



TonEBP regulates *ESR1* transcription and ER-dependent proliferation in ER-positive breast cancer

Hyun Je Kang^{a,b,1}, Hana Song^{c,1}, Hyun Park^{a,1} , Hye-Kyung Park^d, Go Woon Shin^a,
Keoung Sun Son^a, Yeseul Jeong^c, Seung Mi Ko^c, Dougu Nam^a, Hyug Moo Kwon^e,
Soo Youn Choi^{c,f,g,*} 

^a Department of Biological Sciences, UNIST, Ulsan, Republic of Korea

^b Department of Chemical & Systems Biology, Stanford University, Stanford, CA, USA

^c Department of Biology, Jeju National University, Jeju, Republic of Korea

^d Research Institute for Basic Science, Jeju National University, Jeju, Republic of Korea

^e Graduate School of Health Science and Technology, UNIST, Ulsan, Republic of Korea

^f Center for Nutrition-Disease Research & Intervention system, Jeju National University, Jeju, Republic of Korea

^g Interdisciplinary Graduate Program in Advanced Convergence Technology & Science, Jeju National University, Jeju, Republic of Korea

ABSTRACT

Estrogen receptor α (ER α), encoded by *ESR1*, is a central determinant of luminal breast cancer growth, yet the mechanisms sustaining basal *ESR1* transcription remain incompletely defined. Here we identify tonicity-responsive enhancer-binding protein (TonEBP) as a promoter-associated regulator of *ESR1* transcription and ER-dependent proliferation in ER-positive breast cancer. Elevated TonEBP expression correlated with adverse clinical outcomes across independent patient cohorts. In ER-positive cells, TonEBP depletion impaired proliferation, induced G1-phase accumulation, and attenuated ER signaling. A functional TonEBP-binding motif (TonE) was identified within the proximal *ESR1* promoter; its mutation reduced promoter activity, and chromatin immunoprecipitation confirmed TonEBP enrichment at the endogenous locus. Consistently, TonEBP knockdown decreased *ESR1* expression, ER α abundance, and estrogen response element-dependent transcription, while enhancing sensitivity to tamoxifen and doxorubicin.

These findings support a model in which TonEBP sustains ER α expression through promoter-level regulation of *ESR1*, linking stress-responsive transcriptional control to hormone-dependent growth in luminal breast cancer.

1. Introduction

Breast cancer is the most frequently diagnosed malignancy and a leading cause of cancer-related death among women worldwide [1,2]. Approximately 70% of cases are classified as estrogen receptor-positive (ER⁺), in which tumor growth is driven by estrogen receptor α (ER α)-dependent transcriptional programs that regulate proliferation and survival [3,4]. Endocrine therapies targeting ER α signaling, including selective estrogen receptor modulators and aromatase inhibitors, constitute the standard treatment for ER⁺ breast cancer and have substantially improved patient outcomes [5]. However, a significant proportion of patients eventually develop recurrence or progression, indicating that key regulatory mechanisms governing estrogen responsiveness remain incompletely defined [6,7].

ER α expression is a critical determinant of estrogen sensitivity and therapeutic response in ER⁺ tumors. Altered ER α abundance or activity

is frequently associated with aggressive disease and endocrine resistance [4,5]. Although transcriptional and epigenetic regulation of *ESR1*, the gene encoding ER α , has been extensively studied, the upstream transcription factors required to sustain basal *ESR1* expression in breast cancer cells are not fully characterized [8].

Tonicity-responsive enhancer-binding protein (TonEBP), also known as nuclear factor of activated T cells 5 (NFAT5), is a Rel family transcription factor originally identified as a mediator of cellular adaptation to hyperosmotic stress [9]. Subsequent studies have demonstrated broader roles for TonEBP in inflammatory signaling, immune regulation, and metabolic adaptation through the control of gene expression programs governing survival and tissue remodeling [10–12]. In cancer, TonEBP has been implicated in tumor progression and poor prognosis across multiple malignancies, although its functions appear context dependent [13,14]. In breast cancer, accumulating evidence supports its role in aggressive tumor behavior. Network-based analyses have

* Corresponding author. Department of Biology, Jeju National University, Jeju, Republic of Korea.

E-mail address: sychoi@jeju.ac.kr (S.Y. Choi).

¹ Hyun Je Kang, Hana Song, and Hyun Park are co-first authors.

identified TonEBP as a master regulator in inflammatory breast cancer, where increased expression and nuclear localization correlate with highly proliferative and invasive phenotypes [15]. Elevated TonEBP expression has also been associated with reduced responsiveness to neoadjuvant chemotherapy, suggesting a contribution to therapeutic resistance and adaptation to microenvironmental stress [16]. Collectively, these observations establish TonEBP as a multifunctional transcriptional regulator that supports invasion, metastasis, and tumor survival. However, its potential role in modulating hormone receptor signaling in ER⁺ breast cancer remains largely unexplored.

Here, we investigated the clinical significance and functional role of TonEBP in ER⁺ breast cancer. We demonstrate that elevated TonEBP expression is associated with adverse clinical outcomes and that TonEBP promotes proliferation of ER⁺ breast cancer cells. Mechanistically, TonEBP enhances *ESR1* transcription and ER α protein expression, at least in part through a TonEBP-responsive element within the proximal *ESR1* promoter. These findings identify TonEBP as an upstream regulator of ER α and provide a mechanistic link between stress-responsive transcriptional control and estrogen-dependent tumor growth.

2. Materials and methods

2.1. Bioinformatic analysis

Breast cancer transcriptomic datasets were obtained from GEO and TCGA-associated cohorts. Survival analyses were performed using GSE9195 (chemotherapy-treated, $n = 77$) and GSE42568 (untreated, $n = 104$). Patients were stratified by median TonEBP (NFAT5) expression within each dataset. Kaplan–Meier curves were generated for distant metastasis-free survival or overall survival. Statistical significance was assessed using the log-rank test, and hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated where applicable.

