Original Article

ERα promotes *SUMO1* transcription by binding with the ERE and enhances SUMO1-mediated protein SUMOylation in breast cancer

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Background: Estrogen plays a crucial role in the tumorigenesis of breast cancer (BC), and epigenetic modification by SUMOylation is essential for cancer development. However, the mechanism underlying estrogen's actions on protein SUMOylation and its effect on BC development are still incompletely understood.

Methods: *SUMO1* in BC cell lines was verified via real-time quantitative PCR (RT-qPCR) and western blot. Cell proliferation and colony formation assays was also performed to evaluate SUMOylation as mediated by *SUMO1*. Luciferase activity to examine whether E2 promoted the transcription of *SUMO1*, and chromatin immunoprecipitation (ChIP) assay to determine the binding of estrogen receptor alpha (ERα) to SUMO1 were conduction, and an animal model was used to evaluate the effects of E2-ERα-enhanced *SUMO1* transcription.

Results: E2 promoted *SUMO1* mRNA and protein expression levels in a dose- and time-dependent manner in ER-positive BC cells; it exerted no influence on SUMO2/3 expression; in E2-induced *SUMO1* transcription, ERα, but not ERβ, was essential to the process. In addition, E2-ERα upregulated the transcription of SUMO1 by binding with an estrogen-response element half-site (1/2ERE, in the –134 to –123 bp region) of the *SUMO1* promoter, and E2-ERα induced *SUMO1* transcription-enhanced cellular viability in ER-positive BC cells. To further determine SUMOylation as mediated by *SUMO1* in ER-positive BC, we evaluated novel *SUMO1* target proteins such as Ras and demonstrated that E2 increased Ras SUMOylation and cellular proliferation by affecting downstream signaling-pathway transduction. Finally, our data revealed that E2-ERα enhanced *SUMO1* transcription to promote tumor growth in a BC orthotopic tumor model.

Conclusions: Collectively, our results showed that E2 promoted the transcription and protein expression of *SUMO1* via ERα binding to a 1/2ERE in the *SUMO1* promoter, and that E2-ERα also augmented SUMO1-mediated Ras SUMOylation and mediated cellular responses in ER-positive BC. We therefore achieved significant insights into the mechanism involved in ER-positive BC development and provided a novel target for its treatment.

Keywords: Breast cancer (BC); estrogen receptor (ER); Ras; *SUMO1*; SUMOylation

Submitted Jan 30, 2023. Accepted for publication Jul 20, 2023. Published online Jul 31, 2023.
doi: 10.21037/gs-23-39
View this article at: https://dx.doi.org/10.21037/gs-23-39

Introduction

Breast cancer (BC) has one of the highest rates of cancer deaths among women worldwide, with long-term exposure to estrogen considered to be the principal factor leading to its tumorigenesis; estrogen-dependent BC accounts for approximately 2/3 of BC cases (1,2). In addition, clinical research using anti-estrogens or aromatase inhibitors to reduce local and distant recurrence has revealed that estrogen promotes the development of BC (3). The molecular mechanism underlying estrogen-induced BC is hypothesized to be mediated by the combination of estrogen and estrogen receptors (ERs) that bind to the estrogen-responsive element (ERE) of target-gene promoters or regulatory regions. *ESR1* and *ESR2* are different genes that encode ERα and ERβ subtypes, respectively (4), and elevated expression of ERα is observed in ER-positive BC and known to be related to BC growth. In addition, widespread expression of ERβ is found in BC, although its role remains unclear (5).

ERα is overexpressed in approximately 70% of BC cases (6). ERα is a ligand-activated transcription factor consisting of three functional domains of hormone binding, DNA binding and transcription activation. The ligand-binding domain (LBD) is recognized by the E2 (7). The deactivation domains AF-1 and AF-2 synergistically activate ERα. The DNA binding domain (DBD) recognizes estrogen response elements on DNA (8). ERα increases the expression level of carcinogenic proteins, including cyclin D1 and c-myc, and inhibits the level of cell cycle inhibitors, including P21 (9). ERα can also bind to promoters or regulatory regions of target genes that contain incomplete or truncated EREs and activate their transcription (10).

Highlight box

Key findings

- ERα-induced SUMO1 expression plays a key role in the regulation of BC proliferation.

What is known and what is new?

- E2 promoted the transcription and protein expression of SUMO1 via ERα binding to a 1/2ERE in the SUMO1 promoter.
- E2-ERα augmented SUMO1-mediated Ras SUMOylation and mediated cellular responses in ER-positive BC.

What is the implication, and what should change now?

- We need to pay attention to that SUMO1-induced protein SUMOylation, and elucidated its effect on cellular proliferation in BC.

E2-ERα signaling therefore plays a key role in the growth, migration, and invasion of BC cells (11). Because ERα and its signaling pathway play a crucial role in the development and progression of BC, anti-estrogen therapy and targeting ERα signaling pathway are important components of treatment for ERα positive BC patients.

SUMOylation is a vital post-translational modification that is critical to a variety of biologic functions, including cell growth, migration, and metastasis (12). SUMOylation is an enzyme cascade wherein small ubiquitin-related modifiers (SUMOs) are covalently bound to an internal lysine residue of a target protein by the carboxy-terminal glycine of processed SUMO (13). The binding of SUMO to proteins may thus be crucial to protein activity, subcellular localization, and stability (14). For example, increasing evidence reveals that SUMOylation can target various proteins, including nuclear transcription factors, membrane proteins, and cytoplasmic proteins, which are pivotal to BC progression (15). The dysregulation of SUMOylation could result in tumor progression, and is considered as a novel biomarker and possible therapeutic target for cancers (16). *SUMO1*, *SUMO2*, and *SUMO3* are involved in the process of protein SUMOylation (17), and although investigators have previously identified a role for SUMO2 and SUMO3 in BC, the function for *SUMO1* in BC remains arcane (18).

