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Denis Corbeil *Editor*

Prominin-1 (CD133): New Insights on Stem & Cancer Stem Cell Biology

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To my son Xavier

Preface

This book was conceived to celebrate the fifteenth anniversary of the discovery of prominin-1, also known in the medical field as CD133. Since its original description in 1997 in the murine system by Anja Weigmann, myself, Andrea Hellwig and Wieland B. Huttner (Proc Natl Acad Sci USA, 94; 12425–12430), and independently in the human system by David W. Buck and his team (Blood, 90; 5002–5012; 5013–5021), this cholesterol-binding pentaspan membrane glycoprotein has emerged as the object of great attention worldwide. This coincides with the identification and isolation of stem cells from different types of tissue and organ for which prominin-1 has become one of the most valuable cell surface markers with clinical value. Expectations for the development of novel therapies through the replacement and regeneration of damaged or diseased tissues based on isolated stem cells has made this field one of the leading edges on the frontiers of modern medicine. The expression of prominin-1 by putative cancer stem cells has also brought new horizon in cancer treatments, and this molecule may be regarded as a potential target in the eradication of cancers. The medical significance of prominin-1 is also highlighted in the visual system where mutations in the *PROM1* gene cause retinal degeneration.

As a biochemist, my long-standing interest in prominin-1 has brought me in the field of tissue engineering and cellular regeneration to understand the biological basis of tissue formation. The study of the molecular cell biology of prominin-1 in diverse cell types, e.g., epithelial cells, stem cells, and photoreceptors, which reflects by itself the broad tissue distribution of prominin-1, has emphasized divers' phenomena including the organization, remodeling, and dynamics of the plasma membrane as important factors regulating specific properties of the cells. Remarkably, these mechanisms appear conserved despite the considerable difference in cellular function of the cells in question (e.g., stem cell versus photoreceptor). The budding of membrane vesicles containing prominin-1 from the tip of microvilli of polarized epithelial cells and their release during the process of stem cell differentiation, and the organization of photoreceptor cell outer segment are good examples. I believe that the exploration of prominin's function(s) in various model organisms such as

mice, zebra fish, axolotls, and flies would bring more insights, not only for cell biological trends, but also in organogenesis and tissue regeneration.

This book is composed of 15 chapters that will describe, on one hand, the molecular and cellular biology of prominin-1 and other members of the prominin family and, on the other hand, the importance of this molecule in the medical field as a valuable marker of stem and cancer stem cells. The opening chapter by my coworkers and myself presents an overview of the identification of prominin-1, its relation to the widely used AC133 epitope, and the general interest of this molecule in regenerative medicine. This chapter is intended as an introduction to the book and provides molecular details of prominin-1 across species including splice variants, tissue distribution, and certain biochemical properties including its specific subcellular localization in plasma membrane protrusions. It connects directly with the topics elaborated in the subsequent chapters of the initial section constructed as a knowledge base essential to the grasp of the physiological function(s) of this glycoprotein with a particular medical interest: CD133. My colleague Christine A. Fargeas presents prominin-2, the prominin-1 paralog, and the evolution of the prominin family of proteins among the animal kingdom. Then, as prominin-1 is not only tightly associated with plasma membrane protrusions, but also released in association with membrane particles into different body fluids, Anne-Marie Marzesco describes such singularity not only from a cell biology aspect, but also clinically, as prominin-1-containing membrane vesicles might be recognized as potential biomarkers in certain diseases. In the fourth chapter, Elisabeth Knust and her colleagues describe the eminent role of prominin in the retina, which demonstrates not only the significance of prominin-1 in the vision but also its evolutionary conserved function in the maintenance of photoreceptive membranes from humans to flies. Kouichi Tabu and his colleagues present in the next chapter the complex gene regulation of *PROM1* in normal and cancerous tissues. In the following 10 chapters, my colleagues describe new aspects of prominin-1-positive cells and the utility of this molecule as a marker of stem cells and cancer stem cells. Numerous tissues and organs are thus virtually dissected with regards to the expression of prominin-1 and the normal and cancerous cells harboring stem cell properties, i.e., self-renewal and multipotential differentiation capacities. In Chap. 6, Wieland B. Huttner, whose laboratory discovered murine prominin-1, presents with his coworker Alex M. Sykes novel features of neuroepithelial cells, precursors of all neurons of the central nervous system, including the asymmetric cell division. Rupert Handgretinger and Selim Kuçi present in great detail the importance of human prominin-1 (CD133) in the hematopoietic system and bring an update on the use of $CD133^+$ cells in autologous and allogeneic hematopoietic stem cell transplantation. In humans, the clinical relevance of $CD133^+$ cells is not limited to blood system; Benedetta Bussolati and Giovanni Camussi describe, for instance, the expression of CD133 in kidney under normal and pathological conditions. Although the possible use of $CD133^+$ progenitors in human studies presents obvious limitations due to immunological barriers, the in vivo experiments reported in mice suggest a potential role of progenitors in renal regeneration. Mariusz Z. Ratajczak and colleagues present the expression of CD133 by very small embryonic/epiblast-like stem cells (VSELs), and its use for their

