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Original Article

Nrf2 is associated with the regulation of basal transcription activity of the *BRCA1* gene

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***BRCA1* is closely related to the pathogenesis of breast cancer. The activity of *BRCA1* promoter is regulated by transcriptional factors. The transcription factor Nrf2 (Nuclear factor-erythroid-2p45-related factor 2) is a potent transcriptional activator and plays a central role in inducible expression of many cytoprotective genes. In this report, we found that over-expression of Nrf2 stimulated *BRCA1* expression, knockdown of Nrf2 attenuated *BRCA1* expression. Nrf2 also interacted with CBP and p300 to form an active transcription complex, which could bind to the ARE (antioxidant response element) site on the *BRCA1* promoter and activate its transcription by inducing histone acetylation. Our finding could lead to a better understanding of the development of breast cancer.**

Keywords Nrf2; *BRCA1*; promoter; histone acetylation; breast cancer

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Introduction

The breast and ovarian cancer susceptibility gene, *BRCA1*, is identified based on its genetic linkage to familial early onset breast and ovarian cancer syndromes [1]. Mutations in the *BRCA1* are characterized by predisposition to familial breast and ovarian cancer. However, decreased levels of wild-type *BRCA1* expression have been detected in a large percentage of sporadic breast tumors without *BRCA1* gene mutations [2]. It has become more and more apparent that somatic mutations in the *BRCA1* gene do not play an important role in sporadic breast cancer [3,4]. Decreased expression of *BRCA1* in sporadic breast cancer was due to disorders in the gene regulatory processes. Elucidating the regulatory mechanism of *BRCA1* could lead to a better understanding of the development of breast cancer.

The activity of *BRCA1* promoter is regulated by transcriptional factors. The *BRCA1* promoter contains E2F DNA-binding sites that mediate transcriptional activation by E2F1 and repression by RB and/or E2F6 [5,6]. p53 may inhibit *BRCA1* expression by preventing E2F binding to the E2F site [7]. Id4 (Inhibitor of DNA binding 4) and HMGA1 (high mobility group protein A) negatively regulate the *BRCA1* promoter activity [8,9]. 53BP1 (tumor suppressor p53-binding protein 1) can bind to the promoter of *BRCA1* and activates *BRCA1* transcription [10]. It is reported that ERα/p300 complex can be recruited to an AP-1-binding site located in the proximal *BRCA1* promoter and activates *BRCA1* transcription, whereas the over-expression of p53 prevents the recruitment of ERα/p300 complex and represses *BRCA1* promoter activity [11]. The proximal *BRCA1* promoter segment comprises *cis*-acting elements targeted by Sp-binding and CRE-binding proteins that contribute to regulation of *BRCA1* transcription [12]. UHRF1 (Ubiquitin-like with PHD and ring finger domains 1) is associated with epigenetic silencing of *BRCA1* in sporadic breast cancer, which is responsible for regulating *BRCA1* transcription by inducing DNA methylation, histone modifications, and recruitment of transcriptional complex on the *BRCA1* promoter [13]. MyoD and c-myb interact with p300 and P/CAF (p300/CBP-associated factor), forming activating transcriptional complexes which bind to E-box and c-myb sites on the *BRCA1* promoter and activate its transcription by inducing histone acetylation [14]. SRC3 (steroid receptor coactivator 3) and 53BP1 complex occupy the same region of the *BRCA1* promoter, and both are required for *BRCA1* expression in HeLa cells [15].

Nrf2 is a transcriptional factor and so far there is no report whether it can regulate *BRCA1* transcription. The aim of this study is to explore the activity of Nrf2 complex on *BRCA1* transcription. Our results showed that Nrf2 played an important role in activating *BRCA1* transcription

by directly binding to the *BRCA1* promoter, which was helpful to find potential relationship between this protein and breast cancer susceptibility.

Materials and Methods

Patient samples

Ninety fresh-frozen sporadic breast tumors were randomly selected from the pathology archives and tumor bank of the Cancer Hospital, Fudan University (Shanghai). The informed consent forms from the Institutional Review Board of the Cancer Hospital were obtained in advance. The tumor specimens were all invasive ductal carcinomas, according to the WHO tumor classification.

Immunohistochemical detection of Nrf2 and BRCA1

The breast cancer tissues and normal breast tissues from 90 female breast cancer patients were analyzed. Tumor sections were subjected to immunohistochemical staining for Nrf2 and BRCA1. Tissue sections were incubated with mouse monoclonal antibody against human Nrf2 (1 : 100, Santa Cruz Biotechnology, Santa Cruz, USA) and rabbit anti-human polyclonal antibody against BRCA1 (1 : 100; Millipore Corporate, Billerica, USA) overnight at 4°C. The ABC peroxidase method of staining was then employed as described by the manufacturers (Vector Laboratories, Burlingame, USA). The slides were colorimetric detected with 3,3′-diaminobenzidine. For Nrf2 or BRCA1, the reaction was considered positive when more than 15% of the cells showed distinctive nuclear staining, and was considered negative when $<$15% of the cells showed distinctive nuclear staining or unstained.

