Suppression of Telomerase, Reexpression of KAI1, and Abrogation of Tumorigenicity by Nerve Growth Factor in Prostate Cancer Cell Lines

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ABSTRACT

Nerve growth factor (NGF) is expressed in the prostate, where it appears to be involved in the control of epithelial cell growth and differentiation. NGF production is decreased in prostate tumors. However, the role of this neurotrophin in the control of proliferation and progression of prostate cancers is still a matter of investigation.

Prostate adenocarcinomas are telomerase-positive tumors. Chronic exposure of DU145 and PC3 prostate tumor cell lines to NGF resulted in a dramatic down-regulation of telomerase activity. This effect was correlated in terms of concentrations and time with a remarkable down-regulation of cell proliferation both in vitro and in vivo but was not secondary to NGF-induced quiescence. No down-regulation of telomerase activity was, in fact, detectable during serum starvation-induced quiescence. LNCaP cells, which do not express NGF receptors, appear to be insensitive to the actions of NGF.

DU145 and PC3 cells do not express the KAI1 metastasis suppressor gene, which is present in the prostate and is progressively lost during the progression of prostate cancers. Chronic NGF treatment strongly induced the reexpression of this gene in these cell lines, and this effect was correlated with the suppression of their invasive potential in vitro. The data presented here suggest that NGF reverts two metastatic prostate cancer cell lines to slowly proliferating, noninvasive phenotypes characterized by a very low telomerase activity and by the expression of the KAI1 metastasis suppressor gene.

INTRODUCTION

Prostate cancer is the most frequently occurring tumor in men and the second leading cause of male cancer deaths in the United States (1). Although our understanding of the biology of prostate cancer is expanding rapidly, there have been no major advances in the treatment of this tumor, and androgen deprivation remains the cornerstone therapy for patients with advanced disease. Treatment failure and tumor recurrence, however, are very frequent several months or years later (2).

Prostate carcinomas are telomerase-positive tumors (3–6). Telomerase is an unusual RNA-dependent DNA polymerase that uses part of its RNA moiety as a template to synthesize telomeric DNA sequences that are usually lost at each cell division, thus contributing to the maintenance of telomeres. This enzyme is expressed in embryonic cells and in adult male germ-line cells. In mature somatic cells, it is usually undetectable, with the exception of proliferating cells such as activated lymphocytes, hematopoietic stem cells, basal cells of the epidermis, and the intestine (7, 8). Telomere elongation through activation of telomerase seems to be an essential step in tumor immortalization (9–11). Thus, the reactivation of telomerase activity appears to be critical for cell proliferation and correlates with the stabilization of telomere length (7).

Prostatic growth and differentiation are carefully regulated by androgens and other paracrine and autocrine factors secreted by both epithelial and stromal cells (12). Alterations in these regulatory pathways may contribute to the initiation and progression of prostate cancer (12, 13). In particular, one paracrine/autocrine factor regulating prostate function is NGF3 (14–18). NGF belongs to the neurotrophin family and interacts with two receptor proteins: the trkA proto-oncogene endowed with intrinsic tyrosine kinase activity and the M5 75,000 glycoprotein p75NGFR (19). In prostate tissues, NGF is localized in both epithelial and stromal cells (15–18), and its receptors are expressed in epithelial cells (16, 17, 20). Secreted NGF appears to be involved in the control of prostate epithelial growth and differentiation (15). Interestingly, both NGF production and p75NGFR expression are progressively lost in benign prostate hyperplasia, adenocarcinoma, and metastatic cells (18, 21). A dysregulation of the NGF-mediated control of prostate cell growth via p75NGFR could, thus, be associated with the malignant state. This issue appears of critical relevance because it is...
now emerging that NGF is an antiproliferative and differentiation factor for different tumors, including pituitary adenomas (22) and small cell lung carcinoma (23).