prospective immunoisolation. VSELs could provide a therapeutic alternative to the controversial use of human embryonic stem cells. Afterward, three clinically important organs are reviewed. Alessandro Sgambato and colleagues present the current knowledge on CD133 expression in normal and cancer colon tissues, both in humans and mice, and discuss the apparently conflicting data reported. Moreover, the authors devote great attention to the available information about the functional role of CD133 in colon cancer cells. Likewise, Norman J. Maitland and colleagues dissect the current literature regarding prostate cancer stem cells, with specific reference to the expression of CD133 as a stem cell marker to identify and purify stem cells in normal prostate epithelium and prostate cancer. In Chap. 12, Yuichi Hori reveals new facets of pancreatic progenitor cells and cancer stem cells by studying CD133 expression. Because it is also associated with melanoma stem cells, Aurelio Lorico and colleagues elegantly present the importance of CD133 as a therapeutic target. The two final chapters describe the diverse origins and utilizations of $CD133^+$ cells. Peter Donndorf and Gustav Steinhoff give a thorough account of cardiac stem cell therapy, and Yvan Torrente and colleagues discuss potential therapeutic applications of $CD133^+$ cells for degenerative diseases including muscular dystrophies.

Taken as a whole, I have attempted to gather almost all topics of significance to the prominin-1 research field and to its medical weight as a cell surface marker of stem and cancer stem cells. I have been greatly encouraged in this project by positive feedback from worldwide-recognized scientists and physicians working in these fields. Finally, it is my privilege to have the opportunity to edit these chapters and, also on behalf of all coauthors, to thank everyone who has helped us to produce this book.

Dresden, Germany Denis Corbeil

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Part I
Biology of Prominins

Chapter 1
Prominin-1 (CD133): Molecular and Cellular Features Across Species

Denis Corbeil, Jana Karbanová, Christine A. Fargeas, and József Jászai

Abstract Our knowledge of the first member of the prominin family is growing rapidly as the clinical value of prominin-1 (CD133) increases with its ever-wider use as a stem cell marker in normal and cancer tissues. Although the physiological function of this evolutionally conserved pentaspan membrane glycoprotein remains elusive, several studies have revealed new biological features regarding stem cells, cancer stem cells, and photoreceptors. The wide expression of CD133 in terminally differentiated epithelial cells, long overlooked by many authors, has attracted significant interest through the extensive investigation of human PROMININ-1 as a potential target for cancer therapies in various organs. Biochemically, this cholesterol-binding protein is selectively concentrated in plasma membrane protrusions, where it is associated with cholesterol-driven membrane microdomains. Clinically, mutations in the *PROM1* gene are associated with various forms of retinal degeneration, which are mimicked in genetically modified mice carrying either a null allele or mutated form of PROMININ-1. In this introductory chapter, we attempted to review 15 years of prominin-1 study, focusing on its unique protein characteristics across species and the recent developments regarding its cell biology that may shed new light on its intriguing involvement in defining cancer-initiating cells.

