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ORIGINAL ARTICLE

NEMP1 Promotes Tamoxifen Resistance in Breast Cancer Cells

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Abstract

Breast cancer (BC) is a worldwide malignant and a leading death cancer in women. Studies have shown that adjuvant tamoxifen reduces the recurrence rate and metastasis in BC. Even though tamoxifen has been used for the therapy of BC for decades, the resistance of it on BC cells could not be ignored. In this study, we first established a tamoxifen-resistant BC cell line and then demonstrated the overexpression of nuclear envelope integral membrane protein 1 (NEMP1) in the tamoxifen-resistant BC cells. Moreover, through a cell viability assay combined with depletion or overexpression technology, we addressed the important role of NEMP1 for the tamoxifen resistance in BC cells. Importantly, we further revealed that NEMP1 modulated tamoxifen resistance by regulating nuclear receptor coactivator 1 (NCOA1). In general, NEMP1 shows responsibility for the resistance of tamoxifen through regulating NCOA1 in BC cells. These results broaden the understanding of the tamoxifen resistance during the chemotherapy in BC and may provide new therapy method for BC.

Keywords Breast cancer · NEMP1 · NCOA1 · Cell viability · Tamoxifen resistance

Introduction

Breast cancer (BC) is considered to be the major kind of the malignant tumor in women. There are millions of women suffering from the BC and more than 400,000 die of it every year (Coughlin and Ekwueme 2009; Jemal et al. 2010). Up to 70% of BC patients are estrogen receptor (ER)-positive in clinical (Simpson et al. 2005; Tyson et al. 2011). Tamoxifen, a small molecule chemical is a selective estrogen receptor modulator

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(SERM) and has been widely used for the therapy of BC for decades. Emerging studies have suggested that Tamoxifen exhibits a significant therapeutic effect on both primary and metastatic estrogen receptor-positive BC, which has reduced BC mortality by 25–30% over the past 20 years (Dowsett et al. 2005; Jeruss et al. 2008; Musgrove and Sutherland 2009). Even though tamoxifen is one of the most successful molecule drugs for cancer therapy, there is still a relatively high proportion of patients who eventually become resistant to tamoxifen, leading to tumor recurrence and metastasis (Dowsett et al. 2005; Early Breast Cancer Trialists' Collaborative 2005; Early Breast Cancer Trialists' Collaborative et al. 2011; Hayes 2004; Jeruss et al. 2008; Musgrove and Sutherland 2009; Normanno et al. 2005; Shi et al. 2009). The detailed mechanism for the resistance of tamoxifen in BC is still complicated and needed to be further explored.

NEMP1, nuclear envelope integral membrane protein 1, is a novel INM (inner nuclear membranes) protein, which was highly expressed in various cancer types, including breast cancer (Tomczak et al. 2015). Previous studies have reported that NEMP1 can be combined with RanGTP (Shibano et al. 2015) and plays a vital role during the eyes' development of Xenopus laevis co-localized with INM through its evolutionarily conserved C-terminal region (Mamada et al. 2009). However, few studies were reported about the function and regulation of it during the cancer development and tumoregenesis so far. Therefore, the expression pattern and the regulation function of NEMP1 in the BC are needed to be further elucidated.

Emerging studies have shown that the nuclear receptor coactivator 1 (NCOA1) is highly associated with many types of cancer, including colon cancer (Meerson and Yehuda 2016), hepatocellular carcinoma (Jiang et al. 2017), prostate cancer (Luef et al. 2016) and so on. Furthermore, studies also show that NCOA1 was a key regulator in BC and the overexpression of NCOA1 is positively associated with the resistance of tamoxifen therapy (Qin et al. 2011; Qin et al. 2014; Redmond et al. 2009). However, the further regulation mechanism of NCOA1 for the tamoxifen resistance in BC still need to be revealed.

In this study, we first treated the MCF7 cells, an estrogen receptor-positive BC cell line and sensitive to tamoxifen, with 4OH-tamoxifen (an active metabolite of tamoxifen) to obtain a tamoxifen resistant MCF7 cell line which was named as MCF7/TAMR. To reveal the key regulator for the resistance of tomoxifen in the MCF7/TAMR cells, the mRNA and protein levels of NEMP1 were examined in the tamoxifen-resistant MCF7/TAMR cells and the MCF7 cells. A series of function assays were performed to prove that the NEMP1 was responsible for the tumorigenesis of BC and be essential for the tamoxifen resistance in BC cells. Then, the main regulators involving into the BC tumorigenesis were examined to explore the target genes regulated by NEMP1in the tamoxifen resistant MCF7/TAMR cells. Most importantly, the aim of this study is to discover potential therapy target and viable therapeutic strategy for the tamoxifen-resistant patients with BC.