On the basis of these observations, we investigated whether NGF can modify the phenotype of neoplastic cells of prostate origin. In this study, we used three cell lines derived from metastatic localization of primary prostate cancers, namely LNCaP, DU145, and PC3 cells. The results show that chronic NGF treatment of DU145 and PC3 cells remarkably inhibits telomerase activity and induces the reexpression of two proteins, which are progressively lost during the malignant degeneration of prostate cells, such as the p75NGFR (21) and the KAI1 metastasis suppressor gene (24), which is involved in the control of cell-cell and cell-extracellular matrix interactions (25). The impact of these molecular effects is such that they are accompanied by a remarkable reduction of the cell proliferation rate and by the abrogation of the invasive potential in vitro.

MATERIALS AND METHODS

Cell Culture and NGF Treatment. LNCaP, PC3, and DU145 cells were purchased from the American Type Culture Collection (Manassas, VA). LNCaP cells were grown in RPMI supplemented with 10% FCS, 4 mM glutamine, and 100 units/ml penicillin/streptomycin; PC3 cells were grown in Ham’s F-12 medium supplemented with 7% FCS, 4 mM glutamine, and 100 units/ml penicillin/streptomycin; DU145 cells were grown in DMEM supplemented with 10% FCS, 4 mM glutamine, and 100 units/ml penicillin/streptomycin. Media and supplements were purchased from Biochrom KG (Berlin, Germany). Cells were treated with various concentrations (10–100 ng/ml) of 2.5S NGF (Boehringer Mannheim, Milan, Italy) for different times, according to the different experimental procedures.

Measurement of Telomerase Activity. Telomerase activity was determined using the TRAP assay, as described by Piatyszek et al. (26). To perform the TRAP assay, we used 0.2 μg of protein extract (corresponding to ~500–700 cells) for each sample in a 50-μl PCR mixture containing reaction buffer [20 mM Tris-HCl (pH 8.3), 68 mM KCl, 1.5 mM MgCl₂, 1 mM EGTA, and 0.05% Tween 20], 0.1 μg of telomerase substrate oligonucleotide TS 5′-ATCCGGTCAGACAGAGTT-3′, 0.5 μM T4 gene 32 protein (Boehringer Mannheim), 100 μM each dNTP, 2 units of Taq polymerase (Boehringer Mannheim), 2 μCi of [α-32P]dCTP (3000 Ci/mmol; NEN Life Sciences Products, Milan, Italy) in a tube which contained 0.1 μg of reverse primer (CX, complementary to telomeric repeats) 5′-CCCCCTACCTTACCTTACCTT-3′ sequestered at the bottom by a wax barrier. After a 20-min incubation at room temperature, the reaction mixture was heated at 90°C for 90 s and then subjected to 31 PCR cycles (94°C, 30 s; 52°C, 30 s; and 72°C, 45 s) in a thermocycler (Cetus, Emeryville, CA). Pretreatment with RNase (0.5 μg/μl, final concentration) for 30 min at 37°C of untreated cell extracts was performed as an internal control. PCR products were electrophoresed on a 10% acrylamide gel; after fixation, the gel was directly exposed to a Kodak-X-Omat film, and autoradiograms were then scanned using a Magiscan Image Analysis System (Joyce-Loebl, Cambridge, United Kingdom). Relative density was expressed as arbitrary units. The in vitro direct effect of NGF on telomerase activity was analyzed by adding the agent to the reaction mixture at the beginning of the TRAP assay.

[^3H]Thymidine Incorporation. Untreated and NGF-treated cells were detached and plated at the concentration of 8 × 10⁵ cells per well. Eight h later, 0.5 μCi/ml[^3H]thymidine (84.8 Ci/mmol; NEN Life Sciences Products) was added, and cells were incubated for 18 h at 37°C. After three rinses with ice-cold PBS solution, cells were incubated for 10 min at 4°C with 10% trichloroacetic acid, then for 20 min at room temperature with 1 N NaOH and for a further 20 min with 1 N HCl. The two media were collected together and analyzed for radioactivity.