Keywords Cancer • CD133 • Eye • Prominin • Splice variant • Stem cell

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1.1 Introduction

In modern medicine, stem and cancer stem cells are often described as the good and the bad players, respectively, with primitive properties; the former are essential cellular components in the development and maintenance of tissue homeostasis, and the latter appear to cause the initiation and progression of cancer. Therefore, both stem and cancer stem cells are viewed as potential targets for either regenerative therapy or a therapeutic avenue in cancer treatment [1, 2]. To better understand their cell biology, several groups, including ours, are dissecting the molecular and cellular characteristics of a common cell surface marker of these cells referred to as prominin-1 (CD133). Prominin-1 is expressed in a wide range of somatic stem and progenitor cells, including those in the central nervous and hematopoietic system, marks the cancer stem cells of solid tumors and hematopoietic malignancies, and is often associated with chemoresistant subpopulations. In this chapter, we will describe the molecular characteristics of prominin-1 and highlight the novel biological facets of stem cells, cancer stem cells, and photoreceptor cells that have been revealed by the study of prominin-1.

1.2 Identification of Prominin-1

The first member of the prominin family, prominin-1 (alias CD133, PROML1), was identified in 1997 through the characterization of novel monoclonal antibodies (mAbs) and through molecular cloning by two independent groups in Heidelberg (Germany) and Sunnyvale (United States of America) who were searching for novel markers of mouse neural and human hematopoietic stem and progenitor cells, respectively [3, 4]. The 13A4 mAb revealed mouse prominin-1 as a novel protein of plasma membrane protrusions that are present within the apical domain of polarized epithelial cells found in the embryonic neuroepithelium, adult ependymal layer, and proximal tubules of the kidney [3]. The protein was initially referred to as prominin. The AC133 mAb recognized an epitope on the human glycoprotein initially referred to as AC133 antigen, the expression of which appeared to be restricted to CD34-positive hematopoietic stem and progenitor cells [4]. However, Northern blot analysis of human PROMININ-1 provided data discordant with the immunodetection of the AC133 antigen. Notably, the strong mRNA signal on kidneys was incompatible with the lack of immunodetection in this tissue [4, 5].

The question of the relationship between the mouse and human molecules was rapidly raised and sustained by the detection of mouse splice variants and the related transcripts in both species [6, 7]. In a key publication in 2000, the groups that originally studied prominin joined to carefully demonstrate that human AC133 antigen is, like mouse prominin, expressed in epithelial cells and targeted to plasma membrane protrusions [8]. Most importantly, using the colon carcinoma cell line Caco-2 – a widely used model of enterocytic epithelial differentiation – it was demonstrated that AC133 immunoreactivity diminishes upon differentiation, while mRNA levels

increase. This observation led to the hypothesis that AC133 immunoreactivity may be somehow affected by the differential glycosylation state associated with enterocytic differentiation, i.e., the absence of AC133 immunoreactivity detection may not necessarily reflect the absence of protein per se [8]. Further evidence concerning the presence of the human protein in terminally differentiated epithelial cells (e.g., kidneys, mammary glands) was provided by mapping its tissue distribution using an antiserum raised against the PROMININ-1 polypeptide [9]. Along the same line, Karbanová and colleagues reported that, as was demonstrated earlier for its murine counterpart through 13A4 mAb immunohistochemistry [3, 10–12], general expression of PROMININ-1 is widespread in adult human tissues [13]. The analysis of transgenic reporter knock-in mice of the *Prom1* locus led to the same conclusion [14, 15].

