Potential Mechanism for the Effects of Dexamethasone on Growth of Androgen-Independent Prostate Cancer

Kazuo Nishimura, Norio Nonomura, Eiichi Satoh, Yasunori Harada, Masashi Nakayama, Takashi Tokizane, Tatsunari Fukui, Yutaka Ono, Hitoshi Inoue, Masaru Shin, Yuichi Tsujimoto, Hitoshi Takayama, Katsuyuki Aozasa, Akihiko Okuyama

Background: Dexamethasone, a synthetic glucocorticoid, has clinical benefit in patients with hormone-refractory prostate cancer (HRPC), but the mechanisms responsible for its effects are unknown. The nuclear factor-kB (NF-kB)-dependent cytokine interleukin (IL) 6 (IL-6) is thought to stimulate growth of HRPC. Because dexamethasone interferes with NF-kB activation, we determined whether dexamethasone inhibits prostate cancer growth by working through the glucocorticoid receptor (GR) to interfere with NF-kB-IL-6 pathway. Methods: Three human prostate cancer cell lines (DU145, PC-3, and LNCaP) were assessed for GR expression and responsiveness to dexamethasone. Levels of GR, NF-kB, and the cytoplasmic NF-kB inhibitor IκBα were determined by western blotting and of IL-6 by enzyme immunoassay. The subcellular localization of NF-kB was analyzed by immunofluorescence. The effects of dexamethasone (thrice weekly injections of 1 μg/mouse) on DU145 xenografts in nude and severe combined immunodeficient (SCID) mice were evaluated. GR expression in human prostate cancers was assessed by immunohistochemistry. All statistical tests were two-sided. Results: Dexamethasone dose dependently decreased GR levels and inhibited the growth of DU145 and PC-3 but not LNCaP cells (DU145 cells, P<.001; PC-3 cells, P=.009). Dexamethasone increased IκBα protein levels and the cytosolic accumulation of NF-kB in DU145 cells and decreased secreted IL-6 levels to 37 pg/mL (95% confidence interval [CI] = 33 pg/mL to 41 pg/mL), compared with 164 pg/mL (95% CI = 162 pg/mL to 166 pg/mL) secreted by ethanol-treated control cells. Dexamethasone inhibited the growth of DU145 xenografts in nude (P = .060) and SCID (P = .026) mice without affecting GR levels. Eight of 16 human prostate cancers expressed GR at high levels (≥30% GR-positive cells). Conclusion: Dexamethasone inhibited the growth of GR-positive cancers, possibly through the disruption of the NF-kB-IL-6 pathway. [J Natl Cancer Inst 2001;93:1739–46]

Prostate cancer is the second leading cause of cancer-related death in men over the age of 65 years (1). Although the initial growth of prostate cancers is believed to be androgen dependent, prostate cancers, ultimately, become androgen independent and refractory to hormonal therapy. To our knowledge, no therapy has been proven definitively to prolong survival of patients with hormone-refractory prostate cancer (HRPC). Although glucocorticoids have some benefit in men with HRPC (2–5), the subjective and objective response rates varied. Several studies (3–6) that used the glucocorticoids prednisone, hydrocortisone, or dexamethasone to suppress multiple adrenal androgens reported that the serum prostate-specific antigen levels declined more than 50% in 20%–61% of patients with HRPC. This variation may be due, in part, to the type and dose of different glucocorticoids. Recently, we demonstrated the clinical benefits of low-dose (1 mg/day) dexamethasone for patients with HRPC (7). However, the mechanisms by which low-dose dexamethasone affects patients with HRPC are unclear.

Clinical use of glucocorticoids for the treatment of lymphoproliferative disorders and solid tumors (8–10) was prompted by the in vitro observations that glucocorticoids inhibit the proliferation of several cell types. There are at least two potential mechanisms by which glucocorticoids can inhibit proliferation: the induction of growth-inhibitory cytokines or the inhibition of growth-stimulatory cytokines. For example, in the androgen-independent prostate cancer cell line PC-3, dexamethasone inhibited cell growth through the induction of transforming growth factor-β1 (11), whereas in human monocytes, dexamethasone inhibited the production of proinflammatory cytokines, such as interleukin (IL) 1β (IL-1β) and tumor necrosis factor-α (TNF-α) (12).