Cell lines and cell culture

Human breast cancer cell lines, MCF-7 and HCC38 were obtained from American Type Culture Collection (Rockville, USA). MCF-7 and HCC38 cells were maintained in RPMI 1640 (Gibco, Carlsbad, USA) containing 10% fetal bovine serum (FBS, Gibco, Carlsbad, USA), 100 units/ml penicillin, and 100 μg/ml streptomycin (Gibco, Carlsbad, USA) at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air. Cells were checked routinely and found to be free of contamination by *Mycoplasma* or fungi. All the cell lines were discarded after 3 months.

Plasmids

BRCA1 promoter/luciferase construct pGL3-BRCA1 (-1582 to $+36$) was kindly provided by Dr Xingzhi Xu from College of Life Science, Capital Normal University (Beijing, China). CBP and p300 over-expression vectors were kindly provided by Dr Changjiang Xu from Shanghai Innovative Research Center of Traditional Chinese Medicine (Shanghai, China). Nrf2 over-expression vector (pCMV6-XL5-Nrf2) and its control vector (pCMV6-XL5) were purchased from Origene Company (Rockville, USA).

Reverse transcription polymerase chain reaction

Total RNA was extracted from cells or tissue with TRIzol reagent (Invitrogen, Carlsbad, USA) and quantified by UV absorbance spectroscopy. The reverse transcription reaction was performed using the Superscript First-Strand Synthesis System (Invitrogen). The newly synthesized cDNA was amplified by polymerase chain reaction (PCR). The information of the primers that we used was as following: *BRCA1* primers (5′-TTGCGGGAGGAAAATGGGTAGTTA-3′; 5′-GAAGTAGTAAGTGGGAACCGTGT-3′); *Nrf2* primers (5′-TCCCAGGTTGCCCACAT-3′; 5′-AATGCCGGAGTCAGAATC-3′); *GAPDH* primers (5′-GCCAAAAGGGTCATCATCTC-3′; 5′-GTAGAGGCAGGGATGATGTTC-3′). *GAPDH* was used as an internal control. Amplication cycles were as following: 94°C for 3 min, then 33 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 1.5 min, followed by 72°C for 10 min. Aliquots of PCR product were electrophoresed on 1.5% agarose gels, and PCR fragments were visualized by ethidium bromide staining.

Western blot analysis

Cells were washed twice with phosphate-buffered saline (PBS) containing 1 mM phenylmethylsulphonyl fluoride, followed by lysing in mammalian protein extraction buffer (Pierce, Rockford, USA). The lysates were transferred to eppendorf tubes and clarified by centrifugation at 12,000 *g* for 40 min at 4°C. Cell lysates (50 μg of protein) were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were transferred to nitrocellulose. The membranes were incubated in blocking solution consisting of 5% powered milk in PBS plus 0.1% Tween 20 at room temperature for 1 h, then immunoblotted with anti-BRCA1 antibody (1 : 100; Millipore Corporate), anti-Nrf2 antibody (1 : 100; Santa Cruz), or anti-tubulin antibody (1 : 1000; Sigma-Aldrich, St Louis, USA), respectively. The signals were detection by enzyme-linked chemiluminescence according to the manufacturer's protocol (Amersham Pharmacia Biotech, Piscataway, USA).

Chromatin immunoprecipitation assay and ChIP-ReChIP

Chromatin immunoprecipitation (ChIP) assays were carried out according to the manufacturer's protocol (Active motif, Carlsbad, USA). In brief, cells in 150-mm tissue culture dishes were fixed with 1% formaldehyde and incubated for 10 min at 37°C. The cells were then washed twice with ice-cold PBS, harvested, and re-suspended in ice-cold TNT lysis buffer. The lysates were sonicated to shear the DNA to fragments of 200−600 bp, and subjected to

immunoprecipitation with the following antibodies, respectively, Nrf2 (Santa Cruz), acetylated histone H3 (Abcam Inc., Cambridge, USA), CBP (Chemicon, Rosemont, USA), p300 (Millipore), acetylated histone H4, (Millipore), or IgG (Santa Cruz) as negative control. Three micrograms of antibody was used for each immunoprecipitation. The antibody/protein complex were collected by protein G beads and washed three times with ChIP wash buffer (5% sodium dodecyl sulfate (SDS), 1 mM ethylenediaminetetraacetic acid, 0.5% bovine serum albumin and 40 mM $NaHPO_4$, pH7.2). The immune complexes were eluted with 1% SDS and 1 M $NaHCO_3$. And the cross-links were reversed by incubation at 65°C for 4 h in the presence of 200 mM NaCl and RNase A. The samples were then treated with proteinase K for 2 h, and DNA was purified by mini-column, ethanol precipitation, and re-suspended in 100 μl of H_2O. The primers corresponding to the *BRCA1* promoter region −329 and +133 upstream of the transcription start site (sense: 5′-TT
CTTACGACTGCTTTGGAC-3′; antisense: 5′-GGACTCTA
CTACCTTTACCC-3′) (462 bp) were used for PCR to detect the presence of the *BRCA1* promoter DNA. For ChIP-reChIP, briefly, after sonication, chromatin was incubated overnight with 5 μg of Nrf2 antibody or IgG as negative control. After several washings, the beads were incubated with 3 μg of the CBP or p300 antibody overnight, respectively. After washing, Protein−DNA complexes were eluted from beads and treated with proteinase K overnight. DNA was purified with mini-column and the DNA binding to the *BRCA1* endogenous promoter was quantified by real-time PCR using the primers described above.

