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Enhanced EGR1 Activity Promotes the Growth of Prostate Cancer Cells in an Androgen-Depleted Environment

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Abstract During anti-hormonal therapy for prostate cancer, a major clinical problem is the development of androgen-independent disease. The molecular mechanisms underlying the transition to androgen independence are the subject of intense investigation. In many prostate tumors, the activity of the transcription factor EGR1 (early growth response gene 1) is elevated due to overexpression of EGR1 and/or downregulation of the co-repressor, NAB2. We have modeled these alterations by expressing active EGR1 that does not bind NAB co-repressor proteins in human prostate carcinoma cells. We show here that active EGR1 expression enhances the androgen-independent growth of prostate carcinoma cells in vitro and in vivo. Employing RNAi and expression analyses, we show that EGR1 mediates its effects, at least in part, through the AR signaling pathway. These findings support a role for enhanced EGR1 activity in regulating the transition from androgen-dependent to androgen-independent prostate cancer. J. Cell. Biochem. 97: 1292–1299, 2006.

Key words: androgen independence; EGR1; androgen receptor

Prostate carcinoma is the most frequently diagnosed malignancy and the second leading cause of cancer-related death in western countries. Withdrawal of androgens or the peripheral blockade of androgen action remain the main therapeutic options for the treatment of advanced prostate cancer. However, after initial regression, many prostate cancers become androgen-independent and progress with eventual fatal outcome [Denmeade and Isaacs, 2002]. The response rate after castration is 60–90%, with most tumors relapsing within 12–18 months [Mahler and Denis, 1995]. Understanding all the factors involved in mediating the transition of prostate cancers from androgen dependence to independence will facilitate the search for new therapeutic approaches for androgen-independent prostate cancer.

Among the molecular mechanisms offered to explain transition from androgen dependence to independence, two are prominent. These include enhanced androgen receptor signaling which may be caused either by AR mutations allowing the receptor to be activated by new ligands [Taplin et al., 1995], AR gene amplification/overexpression rendering AR sensitive to low concentration of androgen [Koivisto et al., 1998; Linja et al., 2001; Chen et al., 2004], or cross-stimulation of the AR signaling pathway by other growth factors [Craft et al., 1999; Bakin et al., 2003]. The second hypothesis is based on the concept that the growth- and survival-promoting functions of the androgen receptor can be bypassed by

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alternative signaling pathways. This hypothesis is supported by the fact that *AR* gene methylation leads to decreased or absent androgen receptor expression and some oncogenes, such as *c-Myc* and *BCL2* are upregulated in hormone refractory cancers [Raffo et al., 1995; Bernard et al., 2003].

Available evidence indicates that the zinc finger transcription factor EGR1 is involved in promoting prostate cancer progression. Prostate cancer cells and tissues commonly overexpress EGR1 [Thigpen et al., 1996; Eid et al., 1998] and a significant fraction of prostate tumors also downregulate the EGR1 co-repressor, NAB2 [Abdulkadir et al., 2001a]. These observations suggest that prostate cancers have high, unrestrained EGR1 transcriptional activity. In addition, EGR1 levels correlate positively with tumor grade [Eid et al., 1998] and EGR1 expression promotes prostate cancer cell proliferation [Baron et al., 2003; Virolle et al., 2003], while EGR1 deficiency delays progression of prostate carcinomas in two different strains of transgenic mice [Abdulkadir et al., 2001b]. EGR1 overexpression in prostate cancer cells can upregulate some growth factors, such as IGF-II, TGF-β1, and PDGF-A, which have previously been implicated in enhancing tumor progression [Svaren et al., 2000]. The protumorigenic role of EGR1 in prostate cells may not extend to other tumor types, as EGR1 overexpression can suppress the growth of several non-prostatic cell lines such as HT1080 fibrosarcoma cells and glioma cells [Adamson and Mercola, 2002].

In our efforts to explore the basis for the context-dependent role of EGR1 in tumorigenesis, we recently found that EGR1 binds to the AR and modulates AR-mediated regulation of PSA gene expression in prostate carcinoma cells [Yang and Abdulkadir, 2003]. In the present study, we examine the effects of alterations in the EGR1 pathway on androgen-independent prostate cell growth and tumorigenicity. Expression of active EGR1 confers on hormone-sensitive prostate cancer cells LNCaP the ability to grow in low androgen concentrations and form tumors in castrated nude mice. RNAi and expression experiments indicate thatthis effect of EGR1 is mediated at least in part through AR, possibly through the enhancement of AR nuclear translocation. Thus, EGR1 overexpression promotes the transition from androgen-dependence to androgen-independence of prostate cancer by modulating androgen signaling.

