

## Original Article

# The effects of NMIIA protein expression on cell invasion and migration abilities in breast cancer tissues

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Received June 10, 2020; Accepted August 25, 2020; Epub November 15, 2020; Published November 30, 2020

**Abstract:** Objective: The purpose of this study is to explore the effect and possible mechanism of NMIIA protein expression on cell invasion and migration in BC tissues. Methods: 5 human BC cell lines (MDA-MB-231, MCF-7, SUM1315, ZR-75-1, and T47D) were transfected by SiRNA, and the expressions of the NMIIA proteins were determined using Western blotting. The effects of the NMIIA proteins on the proliferation and migration abilities of the MDA-MB-231 cell line were evaluated using cell culture and Transwell assays. Results: An overexpression of NMIIA was seen in all the human BC cells, and this overexpression was more significant in the MDA-MB-231 and SUM1315 cell lines. Compared with the negative control, the invasion and migration rates of the Si-RNA transfected cells were decreased ( $P < 0.05$ ). In addition, the expression of the Wave2 protein was also decreased after the transfection. Conclusion: The high expression of NMIIA in the BC tissues is related to its increased cell invasion and migration abilities, and its mechanism may be related to the wave2 protein and its pathway.

**Keywords:** NMIIA, breast cancer, invasion, migration

## Introduction

Breast cancer (BC) ranks first in the incidence of cancer among women in China, and it is also the second leading cause of cancer deaths among women [1]. The development of BC involves multiple steps and many cell types, and its prevention is still a worldwide challenge. Early diagnosis and timely intervention treatment have improved the survival rate and prognosis of BC patients. As a result of the early interventions, the 5-year survival rate of BC patients has exceeded 80% in some developed countries and regions [2]. A study indicated that gender, age, estrogen levels, family history, gene mutations, and unhealthy lifestyles are the main factors increasing the number of BC patients [3]. In the past decade, many breakthroughs have been made in the treatment of BC through continuous in-depth research on BC's mechanisms and on preventive interventions.

Non-muscle myosin IIA (NMIIA) is a non-muscle myosin encoded by the Myh9 gene [4]. It is a

cytoplasmic myosin widely expressed in the human body and has an important significance for the occurrence of the disease. Many studies have shown that NMIIA can promote the development of various types of cancer [5-7]. Although current research has clarified some functions of NMIIA, its pathogenic mechanism is still unclear. In addition, the effect of NMIIA on BC remains to be further studied.

Therefore, the relationship between NMIIA protein expression and the occurrence of BC is worthy of in-depth study, which has rarely been reported worldwide. In this study, in vitro cell experiments were performed to explore the effect of NMIIA protein expression on cell invasion and migration abilities in BC tissues and to elucidate its underlying mechanism.

## Materials and methods

BC cell lines (MDA-MB-231, MCF-7, SUM1315, ZR-75-1, and T47D) and a human breast epithelial cell line (MCF10A) were purchased from the

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**Table 1.** Primers

Gene	Primer, 5'→3'
NMIIA	Forward: AGAGCTCACGTGCCTCAACG
	Reverse: TGACCACACAGAACAGGCCTG
GAPDH	Forward : CAGCCTCAAGATCATCAGCA
	Reverse: TGTGGTCATGAGTCCTCCA

Chinese Academy of Sciences (Beijing) cell bank.

## Main reagents

Fetal bovine serum (FBS), 0.25% trypsin reagent, modified DMEM medium, DMEM/F12 (Gibco, New York, USA), TRIzol reagent, Lipofectamine 2000 transfection reagent (Invitrogen, Grand Island, New York, USA), a THPCR kit (Toyobo Scientific Research, Tokyo, Japan), GAPDH antibody, rabbit and mouse secondary antibodies, a total protein extraction kit (Biyuntian Biological Company, Nanjing, China), si-NMIIA which was synthesized and identified by Shanghai Jima Pharmaceutical Co., Ltd., Polyclonal NMIIA antibody (Abcam, Cambridge, UK), polyvinylidene fluoride membrane, SDS-PAGE (Sigma-Aldrich, St. Louis, Missouri, USA), and a Cinchonine protein assay kit (Pierce, Illinois, USA).

## Cell culture and grouping

The BC cell lines were cultured in a DMEM medium containing 10% FBS and 1% penicillin-streptomycin. The MCF-10A cells were cultured in a DMEM/F12 medium containing insulin (10 µg/ml), hydrocortisone (0.5 µg/ml), and 5% horse serum. The plate was placed in a 37°C, 5% CO<sub>2</sub> incubator for routine culture.

