Development and Characterization of Nontumorigenic and Tumorigenic Epithelial Cell Lines from Rat Dorsal-Lateral Prostate

David Danielpour, Kenji Kadomatsu, Mario A. Anzano, Joseph M. Smith, and Michael B. Sporn

Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland 20892

ABSTRACT

We have established two new epithelial cell lines (NRP-152, NRP-154), with markedly different properties, from the dorsal-lateral prostate of Lobund/Wistar rats treated with N-methyl-N-nitrosourea and testosterone propionate. NRP-152 cells do not form tumors in athymic mice and retain many of the properties of normal prostatic epithelial cells. They produce prostatic acid phosphatase, have functional androgen receptors, and require the combination of several growth factors in addition to serum for optimal growth. Their growth is stimulated by epidermal growth factor, insulin, dexamethasone, cholera toxin, dihydrotestosterone, and testosterone, and their growth is inhibited by transforming growth factor β1 but not significantly altered by epidermal growth factor, cholera toxin, dihydrotestosterone, retinoic acid, or 1a,25-dihydroxyvitamin D3. Our data suggest that the NRP-152 and NRP-154 cell lines are suitable systems for analysis of normal prostate growth and prostatic carcinogenesis.

INTRODUCTION

Cultures of normal cells under hormonally defined conditions offer useful in vitro model systems for studying the mechanisms of normal cell function and regulation, as well as cellular transformation. However, this is possible only if the normal response patterns and function of cells are preserved when they are separated from their in vivo milieu. Although much effort has been expended in optimizing in vitro conditions for growth and viability of normal adult prostatic epithelial cells in rodents, these specialized cells have a particularly limited growth potential and undergo rapid senescence in culture (1–4). Thus, immortalization of normal prostatic cells has been an important undertaking; however, to date only a limited number of prostatic cell lines are reported to be nontumorigenic (5–7). One of these epithelial cell lines (derived from rat ventral prostate) forms tumors when coinoculated with a fibroblastic line (6). The tumorigenicity of several rat prostate cell lines (immortalized by SV40 large-T antigen) has not been determined (8, 9). Of these cell lines, only one was derived from the dorsal-lateral prostate (9), which is the region of the rat prostate most similar, functionally and structurally, to the human prostate (10–12); this cell line was not characterized with respect to expression of prostatic markers and responsiveness to growth factors and hormones.

Progress in the study of prostatic carcinogenesis has also been limited by the availability of appropriate animal model systems, since spontaneous prostatic cancers are rare in nonhuman mammals (13). One exception, however, is the Lobund/Wistar rat strain, which developed spontaneous prostate and seminal vesicle adenocarcinomas at an incidence of 26% in 26 months (14, 15). Treatment of these animals with MNU2 and TP increases the percentage of tumor incidence and decreases tumor latency (16).

To obtain premalignant as well as malignant prostatic cell lines, we have cultured cells and explants of dorsal-lateral prostates from Lobund/Wistar rats that have been treated with MNU and TP. We have derived and characterized nontumorigenic and tumorigenic epithelial lines; designated them NRP-152 and NRP-154, respectively; and then studied their responsiveness to several hormones and growth regulatory peptides. In this paper, we show that NRP-152 cells are highly responsive to several hormones and growth factors and possess functional ARs. In contrast, NRP-154 cells have less stringent requirements for growth, are less responsive to hormones and growth factors, and lack detectable levels of ARs. NRP-152 cells are exquisitely responsive to retinoic acid and 1,25(OH)2D3, whereas NRP-154 cells are unresponsive to these hormones. Such sensitivity to both retinoids and 1,25(OH)2D3 makes NRP-152 cells suitable for in vitro studies on the mechanism of action of these hormones the potential of which in the chemoprevention of breast and prostate cancer is currently under investigation.

MATERIALS AND METHODS

Materials. Sources were: Lobund/Wistar rats, Harlan-Sprague-Dawley, Inc. (Indianapolis, IN); anti-pan keratin rabbit polyclonal, anti-vimentin, and anti-desmin monoclonal antibodies, New Fuchsins substrate system, and phosphatase-linked rabbit anti-mouse IgG, Kirkegaard and Perry, Inc. (Gaithersburg, MD); MNU, Ash Stevens (Detroit, MI); AR-38 mouse fibrosarcoma cells, Dr. Umberto Saffiotti; keratinocyte growth factor, Dr. Jeffrey Rubin.

