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A Group of Hypermethylated miRNA Genes in Breast Cancer and Their Diagnostic Potential

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Abstract—miRNA genes play an important role in cancer pathogenesis, while they may be suppressed by hypermethylation. Here, we assess the diagnostic potential of a group of hypermethylated miRNA genes (*MIR-124-1*, *MIR-124-3*, *MIR-125B-1*, *MIR-127*, *MIR-132*, *MIR-193a*, and *MIR-34b/c*) in a representative set of 70 breast cancer samples and 17 breast tissue samples from deceased donors with no malignancies. For these seven genes, the methylation status is determined using the methylation-specific PCR. Methylation reached 26–76% in tumor specimens, 1–27% in paired considered normal breast tissues, and 0–18% in breast tissue from deceased donors. By quantitative RT-PCR, reduced expression levels of the investigated miRNAs are detected, with a negative correlation of expression levels with gene hypermethylation. Combinations of three or four hypermethylation biomarkers, namely, *MIR-124-1*, *MIR-125B-1*, *MIR-127*, and *MIR-34b/c* are found suitable for breast cancer diagnostics; with sensitivity (76–93%), specificity (88–100%), and AUC (0.88–0.94). Notably, the *MIR-127* gene was hypermethylated only in the tumor samples of patients with metastases, and, therefore, should be tested as a marker of breast cancer dissemination. These findings may lead to improvement in the management of breast cancer.

Keywords: breast cancer, miRNA genes, hypermethylation, diagnostic markers, metastasis marker, *MIR-127*
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INTRODUCTION

Methylation of regulatory DNA sequences and interaction between miRNA and mRNA of target genes play an important role in the dynamic regulation of gene activity. Aberrations in these mechanisms may result in the dysfunction of cellular signaling pathways, which is observed in malignant tumors [1–3]. In the past decade, interest in the search for new targets of methylation, new regulatory miRNAs, and their target genes involved in oncogenesis continues to increase. These studies determined the progress in the personalized treatment of many malignant tumors [4, 5].

Breast cancer is the most common type of cancer in women: it accounts for 1/10 of all malignant tumors and is considered the most common cause of female cancer death [6]. Every year, as many as 1.6 million cases of breast cancer are recorded in the world, and its prevalence steadily increases [6]. In Russia, more than 70500 new cases of breast cancer were recorded in 2017 [7]. In addition, there is a trend towards younger breast cancer in recent years. The lack of effective diagnosis at the early stages of this disease and the high rate of lethal outcomes necessitate the search for new markers. The prospects of using miRNAs as markers that allow diagnosing breast cancer at an early stage, predict its course, and assess the individual response of the body to therapy are currently widely discussed [8–10].

The factors that modify the expression level of miRNAs (in particular, aberrant methylation of regulatory CpG islands) play a systemic role in the regulation of the function of miRNA target genes [11]. In the past decade, the role of hypermethylation of a wide range of miRNA genes in the development of tumors of various localizations has been established [12]. Epigenomic studies showed that the proportion of the miRNA genes that undergo methylation is several times higher than that of the protein-coding genes. This fact indicates that methylation plays a major role in the deregulation of miRNA genes in tumors and makes hypermethylated miRNAs promising diagnostic markers [13, 14].

Table 1. Clinical and histological characteristics of 70 specimens of breast cancer

Tumor characteristics	Histological type of cancer	Number of samples
	ID-BC	49
	IL-BC	21
Clinical stage	I	9
	II	37
	III	23
	IV	1
Degree of differentiation	Highly differentiated (hd)	6
	Moderately differentiated (md)	51
	Low differentiated (ld)	13
Metastasizing	N_0/M_0	26
	N_{1-2}	44
	M_1	1
Size and degree of tumor invasion	T1	13
	T2	43
	T3	5
	T4	9

Designation: ID-BC, infiltrative ductal breast cancer; IL-BC, infiltrative lobular breast cancer; N_0/M_0, absence of metastases; N_{1-2}, lesion of regional lymph nodes; M_1, distant metastases.

Earlier, we detected aberrant methylation of a large group of miRNA genes, which was involved in ovarian and breast cancer pathogenesis [15–18], and showed the potential applicability of hypermethylated miRNA genes for diagnosing ovarian cancer [19]. In this study, using a representative set of 70 breast cancer samples and 17 breast tissue samples of donors (subjects who died of non-cancerous diseases),we investigated the role of hypermethylation of a groups of miRNA genes (*MIR-124-1*, *MIR-124-3*, *MIR-125B-1*, *MIR-127*, *MIR-132*, *MIR-193a*, and *MIR-34b/c*) and estimated the diagnostic potential of these genes.

MATERIALS AND METHODS

Breast cancer samples were collected and clinically described at the Research Institute of Clinical Oncology, Blokhin National Medical Research Center of Oncology, Ministry of Health of the Russian Federation. In the study, we used paired samples of tumors and histologically unmodified (conditionally normal) breast tissues obtained from 70 patients with breast cancer. The clinical and histological data of the patients are summarized in Table 1.

