Cell Tissue Res (2005) 319: 15–26
DOI 10.1007/s00441-004-1018-z

REGULAR ARTICLE

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Prominin-1/CD133, a neural and hematopoietic stem cell marker, is expressed in adult human differentiated cells and certain types of kidney cancer

Received: 6 August 2004 / Accepted: 5 October 2004 / Published online: 19 November 2004

Abstract Human prominin-1/CD133 has been reported to be expressed in neural and hematopoietic stem/progenitor cells and in embryonic, but not adult, epithelia. This lack of detection of human prominin-1, as defined by its glycosylation-dependent AC133 epitope, is surprising given the expression of the murine ortholog in adult epithelia. Here, we demonstrate, by using a novel prominin-1 antiserum (αhE2), that the decrease of AC133 immunoreactivity observed during differentiation of the colonic adenocarcinoma-derived Caco-2 cells is not paralleled by a down-regulation of prominin-1. We have also shown that αhE2 immunoreactivity, but not AC133 immunoreactivity, is present in several adult human tissues, such as kidney proximal tubules and the parietal layer of Bowman's capsule of juxtamedullary nephrons, and in lactiferous ducts of the mammary gland. These observations suggest that only the AC133 epitope is down-regulated upon cell differentiation. Furthermore, αhE2 immunoreactivity has been detected in several kidney carcinomas derived from proximal tubules, independent of their grading. Interestingly, in one particular case, the AC133 epitope, which is restricted to stem cells in normal adult tissue, was up-regulated in the vicinity of the tumor. Our data thus show that (1) in adults, the expression of human prominin-1 is not limited to stem and progenitor cells, and (2) the epitopes of prominin-1 might be useful for investigating solid cancers.

Keywords Prominin-1 (CD133) · Epithelial cells · Differentiation · Cancer · Human

This study was supported by grants from the Deutsche Forschungsgemeinschaft (SPP 1109, CO 298/2-1 to D.C., Hu 275/7-1 to W.B.H.; SPP 1111, Hu 275/8-2 to W.B.H.) and the Fonds der Chemischen Industrie (to W.B.H.)

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Introduction

Human prominin-1 (CD133; Fargeas et al. 2003a) was originally identified as a novel cell surface marker (AC133 antigen) expressed on a subpopulation of the $CD34^+$ hematopoietic stem and progenitor cells (Miraglia et al. 1997; Yin et al. 1997) that have the capacity to repopulate xenogeneic transplantation models (Yin et al. 1997; de Wynter et al. 1998). This antigen has also been detected in several malignant hematopoietic diseases, including acute and chronic myeloid lymphoblastic leukemias and myelodysplastic syndrome (Waller et al. 1999; Baersch et al. 1999; Buhring et al. 1999; Green et al. 2000; Wuchter et al. 2001). Since its first characterization, several reports have shown that prominin-1 can be used to identify and isolate human stem cells from various sources, including the hematopoietic system and central nervous system (Yin et al. 1997; Uchida et al. 2000; for a review, see Bhatia 2001).

Human prominin-1 displays, on average, 60% amino acid identity to mouse prominin-1 (Corbeil et al. 1998; Miraglia et al. 1998), a 115-kDa glycoprotein originally described as being expressed on the apical surface of neuroepithelial stem cells and in several other epithelia, including kidney proximal tubules (Weigmann et al. 1997). Prominin-1 belongs to a new family of pentaspan membrane glycoproteins expressed throughout the animal kingdom (Corbeil et al. 2001b; Fargeas et al. 2003a, 2003b). Irrespective of the cell type, prominin-1 is specifically associated with microvilli and other plasma membrane protrusions (Weigmann et al. 1997; Corbeil et

al. 2000; Fargeas et al. 2004; Giebel et al. 2004). Prominin-1 is a cholesterol-binding protein, and recent studies involving morphological, biochemical, and genetic approaches suggest a role of this molecule in the morphogenesis and/or physiology of plasma membrane protrusions (Maw et al. 2000; Röper et al. 2000; Corbeil et al. 2001b).

The expression profile of human prominin-1 is generally similar to that of the murine molecule, being expressed in several embryonic epithelia, including neuroepithelial progenitor cells, and adult hematopoietic stem cells (Weigmann et al. 1997; Corbeil et al. 2000). However, a notable exception has been observed (Miraglia et al. 1997; Weigmann et al. 1997; Corbeil et al. 2000). Whereas rodent prominin-1 is highly expressed in the adult kidney (Weigmann et al. 1997; Corbeil et al. 2001a), the same tissue in humans appears to lack prominin-1 (Miraglia et al. 1997). The apparent absence of prominin-1 in human adult kidney is particularly puzzling given the high expression of its mRNA in this tissue (Miraglia et al. 1997; Corbeil et al. 2000) and the isolation of several expressed sequence tag (EST) clones derived therefrom (GenBank accession numbers AI766048, AW614306, BE501788).

The widespread expression of prominin-1 in human embryonic (Corbeil et al. 2000), but apparently not adult, tissues (Miraglia et al. 1997) may be explained, at least in part, because the monoclonal antibody (mAb) AC133 (Miltenyi Biotech) used to detect prominin-1 recognizes a glycosylated epitope (Miraglia et al. 1997), and the glycosylation of this protein may change depending on the state of cellular differentiation (Corbeil et al. 2000). The glycosylation of prominin-1 could also be altered in cells that have undergone malignant transformation. Such alteration of glycosylation of prominin-1 may explain the apparently discordant expression of prominin-1 in patients with acute myelogenous leukemia and myelodysplastic syndrome that have been observed with two mAbs recognizing distinct glycosylation-dependent epitopes (mAbs AC133 and AC141; Green et al. 2000) and could reflect the different stages of differentiation of these malignant cells. The aim of the present study has been to resolve the apparent discrepancy of prominin-1 expression in adult humans by using a novel antibody that recognizes prominin-1 independently of glycosylation.

Materials and methods

Antiserum against recombinant human prominin-1/CD133

The bacterial expression plasmid pGEX-hE2-prominin-1, containing a cDNA fragment encoding part of the second extracellular domain of human prominin-1 fused in-frame to glutathione S-transferase (GST), was constructed by subcloning the *Eco*RI–*Eco*RI fragment (nucleotides 756–1195) derived from the human prominin-1 cDNA (GenBank accession number AF027208; Miraglia et al. 1997) into the corresponding site of pGEX-2T (Amersham Biosciences, Uppsala, Sweden). The GST-hE2 fusion protein encoded by the pGEX-hE2-prominin-1 plasmid was expressed in HB 101 *E. coli*, purified on glutathione Sepharose 4B beads according to the batch method (Corbeil et al. 1999), and eluted from the column at room temperature by addition of 10 mM glutathione in 50 mM Tris–HCl, pH 8.0. Purified GST-hE2 fusion protein was then used to generate the αhE2 antiserum by immunizing a New Zealand white rabbit.

