Intracellular Levels of SGP-2 (Clusterin) Correlate with Tumor Grade in Prostate Cancer

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ABSTRACT

Our previous observations in LNCaP cells in vitro demonstrated an association between apoptotic cell death resistance and SGP-2 (Clusterin) overexpression. Accordingly, we hypothesized that high levels of cellular SGP-2 would aid in identifying biologically aggressive prostate cancer cells with unique survival advantages. To test this hypothesis, 40 archival radical prostatectomy and/or biopsy specimens of varying grades of prostate cancer were subjected to immunohistochemical SGP-2 staining. The resulting epithelial stains were quantified subjectively on a scale of 1–3 by four independent observers. Benign prostatic epithelial cells from young donors served as controls and showed a consistently weak staining intensity. In contrast, prostate cancer specimens showed varying degrees of staining intensity that correlated with a Gleason pattern (P = 0.006). This correlation supports the hypothesis that protection from apoptotic death may account, in part, for biologically aggressive tumor behavior.

INTRODUCTION

Here, we describe the biological significance of SGP-2 (Clusterin) in prostate cancer progression. SGP-2 has been identified as an inhibitor of apoptosis (1). It is a heterodimeric glycoprotein that was first isolated from ram rete testis, and it was so named for its ability to cluster Sertoli cells (2). This protein has been found in a multitude of organisms and is highly conserved. In the human genome, it exists as a single copy, located on chromosome 8 (3). It is present in virtually all body fluids and is expressed by cells lining body cavities. SGP-2 has a multitude of proposed functions, including roles in tissue remodeling, reproduction, lipid transport, complement regulation, and apoptosis (4). In models of apoptosis, SGP-2 expression is markedly elevated (5). Despite the original notion that SGP-2 is merely a marker of programmed cell death, results of our previous studies have suggested a protective role of SGP-2 in apoptosis (1). The protective effect of this protein is not limited to prostate tissue but has been shown in other pathological conditions involving apoptosis, including kidney injury, myocardial infarction, and the negative selection of thymocytes (6–8). In these systems, SGP-2 is associated with surviving cells, not with those undergoing apoptosis. It has been hypothesized that its function is mediated through the stabilization of cell membranes (9, 10).

This study was undertaken to determine whether there is a difference in SGP-2 expression between low- and high-grade prostate carcinomas, as classified by their Gleason pattern. Prostate cancer has a low mitotic rate, compared to those of other malignancies (11, 12). In addition, the apoptotic rate in prostate cancer is significantly lower than the apoptotic index of normal prostate tissue (13). Consequently, the overall growth of prostate adenocarcinoma is, at least in part, determined by its rate of apoptosis. If SGP-2 protects cells from apoptosis, we can expect those cells that express higher levels of this protein to be relatively resistant to programmed cell death. In prostate cancer, those tumors with a higher Gleason score tend to act in a more aggressive manner. Therefore, if a positive correlation is found between levels of SGP-2 and Gleason pattern, then SGP-2 might, at least in part, be a determining factor of this phenotypic trait and may prove useful in the diagnosis and treatment of this disease.

MATERIALS AND METHODS

Prostate Tissue Specimens. Prostate tissue, which was obtained from the archival tissue bank in the Department of Pathology, Northwestern Memorial Hospital, was used in accordance with the approved protocol of the Institutional Review Board of Northwestern University Medical School. All tissues were from either prostate needle biopsy or radical prostatectomy specimens that were stored in paraffin blocks. Specimens were chosen so as to represent the full range of Gleason scores. None of these specimens came from patients who had had any preoperative hormonal ablative therapy in an attempt to down-stage their tumor or to control their obstructive symptoms. Forty archival samples were screened for prostate cancer, revealing 60 distinct regions of frank adenocarcinoma, 17 regions of benign prostatic hyperplasia, and 8 regions of prostatic intraepithelial neoplasia.

