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Zinc finger protein 131 inhibits estrogen signaling by suppressing estrogen receptor α homo-dimerization

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ABSTRACT

Steroid hormone estrogen elicits various physiological functions, many of which are mediated through two structurally and functionally distinct estrogen receptors, ERα and ERβ. The functional role of zinc finger protein 131 (ZNF131) is poorly understood, but it is assumed to possess transcriptional regulation activity due to the presence of a DNA binding motif. A few recent reports, including ours, revealed that ZNF131 acts as a negative regulator of ERα and that SUMO modification potentiates the negative effect of ZNF131 on estrogen signaling. However, its molecular mechanism for ERα inhibition has not been elucidated in detail. Here, we demonstrate that ZNF131 directly interacts with ERα, which consequently inhibits ERα-mediated *trans*-activation by suppressing its homo-dimerization. Moreover, we show that the C-terminal region of ZNF131 containing the SUMOylation site is necessary for its inhibition of estrogen signaling. Taken together, these data suggest that ZNF131 inhibits estrogen signaling by acting as an ERα-co-repressor.

1. Introduction

Estrogen regulates various physiological responses throughout the body [1,2], and its altered signaling contributes to the occurrence and progression of breast cancer [3]. Like all steroid hormones, estrogens diffuse readily across the cell membrane. Once inside the cell, they bind and activate estrogen receptors, which in turn stimulate target gene expression. There are two forms of the estrogen receptor, referred to as ERα and ERβ, each encoded by a separate gene. Although both ERs are widely expressed in different tissue types, ERα predominantly regulates the activity of genes involved in development, reproduction, differentiation, and transformation [4]. Conversely, ERβ has both overlapping and distinct functions from ERα [5,6]. Moreover, ERα is closely associated with the promotion of breast cancer [7].

Estrogen signaling is typically initiated by its binding to ERα. Upon ligand binding, ERα undergoes an activating conformational change that promotes its homo-dimerization; however, in some cell types in which the two forms are co-expressed, ERαβ heterodimers are formed. Homo-dimerization allows the receptor to bind DNA at the estrogen response element (ERE), which is usually located within the promoter of estrogen-responsive genes. Finally, the estrogen–ERα–ERE complex initiates gene transcription related to estrogen signaling [6]. However, some critical reports revealed that ERα homo-dimerization also occurs in the absence of ligand [8,9]. Therefore, ERα homo-dimerization likely occurs in the presence or absence of ligand.

ERα interacts with a variety of cofactors that modify ER action either by enhancing (coactivators) or inhibiting (corepressors) target gene transcription [6]. Compared with co-activators, ERα co-repressors counterbalance estrogen-induced *trans*-activation and represent a potential tumor suppression mode for cells [7]. N-CoR [10] and SMRT [11], the two best-characterized ERα co-repressors, exert their function by recruiting different histone deacetylase (HDAC) protein complexes. In addition to those well-established HDAC-related co-repressors, various other ERα co-repressors, such as TR2 [12], SHP [13], and LMO4 [14], have also been reported.

Zinc finger protein 131 (ZNF131), a member of the zinc finger protein superfamily, was initially identified based on its association with developmental disorders [15]. The ZNF131 gene encodes a 589-amino acid protein, which contains an N-terminal BTB/POZ domain, two nuclear localization signal (NLS) domains, and five zinc finger domains [15–17]. Several zinc finger proteins interact with components of the HDAC co-repressor complexes, including N-CoR and SMRT, via the BTB/POZ domain, and subsequently mediate transcriptional repression [18].

Abbreviations: BTB/POZ, broad-complex, tramtrack, and bric-à-brac/poxvirus and zinc finger; ERα, estrogen receptor α; E2, 17β-estradiol; ERE, estrogen response element; HDAC, histone deacetylase; NLS, nuclear localization signal; SUMO, small ubiquitin-like modifier; ZNF131, zinc finger protein 131.

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Using a high-throughput screening platform created by linking the ERE with a reporter gene, ZNF131 was recently reported to suppress ERα target gene expression [19]. This finding was further supported by our previous report showing that ZNF131 acts as a negative regulator of ERα [20]. Moreover, we demonstrated that small ubiquitin-like modifier (SUMO)-modification potentiates the negative effect of ZNF131 on estrogen signaling [20]. Nevertheless, the detailed molecular and regulatory mechanisms of ZNF131 for ERα inhibition have not been elucidated. In the present study, we identify ZNF131 as a novel ERα binding partner. In addition, we propose that ZNF131 down-regulates estrogen signaling by suppressing ERα homo-dimerization.