We analyzed the samples of malignant tumors of the patients who did not receive specific treatment (radiotherapy or chemotherapy) before surgery. All breast tumors were classified in accordance with the TNM classification of the International Union Against Cancer [21] and histologically verified based on the classification criteria of the World Health Organization [22]. To select the specimens with a high content of tumor cells (at least 70–80%), we performed an additional histological analysis of microsections (3–5 μm) that were stained with hematoxylin and eosin.

In addition, we analyzed 17 breast tissue samples obtained from women who died of non-neoplastic diseases and had no history of cancer (the so-called donors).

Tissue samples were stored at –70°C. Tissue frozen in liquid nitrogen was ground with a SilentCrusher S homogenizer (Heidolph, Germany).

Isolation of DNA and RNA and reverse transcription. High-molecular-weight DNA was isolated from tissue using phenol extraction according to the standard protocols. The total RNA was isolated according to the extraction protocol with the use of guanidine thiocyanate, phenol, and chloroform as described previously [23].

The concentration of the total RNA was determined spectrophotometrically at 260 nm. The quality of RNA was also assessed spectrophotometrically using absorbance coefficients at 260 vs 230 nm and at 260 vs 280 nm. The preservation of RNA was determined by the ratio of band intensities of 28S rRNA and

Table 2. Methylation frequency of seven miRNA genes in breast cancer

miRNA gene	Tumor	Norm	p	Donors
MIR-124-1	**76%** (53/70)	27% (19/70)	1×10^{-8}	12%, 2/17
MIR-124-3	**39%** (27/70)	3% (2/70)	1×10^{-7}	12%, 2/17
MIR-125B-1	**49%** (34/70)	6% (4/70)	1×10^{-8}	0%, 0/17
MIR-127	**30%** (21/70)	1% (1/70)	2×10^{-6}	0%, 0/17
MIR-132	**26%** (18/70)	9% (6/70)	0.01	0%, 0/17
MIR-193a	**59%** (41/70)	13% (9/70)	2×10^{-8}	18%, 3/17
MIR-34b/c	**39%** (27/70)	9% (6/70)	4×10^{-5}	0%, 0/17

Statistically significant hypermethylation frequencies are shown in bold. With allowance for the Benjamini–Hochberg correction for multiple comparisons, $p < 10^{-4}$ values for six genes were statistically significant at $FDR = 0.01$; for the *MIR-132* gene, $p = 0.01$ was significant at $FDR = 0.05$.

18S rRNA by electrophoresis in 1% denaturing agarose gel. Before use, all RNA samples were treated with RNase-free DNase. cDNAs were synthesized from 1 μg of total RNA using M-MuLV reverse transcriptase and random nanomers according to the manufacturer's protocol (Thermo Fisher Scientific, United States).

Quantitative PCR (to assess the changes in the content of seven miRNAs in breast cancer samples) was performed using the cDNA obtained as described in [16]. We used TaqMan MicroRNA Assays (Applied Biosystems, United States): miR-124-3p (Assay ID: 001182), miR-125b-5p (Assay ID: 000449), miR-127-5p (Assay ID: 002229), miR-132-3p (Assay ID: 000457), miR-193a-5p (Assay ID: 002281), miR-34b-3p (Assay ID: 002102) and miR-34c-3p (Assay ID: 241009). For normalization, we used RNU48 (Assay ID: 001006) and RNU6 (Assay ID: 001093). All reactions were repeated thrice. Samples without cDNA were used as a negative control. Data were analyzed using the relative quantification by the ΔΔCt method. Changes in the miRNA level by a factor less than 2($|\Delta\Delta Ct| \le 2$) were regarded as the absence of changes [16].

Bisulfite conversion of DNA and methylation-specific PCR (MS-PCR) was performed as described previously [15, 16]. PCR was performed in a DNA Engine Dyad Cycler T-100 thermal cycle (Bio-Rad, United States) using the oligonucleotides and amplification conditions described in [15–18]. Three to six CpG-dinucleotides in each gene were analyzed. False positive results due to incomplete bisulfite conversion of the DNA were excluded at the primer selection stage by the absence of an MS-PCR product on the DNA that was not treated with bisulfite. Human methylated DNA (#SD1131, Thermo Scientific) was used as a control for the methylated allele, and human DNA (#G1471, Promega, United States) served as a control for the unmethylated alleles. PCR products of different genes were separated simultaneously in 2% agarose gel.

Statistical analysis was performed using Fisher's exact test in the BioStat 6.1 software. Changes were considered significant at $p \le 0.05$. Changes in the level of expression and methylation of the genes with the determination of the Spearman correlation coefficient (r_s) were compared using correlation analysis. Optimal marker systems were selected by the results of ROC (Receiver Operator Characteristic) analysis [24]. The results of multiple comparison were taken into account using the Benjamini–Hochberg correction. Results were considered significant at a false discovery rate (FDR)≤ 0.05.