Development of NRP-152 Cell Line. Lobund/Wistar rats were treated with MNU (30 mg/kg body weight, injected into the intraluminal vein). One week later they received a s.c. implant of TP (50 mg) encased in a Silastic capsule. The TP implants were replaced at 2.5-month intervals. Rats were killed by suffocation in carbon dioxide. The dorsal-lateral prostate was excised sterilely and washed extensively in Hanks' solution and then transferred to a dish containing 2 ml of Hanks' solution supplemented with 10% FBS, where it was minced to <1-mm fragments with fine scissors. Fragments were then transferred to ten 80-cm2 flasks containing 15 ml of GM1. Cells appeared to attach within 1 week, at which time the medium was replaced with GM1 and then changed every 2 days thereafter. At 3–5-day intervals, fibroblasts were removed by a 2–10-mm treatment with trypsin-EDTA at 37°C, and the adherent monolayer was washed with PBS. Epithelial cells were allowed to

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1 To whom requests for reprints should be addressed.

2 The abbreviations used are: MNU, N-methyl-N-nitrosourea; AR, androgen receptor; [3H]R1881, [17α-methyl-3H]methyltrienolone; CT, cholera toxin; Dex, dexamethasone; DHT, dihydrotestosterone; 1,25(OH)2D3, 1α,25-dihydroxyvitamin D3; EGF, epidermal growth factor; FBS, fetal bovine serum; Ins, bovine insulin; PAP, prostatic acid phosphatase; PBS, phosphate-buffered saline; RA, all-trans-retinoic acid; TGF-β, transforming growth factor-β; TP, testosterone propionate; BSA, bovine serum albumin; RT, room temperature; cDNA, complementary DNA; HEPES, 4-(2-hydroxyethyl)1-piperazineethanesulfonic acid; DMEM/F12, Dulbecco's modified Eagle's medium/Ham's F-12 medium; GM1, DMEM/F12 supplemented with 10% FBS, 20 ng/ml EGF, 5 μg/ml Ins, 0.1 μg/ml Dex, 10 ng/ml CT, trypsin-EDTA, 0.05% trypsin-0.53 mM EDTA in Hanks' solution; SFM, serum-free medium.
grow until the flask was near-confluent, at which point epithelial monolayers were detached by treatment with trypsin-EDTA at 37°C. Cells that were detached by trypsinization and those few that remained attached to the culture flasks after trypsinization were washed once with 10 ml of GM1 and cultured separately with 15 ml of GM1. All detached epithelial cells that were passed in this manner died within 1 week. However, one colony of epithelial cells that remained attached after trypsinization continued to grow. Once subconfluent, these cells were passaged by trypsinization with approximately 1–2% viability of the detached epithelial cells. Viability increased upon subsequent passaging; passages 2, 3, and 4 resulted in over 5, 30–50, and 80% viability, respectively. These cells passed much better at higher density than at low density and several attempts at cloning these cells were unsuccessful.

**Development of NRP-154 Cell Line.** Development of the NRP-154 cell line was similar to that of NRP-152, with two modifications: (a) tissue was digested with collagenase (670 units/ml collagenase and 10% FBS in Hanks’ solution) for 1 h at 37°C, passed through 1 mm and 250 μm mesh filters, and after one wash with WAJC 404 (1), supplemented with 10% FBS, followed by two washes with WAJC 404 alone; (b) cells were cultured in a SFM (WAJC 404 supplemented with 200 μg/ml BSA V, 80 μg/ml reduced glutathione, 20 μg/ml human transferrin, 10 μg/ml Ins, 1 μM Dex, 20 ng/ml EGF, 10 ng/ml CT, and 10 ng/ml keratinocyte growth factor), as modified from the system of McKeehan et al. (1) to bypass the problem of stroma growth. After 5 days in culture, medium was changed at 2-day intervals. After an additional 10 days, all epithelial colonies were essentially growth arrested and dying. To revive the remaining colonies, the medium was replaced with GM1. Although most epithelial colonies grew initially, only one colony, which gave rise to the NRP-154 cell line, survived past 3 weeks. Following a 30-min trypsinization, about 30% of the detached cells survived and grew as pure epithelial cells. This constituted passage 1 of NRP-154 cells.

