Pure antiandrogens disrupt the recruitment of coactivator GRIP1 to colocalize with androgen receptor in nuclei

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Abstract We have used confocal microscopy to elucidate the effects of antiandrogens on nuclear localization of the androgen receptor (AR) with its transcriptional coactivator GRIP1. We show that the agonist-activated AR recruits GRIP1 to colocalize with the receptor in the nucleoplasm. By contrast, AR complexed to the antiandrogens hydroxyflutamide and bicalutamide fails to influence nuclear distribution of GRIP1. Likewise, the non-steroidal antiandrogens prevent the agonist-induced AR–GRIP1 colocalization from occurring. Androgen antagonists affect nuclear redistribution of AR–GRIP1 in a fashion that parallels their effects on the transcriptional activity of AR, in that the pure antagonists block GRIP1-dependent activation of AR function, whereas the mixed antagonist/agonist cyproterone acetate promotes both AR-driven redistribution of GRIP1 and activation of AR by GRIP1. **© 2002 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Androgen receptor; Antiandrogen; Coactivator; Transcription; Nucleus

1. Introduction

Androgens are critical for the development and the maintenance of male sexual characteristics and their action is mediated through the androgen receptor (AR) [1] that belongs to the steroid receptor subfamily of nuclear receptors [2]. Nuclear receptors regulate transcription of target genes in response to binding to cognate ligands and consist of three principal domains: a highly variable N-terminal transactivation domain (NTD), a conserved DNA-binding domain and a somewhat more variable C-terminal ligand-binding domain (LBD) [2]. The transcription activation functions (AF) 1 and 2 of nuclear receptors reside in the NTD and the LBD, respectively. The AF2 of AR is very weak compared to other steroid receptors [3–5]. In addition to harboring the strong and hormone-independent AF1, the AR NTD contains conserved FXXLF and WXXLF motifs that are important for the interaction with hormone-occupied LBD [6–9]. The androgen-dependent NTD/LBD interaction is probably direct, but it can be enhanced by binding of coactivator proteins, such as SRC-1 and GRIP1 (TIF2) [6,7]. These two related p160 coactivators have distinct regions that interact with the NTD and the LBD, thereby potentially bridging the NTD/LBD interaction of AR [4,10,11]. The p160 proteins bind to AF2 regions of nuclear receptor LBDs via the LXXLL motifs (NR boxes), whereas regions C-terminal to these motifs are involved in the interaction with AF1 regions of some nuclear receptors, such as AR [4,11,12]. The p160 coactivators are, in turn, capable of recruiting histone acetyltransferases and thereby remodeling chromatin structure, or as is the case with SRC-1, the coactivator itself possesses acetyltransferase activity [10]. These interactions have been shown to result in augmentation of AR-dependent transcription [4,5,11,13–15].

Even though several aspects of the interplay between the agonist-bound AR and its coactivator proteins have recently become less elusive, very little is known of the ways by which antiandrogens affect subnuclear distribution of AR and dynamics of coactivator recruitment by AR. We and others have previously shown that AR antagonists fail to induce a transcriptionally competent conformation in the AR LBD in vitro, but reverse the agonist-elicited conformational change in the LBD [16,17]. In this work, we have visualized by confocal microscopy the effects of antiandrogens on the distribution of AR and GRIP1 at a single cell level. Our results show that the agonist-bound AR is involved in active recruitment of GRIP1 in the nucleoplasm. Furthermore, our assay clearly differentiates between pure agonists and mixed agonists/antagonists in their ability to influence GRIP1 recruitment by AR.

