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Short Communication

Selective Expression of Murine Prostate Stem Cell Antigen in Fetal and Adult Tissues and the Transgenic Adenocarcinoma of the Mouse Prostate Model of Prostate Carcinogenesis

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Prostate stem cell antigen (PSCA) is a GPI-anchored membrane protein whose expression is reportedly up-regulated in a majority of human prostate cancers, including advanced stages and metastases. In this study, we investigate the expression pattern of the murine orthologue of PSCA by *in situ* hybridization in fetal and adult mouse tissues. Murine PSCA is expressed during fetal development in the urogenital sinus, skin, and gastrointestinal tract. The expression in these tissues is restricted to the most superficial cell layer. In the adult mouse, expression is highest in the mucosal lining of the urinary tract. In the normal adult prostate, expression of PSCA is detected exclusively in the secretory epithelium. Examination of PSCA during carcinogenesis of the murine prostate in the transgenic adenocarcinoma of the mouse prostate model showed a markedly increased expression in areas of neoplasia. The transgenic adenocarcinoma of the mouse prostate model may represent a valuable model for the study of PSCA as a potential target for immunotherapy of prostate cancer, despite potential differences in the pattern of expression between mice and humans. ***(Am J Pathol 2001, 158:809–816)***

In the United States, prostate cancer is the most commonly diagnosed nondermatological malignancy in males and the second leading cause of cancer-related death. Although surgical or radiation therapy significantly improves survival of patients with early stages of the disease, the therapeutic options are very limited for advanced cases, particularly for tumor recurrences after hormone ablation.[1–3] A confounding challenge in the development of new therapeutic modalities for prostate cancer is the paucity of appropriate *in vivo* models.

Targeted therapy for prostate cancer is a highly pursued avenue of cancer research with the goal to identify proteins that either show a tissue- or tumor-specific pattern of expression. Prostate stem cell antigen (PSCA) may represent such a protein because it has been reported to be highly expressed in a large percentage of human prostate tumors, including advanced and metastatic cases.[4,5] Structurally, PSCA is a GPI-linked membrane glycoprotein with homology to members of the Thy-1/Ly-6 family of proteins. It is most closely related to Sca2, a cell surface marker for immature lymphocytes.[6] Expression of human PSCA RNA in normal tissues was originally described not only as tissue-specific, but essentially limited to prostatic basal cells. It was inferred from this latter observation and from the structural homology to Sca2 that PSCA may represent a stem cell antigen.

Animal models are of crucial importance for understanding pathogenetic mechanisms and for the development of novel therapeutics. Thus, it is critical to analyze the expression of the murine orthologues of human genes of interest for cancer therapy. Murine and human PSCA are 70% identical at the nucleotide and the amino acid level.[5] Although there are significant anatomical differences in the mouse prostate compared to that of the human there are inherent common characteristics including secretory function and hormonal regulation.[5] One well-established and characterized animal model for human prostate cancer is the transgenic adenocarcinoma of the mouse prostate (TRAMP).[7,8] In the TRAMP model, expression of the SV40 large T antigen is targeted to the prostatic epithelium by the minimal promoter of the rat probasin gene. All male TRAMP mice progress to prostatic intraepithelial neoplasia (PIN) or well-differentiated

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adenocarcinoma of the prostate usually by 8 to 12 weeks of age.[8] The autochthonous TRAMP model is particularly relevant to human prostate carcinoma in that the development of the cancer is specific to the prostatic epithelium and is initially regulated by androgens. Furthermore, metastases to distant sites eventually occur.[9,10]

Here we report the pattern of expression of murine PSCA in fetal and adult tissues, as well as in a murine model for human prostate adenocarcinoma using a sensitive and stringent *in situ* hybridization technique. Our results suggest that the pattern of expression of murine PSCA in normal tissues and in tissues of murine prostatic adenocarcinoma is different from published data on human normal prostate, normal extra-prostatic tissues, and prostatic adenocarcinomas. The potential benefits of a mouse model for the evaluation of therapeutic targets for prostate cancer are discussed.

