® Postepy Hig Med Dosw (online), 2019; 73: 325–331
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 10.10.2018
Accepted: 01.04.2019
Published: 25.06.2019

Expression level of serum circulating miRNA-21, miRNA-10b and miRNA-200c in breast cancer patients with sentinel lymph node metastasis*

Poziom krążących w surowicy miRNA-21, miRNA-10b i miRNA-200c u chorych z rakiem piersi i przerzutem do węzła wartowniczego

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Sebastian Niedźwiecki[1 A B C D E F G], **Janusz Piekarski**[1 B D E], **Bożena Szymańska**[2 B D E], **Zofia Pawłowska**[2 B D E], **Arkadiusz Jeziorski**[1 B D E]

[1]Department of Surgical Oncology, Medical University of Lodz, Poland
[2]Central Scientific Laboratory, Medical University of Lodz, Poland

Summary

Aim: MicroRNAs (miRNAs) act a role in regulation numerous processes crucial for oncogenesis. The aim of the study was to compare the blood serum concentrations of selected microRNAs (miRNA-21, miRNA-10b and miRNA-200c) between breast cancer patients without sentinel lymph node metastasis (Group 1) and those with metastasis (Group 2).

Material/Methods: The serum levels of miRNA-21, miRNA-10b and miRNA-200c were measured with using TaqMan PCR assays performed on a 7900HT Fast Real-Time PCR System in two groups of breast cancer patients: Group 1 – without sentinel lymph node metastasis (32 patients) and Group 2 – with sentinel lymph node metastasis (14 patients).

Results: The mean level of miRNA-200c was noticeably lower in Group 2 than in Group 1. The mean fold change of miRNA-200c level in the metastatic group (Group 2) was approximately 1.3 times lower than that in non-metastatic group (Group 1). However, this result just approached the arbitrary threshold for significance (p = 0.05).

Conclusions: Breast cancer patients with sentinel lymph node metastasis demonstrate diminished levels of circulating miR-200c compared to non-metastatic patients.

Keywords: **miRNA •breast cancer •lymph nodes •metastasis**

GICID 01.3001.0013.2562
DOI: 10.5604/01.3001.0013.2562
Word count: 2699
Tables: 1
Figures: 4
References: 42

*This work was supported by the Medical University of Lodz, Grant No. 502-03/1-34-01/502-14-122.

Author's address: Sebastian Niedźwiecki MD, PhD, Department of Surgical Oncology, Medical University of Lodz, ul. Paderewskiego 4, 93-509 Lodz, Poland; e-mail: sebastian.niedzwiecki@umed.lodz.pl

INTRODUCTION

The presence of axillary lymph node metastasis is one of most important prognostic factors for patients with breast cancer, and axillary lymph node examination is one of the stages in breast cancer diagnosis. The confirmation or exclusion of metastatic lymph nodes is typically based on imaging methods (ultrasonography and/or mammography) correlated with results of biopsy, surgical sentinel lymph node biopsy and finally, histopathological diagnosis of axillary specimens. However, the most reliable approach for identifying metastatic axillary lymph nodes is sentinel lymph node biopsy, which ensures good locoregional control of the disease and saves approximately 60–70% of breast cancer patients, there is the need for axillary lymph node dissection.

Axillary lymph node metastasis is a risk factor for recurrence and poor prognosis. Preoperative or postoperative detection of metastatic axillary lymph nodes affects the choice of the therapeutic procedure, including surgical procedures, neo- and adjuvant chemotherapy and/or radiotherapy, depending on the number of positive lymph nodes and/or presence of isolated tumor cells or micrometastasis. Therefore, axillary lymph nodes should be diagnosed prior to any therapeutic decision.

