Reduced Expression of the Low Affinity Nerve Growth Factor Receptor in Benign and Malignant Human Prostate Tissue and Loss of Expression in Four Human Metastatic Prostate Tumor Cell Lines

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Abstract

In the human prostate, a low affinity (p75) nerve growth factor (NGF) receptor (NGF-R) localizes to the epithelia while a NGF-like protein localizes to the stroma. This NGF-like ligand, derived from prostate stromal cell cultures, has been shown to participate in paracrine mediated growth of a human tumor epithelial cell line (TSU-prl) in vitro. In order to investigate the role of the NGF-R in neoplastic growth we have examined the expression of the NGF-R in normal prostate tissues, benign prostatic hyperplasia tissues, adenocarcinoma tissues, and four metastatic tumor cell lines of the human prostate. In primary epithelial cell cultures of normal human prostate the p75 NGF-R was localized by immunocytochemistry to cytoplasmic vesicles. Furthermore, Western blot analysis of the NGF-R in subcellular fractions of normal prostate tissue identified an M, 75,000 immunoreactive protein in the microsomal fraction under nonreducing conditions of sodium dodecyl sulfate-polyacrylamide gel electrophoresis. However, microsomal preparations of five prostatic adenocarcinoma and five benign prostatic hyperplasia cell cultures of normal human prostate tissue, as well as four human metastatic prostate tumor cell lines, suggest an inverse association of p75 NGF-R expression with the neoplastic progression of the human prostate.

Introduction

Prostatic neoplasia in the form of BPH3 and adenocarcinoma is the leading cause of morbidity and second leading cause of cancer death in men throughout North America (1). The incidence of prostate neoplasia increases with age, becoming prevalent as a clinical entity between 40 and 50 years of age (2). Since the incidence of both BPH and prostate adenocarcinoma increases with age and the modal male population is aging, the clinical prevalence of BPH and adenocarcinoma are projected to dramatically increase morbidity and mortality of men in North America over the next several decades (3). Hence, the high incidence of prostate neoplasia has promoted increased interest in growth regulation of the prostate. A fundamental mechanism of prostate growth regulation is through paracrine interactions between the stroma and epithelium (4, 5). Mesenchyme in the reproductive tract has been shown to induce androgen dependent development and differentiation of epithelium (6). Moreover, androgen dependent soluble paracrine factors secreted by stromal cells have been shown to stimulate epithelial cell proliferation (7). One such paracrine factor in the prostate has been identified as an NGF-like protein (8). This NGF-like protein, secreted by prostate stromal cells, stimulates growth of a prostatic epithelial tumor cell line (8), consistent with a paracrine modulation of prostate tumor epithelial cell growth. The NGF-like protein has been shown to localize in vivo to human prostate stroma, whereas a corresponding low affinity p75 NGF-R localized to the epithelia of the human prostate (9). Curiously, the intensity of p75 NGF-R fluorescence in the adenocarcinoma epithelium appeared reduced in comparison with the normal tissue (9). Hence, the present studies were initiated to characterize the incidence of the p75 NGF-R in normal prostate tissue, adenocarcinoma, and BPH tissue, as well as four human metastatic prostate tumor cell lines.

Materials and Methods

Materials. Media, tissue culture supplies, reagents, and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise described in the text.

