The effect of miRNAs and MALAT1 related with the prognosis of Her-2 positive breast cancer patients with lymph node metastasis

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Primary research

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Abstract

Background: To analyze and screen the miRNAs associated with lymph node metastasis of breast cancer (BC), and to explore the roles of these miRNAs in the proliferation, invasion and prognosis of BC. **Methods:** MicroRNAs associated with lymph node metastasis in Her-2 positive BC was screened by TCGA database. The qRT-PCR was used to verify theses 5 miRNAs in 30 cases of Her-2 positive BC with lymph node metastasis of different degree. The tumor tissue samples were divided into non-lymph node metastasis group, ≤ 3 lymph node metastasis group and > 3 lymph node metastasis group. In addition, 10 cases of paracancerous tissues were considered as paracancerous control group. Pearson correlation analysis was used to analysis the relationship of 5 miRNAs and MALAT1 with Her-2 positive BC patients' clinicopathological characteristics and prognosis. CCK8 and Transwell experiments were used to detect the effects of miR-143 and miR-455 on the proliferation and invasion of Her-2 positive BC cells (MDA-MB-453). **Results:** Five kinds of miRNA (miR-143, miR-196a, miR-455, miR-9 and miR-92a) related with Her-2 positive BC with lymph node metastasis were screened by TCGA database. The detecting results of qRT-PCR showed that the levels of miR-143, miR-196a, miR-9 and MALAT1 increased with the increased number of lymph nodes. The expression level of miR-143 in the group of ≤ 3 lymph nodes metastasis and > 3 lymph nodes metastasis was significantly higher than that in the group of non-lymph nodes metastasis ($P<0.001$), and that in the group of > 3 lymph nodes metastasis was significantly higher than that in the group of ≤ 3 lymph nodes metastasis ($P<0.001$). The expression level of miR-196a in the group of ≤ 3 lymph nodes metastasis and > 3 lymph nodes metastasis was significantly higher than that in the group of non-lymph nodes metastasis ($P<0.001$), and that in the group of > 3 lymph nodes metastasis was significantly higher than that in the group of ≤ 3 lymph nodes metastasis ($P<0.001$). The expression level of miR-455 in the group of ≤ 3 lymph nodes metastasis and > 3 lymph nodes metastasis was significantly lower than that in the group of non-lymph nodes metastasis ($P<0.001$), and that in the group of > 3 lymph nodes metastasis was significantly lower than that in the group of ≤ 3 lymph nodes metastasis ($P<0.001$). The expression level of MALAT1 in the group of ≤ 3 lymph nodes metastasis and > 3 lymph nodes metastasis was significantly higher than that in the group of non-lymph nodes metastasis ($P<0.001$), and that in the group of > 3 lymph nodes metastasis was significantly higher than that in the group of ≤ 3 lymph nodes metastasis ($P<0.01$). Pearson correlation analysis showed that the expression levels of miR-455-5p, miR-196a-5p and MALAT1 were negatively correlated, positively correlated and positively correlated with the pathological stages of Her-2 positive BC, respectively. The results of survival analysis showed that RFS of patients with high expression of miR-196a, miR-92a and MALAT1 was significantly lower than that of patients with low expression ($P<0.05$), and OS and RFS of patients with high expression of miR-9 were significantly lower than those of patients with low expression, while OS and RFS of patients with high expression of miR-455 were significantly higher than those of patients with low expression ($P<0.05$). Cytological experiments showed that up regulation of miR-455 significantly inhibited the proliferation and invasion of BC cells, while down regulation of miR-143 significantly inhibited the proliferation and invasion of BC cells and the expression of MALAT1 ($P<0.05$). **Conclusion:** High expression of miR-143, miR-9, miR-196a, MALAT1 and low expression of miR-455 are related to the degree of lymph node metastasis of Her-2-positive BC patients, indicating poor

prognosis. Down-regulation of miR-455 and up-regulation of miR-143 and MALAT1 can promote the cell proliferation and invasion of Her-2-positive BC.

Background

According to the latest cancer data released in the global cancer statistics report of 2018, breast cancer (BC) has become the highest incidence rate of malignancy in women, and the incidence rate has increased year by year [1]. Epidermal growth factor receptor 2 (Her–2) positive BC is an important subtype. Previous studies have shown that the high expression of Her–2 is closely related to the occurrence, development, invasion and migration of BC [2, 3]. Patients with Her–2 positive BC are prone to lymph node metastasis, which leads to poor prognosis. The median disease-free survival (DFS) and median total survival of patients with Her–2 positive BC are significantly shorter than those with Her–2 negative BC [4–6]. However, the mechanism of lymph node metastasis of Her–2 positive BC patients has not been elucidated. Therefore, to explore the mechanism of Her–2 positive BC with lymph node metastasis is of great significance to improve the prognosis of BC patients.

The existing research data show that miRNA can be used as tumor suppressor gene or oncogene to affect the occurrence, development and prognosis of BC [7–9]. For example, Jiang et al found that the increase of miR–196a can promote the growth and metastasis of BC cells by targeting spred1 [10]. The high metastasis, high proliferation, high mortality and other characteristics of Her–2-positive BC have been widely concerned [11]. Zhang et al showed that MALAT1 promoted the proliferation and migration of Her–2-positive BC [12]. The enhancement of invasion and proliferation will contribute to the lymph node metastasis of BC cells. At the same time, many studies have found that the detection of miRNA is helpful for the prognosis evaluation of Her–2-positive BC [13–15]. Therefore, the study of the regulatory role of miRNA is conducive to reveal the mechanism of Her–2-positive BC occurrence and development, and will help the development of new targeted drugs and improve the prognosis of patients. For example, Hui et al found that increasing the levels of miR–125a and miR–205 could help to improve the efficacy of trastuzumab and paclitaxel in the treatment of Her–2 overexpressed BC [16].

Long-chain non-coding RNA (lncRNA) is another type of non-coding RNA corresponding to miRNA. It has been found that lncrna and miRNA interact with each other, thus affecting the occurrence, development and metastasis of tumor. Zhang et al found that MALAT1 is the core signal molecule promoting the occurrence and metastasis of Her–2-positive BC [12]. Therefore, to explore the regulatory relationship between lncrna and miRNA is helpful to reveal the mechanism of miRNA.

