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Correlation between microRNA-21, microRNA-206 and estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 in breast cancer

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ABSTRACT

Background: Although the function of microRNA-21 and microRNA-206 in breast cancer cells have been investigated in vitro, their association with estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) are not reported.

Methods: ER, PR, HER2, and Ki-67 staining pattern were utilized to classify 75 breast cancer patients recruited. The malignancy was predicted with tumor nodes metastases (TNM) classification. RT-qPCR was performed to detect the relative expression of ER, PR, and HER2 in tumor samples and microRNA-21 and microRNA-206 in the serum. Spearman's correlation analysis was used to determine the association between different molecules. According to the staining pattern, the breast cancer patients were classified into five types.

Results: microRNA-21 was up-regulated in HER2 positive and Basal-like breast cancer types, while microRNA-206 was up-regulated in Luminal A and B types of breast cancer. microRNA-21 expression negatively correlated with the level of ER and PR but positively correlated with HER2 expression and tumor malignancy, while microRNA-206 showed the opposite trend. Neither microRNA-21 nor microRNA-206 showed any significant correlation with the age of the patients.

Conclusion: Both microRNA-21 and microRNA-206 closely correlate with ER, PR, and HER2 expression, which can be considered as clinical biomarkers.

1. Introduction

Breast cancer is among the most common invasive cancers in women, which affects about 12% of women [1]. Current clinical therapy incorporates clinical, pathological, and molecular information [2,3]. In clinical, breast cancer can be staged with the tumor nodes metastases (TNM) system, which is based on the size of the tumor (T), whether or not the tumor has spread to the lymph nodes (N) in the armpits, and whether the tumor has metastasized (M) [4,5]. Hormones receptor status, such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) will indicate prognosis and determine therapy options. Ki-67 is used as a measurement of the proliferative activity of breast cancer cells, while there is some disagreement as to the usefulness of Ki-67 as a prognostic indicator.

Beyond the sole established single molecule biomarker, multigene and multiple microRNA expression pattern analysis has been investigated to decipher the driver pathways and biology process to predict clinical outcome more accurately [4,6]. Studies have shown that microRNA-21 is highly expressed in some breast cancer patients and is associated with breast cancer metastasis and invasion in vitro [7,8]. microRNA-206 showed in vitro inhibitory effects on metastasis and invasion of breast cancer by targeting connexin 43 [9]. However, these experiments were basically in vitro experiments, which do not fully represent the situation of patients in vivo. Therefore, we collected 75 breast cancer patients' tissue and serum samples, grouped them according to ER, PR, HER2, and Ki-67 molecular typing, detected the relative expression of microRNA-21 and microRNA-206 in the samples, and calculated their correlation with ER, PR, HER2, and traditional TMN scores. This investigation will provide a theoretical basis for microRNA-21 and microRNA-206 to be used as the new molecular markers of breast cancer.

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2. Methods & materials

2.1. Participants enrolment

From 2018 January to 2018 May, 75 women diagnosed as breast cancer who underwent surgical treatment were recruited in this investigation in Chongqing Renji Hospital, University of Chinese Academy of Science. The TNM classification was followed by the 8th edition of the staging classification criteria issued by American Joint Committee on Cancer (AJCC) according to the presence and size of the tumor, its metastasis in the axillary lymph nodes, and other factors [10]. This investigation was approved by the Ethics Committee of Chongqing Renji Hospital, University of Chinese Academy of Science, and all the participants signed informed consent forms.

2.2. Immunohistochemistry

Surgery isolated breast tumor tissues without hemorrhage or necrosis were fixed in 4% paraformaldehyde (24 h, room temperature) and embedded in paraffin. In order to compensate for tumor heterogeneity, six sections from the same tumor sample were mixed. Antigen retrieval was performed by microwave-heating. The following primary antibodies were utilized: ER (ER1D5, Immunotech, Marseille, France), PR (PR-AT 4.14, Thermo Fisher Scientific, Inc., Rockford, IL, USA), and Ki-67 (20Raj1, Thermo Fisher Scientific). Histofine® One-Step Polymer Detection System (Nacalai USA, Inc) was used to develop the signal. The Hercep test was performed to determine HER2 status.

