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Original Article

The Expression Levels of Circulating miR-129 and miR-203a in Association with Breast Cancer and Related Metastasis

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Abstract

Background: A significant part of deaths related to breast cancer is the result of invasion to other organs. It is essential to discover new non-invasive biomarkers to improve anticipation of recurrence risk in breast cancer patients. In this study, the plasma levels of miR-129 and miR-203a were evaluated to investigate their diagnostic potential in breast cancer and its metastasis.

Methods: In this case-control study, conducted in Tarbiat Modares University, Tehran, Iran, in 2019, Invasive Ductal Carcinoma blood samples were divided into 3 groups based on their stages as I, II/III, IV. Each group contained 30 individuals. We also recruited 30 normal individuals as a control group. Real-Time PCR was conducted to evaluate miR-129 and miR-203a expression levels. The discriminatory ability of the evaluated plasma miRNAs was assessed by ROC (Receiver Operating Characteristic) curves in breast cancer diagnosis and its metastasis.

Results: MiR-129 and miR-203a expression levels were significantly downregulated in breast cancer. Reducing tendency was observed in the mentioned miRNAs from less to more invasive stages. The expression level of miR-129 was decreased in metastatic than non-metastatic patients and it was significantly related to metastasis. A significant association between miR-129 expression level and lymph node status was also observed (P=0.04). Evaluation of ROC curves revealed that miR-129 and miR-203a were able to discriminate breast cancer fairly and poorly respectively. The ability of miR-129 in the diagnosis of breast cancer metastasis was poor.

Conclusion: MiR-129 and miR-203a may both act as tumor suppressor miRNAs. Our results need further evidence in a large population to be confirmed as diagnostic markers.

Keywords: Breast cancer; Metastasis; Circulating biomarkers; Mirn129 microRNA; Human

Introduction

Breast cancer is the most common type of cancer and the first cause of death related to cancer in women around the world (1). A significant part of deaths related to breast cancer is not due to the primary tumor itself, but are the result of invasion to other organs in the body (2). Metasta-

sis is an early event in breast cancer in which tumor cells are often silent for a long time. This long-term silencing will give us an opportunity for the detection of invasion before tumor cell re-growth and the spread of metastasis to other organs (3). Nowadays determining of solid tumor genetic profile is performed on biopsies, which may fail to show intra-tumoral heterogeneity and restrict the ability to explore genetic modification, which occurs during tumor evolution (4). Considering such problems, it is essential to discover new non-invasive biomarkers to improve anticipation of recurrence risk in BC patients (5).

MicroRNAs (miRNAs) are a group of short non-coding RNAs usually with 22 nucleotides length (6). A group of miRNA molecules that exist in the cell-free portion of the blood or other body fluids are called circulating miRNAs and have been focused tremendously in the field of biomarker discovery (7). Stability and resistance to endogenous RNase degradation and being obtained in a non-invasive manner are among their most important features (8). Recently stable circulating miRNAs identified in biological fluids such as serum or plasma have been used as promising biomarkers in BC (9-11). There is a meaningful association between circulating miRNA expression profile and metastasis in breast cancer, which makes these types of molecules suitable to be used as potential biomarkers in the diagnosis of metastasis (8).

MiR-129 family members are generally known as tumor suppressors which their expression is decreased in several types of tumors (12-14). MiR-129 plays an important role in Epithelial-Mesenchymal Transition (EMT), cell proliferation, apoptosis, autophagy and metastasis (15). Deregulation of miR-129 expression has been found to play important roles in the progression of different types of tumors such as breast cancer and it has also been shown that its expression is associated with patient survival (16-18). MiR-129-5p expression level is often significantly decreased in tissue biopsies of breast cancer (19) and also breast cancer patients with advanced clinical stage (13). It should be important to investigate its expression level changes in plasma samples of breast cancer and also metastatic and non-metastatic BC patients.

MiR-203a has important roles in the regulation of cell apoptosis and proliferation and it also controls angiogenesis and invasion (20). The expression of miR-203a is decreased in different types of cancer and acts as a tumor-suppressive miRNA in different tissues and cell lines (21). miRNA-203a was downregulated in many types of tumors such as breast cancer (22-25). MiR-203a expression is found to be negatively correlated with lymphatic metastasis (26). MiR-203a functional role and mechanistic action are largely unclear in BC (20). In some studies decreased expression levels were observed in metastatic BC cell lines (27) and tissues (28). It is worthwhile to investigate its expression levels in plasma samples of breast cancer and metastatic BC patients too.

