Transcriptional Corepressor SMILE Recruits SIRT1 to Inhibit Nuclear Receptor Estrogen Receptor-related Receptor γ Transactivation

Yuan-Bin Xie, Jeong-Hoh Park, Don-Kyu Kim, Jung Hwan Hwang, Sangmi Oh, Seung Bum Park, Minho Shong, In-Kyu Lee, and Hueng-Sik Choi

From the 4Hormone Research Center, School of Biological Sciences and Technology, Chonnam National University, Gwangju 500-757, the 5Department of Internal Medicine, Chungnam National University School of Medicine, Daejeon 301-721, the 6Department of Chemistry, College of Natural Science, Seoul National University, Seoul 151-747, and the 7Department of Internal Medicine, Kyungpook National University School of Medicine, Daegu 700-721, Republic of Korea

SMILE (small heterodimer partner interacting leucine zipper protein) has been identified as a corepressor of the glucocorticoid receptor, constitutive androstane receptor, and hepatocyte nuclear factor 4α. Here we show that SMILE also represses estrogen receptor-related receptor γ (ERRγ) transactivation. Knockdown of SMILE gene expression increases ERRγ activity. SMILE directly interacts with ERRγ in vitro and in vivo. Domain mapping analysis showed that SMILE binds to the AF2 domain of ERRγ. SMILE represses ERRγ transactivation partially through competition with coactivators PGC-1α, PGC-1β, and GRIP1. Interestingly, the repression of SMILE on ERRγ is released by SIRT1 inhibitors, a catalytically inactive SIRT1 mutant, and SIRT1 small interfering RNA but not by histone protein deacetylase inhibitor. In vivo glutathione S-transferase pulldown and coimmunoprecipitation assays validated that SMILE physically interacts with SIRT1. Furthermore, the ERRγ inverse agonist GSK5182 enhances the interaction of SMILE with ERRγ and SMILE-mediated repression. Knockdown of SMILE or SIRT1 blocks the repressive effect of GSK5182. Moreover, chromatin immunoprecipitation assays revealed that GSK5182 augments the association of SMILE and SIRT1 on the promoter of the ERRγ target PDK4. GSK5182 and adenosival overexpression of SMILE cooperate to repress ERRγ-induced PDK4 gene expression, and this repression is released by overexpression of a catalytically defective SIRT1 mutant. Finally, we demonstrated that ERRγ regulates SMILE gene expression, which in turn inhibits ERRγ. Overall, these findings implicate SMILE as a novel corepressor of ERRγ and recruitment of SIRT1 as a novel repressive mechanism for SMILE and ERRγ inverse agonist.

Estrogen-related receptors (ERRα, ERRβ, and ERRγ) are constitutively active nuclear receptors (NRs) that contain high levels of sequence identity to estrogen receptors (ERs). All the ERR family members bind either as a monomer or a homodimer or as heterodimeric complexes composed of two distinct ERR isoforms to the consensus sequence TCAAGGTCA, referred to as the consensus estrogen-responsive element (ERRE), and as homodimers to the consensus estrogen-responsive element (1–3). Together with ERRα and ERRβ, ERRγ regulates a number of genes involved in energy homeostasis, cell proliferation, and cancer metabolism (3, 4). Targets of ERRγ known to date are PGC-1α (peroxisome proliferator-activated receptor γ coactivator-1α), PDK4 (pyruvate dehydrogenase kinase isofrom 4), retinoic acid receptor α, and cyclin-dependent kinase inhibitors p21 (WAF1/CIP1) and p27 (KIP1) (4–7). The ability of ERRγ to regulate target gene transcription relies on its interaction with coactivators and corepressors. The coactivators GRIP1 (glucocorticoid receptor interacting protein 1), PGC-1α, and corepressors small heterodimer partner (SHP), DAX-1, and RIP140 (receptor interacting protein 140) or NRII1 have been reported to modulate ERRγ activity (5, 8–11). In addition, 4-hydroxytamoxifen and its derivative GSK5182 act as inverse agonists for ERRγ (12–14). However, the deactivation mechanisms by these inverse agonists remain unclear.