2. Materials and methods

2.1. Materials

17β-Estradiol (E2) and the rabbit polyclonal actin antibody were purchased from Sigma–Aldrich. The mouse monoclonal HA antibody was purchased from Covance. Rabbit polyclonal HA, GFP, and mouse monoclonal ERα and GFP antibodies were purchased from Santa Cruz Biotechnology. The rabbit polyclonal V5 antibody was purchased from Abcam. The mouse polyclonal ZNF131 antibody was purchased from Abnova. The HRP-conjugated anti-mouse antibody was purchased from Thermo Scientific. Mouse immunoglobulin G was purchased from Upstate. Mouse monoclonal V5 and HRP-conjugated anti-rabbit antibodies, DMEM, FBS, and LipofectAMINE PLUS were purchased from Invitrogen. Protein A-Sepharose was purchased from Amersham Pharmacia Biotech. All other chemicals were purchased from Sigma–Aldrich.

2.2. DNA constructions

Human ZNF131 isoform 2 (accession number NM_003432) cDNA from HEK293 cells was PCR-amplified and subcloned into modified pRK5-HA (Stratagene) to generate HA-tagged ZNF131 (HA-ZNF131). ZNF131 mutants lacking the first NLS motif (ZNF131-ΔNLS-1) or zinc finger domain (ZNF131-ΔZF1), or with deletion of the C-terminal 177 ($ZNF131^{1-412}$) or 339 amino acids ($ZNF131^{1-250}$), were constructed using PCR. Human ERα (accession number NM_000125) cDNA from MCF-7 cells was PCR-amplified and subcloned into modified pRK5-V5 or pEGFP-C2 (Clontech) to generate V5-tagged ERα (V5-ERα) or GFP-tagged ERα (GFP-ERα), respectively. All cDNA sequences were verified by DNA sequencing (COSMO Genetech). The reporter plasmid pTA-4xERE-Luc was constructed as described previously [20].

2.3. Cell culture and DNA transfection

HEK293 cells were maintained in DMEM supplemented with 10% FBS and 100 U/mL penicillin–streptomycin (Invitrogen). The human breast cancer cell line MCF-7 was maintained in DMEM supplemented with 10% FBS, 100 U/mL penicillin–streptomycin, and 10 μg/mL insulin (Sigma–Aldrich). Twenty-four hours before transfection of these cells, the media was changed to phenol red-free DMEM (HyClone) containing 5% charcoal-stripped FBS (Sigma–Aldrich). DNA transfection was performed using either LipofectAMINE PLUS (Invitrogen) or polyethylenimine (Sigma–Aldrich), according to the manufacturer's instructions.

2.4. Co-immunoprecipitation assays and Western blot analyses

Cells were washed with ice-cold PBS, and lysed in lysis buffer (10 mM Tris, pH 7.4, 1% Nonidet P-40, 150 mM NaCl, 10% glycerol, 20 mM N-ethylmaleimide, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 2 μg/mL leupeptin, 2 μg/mL aprotinin, 5 mM NaF, and 0.5 mM phenylmethylsulfonyl fluoride) supplemented with 0.1% SDS. After incubation for 15 min at 4 °C, the cells were sonicated, and the lysates were clarified by centrifugation at 14,000×*g* for 15 min at 4 °C. For co-immunoprecipitation assays, 250 μg of protein lysate was incubated overnight at 4 °C with 0.5 μg of the indicated antibody with gentle rotation. The mixtures were incubated for 2 h at 4 °C with 30 μL of a 1:1 Protein A-Sepharose bead suspension, and pelleted by centrifugation. The pellets were washed four times with lysis buffer, resuspended in SDS–PAGE sample buffer, and subjected to Western blot analysis as described previously [20].