To date, several studies identified miR-129 and miR-203a dysregulation in breast cancer tissues (13, 28, 29) but no such studies were performed in the Iranian population. As we know, the development of accurate non-invasive biomarkers can reduce the need for a large number of patients to undergo invasive or surgical procedures. In this study, we tried to evaluate the changes in the expression level of circulating miR-129 and miR-203a to explore their association with breast cancer. Furthermore, exploring the expression changes of these miRNAs in different stages can help to investigate their potential association with BC progression.

We aimed to analyze the association of mentioned miRNAs with BC metastasis and intended to exhibit the association between miRNAs expression level and clinicopathological features.

Materials and Methods

Patient samples

This investigation was a case-control study. Samples were obtained from Cancer Institute of Imam Khomeini Hospital, Tehran, Iran in 2019.

All samples were collected from consenting individuals according to the protocols approved by the Ethics Review Board of the Faculty of Medical Sciences of the Tarbiat Modares University.

Peripheral blood (5 ml) was collected from patients with newly diagnosed breast cancer who had received no chemo/radiotherapy before. Patients had no family history of BC in their first-degree relatives or personal history of BC or serious illnesses. The patients were randomly selected (90 women, age range 30–66 yr; mean age 47 yr) diagnosed with different stages of Invasive Ductal Carcinoma (IDC) based on their pathologic reports. The samples included 30 stage I patients as well as 30 stage II/III patients and finally 30 stage IV patients. Therefore, the patients subdivided into three groups: stage I, II/III, IV.

Thirty normal individuals (age range 30–55 yr; mean age 44 yr) were also included in our study. Controls were chosen among individuals with specific criteria such as lifestyle, medical history, smoking, alcohol consumption, etc. The clinicopathological parameters of the IDC samples were determined according to the TNM staging system (30).

Clinicopathological information of Patients

The clinicopathological parameters of the IDC samples were determined according to the TNM staging system. Based on features such as age (<47/≥47), tumor size (≤ 2/>2 cm) and lymph node status (Positive/Negative) patients were subdivided. The clinicopathological features are summarized in Table 1.

Table 1: Clinicopathological information of IDC patients

Variable	*Number of cases (%)*
Age (yr):	
<47	44 (48.88)
≥47	46 (51.11)
T stage:	
T1	38 (42.22)
T2	15 (16.67)
T3	19 (21.11)
T4	18 (20)
Lymph Node Involvement:	
Positive	54 (60)
Negative	36 (40)
Metastasis Status:	
Metastatic	30 (33.33)
Non-metastatic	60 (66.67)
Clinical TNM Staging:	
I	30 (33.33)
II & III	30 (33.33)
IV	30 (33.33)

IDC=Invasive Ductal Carcinoma.

Plasma Isolation and RNA extraction

Blood samples collected in K2 EDTA tubes were centrifuged using a two-step procedure (1,200 xg for 15 min, 16,000 xg for 10 min) to isolate plasma without contamination. Plasma hemolysis was checked by controlling absorbance ratio A414/A375 nm. The hemolysis ratio <2 was considered as hemolysis free plasma. Samples with hemolysis were excluded.

Total RNAs were extracted from 100 µl of plasma using the RNX-Plus solution (Sinaclon, Iran), according to the manufacturer's protocol. During

the procedure, after combining the plasma sample with Denaturing Solution, 3.5µl of miRNeasy Serum/Plasma Spike-In Control (synthetic **C. elegans** miR-39, 1.6 × 10^8 copies/µl, QIAGEN, Germany) was added to each denatured sample.

Reverse transcription and Real-Time PCR

Reverse Transcriptase kit (YTA, Iran) was used to synthesize cDNA using stem-loop primers, following the manufacturer's protocol. Briefly, the following reagents: total RNA, 1 pM stem-loop RT primer, Rnase-free water were added into a nuclease-free tube on ice. They were mixed gently and incubated at 70 °C for 5 min. Then 5x first-strand buffer, dNTPs (10 mM each), RNasin (40U/µl), M-MLV were prepared as cDNA synthesis mix. After adding this mix into the sterile tubes, they were incubated for 30 min at 16 °C and 60 °C for 42 min using PCR system (BIO-RAD, T100™ Thermal Cycler, Germany). Finally, the reaction was terminated by heating at 70 °C for 5 min.