2.2. Cell culture and siRNA transfection

MCF-7 and MDA-MB-231 cells (ATCC) were maintained in DMEM containing 10% FBS and antibiotics at 37 °C with 5% CO₂. For estrogen-response experiments, cells were cultured in phenol red-free DMEM supplemented with 5% charcoal/dextran-stripped FBS for ≥ 48 h prior to stimulation. TonEBP-targeting siRNAs and non-targeting control siRNA were transfected using Lipofectamine RNAiMAX (Invitrogen). Two independent siRNAs were used. Cells were analyzed 24–96 h post-transfection. Knockdown efficiency was confirmed by immunoblotting and qRT-PCR.

2.3. Proliferation and cytotoxicity assays

Cell viability was measured using a colorimetric assay, and cell numbers were determined by direct counting. For hormone stimulation, cells were treated with estrone (E1) or 17 β -estradiol (E2). For drug-response assays, doxorubicin or tamoxifen was applied after siRNA transfection. Cytotoxicity was assessed by lactate dehydrogenase (LDH) release, with total LDH determined following Triton X-100 lysis. DNA synthesis was quantified by EdU incorporation. Cell cycle distribution was analyzed by flow cytometry after ethanol fixation, RNase A treatment, and propidium iodide staining. Experiments were performed with at least three biological replicates.

2.4. Immunoblotting and qRT-PCR

Whole-cell lysates were prepared in RIPA buffer with protease inhibitors. Proteins were separated by SDS-PAGE, transferred to PVDF membranes, and probed with antibodies against TonEBP, ER α , and Hsc70. Signals were detected by chemiluminescence. Total RNA was extracted using TRIzol (Invitrogen), reverse transcribed, and analyzed by quantitative RT-PCR using SYBR Green. Gene expression was

normalized to ACTB using the 2^{- $\Delta\Delta$ Ct} method. Primer sequences are provided in [Supplementary Table 1](#).

2.5. Luciferase reporter assay

Human *ESR1* promoter fragments (–72/+297 and –44/+297) were cloned upstream of a firefly luciferase reporter. TonE mutations were introduced by site-directed mutagenesis. Cells were co-transfected with firefly constructs and a Renilla control plasmid. Luciferase activity was measured 24–48 h post-transfection using a dual-luciferase assay (Promega) and normalized to Renilla activity.

2.6. Chromatin immunoprecipitation (ChIP)-qPCR

ChIP assays were performed using a commercial kit (Millipore). Cells were crosslinked with 1% formaldehyde, lysed, and sonicated (Bioruptor KRB-01) to generate 400–1000 bp DNA fragments. Immunoprecipitation was conducted overnight at 4 °C using anti-TonEBP antibody or normal rabbit IgG control. DNA was purified (QIAquick PCR Purification Kit, QIAGEN) and analyzed by qPCR. Primer sequences are listed in [Supplementary Table 1](#).

2.7. Statistical analysis

Data are presented as mean \pm SD. Two-group comparisons were performed using unpaired two-tailed Student's t-test. Multiple-group comparisons were analyzed by two-way ANOVA followed by Tukey's post hoc test. Kaplan–Meier differences were assessed using the log-rank test. A p value < 0.05 was considered statistically significant.

3. Results

3.1. TonEBP expression is associated with poor clinical outcomes in breast cancer

To assess the clinical relevance of TonEBP expression in breast cancer, we analyzed publicly available transcriptomic datasets derived from TCGA-associated microarray cohorts and performed Kaplan–Meier survival analyses. Patients were stratified into high and low TonEBP expression groups based on median expression levels. Across independent cohorts, high TonEBP expression was consistently associated with significantly worse clinical outcomes compared with low TonEBP expression ([Fig. 1A](#) and [B](#)). In a cohort of breast cancer patients who received systemic chemotherapy (GSE9195, $n = 77$), elevated TonEBP expression was associated with a marked reduction in distant metastasis-free survival (log-rank $P = 0.0059$) ([Fig. 1A](#)). This association persisted despite chemotherapy, suggesting that high TonEBP expression may contribute to metastatic progression and therapy resistance rather than merely reflecting treatment response. In an independent cohort of patients who did not receive chemotherapy (GSE42568, $n = 104$), high TonEBP expression correlated with significantly reduced overall survival (log-rank $P = 0.0113$) ([Fig. 1B](#)). The presence of this association in the absence of therapeutic intervention supports the notion that TonEBP expression is intrinsically linked to aggressive tumor biology.

These data identify TonEBP as a negative prognostic factor in breast cancer and raise the possibility that TonEBP-driven transcriptional programs promote tumor progression.

3.2. TonEBP depletion preferentially suppresses growth of ER-positive breast cancer cells

To determine whether the adverse clinical association of TonEBP reflects a functional role in tumor cell growth, we examined the effects of TonEBP depletion in breast cancer cell lines with distinct hormone receptor status. ER-positive MCF-7 and ER-negative MDA-MB-231 cells were transfected with TonEBP siRNA or scrambled control siRNA.