Small-interfering RNA preparation and transfection

The small-interfering RNA (siRNA) sequence targeting Nrf2 is 5′-GTAAGAAGCCAGATGTTAA-3′. Nrf2 siRNA was synthesized by Shanghai GeneChem (Shanghai, China). The cells in the exponential phase of growth were seeded in six-well plates at a concentration of 5×10^5 cells/well. After incubation for 24 h, the cells were transfected with siRNA specific for Nrf2 and non-targeting siRNA at a final concentration of 100 nM using oligofectamine and OPTI-MEMI reduced serum medium (Invitrogen), according to the manufacturer's protocol. The silencing effect was examined at 48 h after transfection.

Mutagenesis

BRCA1 promoter/luciferase construct pGL3-BRCA1 was used as template. First, the plasmid DNA was methylated with DNA methylase at 37°C for 1 h. Then, the plasmid was amplified in a mutagenesis reaction system with two overlapping primers, one of which contained the target mutation. After linearization, the double-stranded DNA containing the mutation was transformed into wild-type *Escherichia coli*. The host cells circularized the linear mutated DNA, and the McrBC endonuclease in the host cells digested the methylated template DNA, only leaving unmethylated and mutated product. For mutation, the sequence of antioxidant response element (ARE) site **GCAAACTCA** was converted to **GCAAACCTC**.

Luciferase reporter gene assay

MCF-7 cells were seeded in six-well plates at a density of $1-2 \times 10^5$ cells/well and cultured for 24 h. Cells were then co-transfected with *BRCA1* promoter/luciferase construct (0.5 μg/well) and 0.5 μg of pCMV6-XL5, Nrf2 overexpression vector respectively, together with 20 ng of control Renilla luciferase reporter construct pRL-TK (Promega, Madison, USA). The total amount of DNA was adjusted to 1.5 μg per well by the addition of sonicated salmon sperm DNA. Luciferase assays were performed as recommended by the vendor (Promega) and normalized to protein concentration which was determined by the bicinchoninic acid protein assay (Pierce, Rockford, USA). The promoter activity was then expressed as luminescence units, which is the ratio of luminescence counts of cell lysate and the concentration of cell lysate.

Statistical analysis

Statistical analysis was performed using SPSS Software version 13.0 (SPSS, Chicago, USA). Analysis of variance and Student's *t*-test were used to determine the statistical significance of differences between experimental groups. $P < 0.05$ were considered significant and the confidence intervals quoted were at the 95% level.

Results

Nrf2 is positively associated with BRCA1 expression in breast cancer specimens

To study the correlation between Nrf2 and BRCA1, tumor slices from 90 patients were tested with Nrf2 or BRCA1 antibody. Our results revealed a positive association between Nrf2 and BRCA1 ($P < 0.005$) (**Fig. 1**; **Table 1**). In another experiment, we found that BRCA1 and Nrf2 expression were induced under stress of H_2O_2 in MCF-7 cells. H_2O_2 increased the binding of Nrf2/CBP and Nrf2/p300 to the *BRCA1* promoter (Supplementary **Fig. S1**).

Binding status of Nrf2 complex and histone acetylation on the *BRCA1* promoter between HCC38 and MCF-7 cells

The basal level of *BRCA1* gene expression was analyzed in untreated MCF-7 and HCC38 cells by reverse transcription polymerase chain reaction (RT-PCR) and western blot. As shown in **Fig. 2(A,B)**, we found that *BRCA1* expression