Accumulating evidence suggests that estrogen levels correlate with protein SUMOylation (19). It was reported that a tumor's positive nuclear/negative cytoplasmic expression of SUMO proteins, including *PIAS1*, *PIAS4*, and *UBC9*, featured positive expression for ER, and that *PIAS1*, *PIAS4*, and *UBC9* expressions were elevated in an ER-positive MCF-7 cell line compared with an ER-negative MDA-MB-436 cell line (20). Although E2-ER signaling is associated with protein SUMOylation in BC, the molecular mechanism that regulates SUMOylation remains largely undefined. In this study, we demonstrated that ERα activated *SUMO1* gene transcription by binding with the ERE of the *SUMO1* promoter in response to E2, and that ERα-induced *SUMO1* expression was involved in BC development. We present this article in accordance with the ARRIVE reporting checklist (available at https://gs.amegroups.com/article/view/10.21037/gs-23-39/rc).

Methods

Cell culture

MDA-MB-231 and HeLa cells were cultured in DMEM

(Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS). T47D, MCF-7, and SK-BR-3 cells were incubated in RPMI-1640 (Gibco) containing 10% FBS. All cells were cultured at 37 ℃ in 5% CO_2 in compressed air with high humidity.

Plasmids and transient transfection

The SUMO1-reporter construct and expression constructs have been described previously (21). Transient transfections were executed using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). After transfection for 24–48 h, luciferase activity was measured with a Luciferase Reporter Assay System (Promega).

Clinical samples

Tumor tissue samples were obtained from 86 ERα-positive BC patients who underwent surgery at the Affiliated Hospital of Nantong University. This study was approved by the institutional ethics committee of the Affiliated Hospital of Nantong University (ID: 2023-L084). Informed consent was obtained from each participant. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Protein extraction, immunoprecipitation, and western blot

Whole-cell protein extraction and immunoprecipitation were conducted as described previously (22), and western blot analysis used available antibodies. Primary antibodies were generated against *SUMO1* (diluted 1:500, #ab227424, Abcam, Cambridge, UK), ERα (1/200, #ab32063, Abcam), Ras (1/5,000, #ab52939, Abcam), myc (1/1,000, #ab32072, Abcam), GST (1/1,000, #ab111947, Abcam), p-Akt (1/500, #ab8805, Abcam), Akt (1/500, #ab38449, Abcam), p-ERK1/2 (1/1,000, #ab278538, Abcam), ERK1/2 (1/10,000, #ab184699, Abcam), NF-κB p65 (1/1,000, #ab32536, Abcam), and phosphorylated (p-)NF-κB p65 (1/1,000, #ab86299, Abcam); GAPDH (1:10,000, #sc-47724, Santa Cruz Biotechnology, Dallas, TX, USA) was used as an internal control. The protein bands were visualized using an efficient chemiluminescence (ECL) detection kit (Thermo Fisher Scientific).

Real-time quantitative PCR (RT-qPCR)

RNA extraction was performed with TRIzol reagent (Invitrogen), and reverse transcription with a SuperScript First-Strand Synthesis System (Invitrogen). The RT-qPCR amplification was performed on an ABI Prism 7500 system. The relative RNA levels were calculated by the $2^{-\Delta\Delta Ct}$ method.

Chromatin immunoprecipitation (ChIP) assay

We performed ChIP as described previously (23). Each immunoprecipitation reaction contained a small aliquot of lysate with 200 μg of protein, and PCR was used to amplify the precipitated genomic DNA.

Cell proliferation and colony formation assays

Cells were plated in 96-well plates at a density of 1×10^4 cells/well. We measured cellular proliferation by MTT assay according to the manufacturer's protocol for a commercially available kit (KeyGen, Nanjing, China). For colony formation, cells were seeded at a density of 1,000 cells/well, and after 2 weeks, the colonies (>50 cells/colony) were fixed and stained with 0.1% crystal violet and photographed.

Orthotopic tumor model

Male nude mice (4–6 weeks old) were allocated to different groups, and 2×10^6 cells were injected into their right flanks. Four weeks later, the tumors were removed from the dead mice and photographed, and their volumes were calculated as length (mm) × width^2 (mm^2)/2. All nude mice are purchased from the Animal Center of Nantong University. This animal research protocol was approved by the Animal Ethics Committee of Nantong University (ID: P20230222-001), in compliance with institutional guidelines for the care and use of animals.

Statistical analysis

We used SPSS 20.0 to conduct statistical analyses. The differences between the control and experimental groups were analyzed using Student's *t*-test and one-way analysis of variance (ANOVA). $P<0.05$ was deemed to be statistically significant.

