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Prominin-1 (CD133): from progenitor cells to human diseases

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Prominin-1 (CD133) is a pentaspan membrane glycoprotein that binds to plasma membrane cholesterol and concentrates selectively in plasma membrane protrusions. In recent years, this molecule has received considerable interest due to its expression in various progenitors, including those derived from the neural and hematopoietic system, as well as in cancer originating from these systems. Prominin-1 is also the target of mutations leading to retinal degeneration. In the future, prominin-1-positive progenitor cells might become clinically significant, particularly with regard to tissue engineering, and prominin-1 itself might reveal some fundamental cell biological aspects concerning the self-renewal capacity of somatic stem cells.

Biomedical research has recently been focusing on the biological characterization of stem and progenitor cells. Understanding, and eventually controlling, the self-renewal capacity of these cells as well as their ability to differentiate into mature cells could lead to the development of new cell-based therapeutic strategies. A considerable research effort is thus being made in order to identify the key players responsible for the proliferation, self-renewal and differentiation of stem and progenitor cells. The current strategy to isolate these rare cell subpopulations is based mainly on monoclonal antibodies directed against specific cell surface markers.

Searching for such stem cell surface markers, a novel molecule named prominin-1 (also referred to as CD133 [1] and, in the case of the human molecule, as AC133 antigen [2]) has been described in two independent studies as an antigen present on the surface of mouse neuroepithelial progenitors [3] and human hematopoietic stem and progenitor cells [2,4]. Prominin-1 is a cholesterol-binding protein [5] selectively associated with plasma membrane protrusions. Its intrinsic preference for these plasma membrane subdomains motivated the choice of the name ‘prominin’ (from the Latin word *prominere*) for the first characterized member of a novel family of pentaspan membrane glycoproteins [1,3]. This review will focus on the current understanding of this molecule as well as its impact as a marker of primitive cells.

Keywords: AC133 antigen, bone marrow transplantation, cancer, CD133, cholesterol, hematopoietic, lipid raft, progenitors, prominin-1, retinal degeneration, stem cell marker

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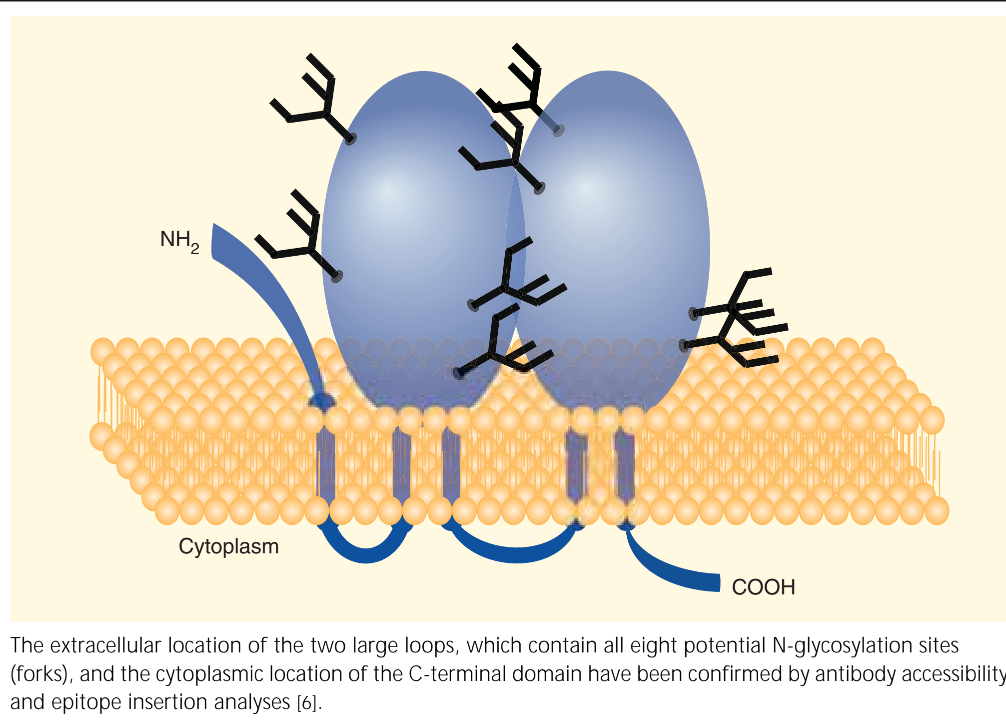
Prominin-1: basic facts

Prominin-1 (CD133) is a 115/120-kDa glycoprotein with a characteristic topology that is unique among the multispan membrane proteins [3,4,6,7]. It contains five membrane-spanning domains, with the N-terminal domain exposed to the extracellular milieu followed by four, alternating short cytoplasmic and large glycosylated extracellular (>250 amino acid) loops and a cytoplasmic C-terminal domain **(Figure 1)** [3,4]. Recently, a second pentaspan membrane glycoprotein related structurally to prominin-1 and encoded by a distinct gene has been characterized in mammals [8]. Predicted prominin-like proteins are found in other vertebrates, including chicken, fish [8] and axolotl [Jászai J and Corbeil D, Unpublished Data], and in invertebrates, including fly and worm [3,8,9]. While occurring throughout metazoan evolution, prominins are absent from unicellular eukaryotes and prokaryotes, suggesting a physiological role related to some aspect of multicellularity. Prominins do not show obvious sequence homology to other known proteins, nor do they display sequence motifs that could provide clues as to their physiological role.