**Culturing Established Lines.** Established cell lines were maintained in GM2 [HEPES-free, and antibiotic-free DMEM/F12 (1:1, v/v) supplemented with 5% FBS, 20 ng/ml EGF, 10 ng/ml CT, 0.1 μM Dex, and 5 μg/ml Ins] in 80-cm² Nunc tissue culture flasks and passaged every 3–4 days at subconfluent density. For passaging, a flask of cells was washed with 5 ml of trypsin-EDTA and treated for 5 to 7 min with trypsin-EDTA (3 ml at 37°C). Cells were dislodged by gentle tapping and then recovered following centrifugation (1000 × g, 5 min) with 7 ml of 10% FBS in DMEM/F12.

**Athymic Mouse Studies.** Cell suspensions trypsinized off confluent monolayers were washed once with DMEM/F12 containing 10% FBS and twice with DMEM/F12 by centrifugation and then resuspended in DMEM/F12 or in DMEM/F12:Matrigel (1:1, v/v) at a density of 5–3×10⁶ cells/ml. Six-week-old intact male athymic mice (five animals/group) were inoculated s.c. in a hindlimb with 0.1–0.2 ml of the cell suspension. DNA synthesis was also measured by the incorporation of [3H]thymidine (18), following a 24-h pulse of 50,000 cells (in 24-well dishes) with 0.25 μCi/well [3H]thymidine.

**Cell Proliferation Assays.** Cell growth was measured by the binding of crystal violet to formalin-fixed monolayers in Costar 6-well dishes (17) and cell enumeration in single-cell suspension with a Coulter Counter. DNA synthesis was also measured by the incorporation of [3H]thymidine (18), following a 24-h pulse of 50,000 cells (in 24-well dishes) with 0.25 μCi/well [3H]thymidine.

**Results**

We have established cell lines from the dorsal-lateral prostates of 2 (No. 152 rat and No. 154 rat) of a group of 8 rats, 5 to 8 months after initial carcinogen treatment. The prostates were transected bilaterally; one lobe was used for histology and the other for cell culture. Histological examination of No. 152 showed no microscopic lesion in the dorsal-lateral region, whereas No. 154 had atypical hyperplasia in this region. In culture, NRP-152 cell monolayers are cuboidal at low cell density, occasionally aligned in parallel curving bundles at high density (Fig. 1, A and B). NRP-154 cells show a different morphology: they are larger and flatter than NRP-152 cells (Fig. 1C).

**Conditions for Optimal Growth of NRP-152 and NRP-154 Cells.** Unlike NRP-154 cells, which were able to grow continuously in DMEM/F12 supplemented with only 10% FBS, NRP-152 cells at passages 3 through 11 were unable to grow in this medium, and additional growth factors and hormones (EGF, Ins, Dex, CT) were required for derivation and maintenance of this cell line. Under optimal growth conditions (GM1) NRP-152 and NRP-154 cells doubled approximately every 17 h. To define the supplements required for growth, we studied the contribution of each of the four supplements in GM1 to the overall growth of these cells after 6 days, using crystal violet staining as a convenient method of approximating cell growth. The presence of PAP was further confirmed by PAP mRNA expression on Northern blots.

**Prostatic Acid Phosphatase Assay.** Prostatic acid phosphatase activity was measured as tartrate-sensitive acid phosphatase using the prostatic acid phosphatase kit from Sigma according to the protocol described in the kit. The presence of PAP was further confirmed by PAP mRNA expression on Northern blots.