Results

ERα induced SUMO1 expression in BC cells

To evaluate the relationship between *SUMO1* and ERs

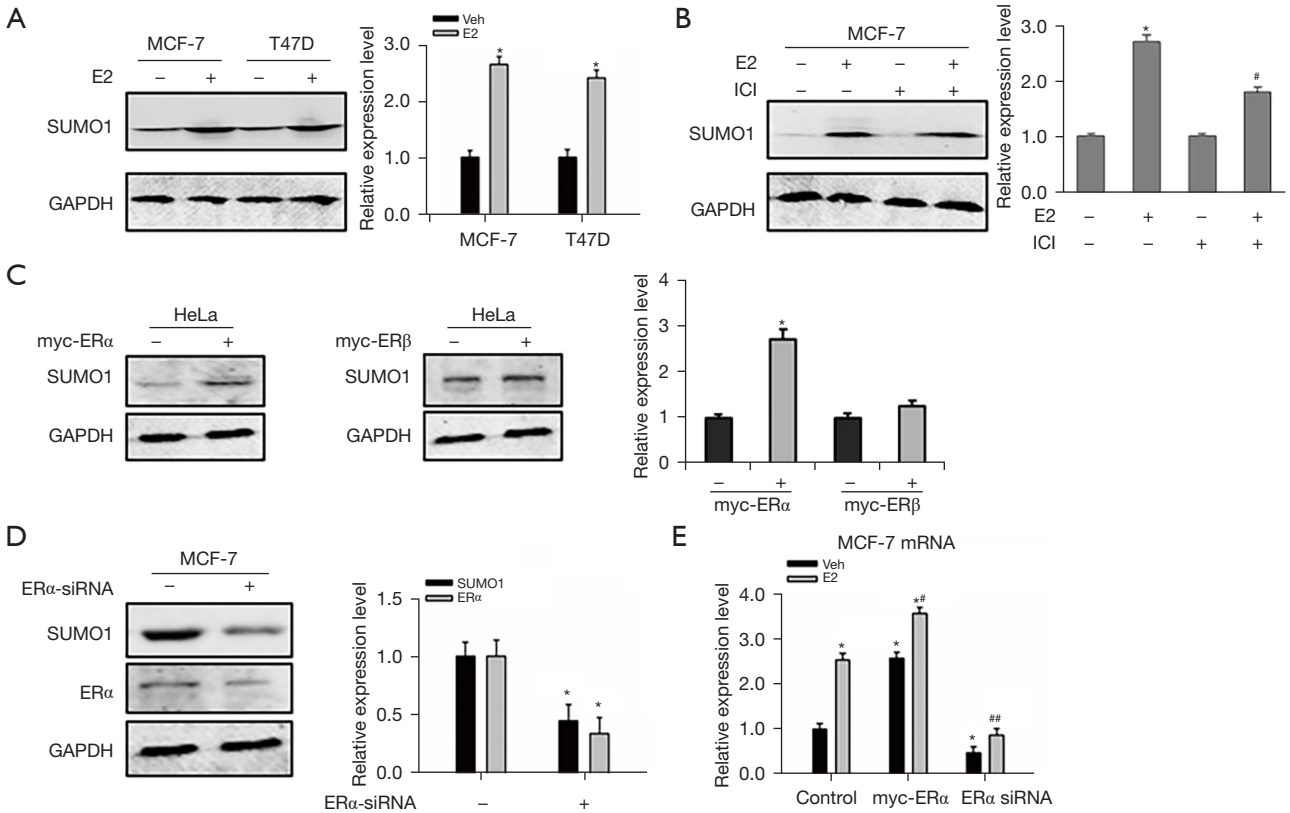


Figure 1 ERα regulation of *SUMO1* expression in BC cells. (A) MCF-7 and T47D cells were treated with vehicle (control) or 1 mM E2 for 12 h, and western blot analysis examined SUMO1 protein expression. *, P<0.05 compared with control group. (B) MCF-7 cells were treated with vehicle, 1 mM E2, or 0.1 mM ICI alone or in combination with E2 for 12 h, and western blot analysis determined SUMO1 protein expression. *, P<0.05 compared with untreated group; #, P<0.05 compared with E2 treated group. (C) HeLa cells transfected with pcDNA3 or pcDNA3-myc-ERα/for 12 h before western blot analysis. *, P<0.05 compared with untransfected group. (D) MCF-7 cells transfected with control siRNA or ERα siRNA for 48 h, and treated with 1 mM E2, before assessment of the expression of *SUMO1* and ERα. *, P<0.05 compared with the untransfected group. (E) MCF-7 cells transfected with ERα to produce overexpression or with ERα siRNA vector, and *SUMO1* mRNA expression determined by RT-qPCR. *, P<0.05 compared with untransfected group; #, P<0.05 compared with E2 treated group; and ##, P<0.05 compared with E2-treated ERα-overexpressing group. ERα, estrogen receptor alpha; BC, breast cancer; RT-qPCR, real-time quantitative polymerase chain reaction; ICI, immune checkpoint inhibitor.

in BC, we examined whether E2 stimulated *SUMO1* expression. The ER-positive MCF-7 and T47D cell lines treated with E2 exhibited increased SUMO1 protein levels in a dose- and time-dependent manner, but exerted no effect on SUMO2 or SUMO3 protein expression (*Figure 1A*, Figure S1A,S1B, Appendix 1). The same treatment of ER-negative MDA-MB-231 and SK-BR-3 cells had little effect on *SUMO1* expression (Figure S1C). Treatment of MCF-7 and T47D cells with the anti-estrogen ICI 182780 partially reversed E2-enhanced *SUMO1* expression (*Figure 1B*, Figure S1D). We also found that *SUMO1* mRNA levels in MCF-7 and T47D cells were elevated in response to E2 in both dose- and time-dependent manner (Figure S1E). When we then assessed whether E2 could promote *SUMO1* expression via ERα or ERβ using HeLa cells that did not contain measurable levels of ERα or ERβ but were transfected with ERα or ERβ, we found that overexpression of ERα increased *SUMO1* expression, but that overexpression ERβ had no effect on HeLa cells (*Figure 1C*). Conversely, knockdown of ERα reduced the expression of *SUMO1* in both MCF-7 and T47D cells (*Figure 1D*, Figure S1F). The effects of ERα on the regulation of *SUMO1* transcription were thus further confirmed. As expected, we noted that knockdown of

