Prominin-1 (CD133) Is Not Restricted to Stem Cells Located in the Basal Compartment of Murine and Human Prostate

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BACKGROUND. Rodent and human prominin-1 are expressed in numerous adult epithelia and somatic stem cells. A report has shown that human PROMININ-1 carrying the AC133 epitope can be used to identify rare prostate basal stem cells (Richardson et al., J Cell Sci 2004; 117:3539–3545). Here we re-investigated its general expression in male reproductive tract including mouse and human prostate and in prostate cancer samples using various anti-prominin-1 antibodies.

METHODS. The expression was monitored by immunohistochemistry and blotting. Murine tissues were stained with 13A4 monoclonal antibody (mAb) whereas human samples were examined either with the AC133 mAb recognizing the AC133 glycosylation-dependent epitope or 80B258 mAb directed against the PROMININ-1 polypeptide.

RESULTS. Mouse prominin-1 was detected at the apical domain of epithelial cells of ductus deferens, seminal vesicles, ampullary glands, and all prostatic lobes. In human prostate, immunoreactivity for 80B258, but not AC133 was revealed at the apical side of some epithelial (luminal) cells, in addition to the minute population of AC133/80B258-positive cells found in basal compartment. Examination of prostate adenocarcinoma revealed the absence of 80B258 immunoreactivity in the tumor regions. However, it was found to be up-regulated in luminal cells in the vicinity of the cancer areas.

CONCLUSIONS. Mouse prominin-1 is widely expressed in prostate whereas in human only some luminal cells express it, demonstrating nevertheless that its expression is not solely associated with basal stem cells. In pathological samples, our pilot evaluation shows that PROMININ-1 is down-regulated in the cancer tissues and up-regulated in inflammatory regions. *Prostate* © 2010 Wiley-Liss, Inc.

KEY WORDS: **CD133; epithelium; prominin-1; proliferative inflammatory atrophy; prostate cancer; stem cells**

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INTRODUCTION

Identification of stem and progenitor cells is an important issue for tissue engineering and stem cell-based therapy, and their prospective isolation relies on the presence of specific cell surface marker(s). In the last decade, prominin-1 (CD133) has gained an enormous attention in the stem and cancer stem cell fields (reviewed in [1,2]). Initially identified in mouse as a novel marker of neuroepithelial progenitor cells [3], it was described in human as the AC133 antigen, a marker of hematopoietic stem and progenitor cells [4]. In addition to the hematopoietic system, human PROMININ-1 has been described to label a large panel of somatic stem and progenitor cells, for instance, those derived from neural tissues [5,6], muscle [7], kidney [8], skin [9], and intestine [10]. As for the human prostate, a minute population of PROMININ-1 (AC133 epitope)-positive progenitors that possess a high proliferative potential in vitro and can reconstitute prostatic-like acini in immunocompromised nude mice was identified in the basal compartment [11]. Similarly, Leong et al. [12] could impressively demonstrate that a single murine prostate stem cell defined by the phenotype $Lin^{-}Sca\text{-}1^{+}prominin\text{-}1^{+}CD44^{+}CD117^{+}$ can generate a prostate after transplantation in vivo.

In addition to stem cells, numerous reports have demonstrated that PROMININ-1 (i.e., AC133 epitope) labels cancer stem cells (reviewed in [1,2,13]). Thus, PROMININ-1 is expressed in malignant cells found in hematopoietic diseases [14–17] and in certain types of solid cancers, for example, brain [18,19], kidney [20,21], and prostate cancers [22,23] as well as various cancer cell lines [24–30]. Moreover, PROMININ-1-positive cancer cells are often associated with chemoresistance [31–33]. It is therefore generally seen as one of the most important marker essential to cancer stem/initiating cells that might be a molecular target for effective cancer therapies [34–36]. Yet, to such end, it is important to keep in mind that most studies actually address specifically the AC133 epitope which is thought to be dependent on the glycosylation profile of PROMININ-1 and sensitive to tissue fixation [5,37,38] (reviewed in [39]) and consequently that the general expression of human PROMININ-1 as such may be more widespread. In the same line, the complex regulation of the expression of its different isoforms (splice variant and/or glycoform) needs to be further investigated [40,41].

In this context, we have demonstrated using either a rabbit antiserum (named αhE2) [20,42] or a novel mouse monoclonal antibody (mAb 80B258) [43] directed against human PROMININ-1 polypeptide that it is expressed far beyond stem cells, like was earlier observed in rodents [3,40,44,45] (reviewed in [1,46]). Indeed, we have detected PROMININ-1 in various human organs or glands such as kidney, pancreas, liver, mammary glands, salivary glands, sweat glands, lacrimal glands, and cervical glands [20,42,43].

Because the molecular (e.g., expression of various splice variants, binding to membrane cholesterol) and cellular (e.g., apical localization in epithelial cells, association with small extracellular membrane vesicles) characteristics of prominin-1 initially demonstrated in rodent tissues [3,40,47,48] are shared by its human counterpart [5,20,49–51], we sought to reevaluate its expression within the prostate in both species (mouse and human). Interestingly, we could demonstrate using 80B258 mAb that the expression of PROMININ-1 in human prostate is not restricted to stem cells located in basal compartment; however, its general expression in this particular organ does not fully mirror the situation observed in mouse.

MATERIALS AND METHODS

Cell Culture and Membrane Preparation

Human colon carcinoma-derived Caco-2 cells were cultured as described [5]. Tissues used in the present study were obtained under the appropriate institutional (Max-Planck-Institute of Molecular Cell Biology and Genetics (murine tissues); Technical University of Dresden (TUD; human tissues)) approval protocol. Samples of human tissues came from the Department of Pathology (TUD) and were anonymous materials with the patient consent that had not been used for genetic analysis. Prostate tissues were acquired from two patients aged 76 and 79, respectively, with prostatic hyperplasia but no cancer. Tissues from adult (4-month-old) C57BL/6 mice and human prostate were used to prepare membrane lysates according to procedures reported previously [44]. Protein concentrations were determined using BCA Protein Assay Reagent (Pierce, Rockford, IL).