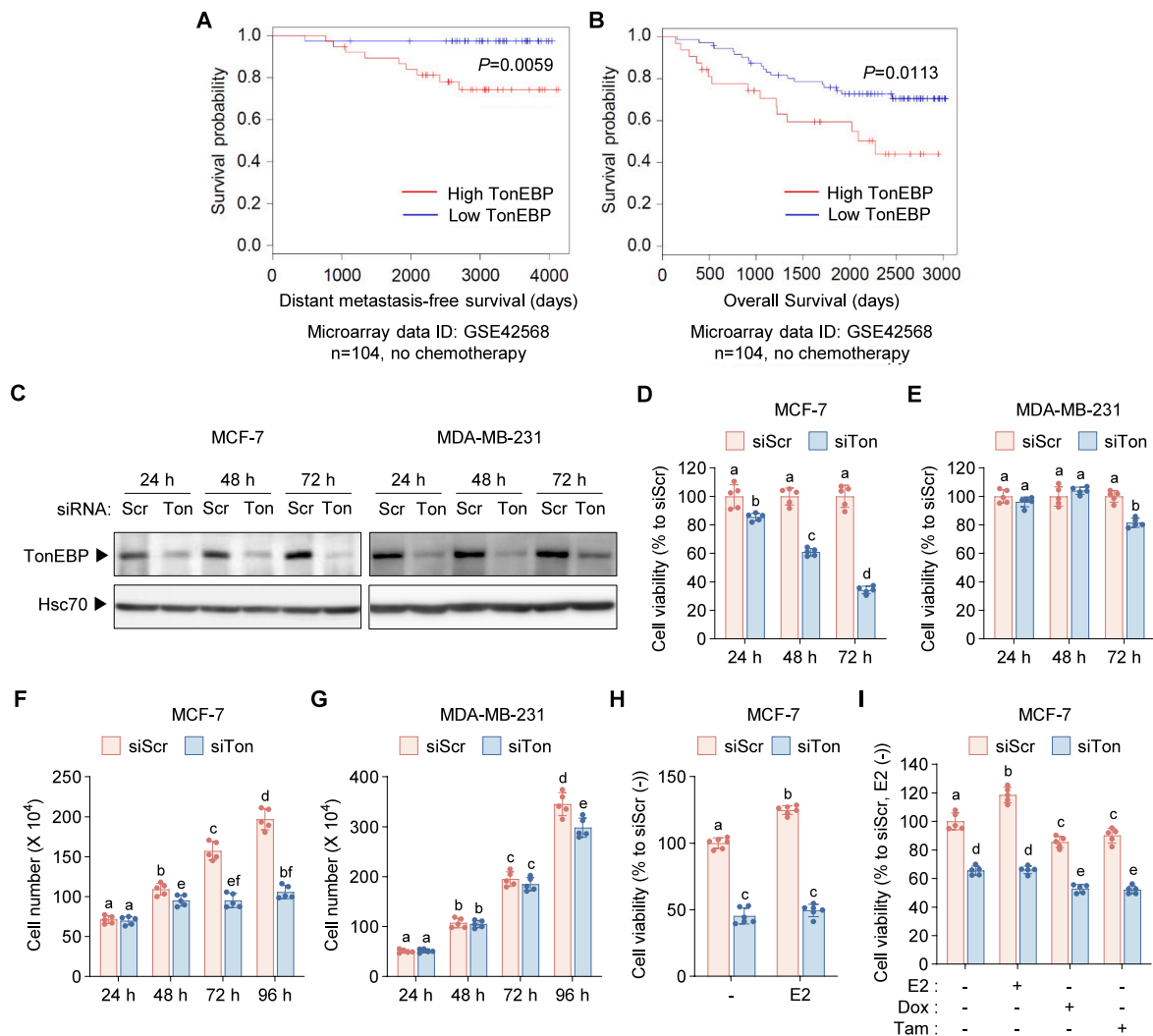


Fig. 1. TonEBP expression correlates with prognosis and regulates breast cancer cell growth

(A, B) Kaplan–Meier analyses of breast cancer cohorts stratified by median TonEBP expression. (A) Distant metastasis-free survival (DMFS) in chemotherapy-treated patients (GSE9195, $n = 77$). (B) Overall survival (OS) in untreated patients (GSE42568, $n = 104$). (C) Immunoblot of TonEBP in MCF-7 and MDA-MB-231 cells transfected with scrambled siRNA (siScr) or TonEBP siRNA (siTon). Hsc70, loading control. (D, E) Cell viability in MCF-7 (D) and MDA-MB-231 (E) cells after siScr or siTon transfection. (F, G) Cell number analysis in MCF-7 (F) and MDA-MB-231 (G). (H) Estrogen (E2)-stimulated proliferation in siScr- or siTon-transfected MCF-7 cells. (I) Viability of siScr- or siTon-transfected MCF-7 cells treated with E2 (0.1 μ M), doxorubicin (0.5 μ M), or tamoxifen (1 μ M). Data are mean \pm SD. Two-way ANOVA with Tukey's test; different letters indicate $p < 0.05$.

Immunoblot analysis confirmed efficient TonEBP knockdown in both cell lines (Fig. 1C). TonEBP depletion markedly reduced cell viability in MCF-7 cells (Fig. 1D), whereas only a modest effect was observed in MDA-MB-231 cells (Fig. 1E). Consistent with these findings, cell number analysis demonstrated a pronounced reduction in MCF-7 cell proliferation (Fig. 1F), while MDA-MB-231 cells exhibited delayed and less robust growth inhibition (Fig. 1G). These results indicate that TonEBP is preferentially required for proliferation of ER-positive breast cancer cells.

We next examined whether TonEBP is involved in estrogen-mediated proliferative signaling. In control MCF-7 cells, 17 β -estradiol (E2) significantly increased cell viability. This estrogen-induced growth stimulation was abolished in TonEBP-depleted cells (Fig. 1H), indicating that TonEBP is necessary for efficient ER-dependent proliferative signaling. To assess therapeutic implications, MCF-7 cells were treated with E2, doxorubicin, or tamoxifen following TonEBP knockdown. As expected, doxorubicin and tamoxifen reduced cell viability. TonEBP depletion further enhanced the inhibitory effects of both agents (Fig. 1I), suggesting that suppression of TonEBP sensitizes ER-positive breast

cancer cells to both cytotoxic chemotherapy and endocrine therapy.