In this study, we used the TCGA database to analyze many miRNAs associated with breast cancer lymph node metastasis. We consulted relevant literature, and performed real-time quantitative PCR (qRT-PCR) detection to further screen and verify miRNAs correlated with lymph node metastasis of Her–2 positive BC. We also evaluated the prognostic value of miRNAs and MALAT1 expression levels in patients with Her–2 positive BC and further verified the effects of the two screened miRNAs (miR–455 and miR–143)

on the proliferation and migration of MDA-MB–453 cells and the regulatory effect on MALAT1. It is hoped to help the mechanism research, treatment and prognosis evaluation of Her–2 positive breast cancer.

1. Method

1.1 Source of cell line and tumor tissue

Cell line: MDA-MB–453 cells (Her–2 positive BC cell line) were purchased from the cell resource center of the Institute of basic medicine, Chinese Academy of Medical Sciences. The cells were cultured in L15 medium containing 10% fetal bovine serum. At 37 ℃ and 5% CO_2, MDA-MB–453 cells were cultured to the confluence rate of 70–85% for 1:3 subculture.

Tumor tissue: 30 cases of patients with Her–2 positive BC admitted to our hospital from March 2011 to June 2013 were selected, including 10 cases of patients with non-lymph node metastasis, <3 lymph nodes metastasis and >3 lymph nodes metastasis, respectively. The average ages of those patients were 53.4 ± 9.25, 57.3 ± 9.68 and 51.7 ± 9.46 years old, respectively. In addition, 10 cases of Her–2 positive BC were selected as the paracancerous control group, the average age of the patients was 51.6 ± 9.36 years old. All the selected patients were confirmed to be breast invasive ductal carcinoma with Her–2 positive by pathological examination, and they did not receive chemotherapy or radiotherapy before the diagnosis. This study has been approved by the ethics committee of our hospital, and the patients provided informed consent. All the selected patients underwent radical surgery. We followed up the patients through telephone, outpatient interrogation and other ways, and the follow-up time was up to December 2018. Overall survival (OS) refers to the time of the end date or the time of death during follow-up. The recurrence free survival (RFS) refers to the time of the first recurrence and metastasis during follow-up. Recurrence and metastasis are defined as patients with distant metastasis (referring to metastasis to lung, brain, bone, liver, etc.) or local recurrence (recurrence in axilla, chest wall or clavicle) during follow-up. Distant metastasis was mainly determined by imaging examination and the condition of metastasis during follow-up. Local recurrence was confirmed by pathological examination.

1.2 miRNA sequencing

The miRNA sequencing was performed on the breast tumor tissues of 30 case of Her–2 positive BC patients and the paracancerous tissues of 10 case of BC patients. This part of the experiment was operated by Hangzhou Baiti Biotechnology Co., Ltd. The main process included total RNA extraction, RNA quality detection, 3 ‘junction, RT primer hybridization, 5’ junction, cDNA synthesis, PCR amplification, library fragment selection, library quality inspection, computer sequencing and data analysis. The sequencing mode is SE50, and the amount of sequencing data is 10M reads.

1.3 Fluorescent real-time quantitative PCR (qRT-PCR) detection

The qRT-PCR was used to detect the relative expression of miRNA and mRNA in cells and tissues. The total miRNA in cells and tissues was extracted according to the instructions of miRNA separation kit, and then the reverse transcription was performed according to the instructions of the reverse transcription Kit (1 min at 42 ℃ and 10 min at 70 ℃), and then the reverse cDNA was quantified. The process is as follows: (1) pre denaturation at 95 ℃ for 40 sec, (2) denaturation at 95 ℃ for 5 sec, (3) reaction at 60 ℃ for 30 sec, each set of three parallel, a total of 40 cycles. U6 considered as internal reference. After that, the relative expression of miRNA and mRNA in the sample was calculated by $2^{-\Delta\Delta}$ method.

1.4 Transfection experiment of mimics and inhibitor

MiR−455 mimics and miR−143 inhibitor were transfected into MDA-MB−453 cells with Lipofectamine 2000, and MDA-MB−453 cells transfected with Lipofectamine 2000 control sequences were used as control groups. After 1−2 days of transfection, cells were collected for subsequent related experiments.

1.5 Transwell experiment

The precooled L15 medium was fully mixed with Matrigel in the proportion of 1:1, and 100 μ l was evenly added to the bottom of the upper chamber, and then incubated for 4 hours. MDA-MB−453 cells of 3×10^4/ well logarithmic growth period was evenly added to the upper chamber to the total volume of 200 μ L. The 500 μ l L15 medium (10% FBS) was added to the lower chamber, and cultured at 37 ℃ and 5% CO_2 for 2 days, 4% POM fixed the cells and then stained; 5 fields were randomly selected for counting.

1.6 CCK8 experiment

The transfected MDA-MB−453 cells in the logarithmic growth stage were made into suspension cells (1×10^6 cells /ml), and then uniformly inoculated into 96 well plates (100 μ L /well). After 24 hours of culture, L15 medium containing 10% CCK8 reagent was added to 100 μ L, and after 2 hours of culture, the light absorption value (482nm) was measured by the whole wave length enzyme scale. The above experiments were repeated three times.

1.7 Statistical methods

Spss22.0 was used for data statistics and analysis. The data were expressed in ± s form and compared with student's t test. Univariate analysis of variance was used for multi group comparison and Turkey test was used for back testing. Pearson correlation analysis was used for the relationship among miRNA,

MALAT1 expression and lymph node metastasis number of patients. Kaplan Meier method was used for drawing. At the same time, log rank test was used to compare the RFS curve and OS curve of BC patients. P<0.05, indicating that the difference was statistically significant.

2. Results

2.1 MiRNA sequencing analysis and comparison of miRNA levels

From TCGA database (download version date 2016/12/16), searched the miRNA sequencing results of BC and paracancerous tissue samples, download the miRNA sequencing results and corresponding clinical information of about 1181 samples (including 1077 BC tissue samples and 104 paracancerous tissue samples). According to the number of lymph node metastases corresponding to clinical samples, we analyzed the correlation between the miRNA's expression value and the number of lymph node metastases in each sample, and screened out 19 miRNAs significantly related to the number of lymph node metastases(P<0.05). The expression of 19 miRNAs is shown in Figure 1a. 5 miRNAs, including miR−143, miR−196a, miR−455, miR−9 and miR−92a, which may be related to Her−2-positive BC metastasis, were screened by referring to relevant literature. Then, further qRT-PCR detection was carried out (Figure 1b and table 2), and the content changes of the above miRNAs in each group were compared.

