Agonist-dependent Repression Mediated by Mutant Estrogen Receptor α That Lacks the Activation Function 2 Core Domain*

Received for publication, July 20, 2001
Published, JBC Papers in Press, August 3, 2001, DOI 10.1074/jbc.M106860200

Dong-Ju Jung‡, Soo-Kyung Lee§§, and Jae Woon Lee‡‡

From the ‡Center for Ligand and Transcription, Pohang University of Science and Technology, Pohang 790-784, Korea and the §Gene Expression Laboratory, The Salk Institute for Biological Studies, San Diego, California 92185

Nuclear receptor corepressor (N-CoR) and silencing mediator of retinoid and thyroid hormone receptors (SMRT) form heterogeneous complexes with various histone deacetylases (HDACs). In this report, we found that ERα-ΔAF2, a mutant estrogen receptor α (ERα) deleted for the C-terminal activation function 2 (AF2) core domain, directs estradiol (E2)-dependent repression and impairs E2-induced transactivation by wild type ERα. This repression required coexpressed BRG1 in SW-13 cells that lack BRG1, the ATPase constituent of the chromatin-remodeling SWI/SNF complex, and was abolished by HDAC inhibitor trichostatin A. We further demonstrated that ERα-ΔAF2 constitutively associates with SMRT but binds DNA in an E2-dependent manner in vivo. These results suggest that ERα-ΔAF2 and similar mutant receptors recently found associated with certain tumors may actively perturb the normal E2 signaling via SWI/SNF, N-CoR/SMRT, and HDAC.

The nuclear receptor superfamily is a group of ligand-dependent transcriptional regulatory proteins that function by binding to specific DNA sequences named hormone response elements in the promoters of target genes (for a review, see Ref. 1). The superfamily includes receptors for a variety of small hydrophobic ligands such as steroids, T3, and retinoids as well as a large number of related proteins that do not have known ligands, referred to as orphan nuclear receptors. The C terminus of the ligand binding domain of these proteins harbors an essential ligand-dependent transcription activation function, activation function 2 (AF2)1 (1), whereas the N terminus of some nuclear receptors includes activation function 1. Genetic studies have implicated that transcription coregulators (or cofactors) with no specific DNA binding activity are essential components of transcriptional regulation that ultimately led to the identification of a series of nuclear receptor-interacting coregulatory proteins (for reviews, see Refs. 2–4). These proteins appear to function by either remodeling chromatin structures and/or acting as adapter molecules between nuclear receptors and the components of the basal transcriptional apparatus.

Transcriptional coactivators of nuclear receptors include the steroid receptor coactivator-1 (SRC-1) family, CAMP-response element-binding protein (CREB)-binding protein/p300, p/CAF, thyroid hormone receptor (TR)-associated protein/vitamin D3 receptor-interacting protein, activating signal cointegrator-1, activating signal cointegrator-2, and many others (2–4). Interestingly unliganded retinoic acid receptor (RAR) and TR bind to their target genes and repress transcription. Silencing mediator of RAR and TR (SMRT) and nuclear receptor corepressor (N-CoR) are known to mediate this repression (2–4). SMRT and N-CoR harbor transferable repression domains that associate with histone deacetylases (HDACs), consistent with the concept that histone hypoacetylation correlates with gene repression. SMRT and N-CoR were originally thought to exclusively act as adaptor molecules between target nuclear receptors and the mSin3HDAC1 complex (5, 6) and were subsequently shown to interact directly with class II HDAC4 and HDAC5 (7). In addition, multiple steady-state N-CoR/SMRT complexes have recently been identified (8–11). Enzymatically active HDAC3 complexes that contained both SMRT and N-CoR were isolated from HeLa nuclei (8, 9). Two multi-protein N-CoR complexes, designated N-CoR-1 and N-CoR-2, were also isolated from HeLa nuclei (10). N-CoR-1 contained HDAC3, the SWI/SNF-related proteins BRG1, BAF 170, BAF 155, and BAF 47/INI1, and the corepressor KAP-1, whereas N-CoR-2 contained predominantly HDAC1 and HDAC2 as well as several other subunits that are found in the Sin3A complex (5, 6). Similarly, Jones et al. (11) reported the presence of at least three distinct N-CoR complexes from Xenopus egg extract: one complex contained Sin3, Rpd3, and RbAp48, the second complex contained a Sin3-independent HDAC, and the third complex lacked HDAC activity. Steroid hormone receptors do not appear to interact with SMRT/N-CoR in the presence or absence of agonists, whereas both the estrogen receptor (ER) and the progesterone receptor can interact with these corepressors in the presence of their respective antagonists (12–15). Similarly, binding of antagonists or the deletion of the AF2 domain is known to enhance the binding of N-CoR/SMRT to TR and RAR (16, 17).