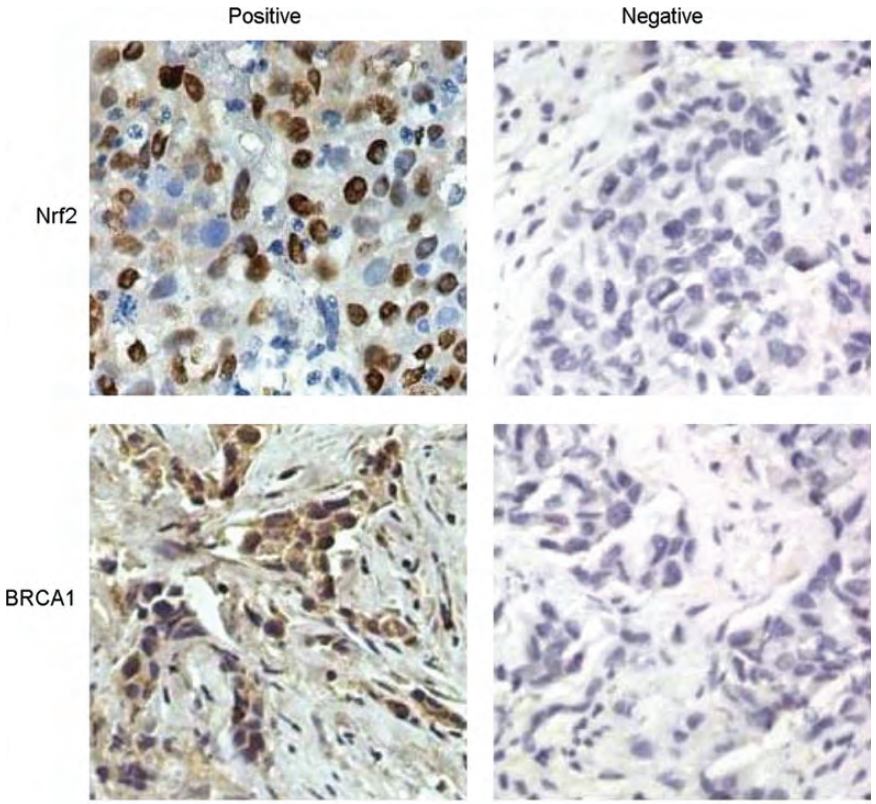

Figure 1 Nrf2 and BRCA1 expression in representative human primary breast tumor tissue samples Immunohistochemical staining was carried out on histological sections in tissue slices of 90 patient's samples with the anti-Nrf2 or anti-BRCA1 antibody (×200).

Table 1 Nrf2 and BRCA1 protein expression are positively associated[a]

	Nrf2	
	Positive	Negative
BRCA1 positive	22	12
BRCA1 negative	10	46

[a]Breast cancer tissue specimens from 90 patients were stained with anti-Nrf2 or anti-BRCA1 antibody.

was very low in HCC38 cells, and *BRCA1* expression was higher in MCF-7 cells than in HCC38 cells.

To determine the potential transcriptional factors binding to the *BRCA1* promoter, MCF-7 and HCC38 cells were subject to ChIP assays. ChIP results demonstrated that the binding of Nrf2, CBP, and p300 was very low in HCC38 cells. However, in MCF-7 cells the recruitment of theses proteins to the *BRCA1* promoter increased significantly [**Fig. 2(C)**].

To determine the potential Nrf2 complex was recruited to the *BRCA1* promoter, we performed ChIP-reChIP assays. Cross-linked and fragmented chromatin was prepared from MCF-7 and HCC38 cells, and sequentially subjected to the first step ChIP with Nrf2 antibody and the second step IP with CBP and p300 antibody, respectively. As shown in **Fig. 2(D)**, the two-step ChIP-reChIP successfully precipitated the *BRCA1* promoter, indicating that Nrf2 could interact with CBP and p300, and formed active transcription complexes on the *BRCA1* promoter. ChIP-reChIP results demonstrated that the recruitment of Nrf2 complex was very low on the *BRCA1* promoter in HCC38 cells. However, the recruitment of theses complexes increased significantly on the *BRCA1* promoter in MCF-7 cells.

Because CBP and p300 are histone acetyltransferase (HAT), they can influence the histone acetylation status in the promoter. Next, we investigated histone acetylation status of the *BRCA1* promoter. ChIPs with antibodies against acetyl-H3, acetyl-H4 were performed in HCC38 and MCF-7 cells. In MCF-7 cells, significant increases of acetyl-H3 and acetyl-H4 on the *BRCA1* promoter were found [**Fig. 2(E)**].

Overexpression of Nrf2 stimulates *BRCA1* expression, recruitment of Nrf2 complexes, and histone acetylation on the *BRCA1* promoter

Nrf2 over-expression vectors were transfected into HCC38 cells, cultured for 6 h then replaced in the RPMI 1640 medium for 48 h. RT-PCR and western blot analysis were performed. As shown in **Fig. 3(A,B)**, we found that over-expression of Nrf2 could activate BRCA1 expression in HCC38 cells.

