

Invited Mini Review

PROM1-mediated cell signal transduction in cancer stem cells and hepatocytes

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Prominin-1 (PROM1), also called CD133, is a penta-span transmembrane protein that is localized in membrane protrusions, such as microvilli and filopodia. It is known to be expressed in cancer stem cells and various progenitor cells of bone marrow, liver, kidney, and intestine. Accumulating evidence has revealed that PROM1 has multiple functions in various organs, such as eye, tooth, peripheral nerve, and liver, associating with various molecular protein partners. PROM1 regulates PKA-induced gluconeogenesis, TGF β -induced fibrosis, and IL-6-induced regeneration in the liver, associating with Radixin, SMAD7, and GP130, respectively. In addition, PROM1 is necessary to maintain cancer stem cell properties by activating PI3K and β -Catenin. PROM1-deficient mice also show distinct phenotypes in eyes, brain, peripheral nerves, and tooth. Here, we discuss recent findings of PROM1-mediated signal transduction. [BMB Reports 2023; 56(2): 65-70]

INTRODUCTION

Prominin-1 (PROM1), also called CD133, is a well-known cancer stem cell (CSC) marker (1-4). PROM1 is a pentaspan membrane protein with two bulky extracellular loops with 8 glycosylation residues and an \sim 50 amino acid long C-terminal tail (5, 6). As a lipid raft protein, PROM1 is known to be expressed mainly in microvilli or membrane protrusions of various stem cells or progenitor cells (5, 7, 8). Since PROM1 was first discovered in 1997 (5, 9), it has been extensively investigated. Because of its discovery in CSCs, it has been studied for decades as a target for cancer therapeutics (1-4, 10, 11). Recently, PROM1 has been extensively investigated in normal organs, such as liver, intestine, central and peripheral nervous system, eyes, and

tooth, using PROM1-deficient mice (12-19). Here, we discuss the precise molecular mechanisms for the physiological functions of PROM1 in different organs.

PROMININ-1 EXPRESSION IN EPITHELIAL AND HEMATOPOIETIC CELLS

PROM1 was first discovered independently in 1997 by two groups, those of Huttner and Buck (5, 9). Huttner's group identified PROM1 in mouse embryonic neuroepithelial cells and adult kidney cortex using a monoclonal antibody (13A4) raised against the mouse neuronal epithelial cells (5). In neuronal epithelial cells and proximal tubule of kidney, the immunoreactivity of 13A4 antigen is preferentially observed in the protrusions or microvilli, rather than the planar membrane of apical phase. These preferential staining patterns led them to name this antigen "Prominin" (in Latin, *prominere* means "prominent"). They found that PROM1 is a novel pentaspan plasma membrane protein with a molecular weight of 115 kDa, containing an N-terminal extracellular domain, two short cytoplasmic loops, two large glycosylated loops, and a long C-terminal cytoplasmic tail. Buck's group identified PROM1 with the different name "AC133" (6, 9). AC133 is identified in CD34-positive hematopoietic stem cells obtained from human fetal liver, bone marrow, and blood, using a monoclonal antibody raised against murine hybridoma cell line AC133.

Northern blot shows that PROM1 is mainly expressed in the brain, heart, colon, kidney, liver, small intestine, placenta, pancreas, and lung (20). To clearly demonstrate the expression pattern of PROM1 in different organs, Gilbertson's group developed LacZ-expressing mice (*pProm1-Cre/ERT2-LacZ*) in different organs under the control of *Prom1* promoter (Table 1, 15). *Prom1* promoter-induced LacZ staining shows that PROM1 is expressed in the epithelial cells of various adult organs, such as brain, retina, lung, kidney, pancreas, and gut. In particular, lineage tracing studies using YFP-expressing mice (*pProm1-Cre/ERT2-LacZ; Rosa26-LoxP-STOP-LoxP-YFP*) in different organs under the control of *Prom1* promoter reveal that PROM1 is expressed at the crypts of the small intestine with LGR5, known as a stem cells marker (Table 1).