Materials and Methods

Mice and Tissue Collection

Male and female adult CD-1 mice between the ages of 10 and 24 weeks were obtained form Charles River Laboratories, Wilmington, MA. Pregnant mice were ordered for the collection of embryos. The day of isolation with copulatory plug was considered day 1 of embryonic development. Fetal tissues examined by *in situ* hybridization included: E10, E13, E14, E15, E17, and E18. Adult tissues examined included: tissues of the male urogenital system, female urinary bladder and kidney, pubescent mammary gland, 14-day pregnant mammary gland, lactating mammary gland, liver, heart, skin, and intestine. All tissues were fixed in 4% formalin and paraffin-embedded. Dr. Norman Greenberg (Baylor College of Medicine, Houston, TX) kindly provided breeder pairs of TRAMP transgenic mice. Urogenital tissues from wild-type littermate C57BL/6 were taken at age 12 and 24 for comparison to age-matched TRAMP transgenic mice. By the age of 12 weeks, TRAMP mice have typically progressed to PIN and or well-differentiated tumors. Nine TRAMP male mice between the ages of 12 to 39 weeks of age were sacrificed and tissues were collected for routine histology and *in situ* hybridization studies. The histological grade of prostate cancer was determined in accordance to previously published studies in the TRAMP model.[10]

Cloning of the Murine PSCA Orthologue

Full-length cDNA for murine PSCA was amplified from mouse 17-day embryo Marathon-Ready cDNA (Clontech, Palo Alto, CA). Primers were designed based on EST sequences present in GenBank. Sense primer (5′ ACT ATG AAG CTT TGC AGC TCA TCC CTT CAC AAT CG 3′) and anti-sense primer in the 3′-untranslated region (5′ GAA TTC GGA TCC ACC ATG AAG ACC GTC TTC TTT CTC CTG CTG 3′) were used resulting in a 420-bp fragment that was subsequently cloned into the polymerase chain reaction (PCR) subcloning vector PCR2.1TOPO (Invitrogen, Carlsbad, CA). Clones were confirmed by DNA sequencing.

In Situ *Hybridization*

PCR primers (upper: 5′ CCT GCT GGC CAC CTA CT 3′ and lower: 5′ CCT TCA CAA TCG GGC TAT 3′) were designed to amplify a 388-bp fragment of murine PSCA. Primers included extensions encoding 27 nucleotide T7 or T3 RNA polymerase initiation sites to allow *in vitro* transcription of sense or antisense probes, respectively, from the amplified products.[11] Five-μm-thick sections were deparaffinized, deproteinated in 4 μg/ml of proteinase K for 30 minutes at 37°C, and further processed for *in situ* hybridization. ^{33}P-UTP-labeled sense and antisense probes were hybridized to the sections at 55°C overnight. Unbound probe was removed by incubation in 20 mg/ml RNase A for 30 minutes at 37°C, followed by a high stringency wash at 55°C in 0.1× standard saline citrate for 2 hours, and dehydration through graded concentrations of ethanol. The slides were dipped in NBT2 nuclear track emulsion (Eastman Kodak, Rochester, NY), exposed in sealed plastic slide boxes containing desiccant for 4 weeks at 4°C, developed, and counterstained with hematoxylin and eosin. The *in situ* hybridization was routinely performed on duplicate sections with sense and antisense probes. Sections hybridized with the sense probe routinely did not show a specific hybridization signal.

