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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