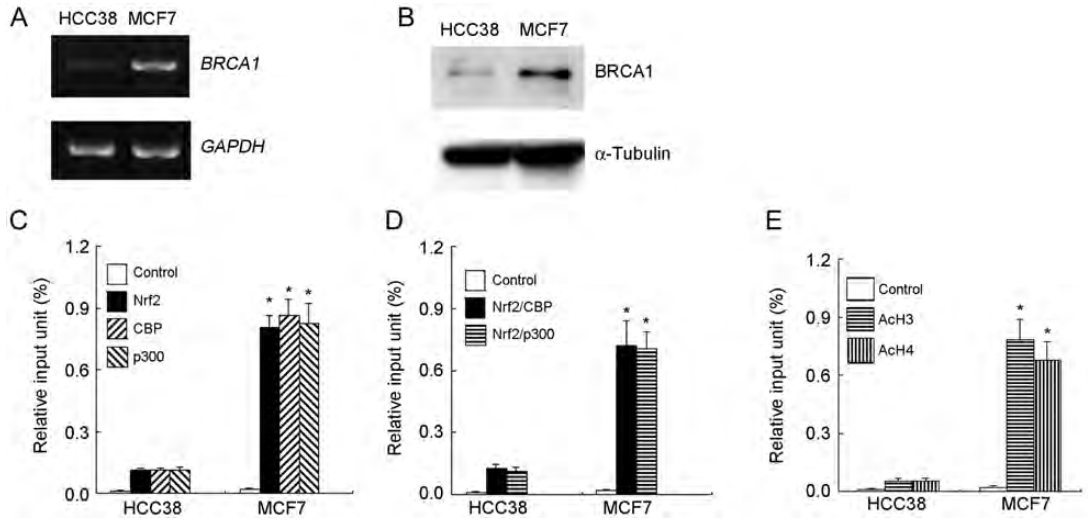

Figure 2 Binding status of Nrf2 complex and histone accetylation on the *BRCA1* promoter in HCC38 and MCF-7 cells (A) Relative *BRCA1* mRNA level was detected in HCC38 and MCF-7 cells. *GAPDH* was used as an internal control. (B) Relative BRCA1 protein level was detected in HCC38 and MCF-7 cells. Tubulin was used as a control. (C) Binding status of Nrf2, CBP and p300 on the *BRCA1* promoter in HCC38 and MCF-7 cells. ChIP assays were performed using antibody against Nrf2, CBP, and p300. $n = 3$, $^*P < 0.05$ vs. control. (D) Detection of Nrf2 complex on the *BRCA1* promoter in HCC38 and MCF-7 cells. ChIP-ReChIP assay was performed. $n = 3$, $^*P < 0.05$ vs. control. (E) Histone accetylation on the *BRCA1* promoter in HCC38 and MCF-7 cells. ChIP assays were performed using antibody against acetyl-H3, acetyl-H4. $n = 3$, $^*P < 0.05$ vs. control.

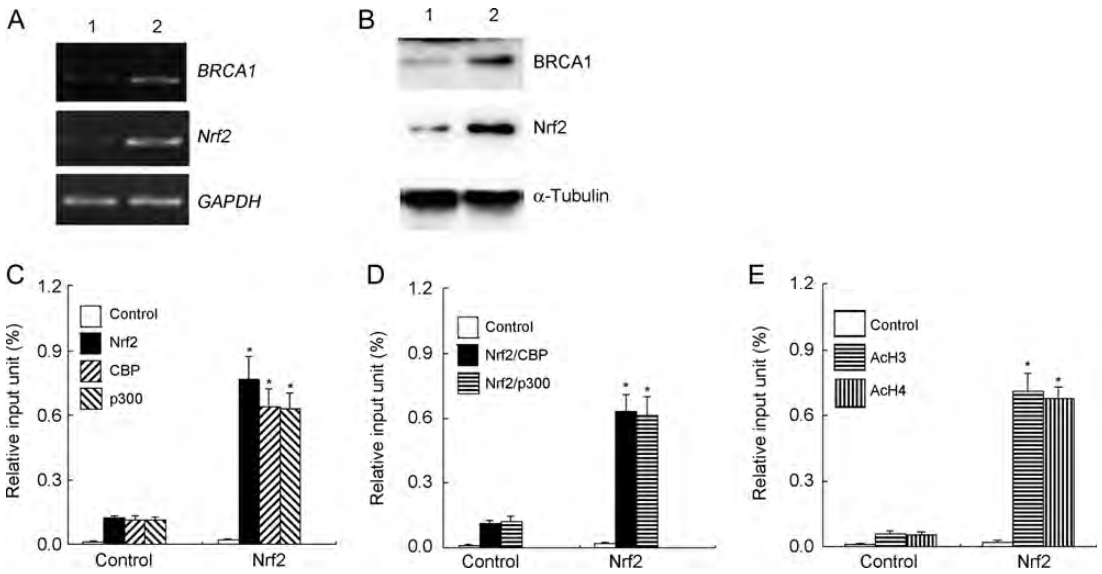

Figure 3 Over-expression of Nrf2 activates *BRCA1* expression in HCC38 cells (A and B) Over-expression of Nrf2 in HCC38 cells activated *BRCA1* expression. pCMV6-XL5 or Nrf2 expression vectors was transfected into HCC38 cells for 72 h and performed reverse transcription polymerase chain reaction (RT-PCR) (A) and western blot analysis (B). 1: HCC38 cells transfected with pCMV6-XL5; 2: HCC38 cells transfected with Nrf2 vector. (C) Binding status of Nrf2, CBP, and p300 on the *BRCA1* promoter in control HCC38 cells and Nrf2 over-expression HCC38 cells. ChIP assays were performed using antibody against Nrf2, CBP, and p300. $n = 3$, $^*P < 0.05$ vs. control. (D) Detection of Nrf2 complex on the *BRCA1* promoter in control HCC38 cells and Nrf2 over-expression HCC38 cells. ChIP-ReChIP assay was performed. $n = 3$, $^*P < 0.05$ vs. control. (E) Histone accetylation on the *BRCA1* promoter in control HCC38 cells and Nrf2 over-expression HCC38 cells. ChIP assays were performed using antibody against acetyl-H3, acetyl-H4. $n = 3$, $^*P < 0.05$ vs. control.

ChIP results demonstrated that over-expression of Nrf2 increased the recruitment of Nrf2, CBP, p300 and histone H3, H4 acetylation on the *BRCA1* promoter [**Fig. 3(C,E)**]. ChIP-reChIP assays demonstrated that over-expression of Nrf2 increased the recruitment of Nrf2 complex on the *BRCA1* promoter [**Fig. 3(D)**].

