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Is there a correlation between miR-301a expression and neoadjuvant chemotherapy efficacy in breast cancer tissue?

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ABSTRACT

Neoadjuvant chemotherapy (NAC) is the standard therapeutic regimen for locally advanced breast cancer. However, clinical physical examination and imaging results fail to accurately assess the treatment response, and postoperative pathological examination has a time lag in response to therapeutic effect which is not conducive to the timely adjustment of treatment strategies. A previous study has shown that miR-301a was associated with invasion and metastasis in breast cancer, and was found to be involved in endocrine therapy resistance; however, evidence regarding the correlation between miR-301a expression and NAC efficacy remains scarce. In this study, 101 patients with locally advanced breast cancer were included. All patients received anthracycline based chemotherapy. The expression level of miR-301a in pretreatment core needle biopsy tissues was determined by real-time polymerase chain reaction analysis. Relevant clinicopathological data were collected, and the correlation between miR-301a expression and NAC efficacy was assessed. Based on our data, miR-301a cannot be used to identify whether breast cancer benefits from NAC, and no correlation was observed between miR-301a expression and clinicopathological characteristics. In conclusion, miR-301a may not be a potential prognostic biomarker of NAC efficacy in breast cancer.

1. Introduction

Neoadjuvant chemotherapy (NAC) has become the standard treatment for locally advanced breast cancer (LABC), offering advantages over traditional adjuvant approaches in patients, including downstaging the primary tumor, rendering breast-conserving surgery more feasible, and monitoring treatment effects *in vivo* [1,2]. Presently, NAC has been shown to improve survival [3]. Pathologic complete response (pCR) is the best outcome for NAC and is increasingly perceived as an essential independent prognostic factor for better overall survival, as well as disease-free survival, in breast cancer [4,5]. Unfortunately, not all patients respond (primary or acquired resistance), and, on average, only 69% of patients achieve pCR or pathologic partial response to NAC [6,7]. Meanwhile, NAC may be detrimental to patients owing to the delay in surgery, with potential for disease progression, particularly in chemoresistant tumors, and an increase in drug toxicity. Response Evaluation Criteria in Solid Tumor version1.1 (RECIST v1.1) is the main clinical method used to assess the tumor response to therapy, while mammography, ultrasonography, and magnetic resonance imaging (MRI) are the most common screening tools, which are noninvasive and highly reproducible. However, monitoring early episodes of response remains flawed in terms of promptness and accuracy. Moreover, positron emission tomography/computed tomography (PET/CT) is considerably expensive to be deemed not the first choice. Surgical pathology is the gold standard for assessing the efficacy of NAC, but it fails to reflect changes in tumors promptly. Considering the limitations of currently

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available prognostic strategies, there is an urgent need for novel, reliable, minimally invasive biomarkers that can be used to monitor the sensitivity to NAC for the clinical management of breast cancer.

Micro-RNAs (miRNAs) are a class of endogenous small non-coding RNAs, with a length of approximately 18–22 nucleotides, which negatively regulate the expression of target genes by mRNA degradation or translational repression [8]. miRNAs are involved in multiple biological processes and are considered as major regulators of various human cancers [9,10], including breast cancer. As an important component of miRNAs, the dysregulation of miR-301a has been previously proposed as a poor prognostic factor in various cancers [11–13], especially in breast cancer [14–18], acting as an enhancement factor of cancer proliferation and metastasis, persistently resulting in poor prognosis. MiR-301a was found to promote radioresistance [19], chemoresistance [20] and resistance to endocrinotherapy [21]. Reportedly, the detection of a single miRNA can be used to predict resistance to multiple therapeutic strategies [22]. miR301a has been utilized as a promising biomarker in breast cancer as it is easily detected in pretherapeutic tumor biopsies, which are generally well-preserved in formalin-fixed paraffin-embedded (FFPE) tissues [23], with easy isolation. Accordingly, miR-301a may be a prospective predictor of response to NAC in breast cancer; however, most evidence has been obtained from preclinical investigations. Furthermore, data on the relationship between miR-301a and the efficacy of NAC are limited, and the precise role of miR-301a in NAC in breast cancer needs to be elucidated. In this study, we aimed to investigate whether miR-301a can be employed to identify patients with breast cancer who respond to NAC in clinical settings.