RESULTS

EGR1 Promotes LNCaP Cell Growth in Low Androgen Concentrations

To recapitulate the high EGR1/low NAB2 expression pattern frequently observed in human prostate cancer [Abdulkadir et al., 2001a], we stably expressed active EGR1 (EGR1I293F; referred to hereinafter as EGR1*), which does not bind the NAB molecules in androgen-sensitive LNCaP prostate carcinoma cells [Yang and Abdulkadir, 2003]. To avoid clonal variations, we used pooled clones of G418-resistant cells. EGR1* expression was confirmed by Western blot analysis in transfected LNCaP cells (Fig. 1A). Further immunoflorescence studies demonstrated higher levels of nuclear EGR1, which co-localized with AR in the EGR1*-transfected cells (Fig. 1B). As previous studies have indicated that EGR1* expression can modulate AR signaling as measured by effects on PSA gene expression [Yang and Abdulkadir, 2003], we examined the consequences of EGR1* expression on androgen-independent prostate cancer cell growth. We performed colony-forming assays in soft agar under varying concentrations of the androgen dihydrotestosterone (DHT). EGR1* expression significantly increased the ability of LNCaP cells to form colonies in soft agar in low DHT concentrations (Fig. 2A).

These results were extended to an in vivo model of androgen-independent prostate cancer. Nude mice were castrated or sham-castrated and 14 days later, LNCaP-Neo or LNCaP-EGR1* cells were implanted into their flanks. While tumor incidence was not affected by EGR1* expression in intact mice, EGR1* had a striking effect on the ability of LNCaP cells to form tumors in castrated animals. Over the course of the experiment, 10 of 16 sites (62.5%) injected with LNCaP-EGR1* cells in castrated animals developed tumors (Fig. 2B). Interestingly, although LNCaP cells do not usually grow in castrated animals [Chen et al., 2004], a tumor developed at 1 of the 16 sites injected in the LNCaP-Neo/castrated group (Fig. 2B). As will be discussed below (see Fig. 5), the development of this "escaped" tumor is most likely a result of spontaneous overexpression of the androgen receptor.

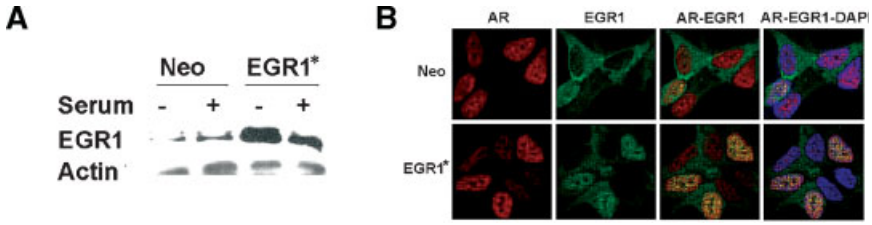

Fig. 1. EGR1 protein expression in LNCaP-Neo and LNCaP-EGR1* cells. **A**: Western blot analysis of EGR1 and actin expression in LNCaP-Neo and LNCaP-EGR1* cells. Cells were stimulated with 20% FBS for 2 h after serum starvation for 48 h. **B**: Immunocytochemical analysis of LNCaP-Neo and LNCaP-EGR1* cells using anti-EGR1 (green) and anti-AR (red) antibodies. Nuclei were stained with DAPI (blue). Images were captured using a confocal microscope.

EGR1 Effects Are Mediated Through the AR Pathway

To determine whether AR signaling is required for EGR1 to promote growth in LNCaP cells grown in androgen depleted conditions, we employed RNA interference targeting the androgen receptor. LNCaP-Neo and LNCaP-EGR1* cells were stably transfected with the pRS/AR construct that expresses AR siRNA or the control pRS vector. As shown in Figure 3A, the pRS/AR transfected cells expressed lower levels of the AR protein. Knockdown of AR expression inhibited the growth of both LNCaP-Neo and LNCaP-EGR1* cells cultured in 1 nM DHT (Fig. 3B,C), demonstrating that the growth-promoting effects of EGR1 are mediated, at least in part, through the androgen receptor signaling pathway.