Materials and Methods

Ethical Approval

All studies were approved by the Ethical Committee in First Affiliated Hospital of Anhui Medical University.

Plasmids and Primers

NEMP1 knock down lentivirus plasmid and the control vector were obtained from GenePharma (Shanghai, China). For the construction of *NEMP1* overexpression plasmid, the *NEMP1* CDS sequence was amplified from MCF7 cDNA and then inserted into Pbabe-puro vector.

Reagents and Antibodies

TRIzol reagent was purchased from Invitrogen (Pleasanton, CA, USA). Quantscript RT Kit and SuperReal PreMix Plus were obtained from TIANGEN Biotech (Beijing, China). The crystal violet and puromycin were bought from Sigma (St. Louis, MO, USA). The ECL was purchased from Pierce (Waltham, MA, USA). The MTT kit was from Sigma.

The antibody for NEMP1 antibody was obtained from Abcam (Cambridge, MA) and NCOA1 antibody was purchased from Sigma. The GAPDH antibody was bought from Sigma. The secondary antibodies for Western blot (goat anti-rabbit IgG and goat anti-mouse IgG) were obtained from Invitrogen.

Cell Culture and Cell Lines Establishment

MCF7 and HEK293T cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured with Dulbecco's Modified Eagle's Medium (DMEM, Life technology, Pleasanton, CA, USA) with 10% fetal bovine serum (FBS, Gibco) at 37 °C, 5% CO_2.

To obtain the MCF7/TAMR cell line resistant for tamoxifen, the MCF7 cells were treated with 1 μM 4OH-tamoxifen for 24 h, after which most of the cells were dead. The cells were subsequently washed with PBS to remove the dead cells and cultured with fresh tamoxifen-free medium for 1–2 days. This drug treatment procedure was repeated for 10 times and then cultured in fresh medium until the sub-clones were formed. Isolated the cell clones and expanded the cells for further studies. To maintain the tamoxifen resistant cells, the MCF7/TAMR cells were kept in the medium with 2 nM tamoxifen. Before experiments, the MCF7/TAMR cells were cultured with tamoxifen-free medium for at least 3 days.

To generate the MCF7 and MCF7/TAMR stable cell lines, cells were infected with lentivirus produced by HEK293T cells with the transfection of the plasmids carrying shNEMP1 or NEMP1 overexpression elements and their control vectors and then selected by puromycin.

Cell Viability Assay

The MTT assay was used to detect the cell viability. Briefly, the MCF7 or MCF7/TAMR stable cell lines were seeded into 96 well plates at a density of 1000 cells/well in triple. After being cultured for 96 h, the data were collected according to the instruction of the MTT kit and OD570 was detected using a microplate reader.

Cell Number Assay

Cells used for the experiment were seeded at 1×10^4 per well into 6-well plate 1 day before the experiment. The cell number in each well was counted with a cell counter (Millipore, Billerica, MA) for three times every 24 h. A total of 5 days of the data were collected. Every group were repeated for at least 3 times.

Colony-Formation Assay

A total of 1000 cells were seeded into one well of the 6-well plates in advance. Cells were fixed with 4% paraform after being cultured for 10 days followed by the incubation of 0.1% crystal violet for 15 min at room temperature. The data were represented as the number of colonies with mean ± SD.

RNA Extraction and Quantitative RT-PCR

The total RNA for experiments was extracted by TRIzol reagent. The reverse transcription of RNA was used by a Quantscript RT Kit. The quantitative RT-PCR used for the determination of mRNA levels was performed with SuperReal PreMix Plus. Gene expression levels were examined in triplicate with indicated primers and normalized to GAPDH.