These findings demonstrate that TonEBP promotes ER-dependent breast cancer cell growth, providing mechanistic support for its association with poor clinical outcomes.

3.3. TonEBP depletion suppresses proliferation without inducing cell death

TonEBP depletion markedly reduced MCF-7 cell numbers. To determine whether this reduction resulted from increased cell death or impaired proliferative capacity, MCF-7 cells were transfected with two independent siRNAs targeting distinct regions of the TonEBP transcript. Immunoblot analyses confirmed efficient and sustained suppression of TonEBP protein beginning at 24 h and persisting up to 48 h (Fig. 2A). MCF-7 cell numbers were significantly reduced from 48 h onward, with growth inhibition maintained through 96 h (Fig. 2B). LDH release assays were performed to distinguish between cytotoxicity and proliferation defects. Basal LDH release into the culture medium was comparable between scrambled control and TonEBP-depleted cells at both 24 h and

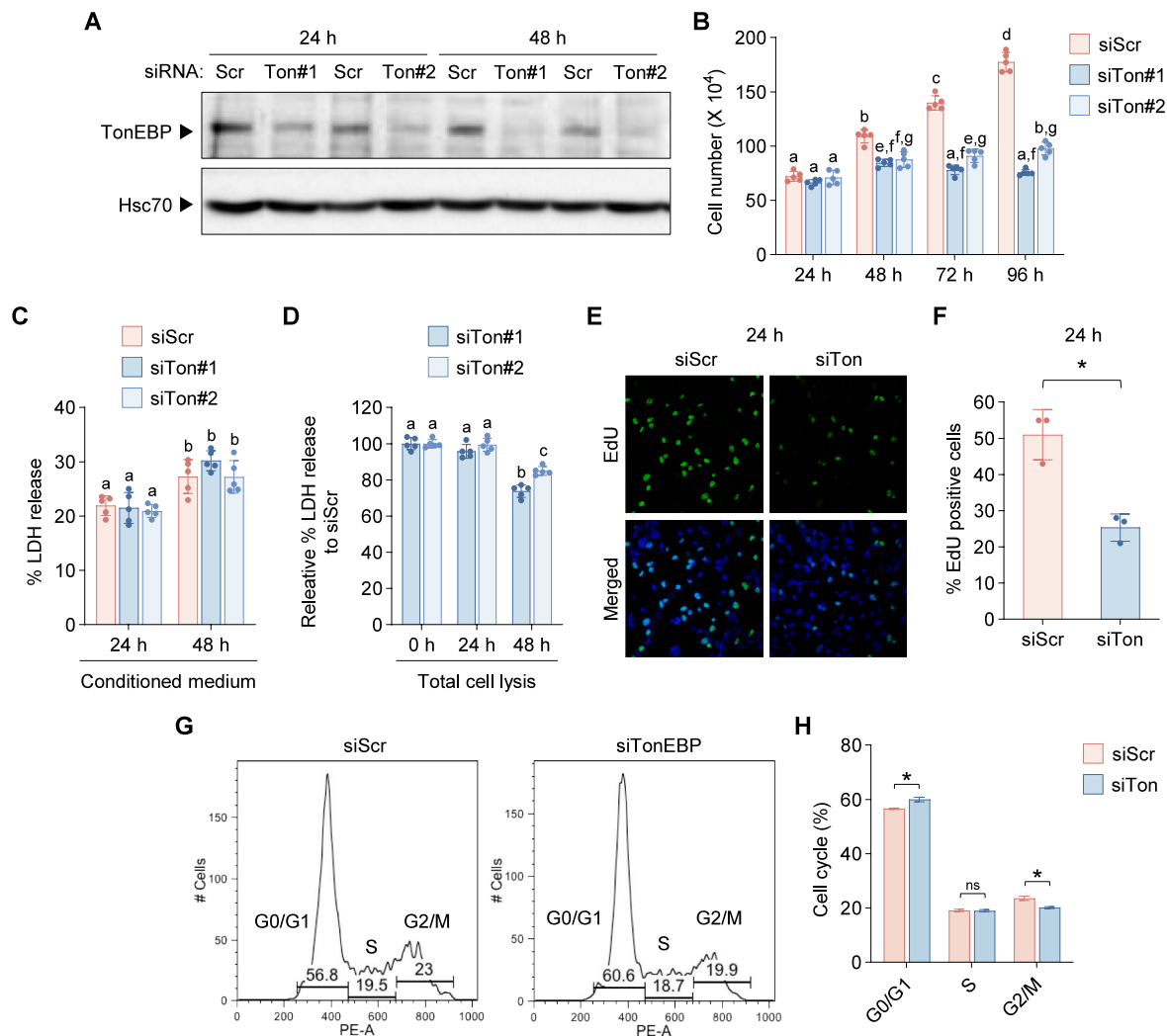


Fig. 2. TonEBP depletion affects proliferation and cell-cycle progression in MCF-7 cells

(A) Immunoblot analysis of TonEBP protein levels in MCF-7 cells transfected with scrambled siRNA (siScr) or two independent TonEBP-targeting siRNAs (siTon#1 and siTon#2). Hsc70 served as a loading control. (B) Cell number up to 96 h post-transfection. (C, D) Basal (C) and total (D) LDH release. (E, F) Representative fluorescence microscopy images of EdU incorporation (green) (E) and quantification of EdU-positive cells (F). (G, H) Cell-cycle profiles (PI staining) (G) and phase distribution (H). Data are mean \pm SD. Student's t-test or two-way ANOVA as appropriate; An asterisk (*) or different letters indicate statistically significant differences ($p < 0.05$). ns, not significant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

48 h (Fig. 2C), indicating no significant increase in cell death. Total LDH release following Triton X-100-mediated lysis was progressively reduced in TonEBP-depleted cells (Fig. 2D), reflecting a decrease in total viable cell mass rather than enhanced cytotoxicity.