Results

Expression of mPSCA in Fetal Tissues

PSCA mRNA expression was examined in multiple stages of embryonic development from E10 to E18. No expression was detected in any histological structure at E10. At E14, PSCA expression was detected in the epithelial lining of the urogenital sinus. Strong expression in the superficial epithelium of the urogenital sinus was seen to be maintained throughout the rest of fetal development (Figure 1, A and B). Expression was detected in the adluminal epithelial cells of the renal pelvis (data not shown). Expression was also observed in the fetal skin from E15 to E17, but neither before or after this time window. Expression within the fetal skin was confined to the outermost epithelial cell layer (Figure 1, C and D). PSCA expression was detected in the mucosal epithelium of the gastrointestinal tract. Particularly high levels of expression were observed in the anal canal (Figure 1, E and F); focal expression was detected in the colon. Again, the hybridization signal in these sites was restricted to the epithelial lining. Significant PSCA expression was observed in the mucosa of the oral cavity, extending into the oropharynx and into the esophagus (Figure 1, G and H). The epithelial cells lining the inner aspect of the amnionic membrane were also positive for PSCA (data not shown). In summary, PSCA expression was limited to epithelial tissues. In multilayered epithelia, PSCA was expressed in the most superficial cell layers.

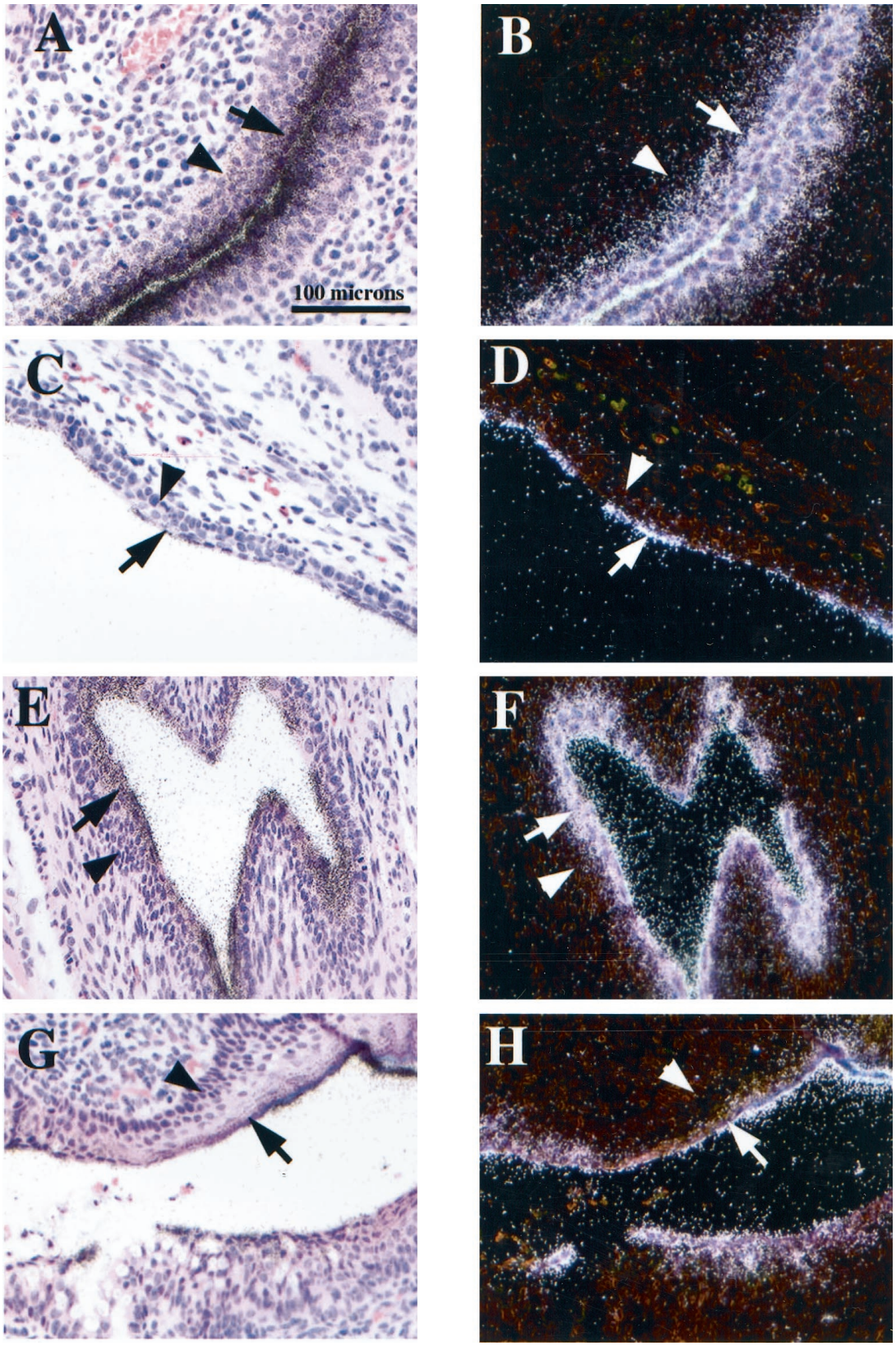

Figure 1. Expression of murine PSCA in fetal tissues by *in situ* hybridization. Bright field (**A**, **C**, **E**, **G**) and dark field (**B**, **D**, **F**, **H**) are shown for each frame. Original magnification ×200 for all images. **A** and **B:** PSCA expression is most intense in the superficial epithelial cells (**arrow**) of the urogenital sinus of an E15 embryo. The more basally located epithelial cells are low or negative for PSCA expression (**arrowhead**). **C** and **D:** Fetal skin is positive for PSCA (**arrow**) at E15, whereas the basal cells are negative (**arrowhead**). **E** and **F:** The squamous epithelium of the embryonic esophagus is very strongly positive for PSCA at E18 (**arrow**). The basal cells of the esophageal epithelium are negative (**arrowhead**). **H** and **G:** The transitional squamous portions of the anal canal show strong signal in the superficial cell layers (**arrow**), basally located epithelial cells are negative (**arrowhead**).