Endoglycosidase Digestion and Immunoblotting

Caco-2 cell detergent extracts corresponding to one-tenth of a confluent 85-mm dish or proteins solubilized from adult mouse and human membranes (20–50 μg protein) were incubated overnight at 37°C in the absence or presence of 1 U peptide-*N*-glycosidase F (PNGase F) according to the manufacturer's instructions (Roche Molecular Biochemicals, Mannheim, Germany). Proteins were analyzed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE; 7.5%) and transferred to poly(vinylidene difluoride) (PVDF) membranes (Millipore Corp., Bedford, MA; pore size 0.45 μm) using a semi-dry transfer system (Cti, Idstein,

Germany) as described [44]. After transfer, membranes were incubated overnight at 4°C in blocking buffer (PBS containing 5% low fat milk powder and 0.3% Tween-20). Mouse and human prominin-1 were then detected using rat mAb 13A4 (1 μg/ml) [3] and mouse mAb 80B258 (1 μg/ml) [43], respectively. Primary antibodies diluted in blocking buffer were incubated for 1 hr at room temperature. Antigen–antibody complexes were revealed using horseradish peroxidase-conjugated secondary antibodies (Dianova) followed by enhanced chemiluminescence (ECL system, Amersham).

PCR Amplification of Prominin-1 (CD133) cDNA

Oligonucleotide primers were designed to amplify all potential PROMININ-1 splice variants from a human prostate cDNA pool (Marathon Ready cDNA kit; BD Biosciences) using polymerase chain reaction (PCR). [Note that, normal prostates pooled from 32 male Caucasians have been used as poly A^+ RNA source.] The first PCR was performed using gene-specific 5′ sense primer I (5′-CTCTGAGGCAGGAGGCACCAAGTTCTA-3′) corresponding to the nucleotide sequence 182–208 of the PROMININ-1 cDNA sequence (GenBank accession number BC012089), and the linker-specific adaptor primer 1 supplied with the Marathon Ready cDNA kit. The nested gene-specific 5′ sense primer II (5′-CCAAGTTCTACCTCATGTTTGGAGGAT-3) matching nucleotides 199–225 of the PROMININ-1 sequence was used with the linker-specific adaptor primer 2 for the second PCR reaction. PCR reactions, performed using Advantage® cDNA Polymerase Mix (Clontech) according to manufacturer's instructions, generated a fragment of about 4 kb. An aliquot of the nested PCR product was purified and subcloned in the pCR®-Blunt II TOPO vector according to the manufacturer's instructions (Zero Blunt TOPO PCR cloning kit; Invitrogen, Groningen, the Netherlands). cDNA inserts were completely sequenced on both strands using Applied Biosystems 3730 Genetic Analyzer.

Immunohistochemistry of Mouse and HumanTissues

Various tissue samples dissected from adult C57BL/6 mouse were fixed by immersion in 4% paraformaldehyde in sodium phosphate buffer, pH 7.4, at 4°C overnight. Tissues were infiltrated with cryoprotectant (30% sucrose in sodium phosphate buffer, pH 7.4), embedded in Tissue-Tek® (Miles, USA) and rapidly frozen on dry ice. Cryosections (10 μm) were mounted on SuperFrost®Plus (Menzel-Glaser, Germany) slides. Samples were washed three times with PBS containing 0.3% Tween-20 (5 min each), and the endogenous peroxidase was then neutralized with 1.0% H_2O_2 for 20 min. After being washed with PBS containing 0.3% Tween-20, cells were permeabilized, and sections were blocked with 10% fetal calf serum and 0.2% saponin in PBS (blocking solution) for 30 min at room temperature. Sections were then incubated sequentially for 1 hr at 37°C with rat mAb 13A4 (10 μg/ml) and horseradish peroxidase-coupled goat anti-rat secondary antibody (1:300; Dianova), all diluted in blocking solution. Color reactions were performed with peroxidase substrate 3,3′-diaminobenzidine (DAB tablet sets, 0.7 mg/ml; Sigma) according to the manufacturer's protocol. Sections were counterstained with Mayer's hematoxylin solution (Merck), dehydrated, and mounted in VectaMount mounting medium (Vector). Stained sections were observed with an Olympus BX61 microscope equipped either with an Olympus DP71 camera or HistoScope High resolution RGB Imaging System (Visitron Systems GmbH, Puchheim, Germany). The images shown were prepared from IPLAB data files (version 3.5) by using Adobe Photoshop software.

Various human samples of prostate cancer (Gleason score ranging 5–10) and non-cancerous prostatic tissues were obtained from anonymous archival tissues. Morphologically normal, that is, without cancer, prostate gland tissues were collected from two independent donors aged 60 and 69, respectively, who had urinary bladder cancer. Samples were fixed in 10% formalin (pH 8.0) for 24 hr at room temperature, dehydrated with increasing concentrations of ethanol (70%, 80%, 2 × 96%, 2 × 100%) for 1 hr each at room temperature, and then treated twice with xylene (Fluka) for 45 min at room temperature. The dehydrated samples were incubated in paraffin at 60°C for 1.5 hr and then for additional 2.5 hr with fresh paraffin. Finally, embedded tissues were stored at room temperature. Thin sections (7 μm) were cut using Leica rotary microtome (MICROM 540HM), mounted on SuperFrost®Plus slides (Menzel-Glaser), and dried overnight at room temperature. They were deparaffinized by two successive xylene treatments (20 min each), hydrated with decreasing concentrations of ethanol (2 × 100%, 96%, 80%, 70%, 40%) for 1 min (each at room temperature), and then rinsed with distilled water (Millipore Corp.) for 5 min. Samples were washed three times with PBS containing 0.3% Tween-20 (5 min each), and the endogenous peroxidase was then neutralized with 1.0% H_2O_2 for 20 min. Tissue sections were permeabilized and stained using either the mouse mAb 80B258 (10 μg/ml) [43] or AC133 (1 μg/ml; Miltenyi Biotec, Gladbach, Germany) or rabbit antiserum αhE2 (1:500) [20] as described previously [20,43]. Immunodetection and counterstaining were performed as described above.

RESULTS

Prominin-1 Is Expressed All Along the Murine Male Reproductive Tract

We have previously demonstrated that distinct mouse prominin-1 splice variants are expressed at the apical membrane of epithelial cells lining the epididymal duct [40]. Here we have pursued our investigation of its general expression all along male reproductive tract by immunohistochemistry and immunoblotting using the rat mAb 13A4 [3]. A strong staining was detected at the apical, but not basolateral, side of epithelial cells located in the ductus deferens (A, arrow), seminal vesicles (Fig. 1B), anterior prostate (coagulating glands; Fig. 1C), lateral prostate (Fig. 1D), ampullary glands (Fig. 1E), ventral and dorsal prostate (see Supplemental Materials Fig. S1). It is interesting to note that within the ventral prostate not all luminal cells exhibit 13A4 immunoreactivity (Fig. S1C,c1–4, arrowhead). Similar data were obtained with another anti-prominin-1 antibody, that is, antiserum αE3 [52] (data not shown). No staining was detected when the primary antibody was omitted (Figs. 1A′–E′ and S1B′,C′).

