The Role of Vitamin D in Normal Prostate Growth and Differentiation

Badrinath R. Koney, Gary G. Schwartz, James S. Aciero, Jr., Michael J. Becich, and Robert H. Getzenberg

Departments of Urology [B. R. K., R. H. G.], Pathology [R. H. G., J. S. A., M. J. B.], and Medicine and Pharmacology [R. H. G.], University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213-2582, and Sylvester Comprehensive Cancer Center, University of Miami School of Medicine, Miami, Florida 33101 [G. G. S.]

Abstract

Although increasing data indicate a role for vitamin D in prostate cancer, little is known about the role of this hormone in the noncancerous prostate. We examined the effect of 1,25-dihydroxyvitamin D₃ (1,25 D) on the growth of noncancerous rat prostates in vivo. Rats were castrated and treated with vehicle (controls), 1,25 D, testosterone, or a combination of both hormones for 2 weeks. Histological examination of the harvested prostates revealed that 1,25 D had a selective regressive effect on epithelial cells in treated rats compared to untreated castrated rats and to normal uncastrated rats. However, 1,25 D stimulated stromal growth in the prostate. The mean prostatic weight of the vitamin D-treated rats was twice that of the untreated rats (0.13 ± SEM 0.005 g versus 0.06 ± SEM 0.006 g). The histological differences were less marked in the testosterone-supplemented animals. A greater degree of cellular differentiation was observed in the rats treated with testosterone and vitamin D compared to rats that received testosterone supplementation alone. Studies of the nuclear matrix composition revealed differences between the testosterone-supplemented and the testosterone and 1,25 D-treated rat prostates. We conclude that in the absence of testosterone, 1,25 D may exert a growth-promoting effect on the prostatic stroma in vivo. In concert with testosterone, it may play an important role in the growth and differentiation of the normal rat prostate.

Introduction

Calcitriol (1,25-Dihydroxyvitamin D₃), henceforth referred to as 1,25 D, is the active hormonal form of vitamin D and is well known for its role in calcium metabolism. Vitamin D was originally classified as a vitamin because supplementation of foods with this substance could prevent the bone disease rickets. After the identification of the chemical structure of 1,25 D, it became apparent that 1,25 D was actually not a vitamin but a steroid hormone (1). Subsequently, 1,25 D has been recognized to be important in modulating the growth and differentiation of various tissues (2). The story of vitamin D, from vitamin to hormone, is a fascinating subject of ongoing biomedical research (3).

Epidemiological data first suggested that vitamin D may play a role in prostate cancer (4). Schwartz and Hulka (4) found that mortality rates from prostate cancer were higher in northern latitudes. Because the major source of vitamin D is casual exposure to sunlight, they postulated that vitamin D maintains the differentiated phenotype of prostatic cells and that inadequate levels of vitamin D could permit the growth of prostate cancer. Hanchette and Schwartz (5) later reported that prostate cancer mortality rates by county within the United States were correlated inverse with the availability of UV radiation (5), lending further support to the vitamin D hypothesis. Men with prostate cancer have been found to have lower serum levels of 1,25 D before the diagnosis of the disease (6). Specific receptors for 1,25 D (VDRs) have been demonstrated in numerous cell types, including breast, kidney, testes, and prostate (7, 8). The antiproliferative and growth-regulatory effects of 1,25 D and its analogues on various cell types, including normal and neoplastic cells, have now been demonstrated by many studies (8–12).

Miller et al. (13) first demonstrated the presence of VDR in the human prostate cancer cell line LnCaP. VDRs have since been found in other prostate cancer cell lines and in normal prostate epithelial and stromal cells grown in culture (8). Studies with human prostate cancer cell lines have shown that vitamin D and vitamin D analogues have antiproliferative effects on many of these cells (13–15). Similar antiproliferative and prodifferentiating effects are evident in primary cultures of normal prostate cells in vitro (8). This effect is also present in vivo; human prostate cancers xenografted in nude mice are inhibited by administration of a vitamin D analogue (16). These data suggest that 1,25 D acts on both normal and neoplastic prostate cells, and this has stimulated therapeutic clinical trials of vitamin D in prostate cancer (17).

Although many studies have examined the effect of 1,25 D on prostate cancer cells, little data are available regarding
the influence of this steroid on noncancerous (normal) prostate growth and differentiation in vivo. The presence of VDR in normal prostatic tissue strongly suggests a role for this hormone in the normal gland. To ascertain the possible role of 1,25 D in the noncancerous prostate, we examined the influence of 1,25 D on the growth and development of the rat prostate. In addition, we studied the interaction of 1,25 D and androgens, which are well known to have a critical role in prostatic growth and development.