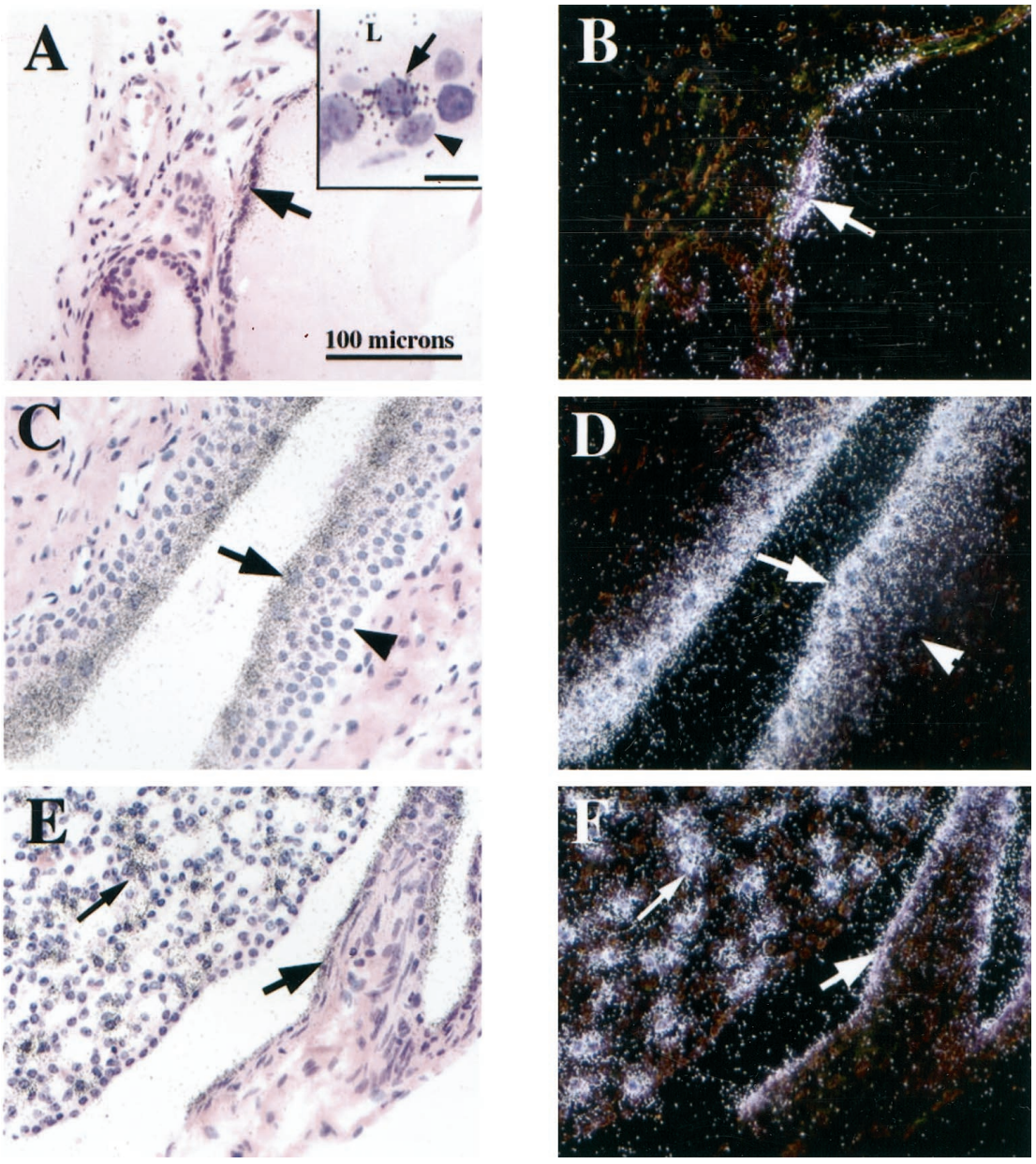

Figure 2. PSCA expression in normal adult mouse tissue by *in situ* hybridization. Bright field (**A**, **C**, **E**) and dark field (**B**, **D**, **F**) are shown for the same frame. Original magnification ×200 for all images, except the **inset** in **A**. **A** and **B:** Areas of PSCA expression in secretory cells of the prostate (**arrow**). **Inset:** A positive secretory cell as shown by the presence of silver grains (**arrow**) adjacent to a negative basal cell (**arrowhead**) of the mouse prostate. L indicates the lumen and the calibration bar represents 5 μm. **C** and **D:** Strong expression in superficial epithelial cells (**arrow**) of the transitional cell mucosa of the urinary bladder. Basally located epithelial cells are negative or express low levels (**arrowhead**). **E** and **F:** Epithelial lining of renal pelvis (**thick arrow**) and scattered tubular epithelial cells of the collecting ducts of the renal papilla (**thin arrow**) are PSCA-positive.

Expression of mPSCA in Adult Tissues

The expression of PSCA in the adult was more restricted compared with the fetus and was essentially limited to urogenital tissues in the male and the urinary tract in the female. Unlike the human gland the murine prostate is composed of several, histologically distinct lobes (anterior, dorsal-lateral, and ventral). Murine PSCA was focally expressed in all three prostatic lobes, with some acini being completely negative and others showing