Real-time polymerase chain reaction (PCR) was then carried out using Real-time PCR System (Applied Biosystems, Step one, USA), instrument according to the manufacturer's protocol. PCR reaction mixture contained 2X SYBR Green qPCR Mix reagent (Biofact, Taiwan), 10 pM of each forward and reverse primer, nuclease-free water and reverse transcription products. Then mixtures were incubated at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 30 sec and 72 °C for 10 seconds. Dissociation was performed as follows: 15 sec at 95 °C, 1 min at 60 °C, and finally 15 sec at 95 °C. The reactions were done in duplicate. Primer sequences used in this study are summarized in Table 2. MiR-1228 and *Cel-miR-39* were used as endogenous and exogenous controls respectively. Finally, the relative expression level of each miRNA was analyzed via the equation $2^{-\Delta\Delta Ct}$.

Table 2: Primer Sequences List

hsa-miR-129	***Stem loop***	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCAAGC*
	Forward	GGACGAGAGCTTTTTGCGGTCTG
hsa-miR-203a	***Stem loop***	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTAGTG
	Forward	CGTGCGGCGTGAAATGTTTAGGA
hsa-miR-1228	***Stem loop***	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACACA
	Forward	TATAATGTGGGCGGGGGCA
Cell-miR-39	***Stem loop***	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAAGCT
	Forward	GTGTCGCTCACCGGGTGTAAAT
Universal reverse primer	***Reverse***	CCAGTGCAGGGTCCGAGGTA

Statistical analysis

Results were analyzed using SPSS software, ver. 25 (IBM Corp., Armonk, NY, USA). The association between miRNAs expression levels and breast cancer and metastasis was calculated using the unpaired t-test. We also performed one way ANOVA and Kruskal-Wallis 1-way ANOVA (k samples) to analyze inter-group association. $P<0.05$ was considered as the criterion for statistically significant differences.

As a diagnostic test, Receiver Operating Characteristic (ROC) curve analysis was established to evaluate the sensitivity and specificity of miRNAs expression levels in plasma of BC and metastatic BC patients. ROC curves and the Area Under the Curve (AUC) with a 95% confidence interval

(95% CI) were generated to identify their discriminatory ability.

Results

The association of MiR-129 expression level with breast cancer

MiR-129 expression levels in Stage I, II/III and IV of Invasive Ductal Carcinoma showed a significant decrease in comparison with normal group with the following results: *P*=0.028, 0.002, 0.001 respectively. Inter-group comparison showed a reduction in expression level from stage I to stage II/III. This difference was not statistically significant (*P*=0.113). Decreased expression was also observed from stage II/III to stage IV, although it was not significant (*P*=0.357). A significant decrease was also found from stage I to stage IV (*P*=0.033). MiR-129 was reduced with BC progression in which it had the lowest expression in stage IV of disease in comparison with other groups.

Based on the data released from qPCR, miR-129 expression level was significantly decreased in plasma samples of BC patients in comparison with normal individuals (*P*=0.003). Therefore, miR-129 was significantly downregulated in breast cancer. Overall, these results indicated that miR-129 expression was associated with breast cancer.

MiR-129 expression analysis in breast cancer metastasis

To investigate the role of miR-129 in breast cancer metastasis, miR-129 expression level in stage I, II/III (as non-metastatic group) was compared with stage IV (as metastatic group). MiR-129 expression level was significantly decreased in the metastatic group (*P*=0.035). Therefore, miR-129 may have suppressive effects on the metastasis of breast cancer.

The association of MiR-203a expression level with breast cancer

Comparison of expression levels among different stages of breast cancer and their changes compared to the normal group indicated that miR-203a was downregulated in each stage compared to the normal group. Although only the difference between stage IV and control group was significant (*P*=0.005) and observed differences between stage I and stage II/III with control were not significant (*P*=0.383, 0.167 respectively). Data related to inter-stage comparison revealed that miR-203a expression level was reduced with BC progression, but this reduction was not statistically significant. Decreased expression was observed from stage I to stage II/III (*P*=0.487) and from stage II/III to stage IV (*P*=0.077).