Cell culture and sample preparation

Chinese hamster ovary (CHO) cells transiently transfected with human prominin-1 cDNA and Caco-2 cells endogenously expressing prominin-1 were cultured as described (Corbeil et al. 2000). $CD34^+$ cells were isolated from G-CSF-mobilized peripheral blood of human healthy donors by using the Direct CD34 Progenitor Cell Isolation Kit (Miltenyi Biotec, Gladbach, Germany). $CD3^+$, $CD19^+$, $CD14^+$, and $CD15^+$ cells were enriched from peripheral blood of human healthy donors by using FACSVantage SE (BD Biosciences; San Jose, Calif.) with appropriate fluorophore-conjugated antibodies ($CD3^+$, $CD19^+$, and $CD14^+$, all from BD Biosciences; $CD15^+$ from Beckman Coulter). Informed consent in all cases was obtained and approved by the institutional review boards. Detergent cell lysates were prepared as described previously (Corbeil et al. 1999). Samples of normal tissue taken some distance (>1 cm) from a tumor region (conventional carcinoma) of kidney were obtained from anonymous archival tissues (Department of Pathology, University of Dresden), and membranes were prepared according to procedures reported previously (Corbeil et al. 2001a). Protein concentrations were determined by using BCA Protein Assay Reagent (Pierce, Rockford, Ill.).

Endoglycosidase digestions and immunoblotting

CHO cell detergent extracts corresponding to one-fifth of an 80%-confluent 60-mm dish, detergent extracts (150 μg protein) derived from Caco-2 cells harvested at various stages of confluency, or membranes from adult human kidney (100 μg protein) were incubated overnight at 37°C in the absence or presence of 10 mU endo-β-*N*-acetylglucosaminidase H (endo H) or 1 U 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 polyvinylidene difluoride membranes (Millipore, Belford, Mass.; pore size: 0.45 μm) by standard procedures (Corbeil et al. 2001a). Immunoblotting was performed essentially as described by Corbeil et al. (2001a) with, as primary antibody, either αhE2 antiserum (1:3,000) or mAb AC133 (1 μg/ml; Miltenyi Biotec, Gladbach, Germany).

Pulse-chase studies and immunoprecipitation

Fourteen-day-old Caco-2 cell monolayers grown on permeable Transwell filters (24-mm Transwell-COL chambers, 0.4-μm pore size) were rinsed once with met/cys-free medium, viz., methionine-free, cysteine-free DMEM supplemented with 1% FCS dialysed against phosphate-buffered saline (PBS), and incubated for 1 h at 37°C in this medium. The cells were then pulse-labeled for 30 min at 37°C with fresh met/cys-free medium containing 350 μCi/ml [^{35}S] Easytag express protein labeling mix (PerkinElmer Life Sciences, Boston, Mass.; 1175.0 Ci/mmol). After the pulse, cells were chased for the indicated times in fresh DMEM supplemented with 5% FCS. At the end of the chase period, the filters were cut into four pieces and incubated in 2 ml ice-cold lysis buffer, viz., 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM TRIS–HCl, pH 8.0, and a protease inhibitor cocktail (Sigma, St. Louis, Mo.), for at least 1 h at 4°C on an end-over-end shaker. Labeled prominin-1 was then immunoprecipitated from the detergent extract obtained after centrifugation (10 min, 10,000*g*, 4°C) by using αhE2 antiserum pre-absorbed to Protein A-Sepharose 4 Fast Flow (Amersham Biosciences, Uppsala, Sweden), followed by SDS-PAGE and phosphoimaging.

In the experiments with unlabeled prominin-1, 10-day-old Caco-2 cell monolayers grown on 100-mm Petri dishes were incubated in 1 ml ice-cold lysis buffer for 1 h at 4°C. Prominin-1 was immunoprecipitated from detergent-cell extracts obtained after centrifugation (10 min, 10,000*g*, 4°C) by using either mAb AC133 (10 μg/ml) or mAb AC141 (10 μg/ml; Miltenyi Biotec) pre-absorbed to Protein G-Sepharose 4 Fast Flow (Amersham Biosciences) and analyzed by immunoblotting with αhE2 antiserum (see above).

Immunofluorescence of Caco-2 cells

Confluent Caco-2 cells grown on glass coverslips were washed with PBS and fixed with 4% paraformaldehyde in PBS for 30 min at room temperature. Coverslips were then rinsed and incubated for 10 min in PBS containing 50 mM ammonium chloride. Fixed cells were incubated in SDS buffer (0.005% SDS and 0.2% gelatin in PBS) for 1 h at room temperature and then washed with PBS containing 0.2% gelatin for 10 min to remove the residual SDS. Cells were then double-labeled for 30 min at room temperature with αhE2 antiserum (1:500) and mouse mAb AC133 (1 μg/ml) followed by Cy3-conjugated goat anti-rabbit IgG (H + L) and Cy2-conjugated goat anti-mouse IgG (H + L; Jackson ImmunoResearch Labs, West Grove, Pa.), all diluted in PBS containing 0.2% gelatin. Coverslips were rinsed and mounted in Moviol 4.88 (Calbiochem). The samples were observed with an Olympus BX61 microscope.

Immunohistochemistry of human adult tissues

Samples from various types of kidney cancer and normal tissues of kidney and mammary glands taken some distance (>1 cm) from tumor regions came from the Department of Pathology (University of Dresden) and were archival materials that had not been used for genetic analysis. They were fixed in 4% buffered formaldehyde (pH 8.0) for 24 h at room temperature, dehydrated with increasing concentrations of ethanol (70%, 80%, 2×96%, 2×100%) for 1 h each at 37°C, and then treated twice with xylene (Carl Roth GmbH+Co, Germany) for 45 min at room temperature. The dehydrated samples were incubated in paraffin for 1.5 h at 60°C and then for an additional 2.5 h with fresh paraffin. Finally, they were embedded in tissue blocks and stored at room temperature. Thin sections (4 μm) were cut, mounted on silanized slides, and dried for 3 h at 37°C or overnight at room temperature. They were deparaffinized overnight by xylene treatment, hydrated with decreasing concentrations of ethanol (2×100%, 95%, 80%, 70%, 45%) for 1 min (each at room temperature), and then rinsed with distilled water (Millipore) for 5 min. The samples were washed with PBS containing 0.3% Tween 20 for 10 min, placed in blocking solution (10% FCS, 0.2% saponin in PBS) for 15 min at room temperature, and then incubated for 1 h at 37°C with αhE2 antiserum (1:500) and mAb AC133 (1 μg/ml) followed by Cy3-conjugated goat anti-rabbit IgG (H + L) and Cy2-conjugated goat anti-mouse IgG (H + L), all diluted in blocking solution. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI; 1 μg/ml; Molecular Probes) for 10 min at room temperature. The sections were rinsed, mounted in Moviol 4.88, and examined with a fluorescence microscope (Olympus BX61). The images shown were prepared from IPLAB data files (version 3.5) by using Adobe photoshop software.

