Cancer Treatment and Research Communications 20 (2019) 100026

Contents lists available at ScienceDirect

Cancer Treatment and Research Communications

journal homepage: www.elsevier.com/locate/ctarc

# Expression of micrornas in molecular genetic breast cancer subtypes

N.N. Kolesnikov [a], Yu.A. Veryaskina [a], S.E. Titov [a,b], V.V. Rodionov [c,d], T.P. Gening [c,*], T.V. Abakumova [c], V.V. Kometova [d], M.Kh. Torosyan [d], I.F. Zhimulev [a]

[a] Institute of Molecular and Cell Biology, Novosibirsk 630090, Russia
[b] Company "Vector-Best", Koltsovo, Russia
[c] Ulyanovsk State University, 432017 Ulyanovsk, Russia
[d] Ulyanovsk Regional Clinical Oncology Center, 432017 Ulyanovsk, Russia

ARTICLE INFO

*Article history:*
Received 4 February 2016
Accepted 12 August 2016

*Keywords:*
Breast cancer
miRNA
ROC-analysis

ABSTRACT

*Background:* It is shown that each type of human malignancies has a unique set of expressed miRNAs, and tumor-specific miRNAs in biological tissues of a patient are stable.

The aim of this study was to determine the differences in the expression of miRNAs in tumor tissue of invasive breast carcinoma compared to normal tissue, as well as to analyze the variable expression of miRNAs in molecular genetic subtypes of breast cancer.

*Methods:* We determined differences in mRNA expression in 35 biopsies of tumor tissue of various molecular genetic subtypes of breast cancer and 35 biopsies of adjacent conventionally normal breast tissue by RT-PCR in real time. We assessed the expression levels of miRNA-21, 221, 222, 155, 205, 20a , 125b and 200a.

*Results:* A significant increase in the level of expression of the oncogenic miRNA-20a ($p=0.000141$) and miRNA-221 ($p=0.037777$) in the triple negative cancer in comparison with the luminal A and luminal B/HER2/neu-negative breast cancer subtypes was established. Assessment of significance of the results was conducted using ROC analysis. For miRNA-221 AUC value was 0.772, for miRNA-20a AUC value was 0.949.

The obtained results suggest the possibility of using the levels of miRNA-21, 155, 205, 125b expression in tumor tissue to assess a malignant potential of a breast carcinoma. The levels of expression of oncogenic miRNA-221 and miRNA-20a are increased in TNBC compared with luminal A and luminal B/HER2/neu-negative breast cancer subtypes, supporting the characteristic of TNBC as the most aggressive subtype of breast cancer. MiRNA-20a is a marker of TNBC compared with luminal subtypes of breast cancer.

*Micro abstract:* To identify markers for breast cancer with triple-negative phenotype, we evaluated expression levels of siRNA-21, 221, 222, 155, 205, 20a, 125b, 200a and 146b in the tumor tissue of 35 patients by RT-PCR. AUC value equal to 0.949 in the ROC-analysis allows us to recommend the miRNA-20a as a marker of triple negative breast cancer to differentiate it from the luminal subtypes.



## 1. Introduction

The discovery of molecules that inhibit protein synthesis in the post-transcriptional level (miRNAs) has provided new opportunities for the determination of specific markers of malignant tumors. MicroRNAs in normal cells regulate the expression of genes which determine almost all of the vital processes [4,6]. Various cells express specific miRNA, depending on the characteristics of their metabolism, type, degree of differentiation and proliferation intensity. The phenotype of a malignant tumor is determined by violations in expression of various genes. It was found that each human tumor type has its own specific set of miRNAs ("signature" of the tumor) [22]. MiRNA database created in 1993 in the 21 version (June 2014) contains information about 2588 human miRNAs [13]. Breast cancer (BC) is a malignancy developing from the epithelial cells of ducts and lobules of the breast. In economically developed countries, breast cancer is the most common form of cancer in women. The highest standardized incidence rates of breast cancer −32% of all newly diagnosed cases of cancer in women - are registered in the United States. In Russia in 2006, the incidence of breast cancer among patients with malignant tumors was 17.8%. However, determination of the molecular tumor markers has prognostic significance. Testing venous blood for tumor markers is not highly specific, so it is not used for primary diagnosis of breast cancer. At the end of the 1990s, the group of tumors