2.3. Real-time qPCR

miRNeasy (Qiagen, Shanghai, China) kit was utilized to extract total RNA from tissue and serum according to the manufacturer's protocol. The concentrations were assayed with NanoDrop 1000 (NanoDrop Technologies Inc., Wilmington, DE). TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA) was applied to perform microRNA-21, microRNA-206, ER, PR, and HER2 specific reverse transcription with 5 ng of total RNA, which was further amplified with the TaqMan microRNA Assay Kit on the StepOne System (Applied Biosystems) with the manufacturer's fast thermal cycling protocol. The comparative C_T ($2^{-\Delta\Delta CT}$) method was utilized to calculate the relative expression and the cut-off value was set up as 2.

2.4. Statistical analysis

Spearman's correlation analysis was utilized to determine the association between different molecular marker. One-way ANOVA analysis followed by a Tukey's post hoc test was used to determine the statistical differences between experimental groups. $P < .05$ was considered to be statistically significant.

3. Results

3.1. microRNA-21 and microRNA-206 demonstrate different expression patterns

Based on previously reported DNA microarray and immunohistochemical (IHC) classification criteria [11,12], breast cancer patients were divided into five groups in this study as indicated in Table 1: luminal A, luminal B(HER2+), luminal B(HER2-), HER2 positive, and basal-like type. The expression of microRNA-21 and microRNA-206 in the serum of 75 breast cancer patients from different group were detected with RT-PCR, and the results demonstrated that microRNA-21 was up-regulated in the HER2 positive and basal-like breast cancer groups (Fig. 1), which is consistent with the fact that microRNA-21 was considered as oncogenic miRNA in breast cancer [13]. While, microRNA-206 was up-regulated in the luminal A, luminal

Table 1
Clinical characteristics of the different group of breast cancer patients.

Type	Breast Cancer				
	Luminal A	Luminal B (HER2+)	Luminal B (HER2-)	HER2 Positive	Basal-like
ER	+	+	+	−	−
PR	+	+/−	+/−	−	−
HER2	−	+	−	+	−
Ki-67	< 14%	0–100%	≥14%	0–100%	0–100%
Number of patients	29	10	8	10	18

Estrogen Receptor, ER; Progesterone Receptor, PR; human epidermal growth factor receptor 2, HER2.

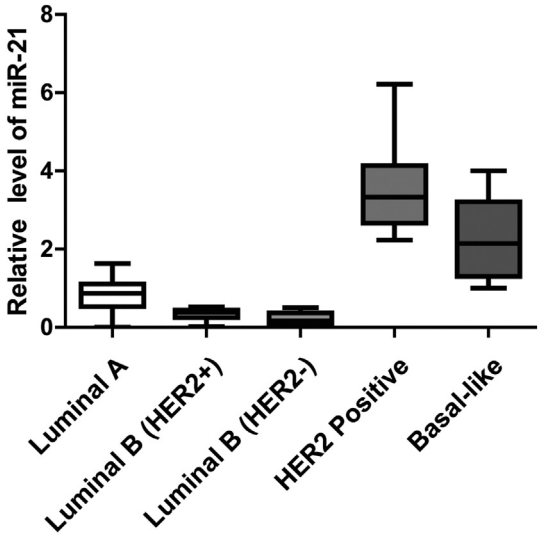

Fig. 1. miR-21 is up-regulated in HER2 positive and Basal-like breast cancer. Box and whisker plots showing the expression level of miR-21 in the serum of 75 breast cancer patients from different groups. The data range is represented by whiskers.

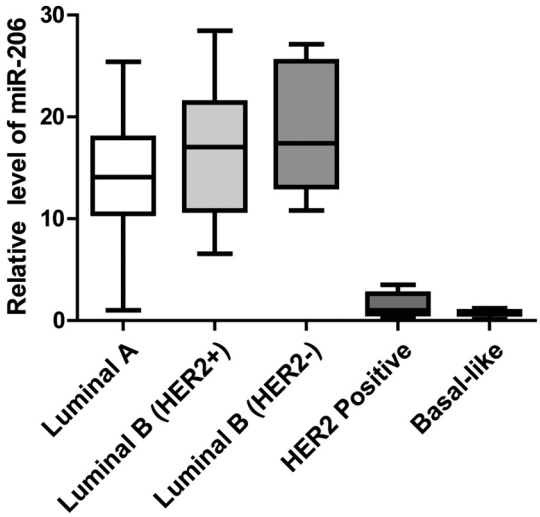

Fig. 2. miR-206 is up-regulated in Luminal A and B type breast cancer. Box and whisker plots showing the expression level of miR-206 in the serum of 75 breast cancer patients from different groups. The data range is represented by whiskers.