SMILE (small heterodimer partner interacting leucine zipper protein), including two alternative translation-derived isoforms, SMILE-L (CREBF; long form of SMILE) and SMILE-S (Zhangfei; short form of SMILE), has been classified as a member of the CREB/ATF family of basic region-leucine zipper (bZIP) transcription factors (15, 16). However, SMILE cannot bind to DNA as homodimers, although it can homodimerize like other bZIP proteins (15, 17). SMILE has been implicated in herpes simplex virus infection cycle and related cellular processes through its association with herpes simplex virus-related host-cell factor and CREB3 (17, 18). SMILE has also been proposed as a coactivator of activating transcription factor 4 (19, 20). This work was supported by National Research Laboratory Grant ROA-2005-000-10047-0 and the Korea Research Foundation Grant KRF-2006-005-J30030.

The on-line version of this article (available at http://www.jbc.org) contains supplemental Tables 1 and 2 and Figs. S1–S4.

1 To whom correspondence should be addressed. Tel.: 82-62-530-0503; Fax: 82-62-530-0506; E-mail: hsc@chonnam.ac.kr.
2 The abbreviations used are: ERR, estrogen receptor-related receptor; CAR, constitutive androstane receptor; GR, glucocorticoid receptor; HNF4α, hepatocyte nuclear factor 4α; ERR, estrogen-response element; SHP, small heterodimer partner; siRNA, small interfering RNA; HA, hemagglutinin; HDAC, histone protein deacetylase; GST, glutathione S-transferase; RT, reverse transcription; qPCR, quantitative real-time PCR; CHIP, chromatin immunoprecipitation; NR, nuclear receptor; LBD, ligand binding domain; TSA, trichostatin A; ColP, coimmunoprecipitation; WT, wild type; ER, estrogen receptor; AF2, activation function-2 domain; bZIP, basic region leucine zipper domain; CREB, cAMP-responsive element-binding protein.
(ATF4/CREB2) (19). Recently, we have reported that SMILE functions as a coregulator of ER signaling and a corepressor of the glucocorticoid receptor (GR), constitutive androstane receptor (CAR), and hepatocyte nuclear factor 4α (HNF4α) (16, 20). However, the detailed roles of SMILE on other NRs still need to be clarified.

Silent information regulator 2 proteins (Sirtuins) are class III histone protein deacetylases (HDACs) and consist of seven members named SIRT1 to SIRT7 in mammals (21). Through deacetylating target proteins, Sirtuins play important roles in cellular processes such as gene expression, apoptosis, metabolism, and aging (21). Of the seven Sirtuins, SIRT1 has been extensively studied. It has been reported that SIRT1 deacetylates and thereby deactivates the p53 and PARP1 protein (poly-ADP ribose) polymerase-1), resulting in promoted cell survival (22, 23). In addition, SIRT1 regulates glucose or lipid metabolism through its deacetylation activity on over 24 known substrates, including FOXO transcriptional factors (24, 25) and p53 (26). In addition, SIRT1 negatively regulates ERRγ transactivation of liver X receptor by SMILE (27).

In this study, we have shown that SMILE negatively regulates ERRγ through direct interaction. We have demonstrated that coactivator competition and recruitment of catalytically active SIRT1 are required for the repression of ERRγ by SMILE. Moreover, ERRγ-specific inverse agonist GSK5182 enhances the interaction of SMILE and ERRγ. siRNA SMILE and siRNA SIRT1 experiments have revealed that SMILE-SIRT1 association is required for the inhibition of ERRγ by GSK5182. In addition, we have observed that ERRγ induces SMILE gene expression in HepG2 cells by directly binding to the SMILE promoter and that SMILE inhibits ERRγ transactivation of its own promoter. Overall, our observations suggest that SMILE acts as a novel corepressor of ERRγ and that ERRγ belongs to a new autoregulatory loop that governs SMILE gene expression.