The biologic actions of glucocorticoids occur in cells that express glucocorticoid receptors (GRs). GRs belong to the family of nuclear hormone receptors that can function as transcription factors once the ligand has bound. Glucocorticoids have been shown to interfere with the transcriptional activity of several transcription factors, including nuclear factor-κB (NF-κB) (13), that are important for the induction of several growth-stimulatory cytokines. Thus, glucocorticoids may inhibit growth-stimulatory cytokines by inhibiting NF-κB activity.

To understand the mechanism by which glucocorticoids have clinical benefit for patients with HRPC, we hypothesized that glucocorticoids act directly through GRs in prostate cancer cells and exert an antitumor effect, in addition to any effects that result from the in vivo suppression of adrenal androgen production. Furthermore, we hypothesized that, because androgen-independent prostate cancer cells are growth dependent on the cytokine IL-6 (14,15), whose transcription is partially dependent on NF-κB (13), glucocorticoids exert their antitumor effect by inhibiting NF-κB activation and the production of NF-κB-dependent cytokines. Here, we tested this hypothesis in vitro with established human prostate cancer cell lines (LNCaP, DU145, and PC-3). Because high-dose (25 μg/mouse per day) dexamethasone may decrease expression of GRs (16–18), we used a low-dose dexamethasone regimen that is considered to be desirable for maintaining sufficient inhibitory effects and for minimizing adverse effects in vivo in a xenograft model of androgen-independent prostate cancer. We also assessed GR expression in prostate cancers from patients, some of whom had received androgen ablation therapy (AAT).

Affiliations of authors: K. Nishimura, N. Nonomura, E. Satoh, Y. Harada, M. Nakayama, T. Tokizane, T. Fukui, Y. Ono, H. Inoue, A. Okuyama (Department of Urology), M. Shin, Y. Tsujimoto, H. Takayama, K. Aozasa (Department of Pathology), Graduate School of Medicine, Osaka University, Suita-City, Japan.

Correspondence and reprint requests to: Kazuo Nishimura, M.D., Department of Urology, Graduate School of Medicine, Osaka University, 2–2 Yamadaoka, Suita-City 565–0871 Japan (e-mail: kazuo@uro.med.osaka-u.ac.jp).

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Methods

Cell Lines

DU145, PC-3, and HeLa cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS). LNCaP cells were maintained in RPMI-1640 medium with 10% heat-inactivated FCS. All cell lines were obtained from the American Type Culture Collection (Manassas, VA).

Tumors and Patients

All patients signed consent forms approved by our institution. Prostate cancer tissue was obtained from a total of 16 patients treated with surgery alone (five patients) or surgery plus preoperative total AAT (11 patients), which consisted of luteinizing hormone-releasing hormone analogue plus antiandrogens for 3–12 months before surgery. Slides of tissue sections stained with hematoxylin–eosin were reviewed by a pathologist (K. Aozasa), who staged the cancers according to the tumor–node–metastasis system of the American Joint Committee on Cancer in 1997 (19).