* Correspondence to: Department of Physiology and Pathophysiology, Russia.
*E-mail addresses:* naum-53@yandex.ru (T.P. Gening), fl-31@mail.ru (I.F. Zhimulev).

http://dx.doi.org/10.1016/j.ctarc.2016.08.006
2468-2942/

in which estrogen and progesterone receptors are not detected and the amplification of HER-2-neu gene is not observed – the so-called triple-negative breast cancer – was identified among malignant breast tumors [15]. Today, it is found that in approximately 80% of cases, triple negative and basal breast cancer concur. But the triple negative breast cancer includes some special histological types. Thus, staining for the basal keratins is not considered to be reproducible enough for widespread use. In practice, clinicians are faced with situations where luminal subtypes of breast cancer characterized by the most favorable course, behave aggressively, so the disease progresses rapidly and often ends in death. Conversely, the most aggressive cancer with triple-negative phenotype can occur indolent for years and do not require adjuvant therapy treatments. All this requires a search for additional molecular genetic markers that can help to individualize the treatment of patients with breast cancer. Opening molecule inhibitors of protein synthesis in the post-transcriptional level – microRNAs (miRNAs) has provided new possibilities in finding specific markers of tumors.

Today, increased expression of 13 miRNAs and reduced expression of 49 miRNAs is established in breast cancer with specificity of 78.8% and sensitivity of 92.5%. The correlation with stage, metastasis, prognosis and survival period is shown. The chosen profile includes miRNA whose role in tumorigenesis is more or less established in tissue cultures, in animal models, in malignant tumors of other localizations. In breast cancer, the oncogenic role is known of the miRNA-21 [20,26], miRNA -155 [20,25,27], miRNA -221 and miRNA -222 [1,19], miRNA -125 [28]. Oncosupressing role is shown for miRNA-200 [11] and miRNA-205 [12]. Tumor-specificity without regard to metastatic disease is shown for miRNA-146 [7]. We could not find data neither on the role of miRNA-20 in breast cancer, nor on variability of expression of miRNAs in tumor tissue in different breast cancer subtypes in the available literature. In connection with the above, the purpose of the present study was to investigate the expression profile of miRNAs in tumor tissue in different phenotypes of breast cancer.

## 2. Materials and methods

The material for the study were 35 biopsies of tumor tissue and 35 biopsies of adjacent conditionally normal tissue, respectively, obtained during radical surgical treatment of 35 patients with a diagnosis of breast cancer in Regional Clinical Oncology Center of Ulyanovsk in 2014. Surgical treatment was carried out without prior exposure to chemoradiation. The average age of patients was 50 ± 5,3 years. Stage of the process was confirmed pathologically after surgery and was determined according to the international TNM classification in the 7 edition of 2010. 11 cases were diagnosed with stage I disease, 17 cases – II stage, 7 cases – III stage. The 26 biopsies of breast tumor tissue revealed luminal A and luminal B / HER2/neu-negative breast cancer; 6 cases had triple negative cancer phenotype; 1 biopsy was diagnosed with luminal / HER2/neu-positive cancer and 2 cases - HER2/neu-positive cancer. Clinical and morphological characteristics of patients and tumors are presented in Table 1.

For storage and transportation of operational material, solution for RNA stabilizing – RNA later was used, allowing to isolate RNA from tissues and cells without freezing in liquid nitrogen. Isolation of total RNA pool was carried out with a set of "Real Best extraction 100" ("Vector-Best", Novosibirsk) in accordance with the manufacturer's instructions. Reverse transcription was performed using specific primers to the miRNAs: miRNA-21, miRNA-221, miRNA-222, miRNA-155, miRNA-205, miRNA-20a, miRNA-125b, miRNA-146b, miRNA-200a. The reverse transcription reaction was carried out using the prepared reaction mixture "Real Best Master mix OT" ("Vector-Best", Novosibirsk). The cDNA obtained in a volume of 3 μl was used directly as the template for PCR. Measurement of expression levels of miRNAs was performed by real time PCR thermocycler CFX96 (Bio-Rad Laboratories, USA) [2]. As a reference gene we used small RNA U6. We have shown that if we take not an individual U6 for each sample, but a median value of U6 and continue to maintain the calculation of the expression of