2.5. Immunocytochemistry

MCF-7 cells were seeded at 60% confluence onto coverslips in 6-well dishes and incubated overnight. Cells were washed with PBS, fixed for 20 min in 4% paraformaldehyde in PBS, and permeabilized for 30 min with 0.2% Triton X-100 in PBS. Cells were blocked with 1% BSA in PBS for 30 min and incubated overnight at 4 °C with mouse monoclonal anti-V5 and rabbit polyclonal anti-HA antibodies. After washing three times with PBS, the cells were incubated for 2 h with Alexa Fluor 488-conjugated anti-mouse and Alexa Fluor 594-conjugated anti-rabbit antibodies (Molecular Probes). To stain the nuclei, cells were incubated for 5 min with 1 μg/mL DAPI in PBS. After washing three times with PBS, the cells were analyzed using confocal microscopy (LSM510 META; Carl Zeiss).

2.6. Luciferase reporter assays

After maintaining MCF-7 cells in phenol red-free DMEM (HyClone) containing 5% charcoal-stripped FBS (Sigma–Aldrich), cells were co-transfected with pTA-4xERE-Luc and pRL plasmids (Promega). The pRL plasmid constitutively expresses *Renilla* luciferase and was used to normalize transfection efficiency. Cells were lysed and analyzed using the Dual-Luciferase Reporter Assay system (Promega). Significant differences in luciferase activity were analyzed using the Student's *t*-test in the Sigma Plot 11 program (Systat Software Inc.).

2.7. Cell growth analyses

MCF-7 cells were maintained for 24 h in phenol red-free DMEM containing 5% charcoal-stripped FBS. After DNA transfection for 24 h, cells were treated for 48 h with vehicle (ethanol) or E2 (10 nM). The number of viable cells was determined using Cell Counting Kit-8 (Dojindo Molecular Technology). Significant differences in cell viability were analyzed using the Student's *t*-test in the Sigma Plot 11 program.

3. Results

3.1. ZNF131 physically interacts with ERα

To investigate the molecular mechanisms for ZNF131-mediated repression of estrogen signaling, we examined whether ZNF131 interacts with ERα using co-immunoprecipitation analyses. HEK293 cells were transfected with plasmids encoding HA-tagged ZNF131 and V5-tagged ERα. The lysates were immunoprecipitated with either the V5 or HA antibody, followed by Western blot with the HA or V5 antibody, respectively. As shown in Fig. 1A, ZNF131 interacts with ERα in HEK293 cells (Fig. 1A). Next, we assessed whether the addition of 17β-estradiol (E2) influences the binding affinity of ZNF131 with ERα in HEK293 cells. As shown in Fig. 1B, the binding affinity of ZNF131 to ERα is rapidly increased in