2. Materials and methods

2.1. Materials

Chemicals were obtained from Sigma (St. Louis, MO, USA). Testosterone (T) was purchased from Makor Chemicals (Jerusalem, Israel). Non-steroidal antiandrogen bicalutamide (BCA) [Casodex, (2*RS*)-4′-cyano-3-(4-fluorophenylsulfonyl)-2-hydroxy-2-methyl-3′-(trifluoromethyl) propionanilide] and hydroxyflutamide (OH-Flu) (4-hydroxy-α,α,α-trifluoro-2-methyl-4′-nitro-*m*-propionotoluidide) were gifts from Zeneca Pharmaceuticals (Macclesfield, UK) and Schering Corp. (Bloomfield, NJ, USA), respectively. Cyproterone acetate (CPA) (6-chloro-1,2-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione-acetate) was from Schering AG (Berlin, Germany). All cell culture reagents were from Gibco Life Technologies (Grand Island, NY, USA). Enhanced green fluorescent protein (EGFP)-AR, its mutants, pSG5-hAR, and pPB(−285/+32)-LUC have been described [18,19]. pSG5-GRIP1 was a gift from Dr. Michael Stallcup (University of Southern California, Los Angeles, CA, USA). pCMVβ was from Clonetech (Palo Alto, CA, USA).

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2.2. Transactivation assays

COS-1 cells (from American Type Culture Collection, ATCC, Ma-

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nassas, VA, USA) were maintained in Dulbecco's minimal essential medium containing penicillin (25 U/ml), streptomycin (25 U/ml), and 10% fetal bovine serum (FBS). LNCaP cells (ATCC) were cultured in RPMI 1640 supplemented with penicillin (25 U/ml), streptomycin (25 U/ml), 2 mM glutamine and 10% FBS. Transfections were performed using FuGENE 6 reagent (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. In brief, 30×10^3 COS-1 cells were seeded on 12-well plates 24 h before transfection. Four hours before transfection, the cells received fresh medium containing 10% charcoal-stripped (CS) FBS, and were transfected 200 ng of reporter plasmid, 20 ng of pCMVβ and 20 ng of pSG5-AR with or without 200 ng of pSG5-GRIP1. Eighteen hours after transfection, the cells received fresh medium containing 2% CS FBS with indicated concentrations of T and/or antiandrogen. After a 30-h culture, the cells were harvested, lysed in reporter lysis buffer (Promega, Madison, WI, USA), and the luciferase (LUC) and β-galactosidase activities were assayed as described [9].

2.3. Microscopic inspection of AR and GRIP1 distribution

COS-1 cells grown on glass coverslips (155×10^3 cells/well) on 6-well plates were transfected with FuGENE reagent with 750 ng of EGFP-AR expression plasmid with or without pSG5-GRIP1, and total DNA amount was balanced to 1.5 μg with empty pSG5. Twenty-four hours after transfection, the cells received fresh medium with 2% CS FBS containing vehicle, T or antiandrogen for 24 h, after which they were fixed with paraformaldehyde (4% in PBS) and permeabilized with Triton X-100 [18]. For hormone withdrawal experiments, the cells received T for 8 h, after which the cultures were washed four times with warm hormone-free medium and maintained in this medium for additional 15 h until fixed. Immunofluorescence labeling was performed with Rhodamine-Red-X-conjugated anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) recognizing monoclonal mouse anti-GRIP1 (a generous gift from Dr. Myles Brown, Dana-Farber Cancer Institute, Boston, MA, USA). Fluorescence analysis was carried out with Bio-Rad MRC-1024 confocal laser system (488 nm excitation for EGFP and 568 nm for rhodamine) connected to Zeiss Axiovert 135 M microscope using a 63×, 1.4 numerical aperture oil immersion objective. Both channels were excited and collected separately and merged for the inspection of colocalization.

3. Results

3.1. Subcellular distribution of AR and GRIP1 and the effect of androgen

AR containing N-terminal fusion to EGFP and GRIP1 were expressed in COS-1 cells, and their subcellular distribution was examined by confocal laser microscopy. In the absence of androgen, AR resides both in the cytoplasm and the nucleus (Fig. 1A). Exposure to 100 nM T brings about complete nuclear transport of AR, and holo-AR shows a pattern comprising hundreds of fine nuclear granules that are distributed throughout the nucleus but excluded from the nucleoli (Fig. 1B). A lower concentration of T (10 nM) elicits the same effect (see Fig. 3). EGFP-fusion part does not influence the subcellular distribution or the amount of the receptor, since non-tagged AR transfected into COS-1 cells, detected with anti-AR antibody, forms similar small foci that are scattered throughout the nucleoplasm but not in the nucleoli (data not shown). Importantly, also endogenous AR in LNCaP cells displays a nuclear distribution that is comparable to that of EGFP-AR (Fig. 1G).