2.1.1 Compared the expression level of miR−143−3p in different group:

Non-lymph node metastasis group and ≤3 lymph node metastasis group were significantly lower than the paracancerous control group (P<0.001), ≤3 lymph node metastasis group and > 3 lymph node metastasis group were significantly larger than non-lymph node metastasis group (P<0.001), > 3 lymph node metastases group was significantly larger than the group with <3 lymph node metastases (P<0.001). There was no significant difference in miR−143−3p expression level between the paracancerous control group and > 3 lymph node metastases group (P > 0.05).

2.1.2 Compared the expression level of miR−455−3p in different group:

Non-lymph node metastasis group, ≤3 lymph nodes metastasis group and 3 lymph nodes metastasis group were significantly lower than paracancerous control group (P<0.001), ≤3 lymph nodes metastasis group and 3 lymph nodes metastasis group were significantly lower than Non-lymph node metastasis group (P<0.001, P<0.01), 3 lymph nodes metastasis group were significantly lower than ≤ 3 lymph nodes metastasis group (P<0.001).

2.1.3 Compared the expression level of miR–196a–5p in different group:

Non-lymph node metastasis group was significantly lower than that in the paracancerous control group (P<0.01), ⩾3 lymph nodes metastasis group was significantly higher than that in the paracancerous control group (P<0.001), ≤ 3 lymph nodes metastasis group and ⩾3 lymph nodes metastasis group were significantly larger than non-lymph node metastasis group (P<0.001), ⩾3 lymph node metastasis group was significantly larger than ≤3 lymph node metastasis group (P <0.001). There was no significant difference among the other groups (P >0.05).

2.1.4 Compared the expression level of miR–9–5p in different group:

⩾3 lymph nodes metastasis group was significantly larger than that of the paracancerous control group (P<0.001), non-lymph node metastasis group and ≤3 lymph nodes metastasis group (P<0.05). There was no significant difference among the other groups (P >0.05).

2.1.5 Compared the expression level of miR–92a–3p in different group:

Non-lymph node metastasis group, ≤3 lymph nodes metastasis group and ⩾3 lymph nodes metastasis group were significantly lower than the paracancerous control group (P<0.001), ≤3 lymph nodes metastasis group and ⩾3 lymph nodes metastasis group were significantly lower than that of non-lymph node metastasis group (P<0.001, P<0.05), and ⩾3 lymph nodes metastasis group was significantly higher than ≤3 lymph nodes metastasis group (P<0.001). There was no significant difference among the other groups (P >0.05).

2.2 Comparison of MALAT1 content in different group:

Non-lymph node metastasis group, ≤3 lymph node metastasis group and ⩾3 lymph node metastasis group had significantly higher expression levels of MALAT1 than the paracancerous control group (P<0.05, P<0.001, P<0.001). The expression level of MALAT1 in the ≤3 and > 3 lymph node metastasis group were significantly higher than that in the non-lymph node metastasis group (P<0.001), and ⩾3 lymph node metastasis group was significantly higher than that in the ≤3 lymph node metastasis group (P<0.01).

2.3 Correlation analysis between related molecular detection indicators and clinical indicators:

Further evaluation of the correlation between the expression levels of 5 miRNAs and MALAT1 and the number of lymph node metastases of patients with Her−2 positive BC, we found that the expression levels of miR−143, miR−196a, miR−9, and MALAT1 were significantly positively correlated with the number of patients with lymph node metastases ($r = 0.8093$, $P<0.0001$; $r = 0.6346$, $P = 0.0002$; $r = 0.4411$, $P = 0.0147$; $r = 0.5857$, $P = 0.0007$); and miR−455 expression level was significantly negatively correlated with the number of patients with lymph node metastases（$r = −0.6498$, $P<0.0001$). However, there was no significant correlation between miR−92a expression and lymph node metastasis degree ($r = −0.0537$, $P = 0.7798$). The correlation analysis results are shown in Figure C. The expression levels of miR−455−5p, miR−196a−5p, miR−9−5p, and MALAT1 were correlated with pathological stages ($P<0.001$); The expression levels of miR−143−3p, miR−455−5p, miR−196a−5p, miR−9−5p and MALAT1 were related to the number of lymph node metastases ($P<0.001$). The expression levels of miR−455−5p and miR−9−5p were related to RFS ($P<0.001$, $P<0.05$); The expression levels of miR−455−5p, miR−196a−5p, miR−9−5p and MALAT1 were correlated with OS ($P<0.05$). The pathological stage of ≤3 lymph node metastasis group was correlated with MALAT1 expression level ($P<0.05$). RFS of 3 lymph node metastasis group was correlated with MALAT1 expression level ($P<0.05$).

2.3 Effect of 5 miRNAs and MALAT1 on the prognosis of Her–2 positive BC patients:

By analyzing the relationship between the expression levels of 5 miRNAs and MALAT1 and the pathological stage of Her–2 positive BC patients, it was found that the expression level of miR–455–5p was negatively correlated with the pathological stage of Her–2 positive BC patients ($r = -0.6393$, $P<0.001$), the expression levels of miR–196a–5p and MALAT1 were positively correlated with the pathological stage of Her–2 positive BC patients ($r = 0.3464$, $P = 0.0286$; $r = 0.3554$, $P = 0.0244$). The results are shown in Figure 2.

Subsequently, we divided patients into high and low expression (H, L) groups based on the median expressions of those miRNA and MALAT1: H-miR–143 group (≥0.955) and L-miR–143 group (<0.955); H-miR–455 group (≥0.625) and L-miR–455 group (<0.625); H-miR–196a group (≥0.89) and L-miR–196a group (<0.89); H- miR–9 group (≥0.93) and L-miR–9 group (<0.93); H-miR–92a group (≥0.835) and L-miR–92a group (<0.835); H-MALAT1 group (≥0.76) And L-MALAT1 group (<0.76). The follow-up results are shown in Figure 3 and Figure 4. It was found that patients with high expression of miR–196a, miR–92a, and MALAT1 had significantly lower RFS than patients with low expression, while those with high expression of miR–455 Patients with RFS were significantly higher than those with low expression ($P<0.05$). The OS of patients with high expression of miR–9 was significantly lower than that of patients with low expression, while the rate of OS of patients with high expression of miR–455 was significantly higher than that of patients with low expression ($P<0.05$).