Immunocytochemical Staining for SGP-2. All pathological specimens were reviewed by one of us (R. O.), and Gleason scores were assigned. If a given specimen contained tumors of more than one grade, then each area was considered separately. Immunocytochemical staining was conducted using

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Clusterin and Prostate Cancer

Table 1  SGP-2 immunostaining intensity in prostate tumor specimens

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<th>Intensity</th>
<th>Gleason’s score</th>
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<tr>
<td></td>
<td>2 (n = 9)</td>
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<tr>
<td>+</td>
<td>33%</td>
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<tr>
<td>++</td>
<td>56%</td>
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<tr>
<td>+++</td>
<td>11%</td>
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a commercial kit (ABC kit; Vector Laboratories, Burlingame, CA). Normal goat serum was used to block the nonspecific binding sites on the slides, which were then incubated in a humidified chamber for 18 h at 4°C with a 1:1000 dilution (10 μg/ml) of an IgG antibody to SGP-2 (Quidel, San Diego, CA). The antigen was visualized by subsequent incubation with biotinylated secondary antibody and an avidin-biotin-horseradish peroxidase complex and a 4-min incubation in diaminobenzidine tetrahydrochloride before counterstaining with Gill’s hematoxylin.

Negative control slides were processed in an identical manner, with substitution of the primary antiserum with nonimmune rabbit IgG at the same concentration as that used for the primary antiserum. No color reaction was observed in any negative control slide.

Photomicrographs were taken through an Olympus Vanox AHBS Research Microscope (Olympus Corp., Woodbury, NJ) using Kodak Daylight 100 Ektachrome Professional film (Eastman Kodak, Rochester, NY).

Staining Intensity. The staining intensity of each slide was reviewed by four independent evaluators (J. M. K., J. A. S., S. S., and S. P.), two of whom had no knowledge regarding Gleason’s scores. Results were scored as “+,” “++,” and “+++,” representing mild, moderate, and heavy staining, respectively. All comparisons of staining intensity were made at X40 magnification. The average pattern of the four viewers for each specimen was then be used in the final statistical analysis.

Statistics. Once a staining pattern was assigned to each specimen, specimens could then be grouped according to grade and staining intensity. The Fisher exact test was used to determine whether or not staining intensity increased with Gleason’s grade. A P of less than 0.05 was considered statistically significant.

RESULTS

Staining Intensity of SGP-2 in Malignant Prostate Tissues. To determine whether or not SGP-2 expression increased with Gleason grade, samples containing frank adenocarcinoma were stained for SGP-2 expression by immunohistochemistry. Staining for SGP-2 was found in the cytoplasm of luminal epithelial cells. No nuclear staining was noted in this study. When observed under higher magnification, the cytoplasmic staining was noted to have a granular pattern, suggesting that SGP-2 may be contained within secretory vesicles. There was a wide variation in staining intensity among the specimens; however, there was a significant positive correlation between SGP-2 staining intensity in carcinoma cells and increases in Gleason’s grade (Table 1: Figs. 1 and 2). Further-more, when a carcinoma demonstrated more than one histological pattern, the staining intensity of each of these areas differed significantly (Fig. 3).

Staining Intensity of SGP-2 in Normal Prostatic Tissue. Prostatic tissues derived from two organ donors (ages 13 and 18) were used for comparison with pathological specimens. All specimens stained very lightly and were uniformly given a + score (Fig. 4).

Staining Intensity of SGP-2 in Benign Prostatic Hyperplasia. Tissue with benign prostatic hyperplasia was obtained from benign areas present in prostates removed for carcinoma as well as from benign glands removed solely for obstructive symptoms. The staining intensity of this group was variable, with the resulting divisions being 35% +, 59% ++, and 6% +++ (Fig. 4B).

Staining Intensity of SGP-2 in Prostatic Intraepithelial Neoplasia. Foci of prostatic intraepithelial neoplasia were selected from specimens with frank carcinoma. The staining intensity varied, with the resulting divisions being 12.5% +, 37.5% ++, and 50% +++ (Fig. 4C).