There is a large and increasing body of experimental and clinical data supporting the existence of variant ER proteins in both normal and neoplastic estrogen target tissues including human breast (for review, see Ref. 18). The functions of these variant ER proteins, either physiological or pathological, remain unclear. However, possible tissue-specific expression suggests that ER variants may have a role in tissue-specific estrogen action. In particular, ER variants lacking internal exons and representing dominant positive and negative activity may be involved in the initiation and/or progression of endocrine-dependent tumors. Interestingly a series of alterations and/or truncations in exon 8, which contains the AF2 core region, were identified with uterine tumor tissues (19).
as well as a very aggressive and poorly differentiated form of breast cancer tissues recently isolated from African-American women (20).

In this report, we demonstrate that a mutant ERα deleted for the C-terminal AF2 core domain (ERα-ΔAF2) directs basal repression and impairs transactivation by wild type ERα, both in an estradiol (E2)-dependent manner, via SWI/SNF, N-CoR/SMRT, and HDAC. Our results suggest that ERα-ΔAF2 and similar receptors associated with certain tumors (19, 20) can perturb the normal E2 signaling, which may play an active role in cancerogenesis.

EXPERIMENTAL PROCEDURES

Plasmids and Ligands—Polymersome chain reaction fragments encoding ERα-ΔAF2 were inserted into EcoRI and XhoI restriction sites of the LexA fusion vector pEG202PL, the B42 fusion vector pBG4-5, and the mammalian expression in vitro translation vector pcDNA3. B42 and LexA fusions to the ERα, mammalian expression vectors for SMRT, N-CoR, BRG1, and ERα, the reporter constructs estrogen response element (ERE)-Luc and LexA-fusions to the ERα, were purchased from Sigma, and trichostatin A was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Cell Culture and Transfection—CV-1 or SW-13 (23) cells were grown in 24-well plates with medium supplemented with 10% charcoal-stripped fetal bovine serum. After 24 h incubation, cells were transfected with 100 ng of β-galactosidase expression vector pBSV-β-gal and 100 ng of ERE-Luc reporter gene along with expression vectors for ERα, ERα-ΔAF2, N-CoR, and BRG1. Total amounts of expression vectors were kept constant by adding decreasing amounts of pcDNA3 to transfections. Twelve hours later, cells were washed and refed with Dulbecco’s modified Eagle’s medium containing 5% fetal bovine serum supplemented with 50 μM of the indicated ligand. Cells were harvested 24 h later, and luciferase activity was assayed as described previously (24), and the results were normalized to the β-galactosidase expression. Consistent results were obtained in more than two similar experiments.

YEAST TWO-HYBRID TESTS—The cotransformation and β-galactosidase assays in yeast were performed as described previously (24). For each experiment, at least three independently derived colonies were tested.

Gel Mobility Shift Assays—Gel mobility shift assays were performed as described previously (24). Radiolabeled ERE oligonucleotides were incubated with in vitro translated ERα and ERα-ΔAF2, and the reaction products were analyzed by native gel electrophoresis and autoradiography as described previously (24).

Limited Proteolyses—ERα and ERα-ΔAF2 were in vitro translated/labeled with [35S]Met, and the digests were done for 10 min at room temperature with 50 μg/ml trypsin in the presence of the increasing amount of the indicated ligands. The reaction products were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis and autoradiography as described previously (25).

Immunoprecipitation and Chromatin Immunoprecipitation—293T cells were transfected with ERα, ERα-ΔAF2, or SMRT expression vector and treated with E2 or TAM for 1 h. Cells were harvested and immunoprecipitated with a monoclonal antibody against SMRT (a kind gift of Dr. Dean Edwards at University of Colorado Health Sciences Center) and blotted with monoclonal antibody against ERα (Santa Cruz Bio-technology). For chromatin immunoprecipitation, soluble chromatin from these cells was prepared and immunoprecipitated with ERα antibody as recently described (26). The final DNA extractions were amplified using pairs of primers that cover the ERE promoter region. The experiments were repeated at least three times and were highly reproducible.