Western Blotting and Protein Extraction

The cells were harvested and lysed in RIPA buffer (150 mM NaCl,50 mM Tris-HCl, 1% SDS 1% Nonidet P-40, 2 mM EDTA, pH = 8.0, and 0.5% sodium deoxycholate) with protease inhibitors. Protein samples were first separated by SDS-PAGE with suitable concentration, followed by the transfer to the nitrocellulose membrane. The membranes were blocked by 5% milk for 1 h with interest proteins at room temperature. Then, the membranes were washed and incubated with specific primary antibody overnight at 4 °C. The membranes were washed and incubated with horseradish-peroxidase-linked secondary antibodies. The protein signals were examined by ECL.

Patient Recruitment and Immunohistochemistry (IHC)

The clinical samples used in the present study were collected from The First Affiliated Hospital of Anhui Medical University with informed consent approved by the

ethic committee at The First Affiliated Hospital of Anhui Medical University. Tissues used for the IHC were firstly fixed with 4% paraform and then embedded in paraffin. Before experiment, the embedded tissues should be cut into sections and then incubated at 65 °C for 30 min. The slides were then put into xylene to deparaffinize and rehydrate in different concentration of ethanol. Boiled the slides for 20 min in antigen retrieval solution, waited until the buffer cooling to the room temperature and incubated with hydrogen peroxide to quench endogenous peroxidase. The slides were blocked with 5% BSA and incubated with primary antibodies overnight at 4 °C. Before adding the diaminobenzidine (DAB), the slides were incubated with HRP-conjugated secondary antibody for 1 h at room temprature. The sections were dehydrated, covered with neutral resin and taken pictures at last.

Statistical Analysis

All experiments in this study were performed in triple. Data were presented as mean ± standard deviation (SD). Student's *t* test, one or two-way ANOVA analysis were used in the study to analyze the significant differences between groups. $P < 0.05$ was considered to have statistical difference.

Results

NEMP1 is Upregulated in Tamoxifen Resistant Breast Cancer Cells

The MCF7 cells were treated with 1 μm tamoxifen for several times to generate a tamoxifen resistant BC cell line. Survival cells were considered to be tamoxifen resistance and named as MCF7/TAMR cells. To further confirm whether MCF7 cells is sensitive to tamoxifen than MCF7/TAMR, a MTT assay was conducted to determine the proliferation ability of MCF7 and MCF7/TAMR cells after being treated with 4OH-tamoxifen in different dosages for 96 h. The results showed that the proliferation ability was higher in MCF7/TAMR cells compared with MCF7 cells with 4OH-tamoxifen treatment (Fig. 1a). Then, to investigate whether NEMP1 involved into the tamoxifen resistance of BC cells, we detected the protein level of NEMP1 in MCF7 and MCF7/TAMR cells. The Western blotting data indicated that the NEMP1 was highly expressed in MCF7/TAMR cells, the tamoxifen resistant cells (Fig. 1b). Furthermore, the mRNA levels of NEMP1 were also examined and data showed that the increase of NEMP1 in MCF7/TAMR cells was not only at the protein level but also at the mRNA level (Fig. 1c). Taken together, all these data suggest that the NEMP1 is upregulated in tamoxifen resistant BC cells and may take part into the resistance of tamoxifen in MCF7/TAMR cells.

Depletion of NEMP1 Reduces the Tamoxifen Resistance of MCF7/TAMR Cells

To reveal the role of NEMP1 on the resistance of MCF7/TAMR cells for tamoxifen, we first established NEMP1 stably depleted MCF7/TAMR cell line and