RNA Extraction, PCR, and Hybridization Conditions. Total RNA was extracted from control and NGF-treated cells by using the guanidinium thiocyanate method (27), and 2 μg of each sample were transcribed into cDNA using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc., Milan, Italy) and oligo(dT)₁₅₋₁₇ as a primer. PCR amplification of each cDNA was performed for 30 cycles under the following conditions. For β-actin amplification, aliquots of each cDNA were amplified in a 25-μl PCR (94°C, 30 s; 60°C, 30 s; and 72°C, 1 min) containing 250 μM each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8), 1.5 mM MgCl₂, 0.01% gelatin, 1.25 units of Taq polymerase (Boehringer Mannheim). 12.5 pmol each of sense oligonucleotide 5′-CAAGAGCTCTGACGGAGAC-3′ (human β-actin residues KDLYANTV) and antisense oligonucleotide 5′-GACGATGGAGGGCCGGCTC-3′ (human β-actin residues DESGPSIV; Ref. 28). PCR products were resolved on 1.2% agarose gel and photographed under UV light. For trkA proto-oncogene amplification, aliquots of each cDNA were used as a template in the 25-μl PCR described above containing 12.5 pmol each of oligonucleotide sense 5′-CTCCTTCCCAGGACAGTT-3′ (encoding human trkA residues SFPA SV) and oligonucleotide antisense 5′-CCATCCTCTGGAC-3′ (encoding human trkA residues LAPEDG; Ref. 29). Reaction temperatures were 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. For p75NGFR amplification, aliquots of each cDNA were used as a template in the 25-μl PCR described above containing 12.5 pmol each of oligonucleotide sense 5′-GGACAGCCAGCCGACTG-3′ (encoding human p75NGFR residues DSQSLH) and oligonucleotide antisense 5′-GGGATGTCAGGGAGAC-3′ (encoding human p75NGFR residues STATSP; Ref. 30). Reaction temperatures were 94°C for 1 min, 53°C for 1 min, and 72°C for 2 min. For KAI1 amplification, aliquots of each DNA were used as a template in a 25-μl PCR described above containing 12.5 pmol each of sense oligonucleotide 5′-CCCCGAACAGGAGACA-3′ (encoding human KAI1 residues PGNRTQ) and antisense oligonucleotide 5′-CCTTGGCCACCTTGAC-3′ (starting 105 bases downstream the stop codon; Ref. 24). Reaction temperatures were 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min.

Aliquots of these reactions were resolved on 1.2% agarose gel and blotted onto nylon membrane (Schleicher and Schuell, Berlin, Germany). Blots were then hybridized, respectively, with HindIII/EcoRI trkA cDNA probe (gift of Dr. M. Zanca, Universidad de Salamanca, Salamanca, Spain), BamHI/BamHI p75NGFR cDNA probe (kindly provided by Dr. R. Dal Toso,
R&C Scientifica, Altavilla Vicentina, Italy), and KAI1 full-length cDNA probe (kindly provided by Dr. J. C. Barrett, NIH, NC). trkA, p75 NGFR, and KAI1 cDNA were labeled with 32P to a specific activity of 10^9 cpm/μg DNA using a multiprime labeling system (Amersham Italia, Milan, Italy). Hybridizations were performed in 50% formamide-6×SSC at 42°C overnight, and each membrane was washed twice for 15 min in 0.1×SSC-0.1% SDS at 60°C. Blots were then exposed to Kodak-X-Omat films.

**In Vitro Invasion Assay.** The invasive potential of DU145 and PC3 prostate cancer cell lines was analyzed as described previously (31, 32). Briefly, polycarbonate filters (8-μm pore size) in 24-transwell chambers were coated with 40 μg of Matrigel (Collaborative Research, Bedford, MA) and incubated 30 min at 37°C. To perform the assay, 10^5 cells were added in DMEM-0.1% BSA medium to the inner side of the transwell. After a 24-h incubation, cells in the inner chamber were removed with a cotton swab. Cells attached to the bottom side of the membrane were fixed with methanol, stained with hematoxylin, and counted.