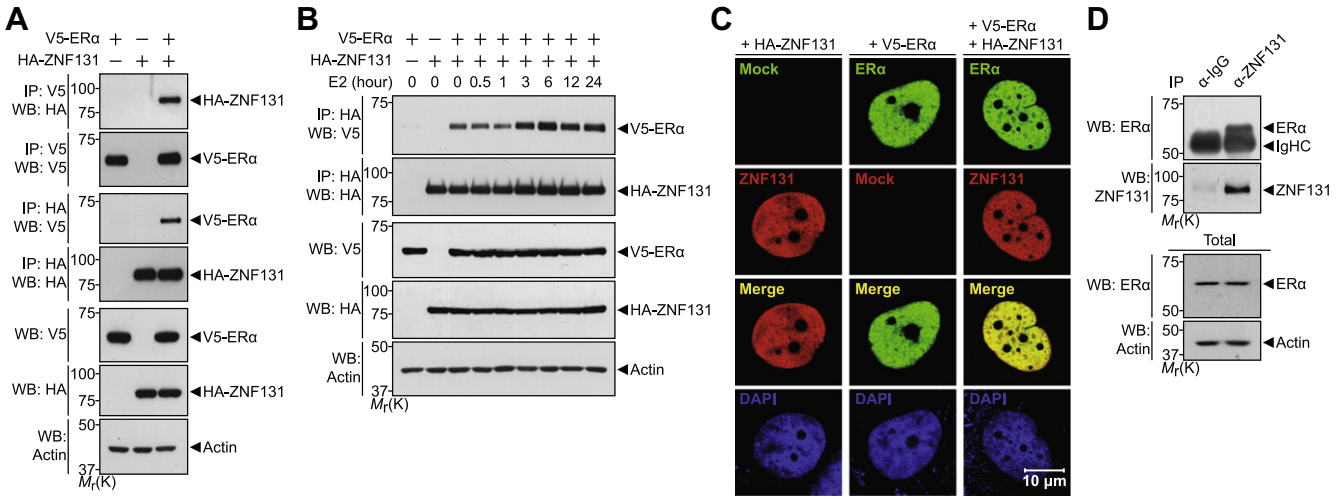

Fig. 1. ZNF131 physically interacts with ERα. (A) HEK293 cells were transfected for 24 h with V5-ERα, in the presence or absence of HA-ZNF131, and immunoprecipitated (IP) with the V5 or HA antibody. Immunoprecipitates were then immunoblotted with the antibodies as indicated. Actin was used as a protein loading control. (B) HEK293 cells were transfected for 24 h with V5-ERα, in the presence or absence of HA-ZNF131, and then treated with E2 (10 nM) for the indicated times. The cell lysates were immunoprecipitated with the HA antibody followed by Western blot (WB) with the indicated antibodies. (C) MCF-7 cells were transfected for 24 h with HA-ZNF131, in the presence or absence of V5-ERα, and analyzed by immunocytochemistry with the HA and V5 antibodies. Expression of HA-ZNF131 (red), V5-ERα (green), and the DAPI-stained nuclei (blue) were analyzed using confocal microscopy. The merged image (yellow) indicates HA-ZNF131 and V5-ERα co-localization. (D) MCF-7 cell extracts were immunoprecipitated with control or ZNF131 antibodies followed by immunoblot with the indicated antibodies. IgHC, immunoglobulin heavy chain; M_r(K), relative molecular mass. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

accordance with the early response to E2 induction (from 0 to 6 h), and is slightly decreased according to the late response to E2 induction (from 6 to 24 h). We immunostained MCF-7 cells transfected with HA-ZNF131 and/or V5-ERα and observed that cells transfected with ZNF131 or ERα alone exhibit diffuse nuclear localization (Fig. 1C, top and middle panels). This confirms previous findings indicating that ZNF131 is a nuclear protein [17,20], and that ERα localizes in the nucleoplasm even in its unliganded state [21–23]. Interestingly, when cells co-expressed ZNF131 and ERα, the proteins were co-localized within the nucleoplasm (Fig. 1C, bottom panel). To prove that endogenous ZNF131 interacts with endogenous ERα, ZNF131- and ERα-positive MCF-7 cell extracts were immunoprecipitated with the ZNF131 antibody followed by Western blot with the ERα antibody. As shown in Fig. 1D, endogenous ZNF131 interacts with endogenous ERα. This result suggests that the ZNF131 and ERα interaction is not an artifact of ectopic DNA transfection (Fig. 1D). Overall, these data show that ZNF131 physically interacts with ERα in mammalian cells.

3.2. ZNF131 inhibits ERα-mediated trans-activation

Because ZNF131 interacts with unliganded-ERα (Fig. 1) and inhibits ERα-mediated *trans*-activation [19,20], it is possible that ZNF131 inhibits estrogen signaling by affecting its initial stages, such as ERα activation. To test this possibility, MCF-7 breast cancer cells were transfected with a luciferase reporter plasmid containing four tandem EREs, in the presence or absence of ZNF131. Kinetic analyses of luciferase reporter activation revealed that ZNF131 significantly inhibits E2-induced expression of estrogen-reporter gene products 30 min after E2 addition, compared with mock-treated control sample ($p < 0.01$, Fig. 2A). However, basal reporter gene expression without E2 treatment is not altered irrespective of ZNF131 (0 min in the Fig. 2A). Interestingly, the inhibitory effect of ZNF131 on estrogen signaling reaches the steady state 3 h after E2 addition (Fig. 2B). Moreover, there was a significant dose-dependent inhibition of ZNF131 on estrogen signaling ($p < 0.001$, Fig. 2C). These data demonstrate that ZNF131 inhibits ERα-mediated *trans*-activation, likely by affecting the early event(s) of estrogen signaling.