Protein expression was next investigated by immunoblotting. The analysis of membrane lysates prepared from lateral prostate and ampullary glands revealed a broad 13A4-immunoreactive band with an apparent molecular mass of 105–120 kDa (Fig. 2, lanes 3 and 5, respectively, bracket). In seminal vesicles, it showed a reduced electrophoretic mobility with a sharp ≈120 kDa band (Fig. 2, lane 7, arrow) compared to the kidney (≈115 kDa band) (Fig. 2, lane 1). Upon removal of *N*-glycans with a PNGase F treatment, a 13A4-immunoreactive band of ≈94 kDa was observed in all tissues (Fig. 2, lanes 2, 4, 6, and 8, arrowhead; data not shown) suggesting that the prominin-1 splice variant expressed in those tissues is s1 (for nomenclature, see Refs. [41,49]) as previously demonstrated for the kidney-derived prominin-1 [3]. The nature of the prominin-1 isoform was confirmed by immunoblotting using a rabbit antiserum (αI3) specific for the cytoplasmic C-terminal domain of the s1 splice variant [53] (data not shown).

Taken together, these results show that the mouse prominin-1.s1 splice variant is expressed in various epithelia distributed all along the male reproductive tract, but with slightly different processing of its complex *N*-glycans. In agreement with a recent study [12], prominin-1 thus appears highly expressed in murine prostate, which sharply contrast to the human situation where it has been reported that only rare cells located in basal compartment expressed it as revealed with AC133 mAb [11].

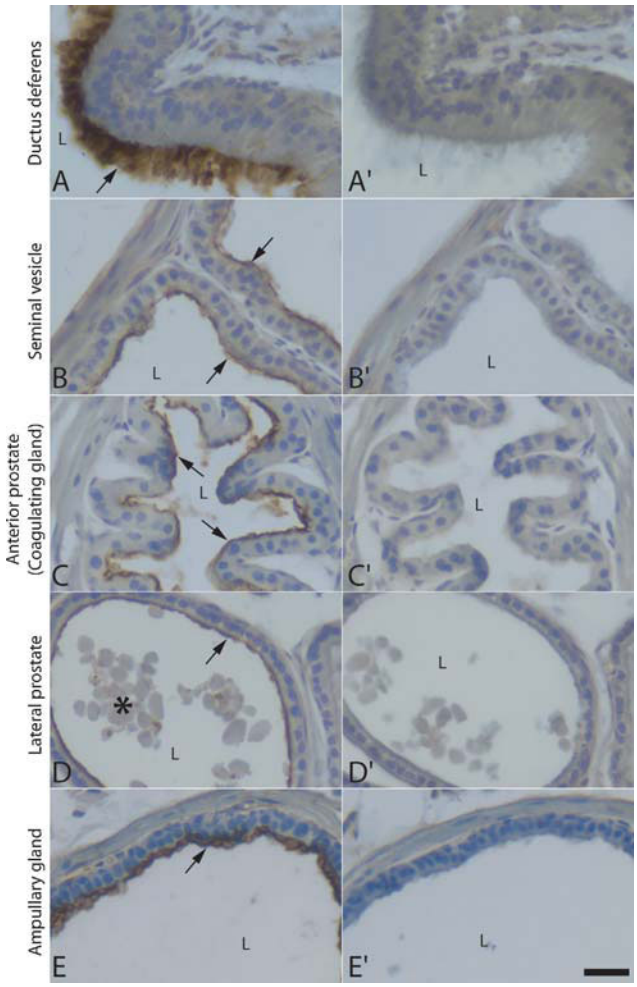

Fig. 1. Immunoperoxidase localization of prominin-1 at the apical domain of epithelial cells lining various tissues of the mouse male reproductive tract. Cryosections of adult murine ductus deferens (**A**,**A**′), seminal vesicles (**B**,**B**′), anterior prostate (**C**,**C**′), lateral prostate (**D**,**D**′), and ampullary glands (**E**,**E**′) were labeled with (A–E) or without (A′–E′) 13A4 mAb followed by peroxidase-coupled goat anti-rat secondary antibody. Sections were counterstained with Mayer's hematoxylin. Arrows indicate the 13A4 immunoreactivity at the apical surface of various epithelia; L indicates the luminal compartment of each tissue; asterisk shows secretion in the lumen of the lateral lobe of the prostate gland. Scale bar, 25 μm.

The Expression of PROMININ-1 Polypeptide in Human Prostate Is Not Limited to Rare Cells Located in Basal Compartment

The apparent discrepancy observed between murine and human prostate prompted us to reinvestigate the expression of PROMININ-1 in the latter species with tools other than mAb AC133, which epitope is thought to be dependent, at least in part, on glycosylation [37]. To that end, we used our newly characterized mouse mAb 80B258 that has been generated against a polypeptide corresponding to amino acid residues

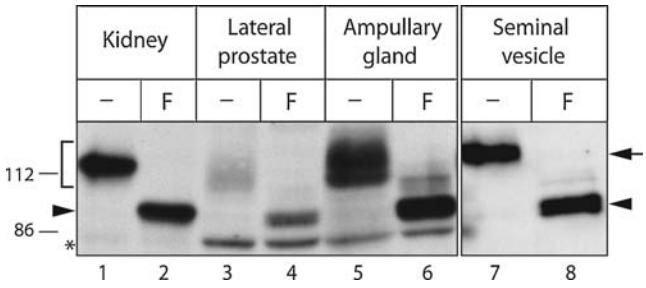

Fig. 2. Characterization of murine prominin-1 in male reproductive tract. Proteins (50 μg) solubilized from adult mouse lateral prostate, ampullary gland, and seminal vesicle membranes were incubated in the absence (–) or presence (F) of PNGase F and analyzed by immunoblotting with 13A4 mAb. For comparison, prominin-1 from adult kidney membranes (50 μg protein) was analyzed in parallel. Bracket, glycosylated 105–120 kDa forms of prominin-1; arrow, glycosylated 120-kDa form of seminal vesicle-derived prominin-1; arrowhead, *N*-deglycosylated, 94-kDa form of prominin-1. Asterisk, PNGase F-insensitive immunoreactive band of unknown identity. Position of prestained apparent molecular mass markers (in kDa) is indicated on the left.