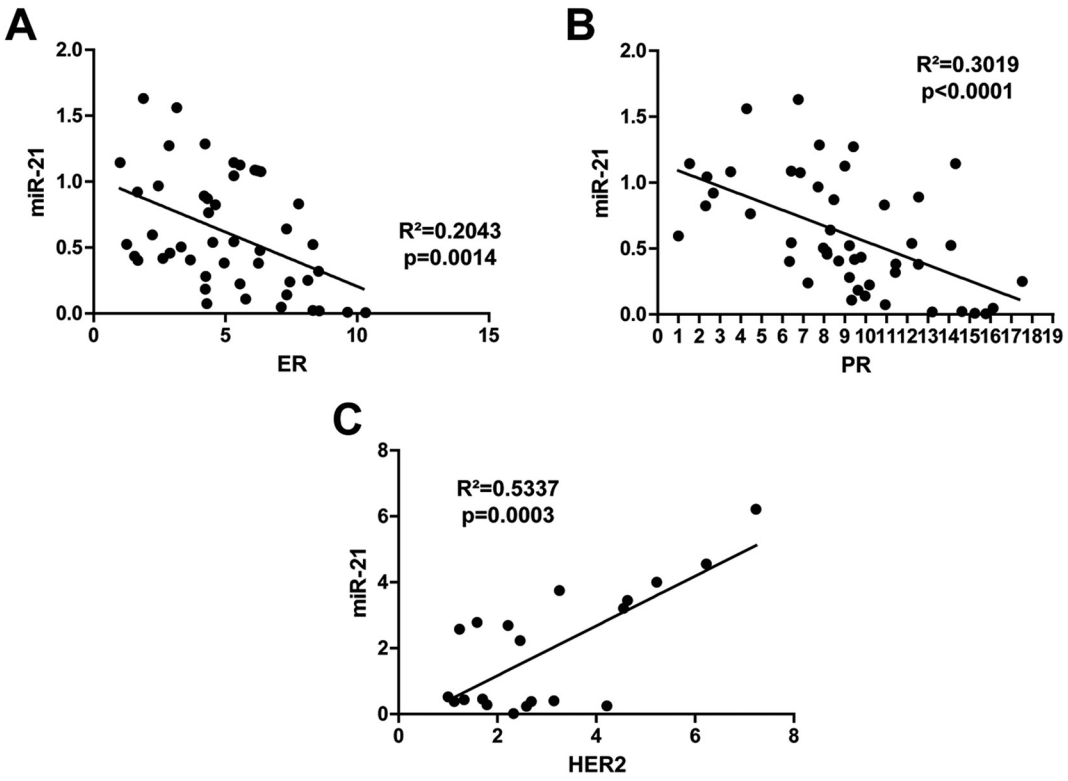

Fig. 3. miR-21 negatively correlates with the expression level of ER and PR but positively correlates with HER2.
The expression levels of miR-21 in the serum and the levels of ER, PR, and HER2 in the tumor tissues of different groups are determined by RT-qPCR. Spearman's correlation analysis of miR-21 with ER (A, in Luminal A, Luminal B (HER2+) and Luminal B (HER2-) groups), PR (B, in Luminal A, Luminal B (HER2+) and Luminal B (HER2-) groups) and HER2 (C, in Luminal B (HER2+) and HER2 positive groups) are showed in each panel. R2 and the *p*-value are also indicated in the panels.

B (HER2+), and luminal B (HER2-) types of breast cancer, and little can be detected in the HER2 positive and basal-like types of breast cancer (Fig. 2).

3.2. microRNA-21 expression negatively correlates with ER and PR but positively correlates with HER2

The relative expression of microRNA-21 in the serum and the levels of ER, PR, and HER2 in the tumor tissues of the different groups were determined by RT-PCR. Spearman's correlation analysis of microRNA-21 with ER in Luminal A, Luminal B (HER2+), and Luminal B (HER2-) groups showed negative correlation (Fig. 3A, $R^2 = 0.2043$, $p = .0014$), and such negative correlation was also observed in microRNA-21 with PR in Luminal A, Luminal B (HER2+), and Luminal B (HER2-) groups (Fig. 3B, $R^2 = 0.3019$, $p < .0001$). While microRNA-21 showed a positive correlation with HER2 in Luminal B (HER2+) and HER2 positive groups (Fig. 3C, $R^2 = 0.5337$, $p = .0003$).

