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Original Article

Long non-coding RNA MALAT1 promote triple-negative breast cancer progression by regulating miR-204 expression

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Abstract: Recent studies demonstrated that lncRNAs have a critical role in the regulation cancer progression and metastasis. However, little is known about the mechanism through which MALAT1 exerts its oncogenic activity, and the interaction between MALAT1 and microRNA remains largely unknown. In the present study, we reported that MALAT1 was up-regulated in triple-negative breast cancer (TNBC) tissues. Knockdown of MALAT1 inhibited proliferation, motility and increased apoptosis in vitro. In vivo study indicated that knockdown of MALAT1 inhibited tumor growth. Patients with high MALAT1 expression had poorer overall survival time than those with low MALAT1 expression. In addition, our findings demonstrate a reciprocal negative control relationship between MALAT1 and miR-204: downregulation of MALAT1 increased expression of miR-204, while overexpression of miR-204 decreased MALAT1 expression. We proposed that MALAT1 exerted its function through the miR-204. In summary, we proposed that MALAT1 may be a target for TNBC therapy.

Keywords: miR-204, MALAT1, triple-negative breast cancer

Introduction

Breast cancer is a common malignancy, with an incidence of more than 1,000,000 new cases and 370,000 deaths annually worldwide [1]. Accounting for about 20% of breast cancers, triple-negative breast cancer (TNBC) refers to breast cancers that do not express the genes for estrogen receptor (ER), progesterone receptor (PR) and the Her2/neu receptor [2]. Treatment of TNBC is challenging because the lack of targeted therapy, aggressive behavior and relatively poor prognosis [3]. Thus, it is urgent to find out new strategy for the treatment of TNBC.

It is well documented that protein-coding genes account for only about 2% of the human genome, whereas the majority of transcripts consist of the non-coding RNAs, including microRNAs and long non-coding RNAs (lncRNAs). MicroRNAs (miRNAs) are small non-coding RNAs of about 22 nucleotides in length that negatively regulate coding gene [4] or lncRNA expression [5]. It is well documented that miRNAs are involved in diverse biological processes, including differentiation, proliferation, apoptosis, and tumorigenesis [6, 7]. MiR-204 has been reported to function as a tumor suppressor in various cancers [8, 9]. A previous report suggests that miR-204 may be a tumor suppressor in breast cancer [10]. However, the underlying mechanism of miR-204 in breast cancer development was still poorly explored. Although it is well known that miRNAs can target a number of protein-coding genes, little is known whether miRNAs/lncRNAs can also target lncRNAs/miRNAs. Recently, a number of literatures documented the regulatory networks between miRNAs and lncRNAs [11, 12].

Unlike the miRNAs, long ncRNAs (lncRNAs) are by definition >200 nt in length. Metastasis Associated Lung Adencarcinoma Transcript 1 (MALAT1), mapped to human chromosome 11q13, was aberrantly up-regulated in multiple cancerous tissues and conferred proliferative and metastatic phenotypes to tumor cells [13-

15]. In the current study, we investigated the clinical significance of MALAT1 on TNBC and confirmed its biological functions by in vitro and in vivo assays. In addition, we also documented the reciprocal regulation of miR-204 and MALAT1.

Materials and methods

Cells culture and TNBC patients tissues

The breast cancer cell lines were purchased from the Cell Bank of the Shanghai Institutes for Biological Sciences (Chinese Academy of Sciences, Shanghai, China).

A total of 129 female breast cancer patients who were diagnosed by histo-pathology in the Affiliated Hospital of Guangdong Medical University from October 2005 to September 2011 were obtained. Specimens were formalin-fixed and embedded in paraffin by standard methodology after obtained during surgery. All breast cancer patients gave written consent for their tissue samples to be used for research purposes. This study was conducted with the approval of the Ethical and Scientific Committees of Guangdong Medical University with the permit number of 778NLSY03.

RNA isolation, reverse transcription, and quantitative real-time PCR

Total RNA from either culture cells or tissue samples was isolated with the use of Trizol reagent (Invitrogen). The samples were reverse transcribed into cDNA with different RT primers by using Revert Ace kit (Takara, Japan). PCR primers of MALAT1 and GAPDH were previously described [16-18]. To detect miR-204 and U6 expression, RNA samples were reverse transcribed into cDNA using Revert Ace transcriptase by specific stem-loop RT primers according to the manufacturer's instruction. The primers used for miR-204 and U6 were described in previous report [19]. Transcript levels were measured against an endogenous control by qPCR using the SYBR Green I fluorogenic dye using the Mastercycler ep realplex system (Eppendorf, Germany).

Lentivirus vector construction and infection

Short-hairpin RNA directed against human lncRNA MALAT1 was ligated into the LV10-CMV-RFP-Puro vector (GenePharma, Shanghai, China). The empty vector was used as a negative control (NC). The viruses were packaged in 293T cells according to standard protocols and the virus particles were harvested 72 h later. Cells were infected with virus particles plus 8 μg/ml Polybrene (Sigma, St Louis, Missouri, USA), followed by selected with puromycin for up to 7 days.

Oligonucleotide transfection

MiR-204 mimic and miR control were purchased from GenePharma (Shanghai, China). Cells transfection was carried out using Lipofectamine 2000.