On the cellular level, the dissemination of cancer cells is regulated by two processes called epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET) [29]. EMT is a mechanism by which epithelial cancer cells change to cells with the ability to invade other tissues and migrate to new areas. After reaching the target organ, the cancer cells undergo the reverse process (MET), allowing metastasis to begin [26]. Moreover, both phenomena are connected with changes in cell structures such as tight junctions and adherens junctions, the loss of E-cadherin and cell polarity, and the expression of mesenchymal markers. These processes are strictly regulated by a range of factors, of which microRNAs (miRNAs) seem to play a crucial role [5]. miRNAs are small, non-coding RNAs molecules, ranging from 18-21 nucleotides, which post-transcriptionally modify gene expression [3]. They play a role in the regulation of numerous processes crucial for oncogenesis, such as proliferation, differentiation and apoptosis. Dysregulated miRNA expression was found in various types of cancer tissue in at least several studies [4, 11]. The studies suggest that miRNAs may play a role in the development of metastasis by influencing EMT-activated signaling pathways and EMT-Transcriptional Factors [16]. Therefore, the use of miRNA as a clinical marker for malignancy has been already proposed [38].

Of the hundreds of miRNAs described in humans, miRNA-21, miRNA-10b and miRNA-200c seem to play the greatest role in the development of breast cancer. miRNA-21 is believed to be one of the most significantly changed miRNAs, usually up-regulated, in breast cancer tissue. The expression of miRNA-21 in breast cancer tissue correlates with lymph node metastasis, tumor diameter and poor prognosis [6, 11, 38].

As noted in *in vitro* studies (metastatic cell lines MDA-MB-231 and SUM1315), miRNA-10b was the first miRNA found to be highly expressed in metastatic breast cancer cells, and is believed to be an initial factor in the process of metastasis [17]. Many studies have reported increased miRNA-10b expression in metastatic breast cancer tissue compared to non-metastatic breast cancer tissue [18].

The third selected microRNA, miRNA-200c, is thought to act by the decreasing activation of ZEB1, an EMT-inducing transcription factor which suppresses cancer cell migration [10]. It has been demonstrated that miRNA-200c is down-regulated in breast cancer tissue, leading to cell migration and the creation of remote metastasis [15]. It was proposed that these above-mentioned miRNAs, acting on different levels on cancerogenesis, might serve as biomarkers of lymph node metastasis in breast cancer patients. The aim of the pilot and initial study was to compare blood serum expression level of selected miRNAs (miRNA-21, miRNA-10b and miRNA-200c) between breast cancer patients without sentinel lymph node metastasis (Group 1) and those with the metastasis (Group 2).

MATERIAL AND METHODS

Forty-six female patients with breast cancer were included into the prospective study. All the patients were operated in the Department of Surgical Oncology, Medical University of Lodz (Poland) in a period of March to December, 2014. The age of the patients ranged from 38 to 72 years (mean age 56.44 years). The patients were diagnosed by core-needle biopsy, and by imaging methods including mammography and ultrasonography. No patients had palpable axillary lymph nodes. Likewise, no symptoms of metastatic axillary lymph nodes were found in imaging studies performed in both study groups. All patients had indications for primary surgical treatment of breast cancer. None of the patients had received any treatment before admission to the department.

The final, postoperative histopathological diagnosis was invasive ductal breast cancer in all patients. The number of patients without sentinel lymph node metastasis was 32 (Group 1) and the number of patients with sentinel lymph node metastasis was 14 (Group 2). The character-

ization and distribution of patient's features in the two groups of breast cancer patients are presented in Table 1.

Blood samples in volume of 5ml of blood were collected from the antecubital vein one day before surgery. The samples were centrifuged and the serum was stored at -80°C.

The project was approved by the Ethical Committee for Scientific Studies at Medical University of Lodz.

MIRNA ISOLATION AND MEASURING

Isolation of miRNAs

Total RNA including circulating miRNA was isolated from blood serum using miRNeasy Mini Kit (Qiagen). Briefly, 1 ml of QIAzol Lysis Reagent was added to 0.2 ml of serum, mixed by vigorous shaking for 10 s and incubated for five minutes at RT to ensure complete dissociation of nucleoprotein complexes. All samples were supplemented with 5 pg Caenorhabditis elegans synthetic miRNA-39 (cel-miRNA-39) used for the normalization of RNA preparation. Aqueous and organic phase separation was achieved by adding 200 µl of chloroform. The mixture was vigorously shaken for 15 s, incubated for 3 min at RT and centrifuged at 14 000 g for 15 min at 40C. Total RNA was precipitated from the upper (aqueous) phase by adding 1.5 volumes of 100% ethanol. Purification of extracted total RNA was performed with miRNeasy columns (Qiagen) according to the manufacturer's instructions. RNA was eluted from the Qiagen columns in 30 µl of RNase-free water.