2.4 Lifetime analysis (survival analysis,Mantel-Cox test)

OS and RFS in the > 3 lymph node metastasis group were significantly lower than those in the non-lymph node metastasis group and ≤3 lymph node metastasis group ($P<0.05$). There was no significant difference between the other groups ($P > 0.05$).

2.5. The role of miR–455 and miR–143 in cell proliferation of breast cancer:

Because miR–143 and miR–455 and the number of lymph node metastases were positively and negatively correlated, respectively, miR–455 and miR–143 were selected for follow-up research. We transfected MDA-MB–453 cells with miR–455 mimics and miR–143 inhibitor, respectively. The qRT-PCR detection showed that miR–455 expression levels in MDA-MB–453 cells significantly increased after transfection with miR–455 mimics, and the expression level of miR–143 in MDA-MB–453 cells after transfection with miR–143 inhibitor was significantly reduced, and the results are shown in Figures 6A and B. The results of the CCK8 experiment (as shown in Figures 6C and 6D) showed that compared with

the control group, transfection of miR–455 mimics and miR–143 inhibitor significantly reduced OD value. It means cell survival rate was significantly reduced (P<0.01).

2.3 Effect of miR–455 and miR–143 on breast cancer cell invasion:

The results are shown in Fig 7. Compared with the MDA-MB–453 + mimics control group, after miR–455 mimics treatment, the cell invasion ability was weakened and there was a significant difference (P<0.01). Compared with the MDA-MB–453 + inhibitor control group, the miR–143 inhibitor treatment reduced the cell invasion ability and there was a significant difference (P<0.01).

2.4 Regulation of miR–455 and miR–143 on MALAT1:

The effects of different expression levels of miR–455 and miR–143 on MALAT1 levels were examined. The results showed that transfection of miR–143 inhibitor significantly reduced the expression level of MALAT1 (P<0.01), and after transfection of miR–455 mimics, the expression level of MALAT1 had no significant change compared with the control group (P > 0.05). See Figure 8.

3. Discussion

The regulatory role of miRNA in Her–2 positive BC has received widespread attention. Multiple studies have shown that miRNA and lymph node metastasis of Her–2 positive BC are closely related and can reflect the prognosis of patients. Therefore, this study used the TCGA database to screen and analyze miRNAs associated with BC with lymph node metastasis, and explored its role in proliferation and invasion and prognosis assessment of BC. We screened 5 kinds of miRNAs related to Her–2 positive BC with lymph node metastasis, including miR–143, miR–196a, miR–455, miR–9 and miR–92a. Laterly, qRT-PCR further verified that there was a positive correlation between the expression levels of miR–143, miR–196a, miR–9, and MALAT1 and the number of lymph node metastases of patients; there was a negative correlation between the expression level of miR–455 and the number of lymph node metastases of patients. There was no significant correlation between miR–92a level and the number of lymph node metastases of patients.

At present, a variety of drugs targeting Her–2 have entered the clinic, such as trastuzumab, pertuzumab, etc. Her–2 positive BC patients have significantly improved their survival, but there are still limited clinical treatment effects for some patients [17, 18]. Therefore, exploring the prognostic indicators of Her–2 positive BC is of great significance for the treatment and the improvement of the prognosis. Firstly, the relationship between the expression level of miRNAs and MALAT1 and the pathological stage of Her–2 positive BC patients were analyzed. It was found that the expression level of miR–455–5p was negatively correlated with the pathological stage of Her–2 positive BC patients. And MALAT1 expression levels were positively correlated with pathological stage in patients with Her–2 positive BC. This indicates that the

abnormal expression of miR–455–5p, miR–196a–5p and MALAT1 may lead to poor prognosis in patients. Existing research data show that miRNAs and lncRNAs as oncogenes or tumor suppressor genes widely affect the occurrence, development and prognosis of various tumors [19, 20]. Our results show that miR–143, miR–92a, miR–9, miR–196a, and MALAT1 are highly expressed, and miR–455 is poorly expressed in patients with Her–2 positive BC who have poor overall and relapse-free survival. It shows that testing these indicators is helpful to understand the prognosis of Her–2 positive BC patients. However, the related regulatory mechanism has not been discussed in depth in this study, and further research is needed in the future.

Because the relationship between miR–455 ($r = -0.6498$, $P<0.0001$), miR–143 ($r = 0.8093$, $P<0.0001$) and the number of lymph node metastases is the most obvious, miR–455 and miR–143 were selected for this study. Considering that cell proliferation and cell invasion are important biological behaviors of Her–2 positive BC, and have a great correlation with lymph node metastasis and prognosis of patients, we studied miR–455 and miR–143 on cell proliferation and invasion of BC.

Cellular function studies show that miR–455 can inhibit the cell proliferation and invasion of Her–2 positive BC, while miR–143 can promote the cell proliferation and invasion of Her–2 positive BC. Tavanafar et al Found that miR–143 can inhibit the proliferation, invasion, and migration of BC cell MDA-MB–468 [21], while Li et al.'S research indicates that miR–455 can promote the invasion and migration of triple-negative BC [22] The above results are contrary to our findings. It may be caused by selected different BC cell types.

Some studies have shown that LncRNA and miRNA can regulate each other and can affect the biological behaviors of cell proliferation, apoptosis, invasion and migration of BC [23, 24]. Jin et al found that MALAT1 and miR–1 regulate each other to promote the development of triple-negative BC [25]. MALAT1 is also involved in the functional regulation of lymph node metastasis, cell invasion and cell metastasis in Her–2 positive BC [12]. Therefore, we explored the relationship between miR–455 and miR–143 expression and MALAT1 levels. This study found that MALAT1 is highly expressed in Her–2 positive BC tissues, and there is a positive correlation between the expression level and the number of lymph node metastases. It was also found that down-regulating miR–143 significantly inhibited the expression of MALAT1 ($P<0.01$), while up-regulating miR–455 had less effect on MALAT1 expression ($P > 0.01$). It was further determined that the abnormal expression of miR–455, miR–143 and MALAT1 will be beneficial to lymph node metastasis in patients with Her–2 positive BC.

This study found that miR–455 low expression and miR–9 high expression is related to RFS in Her–2 positive BC patients. The OS of the Her–2 positive BC patients was bad whose miR–455 level is low, miR–9, miR–196a, and MALAT1 level are high. Both up-regulation of miR–455 and inhibition of miR–143 can inhibit cell proliferation and cell invasion of Her–2 positive BC. The miR–455 and miR–143 provide new targets for the diagnosis and treatment of Her–2 positive BC, and provide data support for the study of pathogenesis and metastasis mechanism of BC. However, the regulatory mechanism of

miR–455 and miR–143 has not been explored in this study, and the follow-up is still the focus of BC research.

