Advances in Brief

A Mechanism for Androgen Receptor-mediated Prostate Cancer Recurrence after Androgen Deprivation Therapy[1]

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Abstract

The development and growth of prostate cancer depends on the androgen receptor and its high-affinity binding of dihydrotestosterone, which derives from testosterone. Most prostate tumors regress after therapy to prevent testosterone production by the testes, but the tumors eventually recur and cause death. A critical question is whether the androgen receptor mediates recurrent tumor growth after androgen deprivation therapy. Here we report that a majority of recurrent prostate cancers express high levels of the androgen receptor and two nuclear receptor coactivators, transcriptional intermediary factor 2 and steroid receptor coactivator 1. Overexpression of these coactivators increases androgen receptor transactivation at physiological concentrations of adrenal androgen. Furthermore, we provide a molecular basis for this activation and suggest a general mechanism for recurrent prostate cancer growth.

Introduction

Prostate cancer is a major cause of cancer-related deaths in American men. In its initial stages, prostate cancer is responsive to androgen deprivation therapy achieved by surgical or medical castration, reflecting a dependence on androgen in tumor cell proliferation in early stage disease. However, subsequent to androgen deprivation therapy, prostate cancers recur and progress to a terminal stage despite reduced circulating testosterone. The AR[3], a member of the steroid receptor family that is activated by testicular androgens, is the major regulatory transcription factor in normal prostate growth and development and in the growth of androgen-dependent prostate cancer. The AR may also contribute to prostate cancer growth during its recurrence in the androgen-deprived patient. A role for AR-mediated gene activation in recurrent prostate cancer is supported by its expression (1, 2) together with the expression of androgen-regulated genes (3). Possible mechanisms for AR reactivation in recurrent prostate cancer include altered growth factor-induced phosphorylation (4–8) and AR mutations (9) that broaden ligand specificity (10). AR gene amplification was observed after androgen depriva-

tion in 30% of recurrent prostate cancers (11). AR overexpression is associated with increased sensitivity to the growth-stimulating effects of low androgen concentrations in recurrent prostate cancer-derived cell lines (12, 13).

Materials and Methods

Immunohistochemistry. Prostate specimens were acquired in compliance with the guidelines of the University of North Carolina at Chapel Hill Clinical Cancer Protocol Review Committee and Institutional Review Board (Chapel Hill, NC). Histological diagnoses were verified by examination of frozen and corresponding formalin-fixed, paraffin-embedded tissues. Samples of BPH from eight men [mean age, 65 (range 58–71) years] and androgen-dependent prostate cancer from eight men [mean age, 59 (range 49–71) years] were obtained from the transition zone and palpable tumor nodules, respectively, in radical prostatectomy specimens. Gleason sum grade was 6 in six men and 7 in two men, and mean serum prostate-specific antigen was 26 (range, 3.5–93) ng/ml. Recurrent prostate cancer was obtained by transurethral resection in eight men who developed urinary retention from a local recurrence of prostate cancer 57 months (mean; range 12–162 months) after androgen deprivation by surgical (four men) or medical (four men) castration. Recurrent tumors were poorly differentiated (Gleason sum, 8 in two men, 9 in four men, and 10 in two men) and serum prostate-specific antigen was 97 ng/ml (mean; range, 0.3–177 ng/ml) despite low serum testosterone (<70 ng/ml) in all of the men. AR gene coding sequence for the prostate cancer samples was wild-type based on denaturing gradient gel electrophoresis and single-strand conformation polymorphism analysis for the entire sequence and direct sequencing of the ligand binding domain (14).

Formalin-fixed, paraffin-embedded sections of BPH and androgen-dependent and recurrent human prostate cancer and CWR22 human tumors propagated in nude mice were antigen-retrieved by heating at 100°C for 30 min in a vegetable steamer using CITRA or AR 10 buffer (BioGenex Laboratories, San Ramon, CA) and cooled for 10 min. Slides were preincubated with 2% normal horse serum for 5 min at 37°C and washed with automation buffer (Fisher Scientific International, Inc., Pittsburgh, PA). Slides were incubated with antihuman monoclonal antibodies for TIF2, SRC1, and amplified in breast cancer-1 (Transduction Laboratories, Lexington, KY) or AR (BioGenex Laboratories) at 1:300 dilutions followed by incubation with antimouse peroxidase-linked secondary antibody at 1:200 dilution. Immunoperoxidase reaction products were detected using diaminobenzidine.