Reverse Transcription–Polymerase Chain Reaction

Complementary DNA (cDNA) was synthesized by use of a reverse transcription–polymerase chain reaction (RT–PCR) kit (Perkin-Elmer Corp., Foster City, CA) from 300 ng of total RNA from each of the prostate cancer cell lines (LNCaP, DU145, and PC-3). PCR was then performed with GR-specific primers (sense 5′-TCCCCTTCTCAACAGCAG-GAT-3′ and antisense 5′-CAATCATTTCTC-CAGCACAT-3′) by a 40-cycle stepdown amplification program that consisted of five cycles of 94 °C for 30 seconds, 69 °C for 45 seconds, and 72 °C for 60 seconds, five cycles of 94 °C for 30 seconds, 64 °C for 45 seconds, and 72 °C for 60 seconds, five cycles of 94 °C for 30 seconds, 59 °C for 45 seconds, and 72 °C for 60 seconds, and 25 cycles of 94 °C for 30 seconds, 54 °C for 45 seconds, and 72 °C for 60 seconds. The primers were designed to span introns (from exons 2 to 4), such that any genomic DNA product would be distinguishable from the 371-base-pair cDNA product by a size difference after the PCR products were separated on a 2% agarose gel and visualized by staining with ethidium bromide. cDNA from HeLa cells was used as a positive control for GR messenger RNA (mRNA) expression. Distilled water was substituted for reverse transcriptase to serve as a negative control. β-Actin primers (sense 5′-GTTGGGGGCGCCCGACGCACA-3′ and antisense 5′-GTCCTTAATGTCAAGCGACGATTTCTCAATG-3′) were used to ensure the integrity of the cDNA samples.

Preparation of Cellular Protein and Western Blots

PC-3 and DU145 cells (5 × 10⁵ cells) were seeded on 100-mm tissue culture dishes. The next day, the medium was changed to DMEM with hormone-depleted 5% charcoal-stripped FCS containing each specific dose of dexamethasone or an equivalent volume of ethanol (0.1%). After 48 hours, the cells were washed twice with phosphate-buffered saline (PBS), collected by scrap-

ing, suspended in lysis buffer (i.e., 50 mM Tris–HCl [pH 7.4], 1% Nonidet P-40, 0.5% sodium deoxycho-
late, 150 mM NaCl, 1 mM phenylmethylsulfonyl-
fluoride, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin, and 1 mM sodium orthovanadate), incubated for 1 hour on ice, and centrifuged at 10,000g for 10 minutes at 4 °C. For GR expression of LNCaP cells, 5 × 10⁵ cells were seeded on 100-
mm tissue culture dishes with RPMI-1640 medium with 10% charcoal-stripped FCS and incubated for 3 days, and the lysate was prepared by the same pro-
cedure. After the determination of the protein con-
centration of the supernatants, the samples were di-
luted in electrophoresis sample buffer (i.e., 100 mM Tris–HCl, 25% glycerol, 2% sodium dodecyl sul-
fate, 0.01% bromphenol blue [pH 6.8], and 5% β-
mercaptoethanol) and boiled for 3 minutes. An aliquot (10 μg of total protein) of each sample was washed in PBS-T, and then the immunoreactive
fluoride by standard electrophoretic techniques. A standard protein marker (Kaleidoscope Prestained Standards; Bio-Rad Laboratories, Hercules, CA) was used to determine the molecular weights of the separated proteins. After blotting, the membrane was blocked with 5% nonfat milk in PBS containing 0.1% Tween-20 (PBS-T) for 12 hours at 4 °C, washed with PBS-T, and incubated with a specific primary antibody (i.e., rabbit polyclonal anti-GR [Santa Cruz Biotechnology, Inc., Santa Cruz, CA] diluted 1 : 1000 in PBS-T; rabbit polyclonal anti-
NF-κB p65 [Santa Cruz Biotechnology, Inc.] diluted 1 : 1000 in PBS-T; rabbit polyclonal IκBα [Santa Cruz Biotechnology, Inc.] diluted 1 : 1000 in PBS-T; or mouse monoclonal anti-β-actin [Sigma Chemical Co., St. Louis, MO] diluted 1 : 5000 in PBS-T) for 1 hour at room temperature. The membrane was washed in PBS-T and then incubated with the ap-
propriate alkaline phosphatase-conjugated or horse-
radish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Inc.) diluted in 1 : 1000 PBS-T for 45–60 minutes. The membrane was washed, dried, and exposed to X-ray film. The bands were visualized by exposing the membrane to 5-bromo-4-chloro-3-indolylphosphate-nitro blue tetrazolium phosphate substrate (Bio-Rad Laborato-
ries, Hercules, CA) (for detection of alkaline phos-
phatase-conjugated antibodies) or to the enhanced chemiluminescence (ECL) substrate per instructions of the manufacturer (Amer sham Pharmacia Biotech, Tokyo, Japan) (for the detection of horseradish per-
oxidase-conjugated antibodies). Membranes were incubated with the ECL reagents were stripped and re-
probed for additional proteins as described in the manufac-
turer’s instructions. An isotype-specific rabbit or mouse immunoglobulin was substituted in place of each primary antibody to serve as a negative control. The relative level of each protein was mea-
sured by densitometry and compared with that of β-actin. The ratio of the level of each protein to β-actin in ethanol-treated control cells was arbi-
trarily given a value of 1.0.