Knockdown of Nrf2 attenuates *BRCA1* expression, recruitment of Nrf2 complexes and histone acetylation on the *BRCA1* promoter

MCF-7 cells were treated with 100 nM of Nrf2 siRNA or non-targeting siRNA for 48 h. RT-PCR and western blot analysis results showed that Nrf2 siRNA inhibited the

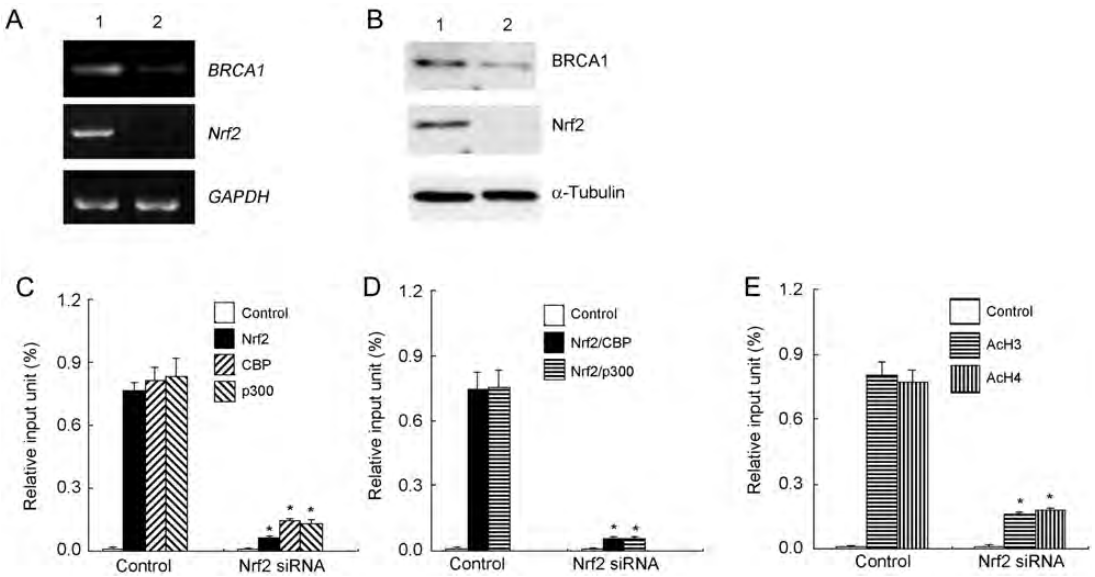

Figure 4 Knockdown of Nrf2 attenuates *BRCA1* expression in MCF-7 cells (A and B) Knockdown of Nrf2 in MCF-7 cells attenuated *BRCA1* expression. MCF-7 cells were treated with 100 nM of Nrf2 siRNA or non-targeting siRNA for 48 h, RT-PCR (A) and western blot (B) analysis were performed. 1, cells transfected with non-targeting siRNA; 2, cells transfected with Nrf2 siRNA. (C) Binding status of Nrf2, CBP and p300 on the *BRCA1* promoter between control MCF-7 cells and MCF-7 cells transfected with Nrf2 siRNA. $n = 3$ experiments, $^*P < 0.05$ vs. controls. (D) Detection of Nrf2 complex on the *BRCA1* promoter between control MCF-7 cells and MCF-7 cells transfected with Nrf2 siRNA. $n = 3$, $^*P < 0.05$ vs. controls. (E) Histone accetylation on the *BRCA1* promoter between control MCF-7 cells and MCF-7 cells transfected with Nrf2 siRNA. $n = 3$, $^*P < 0.05$ vs. controls.

expression of Nrf2 significantly after 48 h transfection [**Fig. 4(A,B)**]. At the same time, we found that knockdown of Nrf2 also significantly inhibited the expression of BRCA1 at mRNA and protein level.

ChIP results demonstrated that knockdown of Nrf2 inhibited the recruitment of Nrf2, CBP, p300, and histone H3, H4 acetylation on the *BRCA1* promoter [**Fig. 4(C,E)**]. ChIP-reChIP assays demonstrated that knockdown of Nrf2 inhibited the recruitment of Nrf2 complex on the *BRCA1* promoter [**Fig. 4(D)**].

Effect of Nrf2 on the *BRCA1* promoter activity

To identify the role of Nrf2 in regulating *BRCA1* transcription, we co-transfected the *BRCA1* promoter/luciferase gene construct with Nrf2 over-expression vector or pCMV6-XL5 in MDA-MB-231 and MCF-7 cells and detected *BRCA1* promoter activity. **Fig. 5(B,C)** showed that the luciferase activity was enhanced by Nrf2 both in MDA-MB-231 and in MCF-7 cells, respectively, indicating that Nrf2 could activate *BRCA1* promoter activity.

To identify the potential-binding site of Nrf2 on *BRCA1* promoter, Nrf2 was co-transfected with either a wild-type (pGL3-BRCA1) or ARE-site-mutated (pGL3-BRCA1mut) BRCA1 construct [**Fig. 5(A)**] into MDA-MB-231 or MCF-7 cells. Although the over-expression of Nrf2 activated the wild-type promoter more than 5 folds, it had no effect on the ARE-site mutant [**Fig. 5(B,C)**], which indicated Nrf2 activated *BRCA1* promoter activity by directly binding to the ARE site of *BRCA1* promoter.