When transfected alone, GRIP1 localizes exclusively in nuclei and resides in round granules that are considerably larger in size and fewer in number than the AR granules (Fig. 1C). The distribution of the scarce nuclear apo-AR population is distinct from that of GRIP1 (Fig. 1D). Interestingly, agonist-occupied AR recruits GRIP1 to redistribute from tens of foci to hundreds of small granules that now colocalize with AR (Fig. 1E). Addition of T in the absence of AR does not influence of the distribution pattern of GRIP1 (data not shown). Removal of androgen from the culture medium has previously been shown to bring about export of AR to the cytoplasm [18,21], and multiple rounds of nucleocytoplasmic recycling of the receptor has been observed in COS-1 cells under conditions of inhibited protein synthesis [21]. Androgen withdrawal breaks down the colocalization of AR and GRIP1 and permits GRIP1 to return to its original distribution pattern (cf. Fig. 1C,F). Thus, the formation of subnuclear complexes containing colocalized AR and GRIP1 is a reversible event that is dependent on receptor occupancy. However, since protein synthesis was not inhibited in this experiment, some of the observed molecules may also represent newly synthesized ones.

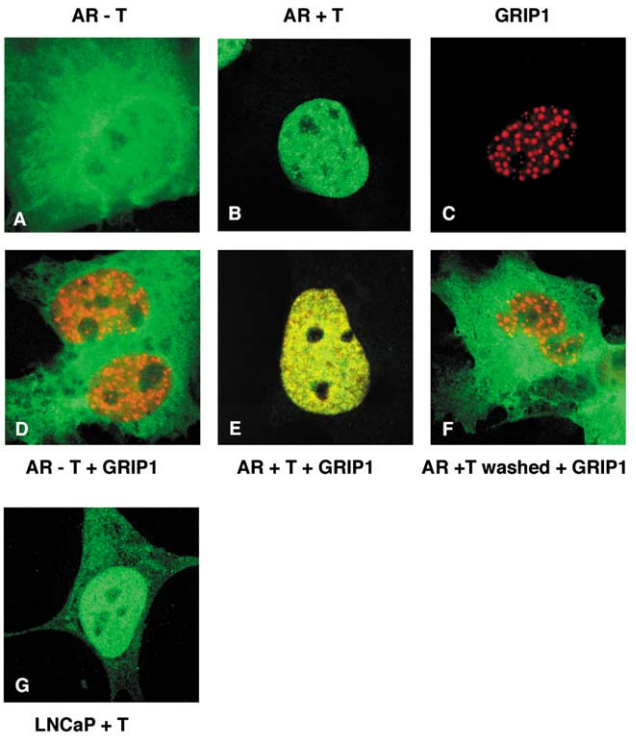

Fig. 1. Holo-AR rearranges nuclear distribution of GRIP1 in COS-1 cells. COS-1 cells grown on coverslips on 6-well plates were transfected with expression vectors encoding pEGFP-AR or without pSG5-GRIP1. After transfection, cells were cultured in 2% CS FBS and exposed to 100 nM T for 24 h as indicated. For immunofluorescence of fixed cells, GRIP1 recognized by monoclonal anti-GRIP1 antibody was detected with Rhodamine-Red-X-labeled secondary antibody. No staining was seen in the absence of the transfected GRIP1 or the primary antibody (data not shown). Cells were analyzed by using Bio-Rad MRC-1024 confocal laser scanning system connected to a Zeiss Axiovert 135M microscope. Images were collected separately for each channel (EGFP at 488 nm and rhodamine at 568 nm excitation) and merged as indicated. A,B: EGFP-AR in the absence and in the presence of T, respectively. C: GRIP1 in the presence of T without cotransfected EGFP-AR. D,E: GRIP1 with cotransfected EGFP-AR in the absence and in the presence of T, respectively. F: EGFP-AR and GRIP1 15 h after T removal. G: Distribution of endogenous AR in LNCaP cells, as detected by using an AR-specific antibody [20] and FITC-conjugated anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories). The LNCaP cells were treated with 100 nM T for 18 h prior to fixation. Endogenous GRIP1 in LNCaP cells was not detectable with the monoclonal anti-GRIP1 antibody.