The human *PROMININ-1* gene is located on chromosome 4 (locus p15.32; see NCBI gene 8842) [10]. It is composed of at least 37 exons [11] that span more than 150 kb, and its structure (i.e., exon/intron boundaries) is strikingly similar to that of the mouse *PROMININ-1* gene (locus 5B3; NCBI gene 19126) [8]. Since the original description of prominin-1 [3], several splice variants affecting the open reading frame (ORF) have been identified in mice [1,6,12] and humans [13,14] and their expression characterized [15]. Many of them exhibit distinct cytoplasmic C-terminal domains, which might indicate various cytoplasmic protein-interacting partners. Further investigation is required to elucidate the biological significance of the use of facultative exons, but given that alternative splicing has been observed with

Figure 1. Topological model of human prominin-1 (CD133).



The extracellular location of the two large loops, which contain all eight potential N-glycosylation sites (forks), and the cytoplasmic location of the C-terminal domain have been confirmed by antibody accessibility and epitope insertion analyses [6].

prominin-2 [8], it appears that a wide variety of related polypeptides can be generated from the two mammalian *PROMININ* genes. Shmelkov and colleagues have reported that human prominin-1 is under the control of five alternative promoters [11]. Factors regulating the *in vivo* expression of prominin-1 remain to be determined, but the mRNA profile suggests that the mouse prominin-1 splice variants are tissue-specific and developmentally regulated [15].

Expression profile of prominin-1 & its AC133 epitope

Tissue distribution

Prominin-1 displays a broad range of expression. In mice, prominin-1 is not limited to neuroepithelial progenitors, but extends to several epithelial and nonepithelial cell types. In early embryos, prominin-1 is expressed in the trophoblast [16]. At later developmental stages, prominin-1 has been detected in epithelia of all three germinal layers [3,6]. In adults, prominin-1 is strongly expressed in the kidney [3], as well as all along the male reproductive tract, including the epididymis [15], the *ductus deferens*, the seminal vesicle and the prostate [Missol-Kolka E and Corbeil D, Unpublished Data]. In all prominin-1-expressing epithelial cells examined, prominin-1 is confined to the apical plasma membrane domain [3]. Prominin-1 is also detected in various nonepithelial cells, such as rod photoreceptor cells [10], bone-marrow-derived $CD34^+$ cells [17], developing spermatozoa found in testis seminiferous tubules [15] and in myelin [Corbeil D, Unpublished Data]. Given the functional diversity of prominin-1-expressing cells, its tissue distribution does not, on its own, provide any obvious clues regarding its physiological role.

In humans, the expression profile of prominin-1 (as deduced from the use of the monoclonal antibody [mAb] AC133, which recognizes a glycosylation-dependent epitope [4]) appears to be limited to developing epithelia, such as those found in the neural tube [17,18], the mesonephron and gut [17], and to various progenitor cells originating from distinct sources. Yet, the glycosylation pattern of prominin-1 might change depending on the tissue examined and/or the state of cellular differentiation [17,19]. Indeed, the use of novel antibodies directed against the human prominin-1 polypeptide allowed the detection of prominin-1 in several adult epithelia [Missol-Kolka E and Corbeil D, Unpublished Data] [19] with an expression profile that is similar to that of the murine ortholog, which is consistent with the widespread tissue distribution of its mRNA as observed either by northern blot analysis [4,17] or by *in situ* hybridization [Jászai J and Corbeil D, Unpublished Data]. Thus, it is important to stress that the mAb AC133 detects only a subpopulation of

human prominin-1 molecules carrying the AC133 epitope, and, consequently, that the AC133 antigen is not necessarily synonymous with prominin-1 [1]. For the sake of simplicity, the term 'AC133' shall be used to describe human prominin-1 bearing the AC133 epitope. Finally, it is worth mentioning that mAb AC141 [2], also directed against human prominin-1, shows a cross reactivity with cytokeratin 18 [20].

Embryonic stem cells

With regard to humans, AC133 expression has been reported for embryonic stem (ES) cell lines [21–23], and its transcripts have been detected in embryoid body-derived cells [24,25]. As for the mouse, ES cell-derived progenitors were demonstrated to express prominin-1 [16]. Although prominin-1 was found to be absent in the undifferentiated cells of the inner cell mass, the founder cells of ES cells, its transcripts were detected in undifferentiated ES cells. Interestingly, both ES-derived cells committed to differentiation and early progenitor cells coexpressed prominin-1 with early lineage markers such as nestin. After induction of neuronal differentiation, some prominin-1/nestin$^+$ cells persisted until terminal stages of differentiation. However, no coexpression of prominin-1 with markers of differentiated neural cells was detected. The somatic stem cell marker prominin-1 therefore appears to be a new tool to define ES cell-derived committed and early progenitor cells [16].