Dual luciferase reporter gene assays

The fragment from MALAT1 containing the predicted miR-204 binding site was amplified by PCR and cloned into a pmirGIO Dual-luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA) and this vector was named wt-MALAT1. To test the binding specificity, the corresponding mutant was created by mutating the miR-204 seed region binding site and this vector was named mut-MALAT1. The luciferase assay was performed by using the dual Luciferase reporter assay system (Promega) 48 h after transfection.

MTT, Boyden and flow cytometry assay

The MTT assay was carried out as previously described [20].

For Boyden assay, the transwell chambers were coated with 100 μl BD Matrigel overnight in cell incubator. Then the cells were added to upper transwell chambers. A medium containing 10% FBS was added to the lower wells. After 48 h incubation, cells were fixed and stained, and the nonmigratory cells were scraped from the upper part of the filter. Then the migrated cells were stained with 0.2% crystal violet solution and counted.

To detect cell apoptosis, the cells were harvested and stained with 7-AAD and Annexin-V-FITC. Flow cytometry data was analyzed by BD FACSDiva software V6.1.3 (BD Biosciences).

Animal studies

All experiment procedures related to animals were approved by the Committee of Animal

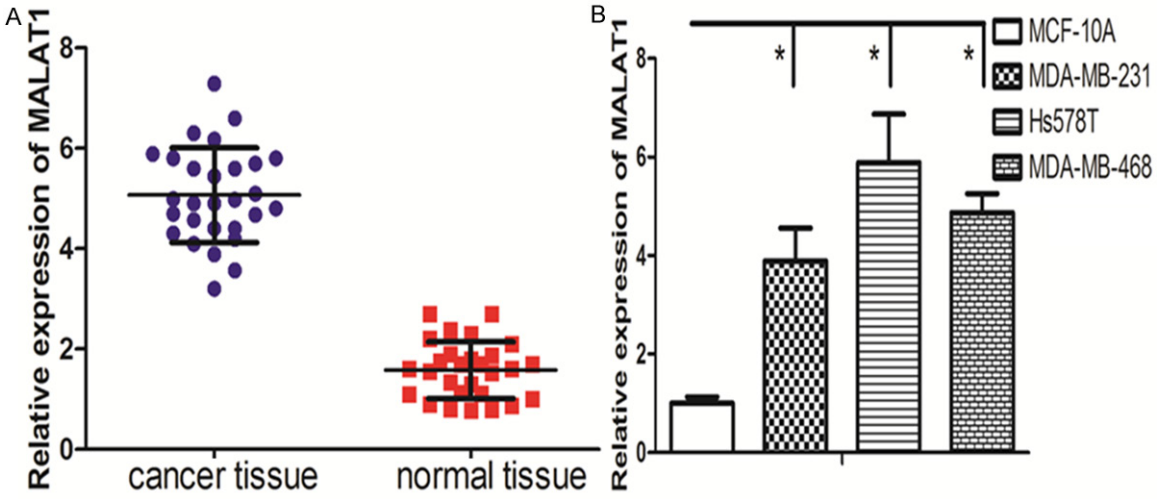

Figure 1. A: MALAT1 was upregulated in TNBC tissues compared with their adjacent normal breast tissues. B: MALAT1 expression in TNBC cell lines was relatively high compared to that in MCF-10A cells.