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PROMININ-1 AS A LIPID RAFT PROTEIN

Lipid rafts are specialized lipid microdomains of the plasma membrane that are rich in cholesterol and sphingolipids, and play a role in a wide variety of important biological processes, such as cell signaling, apoptosis, cell adhesion, and re-organization of the cytoskeleton (21, 22). Most raft proteins, such as glycosylphosphatidylinositol (GPI)-anchored proteins, have Triton X-100 insolubility, because of its tight packaging with cholesterol and glycolipids (23-25). Unexpectedly, Huttner's group found that PROM1 is soluble in Triton X-100, but not in Lubrol WX, another detergent (7). Interestingly, PROM1 is not co-localized with human placental alkaline phosphatase (PLAP), a GPI-anchored protein, but with glycoprotein probe wheat germ agglutinin (WGA), both of which have distinct punctate staining patterns, indicating lipid raft proteins. Thus, Huttner's group suggests that there are two types of lipid rafts, TX-100-insoluble, and Lubrol WX-insoluble lipid rafts; PROM1 belongs to Lubrol WX-insoluble lipid rafts, whereas GPI-anchored proteins belongs to TX-100-insoluble.

PROMININ-1 IN MEMBRANE EXTENSIONS

PROM1 is also found in extracellular vesicles (EVs) and tunneling nanotubes (TNTs) derived from membrane protrusions (26-29). PROM1 is present in EVs originated from the ventricular fluid of the mouse embryonic brain and different cancer cell lines (FEMX-I, Caco-2, Huh7, and HCT116) (26-28). Because PROM1 knockdown in HCT116 cells reduces the amount of EVs, PROM1 might regulate the formation of EVs (28).

TNTs are intercellular communication system with a phospholipid- and cytoskeleton-based structure derived from plasma membrane protrusion (30-32). TNTs transport various intracellular substances, such as ions, vesicles, viruses, proteins, and mitochondria, between distant cells (33). PROM1 is present in TNTs from human CD34-positive primary mouse hematopoietic stem cells, and is transferred to adjacent cells (29). Because

PROM1-positive cells possess more TNTs than PROM1-negative cells, PROM1 could be one of the driving forces to generate TNTs *in vitro*. However, because PROM1 deficiency does not alter the structure of membrane extrusion, such as microvilli in the hepatocytes of mouse liver under electron microscopy (14), the role of PROM1 in TNT formation remains to be solved *in vivo*.

PROM1 is also found in the primary cilia of neuroepithelium from mouse forebrain (34), and epithelium from postnatal mouse incisor tooth (19). Because PROM1 deficiency reduces primary cilia formation and sonic hedgehog (SHH)-stimulated cell growth in mice, PROM1 is required to maintain the stemness of tooth epithelial cells by regulating cilia formation (19). PROM1 is present in the connecting cilium of photoreceptor cells in the eyes, which resides between the outer and inner segments (17). PROM1 deficiency leads to abnormal arrangement of the outer segment of photoreceptor cells, with complete loss of vision in mouse. In addition, point mutation of PROM1 (R373C) is identified from patient with macular degeneration. The transgenic mice expressing PROM1 R373C mutants result in abnormal arrangement of the outer segment of photoreceptor cells (35). These results indicate that PROM1 functions in photoreceptor disk morphogenesis by interacting with a photoreceptor-specific cadherin (PCDH21) and F-actin.

PROM1 regulates the regeneration of axon in peripheral nerves (16). PROM1 is expressed in the neurons of dorsal root ganglion (DRG), and PROM1 deficiency reduces injury-induced axon regeneration in DRG cultures, and in the sciatic nerve. Further studies show that PROM1 promotes axon regeneration in neuron by interacting with activin receptor type 1B (ALK4) and activating SMAD2 signaling. PROM1 in adult mouse brain is expressed along with myelin basic protein (MBP) in white matter, and PROM1 deficiency reduces myelination in the corpus callosum with cognitive impairment, indicating that PROM1 regulates myelination (18). However, the precise mechanism of how PROM1 regulates myelination remains to be solved.