LNCaP-EGR1* Cells Are Resistant to the Androgen Antagonist CPA

To further mimic the clinical circumstances of hormone-refractory disease, we assessed the response of cells to the antiandrogen cyproterone acetate (CPA). LNCaP-Neo and LNCaP-EGR1* cells grown in regular FBS-containing medium were exposed to increasing doses of CPA. We found that the growth of LNCaP-EGR1* cells was not effectively inhibited by treatment with CPA (Fig. 4A) compared to LNCaP-Neo cells. Furthermore, CPA failed to inhibit AR signaling as measured by its effect on DHT-stimulated PSA gene expression in the EGR1*-expressing cells; rather, CPA treatment enhanced DHT-stimulated PSA gene expression (Fig. 4B). Indeed, in the absence of DHT, CPA treatment significantly stimulates PSA

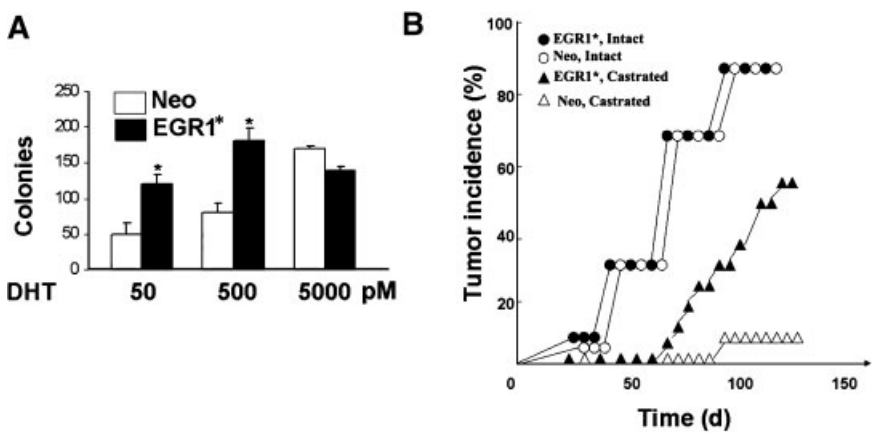

Fig. 2. EGR1 overexpression promotes androgen-independent growth of prostate cancer cell line LNCaP. **A**: Soft agar assay. Cells were cultured in 0.35% agarose in medium containing 10% charcoal/dextran treated FBS (cFBS) containing the indicated concentrations of DHT for two weeks. Colonies were stained with 0.1% crystal violet and counted. (*$P < 0.05$, compared to control cells). **B**: Tumor incidence. Intact (circles, n = 9) or castrated (triangles, n = 8) nude mice were bilaterally inoculated s.c. with LNCaP-Neo (white symbols) or LNCaP-EGR1* (black symbols) cells and monitored for tumor development (cut-off size, 5 mm diameter).