Somatic stem & progenitor cells

In the hematopoietic system, AC133 has received great attention as an alternative to CD34 [26] to identify and isolate progenitor cell populations [4]. AC133 is expressed in a subpopulation of CD34$^+$ progenitor cells [2,4,17] that have the capacity to reconstitute the entire immune system of sublethally irradiated mice [27]. A unique population of CD34-negative cells expressing AC133 with primitive cell properties has also been reported [28,29]. In addition to their hematopoietic capacity, it has been shown that AC133$^+$ hematopoietic progenitors are capable of differentiating *in vitro* into neuronal and endothelial cells [30–32], as well as into myoblasts [33], underlining a much higher plasticity of AC133$^+$ cells compared with CD34$^+$ cells. Clinically, large-scale immunomagnetic selection of AC133$^+$ cells allowed the enrichment of a sufficient number of progenitor cells to perform hematopoietic stem cell transplantation [34], and medical trials with leukemic children have proved the feasibility of AC133$^+$ selection for allogeneic transplantation [35,36]. The authors' laboratories could also show a rapid establishment of donor dendritic cell chimerism after allogeneic transplantation of AC133$^+$-selected cells [37]. Does the immunomagnetic selection, based on AC133, offer any significant advantage to available CD34$^+$ selection techniques? Several studies, including one from this authors' group [Freund D, Oswald J, Felmann S *et al.*, Unpublished Data] [27], have found that CD34$^+$-enriched progenitors and especially the CD34$^+$/AC133$^-$ fraction contained a significantly enriched proportion of erythroid colony-forming cells, whereas the highest content of myeloid colony-forming cells was found in the AC133$^+$-selected cells. These subtle differences of lineage-committed progenitor cells between CD34$^+$ and AC133$^+$ immunomagnetic selection might be significant, and further studies seem indicated to explore their potential clinical relevance.

In the CNS, prominin-1$^+$ (or AC133$^+$ in the case of human-derived cells) progenitor cells have been isolated successfully either from human fetal and post-mortem brain [18,38,39], or more recently, from mouse postnatal cerebellum [40]. It was demonstrated that purified prominin-1$^+$ stem cells could form self-renewing neurospheres and differentiate into astrocytes, oligodendrocytes and neurons *in vitro* [40]. Remarkably, the prominin-1$^+$ cells can generate each of these lineages after transplantation into the cerebellum [40]. Recently, balloon cells associated with focal cortical dysplasia were found to express AC133 among other stem cell markers [41]. Thus, prominin-1$^+$ stem/progenitor cells might be clinically important, particularly with regard to bone marrow transplantation and brain injury/disease [37,42].

Is prominin-1 expressed by other somatic stem cells? Several studies have addressed this issue and shown that AC133 potentially labels human endothelial progenitor cells, as well as those derived from muscle [43], prostate [44], kidney [45,46] and skin [13,47]. For instance, Bussolati and colleagues have immuno-isolated rare AC133$^+$ cells from adult renal interstitium and demonstrated that these cells have the capacity to differentiate into either tubular cells or vascular cells upon the presentation of appropriate cues [45]. Likewise, Richardson and colleagues have identified a small population of prostate AC133$^+$ progenitors located in the basal layer that possess a high proliferative potential *in vitro* and can reconstitute prostatic-like acini in

immunocompromised nude mice [44]. Again, it is important to point out that these rare progenitor cells expressed a specific glycoform of prominin-1 molecules carrying the AC133 epitope, and that the expression of prominin-1 molecules (i.e., its polypeptide) in these particular tissues (i.e., kidney and prostate) is not limited strictly to the progenitor cells [Missol-Kolka E and Corbeil D, Unpublished Observation] [19].

Endothelial progenitor cells have received considerable attention as they may contribute to postnatal vasculogenesis in both physiological (vasculature homeostasis) and pathological (cancer, ischemia) neovascularization [48] and are, therefore, regarded as potential therapeutic agents that could be of considerable interest in regenerative medicine. AC133$^+$ progenitors also display endothelial stem cell potential [49]. The AC133 epitope was found to be expressed in endothelial cell precursors and rapidly lost upon differentiation into mature endothelial cells [31,49]. It therefore constitutes an attractive antigen marker to isolate immature endothelial cells that could contribute to neovascularization in ischemic conditions [50]. AC133 selection from mobilized bone marrow for autologous myocardium regeneration has been used in Phase I clinical trials in the treatment of infarcted myocardium [51–53]. On the other hand, the existence of a correlation between circulating endothelial precursors and different pathological conditions is under intense scrutiny. AC133$^+$ cells may become biomarkers of vascular disease [54,55]. Preliminary data indicating an increase of prominin-1 mRNA level (among other markers) in blood cells of breast carcinoma patients [56], as well as the increase in circulating AC133$^+$ cells in non-small cell lung cancer patients [57], might reflect the contribution of va-sculogenesis to tumor vascularization. Thus, prominin-1 (AC133) may become a molecular target to treat pathological angiogenesis during cancer.