**Table 1**
Clinical and morphological characteristics of patients and brest malignant tumors.

| Characteristic | n | % |
|---|---|---|
| **Age (years)** | | |
| Median (range) | 50 ± 5,3 | |
| < 40 | 1 | 2,9 |
| 41–69 | 29 | 82,8 |
| > 70 | 5 | 14,3 |
| **Tumor location** | | |
| UOQ | 26 | 74,3 |
| LOQ | 3 | 8,5 |
| UIQ | 1 | 2,9 |
| LIQ | 5 | 14,3 |
| Central | 0 | 0 |
| **Histologic grade** | | |
| I | 1 | 2,9 |
| II | 20 | 57,1 |
| III | 14 | 40 |
| **Tumor size, cm** | | |
| Median | 2,3 | |
| Range | 0,7-5,5 | |
| T1b ( ≥ 1) | 3 | 8,6 |
| T1c | 16 | 45,7 |
| T2 | 15 | 42,9 |
| T3 | 1 | 2,8 |
| **Tumor type** | | |
| Ductal | 10 | 28,6 |
| Lobular | 10 | 28,6 |
| Mix (ductal+lobular) | 11 | 31,4 |
| Atypical | 4 | 11,4 |
| **Molecular type** | | |
| Luminal A | 16 | 45,7 |
| Luminal B | 10 | 28,6 |
| Triple negative | 6 | 17,1 |
| Luminal HER2/neu positive | 1 | 2,9 |
| HER2/neu positive | 2 | 5,7 |
| **Metastases in regional lymphatic nodes** | | |
| Yes | 16 | 45,6 |
| No | 18 | 51,4 |
| **TSM prognosis** | | |
| Good | 9 | 25,7 |
| Indeterminate | 12 | 34,3 |
| Bad | 14 | 40 |

NOTE: Tumor size was defined as the size of the invasive component in centimeters. We classified tumor size as a categorical variable, using T stage according to the 2007 TNM classification system.
TSM (Total Score of Malignancy) was counted as a sum of points of following parameters: tumor degree of differentiation (1–3 points), cell polymorphism (1–3 points), mitotic activity (1–3 points), the nature of invasive growth (1–5 points), tumor emboli in stroma vessels (0–3 points) and cell reaction in tumor stroma (0–3 points). All patients were distributed into three prognostic groups depending on the value of TSM: good prognosis (4–13 points), intermediate prognosis (14–15 points) and poor (bad) prognosis (16–20 points).
Molecular type of tumor was defined immunohistochemically according to hormone receptor status, HER2/neu status and ki-67 grade. Luminal A type contains estrogen and progesterone positive, HER2/neu negative, ki-67 lower 20% variants of tumors. Luminal B type contains estrogen and progesterone positive, HER2/neu negative, ki-67 above 20% variants of tumors. Triple negative type includes estrogen and progesterone negative, HER2/neu negative tumors regardless of the ki-67 grade.
Positivity of estrogen and progesterone receptors was defined as at least 10% or more immunostained cells.
Regional lymphatic nodes include axillary and supraclavicular nodes on the side of a tumor.
Abbreviations: UOQ - upper outer quadrant; LOQ - lower outer quadrant; UIQ - upper inner quadrant; LIQ - lower inner quadrant; SLN, TSM - Total Score of Malignancy.

**Table 2**
The final table of the individual and the median U6.

| | mir-21 | mir-221 | mir-222 | mir-155 | mir-205 | mir-20a | mir-125b | mir-146b | mir-200a |
|---|---|---|---|---|---|---|---|---|---|
| U6 individual | 2,29 | −2,46 | −2,07 | 3,63 | −7,89 | −1,08 | −5,55 | −1,18 | 1,60 |
| U6 median | 2,23 | −2,33 | −1,75 | 3,12 | −6,19 | 1,04 | −6,50 | −1,02 | 1,40 |