2. Patients and methods

2.1. Patients

This retrospective study was approved by the ethics committees of the Affiliated Hospital of Zunyi Medical University. As data were obtained from previous clinical diagnosis and treatment, it was deemed that exemption of informed consent would not adversely affect the patients' rights and health, and the requirement for informed consent was waived upon approval of the ethics committee. We collected the clinical data of 101 female patients with LABC undergoing NAC between January 1, 2017 and June 30, 2019 at our hospital. In this study, patients who were confirmed by histology (core-needle biopsy) as breast cancer and received a treatment based on anthracyclines, with no distant metastasis detected, were included. Patients who received any anti-cancer treatment (hormone therapy, chemotherapy, or radiotherapy), had previously been detected with historically malignant tumors, and had incomplete clinicopathological data (age, hormone receptor (HR) status including estrogen receptor (ER) and progesterone receptor (PR), human epidermal growth factor receptor 2 (Her-2) status, proliferation index (Ki-67), T and N stage, molecular subtype, pathological pattern) were excluded. Three weeks after the completion of NAC, patients underwent surgery, except for 5 patients (5.0%) owing to disease progression during NAC. No cases were lost until the last follow-up on April 1, 2020. FFPE biopsy tumor tissue samples used in this study were retrieved from our institute.

2.2. Neoadjuvant chemotherapy

The NAC regimens were as follows: docetaxel + adriamycin + cyclophosphamide (TAC) in 16 patients; docetaxel + epirubicin + cyclophosphamide (TEC) in 63 patients; adriamycin + cyclophosphamide-docetaxel (AC-wP) in 2 patients; epirubicin + cyclophosphamide -docetaxel (EC-wP) in 13 patients; epirubicin + cyclophosphamide-docetaxel + trastuzumab (EC-TH) in 5 patients; epirubicin + cyclophosphamide + fluorouracil (ECF) in 1 patient; epirubicin + cyclophosphamide-docetaxel + carboplatin (EC-PT) in 1 patient.

2.3. RNA isolation and real-time polymerase chain reaction

In total, 101 biopsy specimens were examined by real-time polymerase chain reaction (RT-PCR). Total RNA was extracted from FFPE biopsy tissues using the FFPE RNA Isolation Kit (Magen, Guangzhou, China). Complementary DNA synthesis was carried out using PrimeScript ™ miRNA First- Strand cDNA Synthesis Kit (Thermo Fisher Scientific, CA, USA). miR-301a expression was determined using QuantityNova SYBR® Green PCR Kit (Qiagen, Hilden, Germany) and a microplate reader (Mutiskan™ GO, Thermo Fisher Scientific, CA, USA). U6 snRNA was used as the endogenous control. All steps were performed according to the manufacturer's instructions. Calculations were performed using the $2-\Delta\Delta Ct$ method. The primers used for RT-PCR were designed by Sangon Biotech® (Shanghai, China) as follows:

U6, AACGCTTCACGAATTTGCGT,
U6 forward, 5′-AACGCTTCACGAATTTGCGT-3′,
U6 reserve, 5′-CTCGCTTCGGCAGCACA-3′,
miR-301a, GTCGTATCCAGTGCGTGTCGTGGAGTCGG,
miR-301a forward, 5′-GATGGGCAGTGCAATAGTATTGTCA-3′,
miR-301a reserve, 5′-CAGTGCGTGTCGTGGAGTC-3′.

2.4. Therapeutic evaluation

To monitor the response to NAC, two tools, RECIST v1.1 and Miller-Payne (MP) grading system were employed. RECIST v1.1 based on changes in tumor size, the response to NAC was classified as complete response (CR) if the tumor disappeared completely, partial response (PR) if lesion length reduction was at least 30%, progressive disease (PD) if the lesion length increased by at least 20% or new lesions appear, and stable disease (SD) if there was no PR or PD observed. MP grade is based on the decrease in tumor cellularity between biopsy and mastectomy specimens and was graded as follows: MP1, no significant reduction in malignant cells or no change; MP2, loss of malignant cells $\leq$30%; MP3, a reduction in malignant cells between 30% and 90%; MP4, the disappearance of malignant cells >90%; MP5, no malignant cells were detected.