Because a physiological role of 1,25 D on the prostate in vivo had not been demonstrated previously, our experimental conditions were designed to maximize the opportunity to observe any effect of this steroid. Because testosterone is the major growth regulator in the prostate, we studied rats in which most of the effects of testosterone were removed via castration. Although the adrenal glands are also a source of androgens, the effect of adrenal androgens in rodents is insignificant at the prostatic level (18). Moreover, we used doses of 1,25 D that were clearly pharmacological. We reasoned that if pharmacological doses of 1,25 D do not produce biological responses, then physiological doses are unlikely to be of importance.

We also examined the effect of 1,25 D and testosterone on the NMP composition of normal prostate cells. The nuclear matrix is the scaffolding of the nucleus and consists of residual nucleoli, peripheral lamins, pore complexes, and the internal ribonucleoprotein network (19). It plays a central role in the topological organization of DNA and its replication and transcription (20). We have found previously that the nuclear matrix is tissue-specific and is a signature for tissues including the ventral prostate and seminal vesicle (21). In addition, we have shown that the normal rat dorsal prostate contains at least 10 unique NMPs that are absent in the nuclear matrix of Dunning rat prostate tumors (22). 1,25 D acts by binding to VDR, which in turn acts by regulating the transcription of specific genes (23). Because the nuclear matrix is also involved in regulating gene expression and mRNA transcription, we postulated that differences in 1,25 D exposure may be accompanied by changes in cellular NMP composition.

Results

As noted in "Materials and Methods," all animals were castrated at the beginning of the study. Thirty-six of the 40 rats completed the study. One animal from each group died due to anesthetic complications after castration. Mean gross weights of the prostates and seminal vesicles from the four groups of rats are shown in Table 1. The prostates from rats in group 2 (1,25 D) were visibly larger than those of group 1 (controls; Figs. 1 and 2a). Similarly, the prostatic weights of rats in group 2 receiving vitamin D treatment only were significantly higher than those of the control group (P < 0.0001). The seminal vesicles from the two groups of animals also differed significantly in weight (P < 0.0001; Fig. 1). In contrast, the gross weight of the animals receiving 1,25 D injections was consistently lower than that of the controls, who received sham injections of vehicle only. There was a 14% increase in the mean weight of control animals during the study period, while a decrease of 20% was noted in the mean weights of animals treated with 1,25 D during the same period.

The mean serum calcium levels differed significantly between groups 1 (controls) and 2 (vitamin D; P < 0.0001). Mean serum calcium levels in groups 2 (vitamin D; 13.19 mg/dl ± SEM 0.12) and 4 (vitamin D and testosterone; 12.6 mg/dl ± SEM 0.07) were higher than in groups 1 (controls; 10.05 mg/dl ± SEM 0.17) and 3 (testosterone only; 9.95 mg/dl ± SEM 0.16). Serum calcium levels have been shown to increase in animals given 1,25 D due to its effects on calcium metabolism. Mean serum testosterone levels varied between groups 3 (testosterone only) and 4 (testosterone and vitamin D). Although testosterone levels were higher in group 4 (testosterone and vitamin D; 0.11 ng/ml ± SEM 0.02) than in group 3 (testosterone only; 0.085 ng/ml ± SEM 0.02), this difference was not statistically significant (P > 0.38).

Several variations were observed in the extent of differentiation of the prostates and seminal vesicles as determined by the grading system outlined in "Materials and Methods" (Fig. 2, c-i). The mean grade of epithelial differentiation of prostates from group 2 (1,25 D) rats was significantly lower.
than that of group 1 (control) prostates (1.64 ± SEM 0.13 versus 2.07 ± SEM 0.07; \( P \leq 0.008 \)). The mean grade of stromal differentiation was also lower in prostates from group 2 (1.25 D; 1.42 ± SEM 0.13) compared to those of group 1 (controls), although this difference was not significant (1.71 ± SEM 0.12; \( P \leq 0.14 \)). In the seminal vesicles, the extent of cellular (3.0 versus 3.0) and stromal (2.0 versus 2.0) differentiation was identical in both groups. The histological findings were similar on frozen section and on permanent paraffin-embedded sections. Grading of cytological differentiation was made in comparison and relative to prostates and seminal vesicles from normal untreated (i.e., uncastrated) rats (Fig. 2b). Prostates from groups 3 (testosterone only) and 4 (testosterone and 1.25 D) showed glandular differentiation, with large acini lined by a single layer of columnar epithelium and containing apical secretory granules. The stromal component was also decreased as compared to that seen in prostates from the first two groups. The only difference between the groups was a significantly higher number of secretory cytoplasmic vesicles present in prostates of rats.
receiving 1,25 D treatment in addition to testosterone supplementation. Comparative histological examination of the other organs such as the heart, liver, and kidneys from the two groups of animals revealed extensive calcification of coronary vessels and deposits of calcium in the renal tubules in rats treated with 1,25 D that were absent in the organs from control animals.

