Loss of Uteroglobin Expression in Prostate Cancer: Relationship to Advancing Grade

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ABSTRACT

We have shown previously that the secretory protein uteroglobin (UG) is highly expressed in normal human prostate tissue but this expression is either lost or altered in human prostate cancer cell lines. Treatment of these cell lines with recombinant human UG inhibits their ability to invade human reconstituted basement membrane by up to 90%, implying that the loss of normal UG expression may be related to the invasive potential of prostate cancer. Because invasion represents a critical step in metastasis, the expression patterns of UG could provide a unique and relevant indicator of cancer progression. In this study, we present the immunohistochemical analyses of fresh frozen prostate tissues from surgical specimens taken from 50 patients.

Eight slides per patient were analyzed for UG staining. Slides from 26 patients showed evidence of prostate cancer, whereas slides from the remaining 24 patients showed only benign glands. The results demonstrate UG immunoreactivity in normal prostate, benign prostatic hyperplasia, and prostatic atrophy; low but clearly positive expression in prostatic intraepithelial neoplasia; positive expression in cancerous glands of Gleason's pattern ≤2; and complete loss of UG immunoreactivity in cancerous glands of Gleason's pattern 3 or greater. In addition, the one case of metastatic prostate cancer that we examined, the prostate cancer cells within the lymph node lacked UG expression. These findings suggest that the loss of UG expression may be an indicator of prostate cancer progression and possibly a component of the molecular natural history of prostate cancer, which may have prognostic value.

INTRODUCTION

Adenocarcinoma of the prostate is the most commonly diagnosed form of cancer among men in the United States today. The number of cases diagnosed in 1996 exceeded 260,000, and that figure is expected to climb to around 334,500 in 1997 (1). It is projected that about 41,800 of these diagnosed cancers will result in death due to metastatic progression. Genetic alterations such as the loss of heterozygosity at several loci, including 6p, 7q, 8q, 10q, 11p, 13q, 16q, 17q, and 18q (2–8), the loss of putative metastasis suppressor genes such as KAI-1 (9) and CD44 (10), as well as changes in cell adhesion mechanisms such as E-cadherin/α-catenin (11), are thought to contribute to this progression. However, despite the recent strides made in this area of study, it is generally accepted that the molecular alterations related to the malignant development of PC require better characterization.

One such alteration may involve a protein known as UG. UG is a low molecular weight, homodimeric secretory protein, also known as Clara cell 10 kDa (12) protein, which is found in abundance in human uterus, lung, and prostate (13–15). The UG locus has been localized to a single-copy gene on chromosome 11q23–13.1 (16). This chromosomal region is of interest in several hormonally regulated cancers (17) and has recently been identified as an additional site of loss of heterozygosity in PC (18). We have reported previously that UG expression is markedly decreased or absent in several PC cell lines and that recombinant human UG modulates prostate tumor cell invasiveness, possibly by a mechanism related to the inhibition of the actions of PLA2 in the production of eicosanoids (19, 20).

This study sought to determine the immunohistochemical expression of UG in normal human prostate, BPH, PIN, and PC tissue and to assess the possibility that alterations in UG expression may be associated with prostate cancer.

MATERIALS AND METHODS

Tissue Source. Informed consent was obtained to study tissue from 30 patients who underwent radical prostatectomy for PC, cystoprostatectomy for transitional cell carcinoma, or open prostatectomy for BPH. Because a random sampling of cancer patients was desired, there was no inclusion or exclusion criteria before acquisition of these tissues. It is noteworthy, however, that none of these patients had prior endocrine treatment. Approximately two longitudinal specimens, of 1-cm each, extending from base to apex were excised parallel to the urethra and flash frozen in liquid N2 and stored at −70°C. Lymphatic tissue containing PC was obtained from one laparoscopic pelvic lymph node dissection prior to a planned radical prostatectomy. Of 46 specimens resected for prostatic carcinoma, 26 contained carci-

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The abbreviations used are: PC, prostate cancer; UG, uteroglobin; PLA2, phospholipase A2; BPH, benign prostatic hyperplasia; PIN, prostatic intraepithelial neoplasia.
noma within the tissue samples obtained intraoperatively for UG determination. Tissue from the remaining 24 specimens contained benign prostatic tissue, prostatic atrophy, or fibromuscular tissue. The 26 cases with sampled carcinoma in the intraoperative sample had a similar histopathological tumor pattern to that present in the resected prostate. All 46 prostatectomy cases with preoperative diagnosis of PC contained carcinoma in the final surgical pathological review of the resected organ.

