Advances in Brief

Expression of Sulfated Glycoprotein 2 Is Associated with Carcinogenesis Induced by N-Nitroso-N-methylurea in Rat Prostate and Seminal Vesicle

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Abstract

To understand the molecular mechanism of carcinogenesis in androgen-dependent tumors, we have searched for new markers which are associated with this process. In normal rat prostate and seminal vesicle, sulfated glycoprotein 2 (SGP-2) messenger RNA is barely detectable. However, we have found high levels of SGP-2 expression in the epithelial component of carcinomas of the prostate and seminal vesicle after initiation with N-nitroso-N-methylurea and promotion with testosterone propionate. We have also observed induction of SGP-2 expression in epithelial cells at early stages in carcinogenesis when cytologically malignant cells first begin to appear. SGP-2 has been reported previously to be associated with a variety of models of programmed cell death (apoptosis), including the prostate following castration. Our present findings provide a novel marker for carcinogenesis in the rat prostate and seminal vesicle.

Introduction

Although prostate cancer is the second leading cause of human male cancer death in the United States, little is known about the molecular mechanisms of prostate carcinogenesis. Toward this end, we have been searching for new molecular markers. We have used the Lobund-Wistar rat model in which carcinomas of prostate and seminal vesicle were induced by NMU[2] and TP (1, 2). After screening various molecules, we have found that SGP-2 is expressed at high levels in carcinomas of rat prostate and seminal vesicle.

SGP-2 is referred to under a variety of names: testosterone repressed prostate message 2 (3–5); clusterin (6); complement cytolysis inhibitor (7); SP40-40 (8); and apolipoprotein J (9). This protein is known to be a marker of programmed cell death (apoptosis) and is thought to be involved in the onset of apoptosis (3, 4, 10). Little is known about the precise function of SGP-2 and there are diverse observations regarding the function of SGP-2 in cell death. SGP-2 expression is induced in a variety of models of apoptosis: the prostate after castration (3, 4); the kidney after chronic obstruction of the ureter (4); and the interdigital regions of developing embryos (4). However, SGP-2 has functions which are not associated with apoptosis; it is a major protein which is secreted from Sertoli cells in the testis (11). It is also known that SGP-2 blocks complement mediated cytolysis (8). Thus, it appears that SGP-2 is involved in both cell death and cell survival. Study of the role of SGP-2 in carcinogenesis may provide a clue to the paradox between the above mentioned diverse observations. This is the first report showing that SGP-2 is specifically expressed in malignant epithelium from early to late stages during carcinogenesis in rat prostate and seminal vesicle.

Materials and Methods

Tissue Samples. Three-month-old male Lobund-Wistar rats were given i.v. injections once of NMU (30 mg/kg) and implanted s.c. with testosterone propionate (40 mg) every 2–3 months as described previously (1, 2). Prostate and seminal vesicle were evaluated 5–11 months after NMU injection. Samples for *in situ* hybridization were fixed with 4% paraformaldehyde or 10% formaldehyde, processed for routine histology, embedded in paraffin, and stored at 4°C. Samples for Northern analysis were immediately frozen and stored in liquid nitrogen.

Involuting prostate tissues were used as a positive control for the *in situ* hybridization studies of SGP-2 expression. Untreated normal prostate tissues were also used as a control reference for SGP-2 expression. Both of these tissues were obtained from 3-month-old male Lobund-Wistar rats before or 5 days after castration.

SGP-2 Probes. Complementary DNA was generated from total RNA of the ventral prostate of a Wistar male rat 5 days after castration. Using this complementary DNA as a template, a fragment of rat SGP-2 corresponding to nucleotides 638–1026 was amplified by the polymerase chain reaction. The fragment was cloned into pBluescript KS2 (Stratagene, La Jolla, CA). DNA sequencing revealed that this fragment had the same sequence as previously published (11).