Cancer cells

The heterogeneity in proliferative potential of hematopoietic progenitors is reflected in various cancers where only a small subset of cells proliferate extensively, leading to the concept of cancer stem cells. Their effective isolation occurred initially for acute myeloid leukemia [58]. AC133 is often associated with cell subpopulations with high proliferative potential and is upregulated in various malignant hematopoietic diseases, including acute, chronic myeloid and lymphoblastic leukemias [59–63], but no predominance in any subtype has been determined to date [64]. Although the prognostic value of AC133 expression in leukemia remains uncertain, AC133 is used for leukemia diagnosis and in combination with other markers to characterize the leukemic population and has been used for selection in autologous transplantation of patients with CD34$^+$ leukemia [35].

A similar situation may prevail in solid tumors. AC133 appears to be associated with the subfraction of tumor brain cells responsible for tumor initiation [65–67]. Remarkably, only a rare AC133$^+$ fraction of tumor cells was capable of reproducing the tumor *in vivo* [67]. Similarly, AC133$^+$ populations with high proliferative capacity have been isolated from prostate tumors [68,69]. On the other hand, the physiological expression of human prominin-1 is more widespread than it appears upon detection with mAb AC133 due to differential glycosylation, and the expression of the AC133 epitope appears to be inversely correlated with cell differentiation. The glycosylation of prominin-1 could also change upon malignant transformation of cells expressing it physiologically, such as cells of the kidney. In this context, the observation of an upregulation of AC133 in cells in the vicinity of a conventional kidney carcinoma [19] would be in line with the notion that tumor progression is somehow associated with cell dedifferentiation, such as a change in glycosylation towards a state characteristic of stem/progenitor cells. Thus, prominin-1$^+$/AC133$^+$ 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 prominin-1$^+$/AC133$^-$ cells detected in the tumor region may have lost it. Together, the expression data on the various prominin-1 epitopes could therefore be a useful tool in the diagnosis and monitoring of malignant diseases.

Cell biology of prominin-1

Marker of plasma membrane protrusions

In epithelial cells, prominin-1 is concentrated in microvilli and is absent from the intermicrovillar regions of the plasma membrane [3,17,70]. In epithelial-derived cells, such as photoreceptor cells, prominin-1 is concentrated in the few plasma membrane evaginations at the base of the rod outer segment [10], which are essential precursor structures in the biogenesis of photoreceptive disks. In nonepithelial cells, such

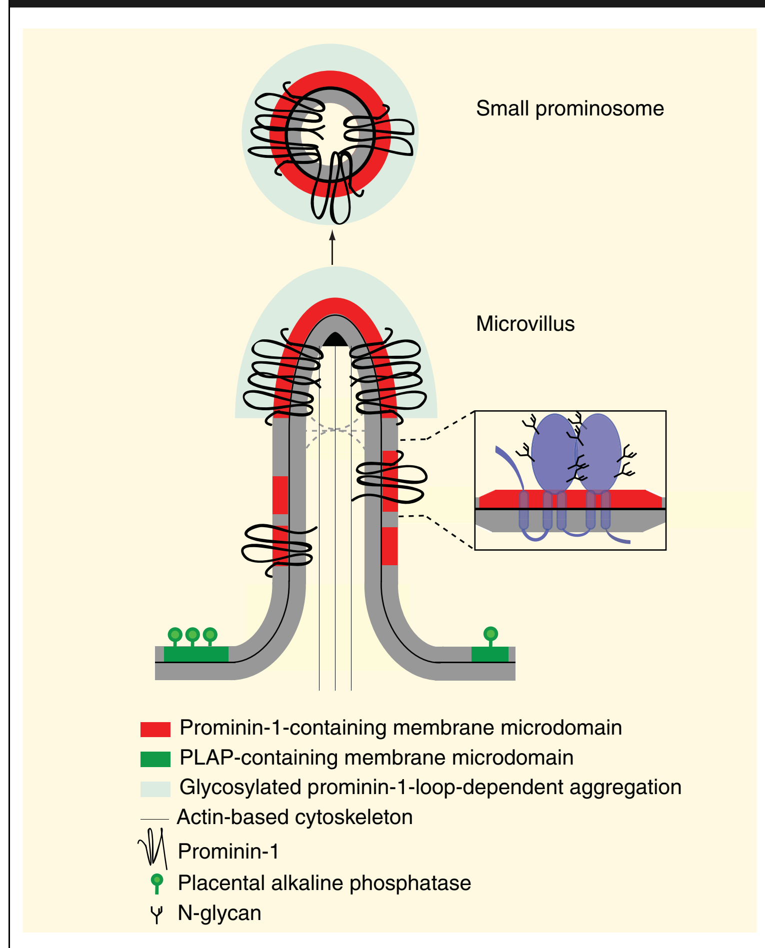
as hematopoietic progenitors, prominin-1 (AC133) shows, depending on the state of the cells, various specific subcellular localizations, which have in common to protrude from the plasma membrane. For instance, in freshly isolated CD34+ hematopoietic progenitors, prominin-1 is enriched in small and sparse plasma membrane protrusions that are distributed over the entire cell surface [17,71]. In migrating hematopoietic progenitors, prominin-1 is concentrated in a rear pole structure called uropod [71]. In hematopoietic progenitors harboring other types of plasma membrane protrusions (e.g., magnupodia), prominin-1 is concentrated therein [Bauer N and Corbeil D, Unpublished Data]. Moreover, when exogenously expressed in fibroblasts by transfection, prominin-1 also exhibits a profound preference for plasma membrane protrusions as it is concentrated in microspikes, filopodia and the leading edge of lamellipodia [3]. Therefore, it appears that, irrespective of the cell type, prominin-1 is associated specifically with plasma membrane protrusions.