Table 1. Mouse models used in PROM1 research

Name	Gene map	Description	References
<i>Prom1</i> knockin mouse (<i>Prom1^{C-L}</i>)	<i>pProm1-Cre/ERT2-LacZ</i>	Tamoxifen-induced Cre recombinase and LacZ expression under the <i>Prom1</i> promoter activity	(15)
PROM1 lineage tracing mouse	<i>pProm1-Cre/ERT2-LacZ; Rosa26-LoxP-STOP-LoxP-YFP</i>	Tamoxifen-induced YFP expression under the <i>Prom1</i> promoter activity	(15)
PROM1 lineage tracing mouse	<i>pProm1-Cre/ERT2-LacZ; Rosa26-LoxP-STOP-LoxP-TdTom</i>	Tamoxifen-induced TdTom expression under the <i>Prom1</i> promoter activity	(13), (14), (42)
PROM1-specific cell ablation mouse	<i>pProm1-Cre/ERT2-LacZ; Rosa26-LoxP-STOP-LoxP-DTA</i>	Tamoxifen-induced Diphtheria Toxin Fragment A (DTA) expression under the <i>Prom1</i> promoter activity: PROM1 ⁺ cells depletion	(42)
Liver-specific <i>Prom1</i> knockout mouse	<i>Alb-Cre; Prom1^{flf}</i>	Liver-specific PROM1 deficiency	(13), (14)
Cholangiocyte-specific <i>Prom1</i> knockout mouse	<i>Krt19-Cre; Prom1^{flf}</i>	Cholangiocyte-specific PROM1 deficiency	(13)

PROMININ-1 IN CANCER STEM CELLS

CSCs are defined as a subpopulation of cancer cells that possess self-renewal, differentiation potential, and resistance to radio and chemotherapy (36). Because of these properties, CSCs are a crucial therapeutic target for cancer. A CD133 (PROM1)-positive cell subpopulation from colon and brain tumor initiates solid tumors in immunodeficient mice, but CD133-negative cell does not (10, 11). Furthermore, serial transplantation of such tumors has been maintained for several generations. In addition, CD133-positive cells from colon cancer show long-term expansion *in vitro*, maintaining CSCs properties.

The tumorigenic potential and stemness of PROM1-positive CSCs are regulated by several signaling pathways. PROM1 regulates cancer cell differentiation in multiple cell lines by stabilizing β -Catenin via interacting with histone deacetylase 6 (HDAC6) (37). The first intracellular loop domain of PROM1 interacts with HDAC6 (Fig. 1). The PROM1/HDAC6 complex induces deacetylation and inhibits the proteasomal degradation of β -Catenin, promoting tumor formation by WNT signaling pathway. However, the physiological role of the PROM1- β -Catenin axis might be debatable, as β -Catenin expression levels are not altered by PROM1 deficiency in mouse liver (Data not shown). PROM1 is upregulated in hepatocellular carcinoma and glioma stem cell upon exposure to hypoxia or interleukin-6 (IL-6) (38, 39). Hypoxia increases glycosyltransferase 8 domain-containing 1 (GLT8D1), which interacts with the first extracellular domain of PROM1 (PROM1-EX1), and prevents the lysosomal degradation of PROM1 via glycosylation (40). Thus, the stabilized PROM1 increases with β -Catenin, and promotes WNT- β -Catenin signaling. Indeed, the peptide from the PROM1-EX1 interferes with the interaction between GLT8D1 and PROM1, abolishing the tumorigenesis of glioma stem cells by inhibiting

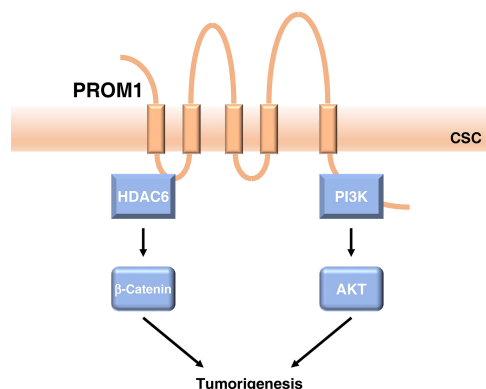


Fig. 1. PROM1 cell signaling in cancer. The first intracellular domain of PROM1 promotes WNT- β -Catenin signaling pathway and tumorigenesis by interacting with HDAC6 (37). The C-terminal cytoplasmic domain of PROM1 activates PI3K-AKT signaling pathway and tumorigenesis by interacting with PI3K p85 (41). CSC, cancer stem cell.