Conclusion

MiRNAs are small ncRNA molecules composed of 21 to 24 nucleotides, which can regulate the expression of post-transcriptional protein-encoding genes and participate in various biological processes. MALAT1 is a kind of lncRNA. Most literature reports suggest that MALAT1 can promote BC. In this study, five miRNAs related to lymph node metastasis were screened from the TCGA database, and two miRNAs were further selected from them for cytological experiments. The expression levels of miRNAs and MALAT1 were detected in tumor tissues of Her–2 positive patients with different degrees of lymph node metastasis. The experimental results found as follows: high expression of miR–143, miR–9, miR–196a, MALAT1 and low expression of miR–455 are related to the degree of lymph node metastasis of Her–2-positive BC patients, indicating poor prognosis. Down-regulation of miR–455 and up-regulation of miR–143 and MALAT1 can promote the cell proliferation and invasion of Her–2-positive BC. Our study may provide a theoretical basis for targeting some miRNAs and MALAT1 in the treatment of Her–2-positive BC.

Declarations

Ethics approval and consent to participate: The Ethics Committee of Zhejiang Cancer Hospital was secured for our research reported, and all authors abided the related rules of Ethics Committee when this study began. All authors abided the ethics in this clinical study. The Ethics Committee of Zhejiang Cancer Hospital approved to publish this paper. The research involving human subjects, human material, and human data have been performed in accordance with the Declaration of Helsinki and have been approved by an appropriate ethics committee of Zhejiang Cancer Hospital.

Consent for publication: All authors declare that we consent for publication.

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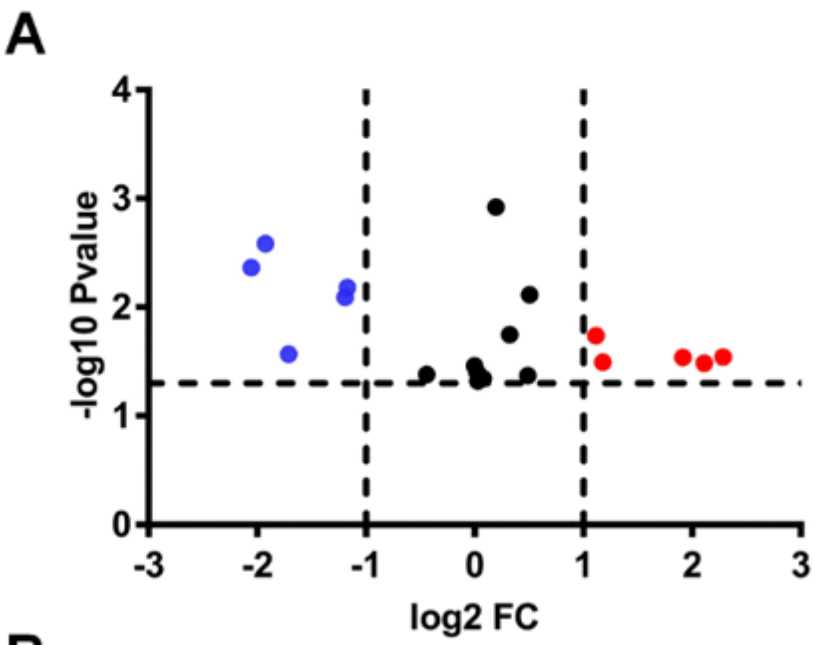
Tables

Due to technical limitations, Tables 1 & 2 are only available for download from the Supplementary Files section.

Figures



C

miRNA	r value	P value
miR-143-3p	0.8093	<0.0001
miR-455-5p	-0.6498	<0.0001
miR-196a-5p	0.6346	0.0002
miR-9-5p	0.4411	0.0147
miR-92a-3p	0.05327	0.7798
MALAT1	0.5857	0.0007

