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NRF3 suppresses the metastasis of triple-negative breast cancer cells by inhibiting ERK activation in a ROS-dependent manner

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Summary. Purpose. Our previous study demonstrated that NRF3 (NFE2L3, Nuclear Factor-erythroid 2-related factor 3) could suppress cell metastasis and proliferation in breast cancer. In this study, we investigated the mechanisms underlying its function in breast cancer.

Methods. In the present study, NRF3 expression and its clinical characteristics in breast cancer were analyzed using public datasets and clinical specimens. After breast cancer cells were overexpressed NRF3, FACS was used to detect the intracellular ROS levels. The migration and invasion activities of NRF3-ectopic expressed breast cancer cells were determined by transwell assay. To validate the role of ROS/ERK axis in the inhibitory effect of NRF3 in cell metastasis, ROS scavenger NAC was also included.

Results. We found that NRF3 mRNA was highly expressed, while NRF3 protein was extremely lowly expressed in breast cancer tissues compared with their normal counterparts, and low level NRF3 was associated with poorer prognosis in patients with triple negative breast cancer (TNBC). More interestingly, over-expression of NRF3 protein significantly increased cellular ROS production and dramatically decreased p-ERK level and cell migration in TNBC cells. Mechanistically, NRF3 protein was found to be mutually regulated by valosin-containing protein (VCP). Strikingly, VCP-knockdown dramatically increased NRF3 protein expression, but NRF3-knockin also decreased VCP expression in return. Moreover, antioxidant NAC treatment effectively increased the level of p-ERK and VCP expression, as well as cell migration and invasion abilities of TNBC cells.

Conclusion. NRF3, a tumor suppressor down-regulated by VCP, could attenuate cell metastasis in TNBC cells by increasing cellular ROS accumulation and subsequently inhibiting the ERK phosphorylation.

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Introduction

Breast cancer is the second main body of cancer-related mortality in females, as well as in China which also has a high incidence rate (Torre et al., 2015; Chen et al., 2016). Triple-negative breast cancers (TNBCs) are those lacking estrogen receptors (ER), gene amplified human epidermal growth factor receptor 2 (HER2), and progesterone receptors (PR). Additionally, they show large metastasis to the viscera and invasion of the node (Al-Bahlani et al., 2017). Due to the metastasis, the prognosis of TNBC remains discontent. Therefore, it is necessary to further demonstrate the molecular mechanism of metastasis in TNBC.

The transcription factor NRF3 (NF-E2-related factor 3 or NFE2L3), comprising NRF1 and NRF2, belongs to the family of Cap 'n' Collar (CNC) (Sykiotis and Bohmann, 2010; Bugno et al., 2015; Chowdhury et al., 2017; Wu et al., 2019). Partly due to the NRF3 knockout mice did not show obvious abnormalities, the physiological roles of NRF3 were unclear (Chevillard et al., 2010, 2011). In the previous study, NRF3 has been reported a physiological relationship in various cancers, for example in gastric cancer (Aono et al., 2019) and breast cancer (Kannan et al., 2015; Sun et al., 2019). Moreover, our previous study illustrated NRF3 expression was down-regulated in human breast cancerous specimens compared with paracancerous tissues (Sun et al., 2019). Furthermore, our previous study also showed NRF3 overexpression inhibited EMT process and the expression of MMPs with breast cancer cells migration and invasion ability decreased, which indicated NRF3 had a negative effect on breast cancer metastasis (Sun et al., 2019). However, the mechanisms underlying the decrease of NRF3 and its anti-cancer functions have not been well investigated.

In the present study, we detected the mRNA and

protein expression profiling in human breast cancer tissues and their normal counterpart. Contradictorily, NRF3 mRNA is upregulated in breast cancer, while NRF3 protein is suppressed. We speculated that protein degradation could explain this contradiction. Previous publications reported that NRF3 could be degraded by several E3 ligases including FBW7, β-TRCP and VCP (Kannan et al., 2015; Chowdhury et al., 2017). To confirm which one is involved in NRF3 protein regulation in breast cancer, siRNAs against these E3 ligases were used to determine their regulation of NRF3 protein expression. Our data revealed that only VCP silence could effectively restore the NRF3 protein expression. This finding suggests the VCP takes part in the NRF3 degradation in breast cancer.

Furthermore, we also examined the ROS level under NRF3 protein overexpression. Unlike its family member NRF2, NRF3 protein increased the intracellular ROS accumulation reversely and inactivated the ERK and downstream pathway. ROS inhibitor NAC not only recovered the activation of ERK but also restored the migration and invasion activities of breast cancer cells with NRF3 overexpression. Collectively, suppression of NRF3 protein in TNBC cells decreased cellular ROS production and enhanced ERK phosphorylation, finally promoting cell metastasis. Our data suggest that NRF3 could provide a potential therapeutic target for TNBC treatment.