WNT- β -Catenin signaling. PROM1 knockdown reduces tumorigenic capacity in human glioblastoma cells by inhibiting PI3K-AKT pathway (41). The phosphorylated 828 Tyr residue in the cytoplasmic C-terminal domain of PROM1 binds to PI3K p85 subunit, and phosphorylates AKT (Fig. 1). Thus, PROM1 Y828F mutant inhibits PI3K-AKT signaling pathway, preventing sphere formation of CSCs, indicating PROM1-PI3K pathways are necessary to maintain CSCs properties. However, insulin-induced phosphorylation of AKT and ERK is not different between *Prom1* wild-type and knockout primary hepatocytes (12). Since the PROM1-PI3K axis study in CSCs is mainly performed in tumors after overexpression and knockdown of PROM1 (41), the physiological role of the PROM1-PI3K axis might be debatable.

PROM1 is upregulated in human hepatocellular carcinoma and mouse tumor derived from diethylnitrosamine (DEN)/CCl₄-treated mice liver (39, 42). Lineage tracing mice (*pProm1-Cre/ERT2-LacZ; Rosa26-LoxP-STOP-LoxP-TdTom*) treated with DEN/CCl₄ show the clonal expansion of PROM1-positive cells (Table 1) (42). The carcinogenesis in DEN/CCl₄-treated mice liver is attenuated by *Prom1*-specific cell ablation in mice (*pProm1-Cre/ERT2-LacZ; Rosa26-LoxP-STOP-LoxP-DTA*) (Table 1). This study reveals that PROM1 is a major driving force in initiating hepatocellular carcinoma.

PROMININ-1 IN OTHER SPECIES

The function of PROM1 is well-characterized in *Drosophila*, of which "*prominin-like (promL)*" is the orthologue of mammalian PROM1. *PromL* is expressed at the apical surface of photoreceptor cells. Because *promL* deficiency shows rhabdomyogenesis and retinal degeneration, *PromL* is required to regulate the apical compartment of photoreceptor cells and their morphogenesis (43). The *PromL* is also expressed and functions in the brain of *Drosophila* (44, 45). *PromL* is expressed at the pars intercerebralis region with insulin-producing cells (IPCs) in the brain of *Drosophila*. Because *PromL* knockdown in IPCs leads to an extended life span and glucose metabolism defects with a reduced AKT phosphorylation, *PromL* regulates longevity and glucose metabolism by insulin-AKT signaling pathway (44). In addition, *promL* knock-down in pan-neuron shows the reduced locomotion activity with a reduced level of dopamine concentration (45). Indeed, *promL* knock-down reduces the level of mRNAs for tyrosine hydroxylase (TH) and DOPA decarboxylase (Ddc), which regulate dopamine synthesis. Thus, *PromL* expressed in dopaminergic neurons controls locomotion of *Drosophila* by regulating dopamine biosynthesis.

From developmental wing imaginal disc epithelium of *Drosophila*, *PromL* is found in microvilli-derived extracellular vesicles along with Hedgehog (Hh), a morphogen required for the development of *Drosophila* wing imaginal disc (46, 47). The knock-down of *promL* shows shorter apical protrusion of epithelium in wing imaginal disc, reducing the expression of de-

capentaplegic (*dpp*), a target gene of Hh. Thus, PromL-containing EVs mediate long-range Hh signaling in wing development of *Drosophila* (46).

PROMININ-1 IN THE LIVER

The liver is a central organ for maintaining the homeostasis of organism through controlling the metabolic process and detoxification (48). The liver is mainly composed of two types of cells: parenchymal cells, and non-parenchymal cells. The parenchymal cells including hepatocytes make up most (~80%) of the liver, and are responsible for its main functions. The non-parenchymal cells are composed of various cell types: cholangiocyte, hepatic progenitor cells, hepatic stellate cells, and Kupffer cells (49). In the liver, PROM1 is mainly known as a hepatic progenitor cell marker, but is also expressed in hepatocytes and cholangiocytes (12-14). The immunofluorescence and electron microscopy of PROM1 show that PROM1 is expressed in microvilli, as well as the plane plasma membrane of mouse hepatocytes. PROM1 deficiency reduces gluconeogenesis in fasted mice by inhibiting glucagon dependent PKA activation in the liver (12). The C-terminal cytoplasmic domain of PROM1 interacts with Radixin, and recruits it to lipid rafts (Fig. 2). Then, Radixin functions as an A-kinase anchoring protein (AKAP), which is a scaffold protein that binds to PKA and its substrates.