scattered, positive cells. PSCA was detected in the luminal cells (Figure 2, A and B); we found no evidence of PSCA expression in the basal cell population (Figure 2A, inset). Strong expression was seen at the juncture between the large prostatic ducts and the urethra. Individual luminal cells within the prostatic acini did not show unique morphological features indicative of expression of PSCA RNA. Seminal vesicle, vas deferens, and testis were completely negative for PSCA expression.

The strongest expression of PSCA in adult tissues was observed in the transitional cell mucosa of the ureter, bladder, and urethra (Figure 2, C and D). As in the PSCA-positive fetal tissues, the signal localized to the most superficial cell layer, whereas the basal cells were completely negative. Furthermore, strong expression of

Table 1. Expression of PSCA in Wild-Type and TRAMP Urogenital Tissues

Animal information				Expression of PSCA in normal and malignant tissues (% positive cells)			
Animal number	Genotype	Age (weeks)	Normal prostate	PIN	Well/moderately differentiated PC	Poorly differentiated PC	Normal urothelium
1	TRAMP	12	<10	50–75	50	N/A	100
2*	TRAMP	21	N/A	25–50	75	<10	100
3	TRAMP	24	<10	50	50–75	N/A	100
4	TRAMP	24	<10	25–50	50–75	N/A	100
5	TRAMP	27	<10	25	50–75	N/A	100
6	TRAMP	29	<10	50	50	N/A	100
7	TRAMP	30	<10	50–75	50–75	N/A	100
8	TRAMP	34	<10	<10	25–50	<10	100
9	TRAMP	35	N/A	10	50–75	N/A	100
10	WT C57B/6	12	<10	N/A	N/A	N/A	100
11	WT C57B/6	12	<10	N/A	N/A	N/A	100
12	WT C57B/6	27	<10	N/A	N/A	N/A	100