RESULTS AND DISCUSSION

E2-dependent Inhibition Mediated by ERα-ΔAF2—To understand the possible role of variant ERs altered in the AF2-containing exon 5 (19, 20) in tumorigenesis, we constructed a mutant ERα that is deleted for the C-terminal 58 amino acids containing the core AF2 region (i.e. ERα-ΔAF2). Interestingly, ERα-ΔAF2 repressed transactivation mediated by the ERE-driven luciferase reporter construct in an E2-dependent manner, whereas the wild type ERα directed E2-dependent transactivation as expected (Fig. 1A). In addition, ERα-ΔAF2 impaired E2-mediated transactivation by the wild type ERα in a dose-dependent and dominant negative fashion (Fig. 1B). These results suggest that ERα-ΔAF2 and similar mutant ERαs associated with tumors (19, 20) can perturb the normal E2 signaling within the cell, which may play a role in the possible tumorigenesis by these receptors.

Constitutive Recruitment of N-CoR/SMRT and E2-dependent DNA Binding by ERα-ΔAF2—To understand how ERα-ΔAF2 achieves this repression, we examined various molecular properties of this mutant receptor. First, LexA fusions to ERα-ΔAF2 and ERα enhanced transactivation mediated by B42 fusions to ERα, ERα-ΔAF2, and ERβ in a ligand-dependent manner (Fig. 2A). It was interesting to note that LexA-ERα stimulated transactivation mediated by B42-ERβ even in the absence of ligand. These results clearly demonstrate that ERα-ΔAF2 binds ligands and still retains the ability to dimerize with ERs. Second, ERα-ΔAF2 was found to bind ERα either as a homodimer or heterodimer with ERα as demonstrated by gel mobility shift assays (Fig. 2B). Next we examined the tertiary structure of ERα-ΔAF2 by using a limited proteolysis experiment (25). Two previously described fragments of 31 and 28 kDa (25) as well as a smaller fragment of ~10 kDa were generated in an E2-dependent manner from the full-length, [35S]Met-labeled ERα when subjected to 50 μg/ml trypsin (Fig. 3A). In contrast, only the 28-kDa fragment was visible when the ER antagonist TAM was used. Interestingly ERα-ΔAF2 produced a pattern similar to ERα/TAM in the presence of either E2 or TAM, producing a fragment of ~28 kDa. Thus, ERα-ΔAF2, in the presence of either E2 or TAM, may adopt a conformation that resembles that of TAM-bound ERα. From these results along with the results in which N-CoR/SMRT binding sites were shown to be enhanced either by antagonists or the deletion of the AF2 domain with TR and RAR (16, 17) and with steroid hormone receptors, observed only in the presence of antagonists (12–15), we hypothesized that ERα-ΔAF2 may recruit corepressor SMRT/N-CoR in an E2-dependent manner. To test this idea, 293T cells cotransfected with expression vectors for the full-length ERα and SMRT either in the absence or presence of E2 or TAM were immunoprecipitated with SMRT...
suspect that ER TAM-dependent manner. Taking all of these results together, we chromatin immunoprecipitation assays. As shown in Fig. 3

ER ligand-dependent repression (Fig. 1). As expected from this prediction, CoR in solution, and this complex is recruited to ERE in a strictly based on the mobility of the retarded band, which runs between ER lane 3

antibody and probed with an antibody against ER and ER and ER lanes 5

by ER

ER exhibited TAM-dependent interactions as reported previously (12–15). Surprisingly, however, ER-ΔAF2 constitutively interacted with SMRT. Similar results were also obtained with the glutathione S-transferase pull-down assays (results not shown). Overall these results were somewhat similar to the case with mutant TR and RAR deleted for the AF2 domain (16, 17). It is important to note that ERα-ΔAF2 was not capable of binding SRC-1 in the glutathione S-transferase pull-down and yeast two-hybrid assays as expected (results not shown). To further examine whether ERα-ΔAF2 binds E2 in an E2-dependent manner in vivo, as it was recently shown with ERα (26), we used chromatin immunoprecipitation assays. As shown in Fig. 3C, both ERα and ERα-ΔAF2 were recruited to ERE in an E2- or TAM-dependent manner. Taking all of these results together, we suspect that ERα-ΔAF2 constitutively associates with SMRT/N-CoR in solution, and this complex is recruited to ERE in a strictly ligand-dependent manner in vivo, leading to the observed E2-dependent repression (Fig. 1). As expected from this prediction, ERα-ΔAF2 also repressed ERE-dependent transactivation in a TAM-dependent manner (results not shown).