DISCUSSION

Results of the present study demonstrated that SGP-2 is expressed in benign and malignant human prostate glands. As the malignant specimens demonstrated more aggressive histological patterns, their staining intensity for SGP-2 increased. Furthermore, the benign specimens showed a faint but consistently positive stain for SGP-2. The persistence of this protein in benign tissues lends credence to the hypothesis that SGP-2 not only protects from apoptotic death in malignant tissues but is also an important physiological protein needed for the mainte-

Fig. 1  SGP-2 immunointensity in varying grades of prostate cancer. Cytoplasmic staining was compared by using the mean results of four different reviewers. A total of 60 cancer specimens were evaluated. The staining intensities of the various grades differed significantly using the Fisher exact test (P = 0.006). □: +; □: ++; ■: +++.
Fig. 2 Immunostaining of SGP-2 in various malignant prostate specimens. A, negative control of moderate grade tumor using sheep IgG. No staining was noted. B, immunostaining of a typical low-grade tumor; this is representative of a + intensity. C, immunostaining of a moderate-grade tumor; this is representative of a ++ intensity. D, immunostaining of a high-grade tumor; this is representative of a +++ intensity. All specimens were compared at ×40 magnification.

Fig. 3 Differential immunostaining intensities of SGP-2 within the same specimen. A, benign tissue showing a staining intensity of +. B, high tumor grade showing an intensity of ++++. Magnification, ×40.

Because it has been previously shown that SGP-2 protects cells from apoptotic death (1, 6–8), increased expression of this protein may impart a survival advantage to a given cell or tissue. Therefore, SGP-2 could be an important factor in determining the aggressive nature of a given prostatic tumor.

To date, increased expression of SGP-2 has been described in four tumor systems. These include human renal carcinoma, human glioma, carcinogen-induced rat prostate carcinoma, and the Shionogi rat mammary carcinoma model (14–17). In these systems, malignant tissues showed higher levels of SGP-2 than did normal controls. In the Shionogi model, not only is there increased expression of this protein compared to that in normal tissues, but persistent high levels of SGP-2 are associated with the progression to an apoptotic-resistant and androgen-independent phenotype (18). To our knowledge, the present study is the...
first report of SGP-2 expression in human prostatic adenocarcinoma.

Here, we have observed that SGP-2 was localized to the cytoplasm of prostate cancer cells. None of our specimens showed any localization of SGP-2 to the nucleus. Using the Shionogi carcinoma model, both cytoplasmic and nuclear staining for SGP-2 were noted. However, nuclear staining was only found in androgen-independent cells (15). A nuclear form of SGP-2 has recently been identified in two epithelial cell lines (19). Whether or not this phenomenon occurs in human prostate cancer remains to be seen.

Although the mechanism of its action remains unclear, it is postulated that SGP-2 is required for cell survival. Prolonged depletion of intracellular SGP-2 will result in apoptosis. Our previous observations showed levels of SGP-2 rise in response to TNF-α (an apoptotic inducer) in the LNCaP cell model. These same cells were found to undergo apoptosis only after depletion of this protein occurred (1). Because similar kinetics of SGP-2 expression were observed in the rat prostate following castration (4, 20), it is proposed that SGP-2 has a similar role in normal prostate tissue as well. Results of the present study indicate that malignant epithelial cells contain more SGP-2 than do benign cells. This suggests that tumor cells will require a larger stress to induce apoptosis than their benign counterparts. This difference in apoptotic threshold may provide these malignant cells with an added survival advantage compared to benign prostatic epithelial cells.

In addition to SGP-2, the Bcl-2 family of proteins is also known to inhibit apoptosis (21–24). However, these proteins differ from SGP-2 in their distribution and possibly in their functional mechanisms. To date, of the apoptotic inhibitory proteins, only Bcl-2, Bcl-λ, and Mcl-1 have been correlated to Gleason pattern (25). Although this correlation has not consistently reached significance in the literature, increased expression of these proteins has consistently correlated with hormone refractory tumors (26, 27). Expression of antiapoptotic proteins appears to be a common feature of aggressive prostate cancers.

In conclusion, these results suggest a positive correlation between staining intensity of SGP-2 by immunohistochemical analysis and Gleason pattern in prostate cancer. If increased levels of this protein protect from apoptosis in a given tumor, then those cells that express SGP-2 at high levels may have a survival advantage. This advantage can, in part, account for their aggressive nature. As we found variability in staining intensity among tumors of the same Gleason pattern, SGP-2 expression may prove to be a prognostic indicator independent of grade. Therefore, SGP-2 may play an important role in determining the phenotypic aggressiveness of prostate cancer. It may ultimately prove to be of prognostic and therapeutic value in the future.

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