Materials and methods

Clinical specimens and cell culture

Ten primary BRCA biopsy specimens and in-paired normal tissues were collected from patients at the time of surgery in Taizhou Central Hospital, and immediately stored in liquid nitrogen for further RNA and protein extraction. All patients provided written informed consent for the use of these clinical materials in research, and the project was approved by the Institutional Ethics Committee of Taizhou Central Hospital.

The human BRCA cell lines MDA-MB-468, MDA-MB-231 and Hs578t were purchased from ATCC (Manassas, VA, USA). All cell lines were cultured in Dulbecco's Modified Eagle's medium (DMEM; Gibco, Life Technologies Corp., Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone, GE Healthcare Life Sciences, Logan, UT, USA), 2 mM L-glutamine (Gibco), 1% penicillin(100 units/ml) and streptomycin (100 μg/ml) (Gibco) and incubated at 37°C, 5% CO_2 in a humidified incubator and passaged at ≥80% confluence by trypsine (Gibco).

Western blotting

After indicated treatment, cultured cells were lysed in RIPA buffer containing 50 mM Tris-HCl at pH 8.0, 150 mM sodium chloride, 2 mM EDTA at pH 8.0, 0.1% Triton-X 100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% protease inhibitor (Sigma, Sigma-Aldrich, Co., Darmstadt, Germany). Keep the supernatant after centrifugation at high speed and prepare the cell lyase with 2x SDS loading buffer. Gel electrophoresis was performed on an acrylamide gel and proteins were transferred onto a PVDF membrane (Bio-rad, Bio-Rad Laboratories, Inc., Hercules, CA). The membranes were blocked with 5% fat-free milk in PBS and incubated with primary antibodies (dilution: 1:1000) against NRF3 (Sigma, HPA05588), ERK (CST, 4695, Cell Signaling Technology, Inc., Massachusetts, USA), p-ERK (CST, 4370), CREB1 (CST, 9197), p-CREB1 (CST, 9198), VCP (CST, 2648) and β-actin (Sigma, A8481) at 4°C overnight. The corresponding horseradish peroxidase (HRP)-conjugated secondary antibody was added and incubated at room temperature for 2h. Signals were visualized after chemiluminescence reaction with HRP substrate.

Quantitative real-time polymerase chain reaction (qPCR)

Total RNA was isolated from cell lines and tumor samples using the TRizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocols. Complementary DNA synthesis was performed using the PrimeScript RT Reagent Kit (TaKaRa, Taka Bio Inc., Osaka, Japan). The expression levels of NRF3 (forward: 5'-CTTACCACTTACAGCCAACT-3'; reverse 5'-TGCCGACAATTTCATCTA-3') and VCP (forward: 5'-TGGAGTTCAAAGT GGTGGAAA-3'; reverse: 5'-ATGGCAGGAGCATTCTTCTCA-3') were evaluated using SYBR green PCR master mix (Applied Biosystems, Foster City, CA) and normalized to β-actin (forward: 5'-AGCACAGAGCCTCGCCTTTGC-3'; reverse: 5'-CTGTAGCCGCGCTCGGTGAG-3'). Real-time PCR amplification was performed in ABI 7300 Real-Time PCR system (Applied Bioscience, Foster City, CA) according to manufacturer's procedure for relative quantification. The standard PCR protocol included initial denaturing at 95°C for 10 min, followed by 40 cycles of denaturing at 95°C for 15 s, annealing and extension at 60°C for 1 min. A DNA dissociation curve was generated to confirm the specificity of amplification. The relative mRNA expression was determined by Relative Standard Curve Method ($2^{-\Delta\Delta Ct}$) using β-actin as a reference. All reactions were run in triplicate.

Assessment of reactive oxygen species (ROS) production

Harvest cells and ensure a single cell suspension by gently pipetting up and down suspension cells. Stain cells in culture media with 20 μM DCFDA and incubate for 30 minutes at 37°C. Once the incubation is completed, do not wash the cells. After staining, the cells are treated with different approaches. Analyze on a flow cytometer, DCF should be excited by the 488 nm laser and should be detected at 535 nm (typically FL1).

Alternatively, the green fluorescence intensity was contrasted by the images captured with Olympus fluorescent microscope.

Plasmid construction and transfection

The coding sequence (CDS) of human NRF3 mRNA was synthesized and subcloned into the pcDNA3.1 vector to construct the overexpression plasmids. The integrity of the respective plasmid constructs was confirmed by DNA sequencing. The plasmid was allowed to form a complex with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) for 20 min at room temperature, and transfection was carried out at 37°C for 24h.

RNA interference of VCP

Short interfering RNAs for VCP and non-targeting siRNA negative control were obtained from Genepharma (Shanghai, China) and the sequences are 5'-GAAUAGAGUUGUUCGGAAU[dT][dT]-3' (siR-VCP-1) and 5'-GGAGGUAGAUAUUGG AAUU[dT][dT]-3' (siR-VCP-2). Cells were transfected with siRNA using Lipofectamine 2000 reagent according to the manufacturer's instructions. Before any treatment, cells were incubated for 24h and the silencing efficiency of the siRNA was determined by Western blotting (WB) assay.