Cell Growth Assay

To assess cell growth, we seeded DU145 and PC-3 cells (1.5 × 10⁵ cells per well) in a 96-well culture plate in DMEM with 5% charcoal-stripped FCS, and incubated to DMEM with 5% charcoal-stripped FCS containing dexamethasone (10⁻⁷ to 10⁻⁶ M) or an equivalent volume of ethanol

Transfection Studies

The pMMTV-Luc reporter, driven by the mouse mammary tumor virus (MMTV) long terminal repeat promoter, was provided by B. O’Malley at Bey-

Tumor localization of NF-κB

Forty-eight hours after treatment with 10⁻⁷ M dexamethasone or 0.1% ethanol control, DU145 cells (2 × 10⁵ cells) grown on a two-well culture slide (Becton Dickinson, Franklin Lakes, NJ) were washed in PBS, then fixed in 3.7% formalin in PBS for 15 minutes at room temperature. The cells were permeabilized with 0.2% Triton X-100 in PBS for 15 minutes at room temperature and blocked with 10% FCS in PBS for 1 hour at room temperature. Immunoreactivity of the p65 subunit of NF-κB was detected with a specific antibody against p65 (Santa Cruz Biotechnology, Inc.) diluted 1 : 100 in PBS for 1 hour at room temperature. The cells were washed in PBS and then incubated with a rhodamine-labeled goat anti-rabbit secondary antibody (Santa Cruz Biotechnology, Inc.) diluted 1 : 100 in PBS for 45 minutes at room temperature in the dark. Immunofluorescent cells were photographed with a Nikon Microphot-FXA photomicroscope (Nikon Corp., Tokyo, Japan). As a negative control, blocking pep-
tide (Santa Cruz Biotechnology, Inc.) for anti-NF-
κB p65 was preincubated with anti-NF-κB p65 ac-
cording to the instructions of the manufacturer. Negative control showed low background immu-
nofluorescence. Only cells showing more intense immunofluorescence than any of the negative con-

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control cells by the same procedure and stained them with hematoxylin for 5 minutes at room temperature.

**IL-6 Assay**

DU145 or PC-3 cells (5 × 10^5 cells) were seeded on 100-mm tissue culture dishes. The next day, the medium was removed and replaced with DMEM containing 5% charcoal-stripped FCS and containing each specific dose of dexamethasone. Control cells were treated with 0.1% ethanol. After 48 hours, the chemiluminescent enzyme immunoassay kit (Fujiレビo Inc., Tokyo, Japan) was used to assay for IL-6 in each culture medium. IL-6 levels from each treatment were evaluated as the mean ± 95% confidence interval (CI) of four separate samples. Two independent assays were performed. This IL-6 assay detects between 0.2 and 1000 pg/mL of human IL-6 and does not cross-react with human IL-1, IL-1β, IL-4, granulocyte colony-stimulating factor, epidermal growth factor, TNF-α, and soluble IL-6 receptor. Human recombinant IL-6 standards (0, 20, 400, and 1000 pg/mL) (Fujirebio Inc.) were used to generate a standard curve.