The expression of PROM1 in the liver is upregulated by various liver injuries, such as choline-deficient, ethionine-supplemented diet (CDE) (50), bile duct ligation (BDL)-induced cholestasis (13), CCl₄ (14, 42), and 2/3 partial hepatectomy (PHx) (14). The immunofluorescence and lineage tracing study

of PROM1 show that the expression of PROM1 is increased mainly in hepatocytes rather than in cholangiocytes, progenitor cells, and stellate cells of BDL and PHx liver (13, 14).

PROM1 deficiency aggravates BDL-induced liver fibrosis (13). The first intracellular domain of PROM1 interacts with SMAD7, a negative regulator of the TGF β -SMAD2/3 signaling, inhibiting SMAD7 ubiquitination (Fig. 2). Thus, the upregulated PROM1 in hepatocytes attenuates liver fibrosis via decreasing TGF β -induced SMAD2/3 phosphorylation. However, PROM1 is expressed only in the periportal region of the liver and aggravates liver fibrosis in the rhesus rotavirus (RRV) model (51), challenging the protective role of PROM1 in liver fibrosis. Lee *et al.*, (13) showed that PROM1 is upregulated mainly in the hepatocytes of BDL liver by multiple methods, such as immunofluorescence and PROM1 lineage tracing analysis. In addition, they elucidated that the function of PROM1 is predominant in hepatocytes over cholangiocytes by analyzing cholangiocyte-specific *Prom1* knockout mice and hepatocyte-specific SMAD7 overexpression mice (Table 1).

Bahn *et al.* found that PROM1 is required for hepatocyte proliferation during liver regeneration after acute liver injury, such as CCl₄ injection or PHx (14). Liver-specific *Prom1* deficiency delays liver regeneration with reduced hepatocyte proliferation. Indeed, the upregulated PROM1 in hepatocytes enhances IL-6 signaling pathway by interacting with an IL-6 signal transducer glycoprotein 130 (GP130). The domain analysis of PROM1 reveals that the first extracellular domain (PROM1-EX1) interacts with GP130 and enhances STAT3 activity by recruiting GP130 to lipid rafts (Fig. 2). Thus, the adenoviral overexpression of GPI-anchored PROM1-EX1 rescues hepatocyte proliferation and liver regeneration in liver-specific *Prom1* knockout mice. This finding suggests that PROM1 plays a central role in liver regeneration via IL-6-STAT3 signaling in response to acute liver injury.

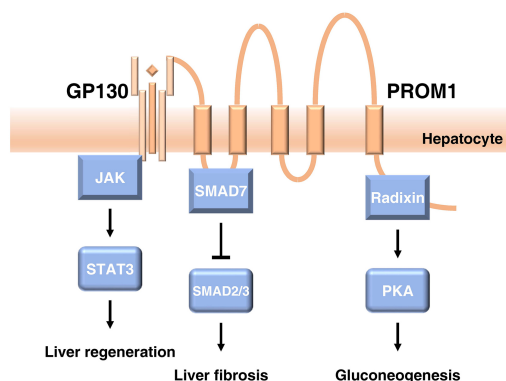


Fig. 2. PROM1 cell signaling in the liver. The first extracellular domain of PROM1 activates IL-6-STAT3 signaling pathway and hepatocyte proliferation by interacting with GP130 (14). The first intracellular domain of PROM1 inhibits TGF β -SMAD2/3 signaling pathway and hepatocyte apoptosis by interacting with SMAD7 (13). The C-terminal cytoplasmic domain of PROM1 enhances glucagon-induced PKA signaling pathway and hepatic gluconeogenesis by interacting with Radixin (12).