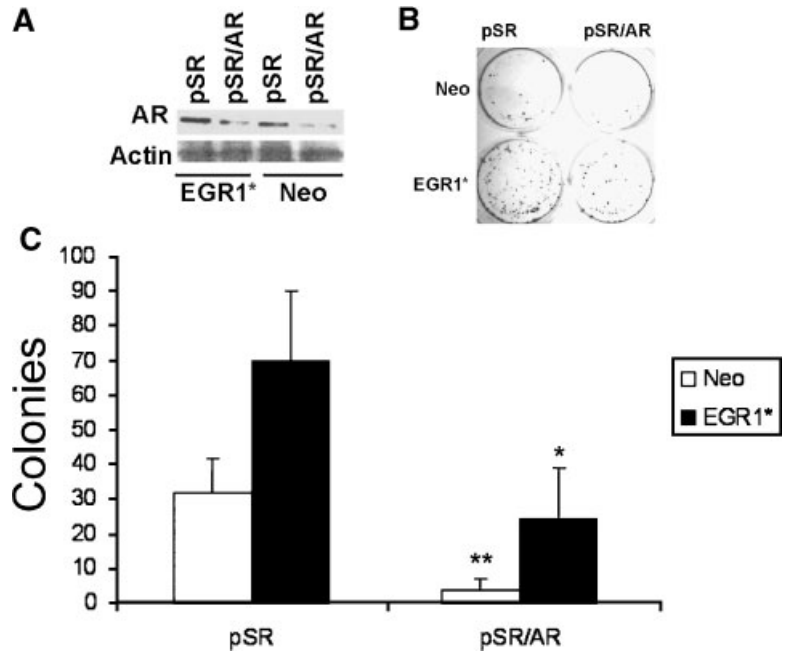

Fig. 3. AR downregulation inhibits EGR1*-stimulated growth of LNCaP cells. **A**: Expression of AR protein in LNCaP-Neo and LNCaP-EGR1* cells stably transfected with pSR (control siRNA) or pSR-AR (AR siRNA). **B**: Colony formation of doubly transfected cells. Cells were cultured under the presence of 1 nM DHT for 2 weeks and stained with 0.1% crystal violet. **C**: Colony numbers in triplicate in Fig. 3B. (*$P < 0.05$, **$P < 0.01$, compared to pSR).

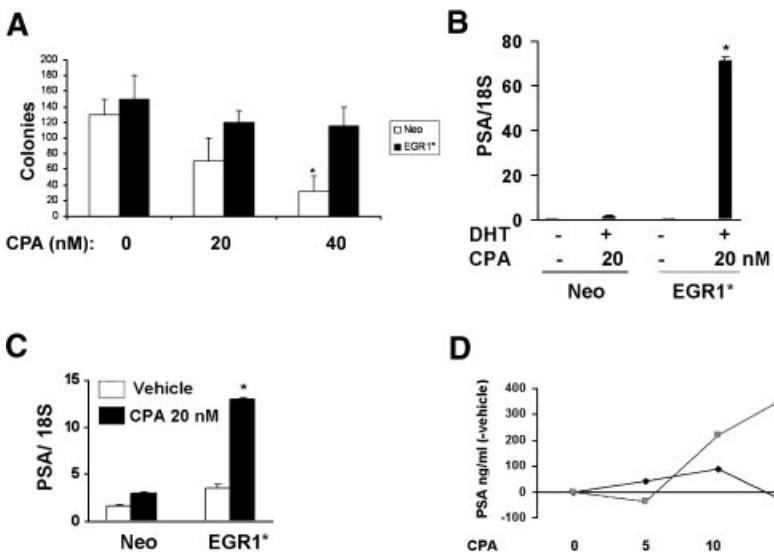

Fig. 4. Effects of the antiandrogen cyproterone acetate (CPA) on colony formation and expression of PSA in control and EGR1* expressing cells. **A**: CPA inhibits colony formation of control (Neo) cells but not EGR1* transfected cells. Cells were seeded into six-well plates at 1×10^4 cells per well and cultured in regular FBS-containing medium with different concentrations of CPA for two weeks. Cells were stained with 0.1% crystal violet. Colony numbers were counted. (*$P < 0.05$, compared to vehicle). **B**: CPA fails to inhibit DHT-stimulated PSA gene expression in LNCaP-EGR1* cells. Cells were grown in cFBS with or without 20 nM CPA for 48 h and then exposed to 5 nM DHT or vehicle for 24 h. PSA mRNA expression was analyzed by qRT-PCR. (* $P < 0.05$, compared to vehicle). **C** and **D**: CPA stimulates expression of PSA mRNA (C) and protein (D) in LNCaP-EGR1* cells. Cells were cultured in cFBS with different concentrations of CPA for 48 h. PSA mRNA was measured by qRT-PCR while PSA protein levels in supernatant were evaluated by ELISA. (*$P < 0.05$, compared to vehicle).

