Prolactin Receptor Expression in the Developing Human Prostate and in Hyperplastic, Dysplastic, and Neoplastic Lesions

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***In situ* hybridization and immunohistochemistry were used to localize and compare the expression of the long form of the human prolactin receptor in fetal, prepubertal, and adult prostate. Results were then compared with hyperplastic, dysplastic, and neoplastic lesions. Both receptor message and protein were predominately localized in epithelial cells of the fetal, neonatal, prepubertal, and normal adult prostate. In hyperplastic lesions the expression of the receptor was unchanged with respect to normal epithelial cells. Irrespective of grade, markedly enhanced expression of the receptor was evident in dysplastic lesions. In lower Gleason grade carcinomas the intensity of receptor signal at the message and protein levels approximated that found in normal prostatic epithelium. However, in foci within higher grade cancers, receptor expression appeared diminished. Results from our study suggest that prolactin action plays a role in the development and maintenance of the human prostate and may also participate in early neoplastic transformation of the gland. Diminution of receptor expression in high grade neoplasms could reflect the emergence of a population of cells that are no longer responsive to the peptide hormone. (Am J Pathol 1999, 154:863–870)**

It has long been thought that prolactin (PRL) influences normal growth, development, and function of the prostate.^{1–6} The possibility that the hormone exerts trophic effects on the prostate has been suggested by numerous past investigations, largely done in rats,^{1–6} and inferred by results from studies in humans which have shown that circulating PRL levels rise sharply around the time of sexual maturation and are significantly elevated in adult

men when compared to prepubertal boys.⁷ In this regard PRL may indirectly influence proliferation in the gland by up-regulating the levels of prostatic androgen receptor.^{8,9} Conversely, androgens may have a regulatory effect on intraglandular PRL synthesis by secretory epithelium as shown by both *in vivo* and organ culture studies of rat¹⁰ and human prostate.¹¹

Indirect evidence of possible PRL involvement in the development of benign prostatic hyperplasia (BPH) and/or carcinoma has come from reports that circulating hormone levels were significantly higher in older men when compared with those found in younger males.^{3,12,13} Moreover, patients with prostate cancer have been reported to have higher levels of plasma PRL than did age-matched controls,^{3,12,13} and high affinity PRL binding sites have been detected in normal, BPH, and neoplastic human prostate.^{3,11,14}

PRL, along with growth hormone, belongs to a superfamily of growth factors.^{15,16} The peptide hormone is known to have highly pleiotropic actions including those related to regulation of growth and differentiation. These broad range of effects are now known to be mediated by the prolactin receptors (PRLr) present in a large number of tissues including the human prostate.^{11,15–17} PRLrs are devoid of intrinsic enzymatic activity^{15,16} but are known to signal intracellularly via the JAK/STAT pathway, as well as the Ras/Raf/MAP kinase cascade.^{15,16} Three isoforms of PRLr (long, intermediate, and short forms), which differ in the lengths of their cytoplasmic domains, have been identified in rat tissues,^{15,16,18} but only the long and an analogous intermediate form of the receptor have been detected in human tissues.¹⁸ Interestingly, among the rat isoforms, both the long and intermediate forms are capable of transducing lactogenic as well as mitogenic signals.^{15,16,18,19} In contrast, the short form does not transduce differentiation signals but can signal cell growth in NIH 3T3 cells.²⁰

As reported for cells in the breast, brain, placenta, and lymphoid cells,¹⁷ Nevalainen et al¹¹ recently demonstrated that PRL is produced locally by secretory epithelia in organ cultures of the human prostate. These find-

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ings indicate that an intraprostatic as well as a pituitary source for PRL exists and together with PRLr constitute an autocrine/paracrine pathway which likely mediates local hormone effects on the gland. In the current study, we used immunohistochemistry and *in situ* hybridization to localize PRLr in the developing and adult human prostate and in hyperplastic, dysplastic (also termed prostatic intraepithelial neoplasia), and carcinomatous lesions of the gland. Our goals were to investigate whether this key component of PRL action is present during prostatic organogenesis and to determine whether its expression is altered in BPH, prostatic intraepithelial neoplasia lesions, and carcinoma. To our knowledge this is the first comprehensive morphological investigation of PRLr expression in the human prostate across a wide spectrum of normal and pathological states. Overall, our findings indicate that PRL likely influences the development of the human prostate and contributes to the maintenance of the adult gland. Our results also suggest that PRL plays a role in early carcinogenesis of the human prostate and that diminished PRLr expression in poorly differentiated cancers may reflect a progressive loss of responsiveness to the peptide hormone in populations of neoplastic cells.