PERSPECTIVE

The function of PROM1 is well-characterized as a signaling molecule maintaining stemness in cancer and regenerative function in liver (13, 14, 37, 41). Although PROM1 is also expressed in other organs such as brain, lung, kidney and gut, the functions in these organs have not been determined. For example, PROM1 might be required for maintaining stemness of crypt epithelial cells of small intestine (15). Thus, it is necessary to study precise signaling mechanism how PROM1 maintains stemness of epithelial cells in different organs.

PROM1 is upregulated in hepatocellular carcinoma and liver at the various conditions (13, 14, 42, 50). It is unclear which factors activate the promoter activity of PROM1 in each condition, although there is some evidence such as STAT3 (39). Thus, it is necessary to investigate which factors increase the expression of PROM1 in response to various liver damages such as CDE diet, cholestasis, reactive oxygen species (ROS), and PHx. PROM1 promoter studies might lead to a broader

understanding of the physiological role of PROM1.

The different domains of PROM1 interact with various signaling molecules. The first extracellular domain interacts with GP130, activating IL-6 signaling (14). The first intracellular domain interacts with HDAC6 and SMAD7 regulating WNT and TGF β signaling, respectively (13, 37). The C-terminal cytoplasmic domain interacts with PI3K and radixin regulating AKT and PKA signaling, respectively (12, 41). Based on this domain study, PROM1 might be a promising target for some gene or peptide therapy. For example, PROM1-EX1 peptide promotes liver regeneration after various liver damages by facilitating IL-6 signaling without tumor development because it lacks C-terminal domain, which regulates PI3K-AKT signaling pathway. The first intracellular domain peptide might interfere the ubiquitination of SMAD7, increasing SMAD7 expressing and then preventing TGF β signaling. Gene or peptide therapy targeting different domains of PROM1 might be novel approaches for liver fibrosis, cancer and transplantation.

SUMMARY

- PROM1 in lipid rafts: PROM1 is localized in lipid rafts of epithelial cells from different organs.
- PROM1 has a crucial role in expanding plasma membrane of different cell types such as cancer, hematopoietic, photoreceptor, nerve and glial cells.
- PROM1/HDAC6 complex regulates cancer differentiation in multiple cancer cells via WNT- β -Catenin signaling pathway.
- PROM1/PI3K complex promotes tumorigenesis in glioblastoma cells via AKT signaling pathway.
- PROM1/Radixin complex regulates hepatic gluconeogenesis via glucagon-induced PKA signaling pathway.
- PROM1/SMAD7 complex protects against liver fibrosis via inhibiting TGF β -SMAD2/3 signaling pathway.
- PROM1/GP130 complex promotes liver regeneration via IL-6-STAT3 signaling pathway.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Liu G, Yuan X, Zeng Z et al (2006) Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer* 5, 67

2. Bao S, Wu Q, McLendon RE et al (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 444, 756-760

3. Florek M, Haase M, Marzesco AM et al (2005) Prominin-1/CD133, a neural and hematopoietic stem cell marker, is expressed in adult human differentiated cells and certain types of kidney cancer. *Cell Tissue Res* 319, 15-26

4. Yin S, Li J, Hu C et al (2007) CD133 positive hepatocellular carcinoma cells possess high capacity for tumorigenicity. *Int J Cancer* 120, 1444-1450

5. Weigmann A, Corbeil D, Hellwig A and Huttner WB (1997) Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. *Proc Natl Acad Sci U S A* 94, 12425-12430

6. Miraglia S, Godfrey W, Yin AH et al (1997) A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood* 90, 5013-5021

7. Roper K, Corbeil D and Huttner WB (2000) Retention of prominin in microvilli reveals distinct cholesterol-based lipid micro-domains in the apical plasma membrane. *Nat Cell Biol* 2, 582-592

8. Corbeil D, Roper K, Hannah MJ, Hellwig A and Huttner WB (1999) Selective localization of the polytopic membrane protein prominin in microvilli of epithelial cells - a combination of apical sorting and retention in plasma membrane protrusions. *J Cell Sci* 112, 1023-1033