In the meantime, the emergence of the prominin family had occurred with the identification and molecular cloning of prominin-2 [16] (for more details concerning prominin-2, see Chap. 2 of this volume by Fargeas) and the characterization of a splice variant in humans [17]. Therefore, in anticipation that human PROMININ-1 may share the characteristics of the murine protein, a unifying nomenclature was proposed to take into account the different paralogs, splice variants, and glycoforms of the prominin family [18]. Moreover, it was stressed that AC133 immunoreactivity may be more restricted than the general expression of human PROMININ-1 (CD133) [18]. The corresponding epitope (AC133 epitope; hereafter AC133) was thought to be dependent on conformation and/or sensitive to changes in glycosylation [5] (reviewed in Ref. [19]).

Here, we will present the molecular and biological characteristics of prominin-1 across species and briefly describe its expression in various tissues and organs, with a special emphasis on those where a medical relevance has been reported.

1.3 Protein Features

The membrane topology of prominin-1 (as of other members of the prominin family) after the cleavage of the signal peptide consists of an N-terminal extracellular domain (EC1), five transmembrane (TM) segments, alternating short intracellular and large extracellular loops (≈20 and >250 amino acid residues, respectively), and an intracellular C-terminal domain (IC3) (Fig. 1.1). A typical prominin-1 molecule comprises approximately 850 amino acid residues depending on the splice variant (see below). The two extracellular loops (EC2 and EC3 domains) contain potential N-glycosylation sites that vary in position according to the animal species from which the prominin-1 sequence is derived [3, 5, 16, 20–23].

The amino acid sequence is poorly conserved among prominin-1 gene products across species. For instance, only 60% identity between primates and rodents was observed [20]. Prominin-1 relatives in other species (fish, amphibian, bird; ≈45%), particularly in invertebrates (worm, fly; <25%), are poorly conserved with mammalian sequences [16, 22–24].

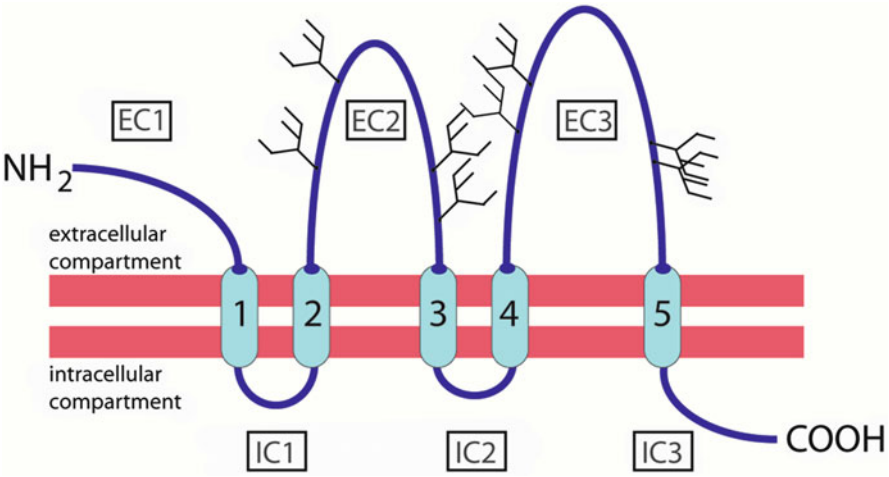

Fig. 1.1 Membrane topology of prominin-1. Prominin-1 is predicted to consist of an extracellular N-terminal domain (*EC1*), five transmembrane domains (1–5) separating two large glycosylated extracellular loops (*EC2* and *EC3*) and two small intracellular loops (*IC1* and *IC2*), and an intracellular C-terminal domain (*IC3*). The locations of the *EC2*, *EC3*, and *IC3* domains have been confirmed by antibody accessibility and epitope insertion analyses [3, 10, 16]. The *EC2* and *EC3* domains contain all of the potential N-glycosylation sites (forks), which vary in position depending on the animal species. Human prominin-1.s2 is illustrated here