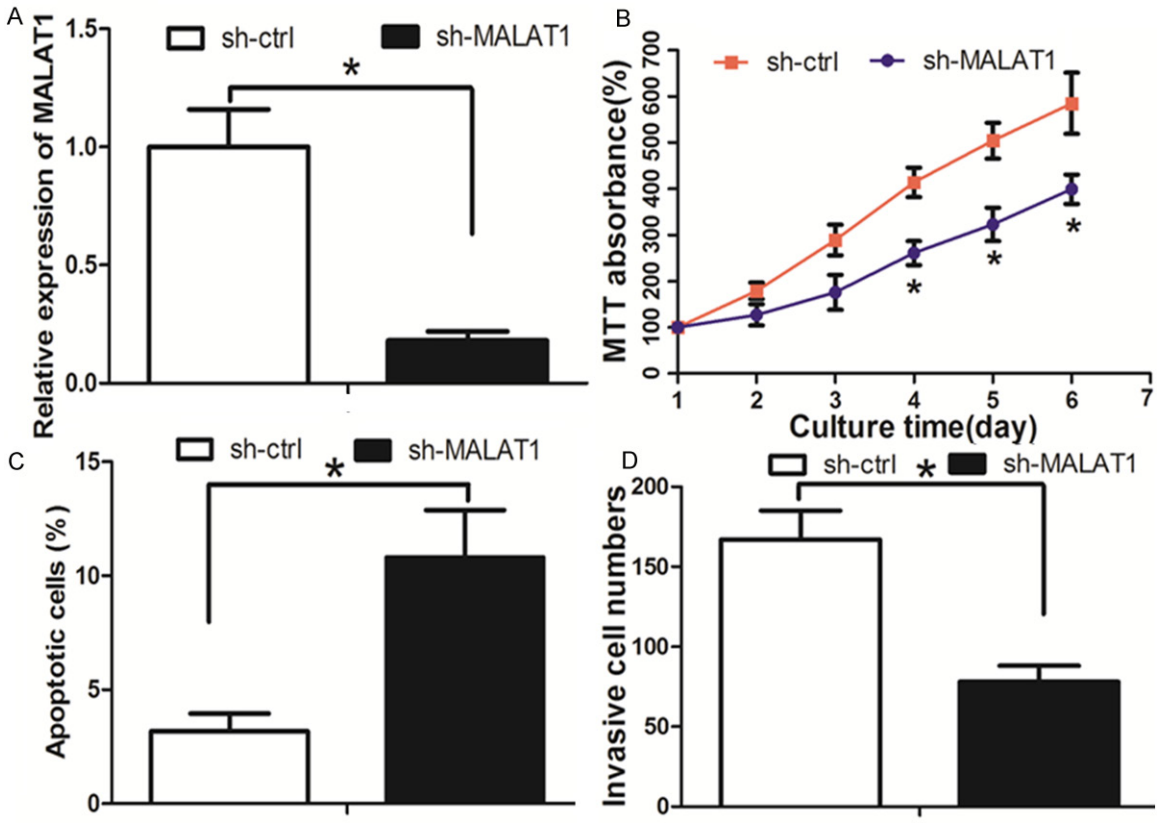

Figure 2. A: Lentivirus sh-MALAT1 was used to knock down MALAT1 in Hs578T cells. B: Knockdown of MALAT1 inhibited Hs578T cell proliferation. C: Knockdown of MALAT1 increased the rate of apoptosis in Hs578T cells. D: The cell invasion ability was decreased in sh-MALAT1 group when compared with sh-ctrl group.

Experimentation and the Ethic Committee of Guandong Medical University (Permit Number: 2014-08-XF818). The nude mice were kept in pathogen free environment with 12-hour light/dark cycle, controlled humidity and temperature. For the in vivo tumor growth studies, 1 ×

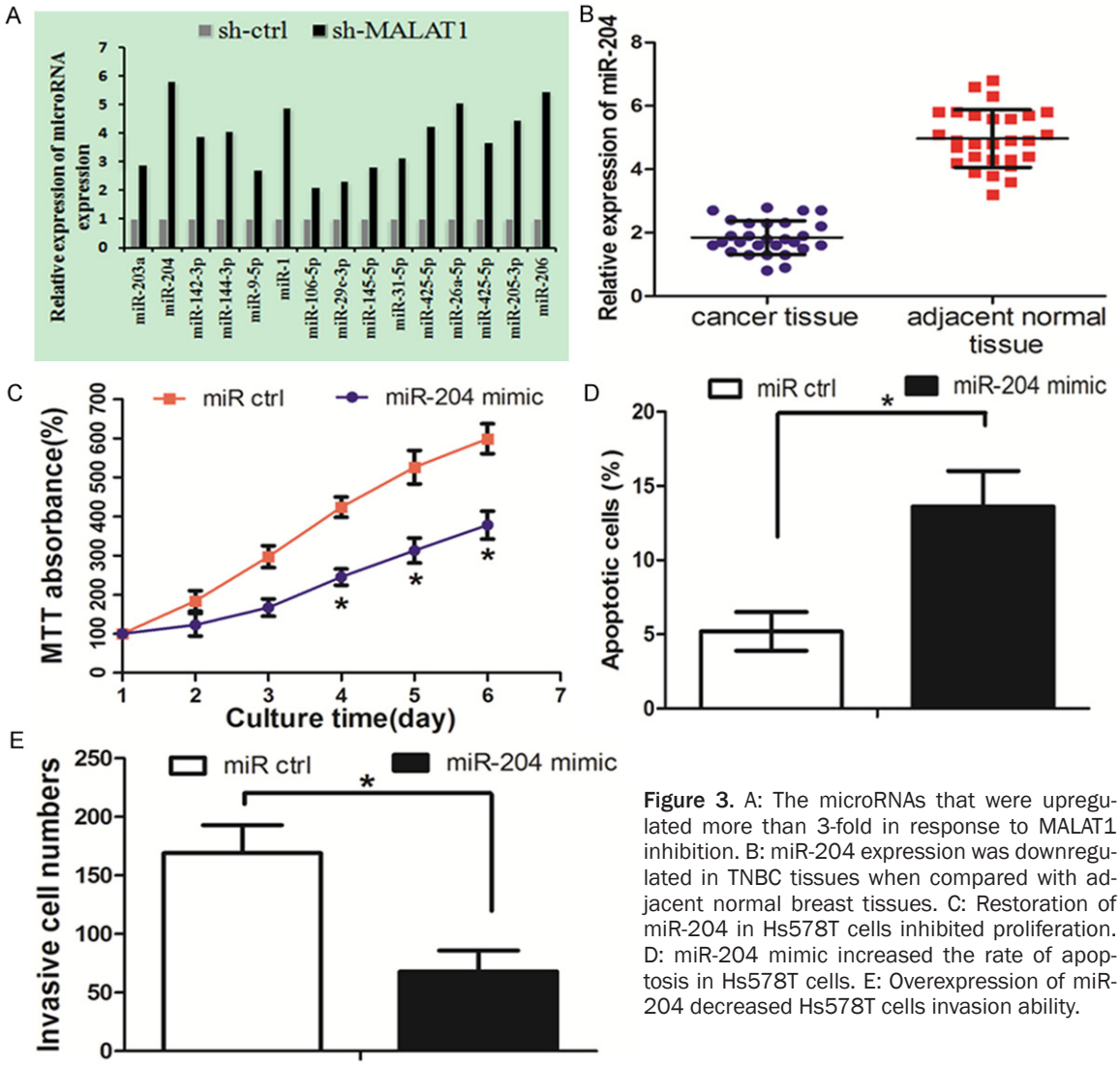

Figure 3. A: The microRNAs that were upregulated more than 3-fold in response to MALAT1 inhibition. B: miR-204 expression was downregulated in TNBC tissues when compared with adjacent normal breast tissues. C: Restoration of miR-204 in Hs578T cells inhibited proliferation. D: miR-204 mimic increased the rate of apoptosis in Hs578T cells. E: Overexpression of miR-204 decreased Hs578T cells invasion ability.