**In Vivo Xenograft Model**

Animal care was in accordance with the laboratory animal guidelines of the Institute of Experimental Animal Sciences in Osaka University Medical School. To establish DU145 tumors in mice, DU145 cells were detached with trypsin and resuspended in DMEM with 10% FCS. Six-week-old male athymic nude or severe combined immunodeficiency (SCID) mice were each given a single subcutaneous injection in the dorsal area of 0.5 × 10^7 cells (nude mice, n = 6) or 1 × 10^7 cells (SCID mice, n = 6) in 0.1 mL of DMEM. Three times a week, the six mice were each given a subcutaneous injection at peri-tumor site of dexamethasone (1 μg per mouse per injection), which had been dissolved in ethanol and diluted 1:2000 in sterile saline immediately before injection. The dexamethasone treatment was started at the time of cell inoculation. Six control mice were given an injection of ethanol, which had been diluted 1:2000 in sterile saline. The tumor volumes were measured weekly with a slide caliper and determined by the following formula: (length × width^2)/2. At the completion of the experiments, the mice were killed, and tumors from the dexamethasone-treated or ethanol-treated SCID mice were removed for immunohistochemical studies.

**Immunohistochemical Analysis**

For the determination of GR expression in tissue sections, surgical specimens of human prostate cancers or DU145 xenograft tumors were fixed in 10% buffered formalin, processed, and embedded in paraffin. Serial tissue sections (5 μm thick) were obtained and mounted on slides that were then deparaffinized, rehydrated, and incubated with 3% (vol/vol) hydrogen peroxide in PBS for 15 minutes at room temperature to inhibit endogenous peroxidase activity. The sections were then rinsed with PBS three times, incubated with a peroxidase-labeled anti-rabbit immunoglobulin antibody (Bio-Rad Laboratories, Richmond, CA) at a dilution of 1:1000 in PBS for 10 minutes at 37 °C, and exposed to avidin–biotin–peroxidase complex (Dako kit; Dako Corp., Carpinteria, CA) according to the instructions of the manufacturer. The sections were then rinsed with 3,3′-diaminobenzidine as the color-developing reagent and counterstained with hematoxylin. An isotype-specific rabbit immunoglobulin was substituted in place of the primary anti-GR antibody to serve as a negative control.

The sections were scanned under a Nikon microscope (Nikon Corp.), at magnifications of ×40, ×100, ×200, and ×400, and the area containing the greatest number of positive cancer cells was selected for each sample. Only cancer cells that showed intense nuclear staining were scored as positive. The number of GR-positive cells per 500 cancer cells was counted and expressed as a percentage. The average percent GR-positive cells among normal epithelial cells in the cancer sections was used to determine the cut point between high and low GR expression. A high level of GR expression was defined as at least 30% GR-positive cells, and a low level was defined as less than 30% GR-positive cells. The intensity of the immunoreactivity was not considered in determining the level of GR expression.

**Statistical Analysis**

The statistical significance of the results was analyzed by unpaired Student’s t test, and P<0.05 was considered to be statistically significant. For multiple comparisons, the level of statistical significance assigned to the P value was adjusted for the total number of comparisons (n), such that P<0.05/n. The level of statistical significance was confirmed by Mann–Whitney U test. All statistical analyses were performed by use of the StatView software (SAS Institute Inc., Cary, NC). All statistical tests were two-sided.

**RESULTS**

**GR Expression Profile and Effects of Dexamethasone on Growth of Human Prostate Cancer Cells In Vitro**

We hypothesized that glucocorticoids act directly through GRs to inhibit tumor growth. To test this hypothesis, we first examined GR mRNA and protein expression in the human prostate cancer cell lines LNCaP, DU145, and PC-3 by RT–PCR and western blot analysis, respectively. GR-specific mRNA and protein were detected in DU145 and PC-3 cells but not in LNCaP cells (Fig. 1, A and B). HeLa cells were used as a positive control for GR mRNA and protein expression (data not shown). We tested whether the cell lines expressed functional GRs with the use of the MMTV luciferase reporter gene assay. Glucocorticoid-dependent MMTV promoter transactivation was observed in DU145 and PC-3 cells but not in LNCaP cells (data not shown).