## Si-RNA transfection

After the cells adhered to the wall, the cultured cells were digested and dispersed. Cell dissociated reagents were used to collect the cells, which were washed with PBS, then resuspended and added to 90 µL of transfection solution, followed by mixing it with 50 pmol of NMIIA specific siRNA oligonucleotide and 4 µl of Lipofectamine 2000, and transferred to 125 µl of DMSM. The cells were then diluted in a growth medium, seeded in tissue culture plates (60 mm), and incubated for 72 h, and then the cells were collected for migration or spreading assays.

## Western blotting

Western blotting was performed according to the standard procedure [8]. The total protein of each group of cells was extracted and stored at -20°C. The rabbit polyclonal NMIIA antibody was diluted to 1:500. The sample was transferred to a microcentrifuge tube, homogenized and then centrifuged (14000 rpm, 4°C, 15 min) to precipitate the protein. The sample separation by electrophoresis was first performed on a 10% SDS-polyacrylamide gel, and then transferred to a polyvinylidene fluoride membrane.

## RT-qPCR analysis

Total RNA was extracted with TRIzol reagent, and the first strand cDNA was synthesized using reverse transcription with a RevertAid first-strand cDNA synthesis kit. The detection condition of the reaction was at 70°C for 5 min. A quantitative measurement was performed using a THPCR kit. The PCR was performed with 12.5 µl 2X qPCR mix with 2.0 µl primer (2.5 µM), and 2.0 µl cDNA and 8 ul double distilled water were added to a 0.2 ml PCR tube. The amplification conditions were 40 cycles of 95°C, 15 min; 95°C, 15 s; 55°C, 30 s; 72°C, 25 s. The internal reference was GAPDH (Table 1).

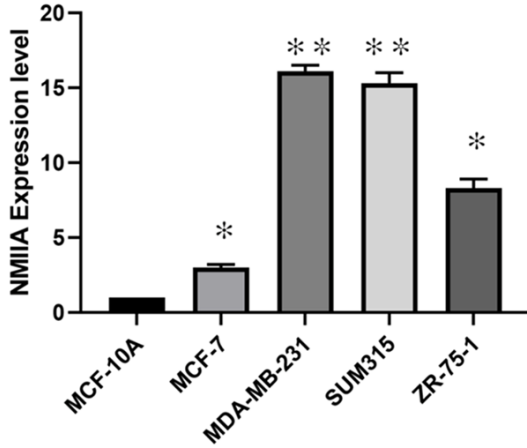
## The transwell cell migration assay in vitro

The cell invasion was measured with a Transwell cell migration assay. 600 µl of DMEM medium containing 10% FBS was added to the 24-well plate. Matrigel was added to the upper chamber (40 µl/well) and then diluted with serum-free DMEM medium. The transfected MDA-MB-231-SiNMIIA and MDA-MB-231-SiNC cells were digested with trypsin, centrifuged at 1000 r/min for 3 min, and suspended at a concentration of 1 × 10<sup>5</sup> cell/ml. A 50 µl suspension was inoculated into the upper chamber. After incubation for 24 h, 10% formalin was used to fix the cells attached to the lower chamber, and the cells were stained with crystal violet for 30 min, and then washed twice with PBS to quantify the cell migration.

## Statistical analysis

SPSS 23.0 software was used for the data analysis, and GraphPad 8.0 was used to create the figures. Each experiment was repeated 3 times. The measurement data were expressed as the mean ± SD and were compared using t

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**Figure 1.** An RT-qPCR analysis of the NMIIA expression levels.

tests. Independent sample t tests were used for the inter-group comparisons, and paired sample t tests were used for the comparisons between the paired samples.  $P < 0.05$  indicates a statistically significant difference.