Cell Lines and Tissue Samples. PC-3, DU-145, and LNCaP prostate epithelial tumor cell lines were obtained from the American Type Culture Collection (Rockville, MD) and the TSU-prl cell line was provided by Dr. John Isaacs (Johns Hopkins University, Baltimore, MD). The human melanoma cell line (A875) was provided by the laboratory of Dr. Moses Chao (Cornell University, New York, NY). The PC-3, DU-145, and TSU-prl cell lines were maintained in RPMI 1640 medium supplemented with antibiotics/antimycotic (100 units/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml Fungizone), 10% FBS, and 10−7 M testosterone. The LNCaP cell line was maintained in Dulbecco's Modified Eagle's Medium/10% FBS and the A875 cell line was maintained in RPMI 1640 supplemented with 10% FBS. The BPH tissue was obtained from transurethral resections of the prostate, prostatic adenocarcinoma and normal prostate tissue were obtained from radical retropubic prostatectomy specimens, renal tissue was obtained from radical nephrectomy specimens, and testis was obtained from orchiectomy specimens at Georgetown University Medical Center. The human prostatic stromal cells and epithelial cells were isolated by enzymatic digestion of tissue samples in a collagenase solution as previously described (8). The prostate stromal cells were cultured in 75 cm2-tissue culture flasks (Costar, Cambridge, MA) maintained in RPMI 1640 containing 10% FBS/T. The normal prostate epithelial cells were plated on glass coverslips and maintained in RPMI 1640 containing a serum-free defined medium consisting of 2 μg/ml insulin, 10 ng/ml epidermal growth factor, 1 μg/ml transferrin, 10−7 M dexamethasone, 2 mM glutamine, 2.5 mg/ml bovine pituitary extract (UpState Biotechnology Inc., Lake Placid, NY), 10 (ng/ml) rat prolactin (NIH), and 10 μg/ml cholera toxin, all of which were also supplemented with 10% FBS/T and dihydrotestosterone (10−7). All cell cultures were incubated at 37°C in 5% CO2/95% air and the media were replaced every second day.
Immunocytochemistry. Cells were grown on glass coverslips to approximately 50% confluence. The cells were washed twice in phosphate buffered saline and incubated in unsupplemented RPMI 1640 for 3 h. The medium was then replaced with fresh unsupplemented RPMI 1640 for another 3 h. Human prostate stromal cell conditioned media were prepared as previously described (8) and added at concentrations of 20 µg/ml, 5 µg/ml, and 1 µg/ml to cultures of the primary epithelial cells and cell lines for a 24-h incubation period. Subsequently, the cells were fixed in ice-cold methanol for 2.5 min, blocked with 3% ovalbumin for 1 h at 37°C to reduce nonspecific binding, and incubated with murine anti-human p75 NGF-R monoclonal antibody (1:80 dilution: Boehringer Mannheim, Indianapolis, IN) for 3 h at 37°C. The coverslips were rinsed with phosphate buffered saline (6 times) and incubated in rhodamine conjugated sheep anti-murine IgG secondary antibody (1:400 dilution; Cappel, Durham, NC) for 1 h. Murine IgG partially purified from whole mouse serum (Cappel) by the method of Jemerson and Margoliash (10) was used as a nonspecific control. Immunocytochemical staining of cell cultures was visualized with a Zeiss photomicroscope fitted with an epifluorescence attachment.

Immunoblot Analysis. Microsomal and cytosolic fractions of cell lines and tissue specimens were obtained after homogenization and sonication by the modified fractionation method of Culty (11). Cytosolic fractions were dialyzed against ice-cold distilled water and lyophilized. The subcellular fractions were resuspended in 50 mM Tris-HCl (pH 7.4) supplemented with 10 mM ethyleneglycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid and 10 µM phenylmethylsulfonyl fluoride. Nonreducing sample buffer was added to 50 µg of each microsomal and cytosolic protein sample. Subsequently, the samples were separated by sodium dodecyl sulfate-polyacrylamide gel (10%) electrophoresis. The protein from each sample was electrotransferred onto nitrocellulose and blocked for 1 h in 5% nonfat dry milk in TBS (20 mM Tris-HCl, 500 mM NaCl, pH 7.5). The membranes were incubated overnight at room temperature with murine monoclonal anti-human p75 NGF-R antibody (1:80 dilution) in TTBS and 1% gelatin (Bio-Rad, Richmond, CA). The membranes were rinsed twice for 10 min with TTBS and reacted with horseradish peroxidase conjugated goat anti-mouse IgG (1:2000) in TTBS containing 1% gelatin (Bio-Rad, Richmond, CA). The membranes were rinsed twice in TTBS and once in TBS. Immunoreactivity was visualized using 4-chloro-l-napthol hydrogen peroxide reaction substrate.

Results

Immunofluorescence. Immunocytochemical localization of the p75 NGF-R in normal prostate epithelial cells and the
The p75 NGF-R was observed in cytoplasmic vesicles of the normal prostate epithelial cells (Fig. 1B). In normal prostate epithelial cells treated with 20 μg/ml hPS protein (Fig. 1C), there appeared to be a greater number of dispersed NGF-R immunostained vesicles than in the untreated cells (Fig. 1B). Substitution of the primary antibody with a murine IgG eliminated p75 NGF-R fluorescence (Fig. 1D). The prostate epithelial tumor cell line TSU-prl (Fig. 1E) treated with 20 μg/ml hPS (Fig. 1F) or in the absence of PS (not shown) showed no immunofluorescence when stained for the p75 NGF-R. The DU-145, PC-3, and LNCaP cell lines also did not show p75 NGF-R immunostaining (not shown). The A875 human melanoma cell line, which overexpresses the p75 NGF-R was used as the positive control (not shown).

**Immunoblot Analysis.** Fig. 2 shows the results of an immunoblot with the p75 NGF-R antibody of microsomal fractions of normal prostate and adenocarcinoma tissue specimens. A M, 75,000 immunoreactive protein was evident in the normal prostate tissue. The prostate adenocarcinoma tissue exhibited reduced expression of the NGF-R protein. Renal tissue was the negative control, whereas testis tissue and the A875 melanoma cell line were the positive controls. In order to further examine the reduced NGF-R expression in the prostate adenocarcinoma, five separate prostatic adenocarcinoma specimens were examined (Fig. 3A). There was a reduction or absence of expression of the p75 NGF-R in all the adenocarcinoma specimens relative to the normal prostate tissue when samples were loaded on an equal protein (50 μg/lane) basis (Fig. 3A). Similarly, there was a reduced expression of p75 NGF-R in all the BPH tissue specimens relative to the normal prostate tissue when samples were loaded on an equal protein (50 μg/lane) basis (Fig. 3B). Microsomal preparations from the prostate tumor cell lines TSU-prl, DU-145, PC-3, and LNCaP showed complete absence of p75 NGF-R immunoreactivity (Fig. 4). No immunoreactivity for the p75 NGF-R was evident in cytosolic fractions of any of the prostate tissue specimens or cell lines examined (not shown).