Materials and Methods

Prostate Specimens

The majority of specimens were selected from a pool of 40 radical prostatectomies done at Stanford Medical Center during the years 1994–1997. Patients ranged from 54 to 71 years of age. Specimens selected for study included 10 samples of lesion-free tissue from the peripheral, central, and transition zones,²¹ 5 BPH specimens, 20 examples of dysplasia of varying grades of severity, and 18 examples of Gleason grades 3–4 carcinoma. The methods used for the collection, fixation, sample selection, and processing of these specimens are the same as previously described.²² In addition archival tissues obtained at autopsy from collections at the Department of Pathology at Tufts University were also studied. They included prostates from two fetuses at 29 and 34 weeks of gestation who died 1 and 7 days, respectively, after premature birth, 2 glands from neonates that were 3 hours old and 1 week of age, and one prostate from a prepubertal individual who was 11 years old.

In Situ Hybridization

The sense and antisense probes used in this study were generated from the H1/H2 human prolactin receptor clone²³ which had been inserted into a pBlueScript vector. The entire 2556-bp sequence of the receptor was digested with the *Bam*HI restriction enzyme to generate a 200-bp fragment which was subcloned into the *Bam*HI site of the pBlueScript vector. The fragment is from the cytoplasmic domain of the long form of the human prolactin receptor, from nucleotides 1029 to 1233. This sequence was chosen because it has no homology with other members of the same family of receptors, such as

the human growth hormone receptor.^{15,16,18} One microgram of the recombinant plasmid vector was linearized by *Xba*I and *Eco*RI enzymes to generate antisense and sense templates, respectively. To generate labeled riboprobes the templates were transcribed using RNA polymerases T7 (antisense) or T3 (sense), NTP labeling mix (the UTP component was 2/3 normal UTP, 1/3 digoxigenin – 11 UTP), and RNase inhibitor. *In vitro* transcription was carried out at 37°C for 1 hour in a 1× transcription buffer (Boehringer Mannheim, Indianapolis, IN).

Formalin-fixed paraffin-embedded sections 5 µm thick were dewaxed, rehydrated, and washed in phosphate-buffered saline. *In situ* hybridization was carried out in an automated instrument (Gen II, Ventana Medical Systems, Tucson, AZ) in which all applications were standardized according to the manufacturer's protocols.

Briefly, the sections were exposed to proteinase K (100 µg/ml in 1 mol/L Tris-EDTA buffer, pH 8) for 8 minutes at 37°C. Prehybridization was carried out in 2× saline sodium citrate (SSC) for 15 minutes at 45°C. Sense or antisense riboprobes in 100 µl of hybridization buffer (50% deionized formamide, 2× SSC, 10% dextran sulfate, 1% SDS and 250 µg/ml denatured herring sperm DNA) were manually applied to the sections. Optimal dilutions of riboprobe in the hybridization buffer generally ranged from 1:100 to 1:300 (concentration: 10–20 pmol/L riboprobe). The Ventana Automated System utilizes a unique "liquid coverslip."

Following a 1-hour hybridization at 42°C, the automated sequence continued with posthybridization washes. The stringency conditions were determined by the duration, temperature, and concentration of the SSC solutions. The highest stringency we used in this study was 0.5× SSC for 20 minutes at 65°C. A blocking solution, which included normal sheep serum and tetramisole (Sigma), was then applied to the sections. This was followed by 20 minutes' exposure to primary antibody (anti-digoxin; Sigma) diluted 1:500 in normal sheep serum/Tris-NaCl buffer. The detection steps used 5 reagents supplied by the manufacturer (Ventana Blue kit). The slides were removed from the machine, stained with nuclear fast red (Rowley Biochemical Institute, Danvers, MA), dehydrated, and coverslipped.