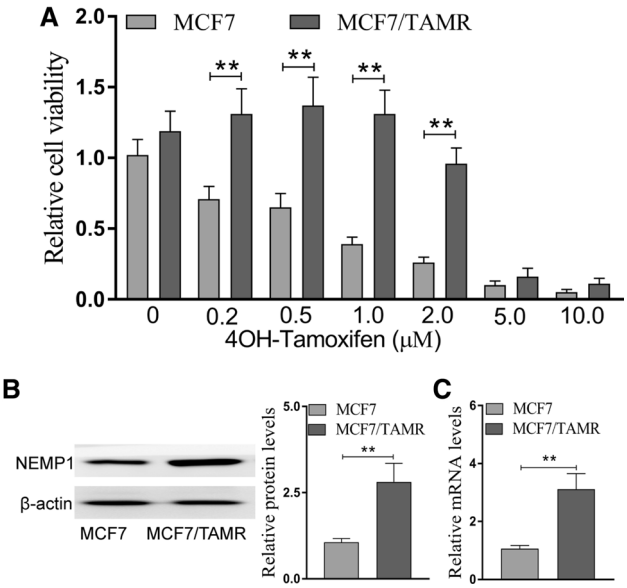

Fig. 1 NEMP1 is upregulated in tamoxifen-resistant breast cancer cells. **a** The proliferation ability is higher in MCF7/TAMR cells compared with MCF7 cells with 4OH-tamoxifen treatment. The MTT assay was performed to determine the proliferation ability of MCF7 and MCF7/TAMR cells after being treated with 4OH-tamoxifen in different dosages for 96 h. **b** The protein level of NEMP1 is increased in MCF7/TAMR BC cells. A Western blot analysis was done in MCF7 and MCF7/TAMR BC cells. Actin served as loading controls. **c** The mRNA level of NEMP1 is upregulated in MCF7/TAMR BC cells. The mRNA level of NEMP1 in MCF7 and MCF7/TAMR BC cells were analyzed by quantitative RT-PCR. GAPDH served as loading controls. Data were presented as mean ± SD of three independent experiments. $^{**}P < 0.01$

examined the knockdown efficiency. A Western blotting result showed that the NEMP1 was downregulated in the shRNA stably expressed cells compared with the control group (Fig. 2a). Then, a total cell number assay was performed to determine whether downregulation of NEMP1 impaired the resistance ability of MCF7/TAMR cells for 4OH-tamoxifen treatment. The results indicated that there was no significant difference of the cell growth between the treatment or un-treatment groups with tamoxifen (Fig. 2b, the red solid line compared with the black solid line). However, after depleting the NEMP1, the cell growth was significantly suppressed when treated with tamoxifen compared with the untreated group (Fig. 2b, the orange dotted line compared with the orange solid line and the green dotted line compared with the green solid line). To further validate the results, we designed a MTT assay to examine the proliferation ability of MCF7/TAMR NEMP1 knockdown cells and similar results were observed. The proliferation ability was not impaired in the control groups after treated with tamoxifen (Fig. 2c, see the first and forth columns), whereas after NEMP1 being knocked down, the cell proliferation ability showed significant suppression in tamoxifen treated group compared with the untreated one (Fig. 2c, the fifth column

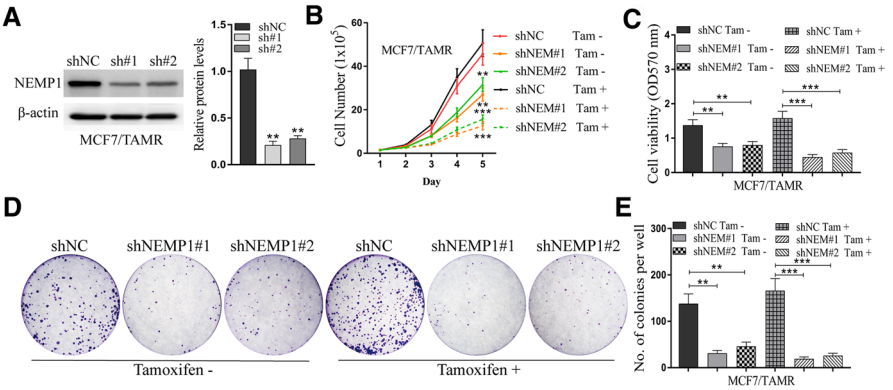

Fig. 2 Depletion of NEMP1 reduces the tamoxifen resistance of MCF7/TAMR cells. **a** Stable depletion of NEMP1. A Western blot shows the NEMP1 expression level in the NEMP1 knocking down stable cell lines. Cells were infected with two shRNA lentivirus against NEMP1 (sh#1 and sh#2). Non-specific RNAs (shNC) was used as a negative control. Actin was used as a loading control. **b** Downregulation of NEMP1 impairs the resistance ability of MCF7/TAMR cells for 4OH-tamoxifen treatment. A total cell number assay was performed to determine the cell growth of NEMP1 knockdown MCF7/TAMR cells (shNEMP1) and the control cells (shNC) in 4OH-tamoxifen treated or untreated group. MCF7/TAMR-shNC and MCF7/TAMR-shNEMP1 cells were grown in 6-well plates and the cell number was determined at the indicated days. **c** Decrease of NEMP1 reduces the tamoxifen resistance in MCF7/TAMR cells. The proliferation ability of MCF7/TAMR NEMP1 knockdown (shNEMP1) or the control (shNC) cells were examined by MTT assay with or without 4OH-tamoxifen treatment for 96 h. **d** A colony forming assay demonstrates the decrease effects of the resistance for tamoxifen in NEMP1 knockdown MCF7/TAMR cells. NEMP1 depletion (shNEMP1) and the control (shNC) cells based on MCF7/TAMR were examined. Tamoxifen+ and tamoxifen− represent that the culture media were treated with or without 1 μM tamoxifen, respectively. **e** A quantitative presentation of the colonies formed from three independent experiments based on MCF7/TAMR cells. Data were presented as mean ± SD of three independent experiments. $*P < 0.05$; $**P < 0.01$