Prominin-1 & membrane lipid microdomains

The specific localization of prominin-1 in microvilli of the apical plasma membrane of epithelial cells [3] is not mediated by interaction with the actin-based cytoskeleton but reflects its ability to interact directly and specifically with plasma-membrane cholesterol and to associate with a characteristic cholesterol-based lipid microdomain [5]. Membrane lipid microdomains (often referred to as lipid rafts) are liquid-ordered domains that are more tightly packed than the surrounding non-raft phase of the lipid bilayer [72]. These membrane lipid microdomains are enriched in cholesterol and sphingolipids and have been implicated in various biological processes, including signal transduction, and interestingly allow the partitioning of membrane-associated proteins into distinct micro-environments, enabling local polarization [73]. Initially, these membrane lipid microdomains were defined biochemically based on their insolubility in a nonionic detergent (Triton® X-100) in the cold. Although prominin-1 was completely soluble in this detergent – unlike placental alkaline phosphatase (PLAP), another apical membrane protein – the authors found that it was a constituent of a distinct type of membrane lipid microdomain that is preserved when using other nonionic detergents, such as Lubrol WX or Brij58 [5]. The prominin-1-containing detergent-resistant membranes exhibited all the characteristics previously reported for Triton X-100-resistant membranes, including floatation in sucrose-density gradients in a cholesterol-dependent manner. Moreover, their size (as revealed by sedimentation) decreased upon cholesterol depletion of the plasma membrane, and such treatment brought about a redistribution of prominin-1 from microvilli over the entire apical plasma membrane [5]. These observations led the authors to hypothesize that the prominin-1-containing detergent-resistant membranes would reflect the existence, at the apical surface of epithelial cells, of novel cholesterol-based lipid microdomains that are distinct from the Triton X-100–insoluble PLAP-containing lipid microdomains in the planar region (**Figure 2**, green segment) and which constitute building units of the microvillar plasma membrane sub-domain (**Figure 2**, red segment) [5,6]. Since then, the proposed involvement of membrane lipid microdomains in the formation of microvilli received further support by the use of atomic force microscopy imaging [74].

What is the relevance of prominin-1 in these particular membrane lipid microdomains? Iglic and colleagues have addressed this issue recently and, based on theoretical models, suggested that prominin-1 itself, due to its specific molecular shape, may form small anisotropic protein-lipid complexes that associate into large membrane lipid microdomains upon curvature-induced accumulation in tubular plasma membrane protrusions [75]. Two scenarios, not mutually exclusive, involving prominin-1 molecules can be envisioned. First, the prominin-1 transmembrane segments – three are unusually long (i.e., 26–28 amino acids) – might increase, by their interaction with the surrounding lipids, the thickness of the plasma membrane, and consequently modify locally the general organization of the lipid bilayer (**Figure 2**, inset). Second, the large prominin-1 extracellular loops carrying glycan moieties may induce the self-aggregation of prominin-1 by homotypic interaction (**Figure 2**, blue segment), which in turn would drive the clustering of small prominin-1-lipid complexes and, hence, potentially change membrane curvature. Beyond all speculations, it is, however, important to point out that prominin-1 alone cannot be viewed as the unique driving-force underlying the formation of microvilli or other plasma membrane protrusions, since its over-expression in a heterologous system does not necessarily result in an increase in these protrusions [3]. Conversely,

Figure 2. Prominin-1-containing membrane microdomain in the microvillar plasma membrane and the formation of the small prominosome.



various plasma membrane protrusions exist without prominin-1. Nevertheless, prominin-1 may be necessary to functionally organize particular plasmalemma protrusions with the appropriate set of lipids and/or proteins. The study of prominin-1 knockout mice is particularly instructive in this regard.

Finally, it is important to note that membrane lipid microdomains, as defined by their insolubility in detergents other than Triton X-100, are not restricted to epithelial cells but are also found in nonepithelial cells, including those of the hematopoietic lineage [Bauer N and Corbeil D, Unpublished Data] [76–79].