By staining with AE1/AE3, which stains epithelial cytokeratin, it was evident that the epithelial component was appreciably decreased in the prostates from group 1 (controls) and group 2 (vitamin D) rats as compared to the normal uncastrated rat prostate ($P \leq 0.43$). The amount of smooth muscle present was higher in group 1 (controls) and 2 (1, 25 D) prostates relative to the normal prostate, as demonstrable by staining with HHHF35 ($P \leq 0.13$). Staining for LCA revealed an almost complete absence of inflammatory cells in the normal, control, and 1,25 D prostates. The stromal component in the prostate tissue determined using quantitative image analysis was higher in group 1 (controls) prostates than normal prostates but was highest in the prostates from 1,25 D-treated rats ($P \leq 0.05$). The stromal component present in prostates from each of the two groups and the normal prostate as calculated by quantitative image analysis are shown in Fig. 3. Staining with Giemsa stain revealed some striking differences. There was a significant increase in the number of mast cells identifiable in stained tissue sections from control and 1,25 D rat prostates compared to the normal rat prostate.

On EM, normal uncastrated rat prostate showed the presence of extensive rough endoplasmic reticulum in tall columnar cells with abundant cytoplasmic secretory vacuoles (Fig. 4a). There was progressive loss of endoplasmic reticulum and secretory vacuoles in control (castrated) and 1,25 D-treated rats (Fig. 4, b and c). These changes were greater in 1,25 D-treated rats as compared to controls. The columnar cells were also not as tall in the controls and 1,25 D-treated rats as compared to normal rats.

Analysis of NMP composition did not reveal any significant differences between 1,25 D-treated and control prostates in the absence of testosterone. The nuclear matrix composition of prostates from groups 3 (testosterone only) and 4 (testosterone and 1,25 D) did show some notable differences. Five proteins present in the nuclear matrix of cells from group 4 (testosterone and 1,25 D) prostates were absent in those from group 3 (testosterone only; Fig. 5, A and B). All of these 5 proteins were different from the 10 abundant proteins that we identified previously in the normal rat dorsal prostate (22). The molecular weights and isoelectric points of these proteins are shown in Table 2.

**Discussion**

Our results indicate that 1,25 D at pharmacological doses has significant effects on prostate growth in vivo. In the absence of testosterone, 1,25 D preferentially stimulates stromal growth. This translates to an overall increase in prostatic weight in castrated rats treated with 1,25 D, largely due to increased stroma. Conversely, 1,25 D has an inhibitory effect on the growth of the epithelium. The difference in the effect of 1,25 D on the stroma and epithelium does not seem to be simply a manifestation of variation in the degree of growth inhibition but an actual regressive effect on the epithelium and a stimulatory effect on the stroma. Previous data suggest that castration inhibits epithelial growth to a much greater extent than stromal growth (24). In the normal rodent prostate, the ratio of epithelium to stroma is 5:1 (24, 25). We found that prostates of rats treated with 1,25 D had a significantly greater amount of stroma than epithelium. This suggests that the inhibitory effect of 1,25 D on the prostate is restricted to the epithelium. The stromal component in the 1,25 D-treated prostates occupied a greater percentage of the total area than that in prostates from normal untreated castrated animals. Likewise, the epithelial component was more prominent in the normal prostates than in the prostates from animals treated with 1,25 D.

As described previously, VDRs have been identified in both prostatic stromal and epithelial cells. It is possible that the present results are explicable by a relative difference in the number of VDRs in the stroma in comparison to the epithelium. Using a polyclonal antibody to the VDR, Johnson et al. (26) demonstrated prominent immunostaining of VDR in the epithelium of rat prostates but not in the stroma. Schliecher et al. (27) demonstrated the presence of intranuclear VDR by nuclear labeling in the ventral and dorsal prostate epithelium and stroma of the mouse prostate. However, the number of VDRs in each component was not reported. In human prostate tissue, the stroma has relatively less VDR than the epithelium (8). Assuming that the different techniques to detect VDRs that were used in these studies are equally reliable at identifying the presence of receptors in the various cell types, there seems to be a species-related difference in receptor localization. Therefore, the effect of 1,25 D does not seem to be related strictly to the presence or number of VDRs in the tissue or cells. We have also found
that using a polyclonal antibody, the VDR is localizable to the nuclear matrix in the rat prostate (28).