compared to the second column and the sixth column compared with the third column). Consistently, the cell colony-formation assay demonstrated that cells knocking down NEMP1 (shNEMP1#1 and shNEMP1#2) formed less colonies treated with tamoxifen compare with the untreated group, whereas the control group (shNC) showed no significant difference with or without tamoxifen treatment (Fig. 2d, e). In summary, all these data suggest that NEMP1 takes part into the tamoxifen resistance of MCF7/TAMR cells and depletion of NEMP1 reduces the tamoxifen resistance of MCF7/TAMR cells.

NEMP1 Causes the Resistance of MCF7 Cells for Tamoxifen

To further confirm that NEMP1 is responsible for the tamoxifen resistance of MCF7/TAMR cells, we constructed a NEMP1 overexpressed MCF7 cells. A Western blotting assay demonstrated that the NEMP1 was highly expressed in the exogenous expression cells compared with the control vector (Fig. 3a). A total cell number assay showed that the cell growth of control cells was suppressed after being incubated with tamoxifen (Fig. 3b, the green line compared with the

red line), however, the inhibition effect of tamoxifen on MCF7 cells was lost when the NEMP1 was overexpressed (Fig. 3b, the black line compared with the blue line). Consistently, the MTT experiment indicated that the MCF7 cell viability was inhibited when tamoxifen was added to the medium (Fig. 3c, see the first and third columns). However, the cell viability showed no significant difference when NEMP1 was overexpressed (Fig. 3c, see second and fourth columns). The colony-formation assay further supported the previous results. MCF7 cells treated with tamoxifen formed less colonies but the colony number of NEMP1 overexpressed MCF7 cells showed no significant difference with or without tamoxifen incubation, in spite of that the NEMP1 overexpression enhanced the colony formation both in tamoxifen treated or untreated groups (Fig. 3d, e). Taken together, all these results put further evidence to prove that NEMP1 causes the resistance of MCF7 cells for tamoxifen.