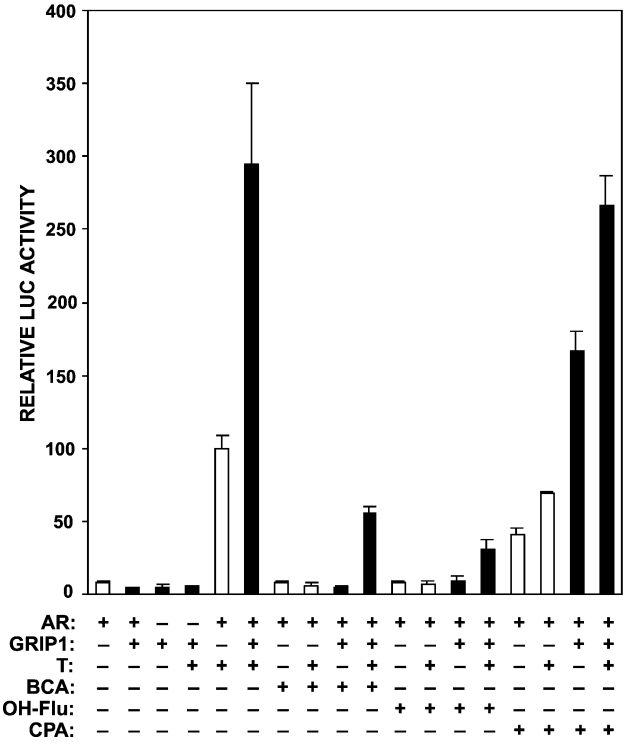

Fig. 2. The pure antagonists OH-Flu and BCA, but not the partial antagonist/agonist CPA, blunt the effect of GRIP1 on AR-dependent transcription. COS-1 cells grown on 12-well plates were transfected with 20 ng of pSG5-AR, 20 ng of pCMVβ, 160 ng of pPB(−285/+32)-LUC with or without 200 ng of pSG5-GRIP1. Twenty-four hours after transfection, the cells received 2% CS FBS containing T (10 nM), OH-Flu (1 μM), BCA (1 μM) or CPA (1 μM), as depicted by the + and − signs, and were cultured for additional 24 h. LUC activities in the cell extracts were adjusted to transfection efficiency using β-galactosidase activity. The activity of wild-type AR in the presence of 10 nM T is set as 100 and the mean ± S.E.M. values of three independent experiments with triplicate samples are shown.

3.2. *Antiandrogens modulate the activation of AR-dependent transcription by GRIP1*

To examine the effect of different antiandrogens on the activation of AR-dependent transcription by GRIP1, COS-1 cells were transfected with AR and GRIP1 expression vectors together with a LUC reporter driven by the natural probasin promoter [pPB(-285/+32)-LUC]. Exposure to T (10 nM) activates the reporter gene by >10-fold, and ectopic expression of GRIP1 increases AR-dependent transcription by ~3-fold, without influencing reporter gene activity in the absence of androgen or AR (Fig. 2). The non-steroidal antiandrogens BCA (1 μM) and OH-Flu (1 μM) are incapable of activating transcription even in the presence of GRIP1, whereas in cells supplemented with CPA (1 μM), GRIP1 increases AR-mediated transcription by 4.5-fold. A 100-fold molar excess of OH-Flu over T (1 μM vs. 10 nM) renders AR transcriptionally inert without ectopic GRIP1 and abolishes ≥90% of the reporter gene activity measured in the presence of GRIP1 (Fig. 2). BCA is somewhat less potent than OH-Flu in inhibiting GRIP1-dependent activation of AR function. The mixed antagonist/agonist CPA, in turn, fails to inhibit significantly the function of agonist-bound AR under the same conditions (Fig. 2).