Prominosomes

Being tightly associated with the plasma membrane via its five transmembrane domains, prominin-1 is nonetheless released into the extracellular milieu. Specifically, prominin-1 is associated with small membrane particles that are found in a variety of external body fluids in the adult, including saliva, urine, seminal fluid and lacrimal fluid [80], reflecting its widespread expression in epithelia. Prominin-1 is also associated with small and large membrane particles present in the ventricular fluid of mouse embryos [80]. The latter prominin-1-containing membrane particles lack other plasma membrane markers such as cadherin, which suggests that their appearance does not simply reflect indiscriminate cell fragmentation but rather a specific release process [80]. Although the cellular mechanism underlying the release of the prominin-1-containing membrane particles, referred to as prominosomes, has not yet been elucidated, the high concentration of prominin-1 at the tip of microvilli [3,80] strongly suggests that the small prominosomes are derived from there. The association of prominin-1 with membrane lipid microdomains is particularly relevant in this context [5]. The clustering of small prominin-1-lipid complexes leading to a large lipid microdomain at the microvillar edge (**Figure 2**, red segment) might create a phase separation with regard to the surrounding 'non-raft' lipid environment (**Figure 2**, grey segment) leading to the budding of these particles (**Figure 2**). Since the different phases in the membrane (i.e., liquid ordered versus liquid disordered) alter the physical properties of the lipid bilayer, the dynamics of membrane budding may be influenced by a lipid/protein-driven formation of large microdomains in the vicinity of the nascent bud [81]. Consistent with this, the prominin-1 molecules associated with the small prominosomes demonstrated the same detergent solubility/insolubility and cholesterol dependence [Marzesco A-M, Janich P, Huttner WB and Corbeil D, Unpublished Data] as those associated with apical plasma membrane protrusions (**Figure 2**, red segment).

What is the physiological significance of these particles? There is currently no understanding of their role. Nevertheless it can be hypothesized that these membrane particles may play a role in intercellular communication by carrying specific signaling molecules. Alternatively, the release of these membrane particles by progenitor cells, (e.g., neuroepithelial [80] and hematopoietic progenitors [Bauer N and Corbeil D, Unpublished Data]) may be a means of disposal of membrane lipid microdomains that allows these cells to modify their stem/progenitor cell properties. The developmental regulation of the prominin-1-containing particles in the ventricular fluid during neurogenesis, as well as their concomitant release with the differentiation of

human colon carcinoma-derived Caco-2 cells, are consistent with (but do not prove) such a function [80].

Irrespective of their function, these body fluid-associated particles constitute an easily accessible source of material for a protein-based diagnosis of human diseases involving prominin-1 [10]. In this context, it will be important to determine the protein and lipid composition of these particles.

Prominin-1-containing membrane lipid microdomains: a key structure determining stem cell fate?

