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ORIGINAL ARTICLE

The Diagnostic Value of Serum miR-129 in Breast Cancer Patients with Bone Metastasis

Chongqiang Wang [1, *], Liwen Pang [2, *], Qiang Shi [1], Xiaocheng Liu [2], Yan Liu [1]

[*] *These authors made equal contributions*
[1] *Department of Ultrasound, Second Affiliated Hospital of Shandong First Medical University, Taian City, Shandong Province, China*
[2] *Ultrasound Department, Zaozhuang Municipal Hospital, Shizhong District, Zaozhuang City, Shandong Province, China*

SUMMARY

Background: **The current study aims to investigate the expression of serum miR-129 in breast cancer patients with bone metastasis and to further study its diagnostic role.**
Methods: **Serum samples of 60 patients with bone metastasis of breast cancer and 60 patients with non-bone metastasis of breast cancer were collected. The expression of serum miR-129 in breast cancer patients was detected by real-time fluorescence quantitative polymerase chain reaction (RT-qPCR). The relationship between miR-129 and bone metastasis or bone pain in breast cancer patients with bone metastasis was analyzed. Receiver operating characteristic (ROC) curve was performed to analyze the diagnostic role of serum miR-129 expression in breast cancer patients with bone metastasis.**
Results: **The expression of miR-129 in serum of breast cancer patients with bone metastasis was significantly lower than that of non-bone metastasis patients. Furthermore, the lower the expression level of serum miR-129 was, the higher the degree of bone metastasis and bone pain was found in breast cancer patients. The ROC curve showed that the area under the curve (AUC) of miR-129 expression in serum for diagnosis of bone metastasis of breast cancer was 0.941 (95% CI: 0.891 - 0.991) with the sensitivity and specificity of 87.5% and 91.7%, respectively.**
Conclusions: **In summary, decreased serum miR-129 in breast cancer patients can be used as a potential diagnostic marker of bone metastasis in breast cancer patients.**
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Correspondence:
Ms. Yan Liu
Department of Ultrasound
Second Affiliated Hospital of
Shandong First Medical University
Taishan Street # 706
Taian City
Shandong Province
China
Email: caichao0606@163.com

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KEY WORDS

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INTRODUCTION

Breast cancer is one of the most common malignant tumors in women all over the world, which is also an important cause of female death [1]. About 1.2 million women worldwide are diagnosed with breast cancer every year, and about half a million people die of breast cancer every year [2]. Bone is the earliest and most common site of metastasis in breast cancer patients [3]. Bone metastasis accounts for 27% to 50% of the breast cancer patients with distant metastasis [3]. In addition, the incidence of bone metastasis in the process of recurrent breast cancer metastasis is 65% - 70% [4]. Once bone metastasis occurs in breast cancer patients, the dis-

ease enters an incurable stage [4]. The median survival time is only 2 years and the 5-year survival rate is only 20% [5]. Therefore, the diagnosis of bone metastasis among breast cancer patients is of great significance. MicroRNA (miR) is a kind of non-coding small single-stranded RNA with a length of 17 - 25 nucleotides [6]. It plays an important role in the occurrence and development of tumors by pairing with the specific base of target genes and down-regulating the expression of target genes. The abnormal expression of miRs is closely related to the occurrence and development of malignant tumors [7]. Therefore, miRs are considered as tumor-specific diagnostic markers and new targets for treatment [8]. miR-129 is widely reported to be down-regulated in breast cancer patients via targeting different target genes, including SOX2 and CBX4 [9,10]. However, whether miR-129 was associated with bone metastasis in breast cancer patients has never been explored. In this study, we analyzed the expression of miR-129 in serum of breast cancer patients with bone metastasis, and explored the diagnostic value of miR-129 in breast cancer patients with bone metastasis.