In vitro migration and invasion assays

The migration and invasion assays were performed as previously described (Justus et al., 2014). Briefly, Chambers (8 μm pore, BD Falcon, BD Biosciences, NY, USA) with or without matrigel (BD Biosciences, NY, USA) were used to investigate invasion and migration respectively. After treatment, cells suspension in 100 μl serum-free medium were placed into the chambers without matrigel for migration assay. For invasion assay, cells suspension in 100 μl serum-free medium were placed into chambers that were coated with matrigel. Then these chambers were put in 24-well plates filled with 600 μl medium containing 20% FBS as an attractant. After incubation for 24 hours, cells in the upper sides were removed and the migrated or invaded

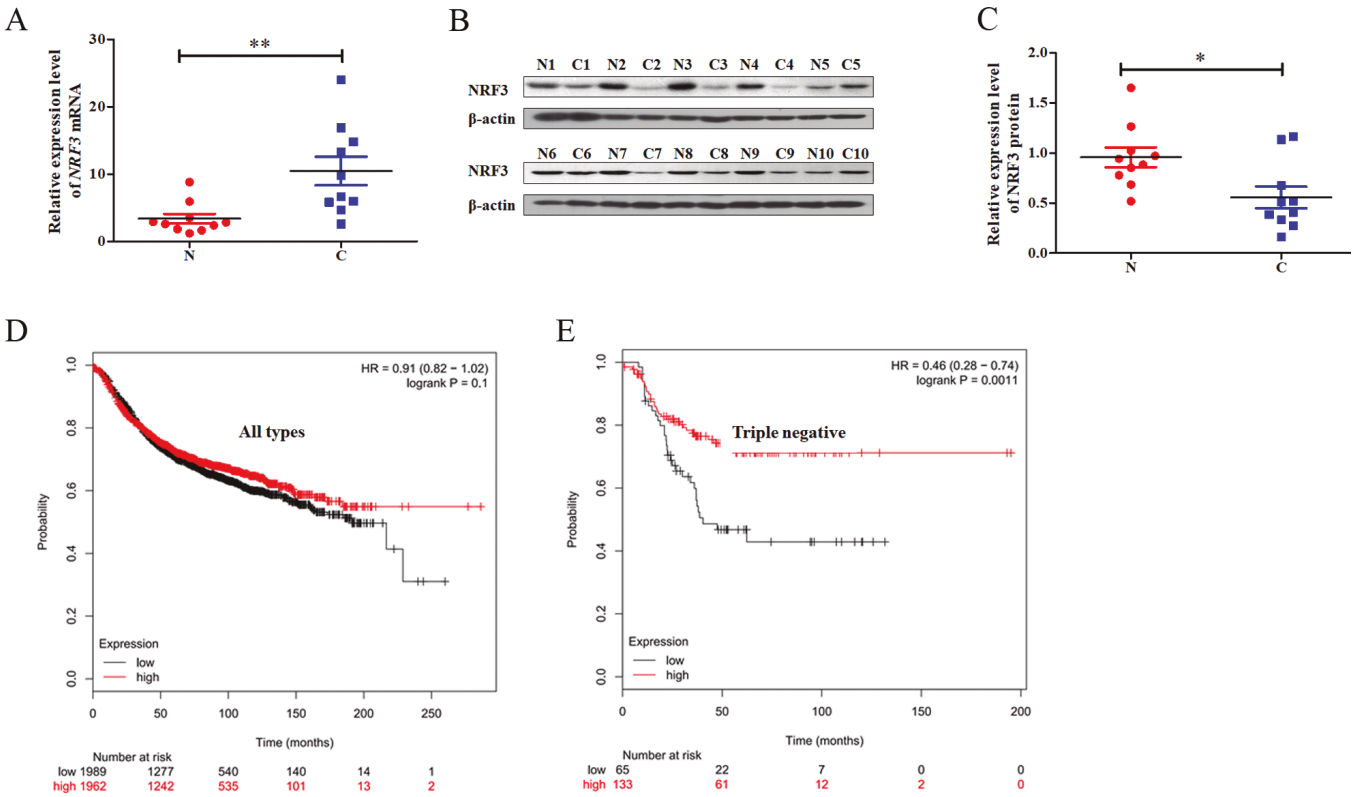

Fig. 1. NRF3 expression profiling and clinical characteristics in human BRCA. **A.** Graph showing NRF3 mRNA expression levels in samples of human BRCA tissues as C and paracancerous tissues as N. Data are presented as mean ± SD, n=10. ***p*<0.01 vs. control. **B, C.** Protein was collected from the samples of human BRCA tissues (as C) and paracancerous tissues (as N) as described in Materials and methods, then subjected to Western blot using NRF3 antibody. β-actin was used as a loading control. Data are presented as mean ± SD, n=10. ***p*<0.01 vs. control. **D.** Correlation between expression of NRF3 and overall survival of BRCA patients. Data were obtained from the TCGA database. **E.** Correlation between expression of NRF3 and overall survival of Triple Negative Breast Cancer (TNBC) patients. Data were obtained from the TCGA database.

cells on the underside of the membrane were fixed with iced methanol and stained with 0.1% crystal violet for 30 min at 37°C and then washed twice with PBS. Stained cells were counted in three independent areas under a microscope.