3.3. microRNA-206 expression positively correlates with ER and PR but negatively correlates with HER2

Spearman's correlation analysis of microRNA-206 with ER in Luminal A, Luminal B (HER2+), and Luminal B (HER2-) groups showed positive correlation (Fig. 4A, $R^2 = 0.4125$, $p < .0001$), and ER also showed positive correlation with PR in Luminal A, Luminal B (HER2+), and Luminal B (HER2-) groups (Fig. 4B, $R^2 = 0.3775$, $p < .0001$). While, microRNA-206 showed a negative correlation with HER2 in Luminal B (HER2+) and HER2 positive groups (Fig. 4C, $R^2 = 0.2577$, $p = .0233$).

3.4. microRNA-21 and microRNA-206 correlate with the malignant signs in breast cancer

In addition to TNM staging, ER, PR, and HER2 status was already utilized to evaluate the prognosis of the disease and make the treatment decision. This investigation showed that microRNA-21 positively corrected with the malignancy of breast cancer based on TNM classification (Fig. 5A–C), while microRNA-206 showed a negative correlation (Fig. 6A–C). Neither microRNA-21 (Fig. 7A, $R^2 = 0.0158$, $p = .2829$) nor microRNA-206 (Fig. 7B, $R^2 = 0.0049$, $p = .5494$) showed any significant correlation with the age of the patients. All of these indicated that both microRNA-21 and microRNA-206 could be utilized as an indicator of breast cancer malignancy.

4. Discussion

In breast cancer clinical practice, ER and PR are the most useful metabolic markers which are utilized to predict the response to hormone therapy. Potential new markers such as HER2 and stearoyl-CoA desaturase-1 (SCD1) are used to predict the response to therapeutic regimens [6]. Thus, the assessment of ER and PR along with HER2 status is among the first approved immunostaining detection to revolutionize individualized breast cancer treatment. As an indicator of cancer cell proliferation, Ki67 is the most useful in hormone-sensitive breast cancers, while non-standardized immunohistochemistry staining and inter-laboratory variability have led the controversial interpretation [14]. It is worth noting that ER, PR, and Ki67 usually alter or change after neoadjuvant therapy and might be different when compared with the primary tumor, while HER2 seems to be more stably expressed. The relationship between such changes and the prognosis after primary systemic therapy is unknown [10,15,16].