Abbreviations: PIN, prostatic intraepithelial neoplasia; PC, prostatic carcinoma; WT, wild type.

Percentage of positive cells was estimated for each tissue type represented on each slide by inspection under low magnification. The large prostatic ducts near the urethral juncture were excluded in the scoring of PSCA expression in the prostate.

*Animal 2 developed a large, poorly differentiated tumor, which enveloped most other prostatic tissues. PIN and well/moderately differentiated PC for animal 2 was only a very small proportion of total tissue.

PSCA was also seen in the kidney and was observed in the surface epithelium of the renal pelvis and in scattered epithelial cells of the collecting tubules of the renal papilla (Figure 2, E and F). The cortex of the kidney including collecting ducts and glomeruli were negative for PSCA expression. Female mice demonstrated the same pattern and intensity of expression in the kidney, the ureter, and the bladder (data not shown). We examined female mammary glands of different developmental and functional stage and were unable to demonstrate PSCA expression. Furthermore, adult skin of either sex is negative. There were no overt differences in pattern or level of expression between the CD-1 and C57BL/6 mouse strains (data not shown).

Expression of PSCA in Prostatic Adenocarcinoma of TRAMP Mice

We examined murine PSCA expression in different stages of progression of prostatic neoplasia in the TRAMP model. We evaluated nine TRAMP animals, all showed morphological evidence of prostatic neoplasia at various stages of tumor progression. The expression of PSCA was increased in the neoplastic areas in all of the animals examined (Table 1). A marked increase in PSCA expression was detected in foci of PIN. These foci were characterized by epithelial crowding or the formation of papillary tufts lined by a mitotically active epithelium with an increased nuclear:cytoplasmic ratio and mild to moderate nuclear pleomorphism (Figure 3A). However, the increased expression of PSCA was not uniformly observed in these foci of PIN (Figure 3B). Areas of invasive adenocarcinoma also demonstrated a significantly increased expression of PSCA compared to normal prostate. Similar to the foci of PIN, the areas of increased expression followed a patchy distribution within an individual tumor, with some areas devoid of any expression (Figure 3, C and D). Cases of PIN and well-differentiated adenocarcinoma showed no obvious correlation between expression of PSCA and morphology of the malignant cells. Areas with identical cellular morphology varied significantly in the level of PSCA expression (Figure 3, E and F). Expression of PSCA was undetectable in the two poorly differentiated cancers analyzed. Within the PSCA-negative areas, glands involved by PIN were observed to have high levels of PSCA expression (Figure 3, G and H) indicating that the lack of a signal in the poorly differentiated tumor was not because of RNA degradation.

Discussion

Our studies demonstrate that expression of murine PSCA is neither prostate-specific or consistent with it being a stem cell marker. The results clearly indicate that PSCA expression in epithelial surfaces is restricted to the luminal cell layer and not, as reported for the human gene, to basal cells. Cells of the adluminal outer layer typically display a high degree of differentiation, but unlike stem cells, low proliferative or self-renewal capability. The designation of this molecule as a "stem cell antigen" is therefore questionable and needs to be reconsidered.