In experiments with immunoperoxidase, sections were deparaffinized by two successive xylene treatments (10 min each) and then hydrated with decreasing concentrations of ethanol. Cells were permeabilized, and sections were blocked in blocking solution for 20 min. Endogenous peroxidase was neutralized with 1% H_2O_2 for 15 min. After being washed with PBS containing 0.3% Tween 20, the sections were incubated sequentially with either αhE2 antiserum (1:500) or mAb AC133 (1 μg/ml) followed by peroxidase-coupled goat anti-rabbit or goat anti-mouse antibody IgG (1:300; Jackson) for 1 h at 37°C. Color reactions were performed with the peroxidase substrate DAB (3,3′-diaminobenzidine tablet sets, 0.7 mg/ml; Sigma) according to the manufacturer's protocol. All sections were counterstained with Mayer's hematoxylin (Merck). Stained sections were observed with an Olympus BX61 microscope.

mRNA expression analysis

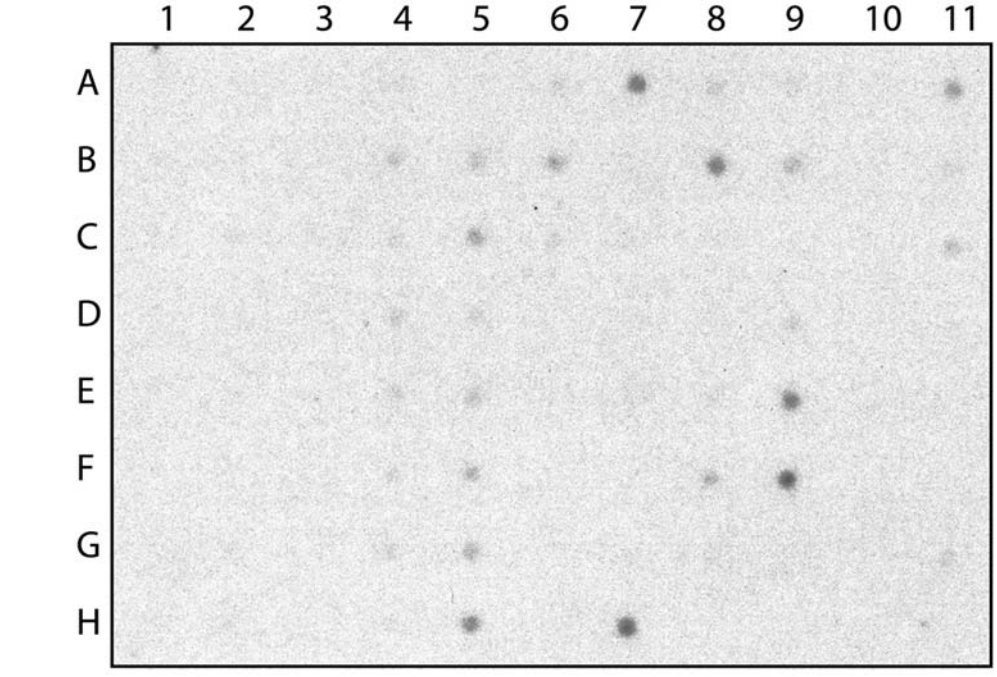
Northern dot blot analysis was performed by using the Human Multiple Tissue Expression (MTE) Array mem-

brane (Clontech, catalog number 7775-1). The amount of poly(A)$^+$ RNA in each dot of the array was normalized by the manufacturer to yield similar hybridization signals for eight housekeeping genes. The amounts of mRNA in each dot varied from 50 ng to 956 ng, and the array allowed the comparative analysis of gene expression in various tissues. The blot was hybridized with a human prominin-1 [α-^{32}P] dCTP-labeled probe (Corbeil et al. 2000) for 6 h at 65°C and washed according to the protocol of Clontech Laboratories (manual PT3307-1). The blot was exposed for 2 days at −80°C.

Results

Prominin-1/CD133 mRNA is widely distributed in adult human tissues

We first examined the relative abundance of human prominin-1 mRNA in 76 different tissues and tumor cell lines by using a multiple tissue expression array (Fig. 1). Human prominin-1 mRNA was found to be strongly expressed in the adult kidney (Fig. 1, A7), mammary gland (F9), trachea (H7), salivary gland (E9), and placenta



	1	2	3	4	5	6	7	8	9	10	11
A	whole brain	cerebellum, left	substantia nigra	heart	esophagus	colon, transverse	kidney	lung	liver	leukemia, HL-60	fetal brain
B	cerebral cortex	cerebellum, right	accumbens nucleus	aorta	stomach	colon, desending	skeletal muscle	placenta	pancreas	HeLa S3	fetal heart
C	frontal lobe	corpus callosum	thalamus	atrium, left	duodenum	rectum	spleen	bladder	adrenal gland	leukemia, K-562	fetal kidney
D	parietal lobe	amygdala	pituitary gland	atrium, right	jejunum		thymus	uterus	thyroid gland	leukemia, MOLT-4	fetal liver
E	occipital lobe	caudate nucleus	spinal cord	ventricle, left	ileum		peripheral blood leukocyte	prostate	salivary gland	Burkitt's lymphoma, Raji	fetal spleen
F	temporal lobe	hippo-campus		ventricle, right	ileocecum		lymph node	testis	mammary gland	Burkitt's lymphoma, Daudi	fetal thymus
G	p. g.* of cerebral cortex	medulla oblongata		inter-ventricular septum	appendix		bone marrow	ovary		colorectal adeno-carcinoma, SW480	fetal lung
H	pons	putamen		apex of the heart	colon, ascending		trachea			lung carcinoma, A549	

Fig. 1 Tissue distribution of human prominin-1/CD133 mRNA. Poly(A)$^+$ RNA from 76 human tissues and cell lines (see *bottom panel*), normalized for various housekeeping genes, was hybridized with a radiolabeled probe specific for prominin-1 (*top panel*)

(B8). Prominin-1 mRNA was also detected in pancreas (B9) and testis (F8) and all along the digestive tract (column 5 and 6 A–C) with the notable exception of the esophagus (A5), in agreement with our previous study (Fargeas et al. 2003b). In fetal tissues, prominin-1 transcript was observed in brain (A11) and kidney (C11). No expression was evident in any of the eight tumor cell lines (column 10). The wide distribution of human prominin-1 mRNA in adult tissues was consistent with previous studies (Miraglia et al. 1997; Corbeil et al. 2000) and the isolation of several EST clones from various adult tissues (see UniGene Cluster Hs.112360 at NCBI; http://www.ncbi.nlm.nih.gov) but contrasted with the apparently restricted expression of the prominin-1 protein as revealed by mAb AC133 (Miraglia et al. 1997).

Generation and characterization of an antiserum directed against the human prominin-1/CD133 polypeptide

To be able to investigate the expression of the prominin-1 protein in adult human tissues with tools other than mAb AC133, we generated a rabbit antiserum, designated αhE2, against a human prominin-1 fragment corresponding to amino acid residues Gly_{240}–Ser_{388}. In CHO cells transiently transfected with human prominin-1 cDNA, immunoblotting with αhE2 antiserum revealed two bands with apparent molecular masses of ≈120 kDa (Fig. 2a, lane 1, arrowhead) and ≈105 kDa (Fig. 2a, lane 1, asterisk). No immunoreactivity was detected when CHO cells were transfected with the expression vector alone (data not shown). The two-band pattern of human prominin-1 was reminiscent of that of mouse prominin-1 ectopically expressed in CHO cells (Weigmann et al. 1997; Maw et al. 2000) and MDCK cells (Corbeil et al. 1999; Röper et al. 2000). The 105-kDa form of human prominin-1 was sensitive to digestion with endo H (Fig. 2a, lane 2) and therefore represented the high-mannose form localized in the endoplasmic reticulum and/or early Golgi compartment. The 120-kDa form of human prominin-1 was resistant to endo H (Fig. 2a, lane 2), indicating that it had passed through the Golgi apparatus. PNGase F treatment converted both the 120-kDa and 105-kDa forms of recombinant human prominin-1 into a product of ≈92 kDa (Fig. 2a, lane 3, arrow), indicating that the αhE2 antiserum recognizes both the *N*-glycosylated-forms and deglycosylated-forms of recombinant prominin-1. Likewise, the αhE2 antiserum also detected human prominin-1 endogenously expressed by the colon-derived epithelial Caco-2 cells (Corbeil et al. 2000; Fig. 2a, lanes 4–6) and leukemia MUTZ-2 cells (Kratz-Albers et al. 1998; data not shown).