10^6 LV-miR-204 or LV-miR-ctrl cells were injected subcutaneously in the upper back of BALB/C-nu/nu athymic nude mice. The length and width of the tumors were measured every 5 days using a digital caliper and tumor volumes were calculated using the formula Volume (mm^3) = L × W^2/2 (length L, mm; width W, mm). Four weeks after injection, mice were anesthetized with chloral hydrate and sacrificed by cervical dislocation. Then the tumors were removed and weighed.

Statistical analysis

SPSS 13.0 software was used to analyze the data. Survival analysis was performed using the Kaplan-Meier method. A log rank test was used to compare different survival curves. A two-tailed Student's t-test was used for the comparison between two independent groups. One-way ANOVA was used to determine the differences between groups. *P* values of <0.05 were considered as statistically significant.

Results

MALAT1 expression was upregulated in TNBC tissues and cell lines

First, we analyzed the relative expression levels of MALAT1 by using real-time qPCR in 38 pairs of human TNBC tissues and adjacent normal breast tissues. The results showed that MALAT1 was upregulated in TNBC tissues compared with their adjacent normal breast tissues (**Figure 1A**). Subsequently, the expression of

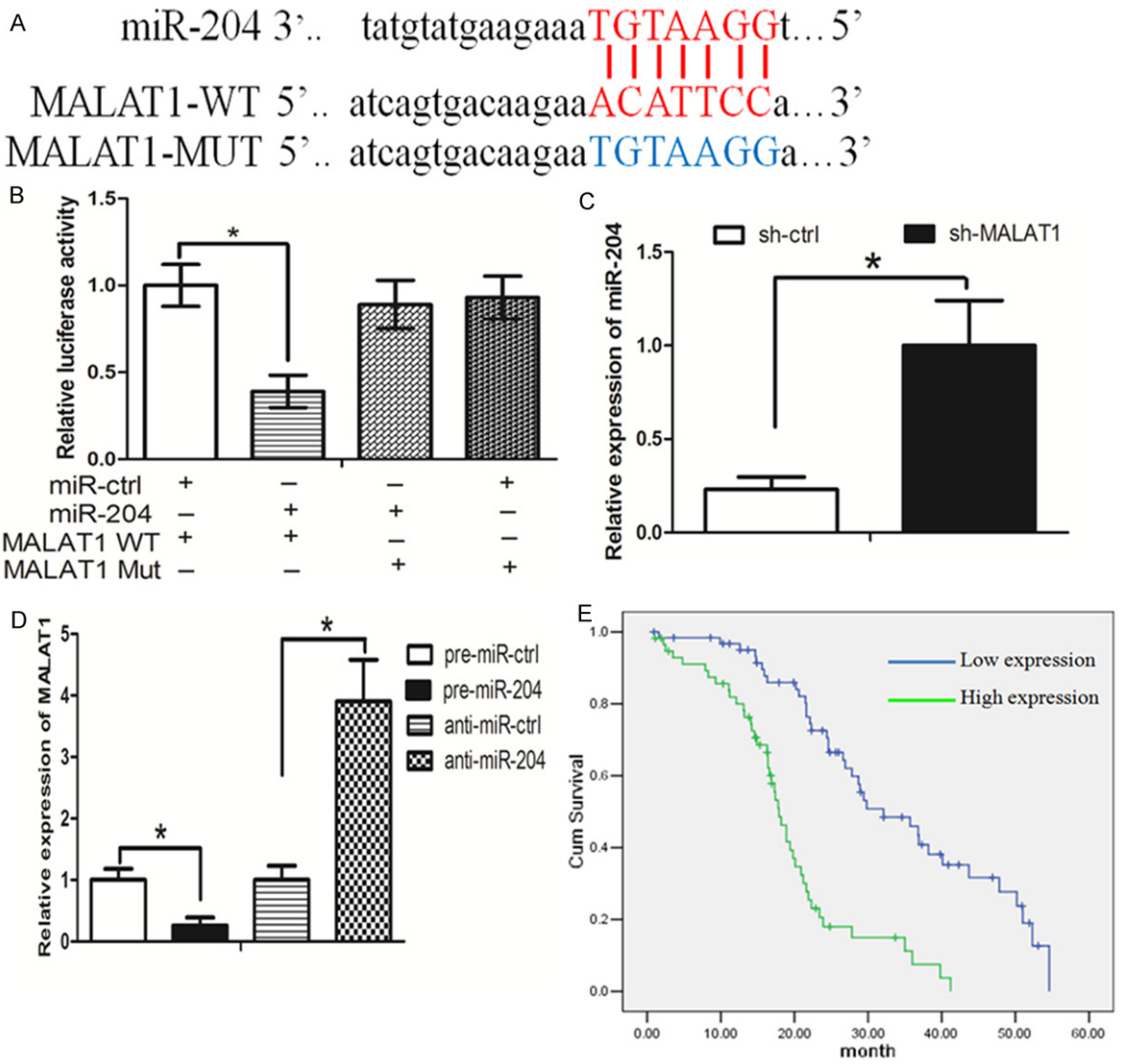

Figure 4. A: The wild-type (wt) MALAT1 and mutant (mut) MALAT1 reporter vector was constructed as indicated. B: miR-204 mimic reduced the luciferase activity of wt MALAT1 reporter vector, but not that of mut MALAT1 reporter vector. C: MALAT1 decreased the expression of miR-204. D: Overexpression of miR-204 decreased MALAT1 expression, while inhibition of miR-204 increased MALAT1 expression. E: TNBC patients with high MALAT1 expression tend to had poorer overall survival time than those with low MALAT1 expression.