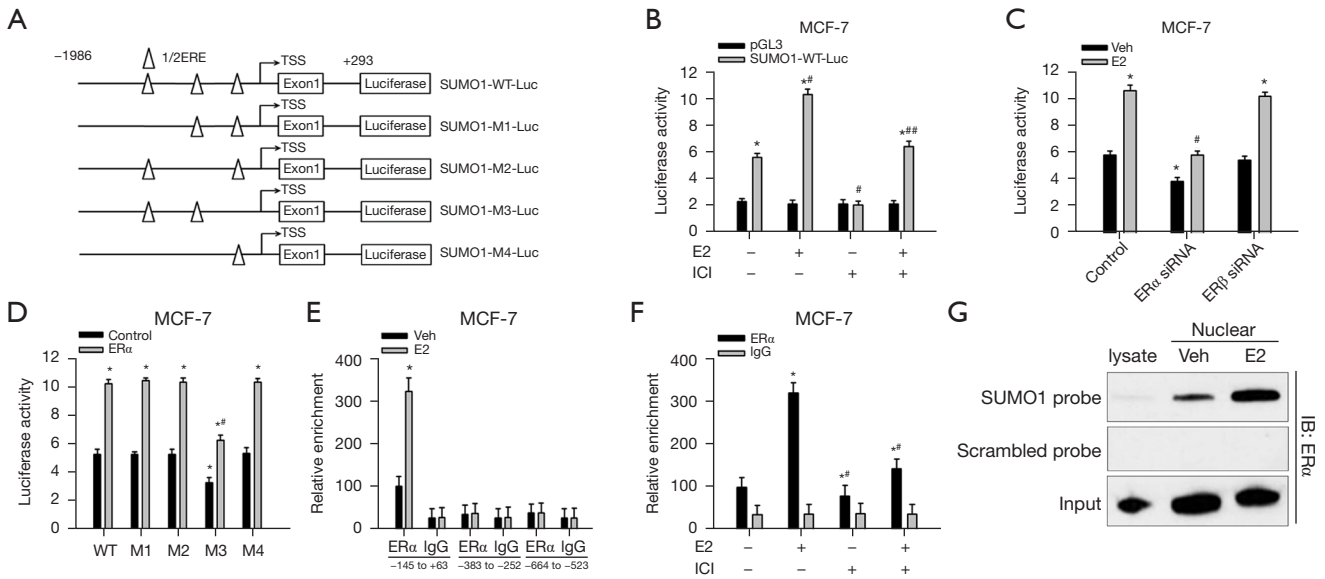


Figure 2 ERα binding to the *SUMO1* promoter regions, and regulating *SUMO1* promoter activity. (A) Schematic illustration of the ERE in the *SUMO1* promoter depicts a *SUMO1* sequence fused to luciferase (SUMO1-WT-Luc) or mutants by fusing the ERE to luciferase (SUMO1-M1-Luc, SUMO1-M2-Luc, SUMO1-M3-Luc, or SUMO1-M4-Luc). (B) MCF-7 cells transfected with SUMO1-WT-Luc and subsequently treated with E2 or ICI separately or in combination. *, P<0.05 compared with untreated group; #, P<0.05 compared with untreated SUMO-WT-Luc group; and ##, P<0.05 compared with E2-treated SUMO-WT-Luc group. (C) MCF-7 cells transfected with ER siRNA vectors for examination of luciferase activity. *, P<0.05 compared with untreated group; and #, P<0.05 compared with E2-treated group. (D) MCF-7 cells transfected with different mutant vectors for examination of luciferase activity. *, P<0.05 compared with untreated SUMO-WT-Luc group; and #, P<0.05 compared with E2-treated SUMO-WT-Luc group. (E) ChIP assay shows recruitment of ERα to the *SUMO1* promoter region. MCF-7 cells were treated with vehicle or 1 mM E2 for 12 h, and subsequently applied ChIP using ERα or IgG antibodies; total input DNA at a 1:10 dilution was designated the positive control. *, P<0.05 compared with Veh group. (F) MCF-7 cells were treated with 1 mM E2 or ICI, and binding between ERα and the promotor detected by ChIP assay. *, P<0.05 compared with untreated group; and #, P<0.05 compared with E2-treated group. (G) Immunoprecipitated ERα and nuclear extract incubated with a *SUMO1* probe or scrambled probe to measure the interaction between the DNA region and ERα by western blot assay. ERα, estrogen receptor alpha; ERE, estrogen-responsive element; WT, wide type; TSS, transcription start site; ICI, immune checkpoint inhibitor; ChIP, chromatin immunoprecipitation.

ERα downregulated *SUMO1* transcription and that ERα overexpression increased *SUMO1* transcription (*Figure 1E*, Figure S1G). Our results therefore proved that *SUMO1* was a target gene of ERα transcription in BC.

ERα bound to SUMO1 promoter regions and regulated SUMO1 promoter activity

To further investigate the regulation of *SUMO1* expression by ERα, the fragments containing the upstream and downstream regions of the transcriptional start site (TSS) were cloned and fused with the luciferase gene to produce a SUMO1-WT-Luc construct (*Figure 2A*). To examine whether E2 promoted the transcription of *SUMO1*, we determined the luciferase activities of MCF-7 and T47D cells transfected with SUMO1-WT-Luc after E2 or immune checkpoint inhibitor (ICI) treatment and observed that in the presence of E2 SUMO1-WT-Luc luciferase activity increased but that ICI lessened this effect (*Figure 2B*, Figure S2A). However, the level of luciferase activity expressed by SUMO1-WT-Luc was unaltered in response to E2 or ICI in MDA-MB-231 and SK-BR-3 cells (Figure S2A). When increasing amounts of ERα-expression plasmids were transfected into ERα-/ERβ-HeLa cells, SUMO1-WT-Luc showed a gene-dosage effect with ERα that was commensurate with the enhanced expression of luciferase activity (Figure S2B). However, the cells transfected with increasing levels of ERβ showed only a small increase in luciferase activity (Figure S2B). In addition, knockdown of ERα attenuated the reporter activity level