Among the two siRNAs tested, TonEBP siRNA #1 was used for subsequent mechanistic studies. EdU incorporation demonstrated that TonEBP knockdown markedly reduced the proportion of EdU-positive cells (Fig. 2E and F), indicating impaired DNA replication and proliferative activity. Flow cytometric analysis at 48 h post-transfection showed accumulation of cells in the G1 phase, with no significant change in S phase and a corresponding decrease in the G2/M population (Fig. 2G and H). These findings indicate that TonEBP is required for efficient G1-to-S phase progression in ER-positive breast cancer cells. TonEBP depletion suppresses ER-positive breast cancer cell growth primarily by inducing G1 cell cycle arrest rather than triggering acute cytotoxicity.

3.4. TonEBP depletion suppresses *ESR1* transcription and disrupts ER-dependent proliferative signaling in MCF-7 cells

ER α signaling is a central driver of G1-S phase progression in ER-positive breast cancer cells. We therefore examined whether TonEBP

regulates ER α expression and ER-dependent transcriptional programs in MCF-7 cells. Immunoblot analysis revealed that siRNA-mediated depletion of TonEBP led to a marked reduction in ER α protein levels as early as 24 h post-transfection, which was sustained up to 48 h (Fig. 3A). To determine whether this reduction occurred at the transcriptional level, *ESR1* mRNA expression was measured by quantitative RT-PCR. TonEBP depletion significantly decreased *ESR1* mRNA levels at both 24 and 48 h (Fig. 3B), indicating that TonEBP contributes to *ESR1* transcriptional regulation rather than merely affecting ER α protein stability.

Consistent with reduced ER α expression, canonical ER target genes were significantly suppressed upon TonEBP knockdown. Expression of luminal ER-responsive markers, including *GREB1* and *PGR*, was decreased (Fig. 3C), demonstrating attenuation of ER transcriptional activity. TonEBP knockdown also significantly reduced expression of *CCND1* and *PCNA* (Fig. 3D), key mediators of G1 progression and DNA replication. In parallel, TonEBP depletion induced upregulation of the CDK inhibitor *CDKN1A* (p21) and the pro-apoptotic gene *PMAIP1* (NOXA) (Fig. 3E), suggesting activation of cell-cycle inhibitory and stress-responsive pathways. Together with the reduction of cyclin D1 expression, these data support a cytostatic mechanism involving