MALAT1 was evaluated in TNBC lines (MDA-MB-231, Hs578T and MDA-MB-468) and normal immortalized MCF-10A cells. It was found that MALAT1 expression in TNBC cell lines was relatively high compared to that in MCF-10A cells (**Figure 1B**).

Knockdown of MALAT1 inhibited the cell proliferation, invasion, and promoted apoptosis of TNBC cells

The significant increase of MALAT1 in TNBC tissues and cell lines prompted us to explore the possible biological significance of MALAT1 in TNBC tumorigenesis. First, the lentivirus sh-MALAT1 was used to knock down MALAT1 in Hs578T cells (**Figure 2A**). The MTT assay revealed that the cell proliferation was decreased in sh-MALAT1 group when compared with sh-ctrl group (**Figure 2B**). Flow-cytometric analysis was performed to determine whether apoptosis was a contributing factor to cell proliferation inhibition. It was found that the apoptosis rate was increased in sh-MALAT1 group when compared with sh-ctrl group (**Figure 2C**). We then evaluated the abilities of cell invasion, which was a significant

Table 1. Clinicopathologic characteristics of the breast cancer patients and MALAT1 expression

Variables	MALAT1			*P* Value*
	All case (n = 129)	Low expression (n = 65)	High expression (n = 64)	
Age (years)				
≤50	54	29	25	0.523
>50	75	36	39	
Tumor size (cm)				
≤2	59	26	33	0.188
>2	70	39	31	
TNM stage				
I-II	77	32	45	0.015*
III-IV	52	33	19	
Distant metastasis				
Yes	54	36	18	0.002*
No	75	29	46	

(*P <0.05).

aspect of cancer progression. The cell invasion ability was decreased in sh-MALAT1 group when compared with sh-ctrl group (**Figure 2D**). Taken together, these results reflected that knockdown of MALAT1 had tumor-suppressive effects that could inhibit cell proliferation and invasion and promote apoptosis in TNBC.

Identification of microRNAs that were targeted by MALAT1

The online software program starbase v2.0 (http://starbase.sysu.edu.cn/mirLncRNA.php) [21] was used to search for miRNAs that have complementary base pairing with MALAT1. The microRNAs that formed complementary base pairing with MALAT1 were shown in Supplementary Table 1. We then examined the expression of these microRNAs in response to MALAT1 knockdown. As found in **Figure 3A**, there was a list of microRNAs that were upregulated more than 3-fold in response to MALAT1 inhibition. We focused on miR-204, which was of the greatest fold-change in response to MALAT1 knockdown. We further examined the expression of miR-204 in 38 pairs of human TNBC tissues and adjacent normal breast tissues used above. The result showed that miR-204 expression was downregulated in TNBC tissues when compared with adjacent normal breast tissues (**Figure 3B**). In addition, restoration of miR-204 decreased TNBC cell proliferation and invasion, and increased the rate of apoptosis (**Figure 3C-E**).

Reciprocal repression of MALAT1 and miR-204

Luciferase reporter constructs were generated to confirm the direct binding between MALAT1 and miR-204. The wild-type (wt) MALAT1 and mutant (mut) MALAT1 was shown in **Figure 4A**. It was observed that miR-204 mimic reduced the luciferase activities of wt MALAT1 reporter vector. On the contrary, luciferase activities in cells transfected with mut MALAT1 and miR-204 mimic were almost comparable to that of control cells (**Figure 4B**). These data suggested that the binding sites are vital for the reciprocal repression of MALAT1 and miR-204.

We next asked whether there was a reciprocal repression between MALAT1 and miR-204. It was found that knockdown of MALAT1 increased miR-204 expression (**Figure 4C**). Interestingly, overexpresion of miR-204 decreased MALAT1 expression, while inhibition of miR-204 increased MALAT1 expression (**Figure 4D**). These data suggest that there was a reciprocal repression between MALAT1 and miR-204.

The relationships between expression of MALAT1 and clinical parameters in TNBC patients

To further investigate the clinicopathological and prognostic significance of MALAT1 levels in TNBC patients, the levels of MALAT1 in a large cohort of 129 TNBC tissues (including the tissues used before) were examined by real-time PCR. The median value of all 129 TNBC samples was chosen as the cut-off point for separating tumors with low-level expression of MALAT1 from high-level expression MALAT1 tumors. Thus, 65 (50.3%) TNBC patients had low-level expression of MALAT1, while 64 (49.7%) TNBC patients had high-level expression of MALAT1. The clinicopathologic characteristics of the TNBC patients and MALAT1 expression are shown in **Table 1**. High expression of MALAT1 correlated with late TNM stage