Direct evidence showing that the αhE2 antiserum recognized the same protein that carried the AC133 and AC141 epitopes (Miraglia et al. 1997) was obtained by immunoprecipitation of prominin-1 with either mAb AC133 or mAb AC141 from Caco-2 cell lysates followed by immunoblotting with αhE2 antiserum (Fig. 2b, lanes 2 and 3, respectively).

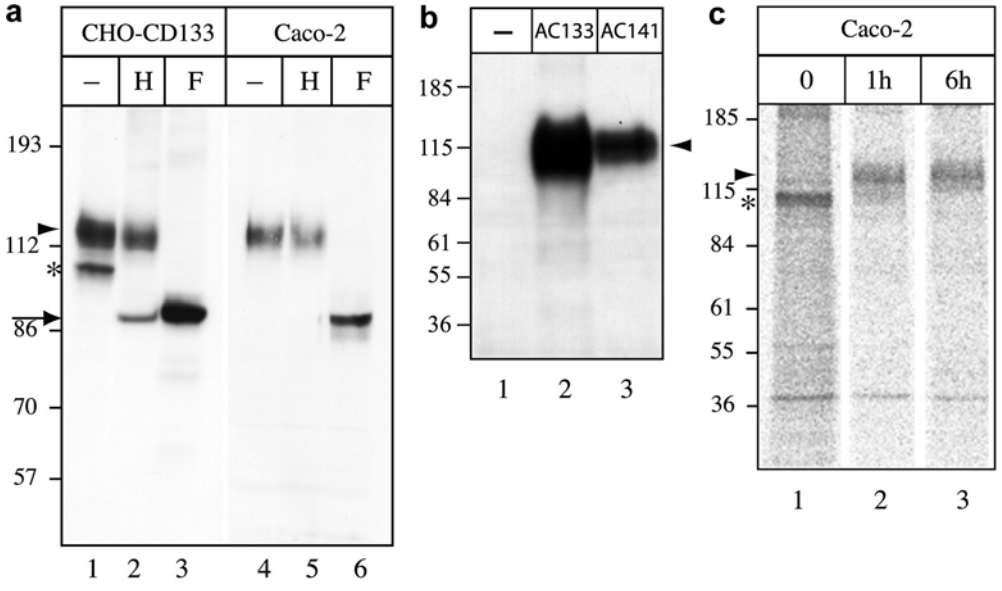


Fig. 2 Characterization of the αhE2 antiserum directed against human prominin-1/CD133. **a** αhE2 antiserum recognizes human prominin-1. Lysates of either prominin-1-transfected CHO cells (CHO-CD133) or Caco-2 cells were incubated in the absence (−) or presence of 10 mU endo H (*H*) or 1 U PNGase F (*F*) and analyzed by immunoblotting with αhE2 antiserum (*arrowhead* endo-H-resistant PNGase-F-sensitive 120-kDa form of human prominin-1, *asterisk* endo-H-sensitive PNGase-F-sensitive 105-kDa form, *arrow* 92-kDa product after *N*-deglycosylation). **b** αhE2 antiserum recognizes the same molecule as the monoclonal antibodies AC133 and AC141. Prominin-1 (*arrowhead*) was immunoprecipitated from a lysate of Caco-2 cells without (−) or with either mAb AC133 (*AC133*) or mAb AC141 (*AC141*) and analyzed by immunoblotting with αhE2 antiserum. **c** αhE2 antiserum immunoprecipitates both the immature and mature forms of prominin-1. Caco-2 cells were pulse-labeled for 30 min with [^{35}S]methionine/cysteine and chased for the indicated times. After immunoprecipitation of prominin-1 with αhE2 antiserum, labeled proteins were analyzed by SDS-PAGE and phosphoimaging (*arrowhead* 120-kDa form of prominin-1, *asterisk* 105-kDa form of prominin-1)

Pulse-chase experiments performed with Caco-2 cells showed that the αhE2 antiserum immunoprecipitated the newly synthesized, 105-kDa form of prominin-1 (Fig. 2c, lane 1, asterisk) and the mature, 120-kDa form of prominin-1 (Fig. 2c, lanes 2 and 3, arrowhead).

In the hematopoietic lineage, expression of prominin-1/CD133 is limited to $CD34^+$ stem and progenitor cells. In agreement with the expression of prominin-1 in hematopoietic stem and progenitor cells (Yin et al. 1997), MACS-isolated $CD34^+$ stem cells derived from mobilized peripheral human blood express the 120-kDa form of prominin-1, as revealed by immunoblotting with αhE2 antiserum (Fig. 3, lane 1, arrowhead). None of the other blood-derived cells enriched by FACS with the corresponding CD marker, such as T cells ($CD3^+$), B cells ($CD19^+$), granulocytes ($CD14^+$), and monocytes ($CD15^+$), show detectable αhE2 immunoreactivity (Fig. 3, lanes 2 to 6).

Expression of the human prominin-1/CD133 protein, but not AC133 immunoreactivity, in differentiated Caco-2 cells

We have previously reported that, in the human colon-derived epithelial cell line Caco-2, the expression of prominin-1, as revealed by AC133 immunoreactivity, is down-regulated after differentiation of the cells (Corbeil et al. 2000). This enterocytic differentiation of Caco-2 cells is a growth-related process that starts about 7 days after the cells reach confluence and is complete within 20–30 days (Pinto et al. 1983). Interestingly, in contrast to the decrease of AC133 immunoreactivity, the prominin-1 transcript appears to be up-regulated under the same conditions (Corbeil et al. 2000), suggesting that the loss of AC133 immunoreactivity reflects either an inhibition of prominin-1 translation or a loss of the AC133 epitope, which is thought to be dependent, at least in part, on glycosylation (Miraglia et al. 1997), because of the alternative processing of asparagine-linked oligosaccharides upon differentiation. Such differential glycosylation has been previously reported (Ogier-Denis et al. 1988). To address this issue, we have analyzed the expression of prominin-1 before and after differentiation of Caco-2 cells by using αhE2 antiserum.