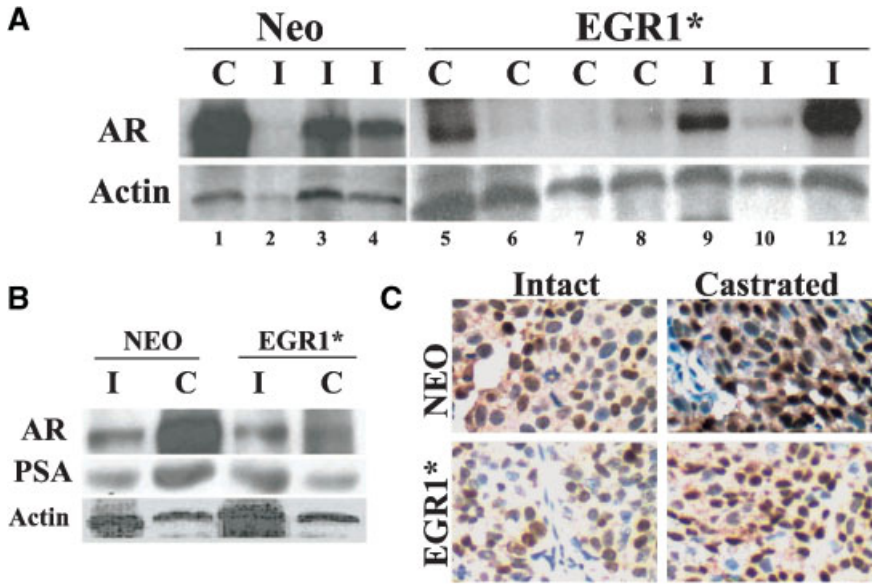

Fig. 5. AR signaling pathway remains active in androgen refractory xenografts from LNCaP-EGR1* cells. **A**: Western blot analysis for AR expression in individual tumors from intact (''I'') or castrated (''C'') mice. Note elevated AR levels in ''escaped'' LNCAP-Neo tumor from castrated animal (lane 1). **B**: Western blot for AR and PSA proteins in tumors from intact (''I'') or castrated (''C'') mice. Extracts from three tumors were pooled for analysis with the exception of castrated LNCaP-Neo sample, which represents the only available single tumor. **C**: Immunohistochemical assay for AR protein expression (brown staining) in xenografts. Nuclei were counterstained with hematoxylin (blue).

mRNA and protein expression in LNCaP-EGR1* cells in a concentration-dependent manner compared to control cells (Fig. 4C,D). It should be noted that the LNCaP-Neo cells used in this study respond to DHT [Yang and Abdulkadir, 2003]. These results indicate that EGR1* expression can convert the antagonist CPA to an agonist, and are reminiscent of the effects of AR overexpression in prostate carcinoma cells, which results in the conversion of antagonists to agonists by altering the recruitment of co-activators/co-repressors to the promoters of androgen target genes [Chen et al., 2004].

AR Signaling Pathway Remains Active in Androgen Refractory EGR1 Xenografts

The results presented thus far indicate that EGR1 overexpression may lead to androgen-independent growth by modulating AR signaling. We, therefore, examined the expression, localization, and activity of the androgen receptor in androgen refractory LNCaP-EGR1* xenografts. Using Western blot analysis, we noticed that the single "escaped tumor" that developed in LNCaP-Neo-injected castrated mice expressed high levels of AR protein (Fig. 5A, lane 1; see also Fig. 2B). Thus, this androgen-independent tumor overexpresses AR, which is a common mechanism for the development of androgen-independent prostate cancer [Chen et al., 2004]. By contrast, androgen refractory EGR1*-expressing xenografts did not show a similar level of AR overexpression, although AR signaling is active in these cells as demonstrated by retention of PSA expression (Fig. 5B) and AR nuclear localization (Fig. 5C). These results are consistent with the interpretation that EGR1* overexpression promotes hormone refractory tumor growth by stimulating the nuclear localization of AR in low androgen conditions.

DISCUSSION

Previous studies indicate that EGR1 has a specific pro-tumorigenic role in prostate cancer [Thigpen et al., 1996; Eid et al., 1998; Abdulkadir et al., 2001a,b; Virolle et al., 2003]. While there are several potential reasons for this effect, involvement of the AR signaling was suggested by recent data showing that EGR1 overexpression modulates AR transcriptional activity [Yang and Abdulkadir, 2003]. In the present study, we identify EGR1 as a factor that promotes androgen-independent prostate

cancer cell growth. In particular, it is striking that EGR1 overexpression confers on LNCaP cells the ability to form tumors in castrated mice. Since EGR1 is overexpressed [Eid et al., 1998] and its repressor NAB2 downregulated [Abdulkadir et al., 2001a] in advanced human prostate cancer, our results suggest that elevated EGR1 activity may contribute to the growth of androgen independent prostate cancer.