Our results differ somewhat from those of Peehl et al. (8), who found that 1,25 D exerted an antiproliferative effect on both normal prostate epithelial and stromal cells grown in vitro. We observed similar effects of 1,25 D on the epithelium, whereas the effects on the stroma were different. However, it is important to note that the effects described by Peehl et
al. were observed in vitro. Cells grown in culture often change a number of their characteristics, including their responsiveness to hormones such as testosterone. The in vivo effects of 1,25 D may be different due to the presence of various other modifying factors that may be absent even in serum-supplemented culture media. The precise effects of 1,25 D on neoplastic cells in vitro are not completely understood.

Other than exerting a growth-promoting effect, 1,25 D also seems to influence the degree of differentiation of the stromal and epithelial components. Compared to control castrated rats (Group 1), castrated rats exposed to 1,25 D showed a lesser degree of differentiation of both prostatic components as measured by our grading system. Although the difference was not large (1.67 versus 2.07), the direction of differentiation was contrary to expectation. This effect was restricted to the prostate; a similar difference was not observed in the seminal vesicles. The variations in the grade of prostatic epithelial and stromal differentiation between groups of rats supplemented with testosterone (Groups 3 and 4) were much more subtle. The effect of testosterone may be so overwhelming that the relatively less dramatic effects of 1,25 D may be overshadowed. Conversely, testosterone may modify the effects of 1,25 D, resulting in a differentiation-promoting effect. This could explain the increased prostatic epithelial differentiation in the prostates of rats receiving both 1,25 D and testosterone, as evidenced by the presence of a greater number of cytoplasmic secretory vesicles (Fig. 2). Miller et al. (29) also found that LnCaP cells were stimulated to proliferate by 1,25 D in the absence of androgens. The interaction of testosterone and 1,25 D in the development of the prostate gland promises to be a fertile area for further research.

The alterations in the NMP composition were only seen in the testosterone-supplemented rat prostates. This could be due to the fact that in the absence of testosterone, 1,25 D predominantly affects stromal growth. During the process of NMP isolation from the cells, the tissues are homogenized. This results in the epithelial cells being stripped off the stroma. The stromal material is then removed. Therefore, the NMPs examined represent those from a cell population that is predominantly epithelial. Thus, the extracted NMPs constitute mainly those from the epithelial cells; hence, alterations in stromal cellular NMPs may go undetected. 1,25 D binds to the intracellular VDR, which is a member of the steroid-thyroid-retinoic acid receptor superfamily and acts to regulate the transcriptional activity of specific genes (30, 31). Hence, it is conceivable that vitamin D could alter the nuclear matrix composition of treated cells because it is also involved in transcriptional regulation. Studies of the osteocalcin gene have revealed interactions between two NMPs and DNA in the promoter region of the gene (32). These NMP-DNA interactions are found close to the vitamin D-responsive sequences of the gene. This raises the possibility that these NMPs, which resemble transcription factors, may be involved in the 1,25 D-mediated regulation of gene transcription. We sought to examine if such interactions may be present in the normal prostate. However, we were unable to demonstrate an alteration in NMPs that could be ascribed solely to 1,25 D.

In summary, we have demonstrated that in the absence of testosterone, pharmacological doses of 1,25 D increase the growth of stroma and decrease the growth of prostatic epithelium. In conjunction with testosterone, 1,25 D increases the differentiation of the prostate. The differentiation-promoting effects of 1,25 D seem to be restricted to prostatic tissue because similar effects are not observed in another sex accessory tissue, the seminal vesicle. Studies are currently underway to further examine the interactions of the hormonal milieu of the prostate. The investigations presented here provide further evidence of a role for 1,25 D in normal prostate biology and may serve as a building block to further understand the potential role of this steroid in the chemoprevention and/or treatment of prostate cancer (33). Future studies of the effect of physiological doses of 1,25 D on the noncancerous prostate are clearly warranted.