3.3. ZNF131 attenuates estrogen signaling by inhibiting ERα homo-dimerization

ERα homo-dimerization is important to promote estrogen-dependent gene expression. Although ERα can exist as a homo-dimer in its unliganded state, its affinity for dimerization increases after the addition of various ligands [8,9]. Because ZNF131 directly interacts with ERα (Fig. 1) and rapidly inhibits estrogen-dependent *trans*-activation of target genes (Fig. 2), we further examined whether ZNF131 could influence ERα homo-dimerization. HEK293 cells were transfected with plasmids encoding HA-tagged ZNF131 and V5/GFP-tagged ERα, and the lysates were immunoprecipitated with the V5 antibody followed by Western blot with GFP antibody. As shown in Fig. 3A, ZNF131 inhibits homo-dimerization of unliganded ERα.

Next, we assessed which ZNF131 domain(s) is critical for the inhibition of ERα-homo-dimerization. Based on functional domain analyses of ZNF131 as well as our previous finding that SUMO modification of ZNF131 at lysine 567 potentiates its negative effect on estrogen signaling, we made several truncation ZNF131 mutants, including deletions of the first nuclear localization signal motif (ZNF131-ΔNLS-1), zinc finger domain (ZNF131-ΔZF1), or the C-terminal region including both the putative coiled-coil region and SUMO targeting site (ZNF131$^{1-412}$ and ZNF131$^{1-250}$). Luciferase reporter assays using these mutants demonstrate that the inhibitory actions of ZNF131-ΔNLS-1 and ZNF131-ΔZF1 on estrogen-dependent reporter expression are not remarkably altered compared to wild-type ZNF131 (designated ZNF131$^{1-589}$). However, the ZNF131$^{1-412}$ and ZNF131$^{1-250}$ mutants exhibit significantly repressed inhibitory action toward estrogen signaling. These results suggest that the C-terminal region of ZNF131 (amino acids 413–589) is important for inhibiting estrogen-responsive *trans*-activation.

Because ZNF131 inhibits ERα homo-dimerization in the unliganded state (Fig. 3A), we further examined whether the C-terminal region of ZNF131 influences homo-dimerization of unliganded-ERα. Consistent with the result shown in Fig. 3C, ERα homo-dimerization is less inhibited by the ZNF131 mutant lacking the C-terminal 177 amino acids (Fig. 3D). Interestingly, the ZNF131

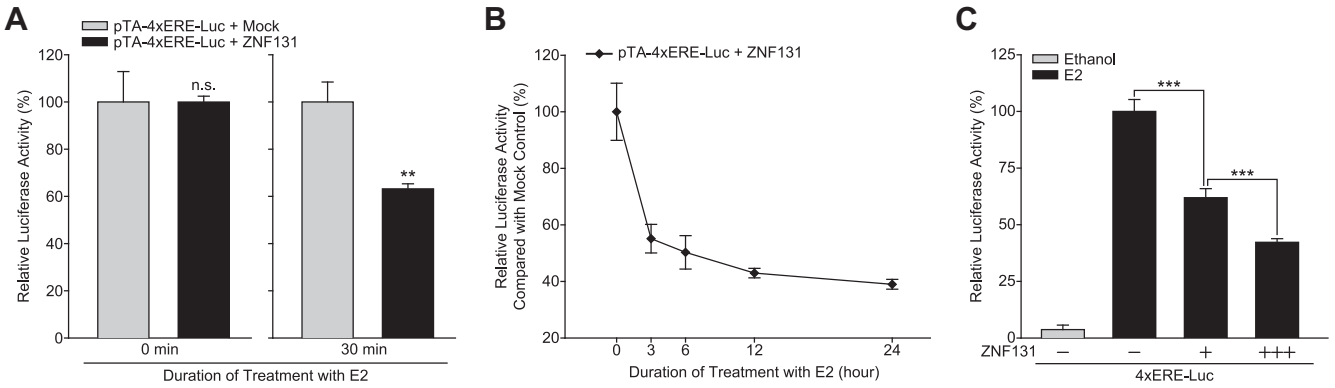

Fig. 2. ZNF131 inhibits ERα-mediated *trans*-activation of estrogen target genes. MCF-7 cells were co-transfected for 24 h with pTA-4xERE-Luc plasmid alone or together with HA-ZNF131, as indicated. (A and B) After cells were treated with E2 (10 nM) for the indicated times, cell extracts were subjected to luciferase assays. (C) After cells were treated for 6 h with vehicle or E2 (10 nM), cell extracts were subjected to luciferase assays. Error bars represent the mean ± S.D. ($n = 3$); n.s., not significant; $^{**}p < 0.01$; $^{***}p < 0.001$.