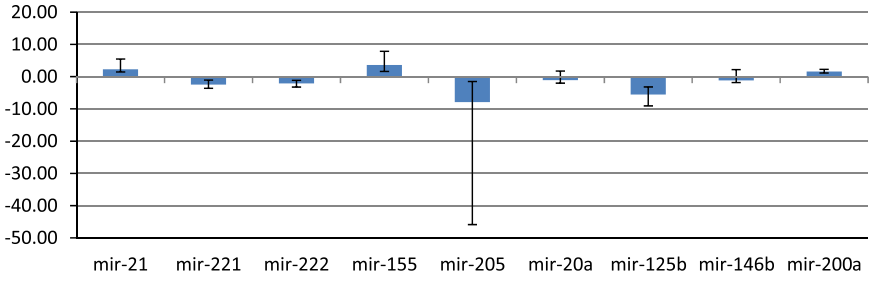


**Fig. 1.** The calculation on the individual U6 value.

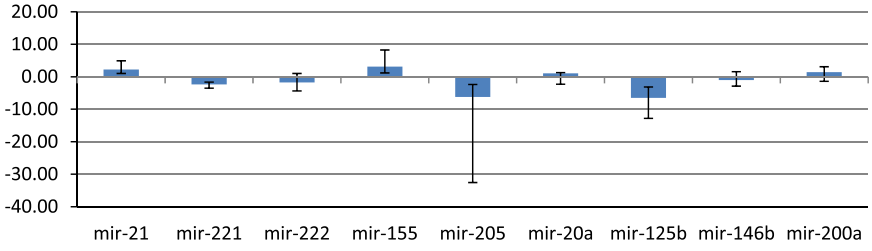


**Fig. 2.** The calculation on the median U6 value.

**Table 3**
Changes in the levels of expression of miRNA in the tumor tissue of luminal A and B luminal/HER2/neu-negative subtypes of breast cancer compared to adjacent morphologically intact tissue.

| | miRNA | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 155 | 21 | 200a | 146b | 205 | 125b | 221 | 222 | 20a |
| Difference | **3.68** | **3.66** | **1.78** | 1.02 | **7.84** | **7.78** | **1.71** | **1.56** | 1.17 |
| Dynamics | increase | | | | Decrease | | | | |
| *P* | **0.000096** | **0.000980** | **0.082627** | 0.744125 | **0.000050** | **0.000020** | **0.006062** | **0.016630** | 0.212682 |

Note: $p < 0.05$ – statistically significant differences between the tumor tissue and adjacent morphologically intact tissue.

**Table 4**
Changes in the level of expression of miRNAs in the tumor tissue in TNBC compared with adjacent morphologically intact tissue.

| | miRNA | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 155 | 21 | 20a | 200a | 146b | 125b | 205 | 222 | 221 |
| Difference | 7.01 | 3.23 | **3.16** | 2.52 | 1.97 | **10.45** | **3.50** | 1.86 | 1.12 |
| Dynamics | increase | | | | | Decrease | | | |
| *P* | 0.111111 | 0.055555 | **0.031746** | 0.095238 | 0.547619 | **0.015873** | **0.007936** | 0.547619 | 0.841269 |

Note: $p < 0.05$ – statistically significant differences between the tumor tissue and adjacent morphologically intact tissue.

microRNAs on the median U6, and not on the value of U6 for each sample, it does not affect the median levels of expression.

of microRNA (Table 2, Figs. 1 and 2).

The PCR reaction was performed in a volume of 30 μl with the prepared reaction mixture "Real Best Master Mix" ("Vector-Best", Novosibirsk) and a solution of forward and reverse primers (5 μm) and a probe (2.5 μm). The levels of miRNAs expression were measured by the method of RT - PCR in real time. Statistical analysis was performed using non-parametric Mann–Whitney *U* test in the Statistica 10.0. In order to evaluate the significance of the differences found, we performed mathematical processing by constructing ROC – curve using the IBM SPSS Statistics 21 software.

## 3. Results and discussion

All the miRNA are divided into oncogenes and oncosupressors depending on their role in carcinogenesis of breast cancer. MiRNA is oncogenic when its target is an oncosupressing gene, and conversely, the miRNA is oncosupressing if its target is oncogene. It should be