Negative controls included sections incubated with the sense probe, pretreated with RNase A, or by omission of probe. Positive controls were surgical biopsy specimens of human breast.

Immunohistochemistry

For these studies we used a monoclonal antibody, B.6, raised against a membrane-enriched fraction of a metastatic human breast cancer line (MCF-7) (a generous gift from Dr. B.K. Vonderhaar, National Cancer Institute). The specificity of this reagent both in its binding characteristics and immunostaining of T47-D human breast cancer cells have been reported.²⁴ In addition, we also used a rabbit polyclonal antibody directed against the complete extracellular domain of the human PRLr, which we termed the CL-AB (a generous gift from Dr. Charles Clevenger, University of Pennsylvania Medical School).

Six-micrometer-thick sections were dewaxed, placed in a 0.01 mol/L citrate buffer (pH 6), and then heated in a microwave oven at high power for 2 or 3 cycles at 5 minutes each. The B.6 antibody was then applied at a dilution of 1:250 and the polyclonal reagent at 1:100. Biotinylated horse anti-mouse or goat anti-rabbit were used as secondary antibodies with the B.6 and polyclonal antibodies, respectively. The remaining immunohistochemical procedures were carried out as previously described.²² For all omission controls, nonimmune or preimmune sera of mouse or rabbit origin were substituted for the primary antibodies at the appropriate dilutions. In addition, we preincubated the polyclonal reagent with the immunizing peptide as a blocking control. The blocking peptide is a chimera between glutathione-S-transferase and the extracellular domain of the human PRLr expressed in *Escherichia coli* (a gift from Dr. Charles Clevenger). Five μ g of the peptide was incubated with 5 μ l of the antibody overnight at 4°C and then applied to the sections. In addition, the specificity of the CL-AB for identifying PRLr in human prostate epithelium was determined by immunoblotting using the prostatic carcinoma cell lines LNCaP and PC-3 (see below). Positive controls were the same surgical biopsy specimens of human breast used for *in situ* hybridization studies.

The intensity of signal at both the mRNA and protein levels were independently evaluated and semiquantitated using a grading scale of 1–4, with 4 representing the highest value, by three of us (I.L., F.M., and M.L.).

Immunoblot

LNCaP, PC-3, and MCF-7 cell lines (American Type Culture Collection) were grown in RPMI 1640 medium, F-12 nutrient mixture (HAM) culture medium, and Dulbecco's modified Eagle's (high glucose) culture medium, respectively, supplemented with 10% heat-inactivated fetal bovine serum and 1% antibiotic-antimycotic (Gibco BRL, Gaithersburg, MD) at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air.

Approximately 1×10^6 cells were trypsinized, washed with phosphate-buffered saline, and protein lysates were extracted with lysis buffer (10% sucrose, 1% Nonidet P-40, 20 mmol/L Tris, pH 8.0, 137 mmol/L NaCl, 10% glycerol, 2 mmol/L EDTA, 10 mmol/L NaF, 1 mmol/L phenylmethylsulfonyl fluoride, and 1 μ g of leupeptin) agitated on ice every 10 minutes for 30 minutes, and centrifuged for 20 minutes at 14,000 rpm. The supernatant was removed and the concentration was determined with a spectrophotometer (Beckman DU 650) using the DC protein assay (BioRad, Hercules, CA).

Western blotting was performed using the Novex transfer system (Novex, San Diego, CA), 12% Tris-glycine pre-cast Novex gel, and HyBond ECL nitrocellulose membrane (Amersham Life Science, Cleveland, OH). Four hundred μ g of lysate was transferred to the membrane, blocked in 5% milk in TBST for 1 hour, and blotted overnight at 40°C with the CL-AB (1:3000 dilution in 5% milk). For detection, the membrane was incubated in horseradish peroxidase-streptavidin goat anti-rabbit sec-

ondary antibody (BioRad) (1:10000 dilution in 5% milk in TBST for 30 minutes at room temperature) and developed with the ECL detection system (Amersham).