MATERIALS AND METHODS

Patient samples

A total of 120 breast cancer patients, aged 35 to 79, with an average age of 48.5 years, were admitted to the Second Affiliated Hospital of Shandong First Medical University from July 2017 to December 2017. All cases were confirmed to be breast cancer by pathological and histological data. All patients with bone metastasis of breast cancer were confirmed to have at least one metastasis, accompanied by pain caused by bone metastasis via the imaging examination method. Among them, there were 60 patients with bone metastasis of breast cancer and 60 patients with non-bone metastasis of breast cancer. All patients had not been treated with surgery, radiotherapy, chemotherapy, and endocrine therapy before blood collection. According to the visual analogue score of bone pain, patients with bone metastasis were divided into three subgroups: mild bone pain group with 0 - 3 scores, moderate bone pain group with 4 - 6 scores, and severe bone pain group with 7 - 10 scores. According to the number and size of bone metastases indicated by bone imaging, patients with bone metastases were divided into three subgroups: 1 - 6 metastases were considered as grade 1; 7 - 20 metastases were considered as grade 2; more than 20 metastases were considered as grade 3. The study was approved by the ethics committee of the Second Affiliated Hospital of Shandong First Medical University. All participants were informed and signed the informed consent form.

Sample collection

The peripheral venous blood of breast cancer patients with bone metastasis and non-bone metastasis were collected by vacuum blood collection without anticoagulant. The blood was centrifuged at 1,500 g at 4°C for 20 minutes. The serum was separated into pre-cooled non-RNA enzyme cryopreservation tubes and quickly frozen in liquid nitrogen.

RNA Extraction and Real-time Fluorescence Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA in serum samples was extracted by RNAVzol LS (Vigorous Biotechnology Beijing Co., Ltd Beijing, China) according to the instructions. Then, 1 μg total RNA was reverse transcribed using Mir-X™ miRNA First Strand Synthesis Kit (638313, Transgen Biotech, Beijing, China). qPCR was detected by SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using an ABI 7500 fluorescence quantitative PCR instrument (ABI). The total volume of the reaction system was 20 μL, including 2.0 μL cDNA, 1.0 μL forward primer, 1.0 μL reverse primer, 10 μL 2 x mix, and 6.0 μL ddH_2O. The amplification conditions of qPCR were: pre-denaturation at 95°C for 5 minutes, then 40 cycles of denaturation at 95°C for 30 seconds and denaturation at 60°C for 30 seconds. U6 was used as an internal reference. Three repeated experiments were carried out for each sample. Relative mRNA expression was normalized to U6 using the $2^{-\Delta\Delta Cq}$ method [11].

Statistical analysis

The data are represented as the mean ± standard deviation (SD). The two-tailed unpaired Student's *t*-tests were used for comparisons of two groups. The one-way ANOVA multiple comparison test (SPSS 20.0) followed by Tukey's post hoc test were used for comparisons of two or more groups. Receiver operating characteristic (ROC) curves were used to assess miR-129 as a biomarker, and the area under the curve (AUC) was reported (IBM SPSS Statistics for Windows, Version 20.0; IBM Corp, Armonk, NY, USA). $p < 0.05$ was considered significant.

RESULTS

Reduced level of serum miR-129 in breast cancer patients with bone metastasis

Compared with breast cancer patients with non-bone metastasis (1 ± 0.69), the level of serum miR-129 in breast cancer patients with bone metastasis showed a significant downward trend (0.23 ± 0.14) (Figure 1).

Serum miR-129 was decreased along with the severity of bone metastasis and bone pain in breast cancer patients with bone metastasis

To further study the relationship between the relative expression of miR-129 and the degree of bone metastasis and bone pain of breast cancer, the patients with bone metastasis of breast cancer were classified and grouped according to the degree of bone metastasis and bone pain. As shown in Figure 2A, lowest serum miR-129 was found in the breast cancer patients with severe