As a family of small noncoding RNAs, microRNAs are sufficiently

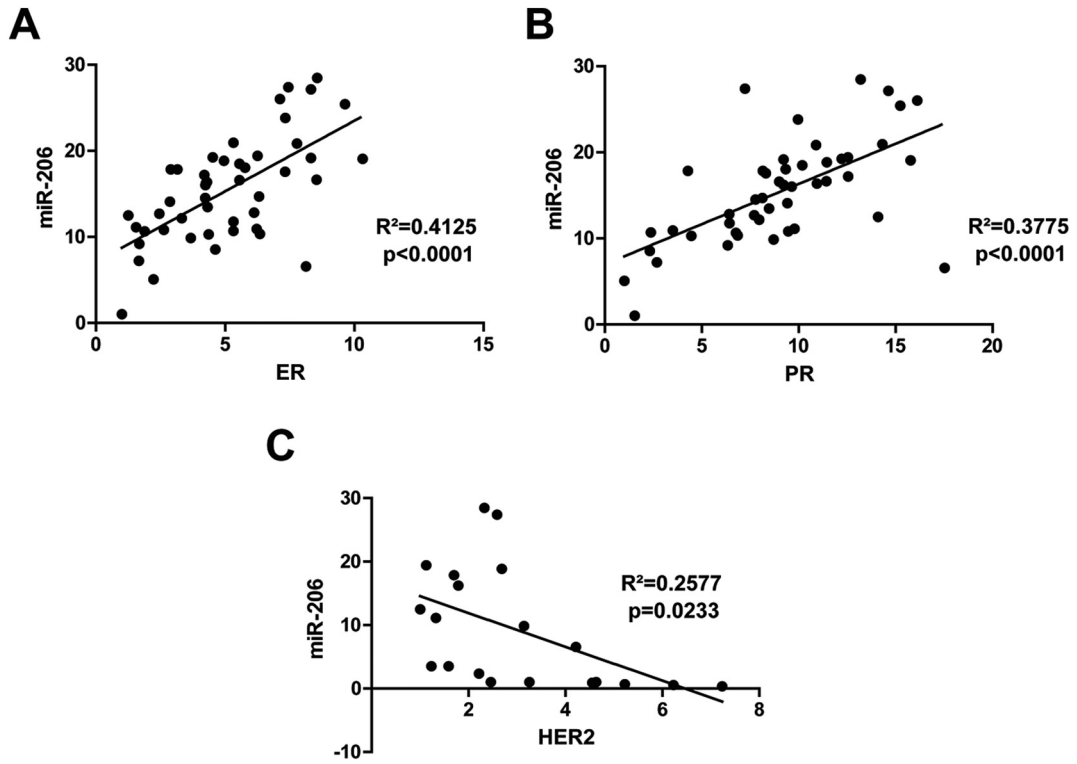

Fig. 4. miR-206 positively correlates with the expression level of ER and PR but negatively correlates with HER2.
The expression levels of miR-206 in the serum and the levels of ER, PR, and HER2 in the tumor tissues of different groups are determined by RT-qPCR. Spearman's correlation analysis of miR-206 with ER (A, in Luminal A, Luminal B (HER2+) and Luminal B (HER2-) groups), PR (B, in Luminal A, Luminal B (HER2+) and Luminal B (HER2-) groups) and HER2 (C, in Luminal B (HER2+) and HER2 positive groups) are showed in each panel. R2 and the p-value are also indicated in the panels.

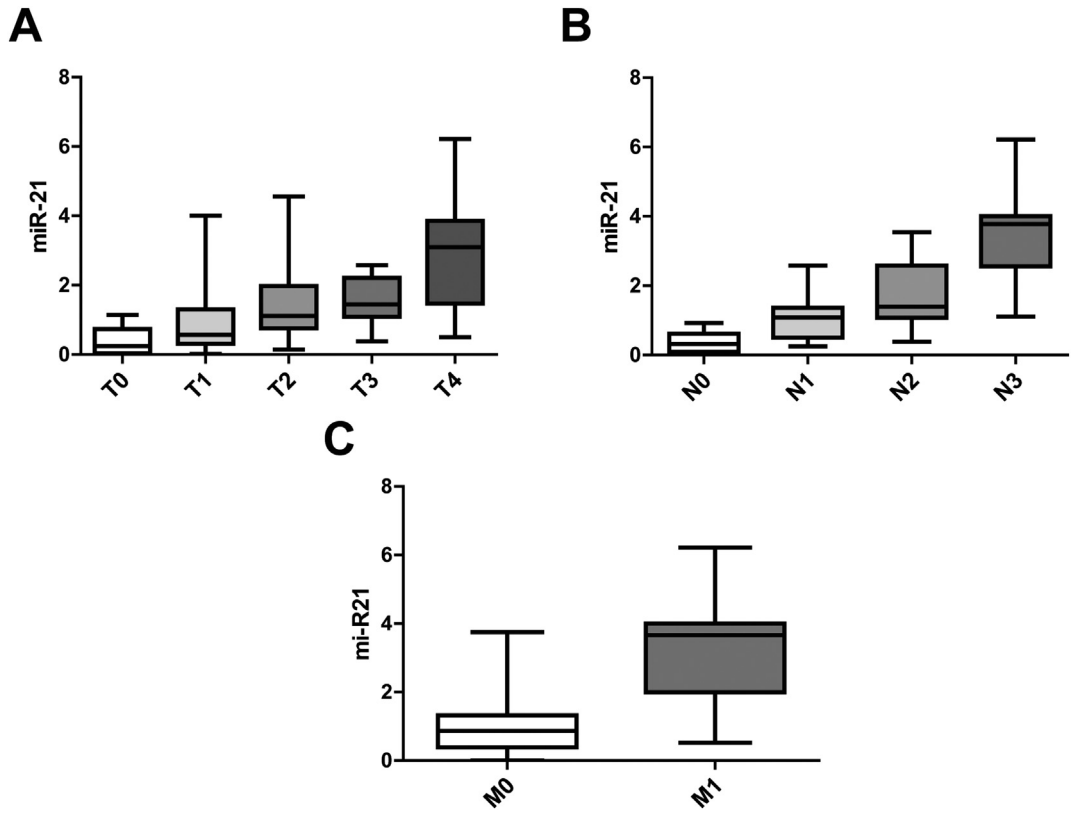

Fig. 5. miR-21 positively correlates with the malignant signs in breast cancer.
Box and whisker plots showing the expression level of miR-21 in the different stage of T (Tumor Size, A), N (Lymph Node Metastasis, B) and M (Distant Metastasis, C). The data range is represented by whiskers.