**Immunohistochemical Procedures.** Two hundred serial sections of 5 μm each were taken from each tissue and placed on silanated slides (two sections per slide). Every tenth slide was stained with hematoxylin and diagnosed by a pathologist to determine which slides contained both normal prostatic glands (as a positive control) as well as adjacent cancerous glands. The remaining slides were stored at −20°C until diagnosis was complete. Selected slides were immunostained as follows. Sections of remaining slides were stored at −20°C until diagnosis was complete. The presence of UG was detected using the ECL system (Dupont NEN, Boston MA). This method of detection was confirmed in histopathological examination of the surgical specimens. Of the 26 patients (15 Caucasians and 11 African-Americans) had no malignancy of the prostate and underwent surgery either for bladder carcinoma (1 Caucasian and 1 African-American) or for BPH (Table 1). These diagnoses were confirmed in histopathological examination of the surgical specimens. Of the 26 patients (15 Caucasians and 11 African-Americans) with carcinoma within the sections we examined, two had associated PIN. Two samples contained only fibromuscular tissue. The remaining 22 specimens tested contained only BPH or prostatic atrophy in the sample. However, the final surgical pathological diagnosis of these radical prostatectomies was adenocarcinoma with Gleason scores ranging from 4 to 10, with 16 patients determined to have moderately differentiated tumors (Gleason score from 4 to 6) and 30 patients with poorly differentiated tumors (Gleason score from 7 to 10). The latter included the patient with lymph node metastasis (Gleason 10).

A total of 400 slides from the 50 specimens was evaluated, and UG expression in each slide was scored by three investigators (A. T. W., J. A. C., and A. S.). The degree of UG expression was graded by assigning a value of 0 to an absence of stain and assigning a value of 3+ to the most positive stain. Slides that stain with intensity between these two extremes were accorded intermediate values. Glands showing an absence of stain were uniformly marked as 0 by all three investigators. Intermediate scores were usually identical and never differed by more than a single value. Two of three scorers were blinded as to the source of the tissue. Immunohistochemical stain was also evaluated and scored according to distribution as focal or diffuse. In cases of suspected minimal disease, tissue sections were obtained from sites adjacent to the localized disease and did not always contain tumor. Of the 50 patient samples examined, we found we were able to analyze slides with cancer from 26. Two of these latter slides contained only cancer, with no evidence of any normal glands, and one contained cancer and PIN with no evidence of any benign glands. Two samples we examined contained only fibromuscular tissue, which lacks staining for UG. Therefore, we found 45 samples that contained benign
Summary of patient characteristics according to age, race, and final pathological diagnosis as determined by surgical pathological examination of the resected organ. Also included in this table is a summary of the differing pathologies seen among the various fields of view in different patients at the time of slide analysis.

<table>
<thead>
<tr>
<th>Detail</th>
<th>n = 50</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Range 35–76 yr</td>
<td>28</td>
</tr>
<tr>
<td>Median</td>
<td>60.8 yr</td>
<td>22</td>
</tr>
<tr>
<td>Race</td>
<td>African-American</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>28</td>
</tr>
<tr>
<td>Final pathological diagnosis*</td>
<td>BPH</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Bladder carcinoma</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>PIN</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>PC Gleason’s grade 5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>PC Gleason’s grade 6</td>
<td>12</td>
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<tr>
<td></td>
<td>PC Gleason’s grade 7</td>
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<td></td>
<td>PC Gleason’s grade 8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>PC Gleason’s grade 9</td>
<td>7</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Slide diagnoses*</td>
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<tr>
<td></td>
<td>Prostatic atrophy</td>
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<tr>
<td></td>
<td>PIN</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>PC Gleason’s pattern ≥3</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>PC Gleason’s pattern ≤2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Fibromuscular tissue only</td>
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</tr>
</tbody>
</table>

* The two patients with bladder carcinoma had a final prostatic diagnosis of BPH. PIN was associated with PC in all five cases; thus n = PC + BPH = 50.

† Patterns of glands and cells in the field of view at the time of slide analysis. The numbers here represent n (patients) of a total of 50 in which these pathologies were observed.