Gly_{240}–Ser_{388} of PROMININ-1 [43], and for the sake of comparison, the AC133 mAb. In agreement with Richardson et al. [11], we could detect AC133 immunoreactivity in rare cells located in basal compartment often at branching folds or in budding regions (Fig. 3A, arrow). Such infrequent cells were also labeled with 80B258 mAb (Fig. 3B, arrow). The nature of these cells was not further studied here. Surprisingly, although the AC133 immunoreactivity appeared confined to the basally located cells [11], 80B258 immunoreactivity was observed, in addition, in some epithelial cells facing the lumen of the acini (Fig. 4; see also Fig. S2). In these cells, the 80B258 immunoreactivity was restricted to the apical plasma membrane (Fig. 4C–E; see higher magnification in panels c–e). In some cases, it appeared also in the upper (i.e., apical) part of the cytoplasm of columnar cells (Fig. 4D,d). It is interesting to note the discontinuous expression pattern of 80B258-positive cells (Fig. 4C,D; for details, see panels c and d, bracket)—a situation similar to the murine ventral prostate (see above, Fig. S1). Moreover, epithelial cells displaying the 80B258 immunoreactivity were frequently atrophic (Fig. 4e). Most of the luminal cells were nevertheless not stained (Figs. 4A,B,a,b and S2, panels 3 and 4). No signal was observed when the primary antibody was omitted (Fig. 4C′–E′,e′).

To confirm that the 80B258 immunoreactivity detected in human prostate samples was related to PROMININ-1 we investigated the actual protein expression by immunoblotting two prostate membrane lysates derived from independent donors (Fig. 5). An 80B258 immunoreactive band was observed at 120 kDa (Fig. 5, lanes 1 and 3, arrow), as for the positive control, that is, human colonic adenocarcinoma-derived Caco-2

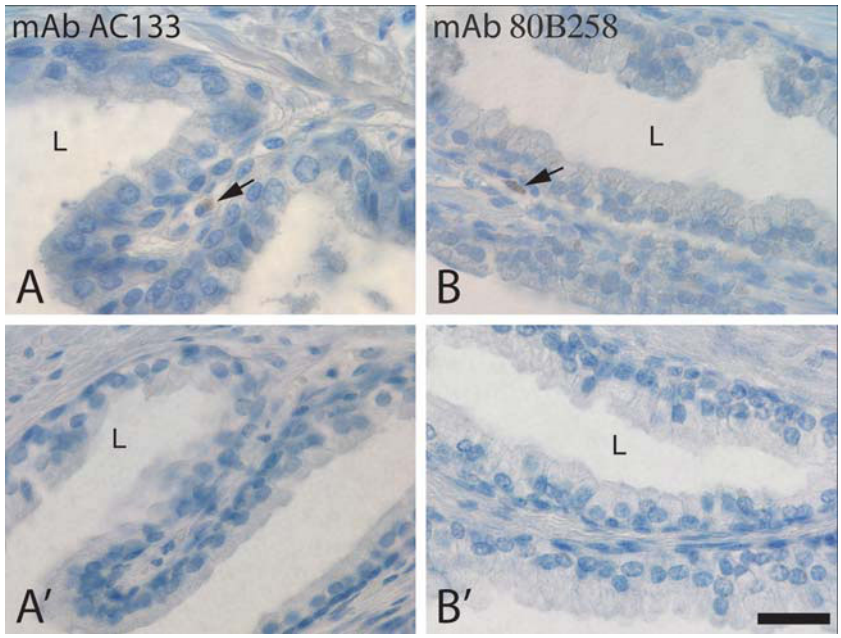

Fig. 3. PROMININ-1 immunoreactivity in rare prostate cells located in basal compartment. Paraffin-embedded sections of a non-cancer human prostate were labeled with either AC133 (**A**) or 80B258 mAb (**B**) or without primary antibody (**A′**,**B′**) followed by the peroxidase-coupled secondary antibody. Sections were counterstained with Mayer's hematoxylin. Arrows indicate either AC133 (A) or 80B258 immunoreactivity (B) in rare single cell located in the basal compartment. Such cells are absent in negative controls (A′,B′). L indicates the luminal compartment. Scale bar, 20 μm.