2.5. Statistics

Statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA). The results are expressed as $\bar{\chi}\pm S$. Pearson's χ^2 tests or Fisher's exact tests were performed to evaluate the relationship between miR-301a expression and clinicopathological features when necessary. A p-value <0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

All patients received anthracycline based treatment for 4 to 8 cycles. In this cohort, the age ranged between 29 and 66 years, with a median age of 47. Ninety patients underwent a modified radical mastectomy, and 6 patients underwent breast-conserving surgery. Nearly half of the tumors were Luminal A and B (n = 54, 53.5%), 26.7% (n = 27) were Her-2 positive, and 19.8% (n = 20) were detected as triple-negative breast cancer (TNBC). Moreover, invasive ductal carcinoma was the most common pathological pattern (n = 88, 87.1%). Overall, 7 patients (6.9%) achieved clinical CR, and 28 (27.7%) patients achieved pCR. In total, 8 patients (7.9%) showed preoperative disease progression, including 3 with distant metastases (1 in the liver, 2 in bones) and 5 with locally advanced tumors (1 in regional lymph nodes, 1 in the skin of the opposite breast, 3 in increased primary mass). Furthermore, 12 patients (11.9%) presented postoperative progression, including 9 with distant metastases (4 in bones, 2 in the lung, 2 in multiple organs, 1 in non-regional lymph nodes), and 3 with locally advanced tumors (1 chest

wall recurrence, 2 in regional lymph nodes). The remaining 81 (80.2%) patients were free of disease progression until the last follow-up time point. (See Table 1).

Furthermore, we assessed the correlation between clinicopathological characteristics and miR-301a expression. No association was determined between the expression of miR-301a and T stage ($p=0.741$), N stage ($p=0.231$), ER status ($p=0.270$), PR status ($p=0.915$), Her-2 status ($p=0.884$), molecular subtype ($p=0.171$), pathological pattern ($p=0.371$), age (<35 years, 35–59 years, >59 years; $p=0.908$), and Ki-67 (every 20% of Ki-67 was classified into a group, $p=0.511$), as shown in Fig. 1.

3.2. Therapeutic response was independent of the miR-301a expression level

In this study, miR-301a expression was independent of the RECIST stage ($p=0.817$). The RECIST stage was sorted into the responsive group (CR + PR), stable group (SD), and unresponsive group (PD), or divided into two groups (CR + PR + SD *vs.* PD), and no significant difference was observed ($p=0.708$, $p=0.666$) (Fig. 2a–c). The expression level of miR-301a was independent of the MP grade ($p=0.906$). No correlation was observed on categorizing the MP grade into the absolute response group (MP5), partial response group (MP2-4), and unresponsive group (MP1), or by dividing it into two groups (MP1 *vs.* MP2-5) ($p=0.638$, $p=0.731$) (Fig. 2d–f) (see Table .2). Finally, we tested the correlation between the

Table 1
Clinicopathological features of patients (n = 101).

Variable	Number (%)	Relative expression of miR-301a (χ̄±S)	*p*-value
Age			0.908
<35 years	6 (5.9)	0.66 ± 0.14	
35–50 years	86 (85.1)	1.34 ± 2.48	
>50 years	9 (8.9)	0.91 ± 0.73	
ER			0.270
(+)	63 (62.4)	1.18 ± 2.39	
(−)	38 (37.6)	1.39 ± 2.19	
PR			0.915
(+)	42 (41.6)	1.38 ± 2.79	
(−)	59 (58.4)	1.18 ± 1.91	
Her-2			0.884
(+)	27 (26.7)	0.82 ± 0.61	
(−)	74 (73.3)	1.42 ± 2.66	
Molecular subtype			0.171
Luminal A	25 (24.8)	1.42 ± 2.24	
Luminal B Her-2 (−)	29 (28.7)	1.19 ± 2.85	
Her-2 (+)HR (+)	10 (9.9)	0.51 ± 0.37	
Her-2 (+)HR (−)	17 (16.8)	1.01 ± 0.66	
TNBC	20 (19.8)	1.77 ± 2.94	
Pathological Pattern			0.371
Invasive ductal carcinoma	88 (87.1)	1.17 ± 1.97	
Non-specific invasive carcinoma	8 (7.9)	2.81 ± 4.96	
invasive lobular carcinoma	3 (3.0)	0.43 ± 0.28	
Mucinous carcinoma	1 (1.0)	0.18	
Papillary carcinoma	1 (1.0)	0.99	
T			0.741
T1	6 (5.9)	0.63 ± 0.45	
T2	57 (56.4)	1.35 ± 2.39	
T3	15 (14.9)	0.61 ± 0.37	
T4	23 (22.8)	1.63 ± 3.00	
N			0.231
N0	16 (15.8)	1.39 ± 1.44	
N1	69 (68.3)	1.20 ± 2.40	
N2	11 (10.9)	1.66 ± 3.24	
N3	5 (5.0)	0.85 ± 0.86	