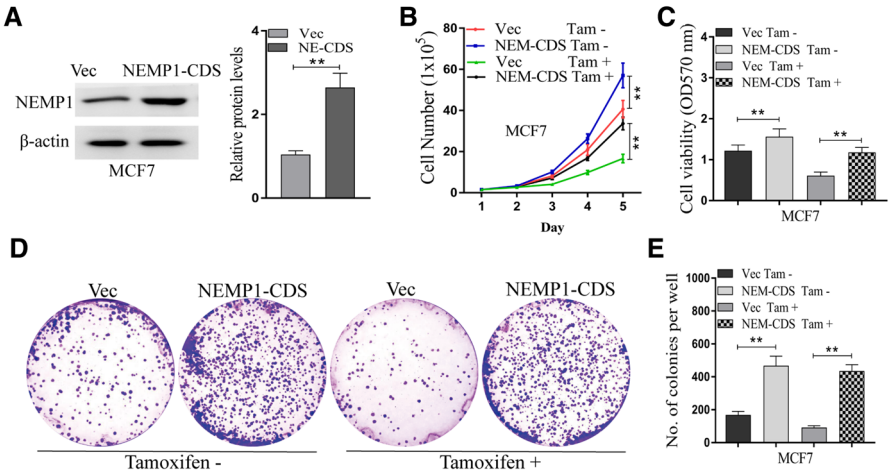

Fig. 3 NEMP1 causes the resistance of MCF7 cells for tamoxifen. **a** A Western blotting assay shows NEMP1 protein level in MCF7 cells with stable overexpression exogenous NEMP1 (NEMP1-CDS). CDS represents coding sequence. Vector (Vec) was used as control. **b** Overexpression of NEMP1 induces the resistance ability of MCF7 cells for 4OH-tamoxifen treatment. A total cell number assay was performed to determine the cell growth of NEMP1 overexpressed MCF7 cells (NEMP1-CDS) and the control cells (Vec) in 4OH-tamoxifen treated or untreated group. Cells were grown in 6-well plates and the cell number was determined at the indicated days. **c** Upregulation of NEMP1 enhances the tamoxifen resistance in MCF7 cells. The proliferation ability of NEMP1 overexpressed MCF7 (NEMP1-CDS) or the control (Vec) cells were examined by MTT assay with or without 4OH-tamoxifen treatment for 96 h. **d** A colony forming assay demonstrates the increase resistant effects for tamoxifen in NEMP1 overexpressed MCF7 cells. NEMP1 overexpression (NEMP1-CDS) and the control (Vec) cells based on MCF7 were examined. Tamoxifen+ and tamoxifen− represent that the culture media were treated with or without 1 μM tamoxifen. **e** The quantitative statistics of the colonies formation from three independent experiments for the (**d**). Data were presented as mean ± SD of three independent experiments. $*P < 0.05$; $**P < 0.01$

NEMP1 Modulates the Tamoxifen Resistance in MCF7/TAMR Cells by Regulating NCOA1 Expression

Studies have shown that the nuclear receptor coactivator 1 (NCOA1) was highly expressed in BC and the overexpression of NCOA1 was positively associated with disease recurrence, metastasis and resistance to tamoxifen therapy (Qin et al. 2014; Qin et al. 2015). To explore the target genes regulated by NEMP1 in tamoxifen resistant BC cells, we examined the mRNA levels of the main regulators HER2 (Krishnamurti and Silverman 2014), EGFR(He et al. 2018), ESR1 (Zhang et al. 2018), MYC (Terunuma et al. 2014), CCND1 (Ullah Shah et al. 2015), PTEN (Kechagioglou et al. 2014), NCOA1 (Qin et al. 2014), BCL2 (Gonzalez-Sistal et al. 2014) and SRC (Li et al. 2014) involving into the BC tumorigenesis. The results indicated that NCOA1 mRNA level was downregulated in NEMP1 depleted MCF7/TAMR cells compared with the control samples (Fig. 4a). Interestingly, the NCOA1 protein level was also decreased in NEMP1 knocking down MCF7/TAMR cells, the tamoxifen resistant BC cells (Fig. 4b). All these data lead us to the conclusion that NEMP1 modulates the tamoxifen resistance in MCF7/TAMR cells by regulating NCOA1 expression.

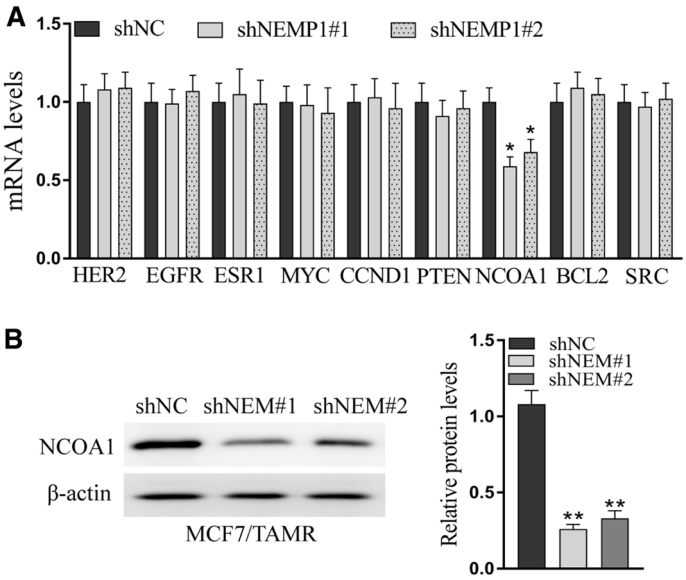

Fig. 4 NEMP1 modulates the tamoxifen resistance in MCF7/TAMR cells by regulating NCOA1 expression. **a** The NCOA1 mRNA level is decreased in NEMP1 depleted MCF7/TAMR cells. The mRNA levels of HER2, EGFR, ESR1, MYC, CCND1, PTEN, NCOA1, BCL2 and SRC were detected by quantitative RT-PCR in NEMP1 depletion (shNEMP#1 and shNEMP1#2) and the control cells (shNC) based on MCF7/TAMR cells. GAPDH was detected as control. **b** The protein level of NCOA1 is decreased in NEMP1 depleted MCF7/TAMR cells. Protein levels of NCOA1 in NEMP1 knockdown (shNEMP1#1 and shNEMP1#2) and the control (shNC) MCF7/TAMR cells was determined by Western blotting. The β-actin was detected as loading control. Data are mean ± SD of three independent experiment and each measured in triplicate (*$P < 0.05$)