**In Vivo Tumorigenicity in Nude Mice.** DU145 cells were transplanted s.c. (4 × 10^6 cells per mouse) into the dorsal region of 20 nude mice (20 g body weight; Charles River Breeding Laboratories, Milan, Italy). After development of tumors, mice were divided into two groups. One group (n = 10) was treated with saline s.c., and the other (n = 10) was treated with s.c. NGF (1 μg/g body weight) once a day for 10 days. Tumor size was measured during a 70-day follow-up period. Tumor volumes were determined using the formula V = A × B × 0.4, where A and B are the larger and the smaller axes of the tumor, respectively. At the end of the follow-up, mice were sacrificed and tumors were weighed.

**Statistical Analysis.** The one-way ANOVA, followed by the Student’s t test, was used for statistical evaluation of differences. IC₅₀s were graphically calculated and represent the mean ± SE of three different experiments run in triplicate.

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**Fig. 1** Telomerase activity in untreated and NGF-treated prostate carcinoma cell lines. A, LNCaP, DU145, and PC3 cells were treated with NGF according to the protocol described in “Materials and Methods.” Untreated and NGF-treated cell extracts (0.2 μg of protein) were subjected to the TRAP assay. PCR amplification products were resolved on a 10% acrylamide gel. After fixation, the gel was exposed to Kodak-X-Omat film, and autoradiograms were scanned using a Magiscan Analysis System. B, quantification of 6-bp ladder signals by densitometric analysis. *P < 0.001 versus untreated cells by Student’s t test.

**Fig. 2** Densitometric analysis of concentration response and time course of NGF-mediated inhibition of telomerase activity in DU145 cells. A, cells were exposed to different concentrations of NGF (10–100 ng/ml) for 8 days. B, time course experiments were performed using 50 ng/ml NGF for different times. To perform TRAP assay, 0.2 μg of protein extract was used for each sample. The gel was exposed to a Kodak-X-Omat film, and the densitometric analysis was conducted using a Magiscan Image Analysis System. Columns, means of three independent experiments, expressed as percentage of telomerase activity; bars, SE. *P < 0.001 versus untreated cells by Student’s t test.
RESULTS

NGF Inhibits Telomerase Activity and Cell Proliferation in DU145 and PC3 Cell Lines. In line with previous observations (3–5), the three cell lines used in this study show high telomerase activity when they are maintained in culture in standard conditions, as demonstrated by the strong signal obtained with the TRAP assay reported in Fig. 1. NGF treatment strongly reduced this enzymatic activity. The effect of NGF on telomerase activity in DU145 cells was concentration dependent, with a maximum at the concentration of 50 ng/ml (Fig. 2A). Analysis of the time course experiments indicated that the maximal effect of NGF was detectable on day 8 of treatment (Fig. 2B). DU145 cells exposed to 50 ng/ml NGF for 8 days exhibited a remarkable reduction of telomerase activity, which was \( \frac{25}{\text{control}} \) as shown by the densitometric analysis of the 6-bp ladder signals (Fig. 1). A lower but significant reduction of telomerase activity was also observed in PC3 cells treated with 100 ng/ml NGF for 14 days. The densitometric analysis of the 6-bp ladder signals showed a reduction of 56% compared to untreated cells (Fig. 1). A direct influence of NGF on telomerase activity was excluded because the presence of 50–100 ng/ml of NGF in the reaction mixture did not modify telomerase signal (data not shown).

Additional experiments were performed to test whether factors capable of inhibiting telomerase activity and/or PCR amplification could be present in the cell extract. Extracts from untreated cells were admixed with those of NGF-treated cells. The results showed that no direct inhibitory activity of telomerase function was present in the extract of NGF-treated DU145 cells (data not shown).