(−): negative, (+): positive; ER: estrogen receptor; PR: progesterone receptor; HR: hormone receptor; Her-2: human epidermal growth factor receptor 2; TNBC: triple-negative breast cancer.

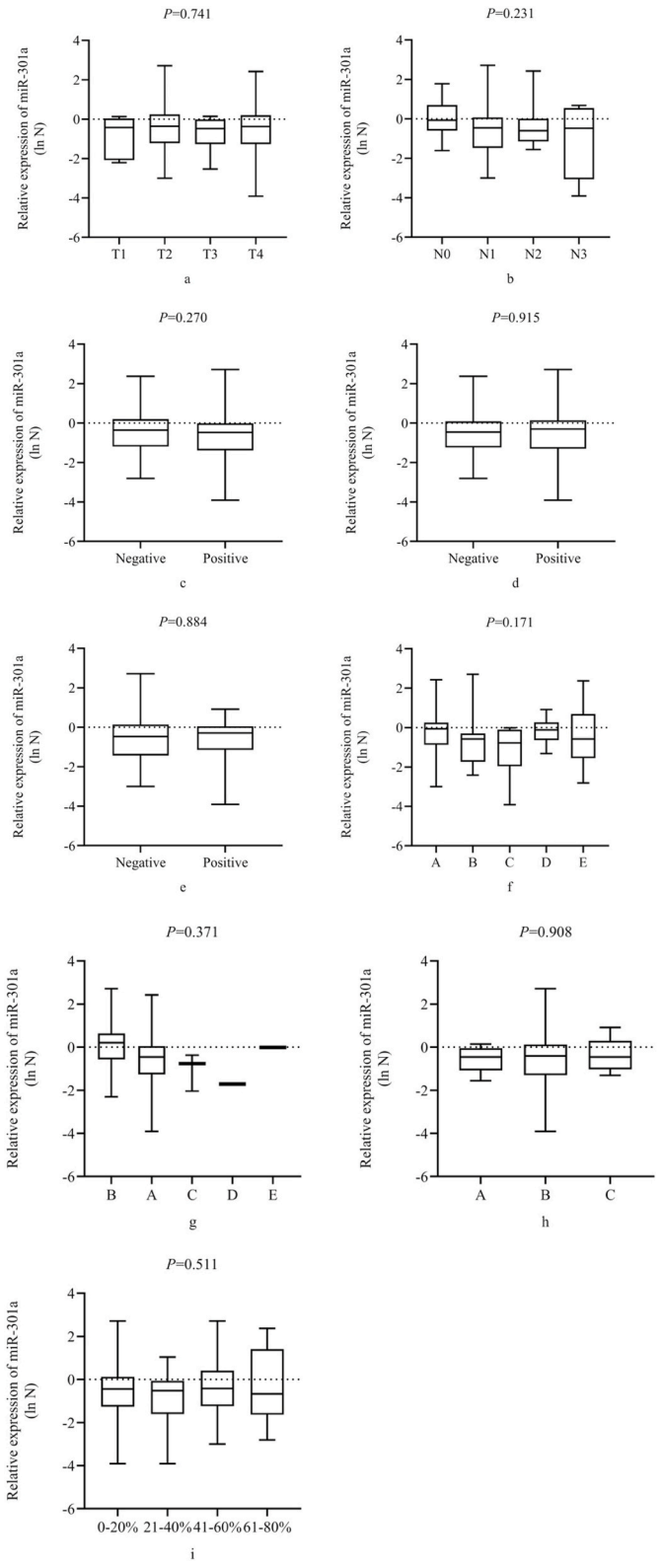

Fig. 1. The correlation between clinicopathological characteristics and the relative expression of miR-301a. a: T stage; b: N stage; c: ER status; d: PR status; e: Her-2 status; f: molecular subtype. A = Luminal A, B=Luminal B Her-2 (−), C=Her-2 (+) HR (+), D = Her-2 (+) HR (−), E = TNBC; g: pathological pattern. A = invasive ductal carcinoma, B = non-specific invasive carcinoma, C = invasive lobular carcinoma, D = mucinous carcinoma, E = papillary carcinoma; h: age groups, A < 35 years, B:35–59 years, C > 59 years; i: the level of Ki-67.