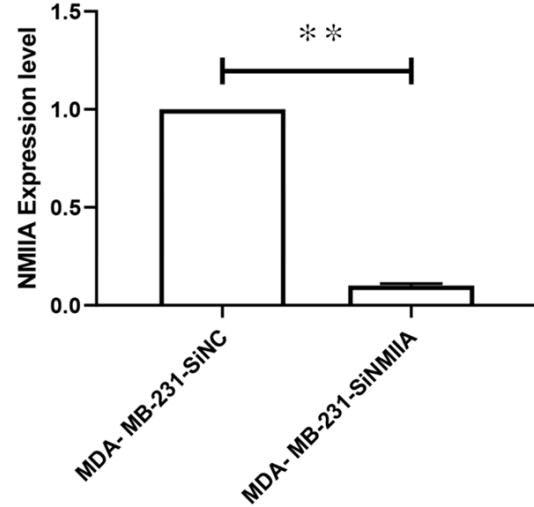
### Results

#### *NMIIA expression is highly expressed in the BC cell line*

Western blotting showed that, compared with the MCF-10A cells, each human BC line showed an increased NMIIA expression, indicating that it was overexpressed in various BC cell lines ( $P < 0.05$ ). The RT-PCR analysis indicated that the expression level of NMIIA increased significantly compared with the expression level of the MCF-10A cells ( $P < 0.05$ ), of which the over-expressions of MDA-MB-231 and SUM1315 were the most significant, which were  $(16.1 \pm 0.4)$  and  $(15.3 \pm 0.7)$ , respectively (**Figure 1**).

#### *NMIIA silencing inhibits the migration of BC cells*

The NMIIA expression was suppressed by SiRNA in the MDA-MB-231 cell line. Compared with the control group, SiNMIIA was  $(0.1 \pm 0.02)$  after the inhibition, showing a significant decrease ( $P < 0.05$ ) (**Figure 2**). The migration rate of the MDA-MB-231 cells  $(346.0 \pm 29.0)$  was significantly lower than that of the non-interfering cells  $(202.0 \pm 33.0)$ , showing a significant difference ( $P < 0.05$ ). The cell invasion of the MDA-MB-231 cells  $(148.2 \pm 24.0)$  was



**Figure 2.** Interference with the NMIIA expressions.

also significantly lower than that of the non-interfering cells  $(196.0 \pm 23.0)$  (**Figure 3**). Compared with the negative control  $(53.5 \pm 5.1)$ , the cell migration and invasion rates were significantly reduced after silencing the NMIIA  $(24.3 \pm 3.1)$  ( $P < 0.05$ ) (**Figure 4**).

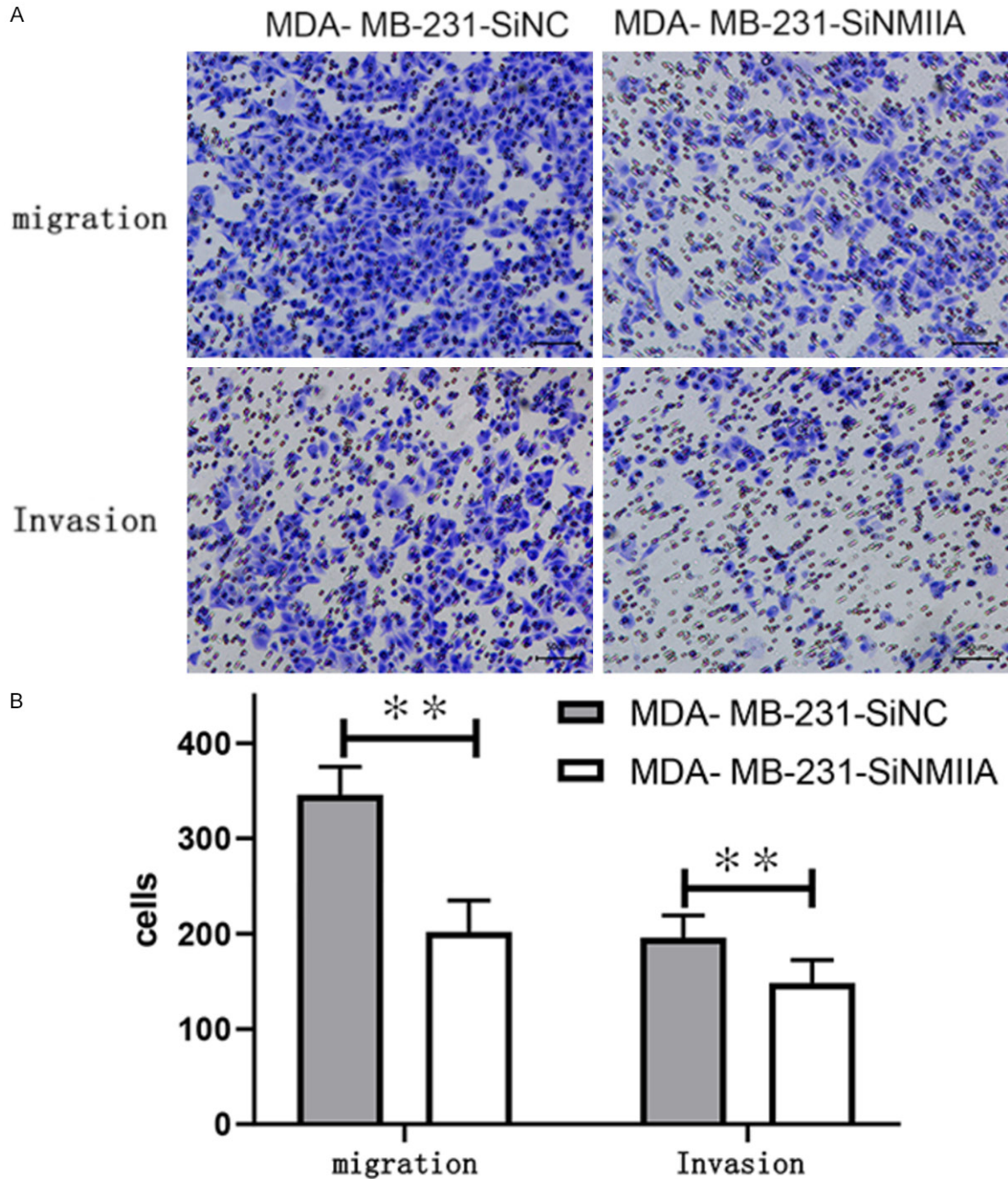
#### *Silencing NMIIA inhibits Wave2 expression*