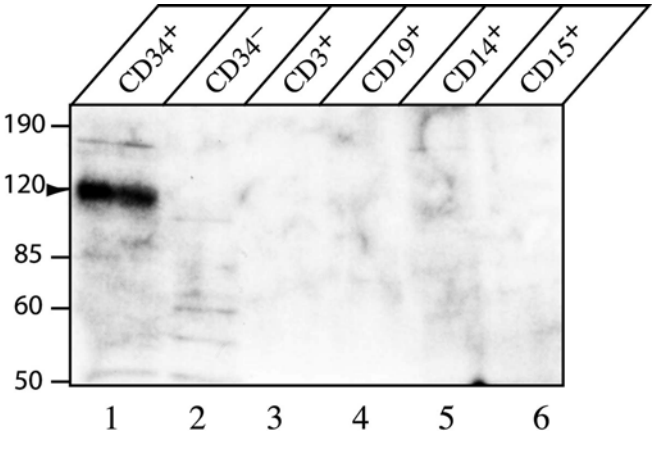


Fig. 3 Expression of prominin-1/CD133 in $CD34^+$ stem and progenitor cells, but not in other cells of the hematopoietic lineage. Lysates of human $CD34^+$ cells (3×10^5 cells) isolated from G-CSF-mobilized peripheral blood of healthy human donors and, as a control, cells derived from the flow-through $CD34^-$ fraction were analyzed by immunoblotting with αhE2 antiserum. Lysates of $CD3^+$, $CD19^+$, $CD14^+$, and $CD15^+$ cells enriched from peripheral blood of healthy human donors by using FACS were also examined (*arrowhead* 120-kDa form of human prominin-1)

Whereas the αhE2 antiserum, upon cell surface labeling of Caco-2 cells, did not recognize the native form of prominin-1 (data not shown), it did reveal, after treatment of fixed cells with a low concentration of SDS (0.005%), the presence of prominin-1 on the apical plasma membrane of both undifferentiated and differentiated Caco-2 cells (Fig. 4a, top and bottom right panels, respectively). The authenticity of this immunostaining was confirmed by the identical pattern observed with the mAb AC133 in undifferentiated cells (Fig. 4a, top panels, see insets) and by its dependence on the presence of prominin-1 cDNA upon transfection of CHO cells (data not shown). As previously reported (Corbeil et al. 2000), AC133 immunoreactivity was down-regulated in differentiated Caco-2 cells (Fig. 4a, left bottom panel).

To corroborate that the persistence of αhE2 immunoreactivity in differentiated Caco-2 cells, as observed by immunofluorescence microscopy, was attributable to prominin-1, we performed immunoblotting experiments with Caco-2 cells harvested at various days after reaching confluence. As shown in Fig. 4b, the 120-kDa form of prominin-1 detected in undifferentiated Caco-2 cells was still observed after differentiation (Fig. 4b, top panel, arrowhead). Quantification revealed that more than 70% of αhE2 immunoreactivity observed in undifferentiated cells (day 2 or 4) persisted in older differentiated cultures (e.g., day 22 or 23; Fig. 4c). Together, these data showed that the loss of AC133 immunoreactivity upon differentiation of Caco-2 cells was not the result of a down-regulation of the prominin-1 protein.

Expression of prominin-1/CD133 in adult human kidney and mammary gland

Given the expression of human prominin-1 in differentiated Caco-2 cells, we investigated its expression in adult human epithelia in vivo. Adult human kidney membranes were analyzed by immunoblotting with either mAb AC133 or αhE2 antiserum. No immunoreactivity was detected with mAb AC133 (Fig. 5a, lanes 1, 2). In contrast, a band with an apparent molecular mass of ≈120 kDa was detected with αhE2 antiserum (Fig. 5a, lane 3, arrowhead). *N*-deglycosylation with PNGase F yielded a band of ≈92 kDa (Fig. 5a, lane 4, arrow). These apparent molecular masses were those expected for the *N*-glycosylated and deglycosylated forms of human prominin-1 (see Fig. 2a), indicating that this membrane glycoprotein was indeed expressed in adult human epithelial tissue.