Immunoblot Analysis. Frozen patient samples and CWR22 tumors (50–100 μg) were pulverized in liquid nitrogen, thawed on ice, and mixed with 1 ml of radioimmunoprecipitation assay buffer containing protease inhibitors [0.15 M NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 5 mM EDTA, 50 mM Tris (pH 7.4), 0.5 mM phenylmethylsulfonyl fluoride, 10 μM pepstatin, 4 μM aprotinin, 80 mg/ml leupeptin, 0.2 mM sodium vanadate, and 5 mM benzamidine] and 1 μM DHT. Tissue was homogenized for 30 s on ice, incubated for 30 min on ice, and centrifuged at $10,000 \times g$ for 20 min twice. Supernatant proteins (50 μg) were electrophoresed on 12% acrylamide gels containing SDS and electroblotted to Immobilon-P membranes (Millipore Corp., Bedford, MA). Immunoblot analysis was performed (3) using the antibodies described above and mouse monoclonal anti-progesterone receptor (Affinity Bioreagents, Golden, CO) and rabbit polyclonal anti-estrogen receptor α (Affinity Bioreagents).

Transcription Assays. AR transcriptional assays were performed in the presence or absence of TIF2 overexpression by measuring luciferase activity in

[3] The abbreviations used are: AR, androgen receptor; TIF2, transcriptional intermediary factor 2; SRC1, steroid receptor coactivator 1; BPH, benign prostatic hyperplasia; DHEA, dehydroepiandrosterone; AF2, activation function 2; GST, glutathione-*S*-transferase; DHT, dihydrotestosterone; LxxLL,L,Leucine,x,any amino acid.

CV1 cells cotransfected with pCMVhAR (100 ng), wild-type or mutant pCMVhAR507–919 (50 ng), pSG5TIF2 (3 μg), and mouse mammary tumor virus-luciferase reporter vector (5 μg) using calcium phosphate precipitation (15). The absence of the metabolism of androstenedione to testosterone was verified by high-pressure liquid chromatography of CV1 cell extracts.

GST Adsorption Studies. GST adsorption incubations were performed (16) using *in vitro*-translated ^{35}S-labeled AR ligand binding domain (amino acid residues 624–919) and bacterial-expressed GST fusion proteins GST-AR1-333 and GST-TIF2 fusion protein containing TIF2 amino acid residues 1143–1464 designated GST-TIF2-M in the presence of 0.2 μM steroids. Equivalent amounts of GST-AR1-333 and GST-TIF2 protein (~2 μg based on Coomassie Blue staining) were loaded on the gels. Approximately 4 μg of the GST-0 control protein was loaded to ensure the absence of nonspecific binding. Autoradiographic signal intensities in the absence of steroid were essentially identical to the GST-0 control that lacked the AR or TIF2 sequence, as shown previously (15, 16).