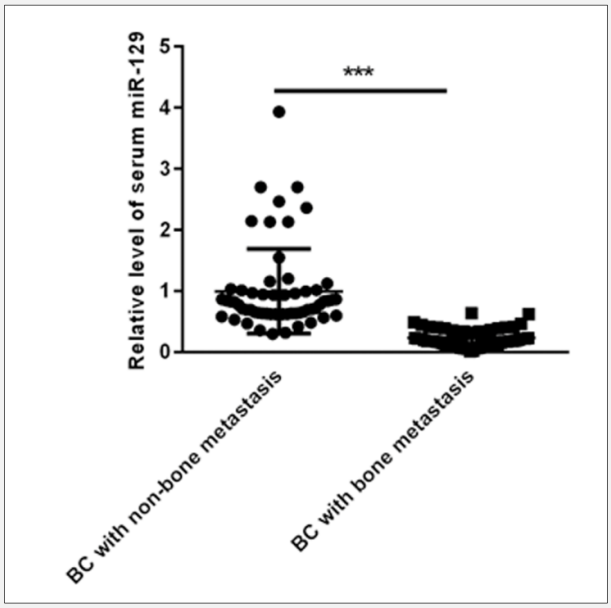

Figure 1. Real-time PCR analysis showed that the level of serum miR-129 was decreased in breast cancer patients with bone metastasis compared with breast cancer patients with non-bone metastasis.

*** **- p < 0.001 vs. breast cancer patients with non-bone metastasis.**

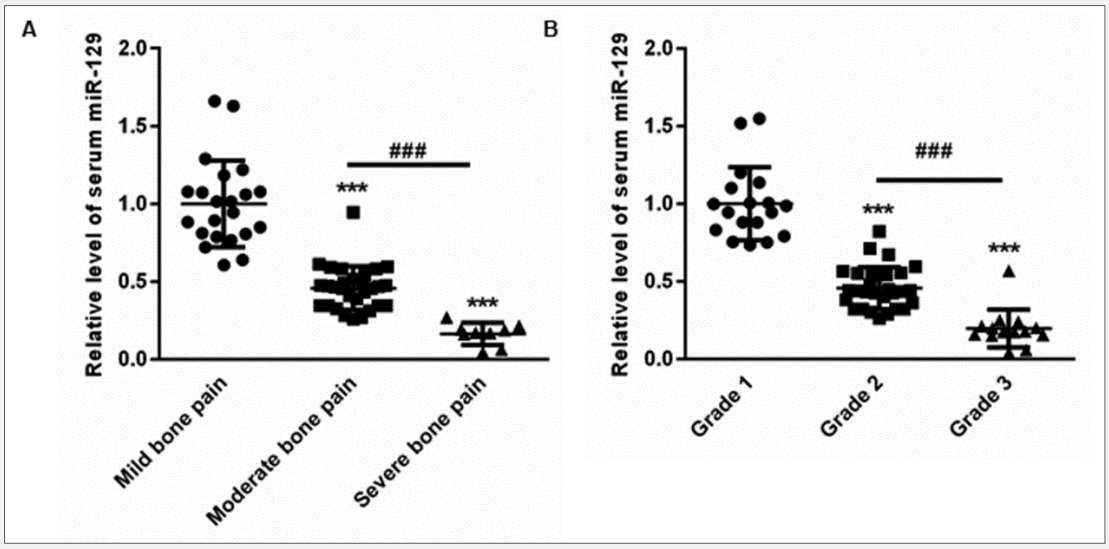

Figure 2. The level of miR-129 was detected according to the degree of bone metastasis and bone pain in breast cancer patients with bone metastasis.

(A) The level of miR-129 was decreased along with the increase of the degree of bone pain. (B) The level of miR-129 was reduced along with the increase of the degree of bone metastasis. *** **- p < 0.001 vs. breast cancer patients with mild bone pain group or grade 1 metastases,** ### **- p < 0.001 vs. as indicated.**

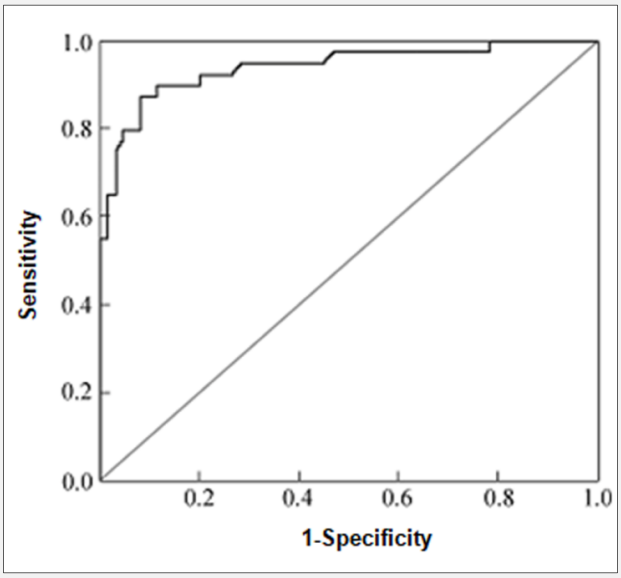

Figure 3. ROC analysis was carried out to analyze the diagnostic value of serum miR-129 in breast cancer patients with bone metastasis.