In Situ **Hybridization.** *In situ* hybridizations were performed essentially as described previously (12, 13). ^{35}S-Labeled riboprobes were generated to a specific activity of 10^9 dpm/μg using the T3/T7 *in vitro* transcription system (Stratagene; pBluescript 2). Probes were digested to an average length of 100
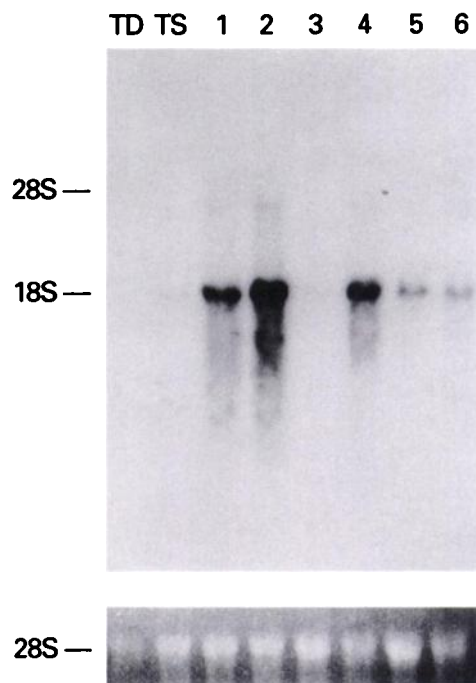