3.3. *Antiandrogens influence nuclear colocalization of AR with GRIP1*

To examine whether subnuclear localization of AR and GRIP1 is reorganized by antiandrogens in a fashion that complies with the transactivation events described above, EGFP-AR was coexpressed with GRIP1 in COS-1 cells, and the effects of antiandrogens on their distribution were visualized. Even though pure antagonists OH-Flu and BCA are known to bring about translocation of AR from cytoplasm to nuclei [18,22], AR complexed to these ligands fails to recruit GRIP1 in the nucleoplasm, as there is no redistribution of the coac-

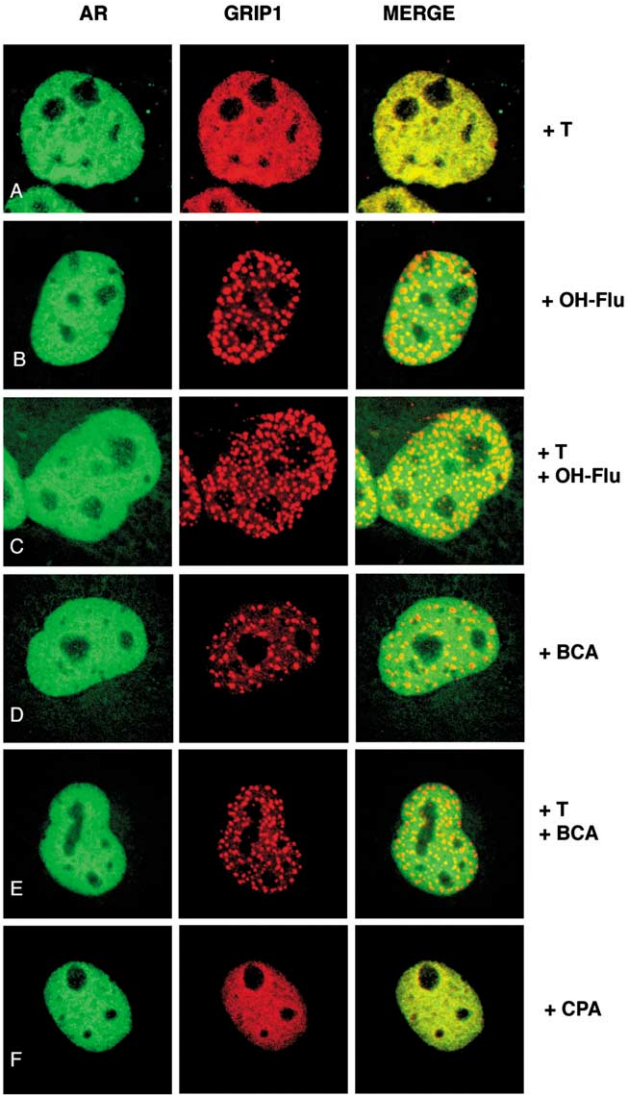

Fig. 3. Pure antiandrogens disrupt the colocalization of AR and GRIP1 in the nucleoplasm. COS-1 cells grown on coverslips on 6-well plates were transfected with 0.75 μg of pEGFP-AR and 0.75 μg of pSG5-GRIP1 expression plasmids. Twenty-four hours after transfection, the cells received fresh medium containing 2% CS FBS with (A) 10 nM T, (B) 1 μM OH-Flu, (C) 10 nM T with 1 μM OH-Flu, (D) 1 μM BCA, (E) 10 nM T with 1 μM BCA, or (F) 1 μM CPA. After a 24-h incubation, the cells were fixed and GRIP1-detected by using monoclonal anti-GRIP1 antibody that, in turn, was recognized by Rhodamine-Red-X-conjugated secondary antibody. Three-hour treatment with BCA or OH-Flu is sufficient to break down the colocalization of holo-AR and GRIP1 (data not shown). Cells were analyzed and images were captured separately for EGFP and rhodamine as described in Fig. 1.