The strongest expression of PSCA is detected in the urothelium of the developing and mature urinary tract. In the adult organism the expression appears continuous from the renal pelvis throughout the urethra. Prostatic expression is patchy, but increases noticeably at the juncture of the large prostatic ducts and urethra. It is tempting to speculate, whether this finding is related to urinary reflux into these structures providing a potential clue to the function of this protein. A common feature of the epithelial surfaces exhibiting PSCA expression is the fact that they line structures that are in continuous contact with fluids, mucous or secretions. This is obviously the case for urothelium, however, this

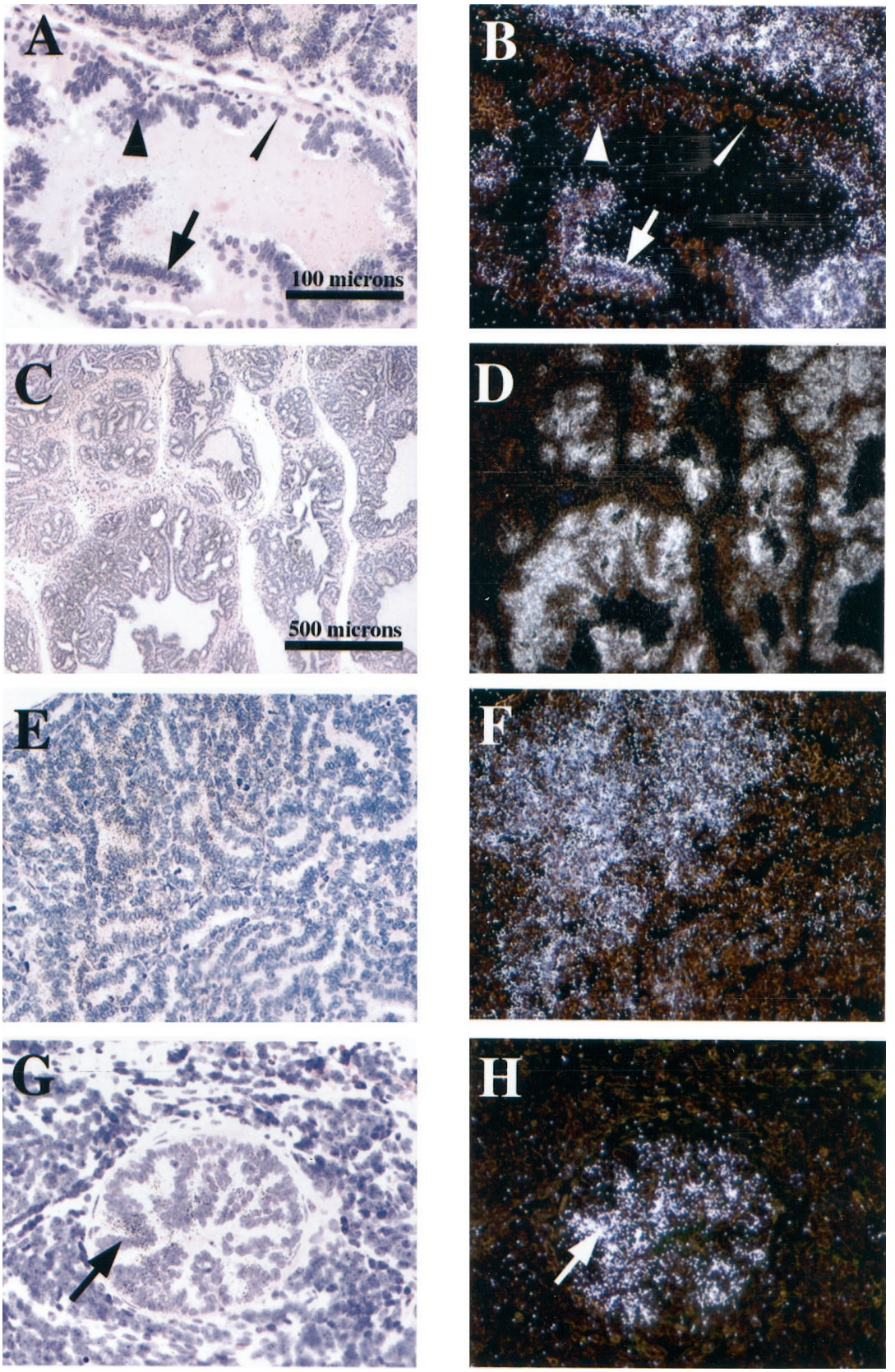

Figure 3. Expression of murine PSCA in prostate tissue of the TRAMP model by *in situ* hybridization. Bright field (**A**, **C**, **E**, **G**) and dark field (**B**, **D**, **F**, **H**) are shown for each frame. **A** and **B:** Prostatic duct with PSCA-positive PIN (**arrow**) and PSCA-negative PIN (**arrowhead**) and normal prostatic epithelia (**thin arrowhead**) (original magnification, ×200). **C** and **D:** Widespread but variable expression in well-differentiated adenocarcinoma (original magnification, ×40). **E** and **F:** Areas of PSCA expression in moderately differentiated adenocarcinoma. PSCA-positive cells are adjacent to morphologically similar PSCA-negative cells. **G** and **H:** PSCA-negative poorly differentiated carcinoma with trapped PSCA-positive duct showing PIN (**arrow**).

notion also holds true for the observation that significant PSCA expression is present in the amnionic membrane, anal canal, oropharynx, esophagus, and skin of the developing fetus. Additional denominators must be at play, because other fluid-exposed epithelial tissues do not express PSCA, such as the fetal tracheobronchial tree. Whether the expression in fetal skin is temporally regulated is difficult to determine at this point as the number of embryos evaluated is small. However it seems that expression of PSCA in skin is no longer required in postnatal, extrauterine life. The pattern of expression within multilayered epithelial surfaces suggests that PSCA may function as a barrier or buffer against external insults. Based on the morphological evaluation it is difficult to determine whether PSCA would be primarily a barrier against chemical, physical, or microbial insults. Further studies, particularly the generation of PSCA knockout mice should clarify the primary function of PSCA in normal tissue.