Data released from qPCR also showed that miR-203a expression level was significantly reduced in plasma samples of breast cancer compared with normal individuals (*P*=0.023). Our data revealed miR-203a was significantly downregulated in breast cancer.

MiR-203a expression analysis in breast cancer metastasis

A non-significant reduced expression in miR-203a was observed in the metastatic group in comparison with the non-metastatic group (*P*=0.070). Therefore, miR-203a might also have suppressive effects on the metastasis of breast cancer investigated in a more precise manner.

Association between miRNAs expression levels and clinicopathological parameters

Based on clinicopathological parameters, samples subdivided into two groups: age (<47/ ≥47), tumor size (≤2/ >2) and lymph node involvement (Positive/Negative). Then the relation between miRNAs expression levels and clinicopathological characteristics was examined.

According to our investigations, no association between miRNAs expression levels and patient's age was observed: miR-129 (*P*=0.82) and miR-203a (*P*=0.39). The expression levels of the mentioned miRNAs did not show a significant association with tumor size in this study: miR-129 (*P*=0.07) and miR-203a (*P*=0.17). In the lymph node status survey, a statistically significant association between miR-129 expression level and lymph node status was observed (*P*=0.04) alt-

hough miR-203a expression levels were not found to be significantly associated with lymph node involvement (*P*=0.13).

Diagnostic performance of miR-129 and miR-203a in BC and BC metastasis.

As shown in Fig. 1, ROC curves were constructed to identify the diagnostic accuracy of miR-129 and miR-203a as non-invasive biomarkers in BC diagnosis and its metastasis.

The ROC curve analysis indicated that miR-129 and miR-203a were able to discriminate BC patients from healthy controls: miR-129 yielded an AUC of 0.751 (95% CI: 0.652–0.850; *P*=0.00) with 71.1% sensitivity and 66.7% specificity; miR-203a yielded an AUC of 0.694 (95% CI: 0.594–0.795, *P*=0.001) with 67.8% sensitivity and 66.7% specificity. Moreover, the combination results showed improved accuracy of two evaluated miRNAs as the AUC reached 0.783 (95% CI: 0.698-0.867, *P*=0.00), the sensitivity and the specificity were 71.1% and 70% respectively (Fig. 1A, B, C).

Analysis of ROC curves in miR-129 revealed that the plasma expression level of this miRNA was able to diagnose BC metastasis with a sensitivity and specificity of 66.7% and 66.7% respectively, AUC of 0.679 (95% CI=0.546-0.812, *P*= 0.006). The ROC analysis also showed that plasma miR-203a expression level couldn't differentiate metastatic BC patients from non-metastatic ones as *P-value* was 0.304 and the ROC curve yielded AUC value of 0.567 (95% CI: 0.447-0.687), with a sensitivity and specificity of 63.3% and 48.3% respectively (Fig. 1D, E).