**Table 5**
Changes in the expression of miRNAs in TNBC compared with luminal subtypes of breast cancer.

| miRNA | Difference | Dynamics | *P* |
|---|---|---|---|
| miRNA - 221 | **43.03** | increase | **0.0377779** |
| miRNA - 222 | 11.3 | Increase | 0.2246853 |
| miRNA - 20a | **6.42** | Increase | **0.0001413** |
| miRNA - 200a | 2.39 | Increase | 0.0965447 |
| miRNA - 155 | 2.33 | Increase | 0.3078420 |
| miRNA - 21 | 1.99 | Increase | 0.3813739 |
| miRNA - 146b | 1.63 | Increase | 0.1185532 |
| miRNA - 125b | 1.89 | decrease | 0.2641339 |
| miRNA - 205 | 1.01 | decrease | 0.8265458 |

Note: $p < 0.05$ – statistically significant differences between the tumor tissue and adjacent morphologically intact tissue.

noted that one miRNA can act either as oncogene, or as oncosupressor depending on the target gene, as well as on the tissue in which it is expressed [9]. We analyzed the expression of nine miRNAs: miRNA-21, -221, -222, -155, -205, -20a, -125b, -146b, -200a in tumor tissue of luminal A and luminal B/HER2/neu-negative breast cancer subtypes compared to the adjacent morphologically unchanged tissue. We obtained statistically significant differences of expression levels for seven miRNA: miRNA-21, -221, -222, -155, -205, -125b, -200a. The most significant differences in tumor tissue and adjacent morphologically intact tissue (more than 3-fold) was observed for two oncogenic miRNAs: miRNA – 155 and miRNA - 21 and two oncosupressing miRNA: miRNA-205 and miRNA-125b (Table 3). Several studies have shown that miRNA-21 is an oncogene and inhibits tumor suppressors PTEN and Pdcd4 [16], and also correlates with the stage of malignancy [3]. Simultaneously, it was shown that miRNA-155 is also oncogenic and is involved in a variety of cellular processes such as regulation of proliferation, migration, invasion, epithelial-mesenchymal transition (EMT) and the immune response [14,18]. Wang et al. showed that in cultured breast cancer cells, overexpression of miRNA-205 inhibits proliferation, increases apoptosis and reduces the invasion of breast cancer cells, characterizing miRNA-205 as a oncosupressor [23]. MiRNA-125b is also an oncosupressor for breast cancer and reduces the proliferative activity of the cells [5,24]. Thus, miRNA-205 and 125b play a key role in the progression and development of breast cancer. It is noticeable that the level of expression of miRNA-200a increases almost 2-fold in tumor samples compared to adjacent morphologically intact tissue (Table 3). MiRNA-200 family regulates the EMT in different types of cancer, and correlates with the presence of lung metastases [10,21].

We assessed the expression of nine miRNAs: miRNA-21, -221, -222, -155, -205, -20a, -125b, -146b, -200a in tumor tissue in patients with triple-negative phenotype of breast cancer (TNBC) compared with the adjacent morphologically intact tissue. We obtained statistically significant differences for the levels of expression of the three miRNAs: miRNA-20a, -205, -125b (Table 4). Numerous studies have shown that miRNA-20a is oncogenic and is overexpressed in breast cancer [8]. We also observed a significant reduction (more than 3-fold) of the expression of 2 oncosupressing miRNAs: miRNA-125b and -205 in tumor tissue as compared to adjacent morphologically intact tissue. As described above, miRNA-205 and miRNA-125b are the key players in carcinogenesis, and their decreased expression is associated with poor prognosis in breast cancer. Furthermore, we observed a tendency to increase the level of expression of oncogenic miRNA-21 and miRNA-155 in the tumor tissue of triple negative breast cancer compared to adjacent morphologically intact tissue.

A comparison of levels in $2^{-\Delta\Delta Ct}$ selected group of miRNAs between patients with luminal subtypes of breast cancer and patients with TNBC was performed (Table 5).

We found that the level of expression of the oncogenic miRNA-221 is 43 times higher in TNBC than luminal A and luminal B/HER2 / neu-negative breast cancer subtypes ($p < 0.05$). MiRNA-221 is an oncogene and adjusts the two key mechanisms in tumor development: inhibits suppressor p27 and promotes EMT by inhibiting E-cadherin. In all probability, the miRNA-221 plays an important role in TNBC [17]. We also found that the level of oncogenic miRNA-20a was increased almost 7 times in TNBC compared with luminal subtypes of breast cancer ($p < 0.001$). Thus, increased expression of oncogenic miRNA-221 and miRNA-20a is a distinctive characteristic of the TNBC and characterizes its more aggressive development. ROC-analysis (Fig. 3) showed that the AUC (Area Under Curve), calculated by the trapezoidal rule for all microRNAs, is more than 0.8, which indicates the high quality of the models. Consequently, miRNA-21, -155, 205, -125b are good classifiers for assessment of malignant potential of breast cancers. ROC-analysis (Fig. 3) also revealed that the AUC for miRNA-221 is 0.772, indicating a high significance of this criterion in understanding the differences between triple-negative breast cancer and luminal subtypes. AUC value for miRNA-20a is 0.949, making it an excellent marker for TNBC.