**RNA Purification and Northern Blot Analysis.** Total RNA was purified using a modification (20) of the method of Chomczynski and Sacchi (21). Ten μg of total mRNA were electrophoresed through 1% agarose-0.66 M formaldehyde gels. Cells were treated with 60 mm NaOH for 20 min, neutralized with 50 mm Tris-HCl (pH 7.4)-10 mm NaCl, for 20 min, and then blotted onto Nytran (pore size, 0.45 μm; Schleicher & Schuell, Keene, NH). After membranes were cross-linked by UV irradiation, they were prehybridized, hybridized, and washed at 65°C as described by Church and Gilbert (22). Hybridization was done with 3 × 10⁶ cpm/ml (10⁻³ dpm/μg DNA) of [32P]cDNA probes that were prepared by random priming (GIBCO BRL, Gaithersburg, MD).

**cDNA Probes.** For the rat AR probe, a 570-base pair cDNA insert from nucleotides 2067–2636 was obtained from Dr. Terry Timme. Rat PAP cDNA probe (411-base pair) was prepared by reverse transcription-polynucleotase chain reaction amplification from nucleotides 481–891, using rat ventral prostate polyadenylated mRNA as a template. The human TGF-β type II receptor cDNA probe (1.7-kilobase insert from the open reading frame) was provided by Drs. Andrew Geiser and Herbert Lin.

**Immunocytochemistry.** Cytoskeletal markers were assayed immunocytochemically using subconfluent monolayers grown in chamber slides. Cells were fixed on these slides for 10 min at −20°C with methanol-acetone (1:1, v/v). Slides were blocked with BB (PBS plus 1% BSA, plus 1% goat serum) for 30 min at RT, treated with primary antibodies or control IgG in BB (BB plus 0.05% Tween 20) for 1 h at RT, followed by 5 μg/ml of phosphatase-conjugated secondary antibodies in BB for 1 h at RT, using extensive washes with PBS-0.05% Tween 20 between each step. Slides were developed using Dako’s New Fuchsin Substrate System.

**Karyotype and Isozyme Analysis.** Karyotype and isozyme analyses were performed by Drs. Ward D. Peterson and Joseph Kaplan at the Cell Culture Laboratory, Children’s Hospital of Michigan, Detroit.

**Mycoplasma and Viral Testing.** The absence of Mycoplasma contamination of NRP-152 and NRP-154 cells was confirmed by an indirect Hoeschst method and by a direct agar method (American Type Culture Collection). The absence of eight viruses (Toollans H-1, GDVII, KRV, PRV, Reo, Sendai, RCV/SDA, and Hantaan) in NRP-152 and NRP-154 cells was confirmed by a rat antibody production test (Microbiological Associates).
Fig. 1. Morphology of NRP-152 and NRP-154 cells grown on plastic. NRP-152 cells (passage 2) at near confluence (A) and confluence (B) and NRP-154 cells (passage 2) at near confluence (C) were grown on plastic in DMEM/F12 supplemented with 10% FBS, 20 ng/ml EGF, 5 µg/ml Ins, 10 ng/ml CT, and 0.1 µM Dex.

were no longer completely growth arrested in serum alone, exhibiting minimal growth from 4 to 6 days and reaching growth arrest by day 7 (Fig. 2C). In contrast, when NRP-154 cells (passage 7) were analyzed as above, growth in wells receiving basal medium alone was 44% of that in complete medium; under these conditions only Ins and Dex significantly stimulated growth over control (data not shown). The addition of 200 µM TGF-β1 to NRP-154 cells caused marked inhibition of growth in both basal and supplemented medium (data not shown).

**Tumor Growth in Athymic Male Mice.** We first tested the tumorigenicity of NRP-152 and NRP-154 cells at passages 8 and 34, using a 3 million-cell inoculum. None of the 10 mice receiving either passage 8 or 34 NRP-152 cells had palpable tumors, even 11 months after inoculation. The positive control AR-38, a mouse fibrosarcoma cell line, formed tumors 1 week after inoculation; these tumors weighed an average of 2.2 ± 1.2 (SD) g by 2 weeks. After 6–7 weeks, palpable masses were observed at the site of inoculation with NRP-154 cells. All five mice in this group had sizable growths, averaging 0.50 ± 0.30 g, 10 weeks after inoculation, that were confirmed histologically as desmoplastic tumors. Because coinoculation of tumorigenic cells with Matrigel has been shown to shorten tumor latency and enhance tumor growth (23), we also tested the tumorigenicity of NRP-152 cells at passage 35, using a large inoculum of 10⁷ cells/0.2 ml in both the presence and the absence of Matrigel. None of the 10 mice in these groups formed tumors after 8 months. The positive control in this experiment, NRP-154 cells without Matrigel, formed tumors in all animals after 7 weeks.