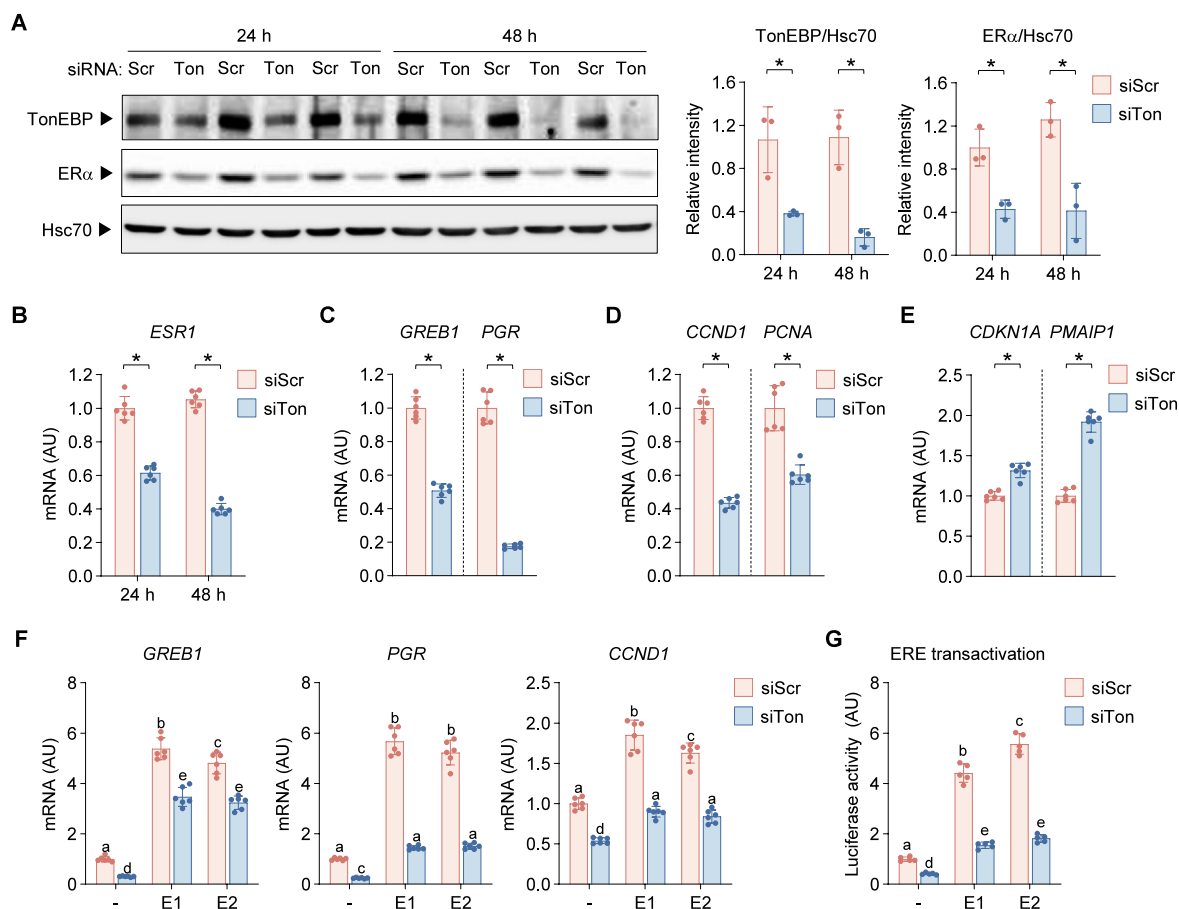


Fig. 3. TonEBP regulates *ESR1* expression and ER-dependent transcription

(A) Immunoblot analysis of TonEBP and ER α protein levels at 24 h and 48 h in MCF-7 cells transfected with scrambled siRNA (siScr) or TonEBP-targeting siRNA (siTon). Densitometric quantification of TonEBP/Hsc70 and ER α /Hsc70 ratios is shown. (B–E) qRT-PCR analysis of *ESR1* (B), *GREB1* and *PGR* (C), *CCND1* and *PCNA* (D), and *CDKN1A* and *PMAIP1* (E). (F) Estrogen-responsive gene expression in siScr- or siTon-transfected cells following treatment with estrone (E1, 10 nM) or estradiol (E2, 10 nM). (G) ERE-luciferase assay following E1 or E2 stimulation. Student's t-test or two-way ANOVA as appropriate; An asterisk (*) or different letters indicate statistically significant differences ($p < 0.05$). AU, arbitrary units.

reinforcement of G1 arrest rather than acute cytotoxicity, consistent with the cell cycle data shown in Fig. 2. To determine whether TonEBP is required for ligand-dependent ER activation, cells were treated with estrogen (E1 or E2). Estrogen stimulation robustly induced expression of ER target genes (*GREB1*, *PGR*) and *CCND1* in control cells (Fig. 3F). This ligand-dependent transcriptional induction was significantly attenuated in TonEBP-depleted cells, indicating impaired ER signaling capacity. Estrogen response element (ERE) luciferase reporter assays further demonstrated that TonEBP knockdown significantly reduced ER transactivation activity following E1 or E2 treatment (Fig. 3G). The decrease in ERE-driven luciferase activity provides direct functional evidence that TonEBP is required for efficient ER α -mediated transcriptional activation at canonical ERE sites.

These findings indicate that TonEBP supports both basal and ligand-induced ER-dependent transcription, thereby sustaining luminal gene expression programs and proliferative signaling in ER-positive breast cancer cells.

3.5. TonEBP directly activates the human *ESR1* promoter through a TonE element

TonEBP depletion significantly reduced *ESR1* mRNA expression (Fig. 3B). We therefore investigated whether TonEBP directly regulates *ESR1* transcription at the promoter level. In silico analysis of the human *ESR1* promoter identified a putative TonEBP-binding consensus sequence (TonE motif) at +47/+57 within the proximal promoter

region (Fig. 4A). Luciferase reporter constructs containing the -72/+297 or -44/+297 regions of the human *ESR1* promoter were generated (Fig. 4A). Site-directed mutagenesis was used to disrupt the TonE sequence (TonE-mt). In MCF-7 cells, wild-type *ESR1* promoter constructs exhibited robust transcriptional activity compared with the basic vector control (Fig. 4B). Mutation of the TonE site significantly reduced promoter activity in both -72/+297 and -44/+297 constructs, indicating that the TonE element is required for full promoter activation.

To determine whether TonEBP occupies the endogenous *ESR1* promoter, ChIP-qPCR assays were performed. TonEBP was enriched at the *ESR1* promoter region containing the TonE site in control cells, whereas this enrichment was markedly reduced upon TonEBP knockdown (Fig. 4C). Together with the reporter assays, these findings indicate that TonEBP associates with the proximal *ESR1* promoter and functionally contributes to transcriptional activation through a TonE-dependent mechanism.

These results support a model in which TonEBP acts as a transcriptional regulator of *ESR1*, linking TonEBP activity to ER α expression and ER-dependent proliferative signaling in ER-positive breast cancer cells.

4. Discussion

In this study, we identify TonEBP as a potential upstream regulator of *ESR1* transcription and ER-dependent proliferative signaling in ER-positive breast cancer. Our findings suggest that TonEBP contributes to the maintenance of ER α expression by enhancing activity of the