‡ See footnote 4 in text.

tissue, of which 42 contained benign glandular hyperplasia and three had prostatic glandular atrophy. Because of the difficulty of both finding and diagnosing PIN on frozen sections, the number of specimens in which PIN was detected remained low. UG expression in benign tissue was predominantly diffuse and moderate in most cases. In 42 cases of histologically confirmed BPH, there was one that stained 3+, 25 that stained 2+, and 19 that stained 1+ for UG. In three cases of prostatic atrophy, two stained 2+ and one stained 1+. All were clearly positive (1+ or greater). The staining pattern was cytoplasmic and tended to be more pronounced at the luminal surface of the acinar cells (Fig. 1, A and B). Immunoreactivity was absent in sections of the prostate that lacked epithelial elements, such as fibromuscular tissue (Fig. 1A). The two cases of PIN examined for the expression of UG were both clearly positive and were scored as 1+ (Table 2, Fig. 1C).

Evaluation of slides containing PC contrasted sharply with normal, BPH, or PIN samples. Twenty of 26 specimens containing cancer did not stain for UG. Each of these cancerous glands was diagnosed as Gleason’s pattern* of 3 or above (Fig. 1, C and D). This absence of stain was consistent among all cancerous glands of this Gleason’s pattern. Five samples contained cancerous glands that were graded with a Gleason’s pattern 1 or 2, and these were positive for UG staining. One sample contained several glands of Gleason’s pattern 3/4 as well as glands that were Gleason’s pattern 2. The Gleason’s pattern 3/4 lacked staining for UG (Fig. 1E, left), whereas the Gleason’s pattern 2 and the normal glands within the sample stained positive for UG (Fig. 1E, right). The prostate tumor cells in the metastatic lymph node likewise registered no signal (Fig. 1F).

Western analysis was performed on prostate tissue from six of the patients in this study presenting with cancers of Gleason’s grade 8 or 9. The UG protein runs at around Mr, 10,000 on an SDS polyacrylamide gel (21). This protein was present in normal tissue lysates but absent in the lysates taken from cancerous tissue (Fig. 2).

**DISCUSSION**

We have previously reported our discovery of abundant expression of UG in the epithelial cells of the human prostate gland (15). In that preliminary description, there was no analysis of the comparative expression of uteroglobin in tissue sections exhibiting different pathological conditions. This study examines UG expression in fresh frozen cellular fields of BPH, PIN, and prostate cancer. We report that UG was absent from prostatic stromal tissue but was clearly expressed in normal glandular epithelial cells, in glandular areas exhibiting BPH and prostatic atrophy, in acinar glands identified as PIN, and in cancerous glands of Gleason’s pattern 2 or lower. In contrast, UG expression in glands of Gleason’s pattern 3 or higher was absent. In addition, in Western analysis of six patient samples of Gleason’s grade 8 or higher, the expression of the UG protein is undetectable as compared to normal tissue. This observation links the loss of UG expression with the pathological pattern of prostatic neoplasia.

The loss of normal UG expression in fresh frozen tissue samples of PC shown here corroborates our observations of human prostatic tumor cell lines derived from metastases (19, 20). The cell lines DU145, TSUp1, and PC-3 were analyzed for UG mRNA expression by Northern blotting and compared with normal tissue. Normal prostatic cells expressed an abundant 600-bp mRNA transcript, whereas UG expression in the cell lines DU145 and PC-3 was absent. In TSUp1 cells, an overexpressed but aberrantly processed (>10-kilobase pair) transcript was detected (20).

The absence of UG expression in prostatic tumor cells is also consistent with reports on the expression of the Clara Cell 10 kDa protein (CC10) gene, which is believed to be UG (21), in lung tissue. Broers et al. (14) and Linnoila et al. (22) showed that CC10 mRNA was abundantly expressed in normal bronchial tissue but was detectable in only 1 of 19 non-small cell lung carcinoma specimens. A large percentage of non-small cell lung carcinoma (primarily adenocarcinomas) exhibit ultrastructural and morphological characteristics indicative of peripheral airway cell origin (22). Because CC10 was a strong positive marker for normal peripheral airway cells, it is likely that the low percentage of CC10-positive tumors was due to the loss of CC10 expression during tumorigenesis. Sandmoller et al. (23) also supported this observation by fusing the 5′ flanking region

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* The Gleason’s number referred to here is that of the particular glands within the field of view at the time of slide diagnosis and refers to the pattern of the individual glands as opposed to the overall Gleason’s grade of the patient.
of the UG gene to SV40 T antigen to target SV-40-induced tumorigenesis to tissues that normally express abundant endogenous UG such as lung, salivary gland, stomach, and prostate. Endogenous production of UG in SV-40 T antigen-transformed cells was clearly detectable during the early stages of tumorigenesis but was undetectable in later stages. This suggests that the UG gene may be transcriptionally silenced as a tumor progresses past its earliest stages.