Fig. 1. Northern blot analysis of SGP-2 expression in grossly visible tumors of rat prostate and seminal vesicle. *TD*, dorsal prostate of a TP control rat; *TS*, seminal vesicle of a TP control rat; *Lanes 1–6*, macroscopically visible tumors of rat prostate and seminal vesicle. Significant expression of SGP-2 is seen in 5 of the 6 tumor samples. Each *lane* contains 20 μg of total RNA. *Bottom*, 28S rRNA stained with ethidium bromide.
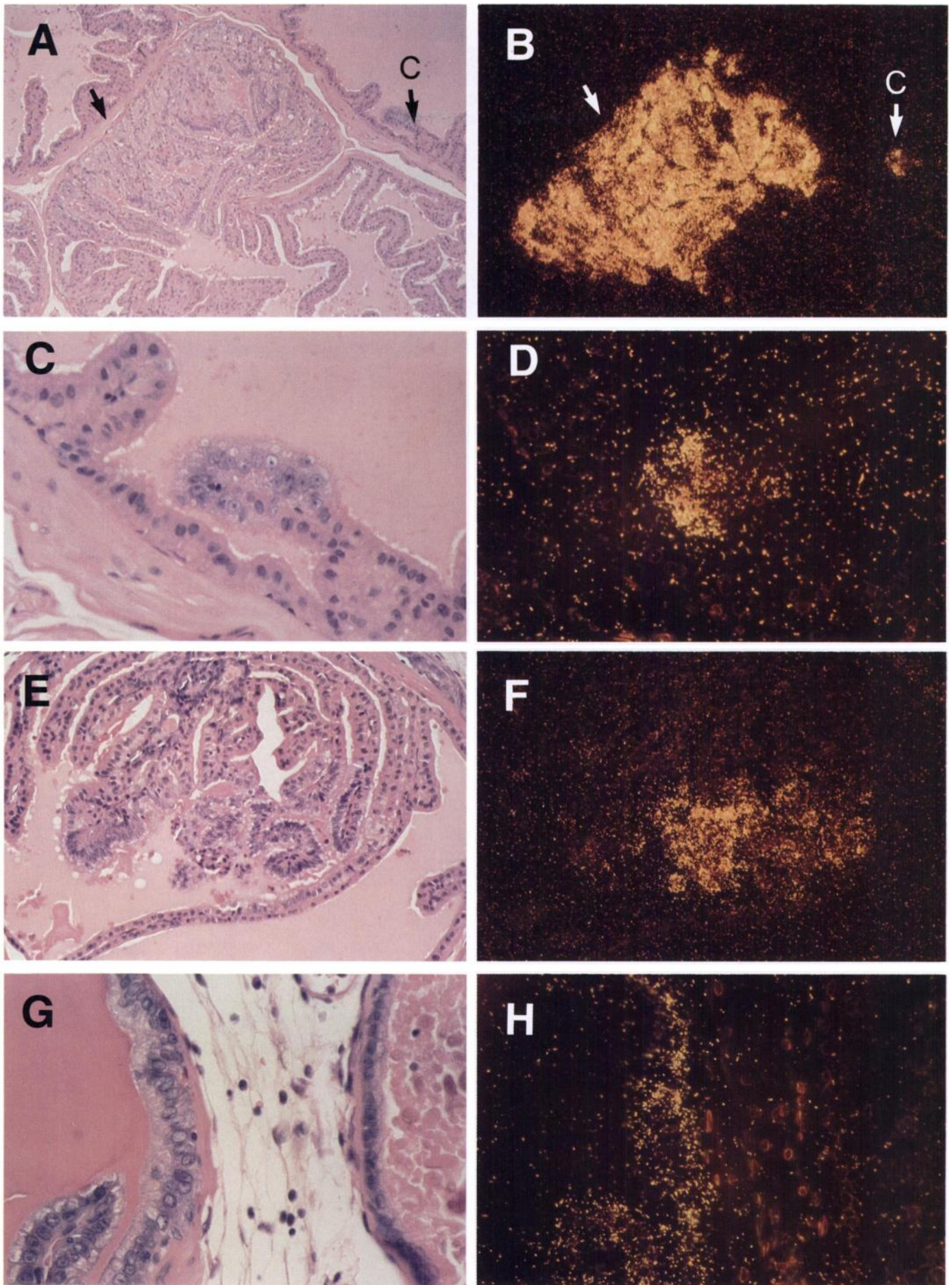
Fig. 2. SGP-2 at early stages in carcinogenesis in rat prostate, showing specific expression of SGP-2 mRNA in cytologically malignant cells but not in surrounding normal cells at early stages in carcinogenesis. Cytologically malignant cells occupy a part of a lobule of the anterior prostate (*A–F*). There are two clusters of cytologically malignant cells in *A* and *B* (*arrows*). *C* and *D*, higher magnification of *A* and *B* (*Arrow c*). In *E* and *F*, cytologically malignant cells accumulate at the tips of the foldings. *G* and *H*, two