However, multiple sequence analysis reveals three common characteristics of prominin proteins: (1) six cysteine residues in the EC2 and EC3 domains are conserved between various prominins, which are likely to form disulfide bridges; (2) a cysteine-rich domain of as yet unknown function is found at the transition of the TM1 and IC1 domains; and (3) a consensus core sequence, i.e., C*X*P*X*(12,13)C*X*(5)[P/S]*X*(4)W*X*(2)h*X*(4)hh*X*h (where *X* stands for any residue; the number of *X* is indicated in parentheses; residues in brackets indicate alternatives for a given position, and h stands for any hydrophobic residue), is observed at the end of the EC3 domain and the beginning of TM5. One of the *Drosophila* prominin sequences constitutes the exception to this rule, as it conforms only partially to the core consensus [24]. Nevertheless, in the absence of known catalytic activity, these sequence characteristics together with the pentaspan membrane topology may help to define the prominin membership of a novel molecule from a particular species. Finally, it is worth mentioning that several prominin-1 molecules exhibit a leucine zipper-like motif in one of the extracellular domains, which may be an indication of an in vivo dimerization process [25], and that prominin-1 (tyrosine residues 828 and 852 within IC3) is a target of Src and Fyn tyrosine kinases in human medulloblastoma cells [26]. The biological relevance of the latter modification remains to be determined.

In mammals, prominin-1 displays only a modest (≈30%) amino acid identity with prominin-2 [16, 27]. Nevertheless, the hydrophobic cluster analysis [28]

reveals several conserved hydrophobic clusters between both proteins, particularly inside the EC2 and EC3 domains, suggesting that the secondary and tertiary structures are highly related (Fargeas and Corbeil, unpublished data). Neither the protein crystal structure of these two loops nor the modeling of the alternative C-terminal domain(s) by nuclear magnetic resonance spectroscopy has been reported.

1.4 Genes, Splice Variants, and Glycoforms

1.4.1 Genomic Organization

The genomic structure of both mammalian prominin paralogs is remarkably similar (introns being concordant in position and phase) and remarkably conserved across species [16, 29]. However, the exonic structure does not correlate with the protein structural domains, as the coding regions of prominin-1 and prominin-2 span 28 and 23 exons, respectively (Fig. 1.2a; note that the numbering of the exons begins with the exon bearing the initial start codon) [16]. Shmelkov and colleagues have described that human PROMININ-1 is under the control of five alternative promoters (named P1–P5), three of which are located in a CpG island and are at least partially regulated by methylation [30, 31]. In certain cancers, aberrant DNA methylation may be an important determinant for the overexpression of PROMININ-1 [32–34]. Up to ten alternative exons that may be included in the 5′-untranslated region have been identified, highlighting complex gene regulation [30]. A potential binding sequence for estradiol receptor has been identified in the *PROM1* gene that was found to be transiently upregulated in uterine luminal epithelium during the implantation process [35]. Readers are invited to look at Chap. 5 by Tabu and colleagues for further details concerning the regulation of the *PROM1* gene.

1.4.2 Prominin-1 Splice Variants

Prominin-1 is subjected to alternative splicing [7, 17, 18, 22, 23, 30, 36, 37]. The functional significance of this variability is not yet understood, but the appearance of certain variants appears to be ontogenetically regulated [37] and/or tissue specific [11, 17]. Currently, 12 alternative splicing variants affecting the open reading frame of mammalian (primates and rodents) prominin-1 have been described in the three extracellular domains or within the IC3 domain (Fig. 1.2b) [11, 29]. As discussed above, a rational nomenclature of the prominin gene products was therefore proposed [18] and updated [29]. In principle, the name of the molecule, i.e., prominin-1 (or prominin-2, prominin-3, etc.), should be followed by a suffix (s) indicating the splice variant, numbered according to the chronology of publication (including database submission in which the sequence is quoted as a distinct splice variant),