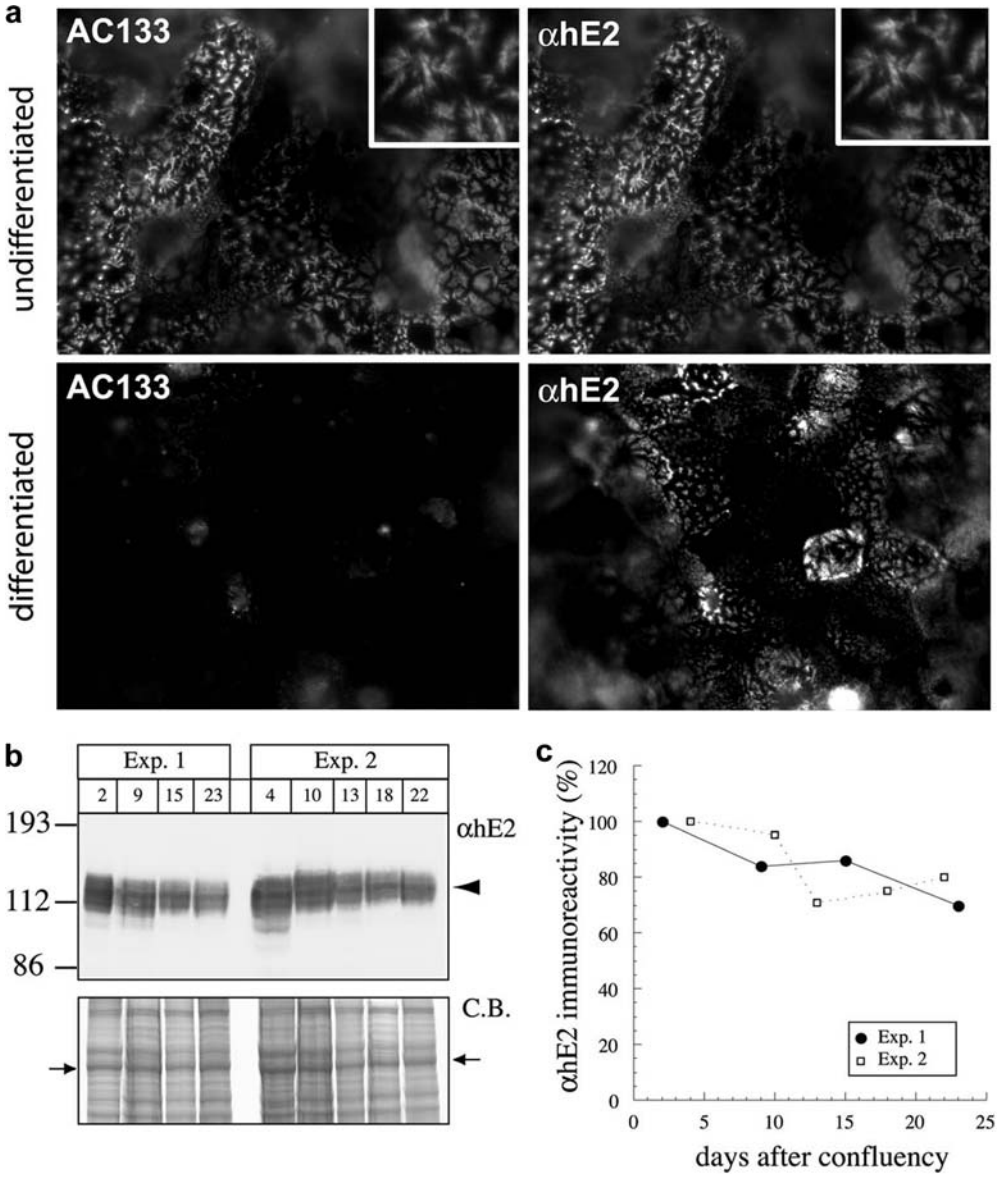


Fig. 4 AC133 immunoreactivity, but not the prominin-1/CD133 polypeptide, is down-regulated upon differentiation of Caco-2 cells. **a** The loss of AC133 immunoreactivity in differentiated Caco-2 cells is not matched by a loss of αhE2 immunoreactivity. Five-day-old (*top panels*) or 20-day-old (*bottom panels*) post-confluent Caco-2 cells grown on glass coverslips were paraformaldehyde-fixed, SDS-treated, double-labeled with mAb AC133 (*left panels*) and αhE2 antiserum (*right panels*), and analyzed by double immunofluorescence. Note the co-localization of AC133 immunoreactivity and αhE2 immunoreactivity at the apical plasma membrane of non-differentiated cells, consistent with both antibodies recognizing the same protein (*insets* high magnification). ×10 (*insets* ×20) **b**, **c** Expression of the prominin-1 polypeptide persists in differentiated Caco-2 cells. Lysates of Caco-2 cells harvested at the indicated days after reaching confluence were analyzed by immunoblotting with the αhE2 antiserum (*top panel*, *αhE2*). In each sample, the total amount of protein (≈150 μg) was visualized by Coomassie-blue staining (*bottom panel*, *C.B.*). Two independent experiments (*Exp. 1, Exp. 2*) were performed, each with a pool of cells as starting material (*arrowhead* 120-kDa form of human prominin-1, *arrows* reference band used for the quantification shown in **c**). **c** Quantification of αhE2 immunoreactivity during differentiation of Caco-2 cells. αhE2 immunoreactivity detected in **b** was quantified by densitometric scanning, normalized to the amount of protein detected by Coomassie-blue staining (reference band indicated by *arrows* in **b**), and plotted as a percentage of the αhE2 immunoreactivity detected in undifferentiated Caco-2 cells, i.e., day 2 and 4 for the first (*Exp. 1*) and second (*Exp. 2*) experiment, respectively

Immunohistological analysis of adult human kidney revealed αhE2 immunoreactivity on the apical, but not basolateral, side of epithelial cells lining proximal tubules (Fig. 5b, αhE2) and the parietal layer of Bowman's capsule (Fig. 5c, αhE2) of nephrons located in the juxtamedullary region of the cortex. The vast majority of nephrons in the outermost region of the cortex were unstained (data not shown). No staining was observed in distal tubules or in collecting duct epithelia. Hence, the expression and localization of prominin-1 in adult human kidney was similar to that in adult mouse kidney (Weigmann et al. 1997). As previously reported (Miraglia et al. 1997), no specific prominin-1 immunoreactivity

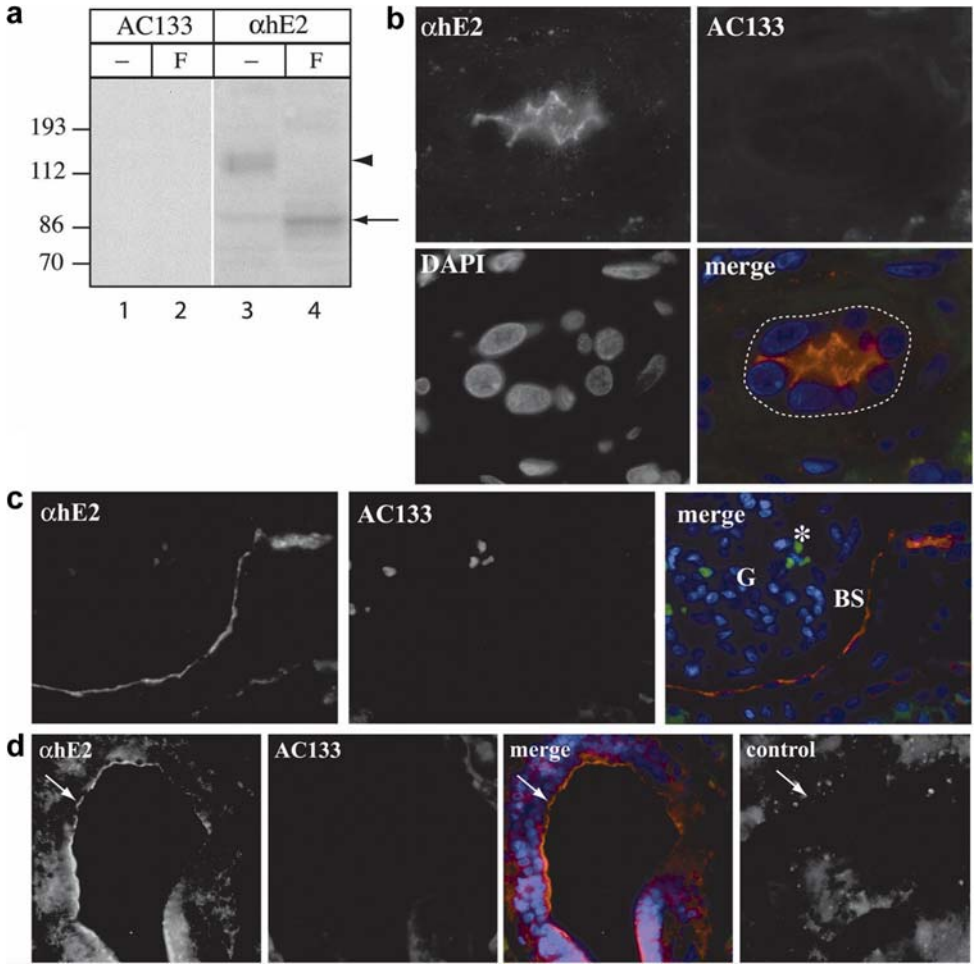


Fig. 5 Characterization and localization of prominin-1/CD133 in adult human kidney and mammary gland. **a** Prominin-1 in adult human kidney is a 120-kDa glycoprotein. Proteins (≈100 μg) solubilized from adult human kidney membranes were incubated in the absence (−) or presence (*F*) of 1 U of PNGase F and analyzed by immunoblotting with either the AC133 (*AC133*) mAb or the αhE2 (*αhE2*) antiserum (*arrowhead* native 120-kDa form of prominin-1, *arrowN*-deglycosylated 92-kDa form of prominin-1). **b, c** Transverse sections of human adult kidney were double-labeled with αhE2 antiserum (*red*, *αhE2*) and mAb AC133 (*green*, *AC133*) and analyzed by immunofluorescence microscopy. Nuclei were stained with DAPI (*blue*). **b** Prominin-1 is detected at the apical side of kidney proximal tubules. In the merged image (*merge*), the basal side of the epithelial cells is indicated by a *dotted line*. ×10. **c** Prominin-1 is present at the parietal layer of Bowman's capsule (*asterisk* background without the primary and secondary antibodies, *BS* Bowman's space, *G* glomerulus). ×10. **d** Transverse sections of mammary gland were double-labeled with αhE2 antiserum (αhE2, *red*) and mAb AC133 (AC133, *green*) or, as a control, without primary antibodies, and analyzed by immunofluorescence microscopy. Nuclei were stained with DAPI (*blue*). Prominin-1 is detected at the apical side of lactiferous ducts in the female human mammary gland (*arrows* apical side of the lactiferous duct). Note that, despite the background of the secondary antibody, apical staining is absent in the control. ×10

could be detected in the adult kidney with mAb AC133 (Fig. 5b, c, AC133; data not shown).