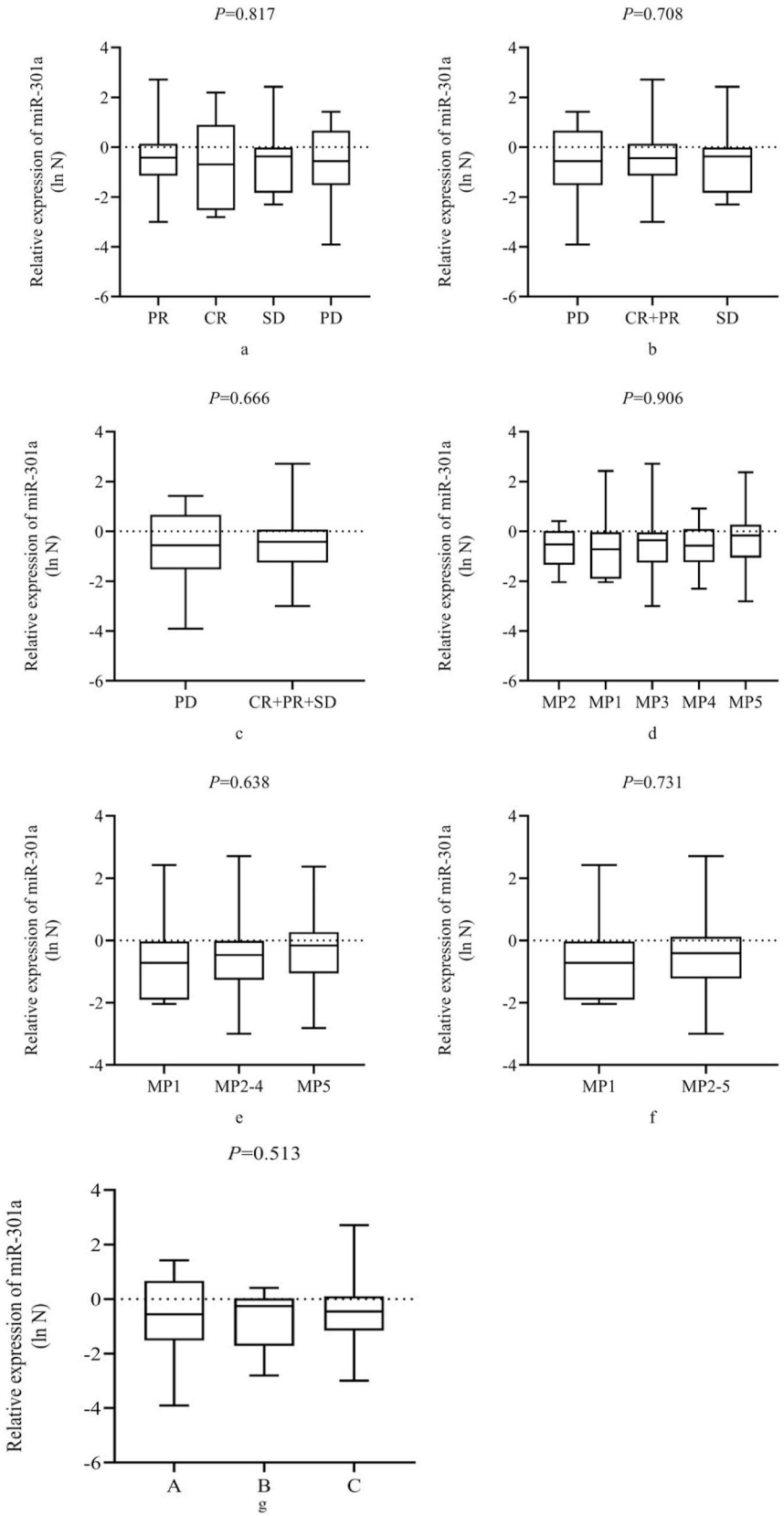

Fig. 2. Correlation between NAC and the miR-301a expression. a–c: the relationship between the relative expression of miR-301a and RECIST; d–f: the relationship between the relative expression of miR-301a and MP; g: A = preoperative progression, B = postoperative progression, C = free of progression. MP, Miller-Payne system.