## 4. Conclusion

The obtained results suggest the possibility of using the levels of miRNA-21, 155, 205, 125b expression in tumor tissue to assess a malignant potential of breast cancers. The levels of expression of oncogenic miRNA-221 and miRNA-20a are increased in TNBC compared with luminal A and luminal B / HER2 / neu-negative breast cancer subtypes, supporting the characteristic of TNBC as the most aggressive subtype of breast cancer. MiRNA-20a is a marker of TNBC compared with luminal subtypes of breast cancer.

### *4.1. Clinical practice points*

It is found that about 80% of the basal and three negative breast cancer coincide. But the triple negative breast cancer includes some histological types, the identification of which by basal keratins staining is not reproducible enough for widespread use. In the clinic, there are situations when the luminal subtypes of breast cancer, characterized by favorable course behave very aggressively, quickly progress and are fatal. Conversely, aggressive cancer with triple-negative phenotype proceeds indolent for years and does not require adjuvant therapy. In connection therewith, molecular genetic markers are needed for breast cancer subtypes differentiation. We found that the expression level of microRNA-221 is 43-fold higher, and level of microRNA-20 is 67 times higher in the tumor tissue of triple negative breast cancer compared to the luminal subtypes of breast cancer.

Formed microRNA expression profiles for a variety of molecular genetic subtypes of breast cancer will allow to individualize the treatment of patients with breast cancer.

## Conflict of interest

The authors declare that they have no conflict of interest.