Statistical analysis

All data are expressed as the means ± S.D. from at least 3 independent experiments. All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS 13.0 (SPSS, Inc., Chicago, IL, USA) software packages. Statistical significance was determined using a two-sided Student's *t* test, and $p<0.05$ was considered significant.

Results

The expression profiling and clinical characteristic of NRF3 in breast cancer

To determine the expression profiling of NRF3 in BRCA, the NRF3 mRNA and protein levels in human breast cancer and the normal tissues were detected by qPCR and WB respectively. qPCR revealed that NRF3 mRNA level was higher expressed in BRCA tissues than in the paracancerous tissues (Fig. 1A). However, the protein level of NRF3 was extremely lower in BRCA tissues than in the paracancerous tissues (Fig. 1B,C). In addition, for all types of BRCA, there is no significant difference in the overall survival rates of breast cancer patients with high or low NRF3 levels according to the dataset Kaplan Meier Plotter (Fig. 1D). However, for the TNBC, patients with higher levels of NRF3 had better overall survival rates (Fig. 1E). Taken together, these data supported that NRF3 was significantly decreased in breast cancer at post-transcriptional level, and associated with better overall survival rates of TNBC patients.

Overexpression of NRF3 protein significantly increased cellular ROS production in TNBC cells

As a member of the CNC family, the transcription factor NRF3 could bind to AREs (antioxidant response elements) for the cellular response to oxidative stress (E.X. ROS) (Kobayashi et al., 1999; Chenais et al., 2005; Kannan et al., 2015). Treatment of TNBC cell lines MDA-MB-468, Hs578t and MDA-MB-231 cells with control vector (pcDNA3.1) or NRF3-expression vector for 24h, promoted the production of ROS detected by flow cytometry (Fig. 2A). As shown in Figure 2B, overexpression of NRF3 protein increased the ROS generation taken by fluorescent microscope in Hs578t cells. We next checked the activation of ERK and CREB1 by transfected Hs578t cells with control vector (pcDNA3.1) or NRF3-expression vector using