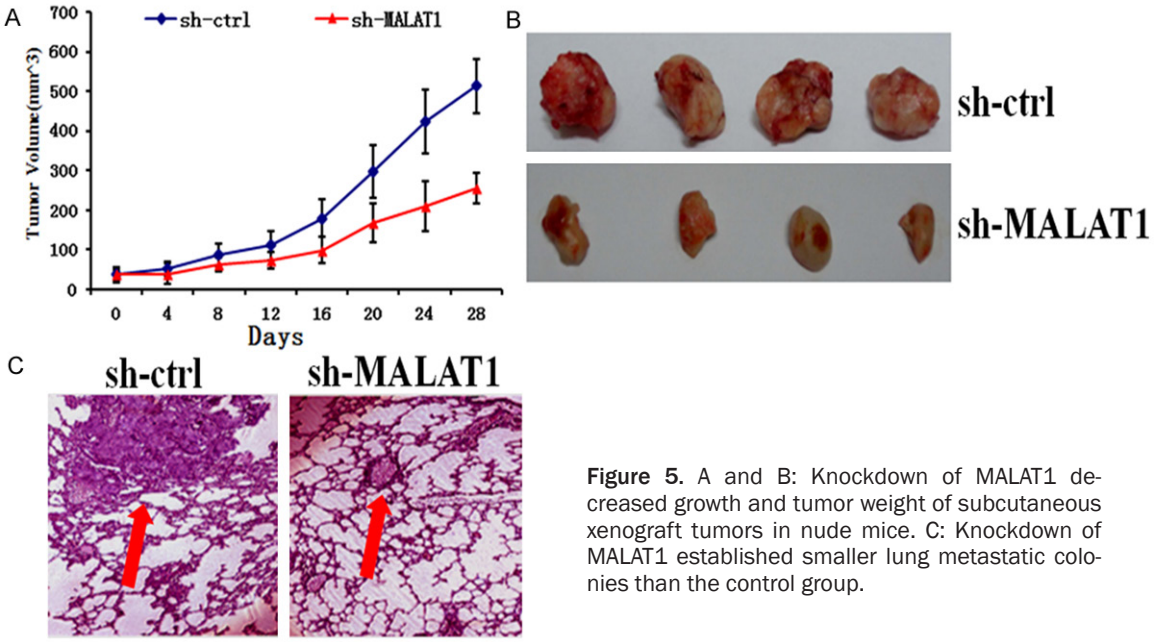

Figure 5. A and B: Knockdown of MALAT1 decreased growth and tumor weight of subcutaneous xenograft tumors in nude mice. C: Knockdown of MALAT1 established smaller lung metastatic colonies than the control group.

(P = 0.015) and higher metastasis (P = 0.002), although there was no significant association between age and tumor size. In addition, we found that TNBC patients with high expression of MALAT1 had poorer overall survival time (**Figure 4E**).

Knockdown of MALAT1 inhibited TNBC cells growth and invasion in vivo

We next investigated whether knockdown of MALAT1 against tumor growth and metastasis in vivo. As expect, knockdown of MALAT1 decreased growth and tumor weight of subcutaneous xenograft tumors in nude mice (**Figure 5A** and **5B**). In the experimental metastasis studies, knockdown of MALAT1 established smaller lung metastatic colonies than the control group (**Figure 5C**). These data revealed that inhibition of MALAT1 inhibited TNBC cells growth and invasion in vivo.

Discussion

Mounting evidence demonstrated that ncRNAs played a significant role in cancer pathogenesis [22, 23]. During the past decade, researches on miRNAs have dominated the field of ncRNA regulation, including in TNBC [24, 25]. However, the role of lncRNAs in TNBC is still largely unknown. Recently, we and other researchers have provided new insights into lncRNAs in TNBC progression [26-28]. MALAT1, mapped to human chromosome 11q13, was originally identified as a prognostic marker for metastasis and patient survival in NSCLC [29]. MALAT1 was demonstrated to promote tumor growth and metastasis through different mechanisms, depending on tissue contexts [30, 31]. However, the role of MALAT1 in breast cancer, especially in TNBC, has not been investigated before. In the present study, we found that MALAT1 was upregulated in TNBC tissues and cell lines. Knockdown of MALAT1 decreased TNBC cell proliferation and invasion, and increased the rate of apoptosis. Inhibition of MALAT1 decreased tumor growth and metastasis in vivo. Further investigation revealed that the MALAT1 expression level was positively associated with late TNM stage and metastasis in TNBC patients. In addition, patients with high MALAT1 expression tend to had poorer over survival time. These data implicated that MALAT1 may serve as an oncogene in TNBC.

Recent studies suggest that lncRNAs may exert functions through targeting miRNAs. Recently, MALAT1 was found to interact with miR-205 in renal cell carcinoma [32]. We explored the interaction between MALAT1 and miR-204 in TNBC. We performed a search for miRNAs that had complementary base pairing with MALAT1 and the expression changes of these microRNAs in response to MALAT1 knockdown were examined. We focused on miR-204, as it is of the greatest fold change. We found that knockdown of MALAT1 increased miR-204 expres-

sion. On the other hand, miR-204 mimic repressed MALAT1 level while miR-204 inhibitor upregulated MALAT1 level. We further explored the mechanism of such a feedback loop. It was found that MALAT1 and miRNA-204 bind to the same RISC complex. Based on the fact that lncRNAs may participate in the 'competitive endogenous RNAs (ceRNA)' regulatory network and act as endogenous miRNA sponges to bind to miRNAs and regulate their function [33, 34], we proposed that MALAT1 may regulate miR-204 expression in such way. Emerging evidence documented that miRNAs mediate post-transcriptional control of gene expression by binding to the 3'-untranslated regions of protein coding genes, we supposed that the way that miR-204 promoted the downregulation of MALAT1 is somewhat similar to the miRNA-mediated silencing of protein-coding genes. MiR-204 has been found to be downregulated in a variety of carcinomas and exhibits tumor-suppressive activity [35, 36]. We proposed that MALAT1 exert its function by negatively regulated miR-204, and thereby promoted TNBC progression.