Several studies have demonstrated the critical role of persistent AR signaling in the development of androgen-independent prostate cancer [Gregory et al., 1998; Koivisto et al., 1998; Chen et al., 2004]. The molecular mechanisms by which the AR signaling pathway remains active in the low-androgen environment of patients treated with anti-hormonal therapy is still unclear. Although AR mutations can cause resistance by altering the response of the receptor such that non-canonical ligands such as estrogen and hydrocortisone, or AR antagonists such as flutamide, behave as agonists, the overall frequency of AR mutations can not account for most cases of hormone-refractory disease [Navarro et al., 2002]. Recent studies show that there is AR mRNA and protein overexpression in most androgen-independent prostate tumors and that AR overexpression can cause androgen-independent growth of prostate cancer cells, further suggesting that "the superactive AR signaling" plays a critical role in the transition of androgen-dependent prostate cancer to androgen-independent prostate cancer [Linja et al., 2001]. Our data indicate that EGR1 mediates its effects on androgen-independent growth through the AR signaling pathway. Knockdown of AR by RNAi inhibits EGR1-mediated growth of prostate cells and LNCaP-EGR1* tumors that developed in castrated animals showed evidence of nuclear AR and AR target gene (PSA) expression. Retention of AR signaling in EGR1 androgen-independent tumors was not achieved by AR overexpression. In contrast to the "escaped" control androgen-independent LNCaP-Neo tumor, which showed evidence of AR protein overexpression, levels of AR in LNCaP-EGR1* androgen-independent tumors were not elevated. Rather, immunohistochemical analyses revealed AR nuclear staining in EGR1* xenografts, suggesting that EGR1 may promote hormone-refractory growth by promoting AR nuclear translocation. Furthermore, our observation that the antiandrogen CPA behaves as an agonist in EGR1-overexpressing cells is consistent with the interpretation that EGR1 works by stimulating AR nuclear translocation. It was recently demonstrated that AR overexpression in prostate carcinoma cells could convert AR antagonists to agonists possibly through mass action by altering the recruitment of co-activators/co-repressors to target gene promoters [Chen et al., 2004]. Using chromatin immunoprecipitation experiments, Chen et al. [2004] showed that in prostate cells overexpressing AR, antagonists led to selective recruitment of co-activators (e.g., SRC-1) to AR target gene promoters. In our LNCaP-EGR1* cells, we hypothesize that EGR1* overexpression may mimic AR overexpression by enhancing AR nuclear translocation as EGR1 was shown to physically interact with AR and to promote AR nuclear translocation [Yang and Abdulkadir, 2003].

The stimulatory effect of AR overexpression on hormone refractory prostate cancer cell growth seems to be mediated through genotropic mechanisms [Chen et al., 2004], indicating that AR nuclear translocation is necessary for the transition to androgen-independence. It is relevant, therefore, that EGR1 physically interacts with AR and can promote AR nuclear translocation [Yang and Abdulkadir, 2003]. Co-immunoprecipitation experiments in LNCaP cells showed interaction of endogenous EGR1 and AR. The interaction was also observed in DU145 cells also after transfection of exogenous AR and was localized using the GST-pull down assay to the AR N-terminal domain [Yang and Abdulkadir, 2003]. Our results, as well as recent findings about the role of the AR in hormone-refractory prostate cancer indicate that molecular alterations affecting AR nuclear translocation may provide a potential pathway to androgen independence in prostate cancer.

Finally, it should be pointed out that the role of EGR1 in prostate cancer clearly extends beyond effects on the AR pathway. In fact, Egr1 deletion significantly delayed prostate tumorigenesis in the Cr2TAg transgenic model of prostate cancer, in which SV40TAg expression in AR-negative prostate neuroendocrine cells leads to tumorigenesis [Abdulkadir et al., 2001b]. Furthermore, siRNA targeted knockdown of EGR1 in AR-negative DU145 cells led to a reduction in Cyclin D2 [Virolle et al., 2003]. Thus, the combined evidence indicates that the EGR1 pathway plays an important role in prostate tumorigenesis through multiple pathways.