RESULTS

Hypermethylated miRNAs as Potential Specific Markers of Breast Cancer

Using a representative sample consisting of 70 paired breast cancer samples (tumor/conditional norm), we analyzed methylation of seven miRNA genes (*MIR-124-1*, *MIR-124-3*, *MIR-125B-1*, *MIR-127*, *MIR-132*, *MIR-193a*, and *MIR-34b/c*) by MS-PCR. Table 2 summarizes the results of determining the frequency of methylation of these genes in breast tissue specimens obtained from 70 breast cancer patients and 17 donors (subjects who died of non-neoplastic diseases).

Table 2 shows that two genes (*MIR-124-1* and *MIR-193a*) were most frequently methylated in tumors (76 and 59%, respectively). However, their methylation was also often detected in the conditionally normal tissue (27 and 13%, respectively) and in the donor breast tissues (12 and 18%, respectively). It was found that four genes (*MIR-125B-1*, *MIR-127*, *MIR-132*, and *MIR-34b/c*) were methylated with a frequency of 26–49% in tumors and 1–9% in the conditional norm, whereas in the breast tissue samples of 17 donors the methylation of these genes was not detected. Therefore, these four genes can be proposed as sufficiently specific diagnostic markers of breast cancer.

Table 3. Frequency of changes in miRNA level in breast cancer

miRNAs	Decrease	Increase	No change
miR-124-3p	**68%** (26/38)	13% (5/38)	18% (7/38)
miR-125b-5p	**76%** (29/38)	8% (3/38)	16% (6/38)
miR-127-5p	39% (15/38)	8% (3/38)	53% (20/38)
miR-132-3p	**50%** (19/38)	5% (2/38)	45% (17/38))
miR-193a-5p	**79%** (30/38)	3% (1/38)	18% (7/38)
miR-34b-3p	**47%** (18/38)	11% (4/38)	42% (16/38)
miR-34c-3p	37% (14/38)	16% (6/38)	47% (18/38)

The high frequencies of unidirectional changes in expression (decrease) are shown in bold; the frequencies of the decreased expression prevailing over the increased expression are underlined.

Table 4. Correlation between changes in methylation status of miRNA gene and miRNA content in analysis of 38 paired breast cancer samples

miRNA gene	miRNA	r_s	p
MIR-124-1	miR-124-3p	0.61	4×10^{-5}
MIR-124-3	miR-124-3p	0.24	0.15
MIR-125B-1	miR-125b-5p	0.77	2×10^{-8}
MIR-127	miR-127-5p	0.50	2×10^{-3}
MIR-132	miR-132-3p	0.60	7×10^{-5}
MIR-193a	miR-193a-5p	0.64	2×10^{-5}
MIR-34b/c	miR-34b-3p	0.51	10^{-5}
MIR-34b/c	miR-34c-3p	0.38	0.02

Spearman correlation coefficient values (rs) are shown. For six rs, values $p \le 2 \times 10^{-3}$ with allowance for the Benjamini–Hochberg correction for multiple comparisons are statistically significant at $FDR = 0.01$; $p = 0.02$ for *MIR-34b/c*/miR-34c-3p is significant at $FDR = 0.05$.

Hypermethylation Contributes to the Suppression of Expression of the Group of miRNA Genes in Breast Cancer

To test the functional role of methylation, we determined the changes in the expression level of the miRNA genes that are hypermethylated in breast cancer. Table 3 shows the frequencies at which the content of mature miRNAs (products of these genes) decreased and increased in a subset of 38 breast cancer samples included in the total set (or collection) of 70 samples. At can be seen in Table 3, the content of all mature miRNAs transcribed from these hypermethylated genes in the breast cancer samples was mostly decreased. For example, miR-124-3p, miR-125b-5p, miR-127-5p, miR-132-3p, miR-193a-5p, miR-34b-3p, and miR-34c-3p were primarily inhibited, which is characteristic of tumor suppressors. This inhibition is associated with the hypermethylation of their encoding genes, as follows from the results of the correlation analysis between the changes in the methylation status and expression, which was performed on the total subset of 38 specimens (Table 4).

We found a strong correlation between the changes in methylation and expression of six of the seven genes; the Spearman correlation coefficient (r_s) was in the range between 0.38 and 0.61, $p \le 0.02$. Only one gene, *MIR-124-3*, showed a weak correlation between the change in the methylation status and expression of miR-124-3p. It can be assumed that the synthesis of miR-124-3p is ensured primarily by the *MIR-124-1*, but not *MIR-124-3*, gene. Thus, we can conclude that hypermethylation of the regulatory regions of the *MIR-124-1*, *MIR-125b-1*, *MIR-127*, *MIR-132*, *MIR-193a*, and *MIR-34b/c* genes significantly contributes to the suppression of expression of these genes and to the synthesis of respective mature miRNAs in breast cancer. These data confirm the involvement of aberrant methylation in the breast cancer pathogenesis.