Next, we tested whether dexamethasone could decrease GR protein levels in DU145 and PC-3 cells. In both cell lines, GR protein levels decreased in a dose-dependent manner after treatment with dexamethasone (at 10^{-8} M or higher doses) (Fig. 1, C). The dose-dependent loss of GR protein levels was more apparent in PC-3 cells than in DU145 cells. For example, in PC-3 cells treated with 10^{-6} M dexamethasone, GR protein levels decreased to approximately 25% (95% CI = 23% to 26%) of the ethanol-treated control levels, whereas in DU145 cells treated with the same concentration, GR protein levels decreased to approximately 59% (95% CI = 56% to 69%).

To determine whether dexamethasone (10^{-9} to 10^{-6} M) affects the growth of prostate cancer cells in vitro, we performed 5-day cell growth assays. Compared with the growth of ethanol-treated control cells, the growth of DU145 cells was statistically significantly lower in those cultures treated with dexamethasone concentrations of greater than 10^{-8} M (10^{-8} M, P = .001; 10^{-7} M, P<.001; and 10^{-6} M, P<.001) (Fig. 1, D). The growth-inhibitory effect was dose dependent between 10^{-9} and 10^{-7} M, with a plateau effect seen at higher concentrations of dexamethasone. By contrast, compared with the growth of ethanol-treated control cells, the growth of PC-3 cells was statistically significantly lower only in those cultures treated with a dexamethasone concentration of 10^{-7} M (P = .009) (Fig. 1, D). The growth of PC-3 cells was not statistically significantly inhibited by 10^{-6} M dexamethasone, presumably because GR levels were decreased by dexamethasone treatment (Fig. 1, C). There was no statistically significant inhibition of growth in LNCaP cells treated with dexamethasone (Fig. 1, D), which corresponded to the absence of GRs in these cells (Fig. 1, A and B). Because of the growth-inhibitory effect in DU145 cells treated with a broad range of dexamethasone concentrations (10^{-10} to 10^{-6} M), we concentrated our efforts on this cell line for subsequent experiments.

**Elucidating the Mechanism of Dexamethasone-Mediated Growth Inhibition: Importance of NF-κB, ILβ, and IL-6**

Glucocorticoids have been shown to cooperate with other transcription factors in modulating gene expression (20). One such transcription factor is NF-κB (13). We assessed the effects of dexamethasone...
on members of the NF-κB transcription factor family to elucidate the mechanism by which dexamethasone may inhibit DU145 cell growth. DU145 cells were treated with dexamethasone (10⁻⁸ to 10⁻⁵ M) for 48 hours, and the protein levels of p65, one of the components of NF-κB, and IκBα, one of the natural cytoplasmic inhibitors of NF-κB, were analyzed by western blot analyses. There was a dose-dependent increase in IκBα levels but not in NF-κB levels in dexamethasone-treated cells (Fig. 2, A). To determine whether the increased levels of IκBα prevented nuclear translocation of NF-κB, we examined the subcellular localization of NF-κB in DU145 cells by indirect immunofluorescence. NF-κB localized to
both the nuclear and cytoplasmic compartments in control ethanol-treated DU145 cells (Fig. 2, B). However, NF-κB localized only to the cytoplasmic compartment, which was accompanied by a loss in localization to the nuclear compartment, in dexamethasone-treated DU145 cells (Fig. 2, B).

NF-κB is a key regulator of several cytokine growth factors, including IL-6 (21). Because IL-6 has been shown to be an autocrine growth factor for prostate cancer cells (14,15), we tested whether dexamethasone affects IL-6 production in DU145 cells. After 48 hours, the mean secretory level of IL-6 in conditioned medium from dexamethasone-treated DU145 cells was 37 pg/mL (95% CI = 33 pg/mL to 41 pg/mL), at least 75% lower (mean difference = 126 pg/mL; 95% CI on the difference = 122 pg/mL to 130 pg/mL) than that in conditioned medium from ethanol-treated control cells (164 pg/mL; 95% CI = 162 pg/mL to 166 pg/mL) (Fig. 2, C). The mean IL-6 levels in the conditioned medium from dexamethasone-treated PC-3 cells decreased to 62 pg/mL, at least 70% lower (mean difference = 179 pg/mL; 95% CI on the difference = 168 pg/mL to 190 pg/mL) than that in conditioned medium from ethanol-treated control cells (242 pg/mL) (data not shown).