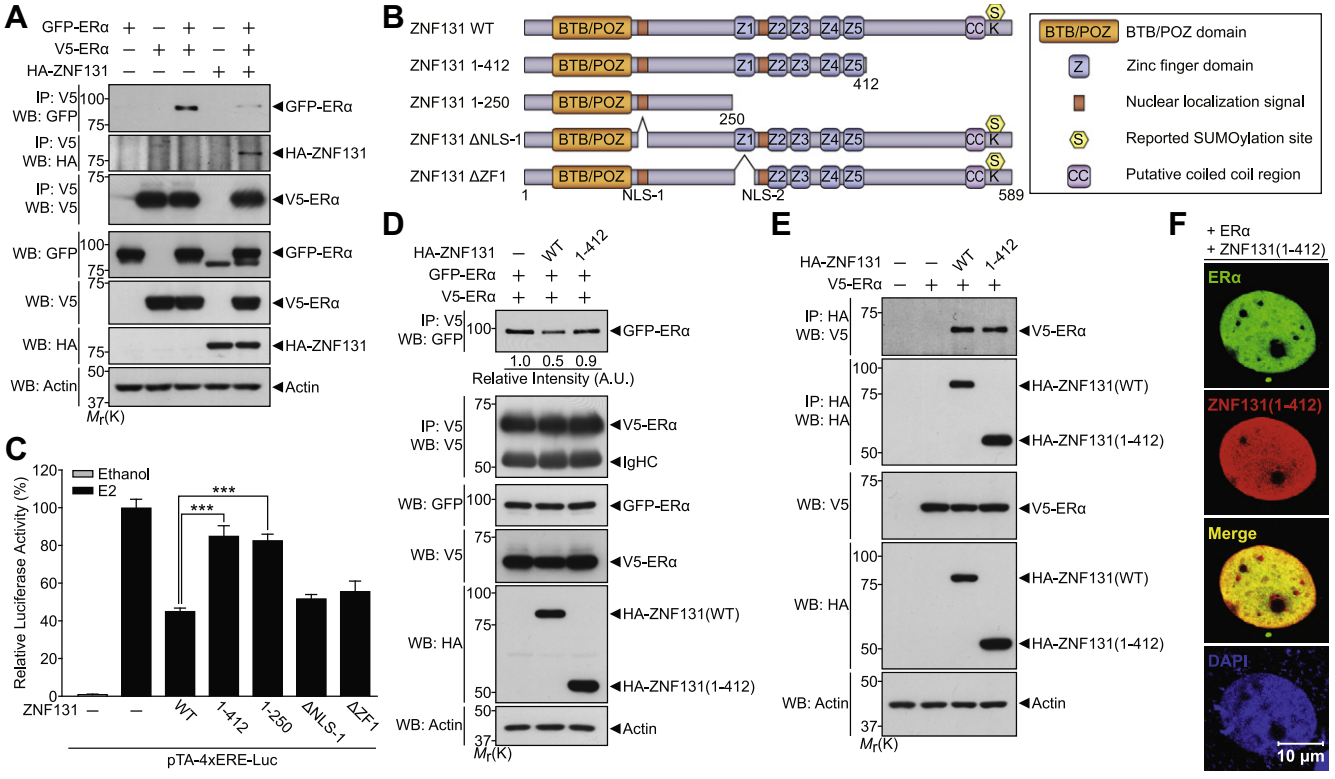

Fig. 3. ZNF131 attenuates estrogen signaling through its C-terminal region by inhibiting ERα homo-dimerization. (A) HEK293 cells were transfected for 24 h with HA-ZNF131, V5-ERα, or GFP-ERα alone or in combination. Cell extracts were immunoprecipitated (IP) with the V5 antibody and immunoblotted with the indicated antibodies. Actin was used as a protein loading control. (B) A diagram of the ZNF131 domains. ZNF131 consists of an N-terminal BTB/POZ domain, two NLS motifs, five central zinc finger domains, and a C-terminal putative coiled-coil region. The reported SUMOylation site (Lys567) is also marked in the C-terminal region. (C) MCF-7 cells were transfected for 24 h with the pTA-4xERE-Luc plasmid alone or together with HA-ZNF131-WT, HA-ZNF131-(1–412), HA-ZNF131-(1–250), ZNF131-ΔNLS-1, or ZNF131-ΔZF1. After cells were treated for 6 h with vehicle or E2 (10 nM), cell extracts were subjected to luciferase assays. (D) HEK293 cells were transfected for 24 h with GFP-ERα and V5-ERα alone or together with HA-ZNF131-WT or HA-ZNF131-(1–412). The cell extracts were immunoprecipitated with the V5 antibody followed by Western blot (WB) with the indicated antibodies. The intensity of the GFP-ERα band was quantified by densitometry using Multi Gauge v3.1 software (Fujifilm Corporation). (E) HEK293 cells were transfected for 24 h with V5-ERα alone or together with HA-ZNF131-WT or HA-ZNF131-(1–412), and immunoprecipitated with the HA antibody. Immunoprecipitates were immunoblotted with the antibodies as indicated. (F) MCF-7 cells were co-transfected for 24 h with V5-ERα and HA-ZNF131-(1–412), and analyzed by immunocytochemistry with the HA and V5 antibodies. Expression of V5-ERα (green), HA-ZNF131-(1–412) (red), and the DAPI-stained nucleus (blue) were analyzed using confocal microscopy. The merged image (yellow) indicates co-localization of HA-ZNF131-(1–412) and V5-ERα. A.U., arbitrary unit; Error bars represent the mean ± S.D. ($n = 3$); IgHC, immunoglobulin heavy chain; M_r(K), relative molecular mass; $^{***}p < 0.001$.