Diagnostic Potential of the Group of miRNA Genes in Breast Cancer

Based on the data on the methylation status of seven miRNA genes in the breast cancer samples (70) and in the donor breast tissue samples (17), two optimal potential diagnostic marker systems for the detection of breast cancer were determined by ROC analysis (Table 5).

The first system consists of three genes whose methylation was not detected in any donor breast tissue sample (see Table 2). The specificity of this system is 100% at a sensitivity of only 76% and Area under ROC-Curve (AUC) value (an integrated characteris-

Table 5. Potential diagnostic systems of miRNA marker genes

Set of miRNA genes	AUC (95% CI)	Criterion	Sn, % (95% CI)	Sp, % (95% CI)	*p*
MIR-125B-1 *MIR-127* *MIR-34b/c*	0.88 (0.79–0.94)	>0	76 (64–85)	100 (80–100)	$<10^{-4}$
MIR-124-1 *MIR-125B-1* *MIR-127* *MIR-34b/c*	0.94 (0.87–0.98)	>0	93 (84–98)	88 (64–98)	$<10^{-4}$

The Area under ROC-Curve (AUC) values, the optimum criterion, the sensitivity (Sn), and specificity (Sp) at a 95% confidence interval (95% CI) are shown.

tic of the system reliability) of 0.88. The addition to this system of the *MIR-124-1* marker with a high frequency of methylation in the tumor samples (76%, Table 2) and a low frequency of methylation in the donor tissues (12%, 2/17) allowed us to obtain the optimum system. This system of four genes is characterized by a high AUC value (0.94) and 93% sensitivity at 88% specificity.

Thus, the system consisting of the four markers can be proposed as a potential diagnostic system. The detection of methylation of at least one of the genes of this system is sufficient to classify a sample with the breast cancer.

MIR-127 Gene Is Hypermethylated Only in Tumor Samples from Patients with Metastases and Is a Highly Specific Marker of Metastatic Breast Cancer

The correlation between the methylation status of each of the seven genes (*MIR-124-1*, *MIR-124-3*, *MIR-125B-1*, *MIR-127*, *MIR-132*, *MIR-193a*, and *MIR-34b/c*) and the clinical and histological characteristics of 70 breast cancer samples was studied. We established the significant correlation of the *MIR-127* gene methylation with later clinical stages ($p < 10^{-4}$), metastasizing (mainly to the regional lymph nodes) ($p < 10^{-4}$), and the tumor size ($p < 10^{-4}$, Fig. 1). A less significant correlation was found between the methylation of this gene and the loss of differentiation ($p \le 0.05$).

It was found that the *MIR-127* gene is methylated solely in the group of tumor samples from the patients with metastases (in half of the cases, 21/44, Fig. 1). The methylation of *MIR-127* was detected in only one conditionally normal sample (1/70, Table 2). This sample was obtained from a patient with the clinical stage III of the disease, a low degree of tumor differentiation, and metastases in two lymph nodes (T2N2M0). A tumor with such a degree of progression may exhibit significant invasion into the surrounding tissues as also as into the regional lymph nodes, and paired adjacent tissue may contain tumor cells, which showed the methylation of this gene in the analysis of the conditionally normal tissue.

Thus, of the seven miRNA genes whose methylation was studied in the set of 70 paired samples, only one, the *MIR-127* gene, was associated with the progression of breast cancer. Moreover, this gene is hypermethylated only in the tumor samples obtained from the patients with metastases. Thus, the *MIR-127*

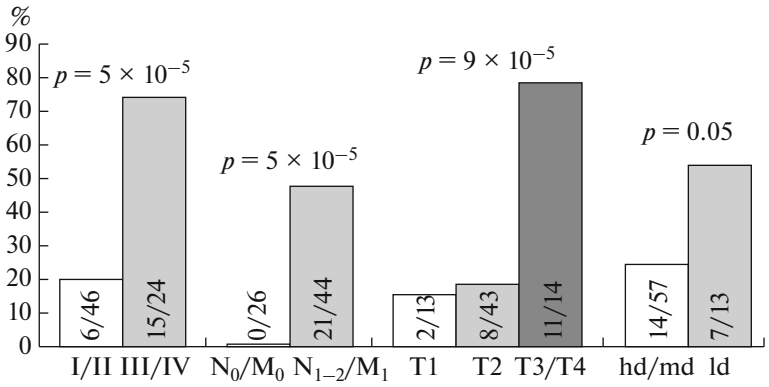

Fig. 1. Correlation between methylation frequency of *MIR-127* gene and breast cancer progression. Clinical stage: I/II vs III/IV. Metastasizing: N0/M0, group without metastases; N_{1-2}/M_1, group with metastases. Size and degree of tumor invasion: T1, T2, T3, T4. Differentiation: hd, highly differentiated; md, moderately differentiated;ld, low differentiated. With allowance for Benjamini–Hochberg correction for multiple comparisons, values $p \le 10^{-4}$ were statistically significant at $FDR = 0.01$.

gene is a potential highly specific marker that can be used to detect or predict metastatic tumors in the patients in which this gene is methylated.