MATERIALS AND METHODS

Plasmid Constructs and Cell Lines

LNCaP-Neo and LNCaP-EGR1* stable cell lines (the latter containing EGR1I293F, which does not bind the NAB repressors, referred to here as EGR1*) were generated as described [Yang and Abdulkadir, 2003]. Pooled G418-resistant clones were used in all experiments. The pRetroSuper retroviral vector (pSR) and the vector with the target sequence used to silence human AR (pSR/AR) were kindly provided by Dr. David H. Beach, University College London and have been described [Bernard et al., 2003]. Phoenix^{TM} retrovirus producer line was obtained from Orbigen, San Diego, CA and maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS. For the generation of Phoenix cells producing pSR or pSR/AR retrovirus, cells were transfected using FuGENE-6 Transfection Reagent. Cells were subjected to selection using 1 μg/ml puromycin 48 h after transfection. Resistant clones were maintained in DMEM medium with 0.5 μg/ml puromycin. For infection of target cells, LNCaP-EGR1* and LNCaP-Neo cells were plated overnight at 100 mm dish. Cells were infected with the supernatant containing pSR or pSR/AR retrovirus using 8 μg/ml polybrene. The infected cells were selected by 1 μg/ml puromycin and 300 μg/ml G418. Resistant cells were maintained in 1640 RPMI with 0.5 μg/ml puromycin and 300 μg/ml G418.

Western Blot and ELISA Analyses

Western blot was done as previously described method [Yang and Abdulkadir, 2003]. The following antibodies from Santa Cruz Biotechnology were used: rabbit anti-human EGR1 antibody (1:500), mouse anti-human AR N-terminal antibody (1:500), and goat anti-actin antibody (1:1,000). Prostate-specific antigen (PSA) concentrations in cell supernatants were determined using an ELISA kit (ANOGEN, Ontario, Canada).

Immunocytochemistry and Immunohistochemistry

For immunocytochemistry, LNCaP-Neo and LNCaP-EGR1* cells were grown in RPMI 1640 containing 5% FBS on coverslips. Cells were fixed and blocked using a previously described method [Yang and Abdulkadir, 2003]. The first primary mouse anti-human AR N-terminal monoclonal antibody was used at 5 μg/ml and incubated for 2 h at room temperature followed by three 10-min washes. A goat anti-mouse IgG antibody conjugated to Alexa Fluor® 594 (Molecular Probes) was used at 5 μg/ml and incubated for 1 h at room temperature followed by three 10-min washes. The second primary rabbit anti-human EGR1 antibody and a florescein goat anti-rabbit antibody (Molecular Probes) were used by the method described above. The coverslips were mounted with mounting medium containing 4′, 6-diamidino-2-phenylindole (DAPI) (Vector Laboratories). The images were analyzed using a Leica DMIRBE Inverted Microscope with a Leica SP-1 Confocal System. Immunohistochemical assay was performed on formalin-fixed, paraffin-embedded pieces of xenografts using TSA^{TM} Biotin System (Perkin Elmer, Boston, MA), according to the manufacturer's protocol. The same mouse anti-human AR N-terminal antibody (1:50) was used.

Colony-Forming Assays

For colony-forming assays, 1×10^4 cells per well were plated in six-well plates. After 1 day, the cells were treated with different concentrations of cyproterone acetate (CPA: Sigma-Aldrich); this treatment was repeated every 2 days. After 2 weeks, cells were washed in PBS, fixed in 0.5% glutaraldehyde (Sigma-Aldrich) and stained with 0.1% crystal violet (Sigma-Aldrich).

Soft Agar Assays

Five thousand cells were resuspended with 3 ml of 0.35% agarose in DMEM medium containing 10% cFBS and different concentrations of DHT. This upper layer was seeded into 60 mm dish coated with 0.5% agarose in DMEM medium with 10% cFBS and different concentrations of DHT. After culture for 2 weeks, cells were stained with 0.1% crystal violet for more than 1 h and the number of foci was counted.

Quantitative RT-PCR Analysis

Conditions and primers for quantitative RT-PCR using SYBR-GREEN have been described previously [Yang and Abdulkadir, 2003].

Animal Studies

Eight-week-old male nude mice (NU/J, Jackson Labs or NSWUN-M, Taconic) were

bilaterally castrated or sham-castrated. Fourteen days after surgery, mice were bilaterally inoculated in their flanks s.c. with 2×10^6 cells in Matrigel (BD Biosciences) at 50:50 volume for a total volume of 200 μl/ injection site. Average tumor burden was calculated with calipers in millimeters as the mean tumor diameter measured in two dimensions. All animal protocols followed approved institutional guidelines.