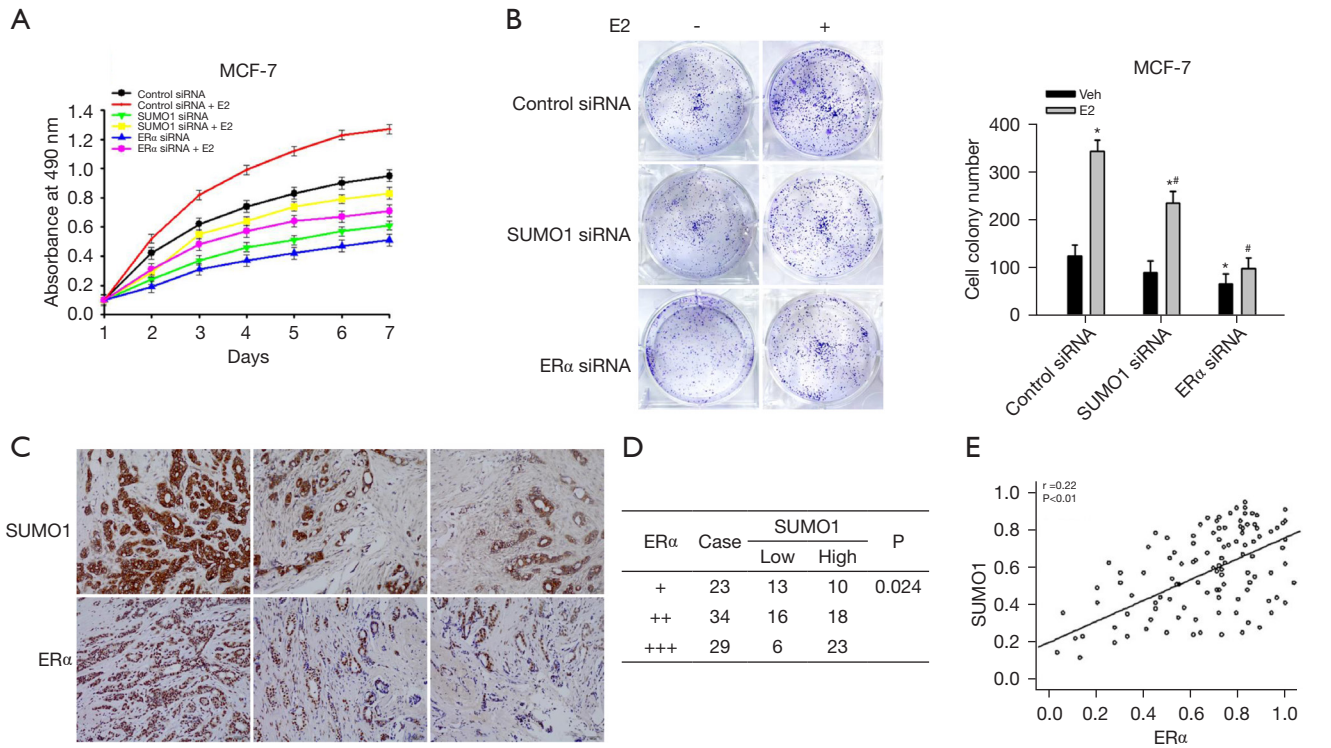
with cellular exposure to E2 in MCF-7 and T47D cells, but exerted less of an effect in ERβ-knockdown cells (*Figure 2C*, Figure S2C). Taken together, these findings revealed that *SUMO1* was regulated by the transcription of ERα. When we searched for ERα-responsive regions in the *SUMO1* promoter, we located three 1/2EREs in the –564 to –553, –343 to –332, and –134 to –123 bp regions with respect to the TSS. To determine which portion of the promoter was most susceptible to ERα, we constructed truncated promoters (SUMO1-M1-Luc, SUMO1-M2-Luc, SUMO1-M3-Luc, and SUMO1-M4-Luc) and transfected them into MCF-7 cells to detect their effects on ERα-reactive activity. Luciferase activity was diminished when the –134 to –123 bp 1/2ERE was deleted, but was less affected when either the –564 to –553 or –343 to –332 bp region 1/2ERE was deleted; and the luciferase activity was increased with ERα overexpression (*Figure 2D*). Furthermore, when we transfected these luciferase vectors with ERα expression vectors into HeLa cells, we noted that overexpression of ERα elevated luciferase activity in MCF-7 and T47D cells, but that the increase in activity was less than in the –134 to –123 bp 1/2ERE-mutant transfected cells (Figure S2D). These findings indicated that this 1/2ERE was vital for ERα activity and drove the luciferase activity of SUMO1. We then implemented a ChIP assay to determine the binding of ERα to *SUMO1* in MCF-7 cells, and demonstrated that when the cells were treated with E2, ERα only bound to region –145 to +63, but not to regions –664 to –523 or –383 to –252 (*Figure 2E*). We also noted binding of ERα to the *SUMO1* promotor, and that E2 enhanced the binding between the *SUMO1* probe and ERα, but reversed it with ICI pretreatment (*Figure 2F*). Besides, we observed that immunoprecipitated ERα bound to the nuclear extract incubated with a *SUMO1* probe (*Figure 2G*). Taken together, our results demonstrated that endogenous ERα bound to the *SUMO1* promoter and induced *SUMO1* gene expression.