DISCUSSION

In this study, we demonstrated a significant role of hypermethylation in the suppression of expression of seven miRNA genes (*MIR-124-1*, *MIR-124-3*, *MIR-125B-1*, *MIR-127*, *MIR-132*, *MIR-193a*, and *MIR-34b/c*) and in the decrease in the content of mature miRNAs in breast cancer. The correlation between the methylation of six of these genes and the synthesis of corresponding mature miRNAs was established. These data suggest that aberrant methylation implements a functional role in the pathogenesis of breast cancer.

Hypermethylation and the decrease in expression of these genes are consistent with the suppressor role of their encoded miRNAs (miR-124, miR-125b, miR-127, miR-132, miR-193a-5p, and miR-34b/c) in breast cancer, which was established mostly with the use of cell cultures [25–30]. A decreased level of expression of these miRNAs is considered as a marker of breast cancer. Furthermore, the correlation of miR-124, miR-125b, miR-127, miR-132, and miR-193a-5p with the breast cancer's progression, invasion, and metastasizing was found [26, 28, 30–32].

We studied the hypermethylation of six miRNA genes as breast cancer markers: the functional role of hypermethylation was confirmed by the correlation between the changes in their methylation and expression.

We created a potential system of markers for diagnosing breast cancer, which is based on the analysis of methylation of miRNA genes (namely, *MIR-124-1, MIR-125B-1*, *MIR-127*, and *MIR-34b/c*). This system is characterized by a high level of sensitivity (93%) and specificity (88%) (AUC = 0.94). These parameters were calculated on the basis of data for a representative sample of 70 breast cancer samples relative to the absolute norm (17 donors without a history of cancer).

The miRNA expression profiles are studied as diagnostic systems (e.g., in cancers of the prostate, bladder, lung, colorectal, and larynx) [33–38]. The proposed panels of markers are characterized by a high degree of sensitivity, specificity, and AUC values (>0.9). Markers based on the methylation status of miRNA genes are also being actively developed. Such panels were proposed, for example, for colorectal, bladder, and prostate cancers [39–41]. As a result of the system analysis of methylation of the suppressor miRNA genes, our group has developed marker systems for diagnosing lung, kidney, and ovarian cancers [19, 42, 43] and, in this study, for diagnosing breast cancer. As far as we know, this is the first study to propose a set of diagnostic breast cancer markers selected based on the analysis of hypermethylated miRNA genes.

Interestingly, we detected the *MIR-127* gene, which is often hypermethylated in tumor samples obtained from the patients with metastases (21 of 44) and is not methylated in any tumor sample from the patients without metastases (0 of 26). Thus, the *MIR-127* gene is a fairly unique highly specific potential prognostic marker of metastatic breast cancer.

The correlation between decreased miR-127 expression and metastatic breast cancer was noted previously [32, 44]. Information about miR-127 targets is scarce: in breast cancer, the *BCL6* protooncogene is regarded as a miR-127 target [32, 44]. The nuclear factor κB (NF-κB) is considered as a putative miR-127 target in hepatocellular carcinoma [45], which confirms the suppressive properties of this miRNA but does not explain its antimetastatic activity. It is surprising that, in lung cancer, miR-127 induces, rather than inhibits, the epithelial–mesenchymal transition [46]. Thus, the molecular mechanism of the antimetastatic activity of miR-127 and the activatory effect of *MIR-127* gene methylation on the dissemination of breast cancer requires further research. At the same time, the content of miR-127 in blood is correlated with the presence of tumor cells circulating in blood, which makes miR-127 a potential non-invasive clinically significant marker [47].

Thus, the revealed features of methylation of seven miRNA genes, the unique prognostic potential of *MIR-127* gene hypermethylation, and the proposed system of diagnostic markers of breast cancer, which is based on the methylation status of miRNA genes, may find clinical application in the development of modern approaches for the diagnosis, prognosis, and selection of treatment for breast cancer.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. This study was performed in compliance with the principles of voluntariness and confidentiality in accordance with the Fundamentals of the Legislation of the Russian Federation on the Protection of Public Health and the Helsinki Declaration of the World Medical Association [20].The permission of the Ethics Committee of the Blokhin National Medical Research Center of Oncology of the Ministry of Health of the Russian Federation and the informed consent of all patients participating in the study were obtained.