Our results also showed that CBP and p300 genes could increase *BRCA1* promoter activity and produce synergy effects with Nrf2 [**Fig. 5(D,E)**].

Discussion

Established functions of BRCA1 include the regulation of cell cycle progression, DNA repair, maintenance of genomic integrity, and transcription regulation [16]. BRCA1 can regulate the transcriptional pathways by interacting with basal transcriptional machinery (RNA helicase A and RNA pol II) [17,18], transcriptional co-activators (CBP and p300) [19], transcriptional co-repressors (HDAC-1/2 and CtIP) [20,21]. It has been reported that BRCA1 can increase the expression of Nrf2-regulated genes [NADPH:quinone oxidoreductase 1 (*NQO1*), Glutathione S-transferase (*GST*)] that are involved in cytoprotective antioxidant and detoxification response, and BRCA1 can be recruited to Nrf2 promoter. These findings suggest that Nrf2 is downstream of BRCA1, and BRCA1 may stimulate Nrf2 transcriptional activity and protect cells against oxidative stress and carcinogens by Nrf2 [22,23]. However, there is no report whether Nrf2 can regulate *BRCA1* transcription.

Nrf2 is a member of CNC (cap-'n'-collar) family of transcriptional factors, and so far, there is no report whether it can regulate BRCA1 transcription. In Kelch-like ECH-associated protein 1 (Keap1)-Nrf2-ARE pathway, Nrf2 is negatively regulated by its cytoplasmic inhibitor Keap1