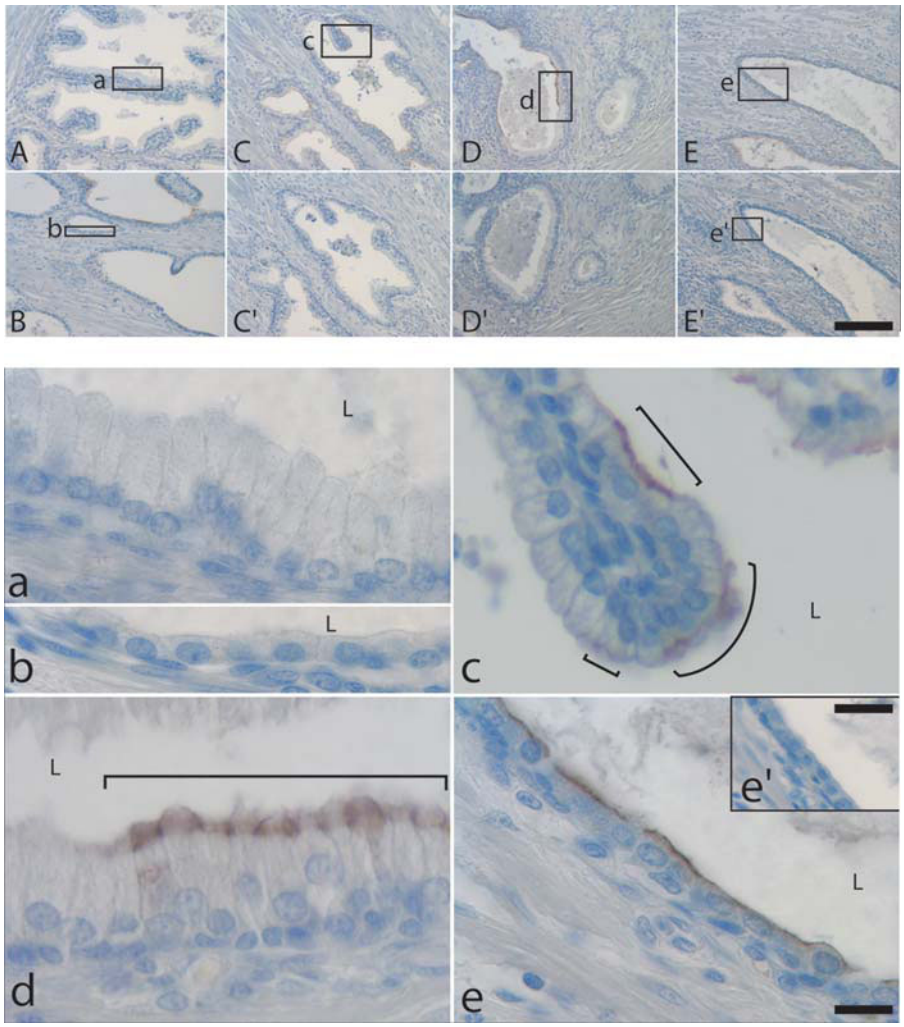

Fig. 4. Immunoperoxidase localization of PROMININ-1 at the apical side of some epithelial cells lining the human prostate. Paraffin-embedded sections of a non-cancer prostate derived from a 69-year-old patient carrying an urinary bladder cancer were labeled with (**A**–**E**) or without (**C′**–**E′**) 80B258 mAb followed by the peroxidase-coupled secondary antibody. Sections were counterstained with Mayer's hematoxylin. Panels identified by a lower case letter (**a**–**e**,**e′**) show a high magnification of the corresponding boxed area in panels A–E and E′. For a lower magnification of panels A–C, see Supplemental Materials Figure S2. Black lines indicate 80B258-positive cells surrounded by unlabeled cells. L, lumen. [Note that similar data were obtained with another individual (60-year-old; not shown).] Scale bars, 150 μm (A–E,C′–E′); 14 μm (a–e); 30 μm (e′).

cell extract (Fig. 5, lane 5). *N*-Deglycosylation with PNGase F yielded a band of ≈92 kDa (Fig. 5, lanes 2, 4, and 6, arrowhead). These apparent molecular masses were those expected for *N*-glycosylated and deglycosylated forms of human PROMININ-1 [5].

To further characterize PROMININ-1 in human prostate we amplified its entire open-reading frame by 3′-RACE (rapid amplification of DNA ends) using a set of gene-specific 5′-primers located downstream the initial start codon, and a cDNA pool derived from normal human prostate (for details, see the Materials and Methods Section). The sequencing of eight individual cDNA clones derived from two independent PCRs revealed the PROMININ-1.s1 splice variant (GenBank accession number AF507034) like in mouse.

Expression of PROMININ-1 in Human Prostate Cancers

Given that PROMININ-1 (AC133 epitope) has been identified in prostate cancer cells having cancer-initiating ability [36], we examined its general expression in human prostate adenocarcinoma by immunohistochemistry using various anti-PROMININ-1 antibodies. Interestingly, an analysis of 18 individual cancer samples revealed no PROMININ-1 immunoreactivity using

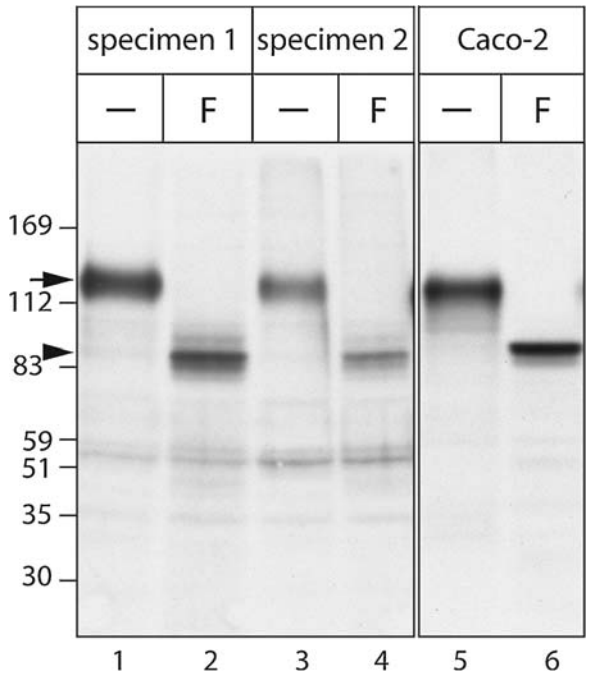

Fig. 5. PROMININ-1 in human adult prostate is a 120-kDa glycoprotein. Proteins solubilized from adult prostate membranes of two distinct individuals (specimen 1, 79-year-old, 30 μg protein; specimen 2, 76-year-old, 20 μg protein) were incubated in the absence (−) or presence (F) of PNGase F and analyzed by immunoblotting using the 80B258 mAb. For comparison, PROMININ-1 endogenously expressed by human Caco-2 cells was analyzed in parallel. Arrow, native 120-kDa form of PROMININ-1; arrowhead, *N*-deglycosylated 92-kDa form of PROMININ-1.

either 80B258 mAb or antiserum αhE2 in cancer (transformed) regions, whatever their Gleason grading (data are summarized in Table I; four examples are shown either in Fig. 6A, panel a1 and F–H or Fig. 7). However in benign glands in the vicinity of the cancer regions, the intensity and frequency of the 80B258 immunoreactivity appeared to be increased (Fig. 6A, panels a2 and a3, arrowhead; see also Fig. S3) compared to the normal prostate (Fig. 4). Indeed, the 80B258-positivity was especially observed in regions of inflammation (Fig. 6A,a3,B, asterisks) and/or where residual acini were compressed by surrounding cancer (Fig. 6C). In all cases, 80B258 immunoreactivity was restricted to the luminal cells (Figs. 6 and S3). Cells located in the stroma were negative (Fig. 6). Some secreted materials found in the glandular lumen were strongly labeled (Fig. 6D,d2), which is not the case of corpora amylacea (Fig. 6E,e). Similar data were obtained with antiserum αhE2 (Fig. 7). Specifically, αhE2 immunoreactivity was detected at the apical side of the luminal cells located in tissues adjacent to the cancer region, where infiltration of leukocytes was often detected (Fig. 7A–C, for details, see legend). The benign acini surrounded by invasive cancer areas were labeled as well (Fig. 7D). It is interesting to observe that PROMININ-1 immunoreactivities (80B258 and αhE2) were frequently detected in *atrophic epithelial cells* (Figs. 6b,d1 and 7a2, respectively; for details, see the legend of Fig. 7) as shown for certain luminal cells in non-cancerous tissues (see above). In contrast, the AC133 mAb did not reveal any positive signal with the exception of rare cells located in basal compartment as observed in normal tissue (Table I; data not shown).