miRNA quantification

Reverse transcription was performed using the TaqMan miRNA Reverse Transcription Kit and miRNA-specific stem-loop primers (Applied Biosystems) according to

Table 1. Clinicopathological characteristics of patients without sentinel lymph node meta-stasis (group 1) and with sentinel lymph node metastasis (group 2). In brackets are shown % of total number of patients in group

	Group 1	Group 2	p Value
Mean age (years)	55.34	57.54	0.362
Menopausal status			0.377
premenopause	3 (9.37%)	1 (7.14%)	
postmenopause	29 (90.6%)	13 (92.85%)	
Diabetes	7 (21.87%)	9 (64.28%)	p <0.05
Ischaemic heart disease	9 (28.12%)	4 (28.57%)	p = 0.365
Hypertension	7 (21.87%)	3 (21.42%)	p = 0.383
Tumor			
T1	12 (37.5%)	5 (35.71%)	p = 0.453
T2	20 (62.5%)	9 (64.28%)	p = 0.476
T3	0	0	
T4	0	0	
Grading			
G1	14 (43.75%)	5 (35.71%)	p = 0.4.32
G2	12 (37.5%)	5 (35.71%)	p = 0.315
G3	6 (18.75%)	4 (28.57%)	p = 0.254
ER			p = 0.389
positive	26 (81.25%)	11 (78.57%)	
negative	6 (18.75%)	3 (21.42%)	
PR			p = 0.311
positive	26 (81.25%)	11 (78.57%)	
negative	6 (18.75%)	3 (21.42%)	
HER2			p = 0.399
positive	26 (81.25%)	11 (78.57%)	
negative	6 (18.75%)	3 (21.42%)	

the manufacturer's protocol. Real-time PCR reactions were carried out with the use of 5 µl of total RNA eluate using and standard TaqMan® MicroRNA Assays (Applied Biosystems): hsa-miR-10b (Assay ID 002218), hsa-miR-21 (Assay ID 000397), hsa-miR-200c (Assay ID 002300) and cel-miR-39 (Assay ID: 000200) as a control. The reactions were run in duplicate, in volume of 20 µl using 10 µl TaqMan Universal PCR Master Mix, 1µl miRNA-specific primer/probe mix and 1.33 µl RT product. The reactions were incubated in a 96-well plate at 95^0C for 10 min, followed by 40 cycles of 95^0C for 15 s and 60^0C for 1 min.

Real-Time PCR analysis

TaqMan PCR assays were performed with the use of a 7900HT Fast Real-Time PCR System (Applied Biosystems) and analyzed using Sequence Detection System 2.3 Software. The automatic cycle threshold (Ct) setting was used for assigning baseline and threshold for Ct determination.

STATISTICAL ANALYSIS

Statistica version 10.0 software was used for the statistical analysis of data. The results were presented as mean values. The Mann-Whitney test, Kruskal-Wallis test and Pearson's linear correlation were used for the correction analysis. The normal distribution of the data was confirmed using the Kolmogorow–Smirnow test with the Lilliefors test. The values were statistically significant at $p < 0.05$.

RESULTS

No significant differences were found between the two groups with regard to age ($p = 0.362$), BMI ($p = 0.353$) or menopausal status ($p = 0.377$). Similarly, no significant differences were found with regard to the level of estrogen ($p = 0.389$), progesterone ($p = 0.311$) or Her2 receptors ($p = 0.399$). No significant link was found between miRNA level and tumor diameter. Although the two groups did not differ significantly with regard to the incidence of hypertension ($p = 0.383$) or ischaemic heart disease ($p = 0.365$), diabetes was significantly more common in Group 2 ($p < 0.05$).