Materials and Methods

Animal Experiments. Forty 90–100-day-old Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 310–320 g were used for all animal experiments. All animals were castrated using a sterile surgical technique on day 0. The rats were divided into four groups. Group 1 (15 rats) served as controls. Group 2 (15 rats) received i.p. injections of 1.25 \( \mu \)g 1,25(OH)\(_2\)D\(_3\) (Blomol, Plymouth Meeting, PA) on alternate days, starting from day 7. The 1,25 D was dissolved in ethanol (1:1) and diluted with PBS (1.25 \( \mu \)l in 500 \( \mu \)l). Group 3 (5 rats) and Group 4 (5 rats) received testosterone supplementation starting from day 9 by means of an osmotic pump implanted s.c. (ALZA, Palo Alto, CA). The pumps were loaded with testosterone (Sigma Chemical Co., St. Louis, MO) suspended in polyethylene glycol 400, which was delivered at a rate of 1000 \( \mu \)g/day. Animals in group 4 also received alternate day i.p. injections of 1 \( \mu \)g of 1,25 D starting day 9. Animals in groups 1 and 3 received sham i.p. injections of the vehicle [100% ethanol in PBS (1 \( \mu \)l in 500 \( \mu \)l)]. To prevent endogenous vitamin D synthesis, all animals were housed in 24-h total darkness. Rats were fed vitamin D-deficient chow containing 0.3% calcium and 0.4% phosphate (Harlan Teklad, Madison, WI). Three representative animals from each of groups 1 (controls) and 2 (1,25 D) were weighed before each injection. The 1,25 D and testosterone treatments were continued until day 21. On day 23, all the animals were sacrificed using CO\(_2\) overdose.

Before sacrificing the animals, blood was collected by cardiac puncture and stored for analysis of serum calcium and testosterone levels. Once sacrificed, the prostates and seminal vesicles were harvested from each animal. The organs were weighed individually. Half of each harvested organ was sent for histopathological examination, and the remainder was used for analysis of NMP composition. Additionally, other organs such as the kidneys, heart, and liver were also harvested from a few animals in groups 1 and 2 and examined histopathologically to assess the systemic effects of the 1,25 D treatment. The prostate and seminal vesicles were also harvested from two untreated and uncastrated normal rats and examined by histopathology. The protocol for this study was approved by the institutional animal care and use committee of the author’s institution.

Pathological Examination. The prostate and seminal vesicles were submitted for frozen section examination, and those from groups 1 and 2 were also embedded in paraffin. For frozen section examination, tissues
were embedded in Tissue-Tek® OCT compound (Miles Inc., Elkhart, IN). Tissue sections were stained with H&E and examined microscopically. The level of differentiation of the prostate and seminal vesicles was analyzed by microscopic examination and quantification of the level of differentiation according to a predetermined scale. The scale graded the level of differentiation based on the presence or absence of cellular components from least differentiated (grade 1) to most differentiated (grade 6). Grade 1 indicated a total absence of cellular cells; grade 2 indicated an increased numbers of basal cells; grade 3 indicated poorly differentiated columnar cells were present; grade 4 indicated undifferentiated columnar cells were present; grade 5 indicated normal differentiation; and grade 6 indicated hypertrophic columnar epithelium. All examinations were performed by a pathologist who was blinded to the rats' treatment group.

EM was performed to determine the presence of intracellular secretory organelles and assess the secretory capacity of the prostatic epithelium from the rats of groups 1 and 2. The procedure for EM was as described previously (34). Paraffin-embedded tissue sections were deparaffinized with xylene and rehydrated with acetone at 4°C in caccodilute buffer. Samples were then fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in cacodylate buffer, rinsed, post-fixed in OsO4, and rinsed again in maleate buffer. En bloc staining was performed with 2% uranyl acetate in succrose-maleate buffer. Samples were rehydrated through acetone, infiltrated, and embedded in epoxy resin. Ultrathin sections were cut on a Reichert Ultracut S microtome, stained with uranyl acetate and Reynold's lead citrate, and examined on a Phillips CM12 transmission electron microscope at 60 kV.

Immunohistochemistry was performed on the formalin-fixed, paraffin-embedded, 5-μm-thick tissue sections of prostates from one normal rat and three rats each from groups 1 (controls) and 2 (1,25 D). Representative tissue sections were stained with Giemsa. Staining was performed for A1/AE3, HHF35, and LCA using an avidin-biotin complex immunoperoxidase method modified from Hsu et al. (35). In short, PBS was used in place of Tris-buffered saline, and 3% H2O2 in methanol was used to block endogenous peroxidases. The dilutions for the primary antibodies were 1:200 for A1/AE3 (Boehringer Mannheim Biochemicals, Indianapolis, IN) and HHF35 (Biogenex Laboratories, San Ramon, CA) and 1:100 for LCA (Dako Corp., Carpinteria, CA). Incubation with primary antibody was performed for 1 h in all cases except HHF35, in which overnight incubation was performed. Antigen retrieval was performed before incubation with A1/AE3 antibody. These stains detect the presence of mast cells (Giemsa), epithelial cells (A1/AE3), smooth muscle (HHF35), and WBCs (LCA).