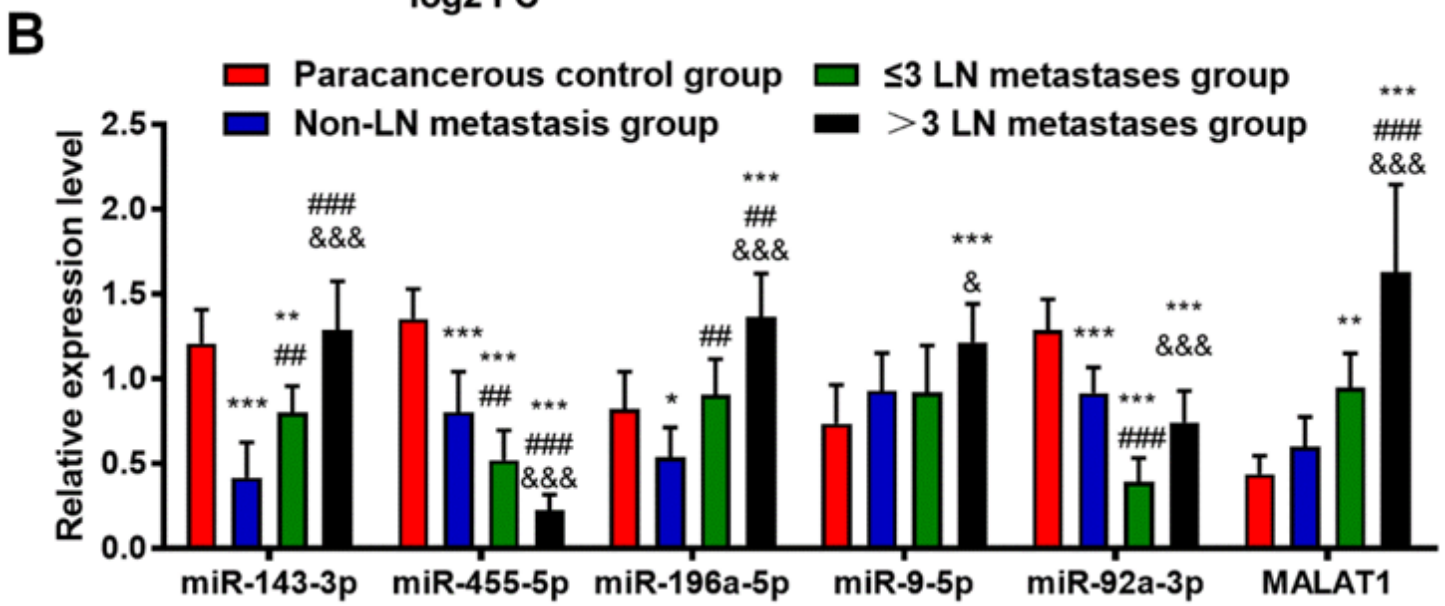


Figure 1

Analysis of the expression of 5 miRNAs and MALAT1 (A) miRNA sequencing data; (B) changes in expression levels of 5 miRNAs and MALAT1 in patients with different degrees of lymph node metastasis. (C) Correlation between the expression levels of 5 miRNAs and MALAT1 and the degree of lymph node metastasis. Note: Statistics are expressed by mean + SEM. Compared with paracancerous control group, * means P<0.05, ** means P<0.01, *** means P<0.001; compared with Non-LN metastasis group, # means P<0.05, ## means P<0.01, ### means P<0.001; compared with ≤3 LN metastasis group, & means P<0.05, && means P<0.01, &&& means P<0.001;

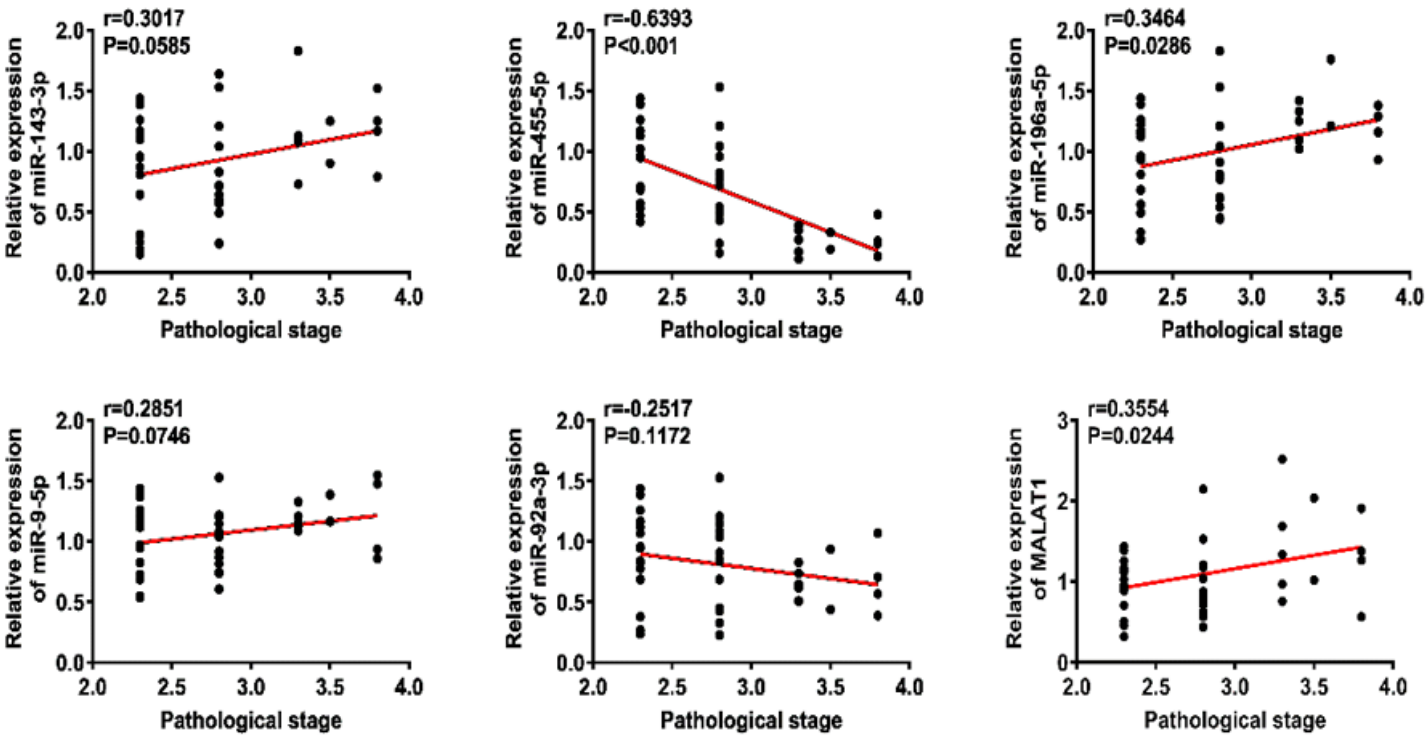


Figure 2

Relationship between the expression levels of 5 miRNAs and MALAT1 and the pathological stage of patients.