**Karyotype and Isozyme Analysis.** The karyotype and isozyme analyses of NRP-152 cells were done at both passage 7 and passage 61 to detect any genotypic drift in culture (NRP-154 was done as passage 7). For both cell lines the electrophoretic mobilities of glucose-6-phosphate dehydrogenase, purine nucleoside phosphorylase, lactate dehydrogenase, and malate dehydrogenase were comparable to those of a normal rat cell control preparation. Karyotype analyses from 100 cells from each passage of NRP-152 demonstrated that the cell line is aneuploid (XXYY), with most chromosomal counts in the hypertriploid range. Karyotype analysis of NRP-154 demonstrated that the cell line is also aneuploid (XY), with 50% of the cells essentially diploid and the remainder in the tetraploid range. For both cell lines structural modification of chromosomes was infrequent.

**Immunocytochemical Staining.** In order to determine the cellular origin of the established cell lines, they were stained with various specific antibodies directed against cytoskeletal proteins. Pan antikeratin polyclonal antibody stained 100% of the cells of both lines, confirming that they represent pure populations of epithelial cells. For NRP-152 cells, antibodies directed against cytokeratins 1, 10, 13, and 18 exhibited no specific staining, whereas anti-cytokeratin 14 stained 100% of the cells. In contrast, NRP-154 cells were positive for
cytokeratins 18, but negative for cytokeratins 1, 10, 13, and 14. Expression of cytokeratins 14 and 18 by NRP-152 and NRP-154 cells, respectively, suggests that NRP-152 cells are derived from basal epithelium and NRP-154 cells from luminal epithelium (24, 25). When stained for other intermediate filaments, both NRP-152 and NRP-154 cells were shown to express vimentin but not desmin and α-actin. The positive controls, BHK-21 baby hamster kidney cells and rat neonatal cardiac fibroblasts stained for desmin and α-actin, respectively.

AR Assay. AR was assayed in whole cells by the specific binding of [3H]R1881 (Fig. 3A). The specific binding of R1881 (2 nM) to NRP-152 cells was about 10-fold higher than that of NRP-154 cells. Northern blot hybridization, using a cDNA probe to rat AR, demonstrated that NRP-152 cells expressed AR mRNA at levels comparable to those in rat prostate in vivo, while no AR transcript was detected in NRP-154 cells (Fig. 3B). NRP-152 cells produced two similar-sized transcripts, one of which comigrated with AR in the seminal vesicle and all lobes of the rat prostate and the other of which migrated slightly faster. The expression of two such AR transcripts has also been observed in other systems (26, 27). The specific binding of [3H]R1881 to NRP-152 cells was saturable at about 1 nM ligand (Fig. 3C). Scatchard analysis of passage 17 NRP-152 cells showed an average of 5300 receptors/cell with a K_d of 53 pM (Fig. 3D). Neither number nor affinity of these sites changed at passage 56. One hundred-fold molar excesses of progesterone, Dex, and β-estradiol competed for <10%, <1%, and 45%, respectively, of the specific binding of 1 nM [3H]R1881 to NRP-152 cells, whereas DHT and testosterone competed for >95% of the specific binding (data not shown). These data confirm the androgen specificity of these high-affinity sites.