Quantitative image analysis microscopy was performed to quantify the histological composition of the prostates in terms of stromal epithelial components. For this procedure, the area of the field being analyzed was calculated initially. This was used as a constant throughout the analysis. For determination of the tissue components, >95% of each of the immunohistochemically stained samples was analyzed to arrive at the most accurate measurements possible. Next, the total tissue area was determined by thresholding all stained cells on the comparable H&E-stained section of each sample, thus eliminating the secretory storage compartments and intrastromal spaces. Subsequent stained slides were thresholded according to the areas staining positive with each specific stain. The area occupied by epithelium, smooth muscle, and stroma as a percentage of the total area in each specimen was calculated and compiled for each slide according to the following formulas:

\[
\text{Epithelium} = \frac{\text{Epithelial area (A1/AE3 positive)}}{\text{Total tissue area}} \times 100
\]

\[
\text{Smooth muscle} = \frac{\text{Smooth muscle area (HHF35-positive)}}{\text{Total tissue area}} \times 100
\]

\[
\text{Stroma} = \text{Total tissue %} - \text{epithelium %} - \text{smooth muscle %}
\]

The sum of the areas occupied by epithelium, stroma, and smooth muscle was equal to 100% of the total area.

Analysis of Nuclear Matrix Composition. The NMPs were isolated from the harvested rat prostatic tissue according to a method that has been described previously (22). Briefly, the tissue was minced into small pieces and homogenized using a Teflon pestle on ice in a 0.5% solution of Triton X-100 containing 2 mM vanadyl ribonucleoside (RNase inhibitor) to release proteins and lipids. The solution was then filtered through a nylon mesh and extracted with 0.25 M ammonium sulfate to release the cytoskeletal elements. DNase treatment at 25°C was used to remove soluble chromatin. The remaining fractions contained intermediate filaments and NMPs. This fraction was disassembled with 8 M urea, and the insoluble components, consisting principally of carbohydrates and extra-cellular matrix components, were pelleted. The urea was dialyzed out, and the intermediate filaments were allowed to reassemble and subsequently removed by centrifugation. The NMPs were then precipitated with ethanol. All solutions contained freshly prepared 1 M phenylmethylsulfonyl fluoride to inhibit serine proteases, 0.3 mM aprotonin, 1 mM leupeptin, and 1 mM pepstatin. The protein concentration was determined by resuspending the proteins and using the Coomassie Plus protein assay reagent kit (Pierce, Rockford, IL) with BSA as standard. For gel electrophoresis, the ethanol-precipitated NMPs were dissolved in sample buffer consisting of 9 M urea, 65 M 3-[l-olamidopropyl]dimethylammonio]-1-propanesulfonate, 2.2 M ampholytes, and 140 M DTT (Oxford Glycosystems, Bedford, MA). The final pellet-containing NMPs represented less than 1% of the total cellular proteins.

High resolution two-dimensional electrophoresis was used to separate the extracted NMPs. This was performed using the Investigator two-dimensional gel system (Oxford Glycosystems, Bedford, MA) as described previously (22). The gels were fixed with 50% methanol and 10% acetic acid. After thorough rinsing and rehydration, gels were treated with 5% glutaraldehyde and 5 M DTT after buffering with 50 M phosphate (pH 7.2). The gels were stained with silver stain (Accurate Chemical Co., Westbury, NY) according to the method of Wray et al. (36). NMP (50 μg) was loaded onto each gel. Only spots clearly and reproducibly observed in the gels were counted as actually representing the nuclear matrix components. The gels were analyzed on the Bioimage two-dimensional analysis system (Bioimage, Ann Arbor, MI).

All statistical analyses were performed using Student’s t test for independent samples (two-tailed).

Acknowledgments
We thank Drs. Candace Johnson and Donald L. Trump of the University of Pittsburgh Cancer Institute for their help with the study and review of the manuscript. We also thank Mara L. Grove and Angela S. Thomas for their help with the pathological studies.

References