lobules of dorsolateral prostate; one contains cytologically malignant cells (*left*) and the other one has only normal epithelium. Each bright field picture shows H & E stain of a section adjacent to one used for *in situ* hybridization (dark field). *A, B,* × 100; *C, D, G, H,* × 400; *E, F,* × 200.
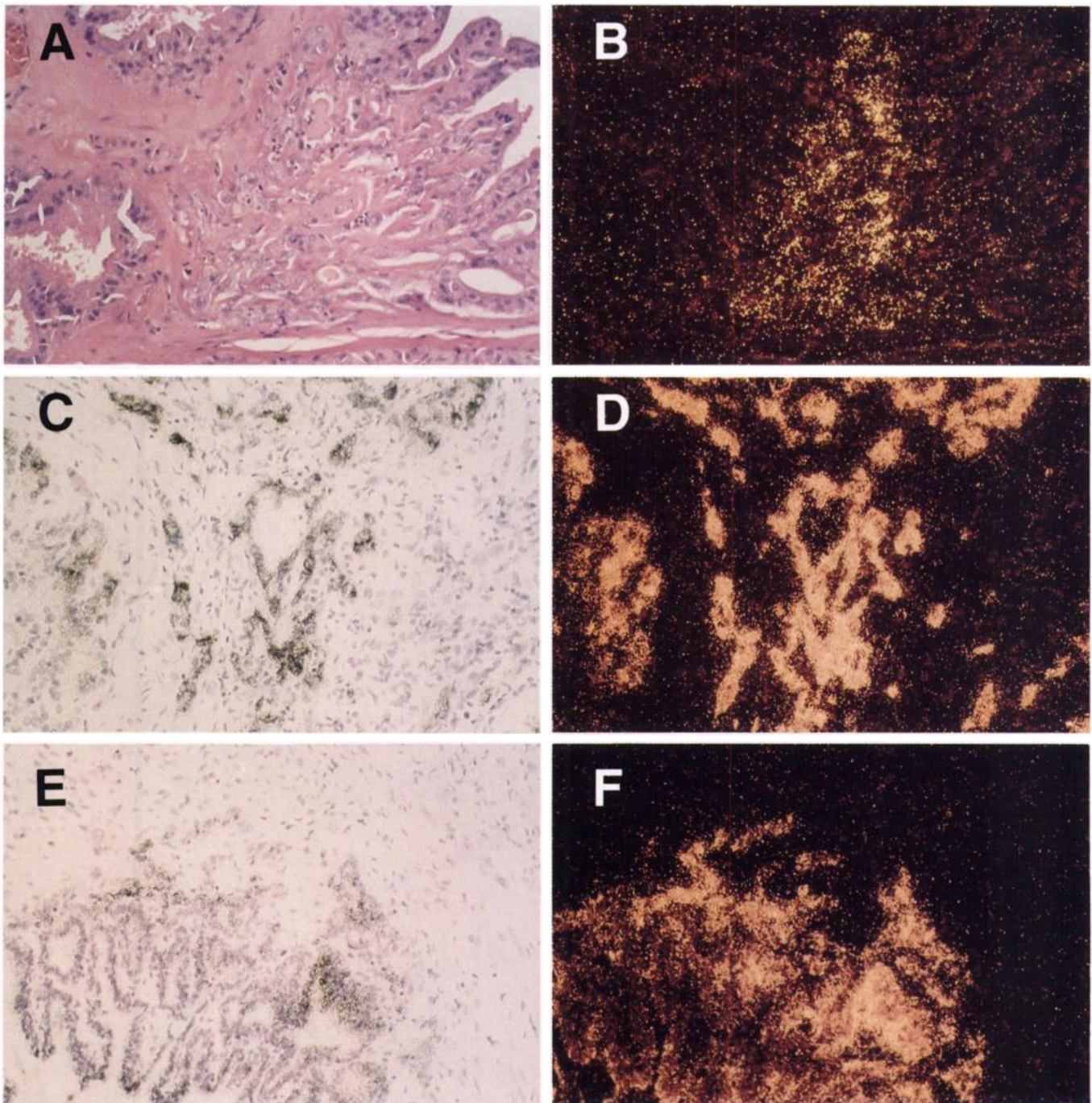
Fig. 3. SGP-2 at later stages in carcinogenesis in rat prostate and seminal vesicle, showing that expression is restricted to the epithelial component of carcinomas. Early invasive lesion in the anterior prostate (A and B). Marked invasion of the underlying stroma in the anterior prostate is shown in C and D; invasion of stroma of the seminal vesicle is shown in E and F. B, D, and F are dark field photomicrographs, while C and E are bright field photomicrographs of in situ hybridizations. A and B are taken from adjacent sections in the paraffin block; C and D, as well as E and F, are taken from the same sections, respectively. A, H & E; C and E, hematoxylin alone. × 200.