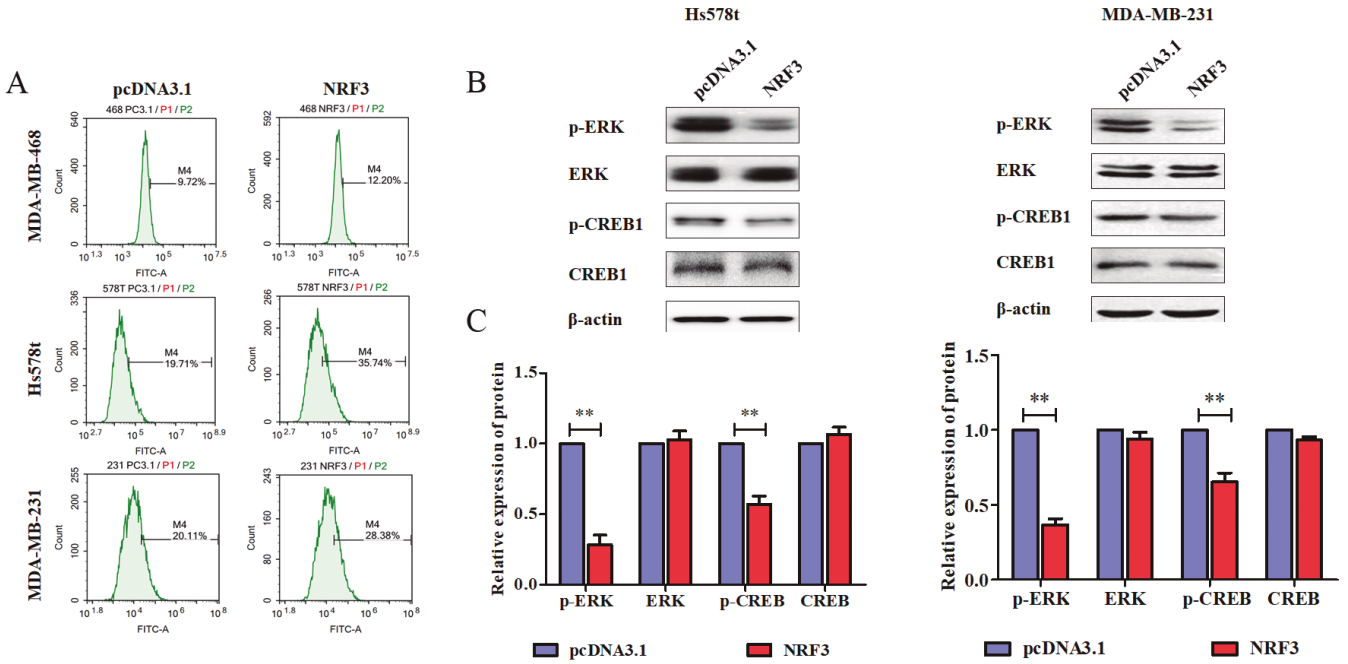

Fig. 2. Overexpression of NRF3 protein significantly increased cellular ROS production in TNBC cells. **A.** MDA-MB-468, Hs578t and MDA-MB-231 were transfected with control vector (pcDNA3.1) or NRF3-expression vector for 24h. Then, the cells were stained with DCFDA and analyzed by flow cytometry for the detection of ROS production. **B.** Hs578t was transfected with control vector (pcDNA3.1) or NRF3-expression vector for 24h. Then, the cells were stained with DCFDA and analyzed by fluorescence microscope for the detection of ROS production. **C.** Hs578t was transfected with control vector (pcDNA3.1) or NRF3-expression vector for 24h. Then, the cells were analyzed by Western Blot for the detection of ERK and the following pathway. The data are derived from the three independent experiments. $^{**}p<0.01$.

WB assay. As shown in Figure 2C, ectopic expression of NRF3 protein effectively decreased the phosphorylation of ERK and CREB1. Taken together, these data showed that NRF3 protein significantly increased cellular ROS production and decreased the phosphorylation of ERK and CREB1 in TNBC.

NRF3 protein attenuated cell migration and invasion abilities of TNBC cells in a ROS-dependent manner

Consistently, overexpression of NRF3 protein significantly decelerated the migrated cells into the bottom of transwell compared to the control group at 24h in both Hs578t and MDA-MB-231 cells (Fig. 3A).To confirm the potential role of ROS in the function of NRF3 protein in TNBC cell metastasis regulation, ROS scavenger NAC was subjected to Hs578t cells transfected with NRF3-expression vector. As shown in Figure 3B, NAC treatment significantly restored the migration and invasion potentials reduced by overexpression of NRF3 protein in Hs578t cells. Finally, NAC treatment also reversed the phosphorylation of ERK and CREB1 in Hs578t cells with NRF3 protein overexpression (Fig. 3C). Therefore, these data also certified that overexpression of NRF3 protein negatively regulated TNBC cell migration and invasion by inhibiting the activation of ERK and phosphorylation of downstream transcriptional factor CREB1 in a ROS-dependent manner at least partially.

NRF3 protein is mutually regulated by VCP in TNBC cells in a ROS-dependent manner

Due to the expression profiling of NRF3 in breast cancer, we speculated that protein degradation was a dispensable factor for NRF3 suppression in breast cancer. In fact, NRF3 could be quickly degraded by HRD1 and VCP, as well as β-TRCP in the cytoplasm under physiological conditions (Chowdhury et al., 2017). To elucidate the underlying molecular mechanism of NRF3 protein regulation, we tested the effects of FBW7, VCP and β-TRCP knockdown on the expression of NRF3 protein in Hs578t and MDA-MB-231 cells. Interestingly, only VCP silence significantly increased the NRF3 protein expression in both Hs578t and MDA-MB-231 cells (Fig. 4A, the data for, FBW7 and β-TRCP were not shown). Moreover, overexpression of NRF3 protein markedly decreased the VCP mRNA and protein