In summary, our findings first revealed that MALAT1 functioned as an oncogene in TNBC and promoted TNBC progression through interacting with miR-204. In addition, our findings deepened the understanding of lncRNA regulatory network and may help to develop potential therapeutic strategy for TNBC.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. The microRNAs that formed complementary base pairing with MALAT1

Name	Mir accession	Gene name	Target sites	Bio complex	Clip read num	Cancer num
hsa-miR-503-5p	MIMAT0002874	MALAT1	1	22	13004	6
hsa-miR-197-3p	MIMAT0000227	MALAT1	2	6	0	5
hsa-miR-92b-3p	MIMAT0003218	MALAT1	1	8	2984	5
hsa-miR-28-5p	MIMAT0000085	MALAT1	1	8	880	5
hsa-miR-25-3p	MIMAT0000081	MALAT1	1	8	2984	5
hsa-miR-370-3p	MIMAT0000722	MALAT1	1	6	0	4
hsa-miR-149-5p	MIMAT0000450	MALAT1	1	6	0	4
hsa-miR-155-5p	MIMAT0000646	MALAT1	1	8	18	4
hsa-miR-378a-3p	MIMAT0000732	MALAT1	1	6	0	4
hsa-miR-23b-3p	MIMAT0000418	MALAT1	2	11	952	4
hsa-miR-506-3p	MIMAT0002878	MALAT1	2	8	7033	4
hsa-miR-135b-5p	MIMAT0000758	MALAT1	1	6	0	3
hsa-miR-129-5p	MIMAT0000242	MALAT1	1	7	1	3
hsa-miR-200c-3p	MIMAT0000617	MALAT1	2	8	759	3
hsa-miR-17-5p	MIMAT0000070	MALAT1	1	7	11	3
hsa-miR-20a-5p	MIMAT0000075	MALAT1	1	7	11	3
hsa-miR-203a	MIMAT0000264	MALAT1	2	7	929	3
hsa-miR-1	MIMAT0000416	MALAT1	2	9	2097	3
hsa-miR-23a-3p	MIMAT0000078	MALAT1	2	11	952	3
hsa-miR-181c-5p	MIMAT0000258	MALAT1	1	6	0	3
hsa-miR-125a-3p	MIMAT0004602	MALAT1	1	7	1	3
hsa-miR-216a-5p	MIMAT0000273	MALAT1	1	10	3465	3
hsa-miR-26b-5p	MIMAT0000083	MALAT1	2	7	1740	3
hsa-miR-185-5p	MIMAT0000455	MALAT1	1	9	5421	3
hsa-miR-206	MIMAT0000462	MALAT1	2	9	2097	3
hsa-miR-455-5p	MIMAT0003150	MALAT1	1	11	126	3
hsa-miR-363-3p	MIMAT0000707	MALAT1	1	8	2984	3
hsa-miR-200b-3p	MIMAT0000318	MALAT1	2	8	759	2
hsa-miR-200a-3p	MIMAT0000682	MALAT1	2	8	2683	2
hsa-miR-429	MIMAT0001536	MALAT1	2	8	759	2
hsa-miR-30e-5p	MIMAT0000692	MALAT1	1	8	2633	2
hsa-miR-30c-5p	MIMAT0000244	MALAT1	1	8	2633	2
hsa-miR-181b-5p	MIMAT0000257	MALAT1	1	6	0	2
hsa-miR-181a-5p	MIMAT0000256	MALAT1	1	6	0	2
hsa-miR-205-5p	MIMAT0000266	MALAT1	3	10	3034	2
hsa-miR-146b-5p	MIMAT0002809	MALAT1	1	4	4873	2
hsa-miR-378c	MIMAT0016847	MALAT1	1	6	0	2
hsa-miR-141-3p	MIMAT0000432	MALAT1	2	8	2683	2
hsa-miR-26a-5p	MIMAT0000082	MALAT1	2	7	1740	2
hsa-miR-135a-5p	MIMAT0000428	MALAT1	1	6	0	2
hsa-miR-4306	MIMAT0016858	MALAT1	1	9	5421	2
hsa-miR-494-3p	MIMAT0002816	MALAT1	1	8	4775	2
hsa-miR-328-3p	MIMAT0000752	MALAT1	1	8	5413	2
hsa-miR-140-5p	MIMAT0000431	MALAT1	2	7	1457	2
hsa-miR-22-3p	MIMAT0000077	MALAT1	1	6	1457	2
hsa-miR-142-3p	MIMAT0000434	MALAT1	1	14	161	2
hsa-miR-181d-5p	MIMAT0002821	MALAT1	1	6	0	2