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REFERENCES

Abdulkadir SA, Carbone JM, Naughton CK, Humphrey PA, Catalona WJ, Milbrandt J. 2001a. Frequent and early loss of the EGR1 co-repressor NAB2 in human prostate carcinoma. Hum Pathol 32:935–939.

Abdulkadir SA, Qu Z, Garabedian E, Song SK, Peters TJ, Svaren J, Carbone JM, Naughton CK, Catalona WJ, Ackerman JJ, Gordon JI, Humphrey PA, Milbrandt J. 2001b. Impaired prostate tumorigenesis in Egr1-deficient mice. Nat Med 7:101–107.

Adamson ED, Mercola D. 2002. Egr1 transcription factor: Multiple roles in prostate tumor cell growth and survival. Tumour Biol 23:93–102.

Bakin RE, Gioeli D, Sikes RA, Bissonette EA, Weber MJ. 2003. Constitutive activation of the Ras/mitogen-activated protein kinase signaling pathway promotes androgen hypersensitivity in LNCaP prostate cancer cells. Cancer Res 63:1981–1989.

Baron V, De Gregorio G, Krones-Herzig A, Virolle T, Calogero A, Urcis R, Mercola D. 2003. Inhibition of Egr-1 expression reverses transformation of prostate cancer cells in vitro and in vivo. Oncogene 22:4194–4204.

Bernard D, Pourtier-Manzanedo A, Gil J, Beach DH. 2003. Myc confers androgen-independent prostate cancer cell growth. J Clin Invest 112:1724–1731.

Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, Sawyers CL. 2004. Molecular determinants of resistance to antiandrogen therapy. Nat Med 10:33–39.

Craft N, Shostak Y, Carey M, Sawyers CL. 1999. 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.

Denmeade SR, Isaacs JT. 2002. A history of prostate cancer treatment. Nat Rev Cancer 2:389–396.

Eid MA, Kumar MV, Iczkowski KA, Bostwick DG, Tindall DJ. 1998. Expression of early growth response genes in human prostate cancer. Cancer Res 58:2461–2468.

Gregory CW, Hamil KG, Kim D, Hall SH, Pretlow TG, Mohler JL, French FS. 1998. Androgen receptor expression in androgen-independent prostate cancer is associated with increased expression of androgen-regulated genes. Cancer Res 58:5718–5724.

Koivisto P, Kolmer M, Visakorpi T, Kallioniemi OP. 1998. Androgen receptor gene and hormonal therapy failure of prostate cancer. Am J Pathol 152:1–9.

Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL, Visakorpi T. 2001. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 61:3550–3555.

Mahler C, Denis LJ. 1995. Hormone refractory disease. Semin Surg Oncol 11:77–83.

Navarro D, Luzardo OP, Fernandez L, Chesa N, Diaz-Chico BN. 2002. Transition to androgen-independence in prostate cancer. J Steroid Biochem Mol Biol 81:191–201.

Raffo AJ, Perlman H, Chen MW, Day ML, Streitman JS, Buttyan R. 1995. Overexpression of bcl-2 protects prostate cancer cells from apoptosis in vitro and confers resistance to androgen depletion in vivo. Cancer Res 55:4438–4445.

Svaren J, Ehrig T, Abdulkadir SA, Ehrengruber MU, Watson MA, Milbrandt J. 2000. EGR1 target genes in prostate carcinoma cells identified by microarray analysis. J Biol Chem 275:38524–38531.

Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, Keer HN, Balk SP. 1995. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. N Engl J Med 332:1393–1398.

Thigpen AE, Cala KM, Guileyardo JM, Molberg KH, McConnell JD, Russell DW. 1996. Increased expression of early growth response-1 messenger ribonucleic acid in prostatic adenocarcinoma. J Urol 155:975–981.

Virolle T, Krones-Herzig A, Baron V, De Gregorio G, Adamson ED, Mercola D. 2003. Egr1 promotes growth and survival of prostate cancer cells. Identification of novel Egr1 target genes. J Biol Chem 278:11802–11810.

Yang S-Z, Abdulkadir SA. 2003. Early growth response gene 1 modulates androgen receptor signaling in prostate carcinoma cells. J Biol Chem 278:39906–39911.