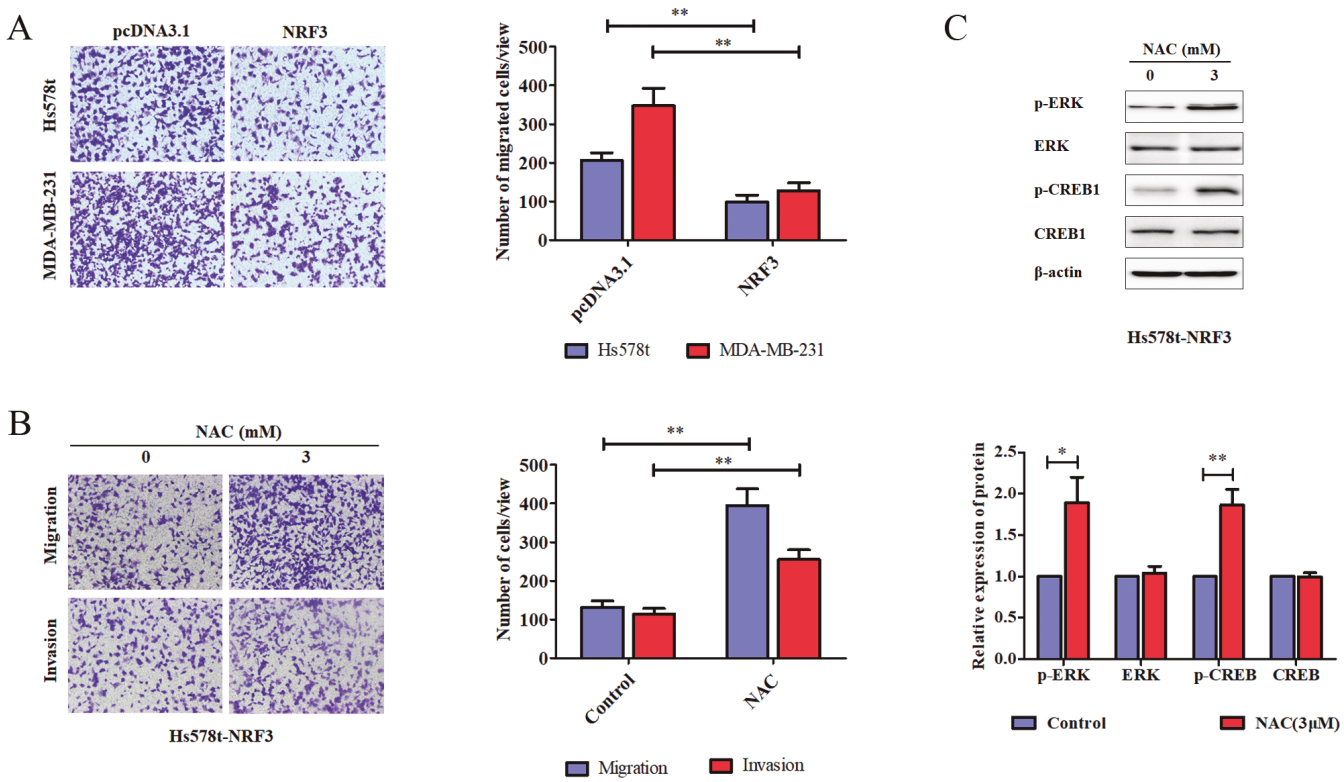

Fig. 3. NRF3 protein attenuated cell migration and invasion abilities of TNBC cells in a ROS-dependent manner. **A.** Hs578t and MDA-MB-231 were transfected with control vector (pcDNA3.1) or NRF3-expression vector for 24h. Then, the cells were analyzed by transwell assay for the detection of cell migration. **B.** Hs578t was transfected with NRF3-expression vector for 24h. Then, the cells treated with or without NAC (3 mM) and the migration and invasion activities were analyzed by transwell assay for another 24h. **C.** Hs578t was transfected with NRF3-expression vector for 24h. Then, the cells were treated with or without NAC (3 mM) and then analyzed by Western blot for detection of the phosphorylation of ERK and CREB1. The data are derived from one of the three independent experiments. *p<0.01, **p<0.01.

levels in Hs578t and MDA-MB-231 cells (Fig. 4B,C). Next, Hs578t and MDA-MB-231 were transfected with NRF3-expression vector for 24h. Then, the cells were treated with or without NAC (3 mM) and then analyzed by qRT-PCR and WB for detection of the mRNA and protein levels of VCP. Treatment of NAC also