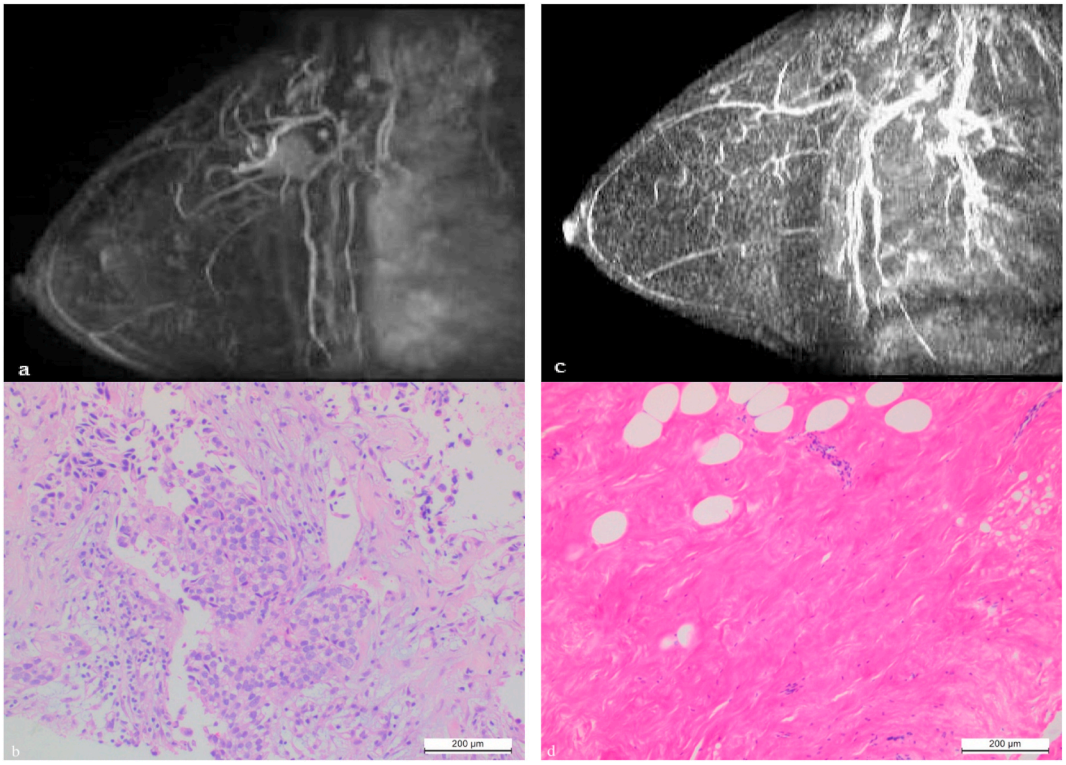

Fig. 3. Comparison chart before and after NAC of a patient who reached MP5 grade. a–b: pre-treatment, c–d: post-treatment, HE × 20.

Table 2
The response to neoadjuvant chemotherapy (NAC) (n = 101).

Variable	Number (%)	Relative expression of miR-301a ($\bar{\chi}\pm S$)	*p*-value
RECIST			0.817
CR	7 (6.9)	1.87 ± 3.21	
PR	61 (60.4)	1.27 ± 2.37	
SD	25 (24.8)	1.11 ± 2.20	
PD	8 (7.9)	1.17 ± 1.41	
MP			0.906
MP1	8 (7.9)	1.81 ± 3.84	
MP2	14 (13.9)	0.66 ± 0.42	
MP3	29 (28.7)	1.45 ± 2.89	
MP4	17 (16.8)	0.82 ± 0.77	
MP5	28 (27.7)	1.54 ± 2.48	
Outcome			0.513
Preoperative progression	8 (7.9)	1.17 ± 1.41	
Postoperative progression	12 (11.9)	0.67 ± 0.50	
No progression	81 (80.2)	1.36 ± 2.52	

CR, complicated response; PR, partial response; SD, stable disease; PD, progressive disease.

expression of miR-301a and the efficacy of NAC among different subgroups, no correlation was observed ($p > 0.05$). There was no statistically significant difference in the expression of miR-301a in the three groups of preoperative progression, postoperative progression and free of disease progression (*p=0.513,* Fig. 2g) (see Fig. 3).