Results and Discussion

We investigated the role of AR and three nuclear coactivators in recurrent prostate cancer by comparing expression levels in tissue specimens of BPH, androgen-dependent prostate cancer, and recurrent prostate cancer after androgen deprivation therapy. Immunoperoxidase staining of TIF2 was more intense in the nuclei of recurrent prostate cancer than in androgen-dependent prostate cancer or BPH (Fig. 1, *b–d*). BPH served as the control for prostate cancer because it is more common than normal prostate tissue in men ≥45 years of age. Tissue morphology showed the characteristic glandular structure of BPH, the small, closely spaced glands of early androgen-dependent prostate cancer, and the poor differentiation of recurrent prostate cancer (Fig. 1). Immunostaining for SRC1 was also more intense in recurrent prostate cancer than in BPH or androgen-dependent prostate cancer (Fig. 1, *f–h*). In contrast, no immunostaining was detected for the nuclear receptor coactivator amplified in breast cancer-1 (data not shown). Immunostaining also indicated high AR expression in recurrent prostate cancer (Fig. 1, *j–l*), and this was supported by immunoblot analysis (Fig. 2, *a–c*). Immunoblots confirmed the striking overexpression of TIF2 in six of eight recurrent cancers (Fig. 2*c*) relative to its barely detectable levels in eight specimens of BPH (Fig. 2*a*) and
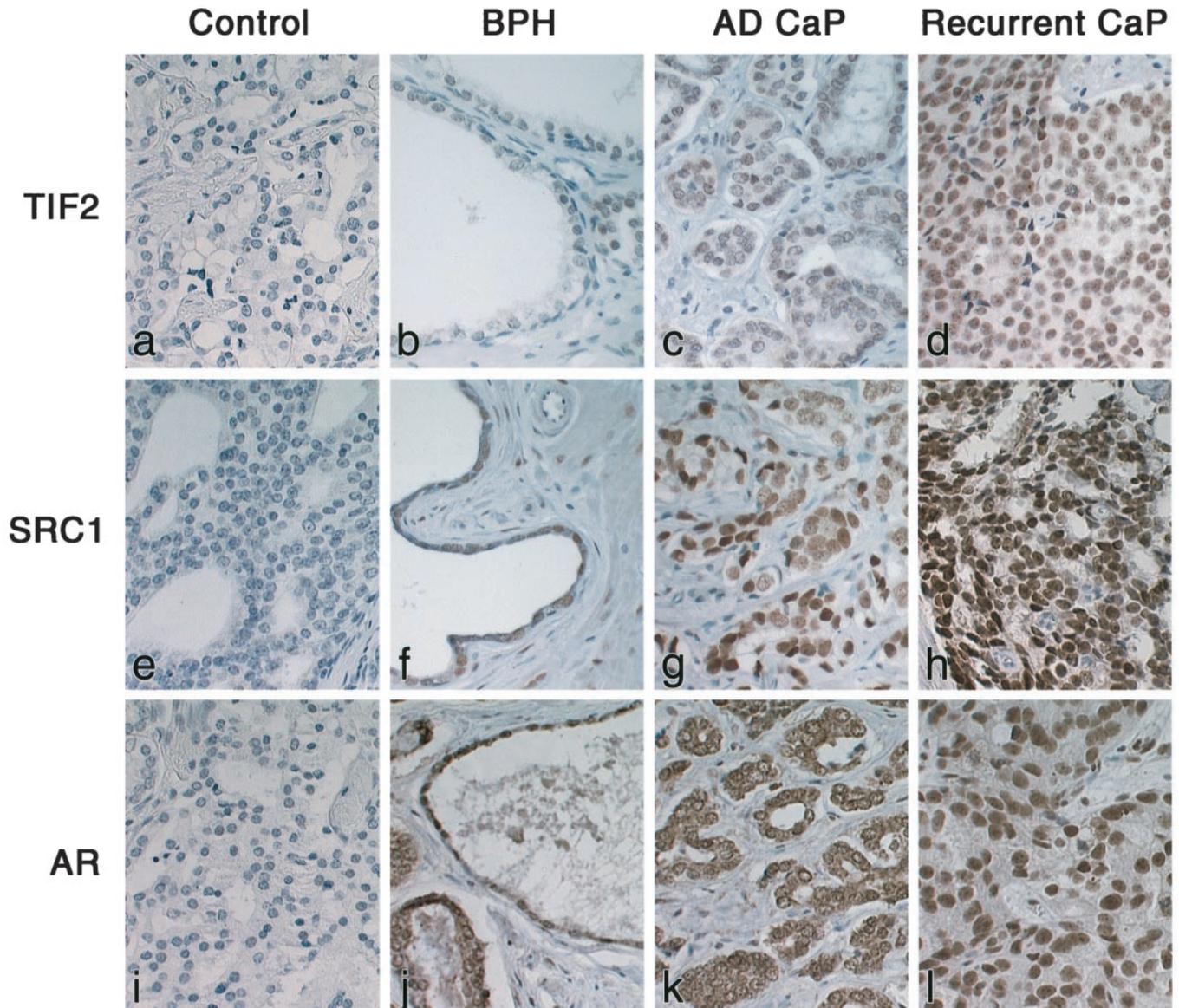