bases by controlled alkaline hydrolysis and used at a final concentration of 4 × 10⁴ cpm/μl. Exposure time (Amersham LM-1 emulsion) was 4–7 days.

Northern Blot Analysis. RNA extraction and Northern blotting were performed as described previously except that nylon membranes were used instead of nitrocellulose membranes (14).

Results

We performed Northern blot analysis to screen a variety of molecules as markers of carcinogenesis in rat prostate and seminal vesicles. We used two control groups; one was treated by saline injection and implanted with testosterone propionate (TP control); the other was treated by saline injection only (saline control). Expression of various molecules was compared in control samples and in macroscopically

visible tumors which were induced by NMU and promoted by TP. Fig. 1 shows that SGP-2 expression is highly induced in 5 of 6 tumor samples, whereas the prostate and seminal vesicle of TP controls express little SGP-2. The tissues of saline controls also express negligible SGP-2 (data not shown).

The Northern blot analysis data encouraged us to examine SGP-2 mRNA by in situ hybridization to evaluate the specific cellular pattern of SGP-2 expression in carcinogenesis. Because SGP-2 expression is known to be induced in rat ventral prostate after castration, we used ventral prostate tissues before and 5 days after castration to check our in situ hybridization technique. We observed a specific expression pattern of SGP-2 which was restricted to epithelium 5 days after

castration (data not shown), in accord with a previous report (4). We then examined SGP-2 gene expression in carcinomas of rat prostate and seminal vesicles and found that SGP-2 expression was spatially and temporally controlled.

Histopathological diagnosis was performed independently by two pathologists. Since macroscopically visible tumors were usually observed approximately 10 months after NMU treatment, we decided to collect tissue samples 5–10 months after NMU treatment and step section the paraffin blocks at 250-μm intervals in order to detect early stages in carcinogenesis. Initially, cytologically malignant cells which have vesicular nuclei, prominent nucleoli, basophilic cytoplasm and loss of polarity are observed as *in situ* lesions. Fig. 2 shows early malignant lesions, each of which occupies only a part of a lobule in the anterior prostate. SGP-2 is specifically expressed only in cytologically malignant cells but not in surrounding normal cells. The same expression pattern is observed in early lesions of the dorsolateral prostate (Fig. 2, G and H).

In later stages, malignant cells occupy a whole lobule and destroy its structure as invasive lesions progress. Eventually malignant cells induce a stromal reaction and form irregular glands. In Fig. 3, *A* and *B*, in a tiny invasive lesion in the anterior prostate, only the invading malignant epithelial cells express SGP-2. The specimen shown in Fig. 3, *C* and *D*, was obtained from a macroscopically visible tumor of the anterior prostate. SGP-2 is again expressed in malignant epithelial cells which form irregular glands but not in the stromal component. Fig. 3, *E* and *F*, shows a lesion of the seminal vesicle in which malignant epithelial cells start to invade into the underlying stroma. SGP-2 expression is observed only in the malignant epithelial component. We have examined, thus far, 8 malignant lesions in anterior prostate, 3 in dorsolateral prostate, and 14 in seminal vesicle at various stages in carcinogenesis, and all of the lesions showed a similar expression pattern of SGP-2 as demonstrated in Figs. 2 and 3. No specific expression was observed in normal rat prostate and seminal vesicles (data not shown).

Discussion

PAP and PSA have been used as markers for human prostate carcinoma. However, PSA and PAP are expressed strongly in normal prostate: the secretory cells lining the prostatic acini; prostatic ducts; and the prostatic urethra (15). Thus, the greatest value of PAP and PSA is identification of prostatic tumors (especially poorly differentiated or undifferentiated tumors) in the bladder neck or in metastatic lesions (16). There has been great need for a marker that is truly specific for prostate carcinoma. Without such a marker, we cannot monitor prostate carcinogenesis in human as well as animal models. We show in this paper that SGP-2 expression is closely associated with carcinogenesis in rat prostate and seminal vesicle. It should be stressed that SGP-2 expression distinguishes cytologically malignant cells or premalignant lesions from normal cells at early stages in carcinogenesis. The present data provide a novel marker for NMU-induced carcinogenesis in rat prostate and seminal vesicle. We are currently studying SGP-2 expression in human prostate cancer. We have previously reported a K-*ras* mutation at codon 12 in tumors of the prostate and seminal vesicle in the Lobund-Wistar rat model (17). The combination of K-*ras* mutation and SGP-2 expression will enable us to study more precisely the process of rat prostate carcinogenesis in this model.

Because the product of the SGP-2 gene is a secretory protein, there is potential to monitor prostate carcinogenesis by measuring serum SGP-2 levels.

SGP-2 may be involved in carcinogenesis not only in the prostate but also in other organs. High level expression of SGP-2 was recently reported in human gliomas (18). Although SGP-2 has previously been reported to be associated with a variety of models of apoptosis (3, 4, 10), further studies are needed to elucidate the role of SGP-2 in carcinogenesis.

Acknowledgments

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