REFERENCES

1. Llinàs-Arias P., Esteller M. 2017. Epigenetic inactivation of tumour suppressor coding and non-coding

genes in human cancer: An update. *Open Biol.* **7** (9). pii: 170152. https://doi.org/10.1098/rsob.170152

2. Musavi Shenas M.H., Eghbal-Fard S., Mehrisofiani V., Abd Yazdani N., Rahbar Farzam O., Marofi F., Yousefi M. 2018. MicroRNAs and signaling networks involved in epithelial-mesenchymal transition. *J. Cell. Physiol.* Nov. 11. https://doi.org/10.1002/jcp.27489
3. Moutinho C., Esteller M. 2017. MicroRNAs and epigenetics. *Adv. Cancer Res.* **135**, 189–220. https://doi.org/10.1016/bs.acr.2017.06.003
4. Stefansson O.A., Esteller M. 2013. Epigenetic modifications in breast cancer and their role in personalized medicine. *Am. J. Pathol.* **183**, 1052–1063. https://doi.org/10.1016/j.ajpath.2013.04.033
5. Bertoli G., Cava C., Castiglioni I. 2015. MicroRNA: New biomarkers for diagnosis, prognosis, therapy prediction and therapeutic tools for breast cancer. *Theranostics*. **5**, 1122–1143. https://doi.org/10.7150/thno.11543
6. Dey S. 2014. Preventing breast cancer in LMICs via screening and/or early detection: The real and the surreal. *World J. Clin. Oncol.* **5**, 509–519. https://doi.org/10.5306/wjco.v5.i3.509
7. *Zlokachestvennye novoobrazovaniya v Rossii v 2017 godu: zabolevaemost' i smertnost'* (Malignant Neoplasms in Russia in 2017: Morbidity and Mortality). Eds. Kaprin A.D., Starinskii V.V., Petrova G.V. Moscow: MNIOI im. P.A. Gertsena Minzdrava Rossii.
8. Takahashi R., Miyazaki H., Ochiya T. 2015. The roles of microRNAs in breast cancer. *Cancers* (Basel). **7**, 598–616. https://doi.org/10.3390/cancers7020598
9. Khordadmehr M., Shahbazi R., Ezzati H., Jigari-Asl F., Sadreddini S., Baradaran B. 2018. Key microRNAs in the biology of breast cancer: Emerging evidence in the last decade. *J. Cell. Physiol.* https://doi.org/10.1002/jcp.27716
10. Campos-Parra A.D., Mitznahuatl G.C., Pedroza-Torres A., Romo R.V., Reyes F.IP., López-Urrutia E., Pérez-Plasencia C. 2017. Micro-RNAs as potential predictors of response to breast cancer systemic therapy: Future clinical implications. *Int. J. Mol. Sci.* **18** (6). pii: E1182. https://doi.org/10.3390/ijms18061182
11. Vrba L., Muñoz-Rodríguez J.L., Stampfer M.R., Futscher B.W. 2013. miRNA gene promoters are frequent targets of aberrant DNA methylation in human breast cancer. *PLoS One*. **8** (1), e54398. https://doi.org/10.1371/journal.pone.0054398
12. Loginov V.I., Rykov S.V., Fridman M.V., Braga E.A. 2015. Methylation of miRNA ganas and oncogenesis. *Biochemistry* (Moscow). **80** (2), 145–162.
13. Kunej T., Godnic I., Ferdin J., Horvat S., Dovc P., Calin G.A. 2011. Epigenetic regulation of microRNAs in cancer: An integrated review of literature. *Mutat. Res.* **717**, 77–84. https://doi.org/10.1016/j.mrfmmm.2011.03.008
14. Piletič K., Kunej T. 2016. MicroRNA epigenetic signatures in human disease. *Arch. Toxicol.* **90**, 2405–2419. https://doi.org/10.1007/s00204-016-1815-7
15. Loginov V.I., Burdennyy A.M., Pronina I.V., Khokonova V.I., Kurevljov S.V., Kazubskaya T.P., Kushlinskii N.E., Braga E.A. 2016. Novel miRNA genes hypermethylated in breast cancer. *Mol. Biol.* (Moscow). **50** (5), 705–709.
16. Pronina I.V., Loginov V.I., Burdennyy A.M., Fridman M.V., Senchenko V.N., Kazubskaya T.P., Kushlinskii N.E., Dmitriev A.A., Braga E.A. 2017. DNA methylation contributes to deregulation of 12 cancer-associated microRNAs and breast cancer progression. *Gene.* **604**, 1–8. https://doi.org/10.1016/j.gene.2016.12.018
17. Loginov V.I., Burdennyi A.M., Filippova E.A., Pronina I.V., Kazubskaya T.P., Kushlinsky D.N., Ermilova V.D., Rykov S.V., Khodyrev D.S., Braga E.A. 2018. Hypermethylation of *miR-107, miR-130b, miR-203a, miR-1258* genes associated with ovarian cancer development and metastasis. *Mol. Biol.* (Moscow). **52**, 801–809.
18. Loginov V.I., Pronina I.V., Burdennyi A.M., Filippova E.A., Kazubskaya T.P., Kushlinsky D.N., Utkin D.O., Khodyrev D.S., Kushlinskii N.E., Dmitriev A.A., Braga E.A. 2018. Novel miRNA genes deregulated by aberrant methylation in ovarian carcinoma are involved in metastasis. *Gene*. **662**, 28–36. https://doi.org/10.1016/j.gene.2018.04.005
19. Braga E.A., Loginov V.I., Filippova E.A., Burdennyi A.M., Pronina I.V., Kazubskaya T.P., Khodyrev D.S., Utkin D.O., Kushlinskii D.N., Adamyan L.V., Kushlinskii N.E.2018. Diagnostic value of a group of microRNA genes hypermethylated in ovarian carcinoma. *Bull. Exp. Biol. Med.* **166** (2), 253–256.
20. World Medical Association. 2013. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *J. Am. Med. Assoc.* **310**, 2191–2194.
21. International Union against Cancer. 2010. *TNM Classification of Malignant Tumours,* 7th ed. Eds. Sobin L.H., Gospodarowicz M.K., Wittekind Ch. Hoboken, NJ: Wiley-Blackwell. https://www.ncbi.nlm.nih.gov/nlmcatalog/101511218.
22. *WHO Classification of Tumours of Female Reproductive Organs,* 4th ed. 2014. Eds. Kurman R.J., Carcangiu M.L., Herrington C.S., Young R.H. Lyon: IARC Press.
23. Pronina I.V., Loginov V.I., Khodyrev D.S., Kazubskaya T.P., Braga E.A. 2012. *RASSF1A* expression level in primary epithelial tumors of various locations. *Mol. Biol.* (Moscow). **46** (2), 260–268. https://doi.org/10.1134/S0026893312010189
24. Göksülük D., Korkmaz S., Zararsiz G., Karaagaoglu A.E. 2016. EasyROC: An interactive Web-tool for ROC curve analysis using R language environment. *The R. J.* **8**, 213–230.
25. Wang Y., Chen L., Wu Z., Wang M., Jin F., Wang N., Hu X., Liu Z., Zhang C.Y., Zen K., Chen J., Liang H., Zhang Y., Chen X. 2016. miR-124-3p functions as a tumor suppressor in breast cancer by targeting CBL. *BMC Cancer.* **16**(1), 826. PMID: 27842510
26. Li Y., Wang Y., Fan H., Zhang Z., Li N. 2018. miR-125b-5p inhibits breast cancer cell proliferation, migration and invasion by targeting KIAA1522. *Biochem. Biophys. Res. Commun*. **504**, 277–282. https://doi.org/10.1016/j.bbrc.2018.08.172
27. Chen J., Wang M., Guo M., Xie Y., Cong Y.S. 2013. miR-127 regulates cell proliferation and senescence by targeting BCL6. *PLoS One.* **8** (11), e80266. https://doi.org/10.1371/journal.pone.0080266