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and ERα interaction is not significantly altered by deletion of the ZNF131-C-terminal region (Fig. 3E). Moreover, when cells co-express the ZNF131 mutant lacking the C-terminal region ($ZNF131^{1-412}$) and ERα, these two proteins predominantly co-localize within the nucleoplasm (Fig. 3F). Overall, these results suggest that the C-terminal region of ZNF131 is necessary for

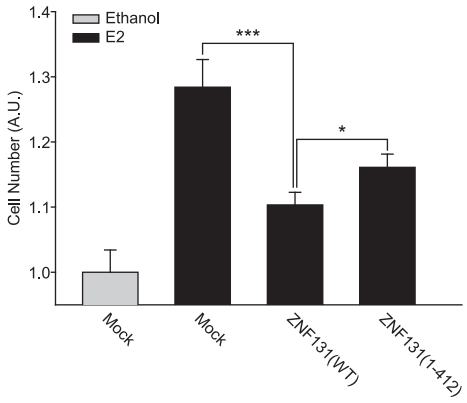

Fig. 4. The C-terminal region of ZNF131 attenuates estrogen-induced cell growth in breast cancer cells. MCF-7 cells were transfected for 24 h with either HA-ZNF131-WT or HA-ZNF131-(1–412), and then treated for 48 h with vehicle or E2 (10 nM). Cell growth was determined by counting the cell number using Cell Counting Kit-8. A.U., arbitrary unit; Error bars represent the mean ± S.D. ($n = 3$); $^{*}p < 0.05$; $^{***}p < 0.001$.

inhibition of ERα homo-dimerization and estrogen signaling, but it does not remarkably influence the physical interaction between ZNF131 and ERα.

3.4. ZNF131 attenuates estrogen-induced breast cancer cell proliferation via its C-terminal region

Based on the physiological role of estrogen to stimulate proliferation in MCF-7 cells [24], we compared the effects of wild-type ZNF131 and its C-terminal mutant on MCF-7 cell growth. Estrogen treatment considerably stimulates the MCF-7 cell growth rate, but this effect is greatly attenuated in the presence of wild-type ZNF131 ($p < 0.001$, Fig. 4). However, this repressive effect on MCF-7 cell growth is disinhibited considerably in the presence of the ZNF131$^{1-412}$ mutant ($p < 0.05$, Fig. 4). This result indicates that the C-terminal region of ZNF131 (amino acids 413–589) plays a critical role in attenuating estrogen-induced growth in MCF-7 cells. Taken together, these results suggest that ZNF131 inhibits ERα homo-dimerization, target gene *trans*-activation, and the consequent estrogen-dependent breast cancer cell proliferation.