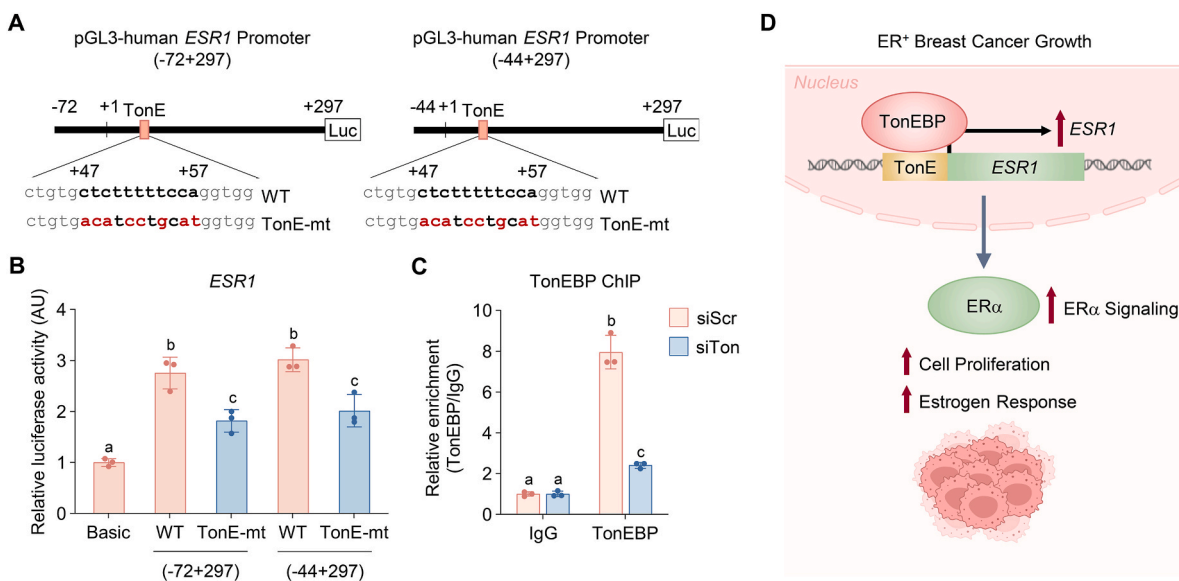


Fig. 4. TonEBP activates the human *ESR1* promoter via a TonE element

(A) Schematic of *ESR1* promoter luciferase constructs (-72/+297 and -44/+297) with TonE site and mutant (TonE-mt). (B) Luciferase activity in MCF-7 cells co-transfected with promoter constructs and siScr or siTon. (C) ChIP-qPCR analysis of TonEBP binding to the endogenous *ESR1* promoter. IgG was used as a negative control. Data are presented as relative enrichment normalized to input. (D) Proposed model of TonEBP-mediated regulation of *ESR1* and ER-dependent proliferation. Data are shown as mean \pm SD. Different letters indicate statistically significant differences ($p < 0.05$). AU, arbitrary units.

proximal *ESR1* promoter through a TonE element (Fig. 4D). These observations expand the spectrum of candidate *ESR1* regulators and implicate TonEBP in the transcriptional control of luminal breast cancer biology. Although ER α expression defines luminal tumors, the transcriptional mechanisms sustaining basal *ESR1* expression remain incompletely understood [8]. Established regulators such as *FOXA1*, *GATA3*, and AP-2 (*TFAP2C*) influence ER chromatin accessibility and transcriptional competence at promoters and enhancers [8,17–21]. Our data position TonEBP as an additional promoter-level regulator that associates with the proximal *ESR1* locus and enhances its transcriptional activity. The identification of a functional TonE element within the proximal promoter region further expands the repertoire of *cis*-regulatory mechanisms contributing to ER α expression.

Functionally, TonEBP depletion preferentially impaired proliferation of ER-positive cells and induced G1-phase accumulation, consistent with attenuation of ER α -driven cell-cycle progression. Suppression of ER target genes and reduced ERE transactivation activity indicate that TonEBP supports both basal and ligand-induced ER signaling. These findings support a model in which TonEBP sustains luminal growth programs through maintenance of the ER α -Cyclin D1 axis. At the same time, given the broader roles of TonEBP in stress adaptation and survival pathways in other tumor contexts [10,13,14], ER-independent mechanisms may also contribute to its growth-promoting effects.

Clinically, elevated TonEBP expression was associated with adverse outcomes across independent cohorts, including reduced distant metastasis-free survival and overall survival. The consistency of this association in both treated and untreated patients suggests that TonEBP reflects intrinsic tumor aggressiveness. In other malignancies, increased TonEBP expression has been linked to metastasis and therapy resistance, further supporting its relevance to aggressive disease biology [13]. The enhanced inhibitory effects observed when TonEBP suppression was combined with tamoxifen or doxorubicin suggest potential therapeutic implications. By sustaining *ESR1* transcription and ER signaling, TonEBP may contribute to maintenance of hormone-dependent growth under therapeutic pressure. Although selective pharmacologic inhibitors of TonEBP are not currently available, disruption of the TonEBP-ER α axis represents a conceptual strategy to modulate ER signaling in luminal breast cancer. Future work should explore feasible strategies to

disrupt the TonEBP-ER α axis, including small-molecule inhibitors, peptide mimetics, or oligonucleotide-based approaches, and evaluate their efficacy in preclinical models of endocrine-resistant breast cancer.

TonEBP is widely recognized as a stress-responsive transcription factor integrating osmotic, inflammatory, and metabolic signals [9,12,22]. Tumor microenvironments are characterized by hypoxia, oxidative stress, and cytokine exposure, conditions that may engage TonEBP-dependent transcriptional programs. Our findings raise the possibility that TonEBP links stress-responsive signaling to hormone-dependent oncogenesis by directly maintaining *ESR1* transcription under dynamic microenvironmental conditions. This study primarily utilized a well-characterized ER-positive model system, and further validation in additional cellular and *in vivo* contexts will strengthen the generalizability of these findings. Nonetheless, combined evidence from promoter mutagenesis, endogenous ChIP enrichment, and functional assays provides a coherent mechanistic framework for TonEBP-mediated regulation of *ESR1*.

In summary, TonEBP emerges as a promoter-associated regulator of *ESR1* transcription that sustains ER α expression and ER-dependent proliferation in luminal breast cancer. By directly linking a stress-responsive transcription factor to control of hormone receptor expression, this study defines a mechanistic axis with potential relevance to tumor progression and therapeutic response in ER-positive breast cancer.

Funding

This work was supported by the research grant of Jeju National University in 2024.

CRedit authorship contribution statement

Hyun Je Kang: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **Hana Song:** Data curation, Formal analysis, Investigation, Validation, Writing – original draft. **Hyun Park:** Data curation, Formal analysis, Investigation, Validation, Writing – original draft. **Hye-Kyung Park:** Investigation, Methodology. **Go Woon Shin:** Data curation, Investigation. **Keoung Sun Son:** Data

curation, Investigation. **Yeseul Jeong**: Investigation, Validation. **Seung Mi Ko**: Data curation, Investigation. **Dougu Nam**: Formal analysis. **Hyug Moo Kwon**: Resources. **Soo Youn Choi**: Conceptualization, Data curation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2026.153559>.

