y, You K, Li Z, Geng L. MicroRNA-106b is involved in transforming growth factor β1-induced cell migration by targeting disabled homolog 2 in cervical carcinoma. *J Exp Clin Cancer Res*. 2016;**35**:11. doi: 10.1186/s13046-016-0290-6. [PubMed: 26769181].
19. Chen S, Chen X, Xiu YL, Sun KX, Zhao Y. Inhibition of ovarian epithelial carcinoma tumorigenesis and progression by microRNA 106b mediated through the RhoC pathway. *PLoS One*. 2015;**10**(5):e0125714. doi: 10.1371/journal.pone.0125714. [PubMed: 25933027].
20. Zeng Q, Jin C, Chen W, Xia F, Wang Q, Fan F, et al. Downregulation of serum miR-17 and miR-106b levels in gastric cancer and benign gastric diseases. *Chin J Cancer Res*. 2014;**26**(6):711-6. doi: 10.3978/j.issn.1000-9604.2014.12.03. [PubMed: 25561770].
21. Yang C, Dou R, Yin T, Ding J. MiRNA-106b-5p in human cancers: diverse functions and promising biomarker. *Biomed Pharmacother*. 2020;**127**:110211. doi: 10.1016/j.biopha.2020.110211. [PubMed: 32422566].
22. Sun W, Lan X, Zhang H, Wang Z, Dong W, He L, et al. NEAT1_2 functions as a competing endogenous RNA to regulate ATAD2 expression by sponging microRNA-106b-5p in papillary thyroid cancer. *Cell Death Dis*. 2018;**9**(3):380. doi: 10.1038/s41419-018-0418-z. [PubMed: 29515109].
23. Badr FM. Potential role of miR-21 in breast cancer diagnosis and therapy. *JSM Biotechnol Biomed Eng*. 2016;**3**(5):1068.
24. Fesler A, Jiang J, Zhai H, Ju J. Circulating microRNA testing for the early diagnosis and follow-up of colorectal cancer patients. *Mol Diagn Ther*. 2014;**18**(3):303-8. doi: 10.1007/s40291-014-0089-0. [PubMed: 24566942].
25. Filipow S, Laczmanski L. Blood circulating miRNAs as cancer biomarkers for diagnosis and surgical treatment response. *Front Genet*. 2019;**10**:169. doi: 10.3389/fgene.2019.00169. [PubMed: 30915102].
26. Testa U, Castelli G, Pelosi E. Breast cancer: a molecularly heterogenous disease needing subtype-specific treatments. *Med Sci (Basel)*. 2020;**8**(1):18. doi: 10.3390/medsci8010018. [PubMed: 32210163].
27. Mehrgou A, Akouchekian M. The importance of BRCA1 and BRCA2 genes mutations in breast cancer development. *Med J Islam Repub Iran*. 2016;**30**:369. [PubMed: 27493913].
28. Kreth S, Hübner M, Hinske LC. MicroRNAs as clinical biomarkers and therapeutic tools in perioperative medicine. *Anesth Analg*. 2018;**126**(2):670-81. doi: 10.1213/ANE.0000000000002444. [PubMed: 28922229].
29. Shi BM, Lu W, Ji K, Wang YF, Xiao S, Wang XY. Study on the value of serum miR-106b for the early diagnosis of hepatocellular carcinoma. *World J Gastroenterol*. 2017;**23**(20):3713-20. doi: 10.3748/wjg.v23.i20.3713. [PubMed: 28611524].
30. Zhu M, Huang Z, Zhu D, Zhou X, Shan X, Qi L, et al. A panel of microRNA signature in serum for colorectal cancer diagnosis. *Oncotarget*. 2017;**8**(10):17081-91. doi: 10.18632/oncotarget.15059. [PubMed: 28177881].
31. Yu LA, Zhang BL, Yang MY, Liu H, Xiao CH, Zhang SG, et al. MicroRNA106b-5p promotes hepatocellular carcinoma development via modulating FOG2. *Onco Targets Ther*. 2019;**12**:5639-47. doi: 10.2147/OTT.S203382. [PubMed: 31406464].
32. Sánchez DB, Canon CA, Torres AP, Velázquez AR, Barrios RG, Espinosa LC, et al. The Promising Role of miR-21 as a cancer biomarker and its importance in RNA-based therapeutics. *Mol Ther Nucleic Acids*. 2020;**20**:409-20. doi: 10.1016/j.omtn.2020.03.003. [PubMed: 32244168].
33. Si H, Sun X, Chen Y, Cao Y, Chen S, Wang H, et al. Circulating microRNA-92a and microRNA-21 as novel minimally invasive biomarkers for primary breast cancer. *J Cancer Res Clin Oncol*. 2013;**139**(2):223-9. doi: 10.1007/s00432-012-1315-y. [PubMed: 23052693].
34. Papadaki C, Stoupis G, Tsalikis L, Monastirioti A, Papadaki M, Maliotis N, et al. Circulating miRNAs as a marker of metastatic disease and prognostic factor in metastatic breast cancer. *Oncotarget*. 2019;**10**(9):966-81. doi: 10.18632/oncotarget.26629. [PubMed: 30847025].