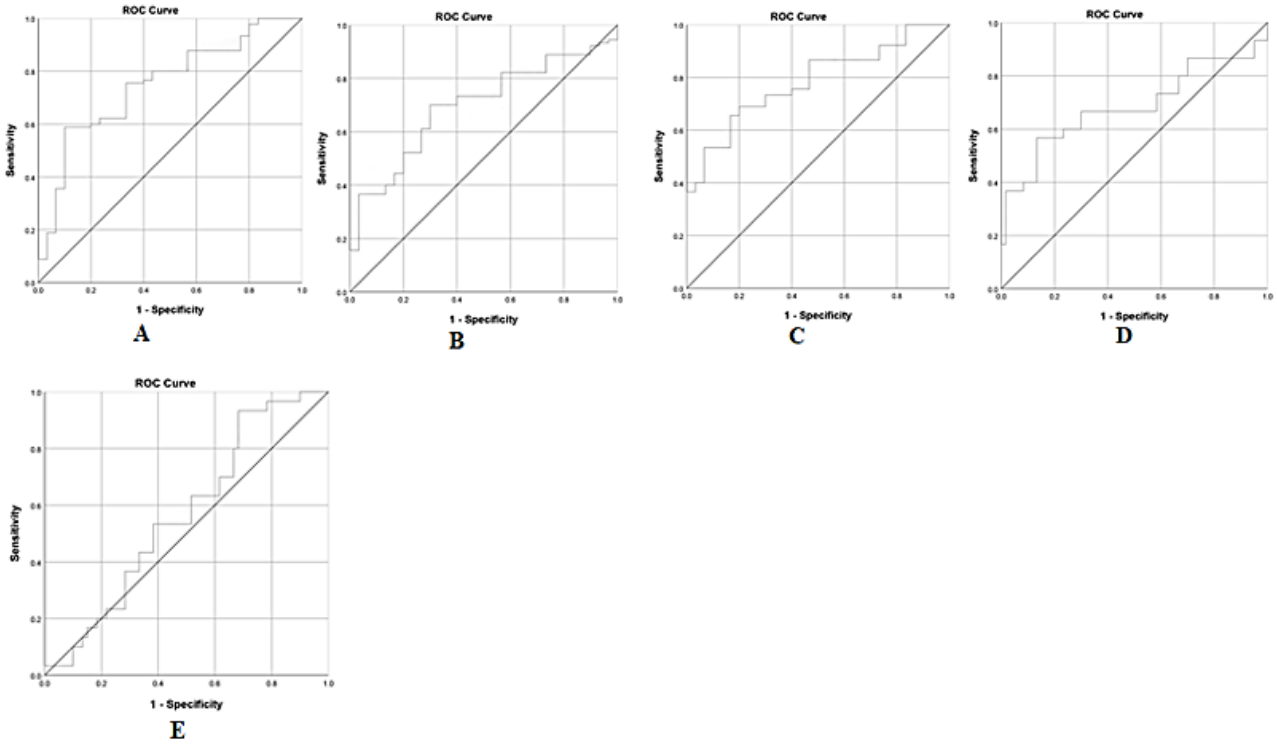

Fig. 1: ROC curve analysis of miR-129 and miR-203a in breast cancer and breast cancer metastasis. ROC curves were generated to evaluate the efficacy of miR-129 (A), miR-203a (B), combination of them (C), in BC diagnosis. A). The AUC was 0.751 (*P*=0.00), B). The AUC was 0.694 (*P*=0.001), C). The AUC reached 0.783 (*P*=0.00). ROC curves were also constructed to evaluate the accuracy of miR-129 (D), miR-203a (E) in breast cancer metastasis diagnosis. D). The AUC was 0.679 (*P*=0.006), E). The AUC was 0.567 (*P*=0.304). ROC= Receiver Operating Characteristic, AUC= Area under the Curve

Discussion

Our result indicated that the expression level of miR-129 was significantly associated with breast cancer. If its expression was downregulated among BC patients compared to the normal groups, this miRNA acts as a tumor suppressor miRNA in breast cancer. These results are in concordance with previous studies verified the downregulation of miR-129 in MCF-7 cell lines and tissues obtained from BC patients (13, 31). In this study, similar result was obtained in plasma samples. As stages of breast cancer progressed, the expression level of miR-129 decreased. Although this observation was not statistically significant except from stage I to stage IV, this regulated reduction can be related to breast cancer progression in our study. Insignificancy the mentioned results may be attributed to the small number of samples who were investigated. The involvement of more individuals may be useful to achieve significant results. Since miR-129 expression was significantly downregulated in the metastatic group rather than the non-metastatic group it can be inferred that, its expression is associated with breast cancer metastasis. The results in this regard are consistent with the results of some previous studies. MiR-129 expression level was associated with advanced clinical stages in breast cancer tissues and its expression will decrease tremendously in more advanced stages. In addition, its reduced expression was associated with poor prognosis and its overexpression suppressed breast cancer proliferation and metastasis (13). The downregulation of miR-129-5p plays a role in breast cancer metastasis (29). In the present study, similar results in plasma samples of metastatic IDC patients were observed. Therefore, it may be used as a diagnostic tool for effective early detection of BC metastasis. There was no significant association between miR-129 expression levels and factors such as age and tumor size. However, the plasma miR-129 expression level was significantly associated with lymph node status. According to the ROC curve analysis, miR-129 had a fair sensitivity and specificity in breast cancer diagnosis. In screening metastatic BC, miR-129 could reach to poor sensitivity and specificity. These results were consistent with qPCR results.