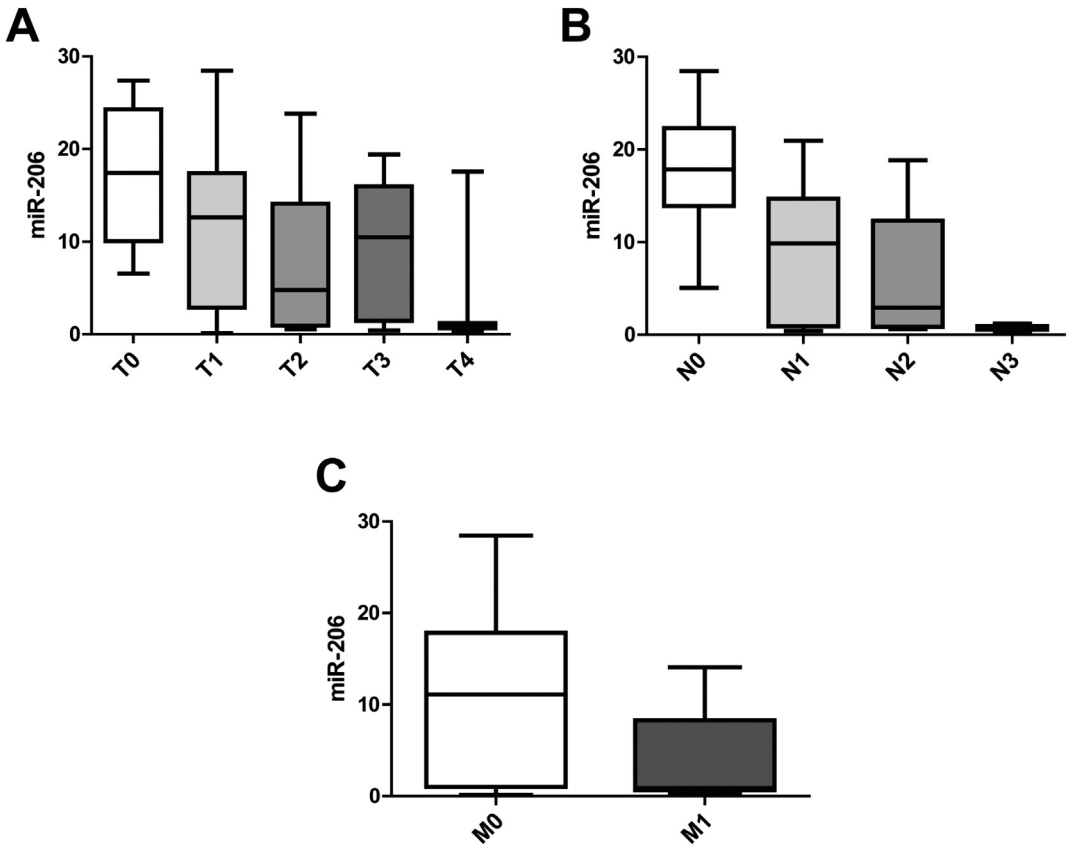

Fig. 6. miR-206 negatively correlates with the malignant signs in breast cancer.
Box and whisker plots showing the expression level of miR-206 in the different stage of T (Tumor Size, A), N (Lymph Node Metastasis, B) and M (Distant Metastasis, C). The data range is represented by whiskers.

stable to serve as clinical prognostic biomarkers to be detected in the serum. It has been assessed that > 50% of these noncoding RNA sequences are located on cancer-associated fragile sites, which can regulate main biological and physiological processes involved in tumorigenesis and development [17]. Our results indicate that microRNA-21 and microRNA-206 show opposite expression pattern and such expressions are correlated with ER, PR, and HER2 expression and the malignancy of breast cancer patients, which will point out the direction for future research in this field.

microRNA-206 can inhibit the expression of ERα through two binding sites in the 3' UTR region [18], while microRNA-206 can also be inhibited by ERα agonists, but not by progesterone or ERβ agonist, all of these suggest the existence of a feedback loop lying between microRNA-206 and ERα. Moreover, microRNA-206 is also testified to induce cell cycle arrest and inhibit estrogen-induced proliferation and motility [19–22]. Since ERα expression dysregulation can be considered to be a hallmark of breast cancers, these findings indicate that miR-206 could be a novel candidate for breast cancer therapy.

microRNA-21 is consistently overexpressed in breast cancer [23] and is considered as oncogenic miRNA to be involved in tumor growth, invasion, and metastasis, which can target tumor suppressor protein tropomyosin 1 (TPM1), programmed cell death-4 (PDCD4), phosphatase and tensin homolog (PTEN), and Maspin [24–27]. In addition, both microRNA-206 and microRNA-21 pathways are involved in the anti-angiogenesis process of the interval exercise treatment with hormone therapy [28]. All of these suggest that microRNA-21 and microRNA-206