TABLE I. Expression of prominin-1 in Various Human Prostate Cancer Samples

			PROMININ-1 immunoreactivity					
			Cytomorphologically normal region in vicinity of cancer area			Cancer/transformed area		
Adenocarcinoma [Gleason grading]	Number of specimens	Age (year)	mAb 80B258	Antiserum αhE2	mAb AC133	mAb 80B258	Antiserum αhE2	mAb AC133
[2+3]	1	68	+	+	−[a]	−	−	−
[3+2]	2	54–67	+	+	−	−	−	−
[3+3]	5	59–73	+	+	−	−	−	−
[3+4]	1	61	+	+	−	−	−	−
[4+3]	1	66	+	+	−	−	−	−
[3+5]	6	50–70	+	+	−	−	−	−
[5+3]	1	64	+	+	−	−	−	−
[5+5][b]	1	71				−	−	−

(+) Presence or (−) absence of prominin-1 immunoreactivity at the apical domain of epithelial cells.
[a]Note that AC133 immunoreactivity is observed only in rare cells located in the basal compartment of epithelium (see Fig. 3).[b]Cancer tissue sample only.

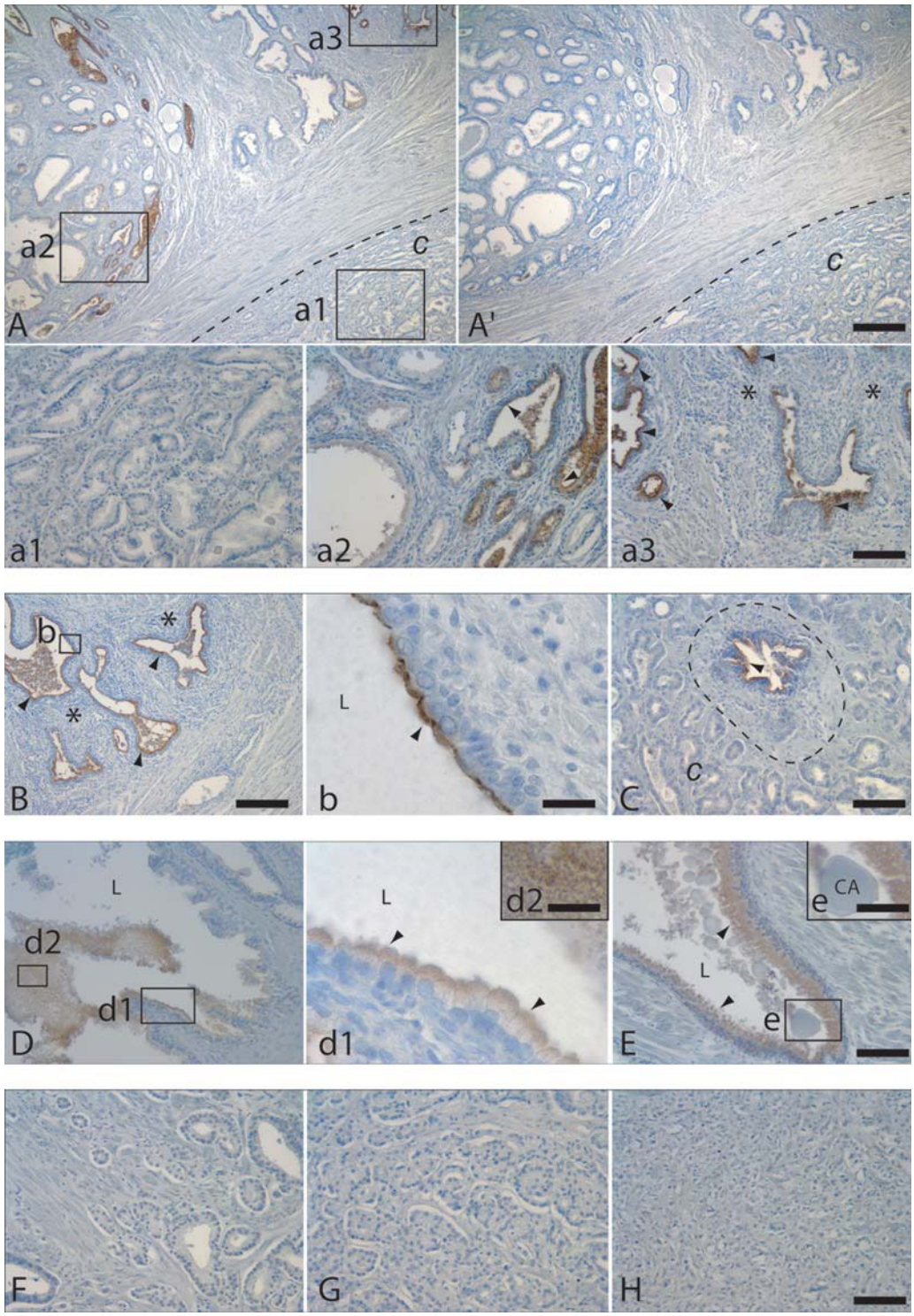

Fig. 6. Immunoperoxidase detection of PROMININ-1 in human prostate adenocarcinoma. Paraffin-embedded sections of various human adenocarcinoma samples were labeled with (**A**–**H**) or without (**A**′) 80B258 mAb followed by the peroxidase-coupled secondary antibody. Sections were counterstained with Mayer's hematoxylin. Samples are derived from prostatic adenocarcinoma exhibiting various Gleason grade; 3 + 2, 67-year-old patient (A,A′,B); 3 + 3, 65-year-old patient (C); 3 + 4, 61-year-old patient (D); 3 + 5, 50-year-old patient (E); 3 + 3, 71-year-old patient (F); 4 + 3, 66-year-old patient (G); 5 + 3, 64-year-old patient (H). Panels identified by a lower case letter (**a1–3**,**b**,**d1**,**2**,**e**) show a high magnification of boxed areas in panels A, B, D, and E. Arrowheads show 80B258 immunoreactivity at the apical side of epithelial cells lining the prostate acini-like structures. Asterisks indicate areas of inflammation. L, lumen; CA, corpora amylacea; c, cancer area is delimitated with dashed line. Scale bars: 350 μm (A,A′); 100 μm (a1–3,C–H); 200 μm (B); 20 μm (b,d1); 12 μm (d2); 55 μm (e).