The cell proliferation rate was also reduced by NGF treatment. Using the \(^{[3]H}\)thymidine incorporation test, we evaluated the effect of NGF on the proliferation rate of the three cell lines. NGF treatment strongly reduced this enzymatic activity. The effect of NGF on telomerase activity in DU145 cells was concentration dependent, with a maximum at the concentration of 50 ng/ml (Fig. 2A). Analysis of the time course experiments indicated that the maximal effect of NGF was detectable on day 8 of treatment (Fig. 2B). DU145 cells exposed to 50 ng/ml NGF for 8 days exhibited a remarkable reduction of telomerase activity, which was \( \frac{25}{\text{control}} \) as shown by the densitometric analysis of the 6-bp ladder signals (Fig. 1). A lower but significant reduction of telomerase activity was also observed in PC3 cells treated with 100 ng/ml NGF for 14 days. The densitometric analysis of the 6-bp ladder signals showed a reduction of 56% compared to untreated cells (Fig. 1). A direct influence of NGF on telomerase activity was excluded because the presence of 50–100 ng/ml of NGF in the reaction mixture did not modify telomerase signal (data not shown). Additional experiments were performed to test whether factors capable of inhibiting telomerase activity and/or PCR amplification could be present in the cell extract. Extracts from untreated cells were admixed with those of NGF-treated cells. The results showed that no direct inhibitory activity of telomerase function was present in the extract of NGF-treated DU145 cells (data not shown).

The cell proliferation rate was also reduced by NGF treatment. Using the \(^{[3]H}\)thymidine incorporation test, we evaluated the effect of NGF on the proliferation rate of the three cell lines. As reported in Fig. 3A, exposure of DU145 cells to NGF resulted in a marked inhibition of cell proliferation. This effect was concentration dependent, with a maximal inhibition at the concentration of 50 ng/ml and an \( IC_{50} \) of 26 ± 5 ng/ml. Inhibition of cell proliferation was detectable on day 4 and
reached its maximum on day 8 of NGF treatment. Interestingly, this effect was correlated in terms of concentration and time with the inhibition of telomerase activity. In PC3 cells, the maximal inhibitory effect of NGF on cell proliferation was evident at the concentration of 100 ng/ml, and after 14 days of treatment, the IC-50 was 62 ± 8 ng/ml (Fig. 3B). On the other hand, both telomerase activity and cell proliferation were insensitive to the inhibitory action of NGF in LNCaP cells (Figs. 1 and 3C). To verify whether telomerase inhibition was a consequence of NGF-induced quiescence, we seeded DU145 and PC3 cells in six-well plates and cultured in standard medium for 24 h. Thereafter, the medium was replaced by serum-free medium to block cell proliferation. Cells were analyzed for proliferation and telomerase activity every 24 h up to 72 h. Incorporation of [3H]thymidine declined rapidly during serum starvation (80% inhibition; Fig. 4). In contrast, no down-regulation of telomerase activity every 24 h up to 72 h. Incorporation of [3H]thymidine declined rapidly during serum starvation (80% inhibition; Fig. 4). In contrast, no down-regulation of telomerase activity was evident at the end of the treatment, when the percentage increase of tumor size was 200 ± 15% in saline-treated mice and 88 ± 7% in NGF-treated animals. On day 18 after the last NGF administration, a 486 ± 15% increase of tumor size was measurable in saline-treated animals, whereas only a 183 ± 10% increase in tumor size was detectable in NGF-treated animals. At this time, saline-treated animals were sacrificed because of the tumor size, whereas observation of NGF-treated mice was continued. Interestingly, tumor growth inhibition was maintained for up to 30 days after the end of NGF treatment (Fig. 7). However, this inhibition was not permanent, and 60 days after the end of NGF treatment, transplanted DU145 cells gradually regained the ability to proliferate (data not shown).

**Fig. 5** Expression of NGF receptors by reverse transcriptase-PCR in untreated and NGF-treated prostate cancer cell lines. Total RNA from control and NGF-treated cells was transcribed into cDNA as described in “Materials and Methods.” Lanes 1 and 2, untreated and NGF-treated LNCaP cells, respectively; Lanes 3 and 4, untreated and NGF-treated DU145 cells; and Lanes 5 and 6, untreated and NGF-treated PC3 cells. Aliquots of each cDNA were amplified by PCR with trkA- and p75NGFR-specific oligonucleotide primers and fragments obtained were blotted onto nylon membranes and hybridized with HindIII/EcoRI trkA cDNA or BamHI/BamHI p75NGFR cDNA probes. Amplification of the β-actin was performed as an internal control.