bone pain (0.07 ± 0.07, n = 9) and lower serum miR-129 was identified in the breast cancer patients with moderate bone pain (0.46 ± 0.14, n = 29), compared with that in mild bone pain group (1.00 ± 0.27, n = 22) (Figure 2A). At the same time, the level of serum miR-129 demonstrated the most significant decline in the breast cancer patients with grade 3 metastases (0.20 ± 0.12, n = 14) and secondary decline in the breast cancer patients with grade 2 metastases (0.45 ± 0.13, n = 28), compared with that in the breast cancer patients with grade 1 metastases (1.00 ± 0.23, n = 18) (Figure 2B).

Diagnostic value of serum miR-129 in breast cancer patients with bone metastasis

ROC curve was used to analyze the diagnostic value of serum miR-129 in breast cancer patients with bone metastasis and non-bone metastasis. ROC curve showed that the AUC of miR-129 in serum of breast cancer patients with bone metastasis and non-bone metastasis was 0.941 (95% CI: 0.891 - 0.991), where the sensitivity and specificity were 87.5% and 91.7%, respectively (Figure 3).

DISCUSSION

The most common distant metastasis of breast cancer is bone metastasis, which causes severe pain, neurological impairment, limb dysfunction, and other serious complications in severe cases [12,13]. Bone metastasis and bone-related events have long plagued patients with advanced breast cancer, which seriously affect the quality of life of breast cancer patients [14,15]. Therefore, more and more attention has been paid to the mechanism, early diagnosis and treatment of bone metastasis in breast cancer [16,17]. At present, a large number of studies have proven that miRs participate in a series of important processes in life activities and play a key role in the occurrence, development, and metastasis of breast cancer, which then provide a new direction for clinical diagnosis, treatment, and prevention of malignant tumors [18-20].

The tumor suppressor role of miR-129 has been widely reported in previous studies [6, 10]. Setijono et al. reported that miR-129 inhibits the progression of breast cancer by targeting lamins [6]. Meng et al. also showed that miR-129 suppresses breast cancer cell proliferation by inhibiting the expression of CBX4 [10]. Additionally, miR-129 is also shown to play key roles in the drug resistance of breast cancer cells [9,21]. For instance, enhanced miR-129-5p expression is shown to sensitize Her-2-positive breast cancer to trastuzumab by inhibiting the expression of rpS6 [21]. In addition, miR-129-5p is also found to suppress Adriamycin resistance in breast cancer by directly reducing the expression SOX2 [9]. These findings indicate the important role of miR-129 in the progression of breast cancer. However, whether they are involved in the progression of bone metastasis of breast cancer has never been explored.

In this study, the expression of miR-129 in the serum of patients with bone and non-bone metastasis of breast cancer was detected. The results showed that the expression of miR-129 in patients with bone metastasis of breast cancer showed a significant downward trend compared with patients with non-bone metastasis of breast cancer. Hence, reduced expression of serum miR-129 is closely related to bone metastasis. More importantly, the higher the degree of bone metastasis and bone pain was, the lower the relative expression of miR-129 was determined. The ROC curve analysis showed that the AUC of miR-129 was 0.941 in breast cancer patients with bone metastasis and non-bone metastasis with the sensitivity and specificity of 87.5% and 91.7%, respectively. It indicated that the expression of miR-129 in serum could differentiate between breast cancer patients with bone metastasis and non-bone metastasis patients and had certain value in the diagnosis of breast cancer patients with bone metastasis.

CONCLUSION

The relative expression of miR-129 in serum of breast cancer patients with bone metastasis was significantly lower than that of non-bone metastasis patients. miR-129 can be used as a potential marker for the diagnosis of breast cancer patients with bone metastasis.

Declaration of Interest:
We declare no conflicts of interest.

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