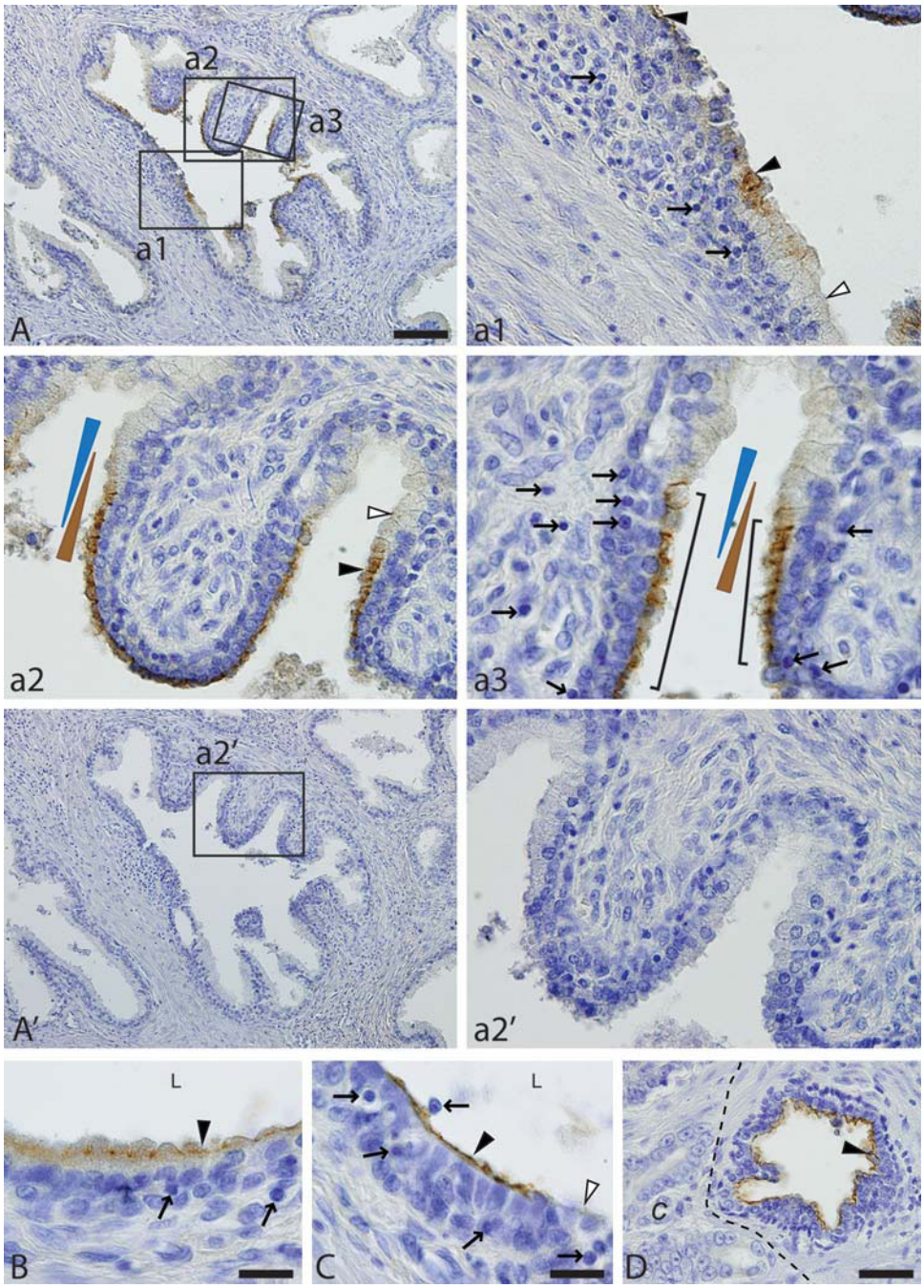

Fig. 7. PROMININ-1 is detected in leukocyte-infiltrated epithelium of benign glands. Paraffin-embedded sections of two human adenocarcinoma samples were labeled with either antiserum αhE2 (**A**–**D**) or pre-immune serum (**A′**,**a2′**) followed by the peroxidase-coupled secondary antibody. Sections were counterstained with Mayer's hematoxylin. Samples are derived from prostatic adenocarcinoma exhibiting two Gleason grade; 3 + 3, 71-year-old patient (A–C,A′); 3 + 4, 61-year-old patient (D). Panels identified by a lower case letter (**a1**–**3**,a2′) show a high magnification of the boxed areas in panels A and A′. Black arrowheads show αhE2 immunoreactivity, which is often found in cell clusters (bracket), at the apical side of epithelial cells lining the acini adjacent to cancer area (not shown). Open arrowheads show negative cells adjacent to the positive ones. Note that in numerous cases, the intensity of αhE2 immunoreactivity (brown triangle) correlates inversely with the cell basal-to-apical height (blue triangle). Black arrows indicate leukocytes (dark nuclei) infiltrated within either epithelium or stroma (a1,a3,B,C). Open arrow indicates a leukocyte found in the lumen (L,C). c, Cancer area is delimitated with dashed line. Scale bars: 100 μm (A,A′); 20 μm (B,C); 50 μm (D).

DISCUSSION

In this study we report two important findings. First, prominin-1 is widely distributed all along the murine reproductive tract. Second, the overall expression of PROMININ-1 in human prostate is not strictly limited to the rare basal stem and progenitor cells as described in an early study using AC133 mAb [11]. Nevertheless, the general expression of prominin-1 in mouse versus human prostate is distinct.

As already demonstrated in various epithelia including those found in distinct regions of the epididymis [40], prominin-1 was found to be concentrated at the apical plasma membrane of all epithelia of the murine reproductive tract investigated reflecting thus its selective localization in particular plasma membrane protrusions, for example, stereocilia (as in the cases of epididymis and ductus deferens) or microvilli (in other tissues) (reviewed in [46,54]). The discontinuous expression pattern specifically observed in the epithelium of the murine ventral lobe, in contrast to the dorsal, lateral, and anterior regions, is consistent with the highly variable density of microvilli on the apical surface between individual cells [55]. The limited expression of prominin-1 protein in this particular prostatic lobe, by comparison to the others, is also coherent with a recent quantitative PCR analysis of its gene expression [12]. Within these epithelia, the glycosylation profile of prominin-1.s1 splice variant seems to vary as previously reported for the testis versus epididymis-associated ones [40]. Such different *N*-glycan modifications in the male genital tract have been previously reported for other glycoproteins such as CAMPATH-1 antigen (CD52) [56], yet the physiological relevance of these glycoforms needs to be determined. Although the physiological function of prominin-1 remains elusive, genetic analyses in human on one hand and a knockout mouse model on the other have linked prominin-1 deficiency with an impairment of the morphogenesis of photoreceptor cell outer segment suggesting that this molecule may play a certain role as an organizer of plasma membrane protrusions [52,57–59]. Similarly, prominin-1 may be involved in the formation and/or stabilization of functional stereocilia and microvilli present in epithelial cells within the male reproductive tract. A further detailed analysis of the prominin-1-knockout mouse model should clarify this issue [59].