Effects of Dexamethasone on DU145 Xenograft Growth

We next determined whether dexamethasone could also inhibit the growth of androgen-independent prostate DU145 tumors in vivo. Because we previously demonstrated the clinical benefits of low-dose dexamethasone for patients with HRPC (7), we wanted to mimic a similar low-dose dexamethasone regimen in the xenograft model. We selected a dose of 1 μg per mouse three times per week because this low dose effectively inhibited the in vivo growth of another type of adenocarcinoma cells (22). Athymic nude mice, which lack T cells, were first given an injection subcutaneously of DU145 tumor cells. On the same day, the mice were started on the regimen of dexamethasone treatments. After 8 weeks, the mean tumor volume in the dexamethasone-treated mice was 333 mm³ (95% CI = 267 mm³ to 399 mm³), statistically significantly
smaller \((P = .006)\) than that in the ethanol-treated control mice \((731 \text{ mm}^3; \text{95\% CI} = 651 \text{ mm}^3 \text{ to } 811 \text{ mm}^3)\) (Fig. 3, A).

To exclude the possibility that dexamethasone may modulate host B-cell immunologic functions, we gave SCID mice, which lack B- and T-cell immunity, a subcutaneous injection of DU145 tumor cells and started them on the regimen of dexamethasone treatments. After 7 weeks, the mean tumor volume in the dexamethasone-treated mice was \(535 \text{ mm}^3\) (95% CI = 375 mm \(^3\) to 695 mm \(^3\)), statistically significantly smaller \((P = .026)\) than that in the ethanol-treated control mice \((1011 \text{ mm}^3; \text{95\% CI} = 846 \text{ mm}^3 \text{ to } 1176 \text{ mm}^3)\) (Fig. 3, B and C). These results suggested that \textit{in vivo} administration of low-dose dexamethasone inhibits the growth of androgen-independent prostate cancers.

\textbf{GR Expression in DU145 Xenograft and Human Prostate Cancers}

To determine whether \textit{in vivo} administration of low-dose dexamethasone alters the level of GRs, after 7 weeks of low-dose dexamethasone treatment, we harvested the DU145 xenografts from the SCID mice and examined GR expression by immunohistochemistry. GRs were readily detected in xenograft tumors from the ethanol-treated control (Fig. 4, A) and low-dose dexamethasone-treated (Fig. 4, B) groups, suggesting that GR levels in DU145 tumor cells were maintained under the long-term administration of low-dose dexamethasone.

Finally, GR expression in human primary prostate cancers was analyzed to determine whether dexamethasone could directly affect human prostate cancer cells. There was wide variability in the proportion of GR-positive cancer cells among the individual cases (Fig. 4, C and D) and within the different lesions from the same patient. Of the prostate cancer specimens examined, 50% (eight of 16) expressed high levels (\(\geq 30\%\) GR-positive cells) of GR. There was no association between GR expression levels and treatment status (surgery versus surgery plus AAT), with six of 11 cancers from patients treated with AAT having high GR expression levels (Fig. 4, E). There was no association between GR expression levels and pathologic tumor stage.

\textbf{DISCUSSION}

The results of this study support the hypothesis that dexamethasone inhibits the growth of prostate cancer cells through intrinsic GRs. However, \textit{in vitro} GR levels are decreased by dexamethasone in a dose-dependent manner \((16,17)\), an observation that we confirmed in DU145 and PC-3 cell lines. The decrease in GR levels may explain the lack of an \textit{in vivo} antitumor effect of high-dose dexamethasone in androgen-independent prostate cancers \((18,23)\). Although the mechanisms by which dexamethasone decreases GR levels are not fully understood, there may be some cell specificity because DU145 and PC-3 cells are both androgen-independent cell lines. In our study, low-dose dexamethasone inhibited the growth of DU145 xenograft tumors in both athymic nude and SCID mice, without apparently altering the level of GR expression. Thus, because GR expression is maintained, low-dose dexamethasone may continue to inhibit the proliferation of prostate cancer cells \textit{in vivo}.