Results

Fetal, Postnatal, and Prepubertal Prostates

As previously described,²² the fetal human prostate at 29–34 weeks of gestation is composed of immature stroma containing branching cords of epithelial cells, some of which are arranged in solid nests and others containing lumens (Figure 1, A and B). In that study, we used high molecular weight cytokeratin immunostaining to show that solid epithelial nests were entirely composed of basal cells.²² With lumen formation basal cells were located along the perimeters of developing acini and ducts, a location found in the postnatal, prepubertal, and adult prostate glands.²²

At all stages of development uniformly strong signals for PRLr message (+4) was strikingly evident in epithelial cells irrespective of whether they were arranged in solid nests or formed lumens (Figure 1A). Faint signals (<1) were also detected in the stroma (Figure 1A).

The immunolocalization of receptor protein in fetal, postnatal, and prepubertal prostates was identical to that found at the message level. Light intensity immunostaining (1–2+) for the receptor was detected in the cytoplasm of both immature luminal and basal cells in these glands (Figure 1B). Thus, when compared with transcript signals staining intensity was reduced in these tissues regardless of whether the B.6 or CL-AB antibody reagent was used (Figure 1B). Stromal staining was rarely seen in these glands and when it was found it was always faint (<1).

Normal Adult Prostate

Localization of PRLr mRNA was evident in all but one of the 25 adult prostate specimens we selected for *in situ* hybridization studies. In all instances hybridization signals were predominately found in the cytoplasm of epithelial cells and approximated the levels of expression intensity found in immature glands (1–2+) (Figure 1C). As was seen in the immature prostates, only a very faint signal was present in the stromal compartment, where it was exclusively localized in the cytoplasm of smooth muscle cells. No consistent differences in either the intensity or in the localization of PRLr mRNA expression was evident between the three anatomical zones of the adult gland (1–2+).

In concert with these findings, the intensity of immunostaining in these three zones mirrored results seen at the message level (Figure 1, D and E). Differences were, however, evident in the localization of receptor staining with the two antibodies. Although both antibodies localized the receptor predominately in the cytoplasm of epithelial cells, the nuclei of basal cells were consistently stained with the CL-AB antibody, a feature less frequently found in secretory cells (Figure 1E). In contrast clear

Figure 1. A and B: Fetal human prostate at 34 weeks of gestation. Cords of epithelial cells are found in solid nests or arranged around a lumen. A: *In situ* hybridization. Strong signals are uniformly evident in epithelial cells. In contrast only faint signals are present in stromal components ($\times 250$). B: Immunostaining of the same fetal gland illustrated in A with the CL-AB reagent. Light to moderate immunostaining is seen in the cytoplasm of basal and luminal cells. Compare the relatively light intensity of immunostaining with the strong hybridization signal present in epithelial cells in A. No immunostaining of stroma is observed in this field but in other areas and in other immature glands faint staining was occasionally detected with either antibody ($\times 250$). C–E: Normal adult prostate from the peripheral zone. C: *In situ* hybridization. Light to moderate signal is evident in the cytoplasm of epithelial cells. No signal is present in stromal cells in this field ($\times 350$). D and E: Immunohistochemical localization of PRLr using the CL-AB reagent in the peripheral zone from the same case illustrated in C. D: The cytoplasm of secretory cells are lightly stained. Basal cell nuclei appear to be moderately stained while those of secretory cells appear unstained. No staining of stromal cells is evident in this field ($\times 250$). E: In this higher power micrograph from the normal peripheral zone in a different case, immunostaining of nuclei of basal and secretory cells is evident ($\times 600$).

immunostaining of nuclei was not well delineated with the B.6 reagent. Light staining of smooth muscle cells (<1) was occasionally observed with the B.6 antibody, which rarely occurred when the CL-AB reagent was used.

Figure 2. A and B: Benign prostatic hyperplasia. A: *In situ* hybridization. The localization of PRLr message is almost exclusively present in hyperplastic epithelial cells within the nodule. The intensity of signal approximates that found in normal epithelium ($\times 250$). B: Immunohistochemical staining with the CL-AB reagent of the same lesion illustrated in A. As was the case for normal epithelial cells, staining intensity is light to moderate. Nuclear staining was infrequent in this example. Note the almost total absence of positivity in the stromal component of the nodule as detected by either *in situ* hybridization or immunohistochemistry ($\times 220$).