**Discussion**

In primary cultures of normal prostate epithelium p75 NGF-R fluorescence localized in perinuclear vesicles. A similar localization of p75 NGF-R has been observed in cholinergic neurons of the human basal forebrain (12). Human prostate stromal cell secretory protein containing a NGF-like protein (8) when added to the primary epithelial cell cultures appeared to stimulate redistribution of the vesicles from the perinuclear region toward the periphery of the cytoplasm, and in addition there appeared to be a greater number of NGF-R immunoreactive vesicles. These results are consistent with a mobilization of the p75 NGF-R toward the cell surface in response to stimulation by the NGF-like ligand. The p75 NGF-R was shown to be expressed in microsomal preparations (membranes) of normal prostate epithelial cells (Fig. 2; Fig. 3, A and B) consistent with our previous work localizing the p75 NGF-R to the prostate epithelium in vivo (9). The p75 NGF-R is absent from the prostate stroma in vivo as determined by Graham et al. (9) and from the human prostatic stromal cell line (Fig. 4) which secretes the NGF-like paracrine growth factor (8). In prostatic adenocarcinomas and BPH tissue specimens, the p75 NGF-R expression was reduced or absent relative to normal prostate tissue. This could be due to partial loss of NGF-R in the individual neoplastic epithelial cells or to a mixture of normal epithelial cells which fully express the NGF-R and neoplastic epithelial cells that have lost the NGF-R expression. In any event, the microsomal preparations of neoplastic tissue specimens exhibited reduced NGF-R expression relative to normal...
prostate. In all the metastatic cell lines examined (TSU-prl, DU-145, PC-3, and LNCaP), the p75 NGF-R expression was completely absent. These results are consistent with previous findings of p75 NGF-R mRNA detected in prostate adenocarcinomas but absent from the mRNA of the DU-145, PC-3, and LNCaP cell lines (13). Hence, there is a gradation in the loss of p75 NGF-R epithelial expression, ranging from the normal prostate where it is fully expressed to a partial loss of expression in prostatic adenocarcinoma and BPH tissues to a complete lack of expression in the four metastatic cell lines examined. Interestingly, the human p75 NGF-R has been mapped as closely distal to the acute promyelocytic leukemia associated chromosome 17 breakpoint at 17q21 (14). Moreover, 17q21 is the locus of a gene(s) for inherited susceptibility to breast cancer (15). This region of 17q contains several candidate genes for oncogenesis which include the truncated form of the epidermal growth factor receptor (erbB2); the estradiol-17-β dehydrogenase gene (edh17); homeobox 2 gene (hox2); a gene associated with lymph node metastasis in primary breast carcinoma (nm23); the gene for the retinoic acid receptor (rara), and the wnt3 gene which is one of the integration sites activated by mouse mammary tumor virus (14). Hence, the cytogenetic locus of the p75 NGF-R in the vicinity of a chromosomal “hotspot” of the human genome may be of significance in the partial and/or complete loss of p75 NGF-R expression in neoplastic and metastatic human prostate tissues and cell lines, respectively.

In the nervous system, the low affinity p75 NGF-R and the trk protooncogene family of high affinity NGF receptors form a receptor complex that in response to the binding of neurotrophins is thought to mediate the differentiation and development of the nervous system (16). This paracrine interaction between the neurotrophins and their corresponding receptor complex is thought to maintain the differentiated phenotype of the neurons. Moreover, the p75 NGF-R receptor may modulate the activity of the trk receptor family (17). Hence, a defect in the expression of the p75 NGF-R may modify the regulation of the trk tyrosine kinase, modifying the paracrine interaction between stroma and epithelia from a differentiative to a proliferative state (18). Indeed, it is intriguing to ponder whether loss of p75 NGF-R expression may up-regulate trk receptor expression. Since loss of p75 NGF-R expression is associated with loss of the differentiated phenotype in neoplastic prostate tissue and tumor cell lines and loss of p75 NGF-R expression is also associated with the enhanced growth of a prostate tumor cell line (TSU-prl), the p75 NGF-R may facilitate differentiation of epithelia in the prostate. Moreover, the partial loss of p75 NGF-R expression in epithelia of BPH and adenocarcinoma tissue and complete loss of p75 NGF-R expression in the metastatic tumor cell lines suggest an inverse association of p75 NGF-R expression with the neoplastic progression of the human prostate.

Acknowledgments

We thank Dr. Martine Culty for her advice regarding the preparation of subcellular fractions.

References

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