References

- [1] M. Arnold, E. Morgan, H. Rungay, et al., Current and future burden of breast cancer: global statistics for 2020 and 2040, *Breast* 66 (2022) 15–23, <https://doi.org/10.1016/j.breast.2022.08.010>.
- [2] J. Kim, A. Harper, V. McCormack, et al., Global patterns and trends in breast cancer incidence and mortality across 185 countries, *Nat. Med.* 31 (2025) 1154–1162, <https://doi.org/10.1038/s41591-025-03502-3>.
- [3] A.G. Waks, E.P. Winer, Breast cancer treatment: a review, *JAMA* 321 (2019) 288–300, <https://doi.org/10.1001/jama.2018.19323>.
- [4] H.J. Burstein, G. Curigliano, B. Thürlimann, et al., Customizing local and systemic therapies for women with early breast cancer: the St. Gallen international consensus guidelines for treatment of early breast cancer 2021, *Ann. Oncol.* 32 (2021) 1216–1235, <https://doi.org/10.1016/j.annonc.2021.06.023>.
- [5] H.J. Burstein, Systemic therapy for estrogen receptor-positive, HER2-negative breast cancer, *N. Engl. J. Med.* 383 (2020) 2557–2570, <https://doi.org/10.1056/NEJMr1307118>.
- [6] A. Ghosh, R. Chaubal, C. Das, et al., Genomic hallmarks of endocrine therapy resistance in ER/PR+HER2- breast tumours, *Commun. Biol.* 8 (2025) 207, <https://doi.org/10.1038/s42003-025-07606-x>.
- [7] H.J. Burstein, C. Lacchetti, H. Anderson, et al., Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: ASCO Clinical practice guideline focused update, *J. Clin. Oncol.* 37 (2019) 423–438, <https://doi.org/10.1200/JCO.18.01160>.
- [8] D.K. Lung, R.M. Reese, E.T. Alarid, Intrinsic and extrinsic factors governing the transcriptional regulation of ESR1, *Horm. Cancer* 11 (2020) 129–147, <https://doi.org/10.1007/s12672-020-00388-0>.
- [9] H. Miyakawa, S.K. Woo, S.C. Dahl, et al., Tonicity-responsive enhancer binding protein, a rel-like protein that stimulates transcription in response to hypertonicity, *Proc. Natl. Acad. Sci. USA.* 96 (1999) 2538–2542, <https://doi.org/10.1073/pnas.96.5.2538>.
- [10] S.Y. Choi, W. Lee-Kwon, H.M. Kwon, The evolving role of TonEBP as an immunometabolic stress protein, *Nat. Rev. Nephrol.* 16 (2020) 352–364, <https://doi.org/10.1038/s41581-020-0261-1>.
- [11] H.H. Lee, S.M. An, B.J. Ye, et al., TonEBP/NFAT5 promotes obesity and insulin resistance by epigenetic suppression of white adipose tissue beiging, *Nat. Commun.* 10 (2019) 3536, <https://doi.org/10.1038/s41467-019-11302-w>.
- [12] J.H. Lee, H. Song, E.J. Yoo, et al., Hepatocyte TonEBP promotes metabolic stress-induced hepatic fibroinflammation involving transcriptional activation of ELR⁺ CXC chemokines, *Cell Death Discov.* (2026), <https://doi.org/10.1038/s41420-026-02978-3>.
- [13] J.H. Lee, J.H. Suh, S.Y. Choi, et al., Tonicity-responsive enhancer-binding protein promotes hepatocellular carcinogenesis, recurrence and metastasis, *Gut* 68 (2019) 347–358, <https://doi.org/10.1136/gutjnl-2017-315348>.
- [14] S.Y. Choi, The roles of TonEBP in the DNA damage response: from DNA damage bypass to R-loop resolution, *DNA Repair* 140 (2024) 103697, <https://doi.org/10.1016/j.dnarep.2024.103697>.
- [15] A. Remo, I. Simeone, M. Pancione, et al., Systems biology analysis reveals NFAT5 as a novel biomarker and master regulator of inflammatory breast cancer, *J. Transl. Med.* 13 (2015) 138, <https://doi.org/10.1186/s12967-015-0492-2>.
- [16] M. Ayers, W.F. Symmans, J. Stec, et al., Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer, *J. Clin. Oncol.* 22 (2004) 2284–2293, <https://doi.org/10.1200/JCO.2004.05.166>.
- [17] J. Eeckhoutte, E.K. Keeton, M. Lupien, et al., Positive cross-regulatory loop ties GATA-3 to estrogen receptor alpha expression in breast cancer, *Cancer Res.* 67 (2007) 6477–6483, <https://doi.org/10.1158/0008-5472.CAN-07-0746>.
- [18] G.L. Powers, P. Rajbhandari, N.M. Solodin, et al., The proteasome inhibitor bortezomib induces an inhibitory chromatin environment at a distal enhancer of the estrogen receptor- α gene, *PLoS One* 8 (2013) e81110, <https://doi.org/10.1371/journal.pone.0081110>.
- [19] G.M. Bernardo, K.L. Lozada, J.D. Miedler, et al., FOXA1 is an essential determinant of ERalpha expression and mammary ductal morphogenesis, *Development* 137 (2010) 2045–2054, <https://doi.org/10.1242/dev.043299>.
- [20] L.A. McPherson, R.J. Weigel, AP2alpha and AP2gamma: a comparison of binding site specificity and trans-activation of the estrogen receptor promoter and single site promoter constructs, *Nucleic Acids Res.* 27 (1999) 4040–4049, <https://doi.org/10.1093/nar/27.20.4040>.
- [21] V. Theodorou, R. Stark, S. Menon, et al., GATA3 acts upstream of FOXA1 in mediating ESR1 binding by shaping enhancer accessibility, *Genome Res.* 23 (2013) 12–22, <https://doi.org/10.1101/gr.139469.112>.
- [22] H.H. Lee, G.W. Jeong, B.J. Ye, et al., TonEBP in myeloid cells promotes obesity-induced insulin resistance and inflammation through adipose tissue remodeling, *Diabetes* 71 (2022) 2557–2571, <https://doi.org/10.2337/db21-1099>.