Fig. 1. TIF2, SRC1, and AR immunoperoxidase staining in human prostate tissue. *a, e,* and *i,* androgen-dependent prostate cancer negative controls lacking primary antibody. *b, f,* and *j,* BPH tissue. *c, g,* and *k,* androgen dependent (*AD*) prostate cancer (*CaP*). *d, h,* and *l,* recurrent CaP. ×200.
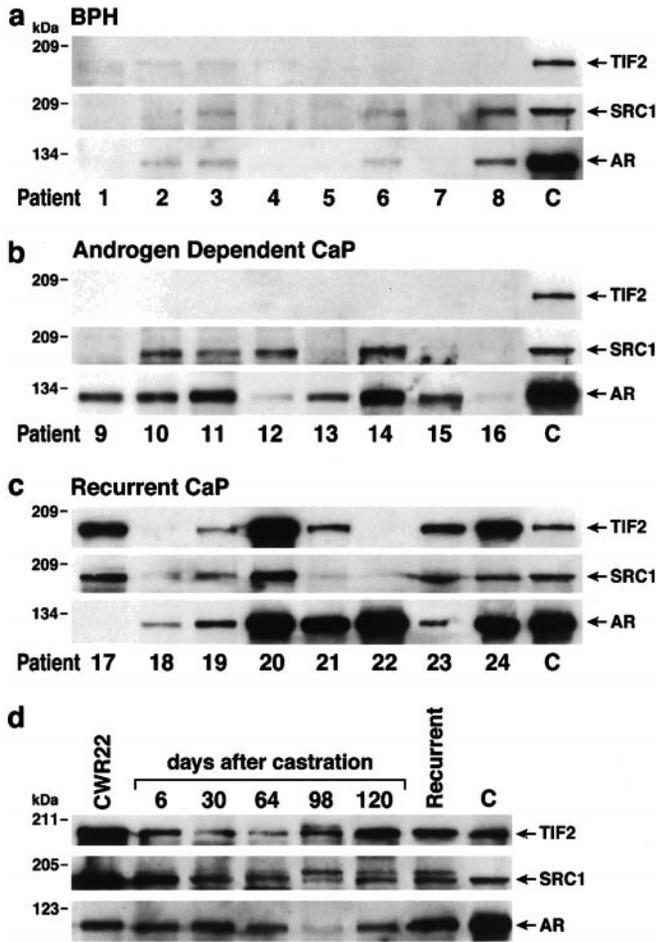
Fig. 2. Immunoblot analysis of TIF2, SRC1, and AR in human prostate tissue. Immunoblotting was performed with TIF2, SRC1, and AR antibodies and lysates from BPH specimens from 8 patients (*a*) androgen-dependent prostate cancer specimens from eight patients (*b*); recurrent human prostate tumor specimens from eight patients (*c*), and CWR22 human prostate xenograft tissue from intact nude mice (CWR22; *d*) and at different stages of tumor progression after the indicated days from castration. Recurrent CWR22 tumor (*recurrent*) was harvested 150 days after castration. Vertical set of samples within *a*, *b*, or *c* were from the same patient. *C*, control protein extracts from monkey kidney COS cells expressing pSG5TIF2, pSG5SRC1, or pCMVhAR. Migration positions of molecular weight standard proteins are indicated in kilodaltons (*kDa*) on the *left*.

eight androgen-dependent prostate cancer tissue lysates (Fig. 2*b*). SRC1 overexpression was evident by immunoblotting in five of eight recurrent cancers (Fig. 2*c*). Thus, a majority of recurrent cancers had high levels of TIF2 and/or SRC1. The increase in TIF2 in recurrent prostate cancer was greater than that of SRC1, inasmuch as SRC1 was also detected in BPH and androgen-dependent prostate cancer. In control immunoblots, we did not detect progesterone receptor α or β in prostate tissue specimens, but estrogen receptor α was detected and no major difference noted between BPH and androgen-dependent or recurrent prostate cancer (data not shown).

We analyzed TIF2 and SRC1 expression in the CWR22 human prostate cancer xenograft that retains the growth characteristics of human prostate cancers. CWR22 prostate tumors regress after androgen deprivation but recur after several months in the absence of testicular androgens (17, 18). Immunoblots showed decreasing TIF2 levels from 6 to 64 days after androgen deprivation (Fig. 2*d*). TIF2 expression increased at 98 and 120 days after castration, which coincided with the onset of recurrent prostate cancer cell proliferation and paralleled increased AR expression in this model. SRC1 was also increased in the recurrent CWR22 tumor (Fig. 2*d*). The results demonstrate that the levels of TIF2 and SRC1 increase with AR expres-

sion during the recurrent growth of prostate cancer cells after androgen deprivation.

Transient overexpression of TIF2 in cotransfection assays increased AR-mediated transactivation of a mouse mammary tumor virus-luciferase reporter gene in the presence of different steroids (Fig. 3*a*). At concentrations of 10^{-9} M, androstenedione, estradiol, and progesterone became potent activators of AR in the presence of TIF2. This concentration of androstenedione is within the physiological range of adrenal androgen in the peripheral blood of human males. Transactivation induced by 10^{-7} M DHEA was also increased by TIF2 expression.