The invasiveness of tumor cells represents one of several important properties necessary for metastasis formation and is related to the loss of factors involved in the control of cell-cell and cell-extracellular matrix interactions. One of these factors is the KAI1 metastasis suppressor gene. This gene encodes for a member of a structurally distinct family of leukocyte surface glycoproteins, which appears to be progressively lost during prostate tumor progression (25). As reported in Fig. 6, DU145 and PC3 cells do not express the KAI1 gene. Exposure of these cell lines to NGF, however, resulted in the reexpression of the mRNA encoding for this metastasis suppressor gene. PCR products derived from both control and NGF-treated cells were resolved on a 1.2% agarose gel and hybridized with the full-length KAI1 cDNA probe. The expected 344-bp band, which was not detectable in PC3 control cells and only slightly evident in DU145 cells, appeared to be strongly induced by NGF treatment in both cell lines. The NGF-induced reexpression of the KAI1 metastasis suppressor gene was accompanied by a significant suppression of the invasive capacity of DU145 and PC3 cell lines. The results of *in vitro* invasion experiments, using a reconstituted basement membrane, are reported in Table 1 and show that the number of penetrating DU145 cells was reduced up to 95% and that the number of PC3 cells was reduced up to 78% by NGF treatment.

**Fig. 6** Induction of KAI1 metastasis suppressor gene expression by NGF in DU145 and PC3 cell lines. Total RNA from control and NGF-treated cells was transcribed into cDNA as described in “Materials and Methods.” Aliquots of each cDNA were amplified by PCR and fragments obtained with KAI1-specific primers were blotted onto nylon membranes and hybridized with cDNA probe.
between NGF and telomerase was excluded by our activity. On the other hand, the possibility of a direct interaction of DU145 and PC3 cells did not modify telomerase ever, here we show that serum starvation that induced quiescence of DU145 and PC3 cells induced a significant inhibition of tumor growth and progression.

The prostate adenocarcinoma cell lines used in this study exhibit a strong telomerase activity (3–5). Chronic NGF treatment of DU145 and PC3 cells induced a significant inhibition of this enzymatic activity. This effect was correlated in terms of concentration and time with NGF-induced inhibition of cell proliferation. In a number of experimental models, it has been shown that proliferating immortal cells repress their telomerase activity when becoming quiescent (reviewed in Ref. 33). However, here we show that serum starvation that induced quiescence of DU145 and PC3 cells did not modify telomerase activity. On the other hand, the possibility of a direct interaction between NGF and telomerase was excluded by our in vitro studies. The observation that NGF does not inhibit either telomerase activity or the proliferation rate in LNCaP cells, that are devoid of NGF receptors, strongly suggests the involvement of a selective interaction of NGF with its receptors in eliciting these effects. Taken together, these observations suggest that a complex mechanism must be involved in the regulation of telomerase activity by NGF.

The loss of metastasis suppressor genes is an important step in the progression of cancer cells from a nonmetastatic to a metastatic phenotype (34). The KAI1 metastasis suppressor gene is highly expressed in the prostate and appears to be down-regulated during the progression of human prostate cancer to a metastatic disease (25). In addition, it has been recently reported that targeted expression of the KAI1 cDNA in colon carcinoma cell lines BM314 and DLD-1 suppressed both their motility and their in vitro invasiveness (35). In line with these observations, the metastatic cell lines DU145 and PC3 do not express the KAI1 gene. NGF treatment, however, induced the reexpression of the KAI1 gene in these cell lines, and this effect appears to be correlated with the inhibition of their in vitro invasive capacity. The data are in line with previous observations showing that NGF reverts human pituitary adenomas (22) and human small cell lung carcinoma cell lines (23) to less severe phenotypes that proliferate slowly and lose their invasive potential in vitro.