A Western blotting analysis was used to evaluate the expression of Wave2 after the NMIIA inhibition. The results of the qRT-PCR quantification showed that after the transfection of Si-RNA, NMIIA was silenced, and it showed decreased wave2 levels in the MDA-MB-231 cells  $(0.24 \pm 0.12)$  compared with the negative control group  $(0.72 \pm 0.26)$  ( $P < 0.01$ ) (**Figure 5**).

### Discussion

BC is the second leading cause of cancer-related deaths [8, 9]. This study evaluated the role of NMIIA in regulating tumor cell invasion and metastasis and predicting prognosis and investigated the expression status of NMIIA in BC at the mRNA and protein levels. The RT-qPCR results showed that the transcription level of NMIIA in the BC cell lines is higher than it is in normal tissue cells, and the Western blotting analysis also confirmed the expression of the NMIIA protein in BC cells compared with the matched normal tissue.

NMIIA is associated with cell migration, covering membrane protrusions, adhesion to the substratum, forward propulsion of cell bodies,



**Figure 3.** Cell invasion and migration.

and the disengagement of the trailing edge [5, 10-12]. NMIIA plays a critical role in the maturation of focal adhesion complexes [13, 14]. The inhibition of NMIIA can cause the disruption of stress fiber formation, which leads to adhesion [15-19] and affects the assembly of adherent plaques [20], thereby disrupting cell retraction and cell migration [21].

In addition, an increased expression of NMIIA was observed in the BC tissues, and the other BC cell lines showed high expressions of NMIIA. Furthermore, the increased expression level of NMIIA was related to the depth of the wall infiltration. In general, these findings suggest that an elevated expression of NMIIA may promote the invasion and metastasis of BC cells.

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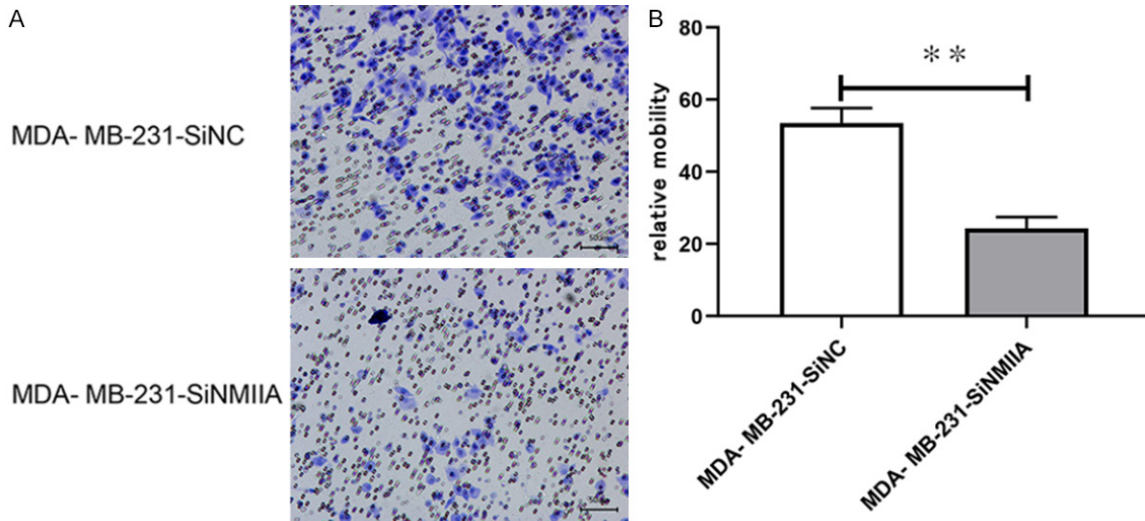


Figure 4. A comparison of the cell migration rates.