The AR DNA and ligand binding domain fragment AR507–919 lacks substantial androgen-dependent transcriptional activity in the presence of DHT or other steroids tested in the absence of overexpressed TIF2 (Fig. 3*b* and data not shown). But with the coexpression of TIF2, AR507–919 is strongly activated by DHT or testosterone
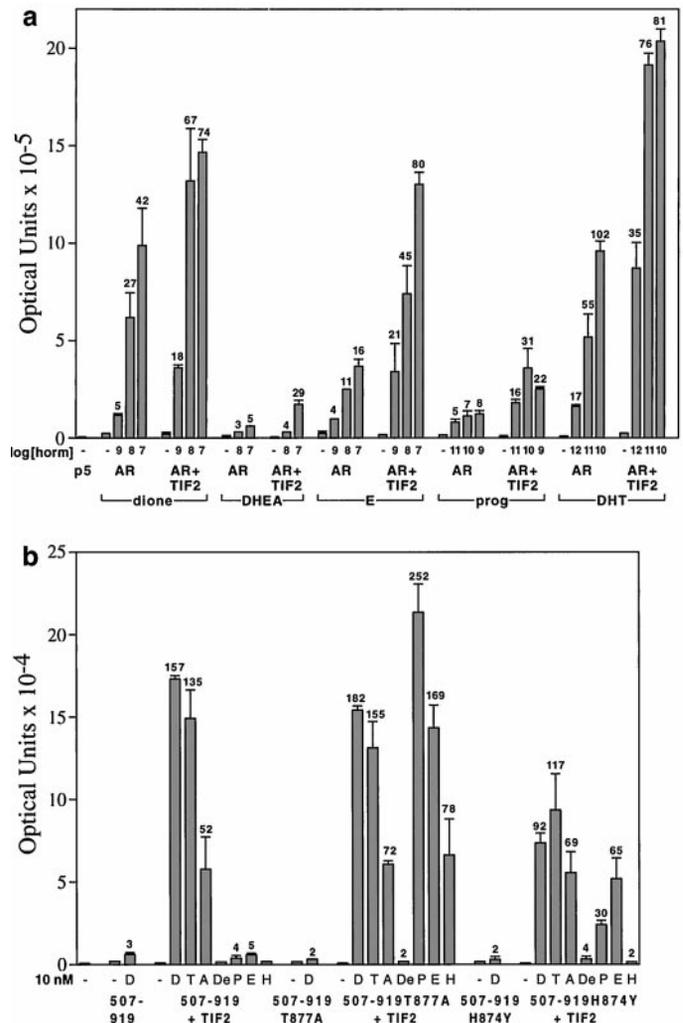

Fig. 3. Effect of TIF2 with AR transcriptional activity. *a,* AR transactivation was determined in the absence and presence of increasing concentrations ($-$log of hormone concentration) of androstenedione (*dione*), DHEA (*DHEA*), 17β-estradiol (*E*), progesterone (*prog*), and DHT (*DHT*) with or without overexpressed TIF2. Fold induction relative to activity in the absence of hormone is shown numerically *above* the bars of a representative of multiple experiments. *b,* transcriptional activity of AR DNA and ligand binding domain fragments with wild-type sequence (*507-919*), the LNCaP cell AR mutation (*507-919T877A*), or CWR22 mutation (*507-919H874Y*) were determined with or without coexpressed TIF2. Activity was determined in the absence and presence of 10 nM DHT (*D*), testosterone (*T*), androstenedione (*A*), dehydroepiandrosterone (*De*), progesterone (*P*), 17β-estradiol (*E*), and hydroxyflutamide (*H*). Fold induction relative to activity determined in the absence of hormone is shown numerically *above* the bars of a representative of multiple experiments.

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(Fig. 3*b*), which supports the observation that an apparently weak interaction of TIF2 with AF2 can be overcome by high TIF2 expression (16). Ten nM androstenedione also increased the transcriptional activity of AR507–919 in the presence of overexpressed TIF2 (Fig. 3*b*) and SRC1 (not shown). Transactivation by ligand binding domain fragment AR507–919 coexpressed with TIF2 required 0.1 nM DHT, 1 nM androstenedione, or 250 nM DHEA (not shown).