9. Yin AH, Miraglia S, Zanjani ED et al (1997) AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* 90, 5002-5012

10. Singh SK, Hawkins C, Clarke ID et al (2004) Identification of human brain tumour initiating cells. *Nature* 432, 396-401

11. Ricci-Vitiani L, Lombardi DG, Pilozzi E et al (2007) Identification and expansion of human colon-cancer-initiating cells. *Nature* 445, 111-115

12. Lee H, Yu DM, Park JS et al (2020) Prominin-1-Radixin axis controls hepatic gluconeogenesis by regulating PKA activity. *EMBO Rep* 21, e49416

13. Lee H, Yu DM, Bahn MS et al (2022) Hepatocyte-specific Prominin-1 protects against liver injury-induced fibrosis by stabilizing SMAD7. *Exp Mol Med* 54, 1277-1289

14. Bahn MS, Yu DM, Lee M et al (2022) Central role of Prominin-1 in lipid rafts during liver regeneration. *Nat Commun* 13, 6219

15. Zhu L, Gibson P, Currle DS et al (2009) Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. *Nature* 457, 603-607

16. Lee J, Shin JE, Lee B et al (2020) The stem cell marker Prom1 promotes axon regeneration by down-regulating cholesterol synthesis via Smad signaling. *Proc Natl Acad Sci U S A* 117, 15955-15966

17. Zacchigna S, Oh H, Wilsch-Brauninger M et al (2009) Loss of the cholesterol-binding protein prominin-1/CD133 causes disk dysmorphogenesis and photoreceptor degeneration. *J Neurosci* 29, 2297-2308

18. Choi MH, Na JE, Yoon YR, Rhyu IJ, Ko YG and Baik JH (2018) Hypomyelination and cognitive impairment in mice lacking CD133 (Prominin-1). *Biochem Biophys Res Commun* 502, 291-298