The miRNA validation revealed mean level of miRNA-21 to be 26,557 ng/µL in Group 1 and (24.644) in Group 2 (Fig. 1), which was not significantly different ($p = 0.224$). The mean levels of miRNA-10b were (33.768) in Group 1 and (32,133) in Group 2 (Fig. 2), which was not significantly different ($p = 0.276$). However, the mean expression level of miRNA-200c was (38.783) in Group 1 and (28.764) in Group 2 (Fig. 3), a concentration approximately 1.3-fold lower in the metastatic group (Group 2) than in the non-metastatic group (Group 1). However, the p-value for this relationship was 0.05, placing the result on the borderline of statistical significance.

Due to more frequent occurrence (significant statistically) of diabetes in Group 2, the correlation between the presence of diabetes and miRNA level was analyzed. The comparison of miRNAs level between diabetic and non-diabetic patients with metastasis revealed only a difference in the level of miRNA-200c: 27.872 in diabetic patients versus 33.456 in nondiabetic patients (Fig. 4), again with a borderline significance ($p = 0.05$). No statistically significant difference was found between the subgroups of diabetic and non-diabetic patients with metastasis with regard to miRNA-21 or miRNA-10b level ($p = 0.348$).

DISCUSSION

Data about the serum level of miRNA-21 in breast cancer patients is rather limited. A meta-analysis of results from 36 studies reported that the specificity and sensitivity of breast cancer detection is 70–90% and 75–90% respectively [22]. Asaga et al. reported a correlation between serum miRNA-21 concentration and visceral and lymph node metastasis in breast cancer, but only for stage IV of breast cancer according to AJCC [41]. A preliminary study performed by Chinese authors revealed significantly higher serum levels of miRNA-21 than miRNA-153 and CEA in breast cancer patients. The authors postulate the use of miRNA-21 as a biomarker of early stage of breast cancer [28]. Other studies indicate the possibility of using miRNA-21 to determine the stage of breast cancer or for early detection of breast cancer [29, 41].

While Volina et al. evaluated miRNA-21 level in 61 serum

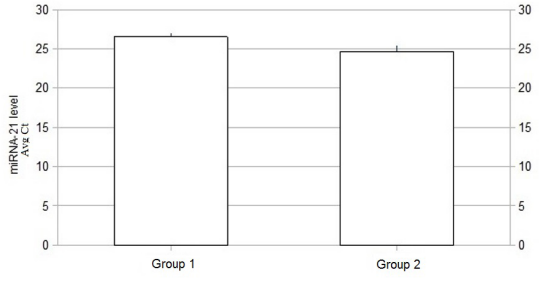

Fig. 1. Expression of miRNA-21 (mean ± standard deviation) in serum of breast cancer patients; (p=0.224)

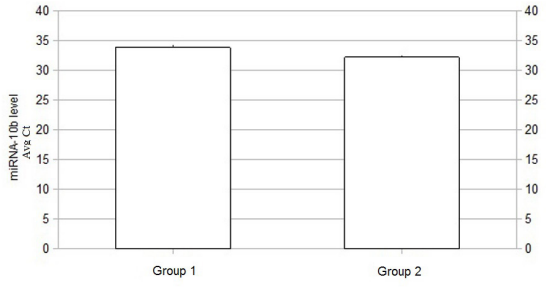

Fig. 2. Expression of miRNA-10b (mean ± standard deviation) in serum of breast cancer patients; (p=0.276)

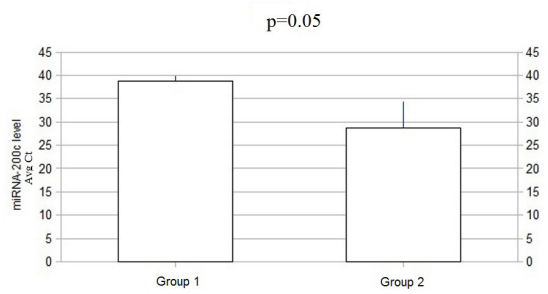

Fig. 3. Expression of miRNA-200c (mean ± standard deviation) in serum of breast cancer patients

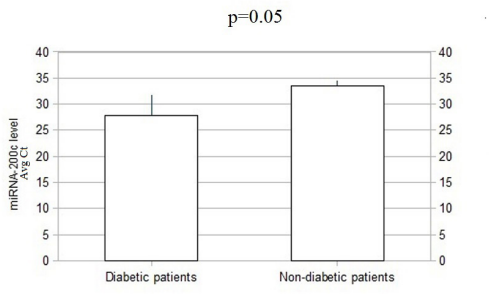

Fig. 4. miRNA-200c serum level among diabetic and non-diabetic patients with sentinel lymph node metastasis

samples from breast cancer patients and healthy controls and demonstrated no significant relationship between miRNA-21level and the presence of breast cancer [34]. However, Enders et al. found that the level of miRNA-21 in a breast cancer patient was decreased after surgery, suggesting a possible relationship between breast cancer and miRNA-21 level [27].