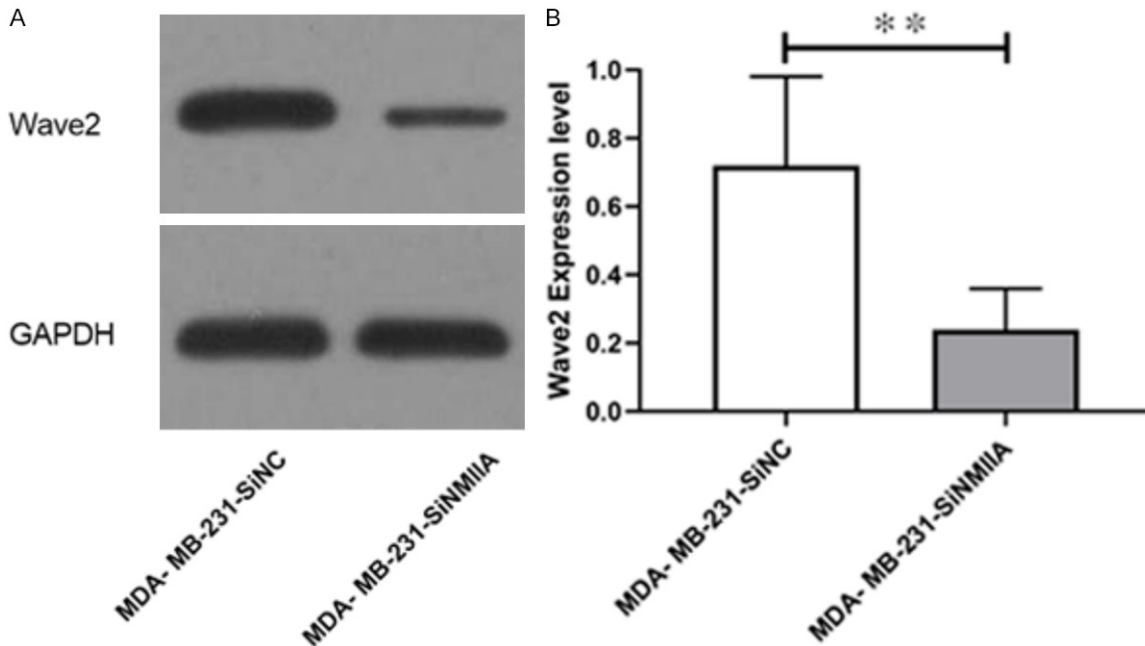


Figure 5. Wave expressions.

At present, several studies have shown that NMIIA is associated with cancer metastasis and invasion, but the underlying mechanism of the overexpression of human NMIIA in human tumors is unclear. A study showed that NMIIA affects the invasion and metastasis of many cancer cells [8]. This result is consistent with another study showing that NMIIA-depleted MDA-MB-231 BC cells have impaired migration [15]. The expression level of light chain kinase activates NMIIA in patients with non-small-cell

lung cancer [16, 22]. The membrane targeting of WAVE2 is a necessary condition for the assembly and formation of adherent plaques on the leading edge of migrating cells [23, 24]. The Wave2 complex targets the insulin-like growth factor [24-27], supplements the end of the microtubule carrying the WAVE2 complex with the stathmin-EB1 complex, and acts on the cell membrane along the microtubule. The WAVE2 complex acting on the cell membrane is connected to PIP3 by IRSp53.

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It was found in this study that the expression level of Wave2 decreased together with the suppression of NMIIA expression, so Wave2 may be its possible mechanism of action, which is consistent with the findings in other studies [28]. In addition, there have been controversial results in the study of NMIIA encoding gene myosin heavy chain 9 (MYH 9). NMIIA encoding gene MYH9 is closely related to the progression and poor prognosis of most solid tumors. MYH9 is also thought to be a suppressor gene that is important for the re-Rho pathway [29]. At present, the relevant research is limited to tissues and needs further exploration.

In summary, NMIIA is highly expressed in BC cells and participates in the migration and invasion of BC cells, which may be regulated by the Wave2 protein pathway.

### Acknowledgements

This work was supported by the Science and Technology Research Project of the Jiangxi Provincial Department of Education (Project no. GJJ180052).

### Disclosure of conflict of interest

None.

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