Requirement of BRG1 and HDAC in ERα-ΔAF2-mediated Repression—Multiple steady-state complexes containing N-CoR/SMRT and distinct HDAC proteins have recently been isolated (8–11). In particular, N-CoR-1 contains HDAC3, the SWI/SNF-related proteins BRG1, BAF 170, BAF 155, and BAF 47/IN1, and the corepressor KAP-1 that is involved in silencing heterochromatin (10). When we examined the transcriptional properties of ERα and ERα-ΔAF2 in SW-13 cells (23) that lack BRG1 and the similar protein hBRM, the -fold repression observed with both receptors was negligible (Fig. 4A). However, the previously described TAM-dependent repression by ERα was slightly increased in the presence of cotransfected BRG1 expression vector and synergistically increased when

BRG1 was coexpressed with N-CoR (Fig. 4A, left). Similarly the E2-dependent repression by ERα-ΔAF2 was also enhanced by cotransfected BRG1 (Fig. 4A, right). As expected, trichostatin A, a HDAC inhibitor, abolished the E2-dependent repression by ERα-ΔAF2 (Fig. 4B). Overall these results suggest that the E2-dependent repression by ERα-ΔAF2 may function through recruitment of a distinct N-CoR complex containing both HDAC and BRG1, such as N-CoR-1 (10), although other similar complexes yet to be identified may also play a role in this repression. Alternatively multiple N-CoR/SMRT complexes (i.e. BRG1 and non-BRG1 complexes) may function in sequence or combination. The SWI/SNF family of nucleosome-remodeling complexes has been shown to play important roles in gene expression throughout eukaryotes via establishing a local chromatin structure that is permissive for increased access of transcription factors for their binding sites. Notably SWI/SNF is required for both transcriptional activation and repression of some genes. Our results, along with the recent results in which BRG1 was shown to be essential for E2-dependent transactivation of ERs (27), suggest that SWI/SNF is also involved with both activation and repression by ER and mutant ERs such as ERα-ΔAF2.

Importantly our results unraveled a novel type of functional transformation in which a specific mutation converted an agonist-dependent transcriptional activator (i.e. ERα) into a potent,
agonist-dependent repressor (i.e. ERα-ΔAF2). With regard to this switch of function by mutations, it is interesting to note that resistance to thyroid hormone can result from an aberrant interaction between mutant TRβ1 and N-CoR/SMRT (28–30). For instance, a natural TRβ1 mutant (G345R) with poor T3 binding affinity, originally isolated from resistance to thyroid hormone (L454S), bound T3 and weakly

ERα-ΔAF2, N-CoR, and BRG1, and the reporter construct ERE-Luc (100 ng) as indicated. Closed, shaded, and open boxes indicate the presence of no ligand, 0.1 μM TAM, and 0.1 μM E2, respectively. 100 nM trichostatin A (TSA) was used as indicated (B). Normalized luciferase expressions from triplicate samples were calculated relative to the LacZ expressions. The experiments were repeated at least three times, and the representative results were expressed as fold-activation (n-fold) over the value obtained with vector alone with the error bars as indicated.

In summary, we found that ERα-ΔAF2 directs E2-dependent repression and impairs E2-mediated transactivation by wild type ERα via N-CoR/SMRT, BRG1, and HDAC. Perturbation of the normal E2 signaling by ERα-ΔAF2 and similar mutant receptors associated with tumors (19, 20) may represent a novel mechanism for the tumorigenesis processes in vivo.

Acknowledgment—We thank Dr. Dean Edwards at Colorado University Health Science Center for SMRT monoclonal antibody.

REFERENCES

1. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1994) Cell 79, 335–355

2. Glass, C. K., Rose, D. W., and Rosenfeld, M. G. (1997) Curr. Opin. Cell Biol. 9, 222–232

3. Mario, N. J., Lanz, R. B., and O'Malley, B. W. (1999) Endocr. Rev. 20, 321–344

4. Lee, J. W., Lee, Y. C., Na, S. Y., Jung, D. J., and Lee, S. K. (2001) Cell. Mol. Life Sci. 58, 289–297

5. Nagy, L., Kao, H. Y., Chakravarti, D., Lin, R. J., Hassig, C. A., Ayer, D. E., Schreiber, S. L., and Evans, R. M. (1997) Cell 89, 373–380

6. Heinzl, T., Lavinsky, R. M., Mullen, T. M., Soderstrom, M., Laherty, C. D., Torchia, J., Yang, W. M., Beard, G., Ngo, S. D., Davie, J. R., Sets. E., Eisenman, R. N., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1997) Nature 387, 43–48

7. Huang, E. Y., Zhang, J., Miska, E. A., Guenther, M. G., Kouzarides, T., and Lazar, M. A. (2000) Genes Dev. 14, 45–54

8. Li, J., Wang, J., Wang, J., Nawaz, Z., Liu, J. M., Qin, J., and Wong, J. (2000) EMBO J. 19, 4342–4350

9. Wen, Y. D., Perzil, V., Staszewski, L. M., Yang, W. M., Krones, A., Glass, C. K., Rosenfeld, M. G., and Seto, E. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 7202–7207