The ability of lower-affinity steroids to induce wild-type AR recruitment of TIF2 was confirmed by GST affinity matrix assays. DHEA, estradiol, androstenedione, and progesterone promoted an interaction between ^{35}S-labeled AR ligand binding domain residues 624–919 and GST-TIF2 residues 624-1141 that contain the 3 LxxLL motifs of TIF2 (TIF2-M, Fig. 4, *Lanes 2, 5,* and *8*). However, these lower-affinity steroids were much less effective than DHT in promoting the interaction between the AR ligand binding domain and AR NH$_2$-terminal fragment 1–333 (Fig. 4, *Lanes 3, 6,* and *9*). Thus, in contrast to DHT, which more readily promotes the interaction between the NH$_2$-terminal and carboxyl-terminal regions of AR, the binding of adrenal androgens and other lower-affinity ligands to the AR ligand binding domain favors recruitment of TIF2. The association of overexpressed TIF2 with AR induced by lower affinity ligands provides a mechanism for AR-mediated transactivation in the absence of circulating testosterone that could account for the growth of recurrent prostate cancer in the androgen-deprived patient.

LNCaP prostate cancer AR mutant T877A (19, 20) and CWR22 prostate cancer xenograft AR mutant H874Y (10) have broader ligand specificities than wild-type AR, which contributes to their activation after androgen deprivation. Here we demonstrate that in the presence of overexpressed TIF2, each of these mutations increases the ability of the adrenal androgens, DHEA and androstenedione, as well as other steroids to activate transcription by the DNA and ligand binding domain fragment AR507–919 (Fig. 3*b*). The broadened ligand specificity caused by these and certain other AR mutations could enhance AR binding of adrenal androgens and increase the recruitment of TIF2 under conditions of androgen deprivation and coactivator overexpression.

The results indicate that a majority of recurrent prostate cancers overexpress TIF2 and SRC1 coincidentally with the onset of recurrent prostate cancer growth. TIF2 overexpression increases the responsive-

ness of AR activation by adrenal androgens that may circulate at sufficiently high concentrations to recruit highly expressed coactivators. Under normal physiological conditions, AR transactivation is induced specifically by testosterone or DHT, causing AF2 in the AR ligand binding domain to interact with the NH$_2$-terminal LxxLL-like sequence ^{23}FQNLF^{27} (15, 16, 21, 22). However, the AF2 hydrophobic surface also forms an overlapping binding site for the LxxLL motifs of the p160 coactivators. Under conditions of lower coactivator expression, AF2 would be occupied by the AR NH$_2$-terminal domain that could have the effect of inhibiting p160 coactivator recruitment by AF2 (15). Ligand-induced interaction of AR AF2 with the p160 coactivators requires their overexpression, which results in increased AR transactivation in transient transfection assays (16, 23). In recurrent prostate cancer growing in the absence of testis androgens, increased coactivator expression could facilitate the induction of AR transactivation by lower-affinity steroids such as the adrenal androgens by shifting the equilibrium toward the formation of AR-TIF2 complexes. Increased AR transactivation induced by lower-affinity ligands seems to result from coactivator-induced AR activity rather than from a change in steroid binding affinity, inasmuch as previous studies indicated that TIF2 overexpression does not alter the dissociation rate of bound androgen.[4] High expression of TIF2 and SRC1 in recurrent prostate cancer increases AR transactivation in response to physiological concentrations of adrenal androgens or other steroids with affinity for AR. Coactivation of AR transactivation may be increased further by the phosphorylation of p160 coactivators (24, 25), thereby linking AR with growth factor signaling pathways. The results provide a mechanism for AR-mediated tumor growth in recurrent prostate cancer.

Acknowledgments

References

1. de Vere White, R., Meyers, F., Chi, S. G., Chamberlain, S., Siders, D., Lee, F., Stewart, S., and Gumerlock, P. H. Human androgen receptor expression in prostate cancer following androgen ablation. Eur. Urol., *31:* 1–6, 1997.

2. Ruizeveld de Winter, J. A., Trapman, J., Vermey, M., Mulder, E., Zegers, N. D., and van der Kwast, T. H. Androgen receptor expression in human tissues: an immunohistochemical study. J. Histochem. Cytochem., *39:* 927–936, 1991.

3. Gregory, C. W., Hamil, K. G., Kim, D., Hall, S. H., Pretlow, T. G., Mohler, J. L., and French, F. S. Androgen receptor expression in androgen-independent prostate cancer is associated with increased expression of androgen regulated genes. Cancer Res., *58:* 5718–5724, 1998.