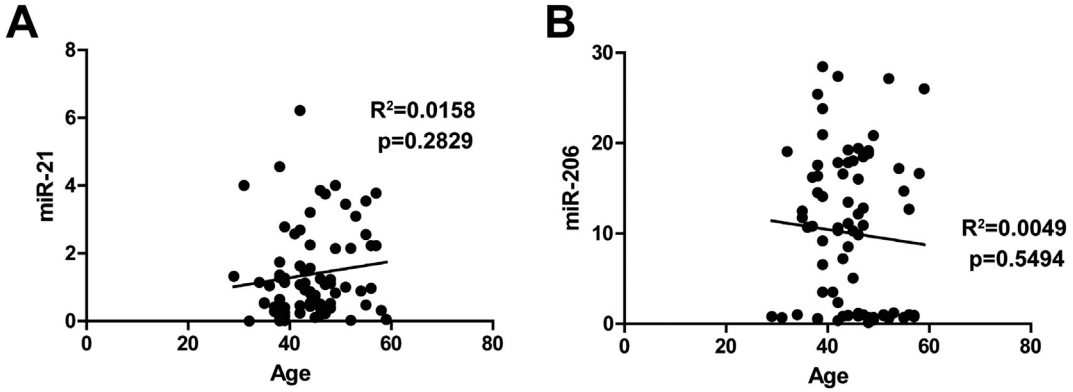

Fig. 7. miR-21 or miR-206 does not correlate with age of the patients.
Spearman's correlation analysis of miR-21 (A) or miR-206 (B) with the age of the patients. R2 and the p-value are also indicated in the panels.

can be utilized as new molecular markers to decipher the malignancy and determine individualized medical treatment.

It is worth noting that there are some limitations for the current study. First, other microRNAs may be involved as well. It would be nice to examine other microRNAs in the future studies. Second, the number of the participants is relatively small. The conclusion would be strengthened if more participants are enrolled. Last, evaluation of other histochemical characters in independent patient could be introduced to further correspond to the expressions of microRNA-21 and microRNA-206.

Despite the need for large-scale clinical data to confirm, microRNA-21 and microRNA-206 may be utilized as clinical biomarkers.

Disclosure of potential conflicts of interest

The authors have declared that no conflicts of interest exist.