4. Discussion

Two previous reports, including one published by our laboratory, have shown that ZNF131 negatively modulates estrogen signaling [19,20]. In the present study, we provide the first evidence that ZNF131 interacts with ERα and consequently inhibits ERα-mediated *trans*-activation by suppressing ERα homo-dimerization. Based on the previous finding that ZNF131 also interrupts ERα-ERE binding [19], the inhibitory mode of ZNF131 on estrogen signaling is similar to that of two previously known co-repressors of ERα, TR2 [12] and SHP [13]. These two proteins directly bind ERα and interrupt
ERα-ERE binding by inhibiting ERα homo-dimerization [12,13].

The ZNF131 protein contains an N-terminal BTB/POZ domain, which is present in numerous developmentally regulated transcription factors and acts as an evolutionary conserved protein–protein interaction domain [16,25]. It has also two tandem double zinc finger domains and one single zinc finger in its central region, which might be involved in central nervous system development [16]. Moreover, ZNF131 possesses a basic classical NLS (NLS-1) and an atypical NLS, which requires additional tandem double zinc finger domains (NLS-2) [17]. Here, we demonstrate that the C-terminal 413–589th region of ZNF131, which includes none of these functional domains, is required for its inhibition of estrogen signaling. Sequence analyses using the online resource SMART (http://smart.embl.de/) further identified a putative coiled-coil domain at the 536–562 region. Interestingly, it is adjacent to the lysine 567 residue that is targeted for covalent SUMOylation, which causes more potent inhibition of estrogen signaling. Because coiled-coil structural motifs are coiled together like the strands of a rope, we speculate that the C-terminal region of ZNF131 may participate in protein binding, particularly with other repressor proteins. Alternatively, other uncharacterized region(s) of ZNF131 play a key role in binding and inhibiting ERα dimerization, which remains to be determined by additional experiments.

Receptor dimerization is necessary for ERα transcriptional activation after binding an ERE [6]. There are two conflicting reports regarding the dimerization kinetics of ERα depending on ligand stimulation and the binding domains during estrogen signaling. For example, Chen et al. revealed that unliganded-ERα could form dimers through the interaction between DNA-binding domains [8]. However, the differential type of receptor dimerization mediated by the ligand-binding domain only occurs upon ligand binding [8]. In contrast, Tamrazi et al. demonstrated that unliganded-ERα exists as a very stable dimer via each ligand-binding domain, and its binding affinity further increased upon the addition of various ligands [9]. Although these results conflict with each other in terms of the dimeric-region of unliganded-ERα, they both proposed that that the ERα dimerization can occur in unliganded ERα. This hypothesis was further supported by our current findings. Our data confirm the ERα homo-dimer is present in its unliganded state, and further indicate ZNF131 inhibits unliganded ERα homo-dimerization. Furthermore, the binding affinity of ZNF131 to ERα increases upon E2 stimulation.

The present study demonstrates that ZNF131 interacts with ERα and significantly inhibits E2-induced *trans*-activation, whereas it does not repress basal ERE reporter gene expression. Similar to ZNF131, nuclear receptors generally interact with their co-repressors to repress target gene transcription, whereas they do not suppress basal transcription [26]. Based on this finding, we propose that ZNF131 acts as an ERα co-repressor in similar manners as TR2 [12] and SHP [13].

N-CoR and SMRT are well-known ERα co-repressor proteins, which bind and recruit HDAC complexes [10,11]. ZNF131 contains an N-terminal BTB/POZ domain that strongly interacts with N-CoR and SMRT. Considering the property of this domain, it is possible that ZNF131 could act as a scaffold protein that recruits other ERα co-repressor proteins, including N-CoR and SMRT. Interestingly, we found that ZNF131 directly binds another ERα co-repressor protein, LMO4 (Y. Oh and K.C. Chung, unpublished data). Taken together, these data suggest that the inhibitory mechanism of ZNF131 on estrogen signaling may be more complex than its role as a simple competitor of the ERα homo-dimer. It will be interesting further to examine the detailed mechanisms of ZNF131 as a transcriptional co-repressor of ERα.

Acknowledgments

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