Effect of Androgens on Cell Growth. Attempts to demonstrate mitogenic effects of androgens on early passage NRP-152 cells in medium containing dialyzed and charcoal-extracted FBS were unsuccessful, since the stripped serum did not support growth of early passage NRP-152 cells over several days. At a later passage, NRP-152 cells (passage 67) exhibited sustained growth (doubling time, 67 h) in the above medium, and a single addition of DHT at 10 nM over 11 days stimulated a 2-fold increase in cell number (data not shown). Growth of NRP-154 cells was not stimulated by DHT under identical conditions. To test early (1–2 days) effects of DHT on cell growth, we assayed for DNA synthesis in a SFM consisting of DMEM/F12, 15 mM HEPES, 0.1% BSA, 10 μg/ml human transferrin, 1× trace element mix, and 20 ng/ml EGF. Using this medium, we showed that as little as 1 nM DHT caused a 2-fold increase in DNA synthesis in NRP-152 cells; similar effects were seen with R1881 (Fig. 4A). These were not nonspecific steroid hormone effects since neither estradiol nor progesterone (10 nM) was mitogenic for these cells, whereas 10 nM DHT or testosterone stimulated DNA synthesis 2.5-fold over control (data not shown). The specificity of this effect was further confirmed by use of the anti-androgen hydroxyflutamide (1 μM), which blocked induction of DNA synthesis by 10 nM DHT (Fig. 4B).
Regulation of Cell Growth by RA and 1,25(OH)\textsubscript{2}D\textsubscript{3}. The effects of retinoids and vitamin D derivatives on inhibiting growth and promoting differentiation of epithelial cells is well documented (32, 33). The effects of RA and 1,25(OH)\textsubscript{2}D\textsubscript{3} on growth of NRP-152 and NRP-154 cells were examined as a function of time. Growth of NRP-152 cells was inhibited by RA as measured by both changes in cell number (Fig. 7) and [\textsuperscript{3}H]thymidine incorporation (Fig. 8) with 50% effective dose of about 1 nM (data not shown). 1,25(OH)\textsubscript{2}D\textsubscript{3} stimulated growth of NRP-152 cells at early time points and inhibited growth at later times (Figs. 7, A and B, and 8). At early time points (24–48 h), 1,25(OH)\textsubscript{2}D\textsubscript{3} also stimulated the incorporation of [\textsuperscript{3}H]thymidine by severalfold with a 50% effective dose of 20 nM (data not shown). In contrast, growth of NRP-154 cells, under conditions identical to those in Fig. 7, was neither stimulated nor inhibited by either RA or 1,25(OH)\textsubscript{2}D\textsubscript{3} (data not shown). Our preliminary results suggest
DISCUSSION

We have developed nontumorigenic and tumorigenic epithelial cell lines from Lobund/Wistar rat dorsal-lateral prostate. Corresponding with the differentiated phenotype of prostatic epithelium, both cell lines produce relatively high levels of PAP, and NRP-152 cells express AR mRNA and possess functional ARs. In contrast, NRP-154 cells show no detectable AR mRNA and do not respond to androgens under identical conditions. Furthermore, similar to primary cultures of prostatic epithelium (1-4, 35, 36), NRP-152 cells require the combination of EGF, Ins, Dex, and CT for optimal growth. NRP-154 cells, however, grow much more autonomously in culture, which is consistent with their tumorigenic phenotype. Although $K_r$-ras mutation at codon 12 is common in prostatic tumors induced by N-methyl-N-nitrosourea (37, 38), neither cell line has this mutation (data not shown).

Table 1

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<tr>
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<th>TAP*</th>
<th>PAP</th>
<th>nonPAP</th>
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<td>NRP-152 cells</td>
<td></td>
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<tr>
<td>Control</td>
<td>34.3 ± 0.5</td>
<td>28.3 ± 0.8</td>
<td>6.0 ± 0.3</td>
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<td>10 nM DHT</td>
<td>23.8 ± 1.5</td>
<td>18.1 ± 0.4</td>
<td>5.7 ± 1.1</td>
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<tr>
<td>NRP-154 cells</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Anterior prostate</td>
<td>12.1 ± 1.3</td>
<td>8.5 ± 0.9</td>
<td>3.6 ± 1.6</td>
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<tr>
<td>Lateral prostate</td>
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<td>0.52</td>
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<td>0.71</td>
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<td>2.45</td>
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* TAP, total acid phosphatase.

that lack of response of NRP-154 cells to 1,25(OH)$_2$D$_3$ may be the result of vitamin D receptor deficiency, since these receptors are expressed at much lower levels in NRP-154 cells relative to NRP-152 cells (data not shown).