SUMO1 expression induced by ERα for BC cell growth

We assessed the effects of decreased *SUMO1* expression on the biologic activities of BC cells. Treatment with E2 promoted the proliferation of MCF-7 and T47D cells with transfection of control siRNA, while in the presence of E2, inhibition of SUMO1 or ERα suppressed cellular proliferation (*Figure 3A*, Figure S3A). We then determined the effects of *SUMO1* silencing on cell-colony formation, and showed that E2 treatment enhanced colony formation of MCF-7 and T47D cells, while in the absence or presence of E2, knockdown of *SUMO1* decreased colony formation compared with the control siRNA-transfected group (*Figure 3B*, Figure S3B). Also, as we expected, downregulated expression of ERα reduced cell-colony formation, and these cells also lost their responsiveness to E2 (*Figure 3B*, Figure S3B). Our findings thus indicated that *SUMO1* expression induced by ERα stimulated cellular proliferation. We also used an immunohistochemical (IHC) assay to ascertain the expression of and relationship between *SUMO1* and ERα in ERα-positive BC samples (*Figure 3C*). With respect to ER-expression levels, 10 samples of *SUMO1* expression were elevated in 23 ER+ patients, 18 samples were augmented in 34 ER++ patients, and 23 samples were high in 29 ER+++ patients (*Figure 3D*). When we analyzed the relationship between *SUMO1* and ERα, we found that their expression levels correlated (*Figure 3E*), which suggested a correlation between *SUMO1* and ERα in ERα-positive BC.

ERα induced SUMO1-mediated protein SUMOylation in BC cells

To further investigate the effects surrounding cell growth as mediated by *SUMO1* expression and to clarify the underlying mechanism of action, we identified aspects of SUMO1-mediated protein SUMOylation. The SUMOylation mediated by SUMO1 was increased in the presence of E2 and reversed when SUMO1 and ERα were knocked down (Figure S4A), and vascular endothelial growth factor receptor (VEGFR) or HIF1α SUMOylation was also increased when cells were treated with E2 and reversed by ERα knockdown (Figure S4B, Figure S5). Immunoprecipitation combined with mass spectrometry was used to investigate novel target proteins of *SUMO1*, and we identified Ras as binding to *SUMO1*. Based on this interaction, we showed that Ras bound to *SUMO1* both *in vitro* and *in vivo*, and that binding was enhanced in the presence of E2 and reduced with ERα knockdown (*Figure 4A*,*4B*). We then used *SENP1* (24) (which removes SUMO from target proteins) and transfected it into cells, which revealed that SUMOylation of Ras was diminished when cells were pretreated with E2 (*Figure 4C*). We also identified the SUMOylation and binding site of Ras as Lys-37, and showed that mutation of this site to glycine decreased SUMOylation (*Figure 4D*). These findings indicated that in BC cells, ERα-induced expression of *SUMO1* was involved in protein SUMOylation, and that Ras may indeed be the target in BC.



ERα	Case	SUMO1		P
		Low	High	
+	23	13	10	0.024
++	34	16	18	
+++	29	6	23	

Figure 3 Promotion of BC cell proliferation by ERα induced-SUMO1 expression. (A) MTT assay in the presence or absence E2 to analyze the viability of cells transfected with control siRNA, *SUMO1* siRNA, or ERα siRNA. (B) Cells transfected with the indicated vectors in the presence or absence of E2, and colonies stained with crystal violet for counting. *, P<0.05 compared with untreated group; and #, P<0.05 compared with E2-treated group. (C) Representative photomicrographs of immunohistochemical staining for ERα and *SUMO1* in BC tissues. Each sample was incubated with antibodies against ERα or *SUMO1*, and a positive or negative reaction was displayed by brown or blue staining, respectively (original magnification ×200). (D) Relationship between ERα and *SUMO1* expression indicated by the BC samples. (E) Correlation between the expression of *SUMO1* and ERα in BC samples. BC, breast cancer; ERα, estrogen receptor alpha.

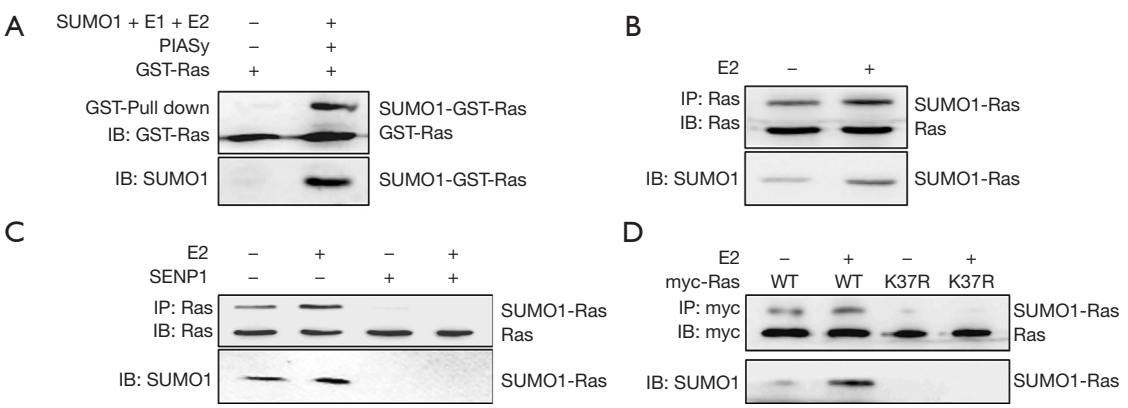


Figure 4 ERα-induced *SUMO1* expression promotes Ras SUMOylation in BC cells. (A) Binding between Ras and *SUMO1* evaluated *in vitro*. (B) MCF-7 cells pretreated with E2 and then lysed to detect the binding between Ras and *SUMO1* using immunoprecipitation. (C) MCF7 cells transfected with SENP1-expression vectors and treated with E2, then lysed before immunoprecipitation to examine the interaction between Ras and *SUMO1*. (D) Mutated SUMOylation sites of Ras transfected into MCF-7 cells for evaluation of SUMOylation of these molecules by immunoprecipitation. ERα, estrogen receptor alpha; BC, breast cancer; WT, wide type.