Whether PSCA plays a role during carcinogenic events is purely speculative at this point. One could consider that expression of PSCA is turned on in proliferative epithelium during tissue repair and incidentally increased in the context of proliferative activity in transformed cells. All of the TRAMP tumors evaluated in this study show, when compared with normal prostate, an increased proportion of PSCA-positive cells. The functional state of the scattered PSCA-positive cells in normal murine prostate is unclear as they are morphologically indistinguishable from adjacent, PSCA-negative cells.

Increased expression of members of the Ly-6 family has been correlated to enhanced malignant potential in some murine tumor cells.[12] Another member of the Ly-6 family, the E48 antigen, may have a role in cell adhesion in both normal and malignant squamous epithelia.[13,14] However, the specific function of these genes in carcinogenesis has yet to be established. The correlation between the levels of PSCA expression and tumor stage and grade for prostate and bladder in human patients is presently controversial. Our own results, which are based on a small number of animals seem to suggest a negative correlation between expression of PSCA and advanced tumor stage in this murine model of prostatic carcinogenesis. Down-regulation of PSCA has been reported for poorly differentiated bladder cancers whereas high-grade and metastatic prostate carcinoma showed increased PSCA expression.[4,15,16] In humans, PSCA is located on chromosome 8q, which is often amplified in metastatic and recurrent prostatic adenocarcinoma and considered to indicate a poor prognosis.[17–19] Interestingly, PSCA is in close proximity to the c-*myc* proto-oncogene, which is amplified in >20% of recurrent and metastatic prostate cancers.[20] Thus, overexpression of PSCA may not necessarily be causative in prostate carcinogenesis, but may occur incidentally in the context of amplification of contributing oncogenes, such as c-*myc*. Most likely, the differences in PSCA expression between the TRAMP tumors and human prostatic adenocarcinoma either indicate species-specific regulation of PSCA expression or are related to the type of carcinogenic event at play.

The rather selective expression of PSCA in fetal and adult tissues makes the mouse a suitable model to determine the physiological function of this protein. Although PSCA is lost in the advanced tumor stages the TRAMP model may represent a potentially useful tool to study PSCA as a potential target for prostate cancer. This model would allow investigators to study potential toxic effects of therapeutics targeting genes that are expressed not only in malignant prostate epithelium, but also in normal epithelial tissues of the urogenital tract or any other organ system.

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