Our findings do not confirm any significant difference in miRNA-21 level between patients with sentinel lymph node metastasis and those without. However, miRNA-21 was suggested as a biomarker for the detection of breast malignancy by other authors. Enders et al. found that the serum concentration of miRNA-21 varied significantly between different groups of cancer patients only in the high stage of the disease (IV according AJCC). The lack of significant results found by us can be caused by the fact that all subjects included into the presented study were at a lower stage of breast cancer (maximum II according AJCC).

The miRNA-10b is widely described as a biomarker of metastasis in breast cancer. It stimulates the prometastatic *RhoC* gene. miR-10b can induce the transformation of cancer cells into metastatic cells [18]. It can be responsible for bone metastasis [19]. Furthermore, high miRNA-10b blood concentration may indicate the presence of metastasis after the surgical procedure [16].

However, Ioro et al. reported a down-regulated expression of miRNA-10b in breast cancer tissue compared to healthy controls [11]. Other studies have reported the under-expression of miRNA-10b in metastatic and non-metastatic lymph node in breast cancer patients, suggesting that miRNA-10b is not an important factor in the metastatic process [8, 25]. Although miRNA-10b is typically under-expressed in breast cancer, miRNA-10b is up-regulated in 50% of metastatic breast cancer tissue cases [20]. The sensitivity and specificity for miRNA-10b as a biomarker of nodal metastasis were found to be 88.9% and 80%, respectively [35]. *In vitro* studies show that inhibition of miRNA-10b expression reduces the possibility of invading and migrating breast cancer cells [14, 17]. Our findings revealed no difference in miRNA-10b level between patients with sentinel lymph node metastasis and without metastasis. This outcome is convergent with a small number of studies reporting that miRNA-10b is rather not an important factor in the metastatic process.

Finally, miRNA-200c is a member of the miRNA-200 family, also consisting of miRNA-200a, miRNA-200b, miRNA-141, and miRNA-429 [2]. It regulates one of the processes in the metastatic spread of breast cancer by inhibiting EMT [36]. Gravgaard et al. reported higher miRNA-200c expression in metastasic tumor tissue compared to primary tumor tissue [9]. The molecular mechanism of miRNA-200c has been widely described with the use of *in vitro* models. It is known that the miR--200c expression alters the features of LY2 cells surface leading to a decrease in cell migration [30].

Some authors have already reported that miRNA-200c has the ability to suppress the oncogenesis of breast cancer stem cells *in vivo* [15]. Berber et al. found that down--regulation of miR-200c was associated with the triple negative breast cancers. The authors suggest the sensitivity and specificity of miR-200c for the prediction of metastatic triple negative breast cancer to be 87.5% and 81.3%, respectively [21]. Results of our study revealed lower serum levels of miRNA-200c in patients with metastatic sentinel lymph nodes. Unfortunately, it was not possible to find a similar study to compare our observation.

Dysregulation of miRNA expression has also been reported in patients suffering from diabetes. In particular, miRNA-10b expression was reported to be decreased in patients with diabetic complications [31]. Dysregulation of miRNA-10b expression in metastatic breast cancer cells was found in an experimental mouse model based on a combination of mouse and human cells [18]. Diabetes with obesity may be also the reason for dysregulated miRNA-21 expression [24]. Furthermore, miRNA-21 plays a role in adipocyte metabolism and has been proposed as a new therapeutic target for diabetes [21]. The miRNA-21 serum levels have been reported to be down--regulated in patients with type 2 diabetes [42]. In our

study only miRNA-200c serum level was dysregulated in diabetic patients compared to non-diabetic patients. It is possible that the metabolic changes caused by diabetes may have an impact on some miRNA serum levels. However, further studies on much larger groups of subjects are required to confirm this result.