10. Underhill, C., Qutob, M. S., Yee, S. P., and Torchia, J. (2000) J. Biol. Chem. 275, 40463–40470

11. Jones, P. L., Sachs, L. M., Wade, P. A., and Shi, Y. B. (2001) Mol. Endocrinol. 15, 720–731

12. Xu, J., Nawaz, Z., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12195–12199

13. Jackson, T. A., Richer, J. K., Bain, D. L., Takimoto, G. S., Tung, L., and Heinzel, T. (1997) Mol. Endocrinol. 11, 693–705

14. Smith, C. L., Nawaz, Z., and O'Malley, B. W. (1997) Mol. Endocrinol. 11, 657–666

15. Lavinsky, R. M., Jepsen, K., Heinzl, T., Torchia, J., Mullen, T. M., Schaffir, R., Del-Rio, A., Lizotte, M., Ngo, S., Gensch, J., Hilsenbeck, S. G., Osborne, C. K., Glass, C. K., Rosenfeld, M. G., and Rose, D. W. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2920–2925

16. Horlein, A. J., Naar, A. M., Heinzel, T., Torchia, J., Gliss, B., Karoukha, R., Ryan, A., Kamei, Y., Soderstrom, M., Glass, C. K., and Rosenfeld, M. G. (1995) Nature 377, 397–404

17. Chen, J. D., and Evans, R. M. (1995) Nature 377, 454–457

18. Murphy, L. C., Dotzlaw, H., Levyge, E., Douglas, D., Coutts, A., and Watson, P. H. (1997) J. Steroid Biochem. Mol. Biol. 62, 583–372

19. Hu, C., Hyder, N. M., Needelman, D. S., and Baker, V. V. (1996) Mol. Cell. Endocrinol. 118, 173–179

20. Koduri, S., Fuqua, S. A., and Polia, I. (2000) J. Cancer Res. Clin. Oncol. 126, 291–297

21. Lee, S. K., Choi, H. S., Song, M. R., Lee, M. O., and Lee, J. W. (1998) Mol. Biochem. Pharmacol. 12, 1184–1192

22. Song, M. R., Lee, S. K., Seo, Y. W., Choi, H. S., Lee, J. W., and Lee, M. O. (1998) Biochem. J. 336, 711–717

23. Leibovitz, A., McComb, W. M., Johnston, D., McCoy, C. E., and Stinson, J. C. (1973) J. Natl. Cancer Inst. 51, 691–697

24. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, A. L., and Struhl, K. (1995) Current Protocols in Molecular Biology, Greene Assoc., New York

25. Emmas, C. E., Fowell, S. E., Hoare, S. A., and Parker, M. G. (1992) J. Steroid Biochem. Mol. Biol. 41, 291–299

26. Shang, Y., Hu, X., Di Renzo, J., Lazar, M. A., and Brown, M. (2000) Cell 103, 843–852

27. Di Renzo, J., Shang, Y., Pleban, M., Sif, S., Myers, M., Kingston, R., and Brown, M. (2000) Mol. Cell. Biol. 20, 7541–7549

28. Yeh, S. M., Chatterjee, V. K., and Privalovsky, M. L. (1997) Mol. Endocrinol. 11, 470–480

29. Liu, Y., Takeshita, A., Misiti, S., Chin, W. W., and Yen, P. M. (1998) Endocrinology 139, 4197–4204

30. Tagami, T., Gu, W. X., Pears, T. P., West, B. L., and Jameson, J. L. (1998) Mol. Endocrinol. 12, 1888–1902

31. Grignani, F., De Matteis, S., Nervi, C., Tomassoni, L., Gelmetti, V., Cicco, M., Fanelle, M., Ruthardt, M., Ferrara, F. F., Zamir, I., Seiser, C., Grignani, F., Lazar, M. A., Minucci, S., and Pelicci, P. G. (1998) Nature 391, 815–818
Agonist-dependent Repression Mediated by Mutant Estrogen Receptor α That Lacks the Activation Function 2 Core Domain
Dong-Ju Jung, Soo-Kyung Lee and Jae Woon Lee

J. Biol. Chem. 2001, 276:37280-37283.
doi: 10.1074/jbc.M106860200 originally published online August 3, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M106860200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 9 of which can be accessed free at http://www.jbc.org/content/276/40/37280.full.html#ref-list-1