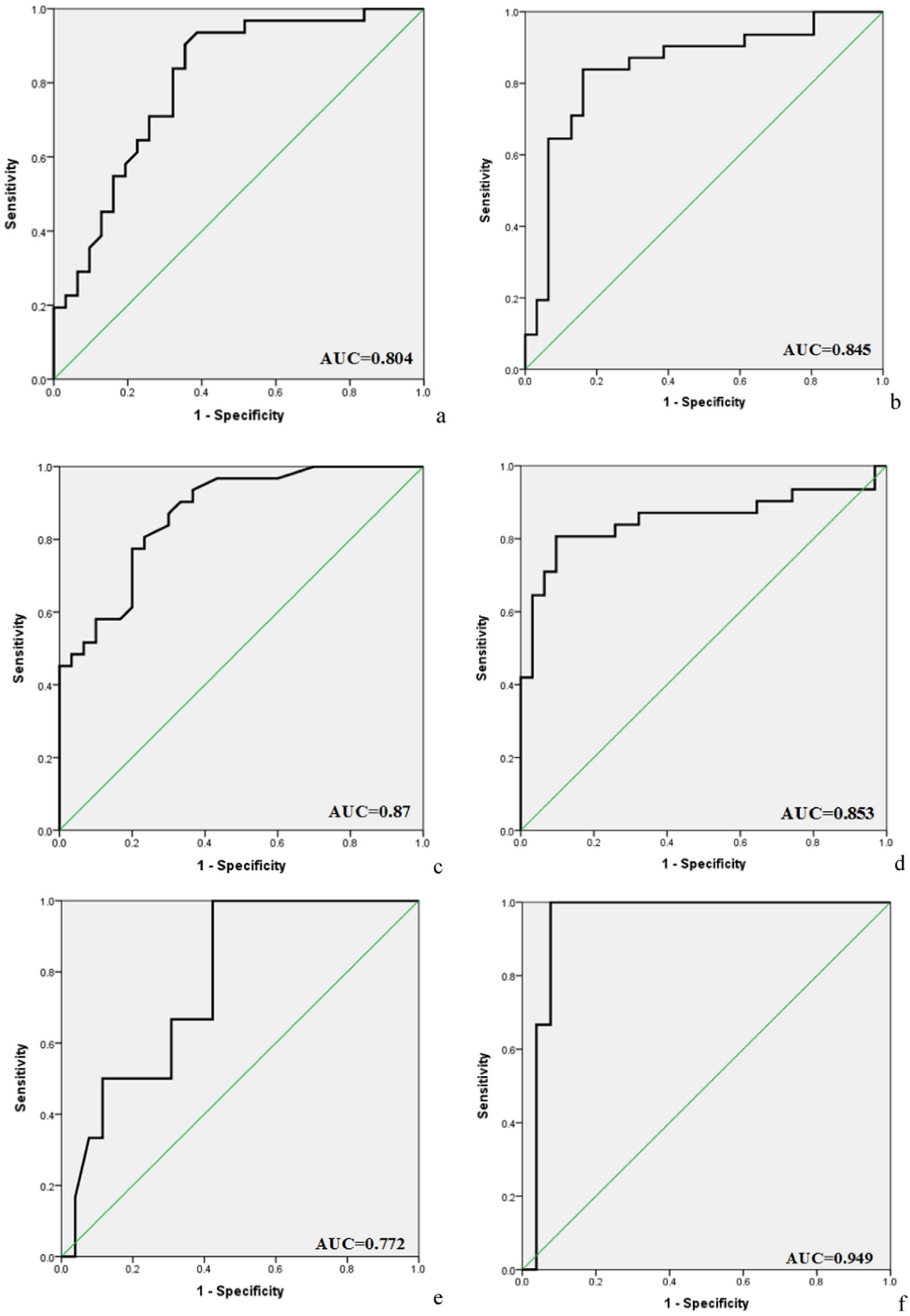


**Fig. 3.** ROC - analysis of miRNA - 21 (a), - 205 (b), - 155 (c), - 125b (d), - 221 (e), - 20a (f) for the differentiation of breast cancer and is adjacent conditional norm.

## Acknowledgments

This work was supported by a grant "Early Diagnosis and Prognosis of Human Cancers Based on microRNA Profiling" - Project 2.1.6. – 2015.