NEMP1 is Highly Expressed in Breast Cancer Patients

To further confirm that NEMP1 is associated with BC, we performed an immunohistochemistry (IHC) assay to detect the NEMP1 level in the clinical samples. The results demonstrated that elevated expression of NEMP1 was found in the BC tissues compared with the adjunct normal tissues (Fig. 5a). Moreover, the mRNA levels of a total of 53 cancer tissues and 18 paired normal tissues were examined by quantitative RT-PCR. Significantly elevated levels of NEMP1 mRNA were found in tumor compared to non-tumor tissues from cancer patients (Fig. 5b). All these data suggest an increase of protein and mRNA levels of NEMP1in the breast tumor tissues, indicating that NEMP1 is highly correlated with the BC.

Discussion

BC is considered to be one of the most prevalent forms of cancers in women and a worldwide malignancy (Coughlin and Ekwueme 2009). Among all the therapy methods for BC, radiotherapy and chemotherapy are two primary medical treatments, however, the resistance of the above-mentioned therapies still remains to be a big challenge in clinic (Lu et al. 2018). Sex-steroid hormones play vital roles in cancer development and mammary gland. Estrogen is a main regulator during the development and progression of BC. Studies have revealed that estrogen ablation or antiestrogen strategy shows positive therapy effects on BC treatment, mainly in estrogen receptors-dependent BC (Clarke et al. 2003). Among the drugs reducing the incidence of BC, tamoxifen, a selective estrogen receptor modulators (SERM), serves as an ER antagonist and a promising therapeutic drug for ER-positive BC (Notas et al. 2015). Despite of the accumulative studies revealing that tamoxifen could dramatically reduce the risk of development of recurrent BC and the metastasis of BC in women, the drug resistance is still a world concern in BC treatment.

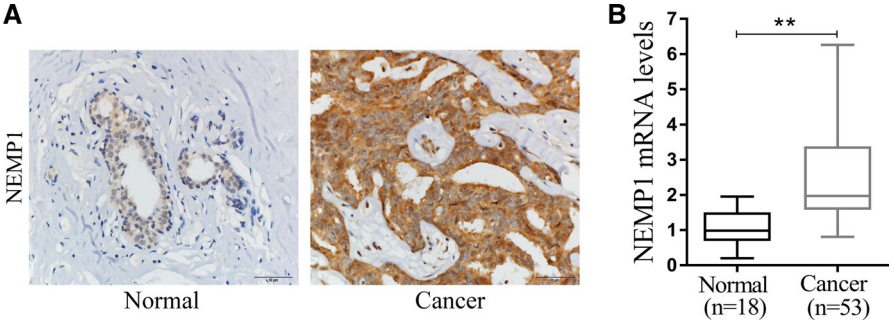

Fig. 5 NEMP1 is highly expressed in breast cancer patients. **a** NEMP1 protein level is upregulated in BC tissue compared with the adjunct normal tissue. An Immunohistochemical (IHC) assay was analyzed of NEMP1 expression in BC tissues. **b** The mRNA level of NEMP1 is increased in BC tissues. The mRNA levels of NEMP1 in normal and tumor breast tissues were detected by quantitative RT-PCR. A total of 18 normal tissues and 53 BC tissues from clinic was examined. GAPDH served as loading controls. Data were presented as mean ± SD of three independent experiments. $^{**}P < 0.01$

Therefore, the detailed mechanism for the tamoxifen resistance of BC is still needed to be explored.