Benign Prostatic Hyperplasia

In the 5 cases of BPH studied we found that PRLr message and protein were predominately localized in epithelial cells and that signal intensities at both levels were comparable to what we observed in normal prostate cells (see above and Figure 2, A and B). As was the case for normal prostatic epithelia, nuclear immunostaining with the CL-AB also occurred in hyperplastic cells. In all BPH lesions, stromal expression of the receptor approximated the faint intensity seen in normal prostate tissue.

Dysplasia and Carcinoma

With rare exception (2 lesions in 2 separate cases) PRLr expression was markedly increased (4+) in dysplastic epithelia when compared with normal adult glandular cells (Figure 3, A–C, E, and F). This finding was consistent at both the mRNA (Figure 3, A–C) and protein levels (Figure 3, E and F). The enhanced (4+) expression was observed with each antibody reagent and occurred irrespective of the grade of the dysplastic lesion (Figure 3, E and F). Interestingly, unlike the situation in epithelia found in normal and hyperplastic glands, the nuclei of dysplastic cells were rarely stained by either antibody reagent (Figure 3, E and F).

Although exceptions were found (2 cases where grade 3 predominated) the intensity of *in situ* hybridization and immunostaining in prostatic carcinomas followed a similar pattern. Thus, in grade 3 carcinomas PRLr signals at both the mRNA and protein levels were consistently uniform throughout the neoplasm and closely approximated the expression observed in normal epithelia (1–2+) (Figure 4, A and C). In contrast, although the intensity of expression in the majority of grade 4 carcinomas was judged to be similar to that found in grade 3 cancers, the higher grade lesions were commonly heterogeneous and contained foci of cells in which the both the intensity of receptor mRNA signals and immunostaining was dimin-

Figure 3. A–G: Dysplasia. **A:** *In situ* hybridization. Note the intense signal present in the multiple foci of high grade dysplasia. An acinus that contains morphologically normal as well as dysplastic cells is present in the center of this field ($\times 220$). **B:** *In situ* hybridization. The differences between the intensity of receptor transcript expression in dysplastic (top) and normal (bottom) cells are well illustrated in these juxtaposed acini ($\times 250$). Also compare the intensity of signal in dysplastic lesions with that seen in normal epithelial cells in the lesion-free peripheral zone illustrated in Figure 1C. **C:** *In situ* hybridization of a high grade dysplastic lesion. This high magnification micrograph exemplifies the intense expression of PRLr message we consistently found in dysplastic lesions irrespective of their grade or anatomical location ($\times 600$). **D:** *In situ* hybridization using the sense probe of the dysplastic lesion illustrated in C. Note the total absence of hybridization in this lesion when the sense probe was used. The same negative results were obtained when the antisense probe was omitted ($\times 350$). Immunohistochemistry of moderate (E) and severe (F) grade dysplastic lesions using the CL-AB reagent ($\times 400$ for both panels). Note the intense cytoplasmic staining in both lesions. Interestingly, the nuclei of dysplastic epithelial cells are unstained. Compare with hybridization and immunohistochemical findings in normal and hyperplastic epithelial cells in Figures 1 and 2. **G:** Competition control in a section of normal peripheral zone. Peptide and the CL-AB were preincubated before application to the section, as described under Materials and Methods; no staining is evident. The same results were obtained when the primary antibody was omitted.

ished ($<1+$) (Figure 4, B and D). Hybridization with the sense probe was consistently negative or greatly reduced when compared with its antisense counterpart in all tissues studied (Figure 3D).

When either of the primary antibodies were omitted from the incubation or peptide competition was performed with the CL-AB reagent, all immunostaining was abolished (Figure 3G). Strong signals (3+) were always detected in epithelial cells of the breast by *in situ* hybridization or by immunohistochemistry with either antibody; all staining was abolished when the control procedures listed above were used.