The multiple tissue expression array analysis (Fig. 1) suggested that the expression of prominin-1 in adult humans might extend to epithelial tissues other than the kidney. To address this issue, we analyzed the expression of the prominin-1 protein in the mammary gland, a tissue with a high prominin-1 mRNA level (Fig. 1, F9). Immunohistochemistry revealed αhE2, but not AC133, immunoreactivity on the apical side of lactiferous ducts (Fig. 5d, αhE2).

Differential αhE2 versus AC133 immunoreactivity in human kidney cancer

The data presented above prompted us to examine the expression of prominin-1 in various types of human kidney cancer by immunohistochemistry. Interestingly, an analysis of 12 individual tumors revealed differential immunoreactivity depending on the site within the cancer tissue and whether the αhE2 antiserum or mAb AC133 was used (Table 1). Five of eight tumors thought to be derived from proximal tubules (conventional and papillary carcinoma) showed αhE2, but not AC133, immunoreactivity in the tumor region, independent of their grading (Table 1, samples 1–8; an example is shown in Fig. 6a, b, region T). Remarkably, in one of them, i.e., sample 3, we

Table 1 Expression of prominin-1 in various human kidney cancer samples (+ presence or − absence of prominin-1 immunoreactivity in the tumor region)

Sample number	Cytomorphology of tumor	Grading	Age (year)	Sex	Prominin-1 immunoreactivity	
					αhE2	AC133
1	Conventional carcinoma (clear cell carcinoma)	G2	66	M	−	−
2		G3	65	M	+	−
3		G2–G3	68	F	+	−[a]
4	Papillary carcinoma	G2	93	F	+	−
5		G1–G2	70	M	+	−
6		G2	55	M	−	−
7		G2	83	F	+	−
8		G2	76	M	−	−
9	Chromophobe carcinoma	G2	65	F	−[b]	−
10		G1	45	F	−	−
11	Collecting duct carcinoma	G2	62	M	−	−
12		G3	74	M	−	−

[a] AC133 immunoreactivity was observed in the vicinity of the tumor region

[b] αhE2 immunoreactivity was found in all tubules of the surrounding normal tissue

also detected AC133 immunoreactivity in the intermediate (I) region between the tumor (T) and cytomorphologically normal (N) tubules (Fig. 6b, region I, arrow). In this particular region, αhE2 and AC133 immunoreactivities were observed at the apical side of the parietal layer of Bowman's capsule (Fig. 6c, top panels, arrows) and proximal tubules (Fig. 6c, bottom panels, arrows). αhE2 immunoreactivity was observed in the cytomorphologically normal region (Fig. 6a, region N, arrows), but no AC133 immunoreactivity was seen (Fig. 6b, region N), as previously demonstrated for the healthy kidney sample (Fig. 5b, c). Both of the chromophobe renal cell carcinomas thought to be derived from distal tubules were negative for the αhE2 antiserum and mAb AC133 in the microscopically defined tumor region (Table 1, samples 9–10), but in one of them, αhE2 immunoreactivity was found in all tubules of the surrounding normal tissue (sample 9; data not shown), whereas in normal kidney, not all tubules were stained (data not shown). Both of the collecting duct carcinomas were negative for the αhE2 antiserum and mAb AC133 in the tumor region (Table 1; samples 11–12).

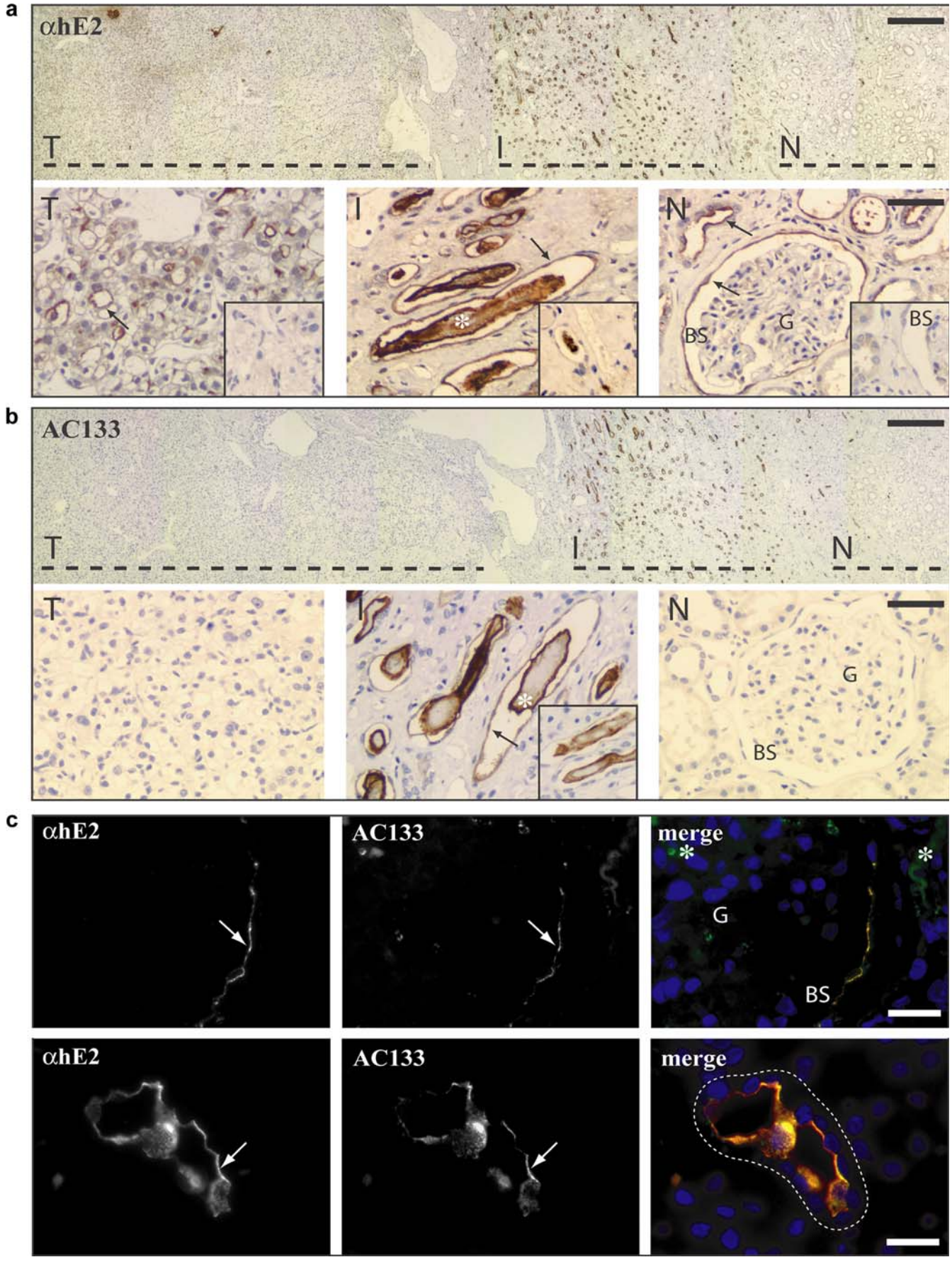
Discussion

Two major points can be made in light of the results of the present investigation. First, the expression of prominin-1 in adult human tissues is more widespread than previously assumed from studies with the mAb AC133. Second, in cancer originating from prominin-1-expressing epithelial cells, the presence or absence of prominin-1 and of the AC133 epitope may provide information about the state of de-differentiation of the tumor.