28. Zhang Z.G., Chen W.X., Wu Y.H., Liang H.F., Zhang B.X. 2014. MiR-132 prohibits proliferation, invasion, migration, and metastasis in breast cancer by targeting HN1. *Biochem. Biophys. Res. Commun*. **454**, 109–114. https://doi.org/10.1016/j.bbrc.2014.10.049

29. Liu X., Feng J., Tang L., Liao L., Xu Q., Zhu S. 2015. The regulation and function of miR-21-FOXO3a-miR-34b/c signaling in breast cancer. *Int. J. Mol. Sci.* **16**, 3148–3162. https://doi.org/10.3390/ijms16023148

30. Xie F., Hosany S., Zhong S., Jiang Y., Zhang F., Lin L., Wang X., Gao S., Hu X. 2017. MicroRNA-193a inhibits breast cancer proliferation and metastasis by downregulating WT1. *PLoS One*. **12** (10), e0185565. https://doi.org/10.1371/journal.pone.0185565

31. Zhang L., Chen X., Liu B., Han J. 2018. MicroRNA-124-3p directly targets PDCD6 to inhibit metastasis in breast cancer. *Oncol. Lett.* **15**, 984–990. https://doi.org/10.3892/ol.2017.7358

32. Wang S., Li H., Wang J., Wang D., Yao A., Li Q. 2014. Prognostic and biological significance of microRNA-127 expression in human breast cancer. *Dis. Markers*. 2014, 401986. https://doi.org/10.1155/2014/401986

33. Huang Z., Zhu D., Wu L., He M., Zhou X., Zhang L., Zhang H., Wang W., Zhu J., Cheng W., Chen Y., Fan Y., Qi L., Yin Y., Zhu W., et al. 2017. Six serum-based miRNAs as potential diagnostic biomarkers for gastric cancer. *Cancer Epidemiol. Biomarkers Prev.* **26**, 188–196. https://doi.org/10.1158/1055-9965.EPI-16-0607

34. Urquidi V., Netherton M., Gomes-Giacoia E., Serie D.J., Eckel-Passow J., Rosser C.J., Goodison S. 2016. A microRNA biomarker panel for the non-invasive detection of bladder cancer. *Oncotarget*. **7**, 86290–86299. https://doi.org/10.18632/oncotarget.13382

35. Daniel R., Wu Q., Williams V., Clark G., Guruli G., Zehner Z. 2017. A panel of microRNAs as diagnostic biomarkers for the identification of prostate cancer. *Int. J. Mol. Sci*. **18** (6). pii: E1281. https://doi.org/10.3390/ijms18061281