**EXPERIMENTAL PROCEDURES**

**Plasmid and DNA Construction**—The plasmids of pcMV-β-gal, pcDNA3-ERRα, -ERRβ, -ERRγ, -ERRγΔA2F, pSG5-HA-ERRγ, pGEX4T-1-ERRγ, and sft4-Luc were described elsewhere (9, 10). (HNF4)8-tk-Luc, pcDNA3-HA-HNF4α, -Pgc-1α, pSG5-HA-GRIP1, pcDNA3-SMILE, -FLAG-SMILE, -SMILE-83Leu, -SMILE-1Phe, pGEX4T-1, pGEX4T-1-SMILE, pEGP-SMILE, pEBG, pEBG-SMILE, and pEBG-SMILE deletion constructs were cloned into SMILE-Luc, the pcDNA3 vector between BglII and XhoI sites. To generate −1131-bp SMILE-Luc, the SMILE promoter region spanning −1131 to −15 bp was PCR-amplified from human genomic DNA and cloned into pGL3-basic vector (Promega) between the SacI and Xhoi sites. −879-bp- and −448-bp-SMILE-Luc were constructed by inserting the PCR fragments into the SacI/Xhoi sites of pGL3-basic vector. The mutant reporters of SMILE-mtERRE1-Luc and SMILE-mtERRE2-Luc were subcloned via site-directed mutagenesis from −1131-bp-SMILE-Luc. The mutated sequences are shown in Fig. 7D. All plasmids were confirmed via sequencing analysis.

**Chemicals and Antibodies**—SIRT1 inhibitors nicotinamide and sirtinol were from Calbiochem; EX527 was purchased from TOCRIS; ERRγ inverse agonist GSK5182 was synthesized according the method described previously (14), and other chemicals were from Sigma. Antibodies used in this work were as follows: anti-FLAG M2 (catalog number 200472-21, Stratagene), anti-HA (12CA5, Roche Applied Science), anti-SMILE (catalog number ab28700, Abcam), anti-GST (sc-33614, Santa Cruz Biotechnology), anti-PGC-1α (H300, sc-13067, Santa Cruz Biotechnology), anti-SIRT1 (catalog number 2493, Cell Signaling Technology), anti-acetyl-histone H3 (Lys-9) (catalog number 9761, Cell Signaling Technology), anti-acetylated lysine (catalog number 9441, Cell Signaling Technology), anti-Myc (catalog number 2276, Cell Signaling Technology), anti-tubulin (catalog number 2146, Cell Signaling Technology), and anti-ERRγ antibodies (catalog number pph6812-00, R & D Systems). The primary antibodies were used at a dilution of 1:1000 in Western blot analysis and at a dilution of 1:200 in immunoprecipitation.

**Cell Culture, Transient Transfection Assay, and Luciferase Assay**—HEK293T (293T, human embryonic kidney), HepG2 (human hepatoma), and HeLa cells (cervical cancer) were obtained from the American Type Culture Collection (ATCC) and cultured according to the manufacturer’s instructions. Transient transfection was performed using Superfect transfection reagent (Qiagen) in 293T cells and Lipofectamine 2000 reagent (Invitrogen) in HepG2 cells. 293T and HepG2 cells were cotransfected with the reporter plasmids (HNF4α-Luc, sft4-Luc, or PDK4-Luc coupled with various expression vectors. The plasmid of cytomegalovirus-β-galactosidase was cotransfected as an internal control, and the total DNA employed in each transfection was adjusted via the addition of an appropriate quantity of pcDNA3 vector. Approximately 36 h post-transfection, the cells were treated with or without chemicals as indicated in the figure legends for 12 h, and then cells were harvested, and the luciferase activity was measured and normalized against β-galactosidase activity as described previously (16, 20). Fold activity was calculated considering the activity of reporter gene alone as 1.