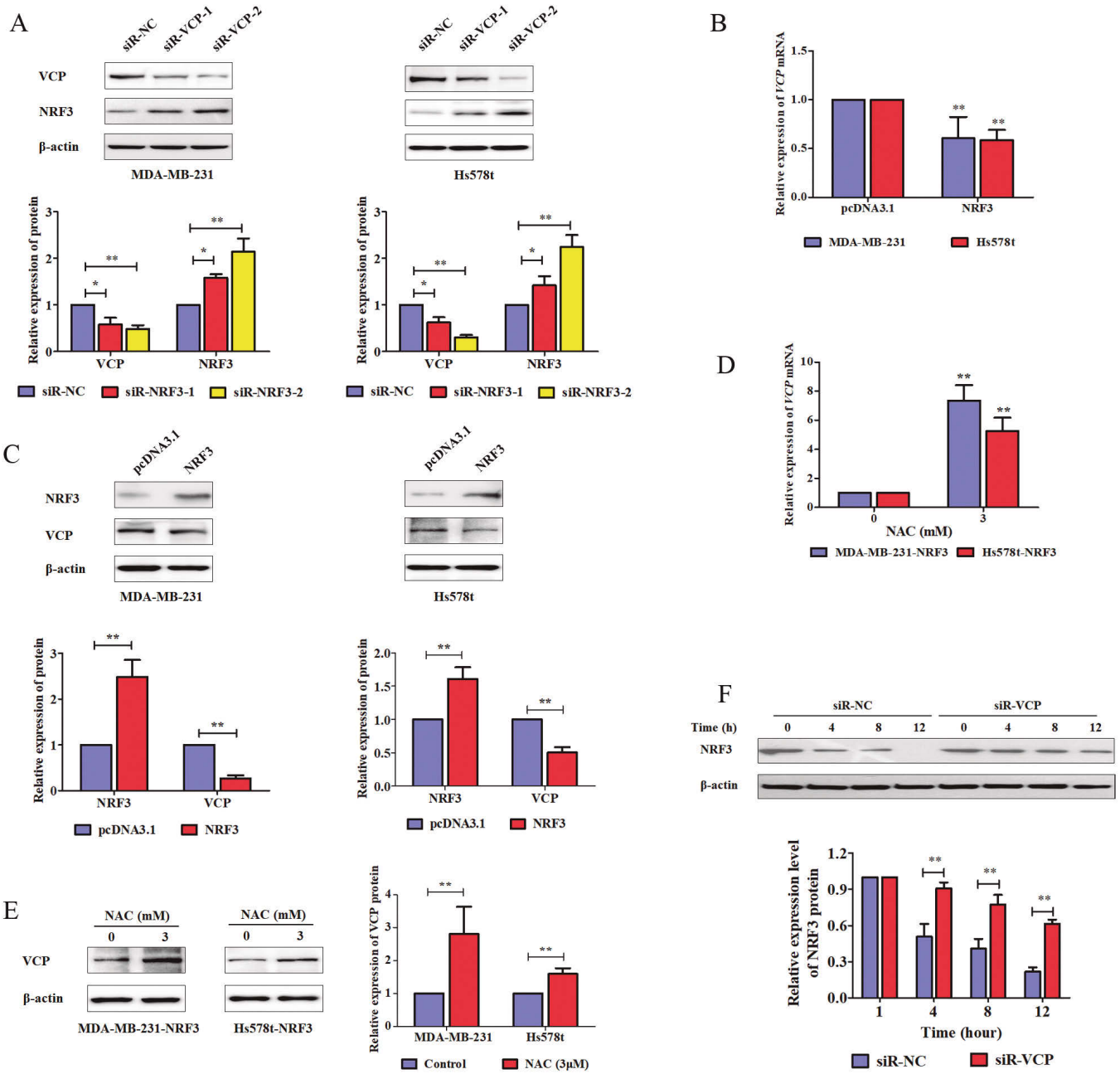

Fig. 4. NRF3 protein is mutually regulated by VCP in TNBC cells. **A.** Hs578t and MDA-MB-231 were transfected with siRNA-negative control or siRNA-VCP-1 and siRNA-VCP-2 for 24h. Then, the cells were analyzed by Western blot for the detection of VCP and NRF3 expressions. β-actin was used as a loading control. **B.** Hs578t and MDA-MB-231 were transfected with control vector (pcDNA3.1) or NRF3-expression vector for 24h. Then, the cells were analyzed by RT-PCR for the detection of VCP mRNA expressions. **C.** Hs578t and MDA-MB-231 were transfected with control vector (pcDNA3.1) or NRF3-expression vector for 24h. Then, the cells were analyzed by Western blot for the detection of VCP and NRF3 expressions. β-actin was used as a loading control. **D.** Hs578t and MDA-MB-231 were transfected with NRF3-expression vector for 24h. Then, the cells treated with or without NAC (3 mM) that analyzed by RT-PCR for detection the mRNA of VCP. **E.** Hs578t and MDA-MB-231 were transfected with NRF3-expression vector for 24h. Then, the cells treated with or without NAC (3 mM) that analyzed by Western blot for detection of the protein level of VCP. β-actin was used as a loading control. The data are derived from three independent experiments. *p<0.01,**p<0.01. **F.** Hs578t cells were transfected with siR-NC or siR-VCP for 24h followed by treatment with 6 μM CHX for indicated time to extract protein to detect the half-life of NRF3 by Western blot. The presentative data was shown from three independent experiments.

effectively restored mRNA (Fig. 4D) and protein (Fig. 4E) levels of VCP in the Hs578t and MDA-MB-231 cells which overexpressed NRF3 protein. Finally, Hs578t cells were transfected with siR-NC or siR-VCP-2 followed by CHX treatment for indicated time, and the half-life of NRF3 protein was determined by WB experiment. As shown in Figure 4F, VCP silence effectively prolonged the half-life of NRF3 protein. In a word, NRF3 protein is mutually regulated by VCP-mediated protein degradation in the Hs578t and MDA-MB-231 cells at least with ROS involved.

Discussion

NRF3 is a CNC family members of transcription factors, which is involved in kinds of cellular processes including inflammation, stress response and carcinogenesis (Chevillard and Blank, 2011). Although hundreds of publications have well defined the functions of its homolog NRF2 in multiple cancer types (Menegon et al., 2016), little is known about the roles and expression profiling of NRF3 in cancer especially in breast cancer. In the current study, we aimed to elucidate the underlying molecular mechanism of NRF3 expression regulation and its anti-tumor function in breast cancer.