tivator (Fig. 3B,D). Importantly, an excess of OH-Flu or BCA efficiently prevents T-induced redistribution of GRIP1 by AR in nuclei (Fig. 3C,E). Nuclear distribution of AR and GRIP1 in the presence of CPA is comparable to that seen with T, and CPA does not elicit any further redistribution of AR or GRIP1 in the nucleoplasm when present together with androgen (Fig. 3F and data not shown). In sum, antiandrogens regulate nuclear redistribution of AR and GRIP1 in a fashion commensurate with their effects on transactivation, in that only pure antiandrogens that abolish activation of AR function by GRIP1 also prevent GRIP1 from colocalizing to the same nuclear foci as AR.

4. Discussion

We have used confocal laser microscopy to study the intracellular dynamics of AR and its transcriptional coactivator GRIP1. This study documents, for the first time, at a single cell level that pure antiandrogens OH-Flu and BCA are able to disrupt the colocalization of AR with GRIP1. The AR–GRIP1 association in nuclei was shown to occur as an agonist-induced recruitment of GRIP1 by AR, i.e. the nuclear distribution of GRIP1 was rearranged from discrete round foci to very fine granular pattern colocalizing with AR. Even though OH-Flu and BCA promoted nuclear translocation of the cytoplasmic AR, they did not permit AR–GRIP1 association to take place, but were capable of disrupting the agonist-induced colocalization. By contrast, the partial agonist/antagonist CPA promoted colocalization of AR and GRIP1. These results are in line with the behavior of the antihormones in transactivation assays; CPA elicited transcriptionally productive AR–GRIP1 interaction, whereas pure antagonists OH-Flu and BCA were not only inactive in this respect but also capable of blocking the effect of GRIP1 on AR-dependent transcription.

Our results are reminiscent of the ligand-specific recruitment of fluorescent protein-fused GRIP1 to estrogen receptor α (ERα)-containing subnuclear domains, in that estradiol and the partial agonist hydroxytamoxifen, but not the pure antagonist ICI 182 780, elicited recruitment of GRIP1 [23]. Colocalization of coactivator SRC-1 with ERα to nuclear matrix-bound foci has been similarly shown to occur in response to estradiol but not to antagonists [24]. However, the behavior of AR with p160 coactivator proteins cannot be inferred from these findings, as many of the molecular features and interactions of AR differ critically from those of ER [3–9]. While this paper was finalized for publication, Saitoh et al. reported agonist-dependent colocalization of fluorescent protein (YFP)-tagged TIF2 with AR in COS-7 cells [25], but the effects of antihormones on the colocalization were not addressed in their study. In contrast to the untagged GRIP1 used in this work, the YFP-TIF2 showed a diffuse nuclear distribution pattern when expressed alone or with apo-AR. The fluorescent protein tag in TIF2 may influence the nuclear distribution of the protein and thus, at least in part, explain this difference.

Nuclear organization appears to provide the architectural framework for the control of transcription [26]. Active transcription has been demonstrated to occur at numerous spatially distinct foci that are associated with the nuclear matrix. Nuclear organization is dynamic [27], and also steroid receptors are continuously attaching to and detaching from the chromatin [28,29]. As shown in the present and two recent studies [21,30], transcriptionally active agonist-occupied AR localizes into a large number (typically 250–400) of nuclear foci that, according to Tomura et al. [30], reside in the boundary region between euchromatin and heterochromatin. The nuclear substructures to which AR and GRIP1 localize are largely unknown, although AR has been shown to be attached to the nuclear matrix [18,21,31], and a granular subpopulation of GRIP1 has been proposed to associate with the ND10 nuclear domains (promyelocytic leukemia (PML) bodies) [32]. The latter domains appear to contain several proteins, including PML gene product and the CREB-binding protein. AR does not colocalize with PML, but interestingly, RNA polymerase II is distributed to hundreds of small foci resembling the holo-AR-containing speckles (our unpublished results), and it is conceivable that these foci represent 'maturation' sites for transcriptionally competent holo-AR-coactivator complexes.