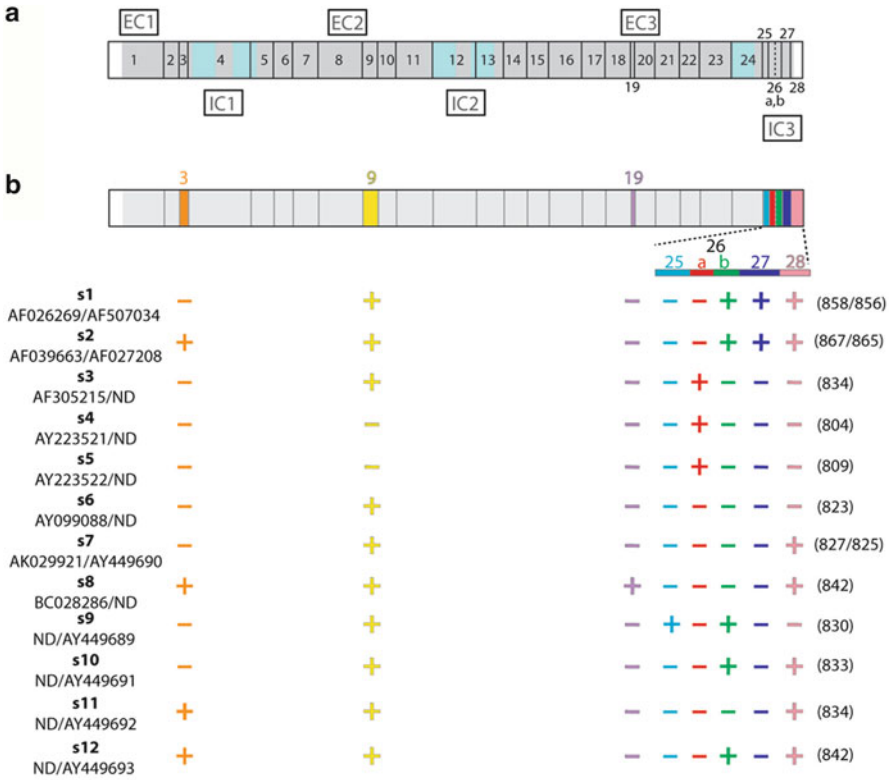

Fig. 1.2 Genomic organization of prominin-1. (**a**) Coding regions appears in *gray*, while the 5′- and 3′-untranslated regions appear in *white*. *Vertical lines* indicate the exon boundaries, and the *blue zones* indicate the predicted transmembrane regions. The numbering of the exons begins with the exon bearing the initial start codon [10, 16, 29]. The various and alternative 5′-untranslated regions were ignored for the sake of simplicity. (**b**) Distinct splice variants of mammalian (mouse and/or human) prominin-1. Facultative exons included in the coding sequences are colored. Splice variants are named according to the prominin nomenclature [18, 29], the GenBank accession number (mouse/human), and the presence (+) or absence (−) of an exon, and the corresponding protein sequence length (number of amino acid) is indicated (ND; not described)

and irrespective of the species. For instance, prominin-1.s1 and prominin-1.s2 refer to the first prominin sequences reported in mouse (prominin) [3] and human (AC133 antigen) [5], respectively, because they differ by the absence or presence of a facultative exon (numbered 3) in the EC1 domain (Fig. 1.2a, b). The use of alternative nomenclature might simply confuse the field; the relationship of the AC133 epitope with the prominin-1 (CD133) protein was already puzzling (see above).