The present study revealed that circulating miR-203a was significantly declined in BC patients compared to controls. MiR-203a has a suppressive effect in BC. The results are consistent with other findings that achieved the same results in breast cancer tissues. They showed lower levels of miR-203a in breast cancer patients compared to the paired normal breast tissues (28). Statistical tests were done to compare plasma miR-203a expression levels between different stages (I, II/III, IV) in IDC patients. As the disease progressed, the expression declined but without statistical significance. Although miR-203 was highly expressed in less-invasive BC cell lines (20). Our results demonstrated the tendency of the expression levels of miR-203a between metastatic cases and non-metastatic ones were slightly different. The observed reduction in the metastatic group was not significant. Therefore, miR-203a expression level might be negatively associated with metastasis. However, miR-203 expression level was reduced in metastatic cell lines (27). Moreover, other study showed a lower expression level of miR-203 in metastatic tissues of breast cancer patients (28). In our study, non-significant results may be attributed to a small number of samples. No significant difference was observed in plasma miR-203a expression level between subgroups defined by clinicopathological parameters (age, tumor size, lymph node status). Using ROC curves analysis, revealed that miR-203a was able to diagnose BC patients from control individuals poorly and it could not reach a significant threshold to distinguish metastatic BC patients from non-metastatic ones. Breast cancer diagnostic ability was improved when miR-129 and miR-203a were combined.

There are still limitations to this study. First, the sample size was relatively small. In some cases, the observed changes were not significant, attributed to a small number of samples. Moreover, in the present study, the primary and metastatic samples were not matched. Examination of the

mentioned miRNAs in matched cases could provide a more comprehensive view of their roles in BC metastasis.

Conclusion

MiR-129 and miR-203a may both act as tumor suppressor miRNAs in BC. The results obtained in this study need further evidence in a large population to be confirmed as diagnostic markers.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflicts of interest

All authors declare that they have no conflict of interest.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011). Global cancer statistics. *CA Cancer J Clin*, 61(2):69-90.
2. Weigelt B, Peterse JL, van 't Veer LJ (2005). Breast cancer metastasis: markers and models. *Nat Rev Cancer*, 5(8):591-602.
3. Thames HD, Buchholz TA, Smith CD (1999). Frequency of first metastatic events in breast cancer: implications for sequencing of systemic and local-regional treatment. *J Clin Oncol*, 17(9):2649-58.
4. Bedard PL, Hansen AR, Ratain MJ, Siu LL (2013). Tumour heterogeneity in the clinic. *Nature*, 501(7467):355-64.
5. Papadaki C, Stratigos M, Markakis G, et al (2018). Circulating microRNAs in the early prediction of disease recurrence in primary breast cancer. *Breast Cancer Res*, 20(1):72.
6. Inns J, James V (2015). Circulating microRNAs for the prediction of metastasis in breast cancer patients diagnosed with early-stage disease. *Breast*, 24(4):364-9.
7. Chim SS, Shing TK, Hung EC, et al (2008). Detection and characterization of placental microRNAs in maternal plasma. *Clin Chem*, 54(3):482-90.
8. Hamam R, Hamam D, Alsaleh KA, et al (2017). Circulating microRNAs in breast cancer: novel diagnostic and prognostic biomarkers. *Cell Death Dis,* 8(9):e3045.
9. Schwarzenbach H, Nishida N, Calin GA, Pantel K (2014). Clinical relevance of circulating cell-free microRNAs in cancer. *Nat Rev Clin Oncol,* 11(3):145-56.
10. Mitchell PS, Parkin RK, Kroh EM, et al (2008). Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A*, 105(30):10513-8.
11. Turchinovich A, Weiz L, Langheinz A, Burwinkel B (2011). Characterization of extracellular circulating microRNA. *Nucleic Acids Res,* 39(16):7223-33.
12. Dyrskjøt L, Ostenfeld MS, Bramsen JB, et al (2009). Genomic profiling of microRNAs in bladder cancer: miR-129 is associated with poor outcome and promotes cell death in vitro. *Cancer Res,* 69(11):4851-60.
13. Yu Y, Zhao Y, Sun XH, et al (2015). Down-regulation of miR-129-5p via the Twist1-Snail feedback loop stimulates the epithelial-mesenchymal transition and is associated with poor prognosis in breast cancer. *Oncotarget*, 6(33):34423-36.