19. Singer D, Thamm K, Zhuang H et al (2019) Prominin-1 controls stem cell activation by orchestrating ciliary dynamics. *EMBO J* 38, e99845
20. Yu Y, Flint A, Dvorin EL and Bischoff J (2002) AC133-2, a novel isoform of human AC133 stem cell antigen. *J Biol Chem* 277, 20711-20716
21. Munro S (2003) Lipid rafts: elusive or illusive? *Cell* 115, 377-388
22. Simons K and Toomre D (2000) Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 1, 31-39
23. Thomas S, Preda-Pais A, Casares S and Brumeanu TD (2004) Analysis of lipid rafts in T cells. *Mol Immunol* 41, 399-409
24. Thomas S, Kumar RS and Brumeanu TD (2004) Role of lipid rafts in T cells. *Arch Immunol Ther Exp (Warsz)* 52, 215-224
25. Korade Z and Kenworthy AK (2008) Lipid rafts, cholesterol, and the brain. *Neuropharmacology* 55, 1265-1273
26. Marzesco AM, Janich P, Wilsch-Brauninger M et al (2005) Release of extracellular membrane particles carrying the stem cell marker prominin-1 (CD133) from neural progenitors and other epithelial cells. *J Cell Sci* 118, 2849-2858
27. Chao OS, Chang TC, Di Bella MA et al (2017) The HDAC6 inhibitor tubacin induces release of CD133⁺ extracellular vesicles from cancer cells. *J Cell Biochem* 118, 4414-4424
28. Kang M, Kim S and Ko J (2019) Roles of CD133 in microvesicle formation and oncoprotein trafficking in colon cancer. *FASEB J* 33, 4248-4260
29. Reichert D, Scheinpflug J, Karbanova J, Freund D, Bornhauser M and Corbeil D (2016) Tunneling nanotubes mediate the transfer of stem cell marker CD133 between hematopoietic progenitor cells. *Exp Hematol* 44, 1092-1112 e2
30. Gerdes HH, Rustom A and Wang X (2013) Tunneling nanotubes, an emerging intercellular communication route in development. *Mech Dev* 130, 381-387
31. Pinto G, Brou C and Zurzolo C (2020) Tunneling nanotubes: the fuel of tumor progression? *Trends Cancer* 6, 874-888
32. Han X and Wang X (2021) Opportunities and challenges in tunneling nanotubes research: how far from clinical application? *Int J Mol Sci* 22, 2306
33. Zhu C, Shi Y and You J (2021) Immune cell connection by tunneling nanotubes: the impact of intercellular cross-talk on the immune response and its therapeutic applications. *Mol Pharm* 18, 772-786
34. Dubreuil V, Marzesco AM, Corbeil D, Huttner WB and Wilsch-Brauninger M (2007) Midbody and primary cilium of neural progenitors release extracellular membrane particles enriched in the stem cell marker prominin-1. *J Cell Biol* 176, 483-495
35. Yang Z, Chen Y, Lillo C et al (2008) Mutant prominin 1 found in patients with macular degeneration disrupts photoreceptor disk morphogenesis in mice. *J Clin Invest* 118, 2908-2916
36. Yang L, Shi P, Zhao G et al (2020) Targeting cancer stem cell pathways for cancer therapy. *Signal Transduct Target Ther* 5, 8
37. Mak AB, Nixon AM, Kittanakom S et al (2012) Regulation of CD133 by HDAC6 promotes beta-catenin signaling to suppress cancer cell differentiation. *Cell Rep* 2, 951-963
38. Soeda A, Park M, Lee D et al (2009) Hypoxia promotes expansion of the CD133-positive glioma stem cells through activation of HIF-1alpha. *Oncogene* 28, 3949-3959
39. Won C, Kim BH, Yi EH et al (2015) Signal transducer and activator of transcription 3-mediated CD133 up-regulation contributes to promotion of hepatocellular carcinoma. *Hepatology* 62, 1160-1173
40. Liu K, Jiang L, Shi Y et al (2022) Hypoxia-induced GLT8D1 promotes glioma stem cell maintenance by inhibiting CD133 degradation through N-linked glycosylation. *Cell Death Differ* 29, 1834-1849
41. Wei Y, Jiang Y, Zou F et al (2013) Activation of PI3K/Akt pathway by CD133-p85 interaction promotes tumorigenic capacity of glioma stem cells. *Proc Natl Acad Sci U S A* 110, 6829-6834
42. Zhou L, Yu KH, Wong TL et al (2022) Lineage tracing and single-cell analysis reveal proliferative Prom1⁺ tumour-propagating cells and their dynamic cellular transition during liver cancer progression. *Gut* 71, 1656-1668
43. Gurudev N, Yuan M and Knust E (2014) chaoptin, prominin, eyes shut and crumbs form a genetic network controlling the apical compartment of Drosophila photoreceptor cells. *Biol Open* 3, 332-341
44. Ryu TH, Yeom E, Subramanian M, Lee KS and Yu K (2019) Prominin-like regulates longevity and glucose metabolism via insulin signaling in drosophila. *J Gerontol A Biol Sci Med Sci* 74, 1557-1563
45. Ryu TH, Subramanian M, Yeom E and Yu K (2022) The prominin-like gene expressed in a subset of dopaminergic neurons regulates locomotion in drosophila. *Mol Cells* 45, 640-648
46. Hurbain I, Mace AS, Romao M et al (2022) Microvilli-derived extracellular vesicles carry Hedgehog morphogenic signals for Drosophila wing imaginal disc development. *Curr Biol* 32, 361-373 e6
47. Ingham PW and McMahon AP (2001) Hedgehog signaling in animal development: paradigms and principles. *Genes Dev* 15, 3059-3087
48. Trefts E, Gannon M and Wasserman DH (2017) The liver. *Curr Biol* 27, R1147-R1151
49. Bale SS, Geerts S, Jindal R and Yarmush ML (2016) Isolation and co-culture of rat parenchymal and non-parenchymal liver cells to evaluate cellular interactions and response. *Sci Rep* 6, 25329
50. Passman AM, Strauss RP, McSpadden SB et al (2021) Maraviroc prevents HCC development by suppressing macrophages and the liver progenitor cell response in a murine chronic liver disease model. *Cancers (Basel)* 13, 4935
51. Zagory JA, Fenlon M, Dietz W et al (2019) Prominin-1 promotes biliary fibrosis associated with biliary atresia. *Hepatology* 69, 2586-2597