In the era of targeted therapy which is accurately customized to the stage of the breast cancer, axillary lymph node metastasis are still an important factor influencing therapeutic methods and prognosis.

Many authors have identified miRNA-21 overexpression in breast cancer tissue, suggesting its crucial role in the development of breast cancer [11, 37]. Therefore, miRNA-21 is considered as a potential diagnostic marker [1]. The up-regulation of miRNA-21 has been proposed as an indicator of greater tumor aggressiveness connected with poorer prognosis [7]. Some studies have demonstrated that high tissue levels of miRNA-21 were correlated significantly with the advanced stage and the presence of lymph node metastasis in patients with breast cancer.

It is possible that miRNA-21 overexpression is acquired during metastatic spread [19]. Zhu et al. suggest that miRNA-21 modulates the expression of multiple metastasis suppressor genes. Overexpressed miRNA-21 can decrease the expression of the genes regulating the cell proliferation [23]. However, the exact molecular mechanism of miR-21 expression alterations in breast cancer tissue remains unclear.

The relationship between circulating miRNAs and human metabolism is not explored enough. Some studies suggest the possibility of miRNA use as biomarkers in the screening and follow-up of cancer patients. The possible source of circulating miRNAs can be either the products of cell disintegration or damage; however, the products of cell secretion may also play a role [33]. As miRNAs are involved in a network of metabolic and oncological disorders, which may influence their serum level, further studies on larger groups of selected patients are needed to confirm the role of circulating miRNAs and the influence of various metabolic factors on their serum level.

CONCLUSIONS

Breast cancer patients with sentinel lymph node metastasis demonstrate lower levels of miR-200c from non-metastatic patients. Type 2 diabetes could be a factor influencing miRNA serum levels. Our research is pilot and initial. Further research on a larger group of patients is needed to elucidate the role of miRNAs in the creation of metastases in breast cancer.

REFERENCES

[1] Asaga S., Kuo C., Nguyen T., Terpenning M., Giuliano A.E., Hoon D.S.: Direct serum assay for microRNA-21 concentrations in early and advanced breast cancer. Clin. Chem., 2011; 57(1): 84–91

[2] Berber U., Yilmaz I., Narli G., Haholu A., Kucukodaci Z., Demirel D.: miR-205 and miR-200c: Predictive micro RNAs for lymph node metastasis in triple negative breast cancer. J. Breast Cancer, 2014; 17(2): 143–8

[3] Caldas C., Brenton J.D.: Sizing up miRNAs as cancer genes. Nat. Med., 2005; 11(7): 712–4

[4] Croce C.M., Calin G.A.: miRNAs, cancer, and stem cell division. Cell, 2005; 122(1): 6–7

[5] Díaz-López A., Moreno-Bueno G., Cano A.: Role of microRNA in epithelial to mesenchymal transition and metastasis and clinical perspectives. Cancer Manag. Res., 2014: 6: 205–16

[6] Fang H., Xie J., Zhang M., Zhao Z., Wan Y., Yao Y.: miRNA-21 promotes proliferation and invasion of triple-negative breast cancer cells through targeting PTEN. Am. J. Transl. Res., 2017; 9(3): 953–61

[7] Gao J., Zhang Q., Xu J., Guo L., Li X.: Clinical significance of serum miR-21 in breast cancer compared with CA153 and CEA. Chin. J. Cancer Res., 2013; 25(6): 743–8

[8] Gravgaard K.H., Lyng M.B., Laenkholm A.V., Søkilde R., Nielsen B.S., Litman T., Ditzel H.J.: The miRNA-200 family and miRNA-9 exhibit differential expression in primary versus corresponding metastatic tissue in breast cancer. Breast Cancer Res. Treat., 2012; 134(1): 207–17

[9] Herrera B.M., Lockstone H.E., Taylor J.M., Ria M., Barrett A., Collins S., Kaisaki P., Argoud K., Fernandez C., Travers M.E., Grew J.P., Randall J.C., Gloyn A.L., Gauguier D., McCarthy M.I. et al.: Global microRNA expression profiles in insulin target tissues in a spontaneous rat model of type 2 diabetes. Diabetologia, 2010; 53(6):1099–1109