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References

[1] A. Mcguire, J.A.L. Brown, C. Malone, R. Mclaughlin, M.J. Kerin, Effects of age on the detection and management of breast cancer, Cancers. 7 (2015) 908–929.
[2] M.T. Weigel, M. Dowsett, Current and emerging biomarkers in breast cancer: prognosis and prediction, Endocr. Relat. Cancer 17 (2010) R245–R262.
[3] A. Nicolini, P. Ferrari, M.J. Duffy, Prognostic and predictive biomarkers in breast cancer: past, present and future, Semin. Cancer Biol. 52 (2017) 56–73.
[4] M.J. Duffy, N. Harbeck, M. Nap, R. Molina, A. Nicolini, E. Senkus, et al., Clinical use of biomarkers in breast cancer: updated guidelines from the european group on tumor markers (egtm), Eur. J. Cancer 26 (2016) 43–51.
[5] S.B. Lee, G. Sohn, J. Kim, I.Y. Chung, J.W. Lee, H.J. Kim, et al., A retrospective prognostic evaluation analysis using the 8th edition of the american joint committee on cancer staging system for breast cancer, Breast Cancer Res. Treat. 23 (2018) 1–10.
[6] M.J. Duffy, Biochemical markers in breast cancer: which ones are clinically useful? Clin. Biochem. 34 (2001) 347–352.
[7] E.K.O. Ng, L. Rufina, V.Y. Shin, J. Hong Chuan, C.P.H. Leung, E.S.K. Ma, et al., Circulating micrornas as specific biomarkers for breast cancer detection, PLoS One 8 (2013) e53141.
[8] M. Han, M. Liu, Y. Wang, Z. Mo, X. Bi, Z. Liu, et al., Re-expression of mir-21 contributes to migration and invasion by inducing epithelial-mesenchymal transition consistent with cancer stem cell characteristics in mcf-7 cells, Mol. Cell. Biochem. 363 (2012) 427–436.
[9] Y. Fu, B.Q. Jiang, Y. Wu, Z.D. Li, Z.G. Zhuang, Hsa-mir-206 inhibits the migration and invasion of breast cancer by targeting cx43, Zhonghua Yi Xue Za Zhi 93 (2013) 2890–2894.
[10] B. Zhou, L. Xu, J. Ye, L. Xin, X. Duan, Y. Liu, The prognostic value of the 8th edition of the american joint committee on cancer (ajcc) staging system in her2-enriched subtype breast cancer, a retrospective analysis, Anticancer Res. 37 (2017) 4615–4621.
[11] M. Fan, R. Krutilina, J. Sun, A. Sethuraman, C.H. Yang, Z.H. Wu, et al., Comprehensive analysis of microrna (mirna) targets in breast cancer cells, J. Biol. Chem. 288 (2013) 27480–27493.
[12] O.N. Torsten, D.H. Forrest, J. Kristin, C. Maggie, K. Gamze, H. Zhiyuan, et al., Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma, Clin. Cancer Res. 10 (2004) 5367–5374.
[13] M.V. Iorio, C.M. Croce, Micrornas in cancer: small molecules with a huge impact, J. Clin. Oncol. 27 (2009) 5848–5856.
[14] D. Mitch, Nielsen TO, A.H. Roger, B. John, R. Charles, C. Jack, et al., Assessment of ki67 in breast cancer: recommendations from the international ki67 in breast cancer working group, J. Natl. Cancer Inst. 103 (2011) 1656–1664.
[15] F. Acevedo, M. Camus, C. Vial, S. Panay, M. Abarca, F. Domínguez, et al., Predictive value of conventional immuno-histochemical biomarkers in breast cancer, Revista Medica De Chile. 143 (2015) 724.
[16] J.B. Nikas, W.C. Low, P.A. Burgio, Prognosis of treatment response (pathological complete response) in breast cancer, Biomark. Insights 7 (2012) 59–70.
[17] M. Khordadmehr, R. Shahbazi, H. Ezzati, F. Jigari-Asl, S. Sadreddini, B. Baradaran, Key micrornas in the biology of breast cancer; emerging evidence in the last decade, J. Cell. Physiol. 234 (2019) 8316–8326.
[18] B.D. Adams, H. Furneaux, B.A. White, The micro-ribonucleic acid (mirna) mir-206 targets the human estrogen receptor-α (erα) and represses erα messenger rna and protein expression in breast cancer cell lines, Mol. Endocrinol. 21 (2007) 1132–1147.
[19] S.F. Tavazoie, A. Claudio, O. Thordur, P. David, W. Qiongqing, P.D. Bos, et al., Endogenous human micrornas that suppress breast cancer metastasis, Nature. 451 (2008) 147–152.
[20] O.D. Elizabeth, L. Ashish, Micrornas and their target gene networks in breast cancer, Breast Cancer Res. 12 (2010) 1–10.
[21] N. Kondo, T. Toyama, H. Sugiura, Y. Fujii, H. Yamashita, Mir-206 expression is down-regulated in estrogen receptor alpha-positive human breast cancer, Cancer Res. 68 (2008) 5004.
[22] Z. Kos, D.J. Dabbs, Biomarker assessment and molecular testing for prognostication in breast cancer, Histopathology. 68 (2016) 70–85.
[23] M.L. Si, S. Zhu, H. Wu, Z. Lu, F. Wu, Y.Y. Mo, Mir-21-mediated tumor growth, Oncogene. 26 (2007) 2799–2803.
[24] L. Qi, J. Bart, L.P. Tan, I. Platteel, T.V.D. Sluis, S. Huitema, 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.
[25] Z. Shuomin, S. Min-Liang, W. Hailong, M. Yin-Yuan, Microrna-21 targets the tumor suppressor gene tropomyosin 1 (tpm1), J. Biol. Chem. 282 (2007) 14328–14336.
[26] 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 (2008) 350–359.
[27] L.B. Frankel, N.R. Christoffersen, A. Jacobsen, M. Lindow, A. Krogh, A.H. Lund, Programmed cell death 4 (pdcd4) is an important functional target of the microrna mir-21 in breast cancer cells, J. Biol. Chem. 283 (2008) 1026–1033.
[28] A. Isanejad, A.M. Alizadeh, S.S. Amani, H. Khodayari, S. Khodayari, V. Khori, et al., Microrna-206, let-7a and microrna-21 pathways involved in the anti-angiogenesis effects of the interval exercise training and hormone therapy in breast cancer, Life Sci. 151 (2016) 30–40.