4. Discussion

Presently, NAC remains the first-line of treatment for LABC, however, only a section of patients responded to NAC [6,7], owing to a lack of early and effective monitoring methods, which, in turn, exposed several patients to overtreatment or undertreatment. In this study, the expression of miR-301a was independent of the RECIST stage and MP grade, high miR-301a expression levels did not indicate a poor therapeutic NAC effect. No correlation was determined between clinicopathological features and miR-301a expression levels, and miR-301a was not found to be a biomarker for relapse and metastasis.

Zheng et al. [14] have reported that in TNBC and non-TNBC tumors, the upregulation of miR-301a indicated a poor prognosis for breast cancer. Lou et al. [24] and Yin et al. [25] have observed that the upregulation of miR-301a promotes breast cancer cell proliferation and invasion. Lettlova et al. [18] have observed that miR-301a is linked to ESR1 expression. Dalmasso et al. [26] have reported that miR-301a is significantly reduced in older patients with breast cancer. Additionally, Terkelsen et al. [27] and McDermott et al. [28] have determined that compared with TNBC, the expression of miR-301a in non-TNBC was not upregulated. To the best of our knowledge, no previous study has investigated the role of miRNA-301 in NAC for breast cancer. Interestingly, in contrast to previous study, in both TNBC and non-TNBC, the level of miR-301a was not associated with a worse outcome. Moreover, no age-related association was observed. Similar to the report of Zheng et al. [14] there was no correlation between clinicopathological features and miR-301a expression levels.

The outcomes determined in the present study are inconsistent with previous investigations, which have reported that miR-301a acts as an enhancement factor for recurrence and metastasis, and thus predicts poor prognosis for breast cancer. On performing a critical review of previous studies, the findings in the present study could be attributed to three reasons. Firstly, we observed that tissue specimens and blood samples were employed in previous investigations, whether it is caused by the different types of samples we employed or the differences between races and regions is unclear. Secondly, depending on the target gene regulated, miR-301a may function as an onco-miRNA, but may not play a role, or a direct role, in the efficacy of NAC in breast cancer. Thirdly, as determined in previous studies miR-301a was observed upregulated in TNBC, most tumors in this cohort were Luminal-like, as our cohort had only 19.8% of patients with TNBC, we were unable to determine a difference in miR-301a expression levels between TNBC and

non-TNBC tumors. However, the correlation between the expression of miR-301a and the therapeutic efficacy of different molecular subtypes has also unobserved, according to our findings we speculated that miR-301a expression is not a regulator of NAC efficacy. Overall, 8.9% of the patients were older, and no age-related alterations in expression were observed. Based on our data, only 7 patients achieved clinical CR, and 28 patients achieved pCR, indicating that molecular changes in cancer are observed before morphological changes. Therapeutic evaluations based on screening tools assessing changes in tumor size lack precision and promptness. Therefore, a high accuracy and low trauma predictive biomarker for response to NAC is urgently needed in clinical settings.

The limitation of this study was the relatively small number of enrolled patients and the retrospective nature of the study, with inherent selection bias; large, multicenter, and prospective studies are warranted to confirm these findings.

Collectively, our findings indicate that the expression of miR-301a cannot be employed to identify patients who can benefit from NAC and is unsuitable as a biomarker for NAC efficacy. The dysregulation of miR-301a and its relationship with breast cancer is complex, and underlying mechanisms remain unclear. Our results can still help in the clinical management of breast cancer to a certain extent.

Author contributions

Shanshan Deng performed the experiment and wrote the manuscript; Tingyou Zhang performed the experiment, organized the data, and reviewed the manuscript; Guojun Yue, Junhua Shi, and Mi Meng collected data and performed statistical analysis; Xi Chen, Shiyun Xing, Xin Tian searched and organized the literature, Xiaorong Yang, Fang Chen collected samples; Ning Li presented the original idea, performed a critical review of the manuscript, and analyzed the literature.

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Declaration of competing interest

The authors report no conflict of interest.

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