[10] Hurteau G.J., Carlson J.A., Spivack S.D., Brock G.J.: Overexpression of the microRNA hsa-miR-200c leads to reduced expression of transcription factor 8 and increased expression of E-cadherin. Cancer Res., 2007: 67(17): 7972–6

[11] Iorio M.V., Ferracin M., Liu C.G., Veronese A., Spizzo R., Sabbioni S., Magri E., Pedriali M., Fabbri M., Campiglio M., Ménard S., Palazzo J.P., Rosenberg A., Musiani P., Volinia S. et al.: MicroRNA gene expression deregulation in human breast cancer. Cancer Res., 2005: 65(16): 7065–70

[12] Korpal M., Lee E.S., Hu G., Kang Y.: The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors *ZEB1* and *ZEB2*. J. Biol. Chem., 2008; 283(22): 14910–14

[13] Kozomara A., Griffiths-Jones S.: miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res., 2011; 39: D152–D157

[14] Liang A.L., Zhang T.T., Zhou N., Wu C.Y., Lin M.H., Liu Y.J.: MiRNA-10b sponge: An anti-breast cancer study in vitro. Oncol. Rep., 2016; 35(4): 1950–8

[15] Ling H.Y., Hu B., Hu X.B., Zhong J., Feng S.D., Qin L., Liu G., Wen G.B., Liao D.F.: MiRNA-21 reverses high glucose and high insulin induced insulin resistance in 3T3-L1 adipocytes through targeting phosphatase and tensin homologue. Exp. Clin. Endocrinol. Diabetes, 2012: 120(9): 553–9

[16] Liu S., Kumar S.M., Lu H., Liu A., Yang R., Pushparajan A., Guo W., Xu X.: MicroRNA-9 up-regulates E-cadherin through inhibition of NF-κB1-Snail1 pathway in melanoma. J. Pathol., 2012; 226(1): 61–72

[17] Ma L.: Role of miR-10b in breast cancer metastasis. Breast Cancer Res., 2010; 12(5): 210

[18] Ma L., Teruya-Feldstein J., Weinberg R.A.: Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature, 2007; 449(7163): 682–8

[19] Mar-Aguilar F., Mendoza-Ramírez J.A., Malagón-Santiago I., Espino-Silva P.K., Santuario-Facio S.K., Ruiz-Flores P., Rodríguez-Padilla C., Reséndez-Pérez D.: Serum circulating microRNA profiling for identification of potential breast cancer biomarkers. Dis. Markers, 2013; 34(3): 163–9

[20] Muluhngwi P., Klinge C.M.: Roles for miRNAs in endocrine resistance in breast cancer. Endocr. Relat. Cancer, 2015; 22(5): R279–R300

[21] Murri M., Insenser M., Fernández-Durán E., San-Millán J.L., Escobar-Morreale H.F.: Effects of polycystic ovary syndrome (PCOS), sex hormones, and obesity on circulating miRNA-21, miRNA-27b, miRNA-103, and miRNA-155 expression. J. Clin. Endocrinol. Metab., 2013; 98(11): E1835–E1844

[22] Negrini M., Calin G.A.: Breast cancer metastasis: a microRNA story. Breast Cancer Res., 2008; 10(2): 203

[23] Ng E.K., Li R., Shin V.Y., Jin H.C., Leung C.P., Ma E.S., Pang R., Chua D., Chu K.M., Law W.L., Law S.Y., Poon R.T., Kwong A.: Circulating microRNAs as specific biomarkers for breast cancer detection. PLoS One, 2013; 8(1): e53141

[24] Ozgün A., Karagoz B., Bilgi O., Tuncel T., Baloglu H., Kandemir E.G.: MicroRNA-21 as an indicator of aggressive phenotype in breast cancer. Onkologie, 2013; 36(3): 115–8

[25] Park S.M., Gaur A.B., Lengyel E., Peter M.E.: The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev., 2008; 22(7): 894–907

[26] Polyak K., Weinberg R.A.: Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat. Rev. Cancer, 2009; 9(4): 265–73