A previous report suggested that a high–molecular weight NGF-like protein produced by both prostate stromal cells and the neoplastic epithelial cell line TSU-pr1 acutely stimulates the proliferation of both stromal and TSU-pr1 cells (25). The different profiles of NGF receptor expression in our experimental conditions and the acute versus chronic treatment could help to explain the apparent discrepancy. Like DU145 and PC3 cells, TSU-pr1 cells do not express p75NGFR and trkA, whereas in the condition used by Djakiew et al. (25), TSU-pr1 cells apparently express only trkA. One possibility is, therefore, that, whereas trkA could mediate a mitogenic effect of NGF, coexpression of both p75NGFR and trkA could be a crucial condition for the antiproliferative effect of NGF. In line with this, evidence suggested that a complex interaction and reciprocal modulation does occur between p75NGFR and trkA receptors (19, 36, 37). In addition, it has been recently published that p75NGFR transfection of TSU-pr1 cells decreased the dose-dependent NGF-mediated proliferation of these cells (38).

On the other hand, it cannot be excluded that the high molecular weight NGF-like peptide described by Djakiew et al. (15) may be a distinct protein with its peculiar biological activ-

### Table 1 In vitro invasiveness of prostate cancer cell lines

<table>
<thead>
<tr>
<th>Cells</th>
<th>Untreated cells/field</th>
<th>NGF-treated cells/field</th>
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<tbody>
<tr>
<td>DU145</td>
<td>157 ± 4.8</td>
<td>9 ± 2.4*</td>
</tr>
<tr>
<td>PC3</td>
<td>119 ± 4.3</td>
<td>26 ± 3.1b</td>
</tr>
</tbody>
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*DU145 cells were treated with 50 ng/ml NGF for 8 days and PC3 cells with 100 ng/ml NGF for 14 days. Untreated and NGF-treated cells were plated on a Matrigel-coated filters. After a 24-h incubation, cells attached to the lower face of the filter were fixed, stained, and counted. Results are the means ± SD of three different experiments run in triplicate. *P < 0.001 vs. untreated cells by ANOVA, followed by the Student’s t test.

**DISCUSSION**

Evidence generated over the past few years suggested that NGF plays a physiological role in the control of prostate epithelial cell growth and differentiation by autocrine/paracrine mechanisms (19–23). Both epithelial and stromal cells appear to be the source of NGF, whereas only epithelial cells express NGF receptors, thus making them the target of this neurotrophin (15–18, 20). Furthermore, the observation that both NGF production and p75NGFR expression are progressively lost in benign prostate hyperplasia, adenocarcinoma, and metastatic cells (18, 21), suggests that this neurotrophin could play a role in prostate tumor growth and progression.

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NGF reverts human pituitary adenomas (22) and human small cell lung carcinoma cell lines (23) to less severe phenotypes that proliferate slowly and lose their invasive potential in vitro.

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On the other hand, it cannot be excluded that the high molecular weight NGF-like peptide described by Djakiew et al. (15) may be a distinct protein with its peculiar biological activ-
ity, belonging to the NGF family. Another interpretation may suggest that the concentrations of NGF used in our experiments could induce a down-regulation of trkA signal transduction pathway. As a matter of fact, the nonspecific trks inhibitor CEP-751 inhibited prostatic cancer growth in vitro, in different animal models (39). Taken together, these different interpretations suggest that the type of response of prostate tumor cells to NGF may be strictly dependent on the pattern of NGF receptors expressed.

In line with the data obtained in vitro, chronic NGF administration to athymic mice xenografted with DU145 cells strongly suppressed the growth of the established tumors, so that 18 days after the end of the treatment the tumor size was remarkably smaller in NGF-treated than in saline-treated mice. Interestingly, the inhibitory effect of NGF on tumor growth persisted for 30 days after the end of treatment, upon which tumor cells gradually regained the ability to proliferate.

In conclusion, the present results suggest that NGF reverts two metastatic prostate cancer cell lines to a less severe phenotype as shown by the observations that: (a) NGF strongly reduces their telomerase activity and keeps their proliferation rate under inhibitory control; (b) NGF induces the reexpression of the KAI1 metastasis suppressor gene and abrogates their in vitro invasive capacity; and (c) NGF-responsive prostate cancer cells reexpress p75NGFR, the progressive loss of which has been proposed as a marker of malignant degeneration of prostate epithelial cells (21).

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