36. Zhu M., Huang Z., Zhu D., Zhou X., Shan X., Qi L.W., Wu L., Cheng W., Zhu J., Zhang L., Zhang H., Chen Y., Zhu W., Wang T., Liu P. 2017. A panel of microRNA signature in serum for colorectal cancer diagnosis. *Oncotarget.* **8**, 17081–17091. https://doi.org/10.18632/oncotarget.15059

37. Zhang H., Zhu M., Shan X., Zhou X., Wang T., Zhang J., Tao J., Cheng W., Chen G., Li J., Liu P., Wang Q., Zhu W. 2018. A panel of seven-miRNA signature in plasma as potential biomarker for colorectal cancer diagnosis. *Gene*. Nov. 17. pii: S0378-1119(18)31197-1. https://doi.org/10.1016/j.gene.2018.11.055

38. Chang Y.A., Weng S.L., Yang S.F., Chou C.H., Huang W.C., Tu S.J., Chang T.H., Huang C.N., Jong Y.J., Huang H.D. 2018. A three-microRNAsignature as a potential biomarker for the early detection of oral cancer. *Int. J. Mol. Sci.* **19** (3). pii: E758. https://doi.org/10.3390/ijms19030758

39. Shimizu T., Suzuki H., Nojima M., Kitamura H., Yamamoto E., Maruyama R., Ashida M., Hatahira T., Kai M., Masumori N., Tokino T., Imai K., Tsukamoto T., Toyota M. 2013. Methylation of a panel of microRNA genes is a novel biomarker for detection of bladder cancer. *Eur. Urol.* **63**, 1091–1100. https://doi.org/10.1016/j.eururo.2012.11.030

40. Toiyama Y., Okugawa Y., Tanaka K., Araki T., Uchida K., Hishida A., Uchino M., Ikeuchi H., Hirota S., Kusunoki M., Boland C.R., Goel A. 2017. A panel of methylated microRNA biomarkers for identifying high-risk patients with ulcerative colitis-associated colorectal cancer. *Gastroenterology.* **153** (6), 1634–1646. e8. https://doi.org/10.1053/j.gastro.2017.08.037

41. Torres-Ferreira J., Ramalho-Carvalho J., Gomez A., Menezes F.D., Freitas R., Oliveira J., Antunes L., Bento M.J., Esteller M., Henrique R., Jerónimo C. 2017. MiR-193b promoter methylation accurately detects prostate cancer in urine sediments and miR-34b/c or miR-129-2 promoter methylation define subsets of clinically aggressive tumors. *Mol. Cancer.* **16**, 26.

42. Rykov S.V., Khodyrev D.S., Pronina I.V., Kazubskaya T.P., Loginov V.I., Braga E.A. 2013. Novel miRNA genes methylated in lung tumors. *Russ. J. Genet.* **49** (7), 782–787. https://doi.org/10.1134/S1022795413070119

43. Loginov V.I., Beresneva E.V., Kazubskaya T.P., Braga E.A., Karpukhin A.V. 2017. Methylation of 10 miRNA genes in clear cell renal cell carcinoma and their diagnostic value. *Cancer Urology.* **13**, 27–33. https://doi.org/10.17650/1726-9776-2017-13-3-27-33

44. Zhao X., Duan Z., Liu X., Wang B., Wang X., He J., Yao Z., Yang J. 2013). MicroRNA-127 is downregulated by Tudor-SN protein and contributes to metastasis and proliferation in breast cancer cell line MDA-MB-231. *Anat. Rec.* (Hoboken). **296**, 1842–1849. https://doi.org/10.1002/ar.22823

45. Huan L., Bao C., Chen D., Li Y., Lian J., Ding J., Huang S., Liang L., He X. 2016. MicroRNA-127-5p targets the biliverdin reductase B/nuclear factor-κB pathway to suppress cell growth in hepatocellular carcinoma cells. *Cancer Sci.* **107**, 258–266. https://doi.org/10.1111/cas.12869

46. Shi L., Wang Y., Lu Z., Zhang H., Zhuang N., Wang B., Song Z., Chen G., Huang C., Xu D., Zhang Y., Zhang W., Gao Y. 2017. miR-127 promotes EMT and stem-like traits in lung cancer through a feed-forward regulatory loop. *Oncogene.* **36**, 1631–1643. https://doi.org/10.1038/onc.2016.332

47. Alunni-Fabbroni M., Majunke L., Trapp E.K., Tzschaschel M., Mahner S., Fasching P.A., Fehm T., Schneeweiss A., Beck T., Lorenz R., Friedl T.W.P., Janni W., Rack B., SUCCESS Study Group. 2018. Wholeblood microRNAs as potential biomarkers in post-operative earlybreast cancer patients. *BMC Cancer.* **18** (1), 141. https://doi.org/10.1186/s12885-018-4020-7

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