Our immunoblot studies confirmed the specificity of the CL-AB for the human prolactin receptor and yield important findings. Thus, the CL-AB detected a strong major band of approximately 85–90 kd in lysates of MCF-7, LNCaP, and PC-3 (Figure 5), which is consistent with the size reported for the long form of the prolactin receptor in the human breast.^{18,24} As expected, among the three cell lines studied, the band staining strongest was detected in lysates of MCF-7 cells which are derived from a carcinoma of the breast, followed by LNCaP and PC-3 cells, respectively. Of particular interest was the finding that the androgen-dependent LNCaP prostate

carcinoma cells yielded a significantly stronger-stained band for the receptor when compared to that observed in lysates of the androgen-independent PC-3 cells.

Discussion

In the current study, we find presumptive evidence that PRL participates in fetal prostate development and likely plays an important role in the physiology of the adult human prostate. Our findings are also in concert with studies that show that PRL exerts proliferative and differentiating effects in the rat prostate.^{1,2,4–6} The precise mechanism(s) by which PRL influences the growth, development, and maintenance of the prostate is currently undefined, but in other tissues it has been reported that the hormone stimulates the expression of a variety of genes that are involved in cell proliferation and differentiation.¹⁶

In our study of both fetal and adult glands the receptor was predominately expressed in epithelial cells. PRLr message and protein were also detected in smooth muscle, albeit signal intensity at both levels was always much less than that observed in epithelia. Receptor transcript

Figure 4. A–D: Carcinoma of the prostate. A and B: *In situ* hybridization, grade 3 carcinoma (A) and grade 4 carcinoma (B). The intensity of signal in the grade 3 carcinoma approximates that seen in normal prostate. The signal appears less intense in this grade 4 carcinoma when compared with its more well differentiated counterpart or the expression in normal prostate ($\times 250$). C and D: Immunohistochemistry using the CL-AB reagent. The results seen with immunostaining in grade 3 (C) and grade 4 (D) carcinomas approximates the level of intensity seen in these lesions by *in situ* hybridization.

and protein were consistently present in the cytoplasm of epithelial cells, but nuclear immunostaining was, however, also evident with the CL-AB reagent. The clear nuclear immunostaining we found with the CL-AB *versus* the B.6 reagent may be due to subtle differences in epitopes recognized by the two antibodies. In this regard the CL-AB reagent is directed against the complete extracellular domain of PRLr and may therefore recognize specific epitopes that are less precisely identified by the B.6 antibody which was produced against whole membranes. While the significance of nuclear localization for the receptor in the prostate is currently unclear, PRLr has been identified in the nuclei of other cells such as rat hepatocytes and Nb-2 lymphocytes.^{25–26} In the case of Nb-2

cells, the action of interleukin-2 induces PRL translocation to the nucleus where it causes the up-regulation of genes involved in the cells entry into S phase.²⁵ In this context, it is of interest to note the consistent nuclear localization of PRLr in prostatic basal cells,²⁷ the major proliferative cell type in the normal prostate,²⁸ and the purported precursor of secretory glandular epithelia.²⁹ Because androgen receptor protein is not immunodetectable in prostatic basal cells^{22,30} our findings may indicate that PRLr, along with other peptide growth factors,^{22,31,32} are involved in mediating the proliferation of these precursor cells. We (I.L. and J.E.M.) had previously localized epidermal growth factor receptor exclusively in basal cells of the fetal human prostate, which, like its adult counterpart, lacked immunohistochemically demonstrable androgen receptor.²² We proposed that the localization of epidermal growth factor receptor in fetal basal cells likely reflects a developing paracrine relationship found later in adult glands between these precursor cells and smooth muscle cells which express transforming growth factor- α , the ligand for the receptor.²² In contrast, the almost exclusive localization of PRLr in both fetal basal and luminal cells suggest that peptide-receptor interactions are restricted to the epithelial cell compartment in the developing gland. In the adult prostate the presence of the receptor in basal and secretory cells together with the reported synthesis of PRL by the latter cells in organ culture¹¹ is consistent with an autocrine/paracrine pathway for the action of the hormone within acinar/duct units of the prostate.