The recent molecular cloning of prominin-1 in nonmammalian vertebrates such as zebrafish (*Danio rerio*), axolotl (*Ambystoma mexicanum*), frogs (*Xenopus laevis*), and birds (*Gallus gallus*) has significantly increased the number of prominin-1 gene products [21–23]. At least 20 potential splice variants (s1 to s20) might exist

Table 1.1 Alternative prominin-1 COOH-termini[§]

Type	Exons included in the coding sequence	COOH-terminal sequence (species depicted)	Splice variant	Species[reference]
A	24, 26b, 27, 28	(H) RRMDSEDVYDDVETIPMKNMENGNNGYHKDHVYGIHNPVMTSPSQH	s1, s2, s13	A^{1}, $H^{2,5}$, $M^{3,6}$, R^{4}
B	24, 26a	(M) RRMDSEDVYDDSSVSGMWHFTL	s3, s4, s5	M^{7}
C	24	(H) RRMDSEDVYDE	s6	F^{8}, H^{7}, M^{7}
D	24, 28	(H) RRMDSEDVYDDPSQH	s7, s8, s11, s15, s18, s21	A^{1}, C^{1}, $H^{7,9}$, $F^{1,10}$, M^{7}, R^{9}
E	24, 25, 26b	(H) RRMDSEDVYDDSSWVTSVQC[b]	s9	H^{9}
F	24, 26b, 28	(H) RRMDSEDVYDDVETIPMKNPSQH	s10, s12, s14	A^{1}, H^{9}, Rh^{9}
G	24, 27, 28	(C) RRMDTEDVYDDMENGNNGYHKEHLYGIHNPVITSSVEQW[c]	s16, s17	C^{1}, D^{11}, M^{12}
H	24, 26b, F27′	(F) RRMDTEDVYDDIETFPMKTIPTYDTMTRFPRASAPPRHADW	s19, s20	$F^{1,13}$, A^{14}
I	24, 26b, 27, F27′	(A) RRMDVYDDVETVPMKNLENGNNGYHNEYLYGIHNPIMTSMSSYDT VNRFPRASAPPRQDD	ND	A^{15}
J	24, F27′	(F) RRMDTEDVYDDIPTYDTMTRFPRASAPPRHADW	ND	F^{16}, A^{17}

[§]Alternative splicing might generate ten distinct cytoplasmic COOH-terminal tails of prominin-1. Exons 25, 26a, 26b, 27, and F27′ are given in cyan, red, green, blue, and pink, respectively. Types D–J are predicted from nucleotide (cDNA, expressed sequence tag (EST), genomic) sequences, in the absence of protein data with specific antibody.

cDNA complementary DNA, *A* amphibian, *C* chicken, *D* dog, *F* fish, *H* human, *M* mouse, *R* rat, *Rh* rhesus, *ND* not determined

Alternative exon F27′ is found in fish and amphibian.

Splice variant s21 is related to zebrafish prominin-1b.

[*]Indicates exon 25 introduces a frameshift on the following exon 26b.

[**]Indicates that exon 27 in dog harbors a stop codon generating a truncated C-terminus in this species (MENGNIGFHRHHSTQTV).

Ref.: 1. Jászai (2011); 2. Yu (2002); 3. Weigmann (1997); 4. Corbeil (2001); 5. Miraglia (1997); 6. Miraglia (1998); 7. Fargeas (2004); 8. McGrail (2010); 9. Fargeas (2007); 10. Fargeas (2003); 11. *Canis lupus* EST DT541441 and DT541268; 12. *Mus musculus* EST EH097137; 13. *Pimephales promelas* EST DT164241; 14. *Xenopus tropicalis* EST CX838199 and CX912260; 15. *Xenopus laevis* EST BJ061920; 16. *Osmerus mordax* EST EL532935, *Pimephales promelas* EST DT181633, *Cyprinus carpio* EST EX822056, *Salmo salar* EST DW541737; 17. *Xenopus laevis* EST CK79685