Despite the fact that antiandrogens have been under intense investigation, the exact molecular mechanism of their action and especially the individual steps in AR signaling that they perturb have remained elusive. Many different steps in AR function – from transactivation to AR mRNA and protein stability, nucleocytoplasmic trafficking, homodimerization, DNA binding, and receptor phosphorylation – are targeted by antiandrogens [18,20,22,33,34]. The physiological role of nuclear receptor corepressors in the function of various antiandrogens is currently unclear. Recently, Dotzlaw et al. [35] detected binding of SMRT to AR complexed with CPA, but not in the presence of BCA or OF-Flu, whereas according to Shang et al. [36], BCA-bound AR indeed recruits both SMRT and N-CoR. However, we have been unable to detect CPA-, BCA- or OH-Flu-induced interaction between AR (fused to Gal4 DNA-binding domain) and SMRT (fused to VP16 activation domain) in COS-1 cells by using two-hybrid assays (our unpublished results).

Even though the nuclear distribution pattern of endogenous GRIP1 may differ from that of the transfected protein, our findings indicate clearly that the pure antiandrogens OH-Flu and BCA, but not the partial agonist/antagonist CPA, can attenuate AR function by releasing GRIP1 – probably the related p160 nuclear receptor coactivators as well – from the receptor-containing subnuclear sites. These data have important implications in the development of next generation AR antagonists and novel therapeutic modalities for recurrent prostate cancer in which GRIP1 is often overexpressed [37].

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References

[1] Quigley, C.A., De Bellis, A., Marschke, K.B., el-Awady, M.K., Wilson, E.M. and French, F.S. (1995) Endocr. Rev. 16, 271–321.
[2] Beato, M., Herrlich, P. and Schütz, G. (1995) Cell 83, 851–857.
[3] Moilanen, A., Rouleau, N., Ikonen, T., Palvimo, J.J. and Jänne, O.A. (1997) FEBS Lett. 412, 355–358.
[4] Ma, H., Hong, H., Huang, S.M., Irvine, R.A., Webb, P., Kush-