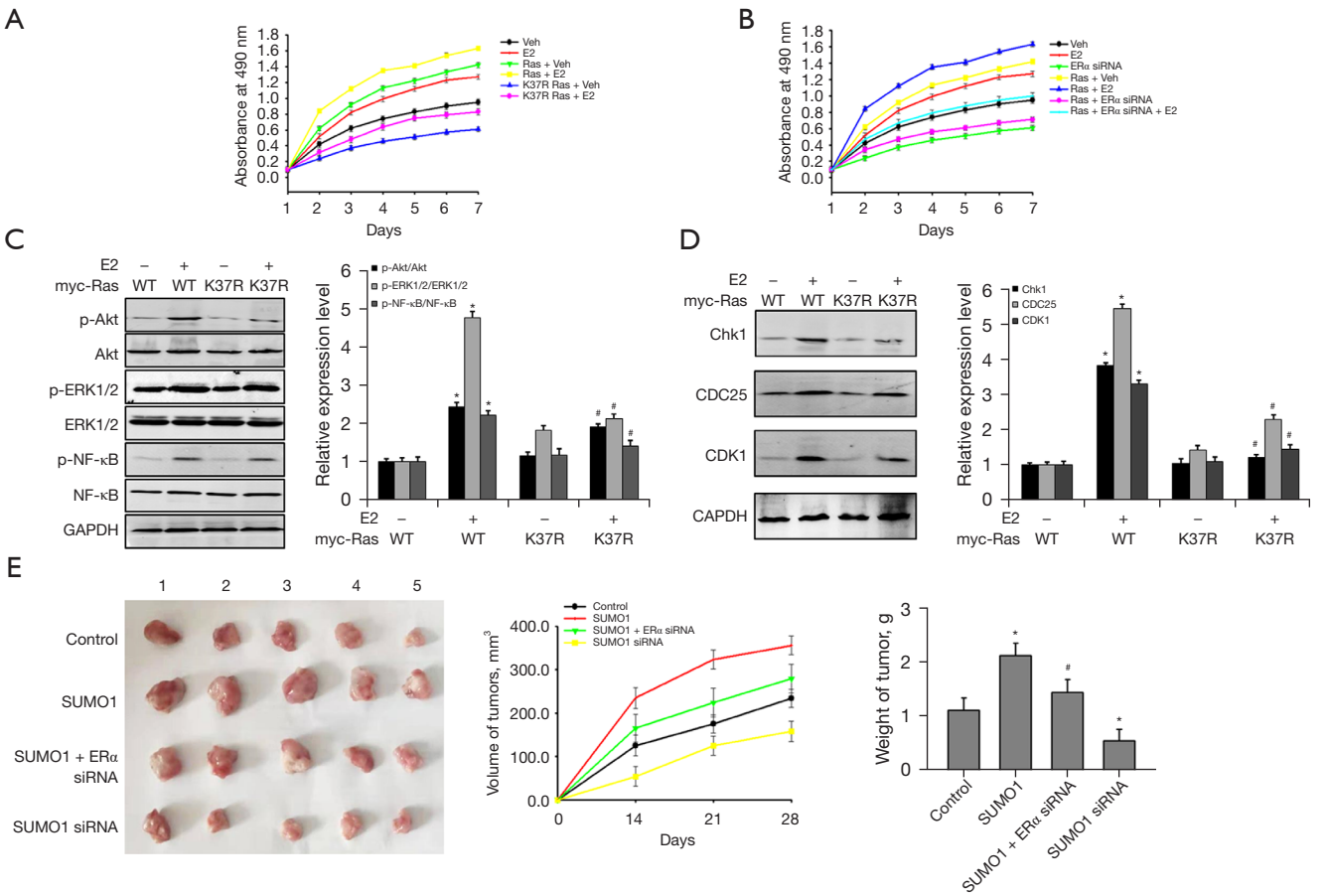


Figure 5 RAS SUMOylation promotes cellular proliferation in BC. (A) Cells were seeded in steroid-depleted medium for 2 days and transfected with WT or mutant RAS vector, followed by MTT assay. (B) Transfected cells were treated with E2 or siRNA ERα and then seeded in steroid-depleted medium for 7 days, followed by MTT assay. (C,D) MCF-7 cells transfected with SUMOylation site-mutant Ras for evaluation of the expression and activation of the cognate signaling pathway by western blot analysis. *, P<0.05 compared with untreated WT group; and #, P<0.05 compared with untreated K37R group. (E) Control, SUMO1-expressing, or SUMO1-knockdown cells (2×10^6) were injected into the right flanks of nude mice, and the volume and weight of tumors were ascertained after transplantation. *, P<0.05 compared with control group; and #, P<0.05 compared with SUMO1-overexpressing group. BC, breast cancer; WT, wide type; ERα, estrogen receptor alpha.