In our current study we found that the expression of PRLr appeared unchanged in BPH specimens when compared with normal glands. In contrast, markedly strong hybridization signals and immunostaining were evident in dysplastic epithelial cells irrespective of the grade of the lesion. Since dysplasia is a putative precursor of carcinoma,^{33,34} PRL action may therefore play an important role in early carcinogenesis of the gland. Support for this concept comes from both organ culture studies of human¹¹ and rat³⁵ prostate and from our *in vivo* studies in the Noble rat sex hormone-prostatic carcinoma model.³⁶ The organ culture studies demonstrated that both rat and human prostate explants, supplemented with PRL, underwent proliferative changes that closely resembled cribriform dysplastic lesions found in the human gland.³⁴ Moreover, using the Noble rat we (I.L. and S.M.H.) recently reported that the testosterone and estradiol-17 β treatment, used to induce prostatic dysplasia in this animal model,³⁷ caused hyperprolactinemia and that the administration of bromocriptine blocked both the increase in circulating PRL and the development of the prostatic lesions.³⁶ Results from the aforementioned studies taken together with our current findings in dysplastic human prostate lesions indicate that PRL may play a significant role in carcinogenesis of the gland as has been proposed for the rodent and human mammary gland.^{38,39} Moreover, the finding of an autocrine/paracrine pathway for localized PRL action in epithelial cells of human breast and prostate may explain past clinical findings in which inconsistencies in the association be-

Figure 5. Immunoblot. The blot illustrates a single band of approximately 85–90 kD detected in lysates of MCF-7, LNCaP, and PC-3 cells. Note the intense band detected in the MCF-7 cells and compare this with the less intense bands present in LNCaP and PC-3 cells, respectively. Molecular weight markers are illustrated on the left.

tween hyperprolactinemia and carcinoma of both tissues have been reported.^{3,6,40}

In addition to its possible enhancement of early prostatic carcinogenesis, diminished PRL responsiveness may also influence the progression of the neoplastic process. In this regard, we found that PRLr expression was heterogeneous and was often notably diminished in poorly differentiated foci within high grade carcinomas when compared with areas of lower grade cancer in the same neoplasm. Interestingly, the results of our immunoblot studies are consistent with the findings at the tissue level and support the concept that diminution of receptor expression is a feature of less differentiated prostatic carcinomas. In addition, results from a past study had shown that the growth of the transplantable, well differentiated, androgen-dependent rat prostatic carcinoma line R3327-3219 was significantly enhanced by pituitary graft-induced hyperprolactinemia.⁴¹ Moreover, the highest growth rates in this tumor line occurred when the hyperprolactinemic castrated rats were given testosterone. This was in marked contrast to the poorly differentiated androgen-independent subline 150, whose growth was unaffected by the hyperprolactinemic state. These investigators concluded that the enhanced growth of R3327-3219 in the testosterone-treated rats may have been due to the synergistic interaction between PRL and the androgen. Recent findings in transgenic mice by Wennbo et al⁴² support the concept that a synergistic interaction between androgen and PRL plays an important role in the pathogenesis of abnormal growth of the prostate. These workers reported that transgenic mice, which overexpress PRL and have elevated plasma levels of testosterone, consistently develop hyperplasia of the gland. PRL may however have a direct proliferative and differentiating effect on the gland that are independent of androgen action, as demonstrated by studies using organ cultures of the rat³⁵ and human¹¹ prostate, which were not supplemented with male hormones. In either case, the decreased expression of PRLr we observed in areas of poorly differentiated high grade carcinomas may reflect the emergence of cells that are no longer responsive to the action of PRL.

In summary, results from our current studies and those of Nevalainen et al,¹¹ who have reported that PRL is locally produced by human prostate epithelium, suggests that the hormone is involved in modulating the normal growth, differentiation, and maintenance of the human prostate. Our findings of enhanced and diminished PRLr expression in dysplastic and poorly differentiated carcinomas respectively may indicate that the hormone plays a role in early carcinogenesis of the gland but that with progression, prostatic cancers become increasingly independent of its action. Finally, it should be noted that our investigation has focused only on the expression of the long form of the receptor and that the possibility exists that other isoforms of PRLr may be present in normal and diseased prostatic epithelia which may be involved in the pathogenesis of neoplasia in the gland.

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