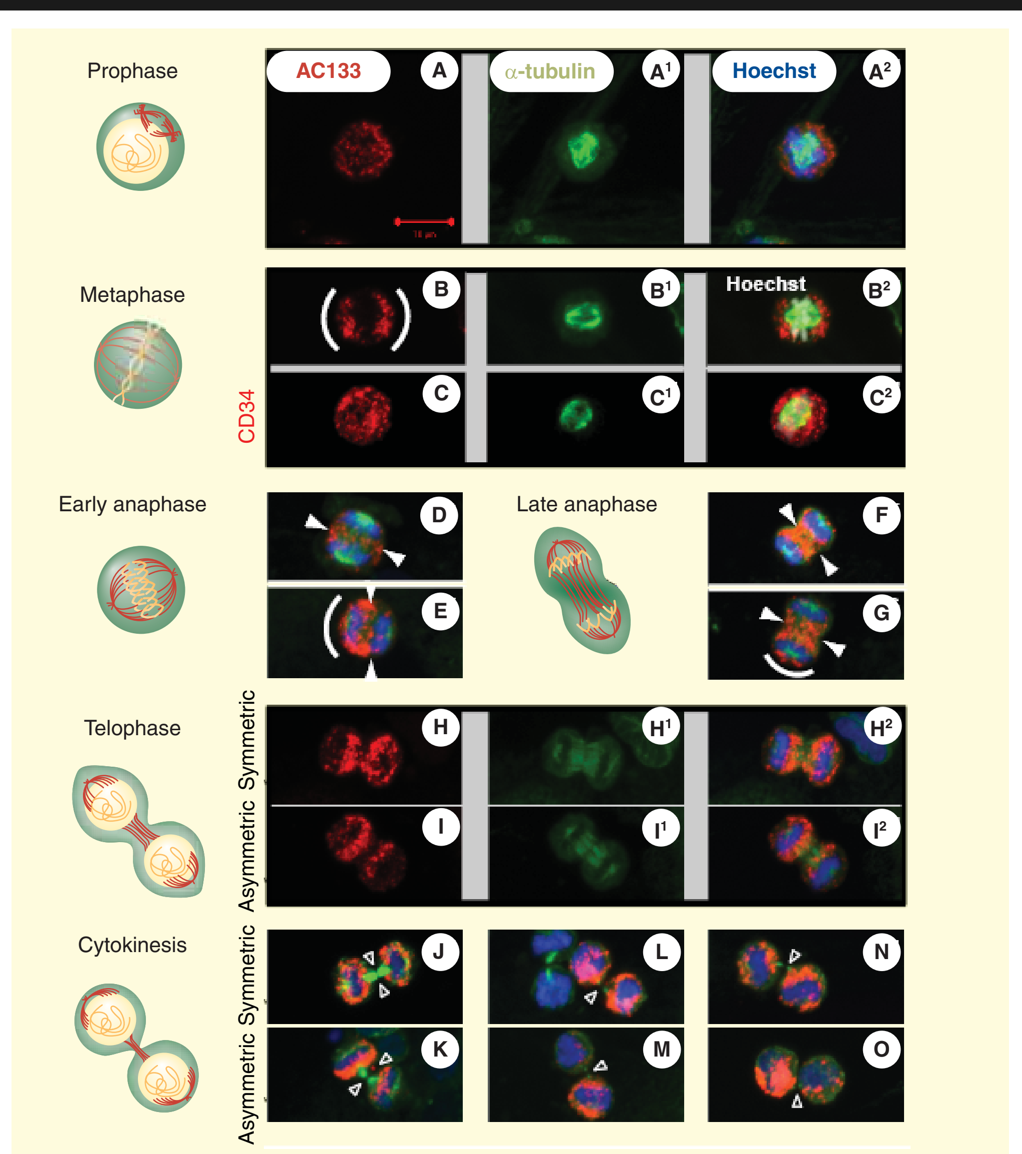
The self-renewal capacity of stem and progenitor cells relies on both extrinsic and intrinsic determinants, for instance involving specific signaling pathways [82]. Although a possible role of prominin-1 in the determination of cell fate remains to be investigated [16], and despite the unknowns as to its molecular function, its association with cholesterol-based lipid microdomains deserves particular attention. Lipid microdomains have been implicated in several signaling cascades by allowing the formation of active transduction complexes [83]. The presence of prominin-1-containing membrane lipid microdomains might be necessary to maintain certain stem cell properties, and their loss might contribute to differentiation [84]. In view of the current data, at least three models can be hypothesized to explain the 'loss' of these plasma membrane microdomains. First, upon reduction of the cholesterol content of the plasma membrane, the membrane lipid microdomains might disperse, and therefore the signaling complexes. Second, these microdomains could be eliminated from the plasma membrane either by selective release as small membrane particles, such as the small prominosomes described above [80], or by endocytosis into multivesicular bodies and eventual release with exosomes [85]; although the latter would imply that prominin-1 is also found in exosomes, which has not been reported [80]. Third, they could be distributed asymmetrically during cell division as previously observed during neurogenic divisions of mammalian neuropithelial cell progenitors where the apical plasma membrane, which selectively hosts prominin-1-containing membrane microdomains, is distributed asymmetrically [86]. A similar phenomenon was observed with hematopoietic progenitors cultured on primary mesenchymal progenitors as the feeder cell layer [87]. As summarized in **Figure 3**, prominin-1 (AC133) displays various localizations in mitotic hematopoietic progenitors. For instance, while AC133 appears to be distributed randomly over the cell surface in prophase (**Figure 3A–A²**), it becomes concentrated in surface domains corresponding to the spindle pole areas during metaphase (**Figure 3B–B²**, white lines), a phenomenon that is rarely observed with CD34 (**Figure 3C–C²**). In early and late anaphase, AC133 is concentrated towards the cleavage furrow (**Figure 3D–G**, arrowheads) and, interestingly, also remains clustered at one pole of the dividing cell (**Figure 3E & G**, white line). In telophase, the authors could observe under these experimental conditions that AC133 is either equally (**Figure 3H–H²**, symmetric), or unequally (**Figure 3I–I²**, asymmetric) distributed between the two nascent daughter cells. This phenomenon may persist through cytokinesis (**Figure 3J–O**). Future studies are required to determine the cell-biological consequence of such asymmetric distribution of AC133, but it becomes clear that the cholesterol-binding prominin-1, as a marker of a characteristic membrane lipid microdomain, may reveal new aspects of these microdomains for stem cells.

Prominin-1 & retinal degeneration

Photoreceptor cell dystrophies are one of the most common causes of inherited blindness in the world. The integrity of these cells, particularly their outer segment, which contains photoreceptive disks, is maintained by several structural proteins, including peripherin. These membranes have a high turnover rate. The disks of vertebrate photoreceptors (e.g., rods) are generated at the base of the outer segments initially by flattened evagination of the plasma membrane with subsequent rim formation and membrane fusion resulting in release of the newly formed disks into the cytoplasm [88]. These morphological processes, which are associated with complex changes in membrane curvature, are still poorly understood mechanistically.

A homozygous single-nucleotide deletion (nt 1878) in the human *PROMININ-1* gene was identified in an Indian pedigree with an autosomal-recessive retinal degeneration [10]. This genetic alteration was predicted to cause a frameshift, starting at codon 614, resulting in premature termination of translation. The corresponding truncated prominin-1 protein does not reach the cell surface but is degraded in the endoplasmic reticulum [10]. Other mutations in the *PROMININ-1* gene, associated with

Figure 3. Prominin-1 (AC133) distribution in mitotic hematopoietic progenitors.