hsa-miR-217	MIMAT0000274	MALAT1	2	20	12989	2
hsa-miR-216b-5p	MIMAT0004959	MALAT1	2	8	96	2
hsa-miR-425-5p	MIMAT0003393	MALAT1	1	6	0	2
hsa-miR-143-3p	MIMAT0000435	MALAT1	1	7	1198	2
hsa-miR-145-5p	MIMAT0000437	MALAT1	1	6	0	2
hsa-miR-146a-5p	MIMAT0000449	MALAT1	1	4	4873	2
hsa-miR-590-3p	MIMAT0004801	MALAT1	2	7	936	2
hsa-miR-876-5p	MIMAT0004924	MALAT1	1	6	0	2
hsa-miR-873-5p	MIMAT0004953	MALAT1	1	7	801	2
hsa-miR-204-5p	MIMAT0000265	MALAT1	1	8	845	2
hsa-miR-23c	MIMAT0018000	MALAT1	2	11	952	2
hsa-miR-20b-5p	MIMAT0001413	MALAT1	1	7	11	2
hsa-miR-106a-5p	MIMAT0000103	MALAT1	1	7	11	2
hsa-miR-194-5p	MIMAT0000460	MALAT1	1	6	0	1
hsa-miR-202-3p	MIMAT0002811	MALAT1	1	9	2478	1
hsa-miR-3167	MIMAT0015042	MALAT1	1	6	0	1
hsa-miR-320d	MIMAT0006764	MALAT1	2	7	1198	1
hsa-miR-1297	MIMAT0005886	MALAT1	2	7	1740	1
hsa-miR-92a-3p	MIMAT0000092	MALAT1	1	8	2984	1
hsa-miR-485-5p	MIMAT0002175	MALAT1	1	7	1	1
hsa-miR-154-5p	MIMAT0000452	MALAT1	1	7	1510	1
hsa-miR-211-5p	hsa-miR-211-5p	MALAT1	1	8	845	1
hsa-miR-124-3p	MIMAT0000422	MALAT1	2	8	7033	1
hsa-miR-1271-5p	MIMAT0005796	MALAT1	2	9	5601	1
hsa-miR-30a-5p	MIMAT0000087	MALAT1	1	8	2633	1
hsa-miR-93-5p	MIMAT0000093	MALAT1	1	7	11	1
hsa-miR-106b-5p	MIMAT0000680	MALAT1	1	7	11	1
hsa-miR-96-5p	MIMAT0000095	MALAT1	2	9	5601	1
hsa-miR-383-5p	MIMAT0000738	MALAT1	1	22	13004	1
hsa-miR-491-5p	MIMAT0002807	MALAT1	1	7	937	1
hsa-miR-32-5p	MIMAT0000090	MALAT1	1	8	2984	1
hsa-miR-374b-5p	MIMAT0004955	MALAT1	1	13	938	1
hsa-miR-374a-5p	MIMAT0000727	MALAT1	1	13	938	1
hsa-miR-224-5p	MIMAT0000281	MALAT1	2	11	952	1
hsa-miR-101-3p	MIMAT0000099	MALAT1	1	22	13004	0
hsa-miR-320b	MIMAT0005792	MALAT1	2	7	1198	0
hsa-miR-346	MIMAT0000773	MALAT1	1	6	0	0
hsa-miR-708-5p	MIMAT0004926	MALAT1	1	8	880	0
hsa-miR-613	MIMAT0003281	MALAT1	2	9	2097	0
hsa-miR-376a-3p	MIMAT0000729	MALAT1	1	8	4737	0
hsa-miR-376b-3p	MIMAT0002172	MALAT1	1	8	4737	0
hsa-miR-544a	MIMAT0003164	MALAT1	1	7	729	0
hsa-miR-422a	MIMAT0001339	MALAT1	1	6	0	0
hsa-miR-144-3p	MIMAT0000436	MALAT1	1	6	0	0
hsa-miR-338-3p	MIMAT0000763	MALAT1	3	11	3574	0
hsa-miR-320c	MIMAT0005793	MALAT1	2	7	1198	0
hsa-miR-150-5p	MIMAT0000451	MALAT1	1	7	2087	0
hsa-miR-519d-3p	MIMAT0002853	MALAT1	1	7	11	0
hsa-miR-499a-5p	MIMAT0002870	MALAT1	1	7	5	0

hsa-miR-378b	MIMAT0014999	MALAT1	1	6	0	0
hsa-miR-367-3p	MIMAT0000719	MALAT1	1	8	2984	0
hsa-miR-3139	MIMAT0015007	MALAT1	1	8	880	0
hsa-miR-320a	MIMAT0000510	MALAT1	2	7	1198	0
hsa-miR-30b-5p	MIMAT0000420	MALAT1	1	8	2633	0
hsa-miR-30d-5p	MIMAT0000245	MALAT1	1	8	2633	0
hsa-miR-384	MIMAT0001075	MALAT1	4	7	873	0
hsa-miR-378f	MIMAT0018932	MALAT1	1	6	0	-1
hsa-miR-4429	MIMAT0018944	MALAT1	2	7	1198	-1
hsa-miR-4262	MIMAT0016894	MALAT1	1	6	0	-1
hsa-miR-378i	MIMAT0019074	MALAT1	1	6	0	-1
hsa-miR-378d	MIMAT0018926	MALAT1	1	6	0	-1
hsa-miR-378h	MIMAT0018984	MALAT1	1	6	0	-1
hsa-miR-378e	MIMAT0018927	MALAT1	1	6	0	-1
hsa-miR-4465	MIMAT0018992	MALAT1	2	7	1740	-1
hsa-miR-4644	MIMAT0019704	MALAT1	1	9	5421	-1
hsa-miR-4770	MIMAT0019924	MALAT1	1	7	1198	-1