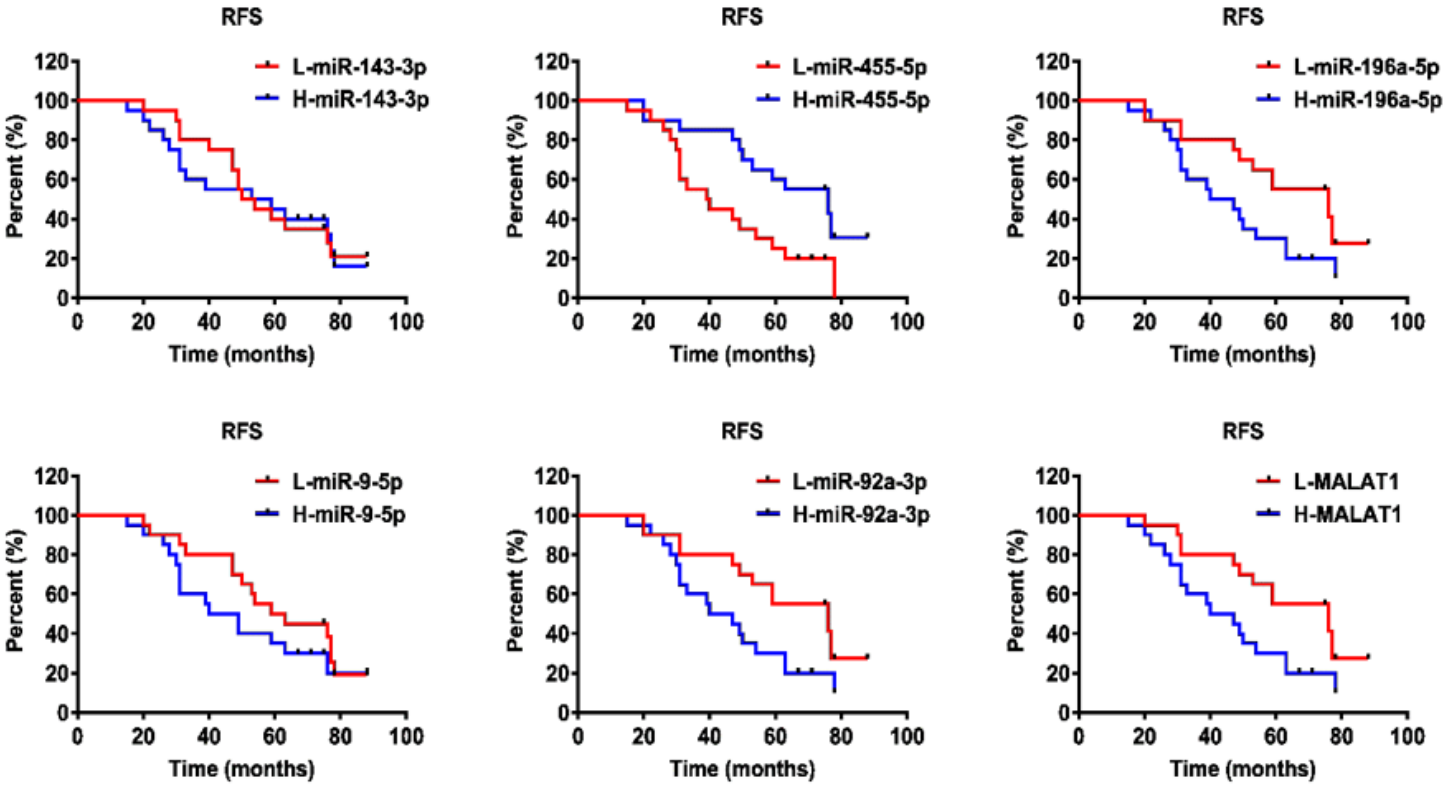


Figure 3

Effect of high and low expression levels of 5 miRNAs and MALAT1 on patient RFS

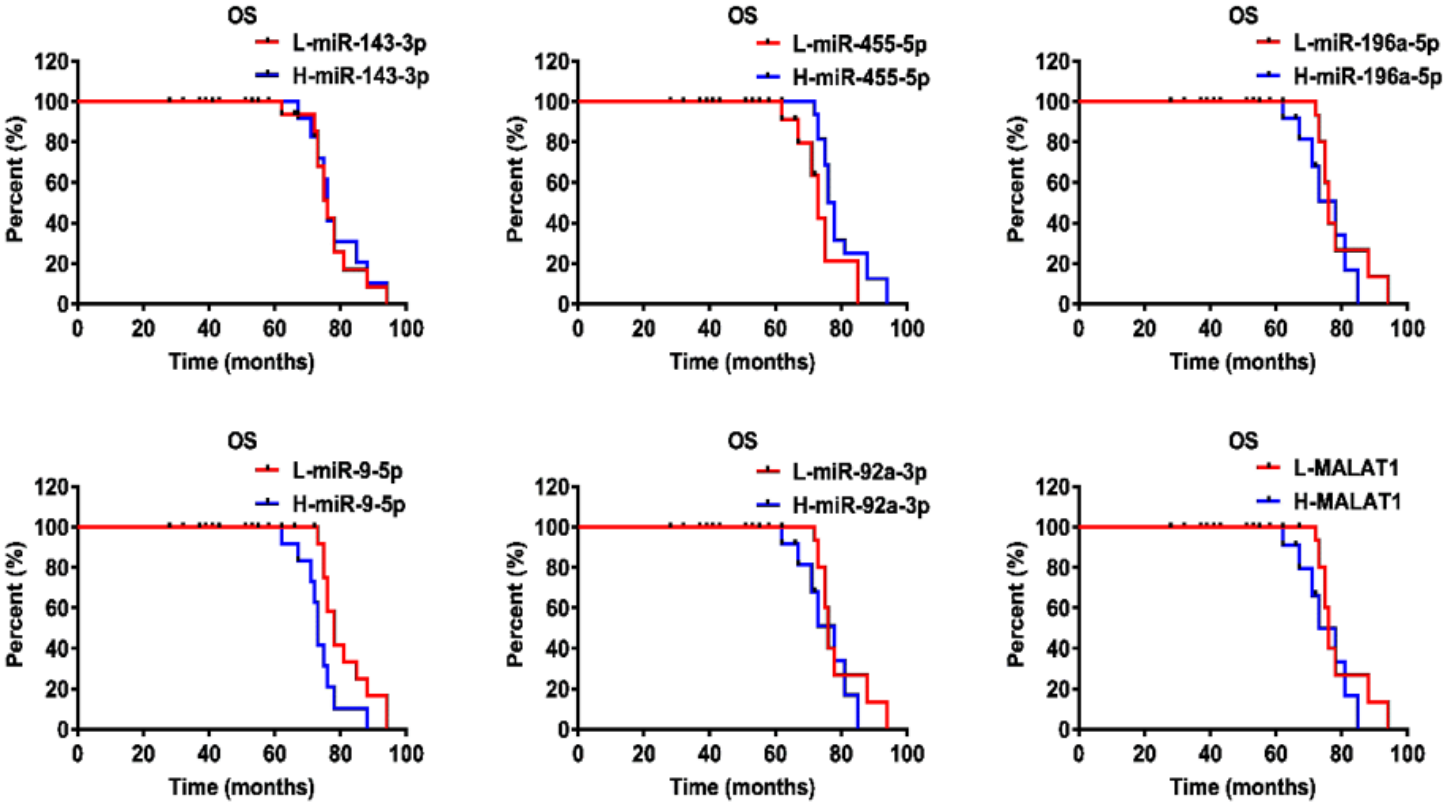


Figure 4

Effect of high and low expression levels of 5 miRNAs and MALAT1 on patient OS

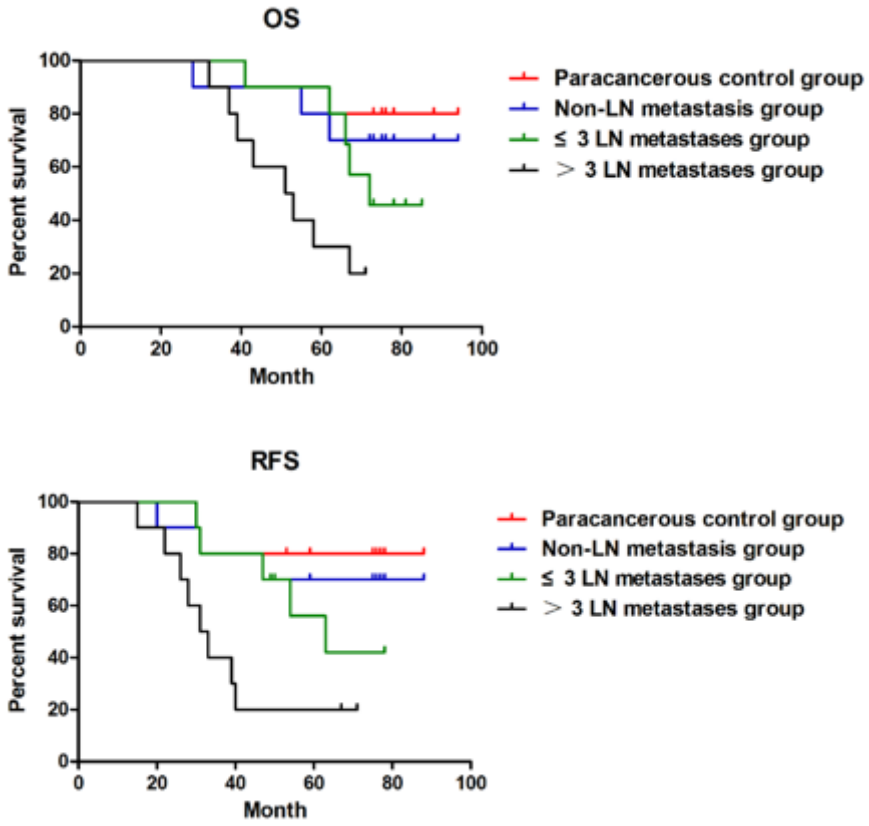


Figure 5

OS and RFS in the different groups

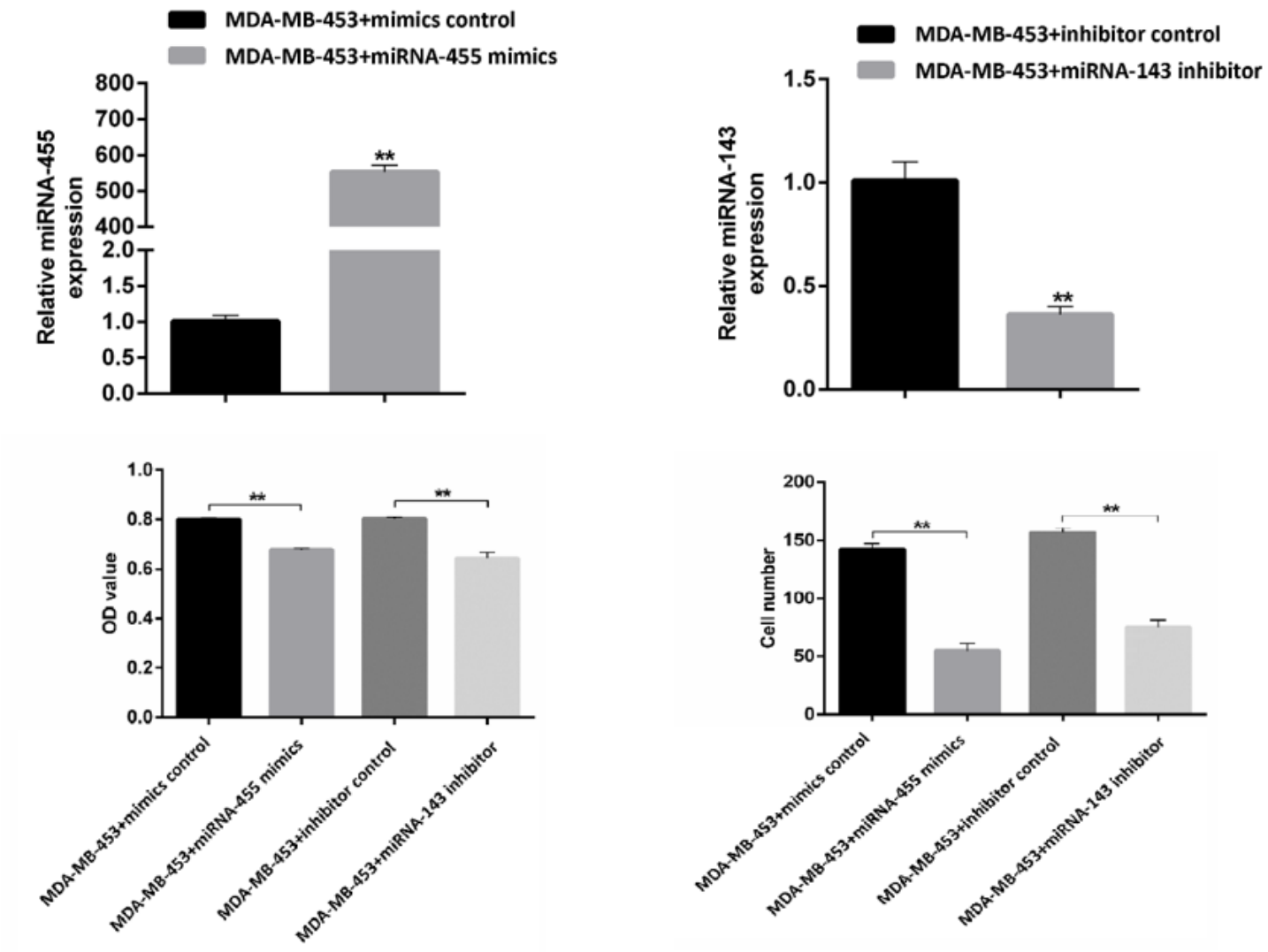


Figure 6

Effect of miR-455 and miR-143 on cell proliferation of breast cancer. (A) miR-455 expression after transfection with miR-455 mimics; (B) miR-143 expression after transfection with miR-143 inhibitor; (C) Effects of miR-455 and miR-143 on cell proliferation of BC; (D) Effects of miR-455 and miR-143 on the number of BC cells. Note: ** means $P<0.01$, statistics are expressed by mean + SEM, each group is repeated 3 times;

Supplementary Files

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- Tables1and2.docx