To our knowledge, only one previous study \((24)\) addressed GR status in human prostate cancer. The investigators of that study reported that, in radical prostatec-
In a DU145 xenograft tumor from a SCID mouse that was treated with ethanol, GR expression was visualized with an avidin–biotin–peroxidase detection kit by use of 3,3'-diaminobenzidine as a substrate. GR expression in a representative section of a DU145 xenograft tumor from a SCID mouse that was treated with ethanol as the vehicle control. GR expression in a representative section of a DU145 xenograft tumor from a SCID mouse that was treated with low-dose dexamethasone. A representative section showing low GR expression (defined as <30% GR-positive cells) in a human primary prostate cancer. Original magnification ×200 for all micrographs. Summary of results of GR expression analysis in human primary prostate cancers. The results are presented according to level of GR expression (low [≤30% GR-positive cancer cells] versus high [>30% GR-positive cancer cells]), preoperative androgen ablation therapy (AAT), and pathologic stage (≤pT2 versus >pT3).

The mechanism responsible for the antiproliferative effect of glucocorticoids is poorly understood. One mechanism is a direct effect on cell cycle regulatory proteins. The glucocorticoid–GR complex mediates cell cycle arrest in several cell types, including human osteosarcoma cells, human cervical carcinoma cells, rat hepatoma cells, and human lymphocytes (25–28). When we analyzed the cell cycle distribution of dexamethasone-treated DU145 cells, we found that cells accumulated in the G1 phase, which was associated with increased levels of the cell cycle regulatory protein p27 but not p21 (data not shown). Because GR-mediated growth arrest is cell type specific (25), further study is required to clarify the involvement of p27 and p21 in prostate cancer cells.

A second mechanism by which glucocorticoids may exert their antiproliferative effects is through the modulation of growth-related cytokines or their transcriptional regulators. The transcription factor NF-κB is constitutively activated in DU145 cells (29), and its activation is implicated in the control of cell proliferation and apoptosis in many tumors (30–32). Because glucocorticoids are potent inhibitors of NF-κB activation in cultured cells (33), inactivation of NF-κB may be an essential mechanism involved in GR-mediated growth inhibition of DU145 cells. Our data indicate that, in response to dexamethasone, NF-κB accumulates in the cytosol of treated cells. This accumulation is accompanied by an increase in IκBα protein levels and a decrease in secreted IL-6 levels, an NF-κB-inducible gene. Thus, because IL-6 has been implicated as an autocrine growth factor for androgen-independent prostate cancer cells (14,15), the antiproliferative effects of dexamethasone may be mediated through the inhibition of an NF-κB–IL-6-dependent pathway. Consistent with previous reports (14,15), the addition of antibodies to IL-6 inhibited the growth of DU145 cells in vitro (data not shown). A recent study (34) showed that PC-3 xenograft tumors regressed after the in vivo administration of antibodies to IL-6. Moreover, patients with HRPC were reported to have elevated serum IL-6 levels (35). We measured IL-6 levels in serum from patients with HRPC who had been treated with low-dose dexamethasone therapy (7) and found that the mean IL-6 levels declined from 5.4 pg/mL (95% CI = 1.9 pg/mL to 8.9 pg/mL) to 1.2 pg/mL (95% CI = 0.9 pg/mL to 1.5 pg/mL). Taken together, these results and observations suggest that the inhibition of IL-6 production by the cancer cells may be a major mechanism responsible for dexamethasone-mediated antitumor activity against HRPC.

Although the growth-inhibitory effect of dexamethasone alone is not dramatic, combinations with other chemotherapeutic agents may enhance its antiproliferative activity. Because NF-κB activation is implicated as a principal mechanism of inducible tumor chemoresistance (36), NF-κB inactivation by dexamethasone may substantially augment and prolong chemosensitivity. Therefore, our results may provide a basis for developing improved protocols for dexamethasone
therapy alone or in combination with chemotherapy agents for treating patients with HRPC.

REFERENCES


NOTES

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