4. Culig, Z., Hobisch, A., Cronauer, M. V., Radmayr, C., Trapman, J., Hittmair, A., Bartsch, G., and Klocker, H. Androgen receptor activation in prostate tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res., *54:* 5474–5478, 1994.

5. Nazareth, L. V., and Weigel, N. L. Activation of the human androgen receptor through a protein kinase A signaling pathway. J. Biol. Chem., *271:* 19900–19907, 1996.

6. Craft, N., Shostak, Y., Carey, M., and Sawyers, C. L. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/*neu* tyrosine kinase. Nat. Med., *5:* 280–285, 1999.

7. Abreu-Martin, M. T., Chari, A., Palladino, A. A., Craft, N. A., and Sawyers, C. L. Mitogen-activated protein kinase kinase kinase 1 activates androgen receptor-dependent transcription and apoptosis in prostate cancer. Mol. Cell. Biol., *19:* 5143–5154, 1999.

8. Sadar, M. D., Hussain, M., and Bruchovsky, N. Prostate cancer: molecular biology of early progression to androgen independence. Endocr. Relat. Cancer, *6:* 487–502, 1999.

9. Newmark, J. R., Hardy, D. O., Tonb, D. C., Carter, B. S., Epstein, J. I., Isaacs, W. B., Brown, T. R., and Barrack, E. R. Androgen receptor gene mutations in human prostate cancer. Proc. Natl. Acad. Sci. USA, *89:* 6319–6323, 1992.

10. Tan, J. A., Sharief, Y., Hamil, K. G., Gregory, C. W., Zang, D. Y., Sar, M., Gumerlock, P. H., de Vere White, R. W., Pretlow, T. G., Harris, S. E., Wilson, E. M., Mohler, J. L., and French, F. S. Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human prostate cancer xenograft CWR22 and LNCaP cells. Mol. Endocrinol., *11:* 450–459, 1997.
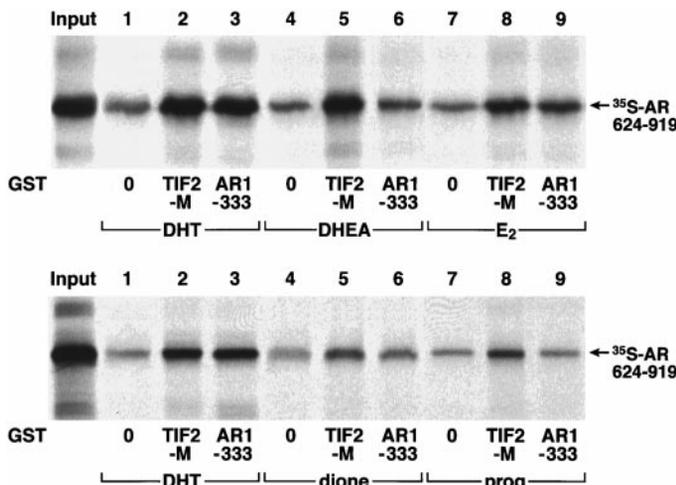
Fig. 4. Steroid dependence of TIF2 interaction with AR. GST affinity matrix assays of AR ligand binding domain interactions with a TIF2 fragment and AR NH$_2$-terminal domain fragment. Incubations included 0.2 μM DHT, DHEA, 17β-estradiol (E$_2$), androstenedione (*dione*) or progesterone (*prog*) and ^{35}S-labeled AR ligand binding domain residues 624–919 with GST-AR1-333 and GST-TIF2 (*1143–1464*; *GST-TIF2-M*). Autoradiographic signal intensities in the absence of added steroids was identical to the GST-0 control (data not shown).

11. Visakorpi, T., Hyytinen, E., Koivisto, P., Tanner, M., Keinanen, R., Palmberg, C., Palotie, A., Tammela, T., Isola, J., and Kallioniemi, O. P. *In vivo* amplification of the androgen receptor gene and progression of human prostate cancer. Nat. Genet., *9:* 401–406, 1995.

12. Gregory, C. W., Johnson, R. T., Mohler, J. L., French, F. S., and Wilson, E. M. Androgen receptor stabilization in recurrent prostate cancer is associated with hyper-sensitivity to low androgen. Cancer Res., *61:* 2892–2898, 2001.