## References

[1] M. Bandopadhyay, A. Banerjee, N. Sarkar, R. Panigrahi, S. Datta, A. Pal, S. P. Singh, A. Biswas, S. Chakrabarti, R. Chakravarty, Tumor suppressor micro RNA miR-145 and onco micro RNAs miR-21 and miR-222 expressions are differentially modulated by hepatitis B virus X protein in malignant hepatocytes, BMC Cancer 14 (2014) 721.
[2] C. Chen, D.A. Ridzon, A.J. Broomer, et al., Real-time quantification of microRNAs by stem-loop RT-PCR, Nucleic Acids Res. 33 (20) (2005) 179.
[3] J. Chen, X. Wang, MicroRNA-21 in breast cancer: diagnostic and prognostic potential, Clin. Transl. Oncol. 16 (3) (2014) 225–233.
[4] A. Esquela-Kerscher, F.J. Slack, Oncomirs - microRNAs with a role in cancer, Nat. Rev. Cancer 6 (4) (2006) 259–269.
[5] A. Feliciano, J. Castellvi, A. Artero-Castro, et al., miR-125b acts as a tumor suppressor in breast tumorigenesis via its novel direct targets ENPEP, CK2-α, CCNJ, and MEGF9, PLoS One 8 (10) (2013) 76247.
[6] R.C. Friedman, K.K. Farh, C.B. Burge, D.P. Bartel, Most mammalian mRNAs are conserved targets of microRNAs, Genome Res. 19 (1) (2009) 92–105.
[7] D.R. Hurst, M.D. Edmonds, D.R. Welch, Metastamir: the field of metastasis-regulatory microRNA is spreading, Cancer Res. 69 (19) (2009) 7495–7498.
[8] K. Kim, G. Chadalapaka, S.O. Lee, et al., Identification of oncogenic microRNA-17-92/ZBTB4/specificity protein axis in breast cancer, Oncogene 31 (8) (2012) 1034–1044.
[9] N.N. Kolesnikov, S.E. Titov, Yu.A. Veryaskina, et al., MicroRNA evolution and cancer, Cytology 55 (3) (2013) 159–164.
[10] M. Korpal, B.J. Ell, F.M. Buffa, et al., Direct targeting of Sec23a by miR-200s influences cancer cell secretome and promotes metastatic colonization, Nat. Med. 17 (9) (2011) 1101–1108.
[11] M. Korpal, E.S. Lee, G. Hu, Y. Kang, 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. 283 (22) (2008) 14910–14914.
[12] N.I. Kosorotikov, V.S. Chorniy, MiRNA expression in different types of breast tumors, Sib. J. Oncol. 1 (2012) 83–84.
[13] A. Kozomara, S. Griffiths-Jones, miRBase: annotating high confidence microRNAs using deep sequencing data, Nucleic Acids Res. 42 (2014) D68–D73.
[14] S. Mattiske, R.J. Suetani, P.M. Neilsen, D.F. Callen, The oncogenic role of miR-155 in breast cancer, Cancer Epidemiol. Biomark. Prev. 21 (8) (2012) 1236–1243.
[15] R.R. Parikh, D. Housman, Q. Yang, D. Toppmeyer, L.D. Wilson, B.G. Haffty, Prognostic value of triple-negative phenotype at the time of locally recurrent, conservatively treated breast cancer, Int. J. Radiat. Oncol. Biol. Phys. 72 (4) (2008) 1056–1063.
[16] L. Qi, J. Bart, L.P. Tan, et al., Expression of miR-21 and its targets (PTEN, PDCD4, TM1) in flat epithelial atypia of the breast in relation to ductal carcinoma in situ and invasive carcinoma, BMC Cancer 9 (2009) 163.
[17] Rounak Nassirpour, P. Mehta Pramod, M. Baxi Sangita, Min-Jean Yin, miR-221 promotes tumorigenesis in human triple negative breast cancer cells, PLoS One 8 (4) (2013) 62170.
[18] Ryan M. O'Connell, D. Taganov Konstantin, P. Boldin Mark, Cheng Genhong, Baltimore David, MicroRNA-155 is induced during the macrophage inflammatory response, Proc. Natl. Acad. Sci. USA 104 (5) (2007) 1604–1609.
[19] M.Y. Shah, G.A. Calin, MicroRNAs miR-221 and miR-222: a new level of regulation in aggressive breast cancer, Genome Med. 3 (8) (2011) 56.
[20] M.A. Taipov, Z.N. Nikiphorova, K.P. Laktionov, N.E. Levchenko, Shevchenko VE. MicroRNA MiR-21 and MiR-155 are new markers for brest cancer, Tumors Female Reprod. Syst. 3-4 (2013) 6–11.
[21] J.P. Thiery, H. Acloque, R.Y. Huang, M.A. Nieto, Epithelial-mesenchymal transitions in development and disease, Cell 139 (5) (2009) 871–890.
[22] S. Volinia, G.A. Calin, C.G. Liu, et al., A microRNA expression signature of human solid tumors defines cancer gene targets, Proc. Natl. Acad. Sci. USA 103 (7) (2006) 2257–2261.
[23] Z. Wang, H. Liao, Z. Deng, P. Yang, N. Du, Y. Zhanng, H. Ren, miRNA-205 affects infiltration and metastasis of breast cancer, Biochem. Biophys. Res. Commun. 441 (1) (2013) 139–143.
[24] Y. Zhang, L.X. Yan, Q.N. Wu, et al., miR-125b is methylated and functions as a tumor suppressor by regulating the ETS1 proto-oncogene in human invasive breast cancer, Cancer Res. 71 (10) (2011) 3552–3562.
[25] S.R. Zheng, G.L. Guo, W. Zhang, G.L. Huang, X.Q. Hu, J. Zhu, Q.D. Huang, J. You, X.H. Zhang, Clinical significance of miR-155 expression in breast cancer and effects of miR-155 ASO on cell viability and apoptosis, Oncol. Rep. 27 (4) (2012) 1149–1155.
[26] S. Zhu, M.L. Si, H. Wu, Y.Y. Mo, MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1), J. Biol. Chem. 282 (19) (2007) 14328–14336.
[27] S. Zhu, H. Wu, F. Wu, D. Nie, S. Sheng, Y.Y. Mo, MicroRNA-21 targets tumor suppressor genes in invasion and metastasis, Cell Res. 18 (3) (2008) 350–359.