First of all, to determine the expression profiling of NRF3, it's the mRNA and protein levels were detected in in-paired breast cancer tissues and paracancerous tissues by qRT-PCR and WB assays. Interestingly, NRF3 mRNA level is higher expressed in BRCA tissues than the paracancerous tissues. However, the protein level of NRF3 is extremely lower in BRCA tissues than the paracancerous tissues. We speculated that the mRNA of NRF3, as a member of CNC family, could be highly expressed in breast cancer like NRF2 (Zhang et al., 2018), while its protein was suppressed as it could negatively regulate the ROS production, and the excess ROS could be toxic for cancer cells (Pavithra et al., 2018). Our data suggested that the suppression of NRF3 in breast cancer probably was due to protein degradation. Furthermore, the clinical characteristic of NRF3 in breast cancer was analyzed using the public dataset Kaplan Meier Plotter. For all types of BRCA, there is no significant difference in the overall survival rates in patients with higher or lower NRF3 levels. However, for the TNBC, patients with higher levels of NRF3 had better overall survival rates. Therefore, the TNBC cell lines including MDA-MB-468, MDA-MB-231 and Hs578t cells were chosen for further experiments.

Secondly, we tried to determine the role of NRF3 in the intracellular ROS production in TNBC cell lines by DCFDA staining. Surprisingly, ectopic expression of NRF3 protein significantly increased the intracellular ROS production, which is consistent with the previous publication (Sankaranarayanan and Jaiswal, 2004). It is well known that there is a tight connection between oxidant stress and inflammation, and their abnormality could finally contribute to the carcinogenesis of many cancer types including breast cancer (Reuter et al., 2010). Previous studies have also illustrated that intracellular ROS generation elicited ERK phosphorylation (Espinosa et al., 2006; Wong et al., 2010; Shan et al., 2017). However, excessive levels of ROS are cytotoxic for cancer cells (Chio and Tuveson, 2017; Vucetic et al., 2017). More interestingly, the overexpression of NRF3 protein effectively suppressed the activation of ERK and the phosphorylation of downstream transcriptional factor CREB1 in Hs578t cells. We also proved that NRF3 protein negatively regulated TNBC cell migration and invasion. More importantly, the ROS scavenger NAC not only recovered the migration and invasion activities of breast cancer cells, but also effectively increased the ERK and CREB1 phosphorylation attenuated by NRF3 protein. All these data implied that NRF3 protein might play an anti-cancer effect by inhibiting ERK signaling pathway with downstream transcriptional factor in a ROS-dependent manner.

NRF3 has a quick half-life period and is stabilized by inhibiting proteasomal degradation (Nouhi et al., 2007). The proteasome degrades substrate proteins that are binding to the polyubiquitin chain degradation signal dependent on the E3 ubiquitin ligase. Previous studies have shown that FBW7, β-TRCP and VCP are the E3 ligase mediating the degradation of NRF3 (Kannan et al., 2015; Vekaria et al., 2016; Chowdhury et al., 2017). To further elucidate the mechanism for regulation of NRF3 expression, the influence of silencing three E3 ligases using siRNA on NRF3 expression was assessed. We found that only the knockdown of VCP but not FBW7 or β-TRCP could effectively upregulate the NRF3 protein expression in TNBC cells. More importantly, VCP silence could effectively prolong the half-life of NRF3 protein. Conversely, exogenous NRF3 protein expression had the opposite effect, decreasing VCP levels in TNBC cells. In addition, treatment of NAC increased both mRNA and protein levels of VCP in TNBC cells which exogenous NRF3 expression. Our data suggested that NRF3 protein could inhibit the VCP expression at the transcriptional level in a ROS-dependent manner.

Nevertheless, our study has a few limitations. This study explored the role and mechanism of NRF3 expressions in breast cancer cells through its ROS production, ERK downstream pathway and the effects of VCP and NAC scavenger treatment, however, it was unable to further explore in-depth mechanisms such as a visual representation of ROS levels, other VCP inhibitors effects and specific regulatory connection to NRF3 by luciferase assay, and localization and other downstream transcriptional factors to NRF3 protein. Henceforth, more studies are needed to elucidate these specific roles and mechanisms of NRF3 and further validate our findings in breast cancer by conducting *in*

vivo and *in vitro* experiments.

In summary, our findings suggest that NRF3 could act as a tumor suppressor in breast cancer especially in TNBC, in which higher levels of NRF3 had better overall survival rates. NRF3 expression is suppressed at the protein level mediated by VCP-induced degradation. NRF3 could negatively regulate the metastasis of TNBC cells by inhibiting ERK pathway and subsequently the expression of VCP by elevating intracellular ROS production. Therefore, the NRF3 may be a novel molecular therapeutic target and/or predictive factor for the treatment of TNBC.

Data Availability. The data used to support the findings of this study are included within the article.

Conflicts of Interest. No conflict of interest exists in the submission of this manuscript, and the manuscript is approved by all authors for publication.

Ethics statement. This study was approved by the Ethics Committee of Taizhou Central Hospital (Taizhou University). Written informed consent was obtained from individuals in the study.

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