14. Tsai KW, Wu CW, Hu LY, et al (2011). Epigenetic regulation of miR-34b and miR-129 expression in gastric cancer. *Int J Cancer,* 129(11):2600-10.
15. Gao Y, Feng B, Han S, et al (2016). MicroRNA-129 in Human Cancers: from Tumorigenesis to Clinical Treatment. *Cell Physiol Biochem*, 39(6):2186-202.
16. Kang HS, Kim J, Jang SG, et al (2014). MicroRNA signature for HER2-positive breast and gastric cancer. *Anticancer Res*, 34(7):3807-10.
17. Zhang J, Li S, Yan Q, et al (2013). Interferon-beta induced microRNA-129-5p down-regulates HPV-18 E6 and E7 viral gene expression by targeting SP1 in cervical cancer cells. *PloS One*, 8(12):e81366.
18. Kang M, Li Y, Liu W, et al (2013). MiR-129-2 suppresses proliferation and migration of esophageal carcinoma cells through downregulation of SOX4 expression. *Int J Mol Med,* 32(1):51-8.
19. Xiao G, Li X, Li G, et al (2017). MiR-129 blocks estrogen induction of NOTCH signaling activity in breast cancer stem-like cells. *Oncotarget*, 8(61):103261-73.
20. He S, Zhang G, Dong H, Ma M, Sun Q (2016). MiR-203 facilitates tumor growth and metastasis by targeting fibroblast growth factor 2 in breast cancer. *Onco Targets Ther,* 9:6203.
21. Liu S, Feng P (2015). MiR-203 determines poor outcome and suppresses tumor growth by targeting TBK1 in osteosarcoma. *Cell Physiol Biochem,* 37(5):1956-66.
22. He JH, Li YM, Li YG, et al (2013). Hsa-miR-203 enhances the sensitivity of leukemia cells to arsenic trioxide. *Exp Ther Med*, 5(5):1315-21.
23. Zhang F, Yang Z, Cao M, et al (2014). MiR-203 suppresses tumor growth and invasion and down-regulates MiR-21 expression through repressing Ran in esophageal cancer. *Cancer Lett*, 342(1):121-9.
24. Furuta M, Kozaki KI, Tanaka S, Arii S, Imoto I, Inazawa J (2010). MiR-124 and miR-203 are epigenetically silenced tumor-suppressive microRNAs in hepatocellular carcinoma. *Carcinogenesis*, 31(5):766-76.
25. Taube JH, Malouf GG, Lu E, et al. Epigenetic silencing of microRNA-203 is required for EMT and cancer stem cell properties (2013). *Sci Rep*, 3:2687.
26. Tang R, Zhong T, Dang Y, Zhang X, Li P, Chen G (2016). Association between downexpression of MiR-203 and poor prognosis in non-small cell lung cancer patients. *Clin Transl Oncol*, 18(4):360-8.
27. Zhang Z, Zhang B, Li W, et al (2011). Epigenetic Silencing of miR-203 Upregulates SNAI2 and Contributes to the Invasiveness of Malignant Breast Cancer Cells. *Genes Cancer*, 2(8):782-91.
28. Zhao S, Han J, Zheng L, Yang Z, Zhao L, Lv Y (2015). MicroRNA-203 Regulates Growth and Metastasis of Breast Cancer. *Cell Physiol Biochem*, 37(1):35-42.
29. Meng R, Fang J, Yu Y, et al (2018). MiR-129-5p suppresses breast cancer proliferation by targeting CBX4. *Neoplasma,* 65(4):572-8.
30. Edge S BD, Compton C, Fritz A, Greene F, Trotti A (2009). *AJCC Cancer Staging Manual.* Springer.
31. Luan QX, Zhang BG, Li XJ, Guo MY (2016). MiR-129-5p is downregulated in breast cancer cells partly due to promoter H3K27m3 modification and regulates epithelial-mesenchymal transition and multi-drug resistance. *Eur Rev Med Pharmacol Sci,* 20(20):4257-65.