NEMP1 was previously reported as a key regulator for the development of Xenopus laevis, combined with RanGTP (Mamada et al. 2009; Shibano et al. 2015). Moreover, NEMP1 was also found to play essential roles in the papillary and anaplastic thyroid cancers (Iacobas et al. 2018). However, it is still unknown that whether NEMP1 associates with BC or participates in the tamoxifen resistance of BC. In the current study, to answer whether NEMP1 is important for the tamoxifen resistance in BC, we first assessed the dose-dependent anti-proliferative effect of tamoxifen on ER-positive human MCF7 cells and established a tamoxifen resistant breast cancer cell line which was named it as MCF7/TAMR. To our surprise, a Western blotting assay demonstrated that NEMP1 was highly expressed in the tamoxifen resistant MC7 cells and we further found that NEMP1 was also elevated in the BC tissues. Moreover, to explore whether NEMP1 take part into the regulation of BC for the resistance of tamoxifen, we knocked down the NEMP1 in MCF7/TAMR. There is no difference of the cell proliferation ability in the control MCF7/TAMR after being incubated with tamoxifen compared with the untreated group, which means that the established MCF7/TARM cell lines is successfully resistant to the tamoxifen. However, the cells proliferation ability is remarkably suppressed in NEMP1 depleted MCF7/TAMR cells when treated with tamoxifen, which indicates that the NEMP1 is responsible for the tamoxifen resistance in MCF7/TAMR cells. At the same time, we also found that knocking down the NEMP1 still leads to the inhibition of cell growth without the treatment of tamoxifen, which probably because of that NEMP1 is an oncogene. Similarly, the cell viability of MCF7 cells is inhibited after being treated with tamoxifen, which suggests that the MCF7 is a tamoxifen sensitive cell line. Furthermore, the cell viability shows no difference in the NEMP1 overexpressed MCF7 cells treated with tamoxifen compared with the untreated group. This phenomena implies that NEMP1 induces the tamoxifen resistance for MCF7 cells. Taken together, all these results lead us to the conclusion that NEMP1 is highly associated with BC and responsible for the resistance of tamoxifen in MCF7 cells.

Recently, studies have shown that the nuclear receptor coactivator 1 (NCOA1) was closely related with many types of cancers (Jiang et al. 2017; Luef et al. 2016; Meerson and Yehuda 2016). Especially, NCOAl was also be addressed as a key regulator in breast cancer and positively associated with the tamoxifen resistance in BC (Redmond et al. 2009). In conclusion, based on the hypothesis that NEMP1 and NCOA1 are two potential regulators for the progression of BC, we want to revel the detail mechanism of these two genes in BC development. To investigate the target genes NEMP1 regulated during the progression of tamoxifen resistance, we screened numerous important regulators for the development of BC. A quantitative RT-PCR shows that NCOA1 is regulated by NEMP1. The protein and mRNA levels are decreased in the NEMP1 downregulated MCF7/TAMR cells. These results tell us that NEMP1 modulates tamoxifen resistance of MCF7/TAMR cells by the regulation of NCOA1.

In summary, we successfully established a tamoxifen resistant MCF7//TAMR cell line and firstly found that NEMP1 was highly expressed in the tamoxifen resistant

breast cancer cells and breast cancer tissues. Moreover, knocking down the NEMP1 protein level significantly leads MCF7//TAMR cells lost the resistance for tamoxifen treatment. Consistently, the overexpression of NEMP1 induces the tamoxifen resistance in MCF7 cells. More importantly, we revealed that the target gene NEMP1 regulated for tamoxifen resistance in BC cells mainly through NCOA1. Our findings may broaden the understanding of the drug resistance during the chemotherapy procedure in BC and may provide new therapy target for BC.

Upon the hormone binding to the nuclear receptors, such as ERs, progesterone receptors (PR) and androgen receptors (AR), the conformation of the receptor will change and the receptor will form a homodimer (Jia et al. 2015). When a coactivator interacts with the homodimer, the homodimer binds to the hormone response elements and promotes the transcription activity of hormone-dependent genes (Nwachukwu et al. 2016). Previous studies have shown that NCOA1 was a coactivator in nuclear. In this study, we prove that NCOA1 could be regulated by NEMP1. In this paper, we could not explain how NEMP1 regulate the NCOA1 and more studies should be performed to explore it. Maybe the NEMP1 could have interaction with nuclear receptor and then regulate the NCOA1 or NEMP1 could directly bind with NCOA1 and regulate the transcription of it. Nonetheless, further studies are needed to explore the detailed regulation network for tamoxifen resistance in BC.

Conclusion

1. NEMP1 is highly expressed in tamoxifen resistant BC cells and BC tissues and responsible for the resistance of tamoxifen in BC cells.
2. NEMP1 modulates the tamoxifen resistance by regulating the NCOA1.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and Animal Rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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