ner, P.J., Coetzee, G.A. and Stallcup, M.R. (1999) Mol. Cell. Biol. 19, 6164–6173.
[5] Alen, P., Claessens, F., Verhoeven, G., Rombauts, W. and Peeters, B. (1999) Mol. Cell. Biol. 19, 6085–6097.
[6] He, B., Kemppainen, J.A. and Wilson, E.M. (2000) J. Biol. Chem. 275, 22986–22994.
[7] Langley, E., Zhou, Z.X. and Wilson, E.M. (1995) J. Biol. Chem. 270, 29983–29990.
[8] Doesburg, P., Kuil, C.W., Berrevoets, C.A., Steketee, K., Faber, P.W., Mulder, E., Brinkmann, A.O. and Trapman, J. (1997) Biochemistry 36, 1052–1064.
[9] Ikonen, T., Palvimo, J.J. and Jänne, O.A. (1997) J. Biol. Chem. 272, 29821–29828.
[10] Leo, C. and Chen, J.D. (2000) Gene 245, 1–11.
[11] Bevan, C.L., Hoare, S., Claessens, F., Heery, D.M. and Parker, M.G. (1999) Mol. Cell. Biol. 19, 8383–8392.
[12] Heery, D.M., Kalkhoven, E., Hoare, S. and Parker, M.G. (1997) Nature 387, 733–736.
[13] He, B., Kemppainen, J.A., Voegel, J.J., Gronemeyer, H. and Wilson, E.M. (1999) J. Biol. Chem. 274, 37219–37225.
[14] Slagsvold, T., Bentzen, T., Kraus, I., Palvimo, J.J. and Saatcioglu, F. (2000) Mol. Endocrinol. 14, 1603–1617.
[15] Thompson, J., Saatcioglu, F., Jänne, O.A. and Palvimo, J.J. (2001) Mol. Endocrinol. 15, 923–935.
[16] Kallio, P.J., Jänne, O.A. and Palvimo, J.J. (1994) Endocrinology 134, 998–1001.
[17] Kuil, C.W., Berrevoets, C.A. and Mulder, E. (1995) J. Biol. Chem. 270, 27569–27576.
[18] Poukka, H., Karvonen, U., Yoshikawa, N., Tanaka, H., Palvimo, J.J. and Jänne, O.A. (2000) J. Cell Sci. 113, 2991–3001.
[19] Palvimo, J.J., Reinikainen, P., Ikonen, T., Kallio, P.J., Moilanen, A. and Jänne, O.A. (1996) J. Biol. Chem. 271, 24151–24156.
[20] Karvonen, U., Kallio, P.J., Jänne, O.A. and Palvimo, J.J. (1997) J. Biol. Chem. 272, 15973–15979.
[21] Tyagi, R.K., Lavrovsky, Y., Ahn, S.C., Song, C.S., Chatterjee, B. and Roy, A.K. (2000) Mol. Endocrinol. 14, 1162–1174.
[22] Jenster, G., Trapman, J. and Brinkmann, A.O. (1993) Biochem. J. 293, 761–768.
[23] Schaufele, F., Chang, C.Y., Liu, W., Baxter, J.D., Nordeen, S.K., Wan, Y., Day, R.N. and McDonnell, D.P. (2000) Mol. Endocrinol. 14, 2024–2039.
[24] Stenoien, D.L., Mancini, M.G., Patel, K., Allegretto, E.A., Smith, C.L. and Mancini, M.A. (2000) Mol. Endocrinol. 14, 518–534.
[25] Saitoh, M., Takayanagi, R., Goto, K., Fukamizu, A., Tomura, A., Yanase, T. and Nawata, H. (2002) Mol. Endocrinol. 16, 694–706.
[26] Dundr, M. and Misteli, T. (2001) Biochem. J. 356, 297–310.
[27] Misteli, T. (2001) Science 291, 843–847.
[28] McNally, J.G., Muller, W.G., Walker, D., Wolford, R. and Hager, G.L. (2000) Science 287, 1262–1265.
[29] Stenoien, D.L., Patel, K., Mancini, M.G., Dutertre, M., Smith, C.L., O'Malley, B.W. and Mancini, M.A. (2001) Nat. Cell Biol. 3, 315–323.
[30] Tomura, A., Goto, K., Morinaga, H., Nomura, M., Okabe, T., Yanase, T., Takayanagi, R. and Nawata, H. (2001) J. Biol. Chem. 276, 28395–28401.
[31] van Steensel, B., Jenster, G., Damm, K., Brinkmann, A.O. and van Driel, R. (1995) J. Cell Biochem. 57, 465–478.
[32] Baumann, C.T., Ma, H., Wolford, R., Reyes, J.C., Maruvada, P., Lim, C., Yen, P.M., Stallcup, M.R. and Hager, G.L. (2001) Mol. Endocrinol. 14, 485–500.
[33] Wang, L.G., Liu, X.M., Kreis, W. and Budman, D.R. (1999) Biochem. Biophys. Res. Commun. 259, 21–28.
[34] Waller, A.S., Sharrard, R.M., Berthon, P. and Maitland, N.J. (2000) J. Mol. Endocrinol. 24, 339–351.
[35] Dotzlaw, H., Moehren, U., Mink, S., Cato, A.C., Iniguez Lluhi, J.A. and Baniahmad, A. (2002) Mol. Endocrinol. 16, 661–673.
[36] Shang, Y., Myers, M. and Brown, M. (2002) Mol. Cell 9, 601–610.
[37] Gregory, C.W., He, B., Johnson, R.T., Ford, O.H., Mohler, J.L., French, F.S. and Wilson, E.M. (2001) Cancer Res. 61, 4315–4319.