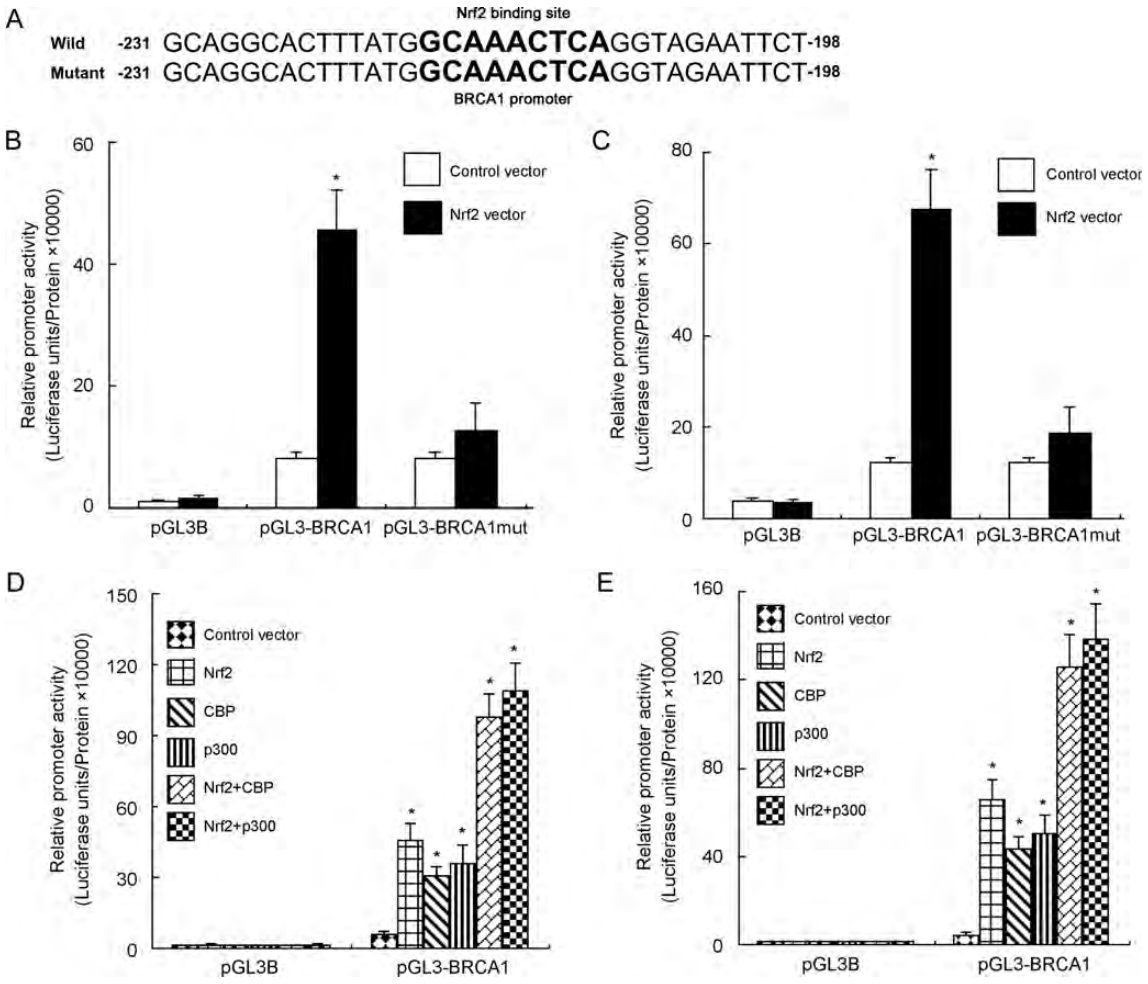

Figure 5 Effect of Nrf2 complex on *BRCA1* promoter activity (A) Model depicting binding of Nrf2 transcriptional complexes to the ARE site on the *BRCA1* promoter. Nrf2 interacts with CBP and p300, and forms an activating transcriptional complex which can bind to ARE site on the *BRCA1* promoter and activate its transcription. (B, C) Nrf2 requires an intact Nrf2 site for *BRCA1* promoter activation in MDA-MB-231 (B) or MCF-7 (C) cells. $n = 3$ experiments, $^*P < 0.05$ vs. controls. (D, E) Nrf2 complex is required for *BRCA1* promoter activation in MDA-MB-231 (D) or MCF-7 (E) cells. Luciferase activity was determined and normalized to protein concentration. $n = 3$, $^*P < 0.05$ vs. controls.

under basal conditions. Keap1 inhibits Nrf2 by binding to Nrf2 and promoting its degradation [24]. On an event of oxidative stress, Nrf2 dissociates from Keap1, translocates into the nucleus, and binds to ARE sequence to activate transcription of a variety of genes. The majority of these genes encodes Phase II detoxification enzymes and oxidative stress-responsive-related proteins, such as NQO1, heme oxygenase 1 (HO-1), GSTs, UDP-glucuronosyl transferases (UGTs), superoxide dismutases, protecting cells against DNA damage, and mutagenesis induced by ROS and carcinogens [25,26]. It has been demonstrated that Nrf2 can protect normal cells from transforming into cancer cells [27].

In our study, IHC experiments demonstrated that MyoD expression and BRCA1 expression were correlated ($P < 0.005$). Bioinformatic analysis of the 5′-flanking region of the human *BRCA1* gene showed that there existed an ARE site in the *BRCA1* promoter region from −218 to −210 bp, which indicated that Nrf2 might bind to *BRCA1* promoter and regulate *BRCA1* transcription. So far, there is no report whether Nrf2 can bind to *BRCA1* promoter and regulate *BRCA1* transcription and its function, especially, whether Nrf2 plays important roles in controlling breast tumorigenesis by regulating histone modifications on *BRCA1* promoter.

It has been reported that *BRCA1* is lowly expressed in HCC38 cells, but *BRCA1* is highly expressed in MCF-7 cells [28]. In this study, our data were consistent with the previous report. To identify the potential roles of Nrf2 complexes in regulating *BRCA1* expression, we performed ChIP and ChIP-rChIP assays in HCC38 cells and MCF-7 cells. We found that Nrf2 could interact with CBP and p300, and formed Nrf2 active transcription complex on the *BRCA1* promoter.

In the Nrf2 complex on the *BRCA1* promoter, CBP and p300 are HAT and key transcriptional co-activators [29]. CBP and p300 are believed to catalyze acetylation of

histones, aid chromatin remodeling, and promote target gene transcription [30,31]. Our experiments showed that histone H3 and H4 were highly acetylated on *BRCA1* promoter in MCF-7 cells. However, H3 and H4 acetylation were very low on *BRCA1* promoter in HCC38 cells. These results indicated that *BRCA1* expression was closely associated with epigenetic status of its promoter.

In the following experiments, we found that over-expression of Nrf2 in HCC38 cells could activate *BRCA1* expression. Over-expression of Nrf2 increased the recruitment of Nrf2 complex and histone H3, H4 acetylation on the *BRCA1* promoter. These results indicated that Nrf2 could stimulate *BRCA1* expression by recruiting of Nrf2 complex and inducing histone acetylation on the *BRCA1* promoter in HCC38 cells.

We also found that knockdown of Nrf2 could attenuate *BRCA1* expression by inhibiting the recruitment of Nrf2 complex and histone acetylation on the *BRCA1* promoter region in MCF-7 cells. Based on the above experiment results, we hypothesized that Nrf2-induced epigenetic changes involving histone modifications and chromatin remodeling were responsible for *BRCA1* expression.

To further confirm the roles of Nrf2 and their related transcriptional factors in regulating *BRCA1* transcription, we performed luciferase assay. Our results demonstrated that Nrf2 could activate *BRCA1* promoter activity. We found an ARE site from −218 to −210 bp on the *BRCA1* promoter. Point mutant of ARE site on the *BRCA1* promoter construct abrogated the activation effect of Nrf2 on *BRCA1* promoter activity, indicated that Nrf2 activated *BRCA1* promoter activity by directly binding to the ARE site of *BRCA1* promoter.

In a summary, in this study we found that over-expression of Nrf2 stimulated *BRCA1* expression, which is through interacting with CBP and p300, formed an active transcription complex binding to ARE site on the *BRCA1* promoter. At the same time, histone acetylation was increased, which is helpful for activating the transcription of *BRCA1*. Knockdown of Nrf2 attenuated *BRCA1* expression. Regulation of *BRCA1* expression by Nrf2 complexes may be part of an integral signaling pathway that determines and explains breast cancer susceptibility.

Supplementary Data

Supplementary data are available at *ABBS* online.

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