SUMO1-mediated protein SUMOylation associated with cellular proliferation in BC cells

Although investigators have determined that Ras occupies a critical role in cellular proliferation, whether its SUMOylation mediated by *SUMO1* is involved in this process remains unknown. Because Ras overexpression in MCF7 cells increased their proliferation rate, we transfected mutant Ras into the cells to further ascertain the role of SUMOylation in the process, and observed that the proliferation induced by Ras was reversed when SUMOylation sites were mutated (*Figure 5A*). This finding indicated that Ras SUMOylation was essential to cell proliferation. When cells were treated with E2 or underwent ERα knockdown, Ras-mediated cell proliferation was enhanced in cells pretreated with E2, but was circumvented with ERα knockdown in the presence of E2 (*Figure 5A,5B*). We also evaluated signal transduction-pathway activation in these cells, and demonstrated that activation of *RAS-mediated nuclear factor kappa B* and *CDK1* signaling was increased when cells were incubated with E2, and that it was further enhanced when the SUMOylation sites were mutated (*Figure 5C,5D*). To gain further insights into the ERα induction of SUMO1-mediated protein

SUMOylation in BC, we examined tumor growth in BC *in vivo*. We found that the MCF-7 line that stably expressed *SUMO1* grew more rapidly, that the cell line that stably expressed *SUMO1* siRNA grew more sluggishly, and that SUMO1-induced tumor growth was suppressed with ERα knockdown (*Figure 5E*), findings that indicated that E2-ERα enhanced cellular proliferation via increased SUMO1-mediated RAS SUMOylation.

Discussion

Intriguingly, women who are chronically exposed to artificial light at night or are engaged in work that may change their circadian or menstrual rhythms, are more likely to develop BC, with E2 known to be a prominent factor in the effect (25), and numerous reports have shown that the tumorigenesis of BC is related to signaling pathways such as protein SUMOylation (26). It is therefore of paramount importance to analyze the signal transduction of estrogen, which is crucial to several cellular processes and to the pathogenesis of BC (27). In the present study, we found that E2-ERα activated *SUMO1* expression at the transcriptional level, and that ERα-induced *SUMO1* expression was involved in the proliferation of BC cells. In addition, *SUMO1* can bind to Ras, inducing subsequent protein SUMOylation and BC development. Collectively, our findings indicated that *SUMO1* was the target of ERα, and that protein SUMOylation regulated BC development (Figure S6).

ERα-positive BC can be controlled by ERα modulators such as tamoxifen (28). However, acquired resistance to tamoxifen is common, making it an important clinical issue in BC treatment. Understanding the dysregulation of ERα signals will help develop new strategies for treating cancer patients. A previous study has shown that the ubiquitin proteasome system (UPS) is involved in the regulation of ERα stability (29). E3 ubiquitin ligases induce 26S proteasome mediated ERα degradation by increasing polyubiquitin to ERα lysine residues (30). However, further research is needed to explore the exact mechanism of ERα dysfunction. We demonstrated that a higher proportion of ERα-positive BC samples possessed higher SUMO1 protein levels, indicating that *SUMO1* transcription may be upregulated in ERα-positive tumors (31,32). Owing to the ambiguity in tumor grades recorded for the tissue samples, a correlation between tumor grade and *SUMO1* expression could not be established. Timing of tumor resection may be key to studying the circadian rhythm that influences SUMO1 protein actions, but this endpoint was not fully recorded in our investigation (33). We concluded that the MCF-7 and T47D cell lines exhibited SUMO1 mRNA and protein levels that were specifically affected by ERα (34). The differences in E2 response between MDA-MB-231 (ERβ-positive/ERα-negative cells) and T47D cells (ERα-positive/ERβ-negative cells) revealed that ERβ may wield a less potent regulatory effect on the *SUMO1* response to E2 than to ERα (35), and this was confirmed by the absence of changes in SUMO1 protein levels in T47D cells that overexpressed ERβ (36). These results were also consistent with the experimental data we collected using a reporter gene. ICI and E2 competed for ERα binding, promoting ERα degradation, and thus disrupted its location in the cell nucleus and subsequent dimerization.

Ras is a major oncogene in the mammalian *Ras* gene family, encoding a protein (*RAS*) that belongs to the small-GTPase superfamily, and plays an essential role in tumorigenesis (37-39). In this study, we found that Ras can complex with *SUMO1* and promote cellular proliferation via enhanced SUMOylation, and we additionally identified the SUMOylation site of the two proteins. SUMOylation is essential in the development of BC, and several oncogenes have been identified in the SUMOylation process (40). Our findings have thus critically supplemented the available information regarding SUMO1-induced protein SUMOylation, and elucidated its effect on cellular proliferation in BC.

Conclusions

In conclusion, our findings reinforce the concept that ERα-induced *SUMO1* expression is vital to the regulation of BC proliferation; and that targeting ERα-SUMO1 to attenuate protein SUMOylation may be a novel therapeutic inhibitor of BC development.

Acknowledgments

Funding: This work was supported by grants from the Postgraduate Research & Practice Innovation Program of Jiangsu Province to NZ (No. KYCX21_3110), the Research Project of Maternal and Child Health of Jiangsu Province to ZH (No. F201953), and the Science and Technology Project of Nantong to ZH and SF (Nos. JC2020067, MS2021032, H2019049, and MS2022018).

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://gs.amegroups.com/article/view/10.21037/gs-23-39/rc

Data Sharing Statement: Available at https://gs.amegroups.com/article/view/10.21037/gs-23-39/dss

Peer Review File: Available at https://gs.amegroups.com/article/view/10.21037/gs-23-39/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://gs.amegroups.com/article/view/10.21037/gs-23-39/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the institutional ethics committee of the Affiliated Hospital of Nantong University (ID: 2023-L084). Informed consent was obtained from each participant. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The animal research protocol was approved by the Animal Ethics Committee of Nantong University (ID: P20230222-001), in compliance with institutional guidelines for the care and use of animals.

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Cite this article as: Wang Q, Zhang N, Yang X, Feng S, Wang F, Zhang W, He Z. ERα promotes *SUMO1* transcription by binding with the ERE and enhances SUMO1-mediated protein SUMOylation in breast cancer. Gland Surg 2023;12(7):963-973. doi: 10.21037/gs-23-39