Recent evidence suggests that effects of RA and 1,25(OH)$_2$D$_3$ on inhibition of growth may be synergistic (34). We examined the potential synergism of 1 μM RA and 10 nM 1,25(OH)$_2$D$_3$ on growth of NRP-152 cells in DMEM/F12 supplemented with 20 ng/ml of EGF and FBS as assayed by the incorporation of [³H]thymidine (³H-TdR) was determined. All values represent the average of triplicate determinations ± SD (bars).
The NRP-152 cell line offers a suitable and unique system for studying early events during carcinogenesis: (a) the dorsal-lateral prostate is believed to be functionally and structurally more similar to human prostate than rat ventral prostate (10–12); (b) carcinomas can be induced in the dorsal-lateral prostate in several rat models (38–40); (c) NRP-152 is the only nontumorigenic prostatic cell line reported to have functional ARs (5–7); (d) previously described human and rat dorsal-lateral prostatic cell lines that are nontumorigenic have often been immortalized by overexpression of SV40 large-T antigen (5, 7–12), a viral product that has not been reported to be involved in the etiology of prostate cancer in any system. In contrast, the NRP-152 cell line has been derived from animals that undergo spontaneous prostate adenocarcinoma. Thus, although the immortalizing gene alteration(s) of this cell line is as yet undefined (in contrast to the SV40 immortalized lines), NRP-152 cells are more likely to be representative of a preneoplastic state than any of the immortalized prostatic cell lines.
It has been shown recently that although prostatic tumors initially express elevated levels of PAP, such levels drop with tumor progression toward a more malignant state (46). Our demonstration of elevated levels of PAP in NRP-152 cells relative to the whole prostate and in NRP-154 cells is consistent with increased PAP expression occurring as an early event in carcinogenesis. The increased growth potential of more malignant prostate tumors may be explained by reduced cellular PAP, which has been shown to be the major phosphotyrosine phosphatase in human prostate (47), and is believed to dephosphorylate phosphotyrosine residues that are essential for growth factor signaling (48). This model has recently been tested by transfection of malignant prostatic carcinoma cells with a PAP expression construct, which elevated PAP levels and inhibited growth of the transfectants (46). By this mechanism, reduction of PAP levels by DHT in NRP-152 (Table 1) and LNCaP (49) cells may stimulate growth and thereby account for the mitogenic action of androgens on these cells.

There is strong evidence that TGF-βs play an important role in prostate development and function. TGF-β1 not only inhibits growth of prostatic epithelial cells (29–31) but may also be responsible for apoptosis in the prostate since its mRNA expression is dramatically elevated in regressed rat prostates following castration (50). Here, we show that TGF-β1 not only inhibits growth of NRP-152 and NRP-154 cells but also induces death of NRP-154 cells. The apparent greater sensitivity of NRP-154 cells than of NRP-152 cells to TGF-β1, which is consistent with the selective loss of luminal epithelium during apoptosis of the prostate (24), may be explained by the greater abundance of mRNA for TGF-β type II receptors in NRP-154 cells (data not shown), as recent evidence supports that this class of TGF-β receptors mediate inhibition of growth by TGF-β (51). The great sensitivity of NRP-154 cells to TGF-β further demonstrates that loss of sensitivity to TGF-β is not an early event of carcinogenesis in this system.

The use of steroids and retinoids in the chemoprevention of cancer has recently attracted much attention. The high sensitivity of NRP-152 cells to both RA and 1,25(OH)2D3 makes these cells suitable for studying the mechanism of action of these hormones. We have shown here that inhibitory effects of RA and 1,25-(OH)2D3 can be synergistic, rather than additive, which is consistent with recent findings in human breast cancer cells (34). Recently, we have also demonstrated that inhibition of growth by RA in NRP-152 cells is mediated via induction of TGF-βs (52), as reported for neonatal mouse keratinocytes (53). These new data suggest that TGF-βs may play a major role in prostatic responsiveness to retinoids and the potential ability of retinoids to function in the chemoprevention of prostate cancer.

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