[27] Radojicic J., Zaravinos A., Vrekoussis T., Kafousi M., Spandidos D.A., Stathopoulos E.N.: MicroRNA expression analysis in triple-negative (ER, PR and Her2/neu) breast cancer. Cell Cycle, 2011; 10(3): 507–17

[28] Roth C., Rack B., Müller V., Janni W., Pantel K., Schwarzenbach H.: Circulating microRNAs as blood-based markers for patients with primary and metastatic breast cancer. Breast Cancer Res., 2010; 12(6): R90

[29] Salahandish R., Ghaffarinejad A., Omidinia E., Zargartalebi H., Majidzadeh-A K., Naghib S.M., Sanati-Nezhad A.: Label-free ultrasensitive detection of breast cancer miRNA-21 biomarker employing electrochemical nano-genosensor based on sandwiched AgNPs in PANI and N-doped graphene. Biosens. Bioelectron., 2018; 120: 129–36

[30] Sathyapalan T., David R., Gooderham N.J., Atkin S.L.: Increased expression of circulating miRNA-93 in women with polycystic ovary syndrome may represent a novel, non-invasive biomarker for diagnosis. Sci. Rep., 2015; 5: 16890

[31] Tang Y., Zhou X., Ji J., Chen L., Cao J., Luo J., Zhang S.: High expression levels of miR-21 and miR-210 predict unfavorable survival in breast cancer: a systemic review and meta-analysis. Int. J. Biol. Markers. 2015; 30(4): e347–358

[32] Thiery J.P., Acloque H., Huang R.Y., Nieto M.A.: Epithelial-mesenchymal transitions in development and disease. Cell, 2009; 139(5): 871–90

[33] Valadi H., Ekström K., Bossios A., Sjöstrand M., Lee J.J., Lötvall J.O.: Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat. Cell Biol., 2007; 9(6): 654–9

[34] Volinia S., Galasso M., Sana M.E., Wise T.F., Palatini J., Huebner K., Croce C.M.: Breast cancer signatures for invasiveness and prognosis defined by deep sequencing of microRNA. Proc. Natl. Acad. Sci. USA, 2012; 109(8): 3024–9

[35] Wellner U., Schubert J., Burk U.C., Schmalhofer O., Zhu F., Sonntag A., Waldvogel B., Vannier C., Darling D., zur Hausen A., Brunton V.G., Morton J., Sansom O., Schüler J., Stemmler M.P.: The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat. Cell Biol., 2009; 11(12): 1487–95

[36] Wu J.H., Gao Y., Ren A.J., Zhao S.H., Zhong M., Peng Y.J., Shen W., Jing M., Liu L.: Altered microRNA expression profiles in retinas with diabetic retinopathy. Ophthalmic Res., 2012; 47(4): 195–201

[37] Wu K., Li L., Li S.: Circulating microRNA-21 as a biomarker for the detection of various carcinomas: an updated meta-analysis based on 36 studies. Tumour Biol., 2015; 36: 1973–81

[38] Yan L.X., Huang X.F., Shao Q., Huang M.Y., Deng L., Wu Q.L., Zeng Y.X., Shao J.Y.: MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA, 2008; 14(11): 2348–60

[39] Yanaihara N., Caplen N., Bowman E., Seike M., Kumamoto K., Yi M., Stephens R.M., Okamoto A., Yokota J., Tanaka T., Calin G.A., Liu C.G., Croce C.M., Harris C.C.: Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell, 2006; 9(3): 189–98

[40] Yerukala Sathipati S., Ho S.Y.: Identifying a miRNA signature for predicting the stage of breast cancer. Sci. Rep., 2018; 8: 16138

[41] Zhao F.L., Hu G.D., Wang X.F., Zhang X.H., Zhang Y.K., Yu Z.S.: Serum overexpression of microRNA-10b in patients with bone metastatic primary breast cancer. J. Int. Med. Res., 2012; 40(3): 859–66

[42] Zhu S., Wu H., Wu F., Nie D., Sheng S., Mo Y.Y.: MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. Cell Res., 2008; 18(3): 350–9

The authors have no potential conflicts of interest to declare.