Until now, all molecular and cellular characteristics of mouse prominin-1 and its human ortholog including their tissue distribution have appeared as the mirror image of one another. Here, we demonstrated that this does not hold true for the prostate as only a sparse subpopulation of human luminal cells appears to express PROMININ-1—yet the same splice variant (i.e., s1)—(for an overview, see Fig. S2). This discontinuity in expression appears even within one given acinus and is reminiscent of the pattern observed in the murine ventral prostate that has no anatomical or histological homolog in man [60]. The mechanism underlying the differential expression of prominin-1 between the human and rodent is unknown but it might somehow reflect their histological differences. For instance, human prostate has a continuous layer of basal cells between the secretory cells and the basement membrane, whereas mouse has fewer basal cells and a discontinuous layer around the glands [61]. In this respect, prominin-2 (i.e., prominin-1 paralog) also displays a distinctive cellular localization within the prostate since its exclusively basal localization in human is neither evident in mouse nor in rat [62,63].

In human, both PROMININ molecules thus appear to have a distinct distribution within prostate glands; PROMININ-2 being strongly expressed in all cells located in the basal compartment [62], whereas PROMININ-1-positive cells appear as two distinct minute subpopulations; one located in the basal compartment as previously reported [11] and the other distributed within the columnar epithelial secretory cells (Fig. 4). The lack of detection of the latter subpopulation by AC133 mAb might be explained either by a differential *N*-glycosylation pattern of PROMININ-1 molecules (see the Introduction Section) or the inaccessibility of the AC133 epitope in luminal cells. Its detection might require some special and/or drastic techniques for antigen retrieval as demonstrated for other epithelia [38]. In any case, our data indicate that PROMININ-1-negative cell populations isolated using AC133 mAb should be analyzed with caution [64]. What could be the physiological relevance of rare PROMININ-1-positive cells within the luminal epithelium? The answer to this question is still open. However, in light of a recent publication describing rare luminal cells susceptible to cellular oncogenic transformation which are expressing the homeobox gene *Nkx3-1* in the absence of testicular androgens (castration-resistant Nkx3-1-expressing cells; CARNs) and acting as alternative source of stem cells in rodent models [65], it is tempting to speculate that luminal PROMININ-1-positive cells might represent a second source of stem cells in human prostate. It might be more than a coincidence that they are often found in cluster as described for CARNs in mice [65].

Diagnosis of prostate diseases at early stages by using biomarkers in the blood is still under investigation. No ideal biological marker that can determine the diagnosis and the clinical stage, inform about high-risk patients and eventually monitor the treatment has emerged yet. Due to the disease heterogeneity [66], which ranges from inflammation and benign hyperplasia to carcinoma and metastatic cancer, a

single marker is not feasible for clinical diagnosis. The lack of PROMININ-1 in transformed regions and its up-regulation in surrounding areas that we have observed in various adenocarcinoma samples may find, in conjunction with other markers, some diagnostic application. Interestingly, a similar phenotype is also observed in certain cancers affecting salivary glands (J.K. and D.C., unpublished work) suggesting a common background in the expression of PROMININ-1 in diseases associated with particular glands. Are the PROMININ-1-positive cells in the peripheral areas of tumor in process of oncogenic transformation? How is the PROMININ-1 gene down-regulated upon complete transformation? How is the PROMININ-1 gene regulated as such? Answering these questions might shed light onto the development and/or progression of cancers as this molecule is expressed, not only in rare cancer stem cells, but also in numerous embryonic (developing) epithelia in mouse and human [3,5]. The potential sensitivity of luminal PROMININ-1-positive cells to oncogenic transformation would also be consistent with the notion that human prostate cancer cells exhibit a luminal phenotype [67]. Moreover, the particular ''shrunken'' morphology of certain PROMININ-1-positive cells found in both normal and tissues adjacent to the cancer region is characteristic of lesions known as proliferative inflammatory atrophy (PIA) [68,69]. Such stressed cells might play a role not only in tissue repair but also give rise to cancer. In this context, it would be interesting to determine whether PROMININ-1-positive cells co-express for instance caretaker genes (e.g., π-class glutathione *S*-transferase) that are up-regulated in PIA, and/or undergo an intensive proliferative process [68]. Finally, it is of note that in contrast to a recent report using an alternative anti-PROMININ-1 antibody [70] but in keeping with an earlier study [23] we did not detect any PROMININ-1 immunoreactivity in the prostatic stromal compartment. Obviously, further analysis of its general expression using various anti-PROMININ-1 antibodies as well as a larger pool of samples is needed. Thus, it appears important to complete an exhaustive catalog of its expression and regulation (e.g., hypermethylation level of CpG islands within PROMININ-1 promoter [71]) in healthy and affected individuals before initiating a targeting therapeutic intervention based on it [72].

Lastly, the detection of PROMININ-1 in the glandular lumen (e.g., Fig. 6D) deserves a particular comment. Being tightly associated with plasma membrane protrusions, prominin-1 is nevertheless released in various body fluids including saliva, urine, and seminal fluid [45,48,51,73]. Therein, PROMININ-1 is associated with small membrane vesicles that could be recovered upon high-speed centrifugation [48]. Both primary cilium and microvilli have been demonstrated to be the membrane donors of these extracellular vesicles [51,74]. Whether PROMININ-1 is associated with the cytoplasmic vesicles referred to as prostasomes [75,76] remains to be determined. The cytoplasmic detection of PROMININ-1 in the apical part of the prostatic epithelial cells would be consistent with it. Indeed, PROMININ-1 has been already demonstrated to be located, in addition to plasma membrane protrusions, within the cytoplasmic compartment [77]. In any scenarios, its detection in the seminal fluid may provide certain information concerning the progression of prostate diseases as recently shown for those from central nervous system [50]. Thus, PROMININ-1-containing membrane vesicles found in the seminal fluid might become a potential biomarker, and their full characterization for instance at the proteomic level is needed in order to formally establish their cellular and tissue origin.

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