13. Culig, Z., Hoffmann, J., Erdel, M., Eder, I. E., Hobisch, A., Hittmair, A., Bartsch, G., Utermann, G., Schneider, M. R., Parczyk, K., and Klocker, H. Switch from antagonist to agonist of the androgen receptor blocker bicalutamide is associated with prostate tumour progression in a new model system. Br. J. Cancer, *81:* 242–251, 1999.

14. Lubahn, D. B., Brown, T. R., Simental, J. A., Higgs, H. N., Migeon, C. J., Wilson, E. M., and French, F. S. Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity. Proc. Natl. Acad. Sci. USA, *86:* 9534–9538, 1989.

15. He, B., Kemppainen, J. A., and Wilson, E. M. FXXLF and WXXLF sequences mediate the NH_2-terminal interaction with the ligand binding domain of the androgen receptor. J. Biol. Chem., *275:* 22986–22994, 2000.

16. He, B., Kemppainen, J. A., Voegel, J. J., Gronemeyer, H., and Wilson, E. M. Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH_2-terminal domain. J. Biol. Chem., *274:* 37219–37225, 1999.

17. Wainstein, M. A., He, F., Robinson, D., Kung, H. J., Schwartz, S., Giaconia, J. M., Edgehouse, N. L., Pretlow, T. P., Bodner, D. R., Kursh, E. D., Resnick, M. I., Seftel, A., and Pretlow, T. G. CWR22: androgen-dependent xenograft model derived from a primary human prostatic carcinoma. Cancer Res., *54:* 6049–6052, 1994.

18. Nagabhushan, M., Miller, C. M., Pretlow, T. P., Giaconia, J. M., Edgehouse, N. L., Schwartz, S., Kung, H. J., de Vere White, R. W., Gumerlock, P. H., Resnick, M. I., Amini, S. B., and Pretlow, T. G. CWR22: the first human prostate cancer xenograft with strongly androgen-dependent and relapsed strains both *in vivo* and in soft agar. Cancer Res., *56:* 3042–3046, 1996.

19. Harris, S. E., Harris, M. A., Rong, Z., Hall, J., Judge, S., French, F. S., Joseph, D. R., Lubahn, D. B., Simental, J. A., and Wilson, E. M. Androgen regulation of HBGF-1 (α FGF) mRNA and characterization of the androgen receptor mRNA in the human prostate carcinoma cell line- LNCaP/A-Dep. *In:* J. P. Karr, D. S. Coffey, R. G. Smith, and D. J. Tindall (eds.), Molecular and Cellular Biology of Prostate Cancer, pp. 315–330. New York: Plenum Publishing Corporation, 1991.

20. Veldscholte, J., Ris-Stalpers, C., Kuiper, G. G., Jenster, G., Berrevoets, C., Claassen, E., van Rooij, H. C., Trapman, J., Brinkmann, A. O., and Mulder, E. A mutation in the ligand binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens. Biochem. Biophys. Res. Comm., *173:* 534–540, 1990.

21. Langley, E., Zhou, Z. X., and Wilson, E. M. Evidence for an antiparallel orientation of the ligand-activated human androgen receptor dimer. J. Biol. Chem., *270:* 29983–29990, 1995.

22. Langley, E., Kemppainen, J. A., and Wilson, E. M. Intermolecular NH_2-/carboxyl-terminal interactions in androgen receptor dimerization revealed by mutations that cause androgen insensitivity. J. Biol. Chem., *273:* 92–101, 1998.

23. Ma, H., Hong, H., Huang, S. M., Irvine, R. A., Webb, P., Kushner, P. J., Coetzee, G. A., and Stallcup, M. R. Multiple signal input and output domains of the 160-kilodalton nuclear receptor coactivator proteins. Mol. Cell. Biol., *19:* 6164–6173, 1999.

24. Rowan, B. G, Weigel, N. L., and O'Malley, B. W. Phosphorylation of steroid receptor coactivator-1. Identification of the phosphorylation sites and phosphorylation through the mitogen-activated protein kinase pathway. J. Biol. Chem., *275:* 4475–4483, 2000.

25. Rowan, B. G., Garrison, N., Weigel, N. L., and O'Malley, B. W. 8-Bromo-cyclic AMP induces phosphorylation of two sites in SRC-1 that facilitate ligand-independent activation of the chicken progesterone receptor and are critical for functional cooperation between SRC-1 and CREB binding protein. Mol. Cell. Biol., *20:* 8720–8730, 2000.

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