Specifically, the expression of prominin-1 protein in adult humans is not limited to the stem and progenitor cells (Miraglia et al. 1997; Yin et al. 1997). Like murine prominin-1 (Weigmann et al. 1997; Maw et al. 2000), human prominin-1 is expressed in epithelial cells (this study) and cells derived therefrom, such as photoreceptor cells (M. Florek and D. Corbeil, unpublished). The glycosylation-dependent AC133 epitope of human prominin-1 appears to be present only on a subset of molecules, i.e., those specifically expressed in hematopoietic stem and progenitor cells (Yin et al. 1997), embryonic epithelia (Corbeil et al. 2000; Uchida et al. 2000), and cells de-differentiating in the process of malignant transformation (Green et al. 2000; this study), but not in differentiated adult epithelia, such as kidney (Miraglia et al. 1997; this study; Fig. 7). Consistent with this, the AC133 epitope is present on undifferentiated Caco-2 cells (Corbeil et al. 2000) but is lost upon the differentiation of these cells, although the expression of the prominin-1 protein as such persists (Fig. 4). Therefore, the term "AC133 antigen" should not be used synonymously with prominin-1 or CD133 (Fargeas et al. 2003a). Nonetheless, within the hematopoietic lineage, the human prominin-1 protein is exclusively expressed by primitive stem and progenitor cells (Fig. 3) and does indeed constitute a cell surface hematopoietic stem cell marker, as originally concluded from the expression of the AC133 antigen (Miraglia et al. 1997; Yin et al. 1997).

Together with previous studies, the present data indicate that human and mouse prominin-1 share the same properties, i.e., (1) a pentaspan membrane topology (Miraglia et al. 1997; Weigmann et al. 1997); (2) a widespread distribution in embryonic and adult tissues (Weigmann et al. 1997; Yin et al. 1997; Maw et al. 2000; the present study); (3) their targeting to the apical domain of epithelial cells (Weigmann et al. 1997; Corbeil et al. 1999, 2000), and (4) their concentration in plasma membrane protrusions (Weigmann et al. 1997; Corbeil et al. 2000). Thus, human and mouse proteins clearly represent an orthologous pair, and the open question regarding their relationship (Corbeil et al. 1998; Miraglia et al. 1998) has been answered. Hence, the murine model, including the valuable mAb 13A4 directed against mouse prominin-1 (Weigmann et al. 1997), should constitute a useful and complementary system for studying hematopoiesis.

In both human and mouse, prominin-1 is confined to the brush-border membrane of the kidney proximal tubules and is absent in the distal tube and collecting duct epithelia. The lack of prominin-1 in these epithelial cells, which also contain microvilli, is surprising considering the postulated function of prominin-1, i.e., organizing functional plasma membrane protrusions (Corbeil et al. 2001b). However, we have recently identified prominin-2, a paralog of prominin-1 (Fargeas et al. 2003b). Prominin-2 shows a similar tissue distribution to prominin-1, being highly expressed in the adult kidney and detected all along the digestive tract and in various other epithelia (Fargeas et al. 2003b). Preliminary data from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◀ **Fig. 6** Expression of prominin-1/CD133 and its AC133 epitope in a conventional carcinoma. **a**–**c** Transverse sections of a human kidney sample were labeled with either αhE2 antiserum (**a**) or mAb AC133 (**b**) followed by the peroxidase-coupled secondary antibody and counterstaining with hematoxylin or were double-labeled (**c**) with both αhE2 antiserum (*red*) and mAb AC133 (*green*) and observed by immunofluorescence microscopy (*arrows* prominin-1 immunoreactivity, *asterisks* background observed without the primary and secondary antibodies, *BS* Bowman's space, *G* glomerulus). In **c**, nuclei were stained with DAPI (*blue*). **a**, **b** Sample tissue was divided in three cytomorphologically regions, i.e., tumor (*T*), intermediate (*I*), and normal (*N*) regions from which a high-magnification image is shown. Negative controls, i.e., pre-immune serum (**a**) or without the primary antibody (**b**), are shown in the *insets*. **c** The intermediate region is shown at high magnification (*dotted line* the basal side of the epithelial cells lining the proximal tubule). *Bars* 350 μm (**a**, **b**, *top panel*,; 50 μm (**a**, **b**, *bottom panel*), 30 μm (**c**)

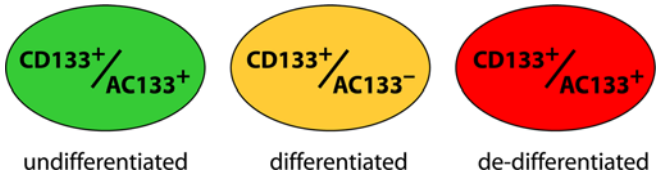

Fig. 7 Expression of the glycosylation-dependent AC133 epitope of prominin-1/CD133 according to the status of cell differentiation —a model

epithelial Madin–Darby canine kidney cells transfected with prominin-2-GFP have revealed that prominin-2 is also found in microvilli (M. Florek and D. Corbeil, unpublished). Therefore, the absence of prominin-1 in a given epithelial cell type does not necessary correspond to the lack of a prominin molecule. Prominin-2 may exert a similar function and hence compensate for the lack of prominin-1 in certain epithelia. Further studies are needed to determine the complete expression pattern of prominin-2 and eventually the role of prominins in kidney physiology.

Given the apparently inverse correlation between the expression of the AC133 epitope and cell differentiation, we have investigated the possibility of exploiting immunoreactivity to αhE2 and AC133 as indicators of de-differentiation of cells endogenously expressing prominin-1. With regard to human kidney cancer, our pilot screen shows that prominin-1, as detected by αhE2 immunoreactivity, is expressed in several cancers thought to be derived from proximal tubules, e.g., conventional carcinoma. This differential expression profile of prominin-1 might provide new information about the biology of tumors arising from one histological family. In one particular case, the up-regulation of the AC133 epitope in cells in the vicinity of a conventional carcinoma is particularly interesting as it is consistent with the notion that tumor progression is associated with cell de-differentiation, such as a change in glycosylation toward a state characteristic of stem/progenitor cells as in the present case of the AC133 epitope (see Fig. 7). The AC133-positive cells may represent a unique population of cancer stem cells that possess the ability to proliferate and maintain their self-renewal capacity extensively, whereas the αhE2-positive cells detected in the tumor region may have lost this ability and are therefore negative for AC133 immunoreactivity. This agrees with the cancer stem cell hypothesis that suggests that not all cells in a tumor have the same capacity for proliferation (Reya et al. 2001). Expression data on the AC133 epitope could therefore be useful tools in the diagnosis and monitoring of malignant disease. Clearly, the clinical significance of prominin-1 and the AC133 epitope is not limited to the hematopoietic field (Handgretinger et al. 2003; Bornhäuser et al. 2004). Large-scale studies are warranted to determine the application of prominin-1 with its various epitopes as a novel prognostic and/or predictive clinical marker.

Acknowledgements We thank D. Buck for the human prominin-1 cDNA, G. Baretton for human tissue samples, I. Peterson for her excellent sample preparation, I. Nüβlein for her valuable assistance with FACS, and S. Langer for valuable technical assistance with some immunohistochemistry experiments.

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