The significance of the loss of UG expression in tumor cells is presently unknown. During development and in the adult genitourinary tract, its expression is a function of hormone receptor expression and hormonal status. Therefore, it is possi-
able that the loss of UG expression may be an indicator of the stage of differentiation of an evolving tumor. On the other hand, there is a growing body of evidence that indicates that loss of UG expression may be a functional component in the molecular natural history of prostate cancer. We have reported that treatment of human prostatic tumor cell lines (which lack normal endogenous UG) with submicromolar nontoxic doses of recombinant human UG results in inhibition (by up to 90%) of the ability of those cell lines to invade through reconstituted basement membrane in response to fibroblast-conditioned medium (19). The ability of UG to inhibit invasiveness suggests that the loss of normal UG expression may correlate with the acquisition of metastatic potential because invasion of the local tissue matrix is a necessary component of metastasis. Furthermore, the genetic or pharmacological reconstitution of UG to UG-deficient cells may have therapeutic value in suppressing metastasis. Experiments to test this hypothesis are in progress.

Although the existence of UG has been known for almost 30 years (24), its physiological function and mechanism of action remains unknown. We have found that recombinant UG inhibited fibroblast-conditioned medium-stimulated tumor cell invasion but did not affect simple cell motility. Therefore, it is possible that the ability of UG to interfere with cell motility may be cell type specific or may itself be altered as a function of tumorigenicity. It has also been shown that UG noncompetitively inhibits the activity of PLA2 in vitro (25, 26), which may account for the potential anti-inflammatory activity of UG, but it is not known whether this inhibition occurs in vivo. We have shown that treatment of prostate tumor cell lines with recombinant UG inhibits the biphasic release of arachidonic acid that was also induced by fibroblast conditioned medium (19). This may be due to inhibition of cell-membrane associated PLA2 activity, but presently the relationship between the inhibition of arachidonic acid release and the inhibition of invasion is merely correlative. It remains to be seen whether either intracellular or extracellular metabolites of arachidonic acid are the proximate activators of invasive motility in these cells, or whether, alternatively, UG acts through a specific receptor and signaling mechanism to regulate genes governing invasive motility. An alternate mechanism is suggested by the observation by Zhang et al. (27) that UG binds to fibronectin. Thus, it is possible that UG binding to fibronectin may also modulate the role of fibronectin in cell-matrix attachment and invasive motility.

With an aging population, PC will continue to have an impact on health care well into the next century. Over 50% of patients undergoing radical prostatectomy have locally advanced disease, and 25% of these will eventually exhibit disease progression. Clearly, there is a need to develop prognostic markers that will help predict individual outcome and thereby direct appropriate therapy. If UG proves to be an autocrine/paracrine inhibitor of localized cellular invasiveness, then the degree of loss of UG expression in biopsy specimens may be predictive of increased potential for metastasis. In contrast, positive expression of UG, such as we observed in PIN, may indicate an earlier stage in the continuum from neoplastic change to wide dissemination. Differential UG expression by PIN may distinguish those cases with a predilection for invasion from those with a less aggressive course. This hypothesis is presently being tested by expanding this study to include archived specimens, where PIN can be readily selected from among paraffin sections used for pathological diagnosis.

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REFERENCES


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*Fig. 2. UG expression in human prostate tissue as determined by Western analysis. The UG protein runs as a Mr 10,000 band on a 15% SDS-polyacrylamide gel (N). In five patient samples of Gleason’s grade 9 (G9) and one of Gleason’s grade 8 (G8), there is no detectable UG expression.*

Table 2 Staining for UG expression

<table>
<thead>
<tr>
<th>Staining Pattern</th>
<th>Low Positive</th>
<th>Moderate Positive</th>
<th>Strong Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign (0)</td>
<td>19</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>PIN</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer (≤2)</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cancer (≥3)</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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*N. A. Weeraratna, J. Leyton, M. J. Manyak, and S. R. Paterno, manuscript in preparation.*


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