CD34+ MACS-immuno-isolated cells from leukapheresis product were cultured for 3 days on primary mesenchymal progenitors prior to 24 h incubation with (C–M) or without (A, B, N, O) nocodazole for cell synchronization. Cells were then fixed with PFA, permeabilized with saponin and double-labeled with mouse mAb AC133 (red, Miltenyi Biotec) (or mAb 581 against CD34 [BD Pharmingen] red) and rat mAb anti-α-tubulin (Serotec, green) followed by appropriate cy3- and cy2-conjugated secondary antibodies before double immunofluorescence analysis using confocal microscopy. A composite picture from nine optical sections is shown. Nuclei were visualized with Hoechst (A^2, D–G, H^2, I^1, J–O, blue; B^2, C^2, white). White lines indicate the AC133 clustering at cell poles. Solid and outlined white arrowheads indicate the cleavage furrow and midbody, respectively.

autosomal dominant macular dystrophy, have been reported [89]. The importance of prominin-1 for photoreceptor function is substantiated by data from a murine model. In collaboration with Peter Carmeliet and colleagues, Belgium, whose laboratory has generated a prominin-1 knockout mouse line, the authors have recently observed that prominin-1 deficiency leads to progressive retinal degeneration [90]. In young prominin-1$^{-/-}$ animals, the outer segments show a complete disorganization, although the formation of plasma membrane protrusions is not impaired completely, whereas in older animals, the entire photoreceptor layer is absent [90]. Given that within the retina, prominin-1 is concentrated selectively in membrane evaginations, the lack of

Executive summary

Molecular biology of prominin-1 (CD133)

- The human *PROMININ-1* gene is located on chromosome 4 (locus p15.32).
- Prominin-1 is a plasma membrane cholesterol-binding pentaspan glycoprotein.
- Prominin-1 is associated selectively with plasma membrane protrusions, in a cholesterol-dependent manner.
- Prominin-1 is concentrated in membrane lipid microdomains.
- Prominin-1 is associated with small membrane particles that are released into several body fluids including saliva, urine, seminal fluid, lacrimal fluid and ventricular fluid.
- AC133 represents a specific glycoform of human prominin-1.

Expression profile

- Prominin-1 is expressed by various developing epithelia, where it is found selectively at the apical plasma membrane domain.
- It is found in embryonic stem cell-derived progenitors.
- Various somatic progenitor cells, including those of the neural and hematopoietic system, express prominin-1.

Hematopoietic progenitors

- AC133 is expressed on a subpopulation of $CD34^+$ progenitor cells that have the capacity to reconstitute the entire immune system of sub-lethally irradiated mice.
- $AC133^+$ hematopoietic progenitor cells are capable of differentiating *in vitro* into neuronal, endothelial cells and myoblasts, reflecting a much higher plasticity of $AC133^+$ cells compared with $CD34^+$ cells.
- A large-scale immunomagnetic selection of $AC133^+$ cells allowed the enrichment of a sufficient number of progenitor cells to perform hematopoietic stem cell transplantation.
- Clinical trials with leukemic children have proved the feasibility of $AC133^+$ selection for allogeneic transplantation.

Neovascularization

- AC133 is being selected from mobilized bone marrow for autologous myocardium regeneration in the treatment of infarcted myocardium (Phase I clinical trials).
- $AC133^+$ cells may become biomarkers of vascular disease.

Diseases

- A single nucleotide deletion (G1878) in the human *PROMININ-1* gene causes retinal degeneration.
- AC133 is often associated with cell subpopulations showing high proliferative potential and is upregulated in various malignant hematopoietic diseases.
- AC133 expression identifies tumor-initiating cells in brain tumors.

its appearance at the photoreceptor cell surface may perturb the appropriate lipid–protein composition of these nascent membrane protrusions, thereby somehow impairing either the rim formation or their fusion, which precede the generation of a new photoreceptor disk.

Supporting the lack of other pathological symptoms in the affected individuals [10], no other obvious phenotype was observed in prominin-1$^{-/-}$ animals [90], thus suggesting that the lack of prominin-1 may be compensated by prominin-2 in tissues other than the retina, which is devoid of prominin-2 [8].

Conclusions

Prominin-1 (CD133), initially identified as a cell surface marker of neuroepithelial progenitors in mice and hematopoietic stem cells in humans, has rapidly become a general marker used to define self-renewing cell subpopulations in various tissues. It has proven a valuable tool for the purification of bone marrow-derived stem cells. By enabling the isolation of cancer-initiating cells, it has revealed the parallel between the CNS and hematopoetic system and has made a case for the implication of cancer stem cells in tumor genesis. It thus contributes to the outline of new prospects for more effective cancer therapies by targeting tumor-initiating cells. The molecular function of prominin-1 still needs to be defined, but its specific interaction with cholesterol in plasma membrane lipid microdomains together with the fact that it is necessary to the proper organization of the rod photoreceptor outer segment favors the idea that it might be related to membrane organization.

Future perspective

The growing body of reports using prominin-1 (CD133) as an early cell marker is essentially a reflection of the recent interest in stem cells and the urge to define both normal and neoplastic lineage pathways, which is critical to the development of proper cellular therapies. The clinical relevance of prominin-1 in this respect would be:

- Prominin-1 (or its various epitopes) might become a biomarker for diagnosis and monitoring of malignant diseases
- This protein represents a potential molecular target in treatment of pathological angiogenesis (e.g., cancer)
- A potential target to eliminate cancer stem cells
- A specific marker of pluripotent progenitors for tissue engineering and cell-based therapies
- A good candidate gene for *in vivo* gene transfer therapy for retinal disease

The specific release of prominin-1-containing membrane particles into various body fluids is intriguing and deserves further study; however, irrespective of this, prominosomes already appear to be an interesting basis for the potential diagnosis of prominin-1-related human diseases.

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