This table is modified from Ref. [22]

across species. Moreover, in certain fish, a duplication of the prominin-1 gene (e.g., zebrafish, Fugu (*Takifugu rubripes*); named prominin-1a and prominin-1b), which likely arose from the whole-genome duplication within the teleost lineage that resulted in multiple copies of many genes [38], adds a further twist to the complexity of splice variants. Nevertheless, the fish prominin-1 co-orthologs are not affected by alternative splicing to the same extent. For prominin-1b, only one splice form has been identified and is designated as prominin-1b.s21 [22]. Remarkably, at least ten alternative C-termini (Table 1.1; referred to as type A to J) can be generated by intron retention, exon skipping, or the use of a cryptic acceptor site [11, 22, 29].

Some prominin gene products exhibit distinct potential PSD95/Dlg1/ZO-1 (PDZ)-binding domains [29], which may indicate unidentified cytoplasmic PDZ domain-containing protein-interacting partner(s) [39]. Such interactions might regulate the targeting and/or retention of prominin-1 into specific subdomains of the plasma membrane that are characterized by a membrane curvature (e.g., microvillus, leading edge of lamellipodium) [27, 40]. Only tyrosine 828 found in the tyrosine phosphorylation consensus site (R/K)xxx(D/E)xxY is conserved among these prominin-1 splice variants [29]. Further studies are necessary to

elucidate the biological relevance of the use of facultative exons, but it appears that a variety of prominin-1-related polypeptides can be created that indirectly highlight alternative and/or complementary function(s).

1.4.3 Glycoforms

In general, N-linked glycans represent approximately 15–20% of the apparent molecular mass (ca. 112–120 kDa) of prominin-1 [3, 5, 8, 11]. The terminal N-glycan moieties of human PROMININ-1 are sialylated [41], while its polypeptide does not appear to carry any O-linked glycan [42]. Mak and colleagues have demonstrated by the heterologous expression of PROMININ-1 mutants that the glycosylation of each potential N-glycan site is not essential for its intracellular transport to the cell surface, but are collectively necessary [43]. In the murine reproductive tract, such as the epididymis and testis, biochemical analysis of prominin-1 suggested that an individual splice variant could exist in several distinct glycoforms [11]. In the absence of catalytic activity or a signaling pathway directly modulated by prominin-1, it is difficult to estimate the physiological significance of this differential posttranslational modification.

As mentioned above, the lack of AC133 immunoreactivity in Caco-2 cells upon their spontaneous enterocytic differentiation, which is concomitant with an alteration of the general glycosylation pattern of intestinal cells, has suggested a link between the accessibility of AC133 and glycosylation status [8]. Similarly, Hemmoranta and colleagues have observed differences in the glycan profiles of AC133-positive and AC133-negative hematopoietic stem and progenitor cells [44]. Whether and how alternative splicing, such as generation of the exon 3 that distinguishes PROMININ-1.s1 from PROMININ-1.s2 (named AC133-2 and AC133-1, respectively, in [17]), affects the recognition of the glycoprotein by the AC133 mAb is currently unknown. Indeed, the exact location of AC133 within human PROMININ-1 is unknown, but it may reside in the second half of the EC3 domain [45]. The differential glycosylation of PROMININ-1 among various human tissues may explain, at least in part, why its transcript is broadly detected, whereas the AC133 immunoreactivity appears to be limited to cells harboring stem cell properties, with some exceptions such as the differentiated cells found in the pancreas [46, 47] and the cones within the retina (see Chap. 4). Moreover, it is important to keep in mind that AC133 may also be masked under certain circumstances, particularly when prominin-1 is embedded in a specific cholesterol-dependent membrane microdomain where protein-lipid and protein-protein interactions are engaged (see below). Thus, a number of parameters other than differential glycosylation may interfere with AC133 detection, and some caution thus needs to be taken, particularly with the immunohistological preparation of samples [9, 13, 46, 48] (reviewed in Refs. [49, 50]). In addition to immunohistochemistry detection, it might be useful to use alternative