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Abstract

Prominin–1, 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 PROM has multiple functions in various organs, such as eye, tooth, peripheral nerve, and liver, associating with various molecular protein partners. Prominin–1 regulates PKA-induced gluconeogenesis, TGFβ-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-deficienct mice also show distinct phenotypes in eyes, brain, peripheral nerves, and tooth. Here, we discuss recent findings of PROM1-mediated signal transduction.

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 ~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.

1. 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 [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.

2. 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.

3. 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.

4. 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 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)/CCl_4-treated mice liver [39, 42]. Lineage tracing mice (*pProm1-Cre/ERT2-LacZ; Rosa26-LoxP-STOP-LoxP-TdTom*) treated with DEN/CCl_4 show the clonal expansion of PROM1-positive cells [42]. The carcinogenesis in DEN/CCl_4-treated mice liver is attenuated by *Prom1*-specific cell ablation in mice (*pProm1-Cre/ERT2-LacZ; Rosa26-LoxP-STOP-LoxP-DTA*). This study reveals that PROM1 is a major driving force in

initiating hepatocellular carcinoma.

5. 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 rhabdomere dismorphogenesis 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 knock-down 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 decapentaplegic (dpp), a target gene of Hh. Thus, PromL-containing EVs mediate long-range Hh signaling in wing development of *Drosophila* [46].

6. 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], CCl_4 [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-induced 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 TGFβ-SMAD2/3 signaling, inhibiting SMAD7 ubiquitination (Fig. 2). Thus, the upregulated PROM1 in hepatocytes attenuates liver fibrosis via decreasing TGFβ-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.

Bahn et al. found that PROM1 is required for hepatocyte proliferation during liver regeneration after acute liver injury, such as CCl_4 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.

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 TGFβ 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 TGFβ 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 TGFβ-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 disclose no conflicts

Figure Legends

Figure 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.

Figure 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 TGFβ-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].

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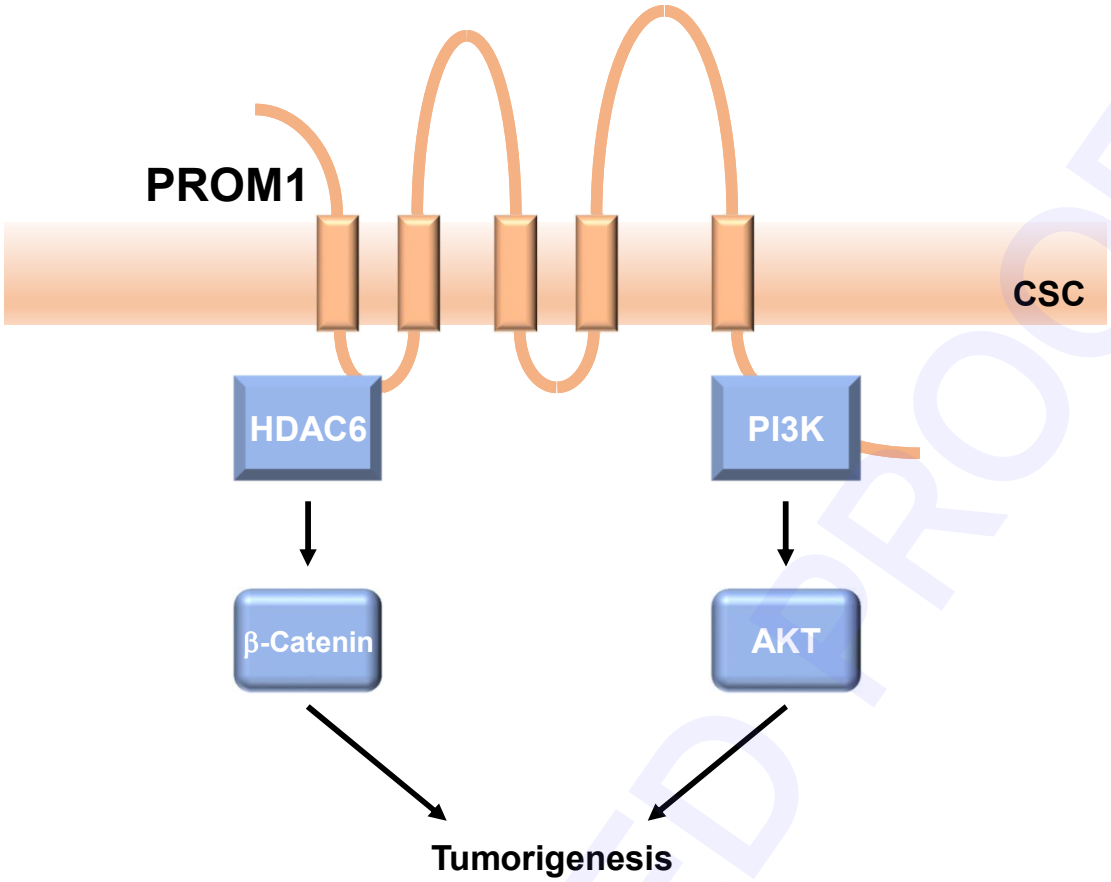

Figure 1

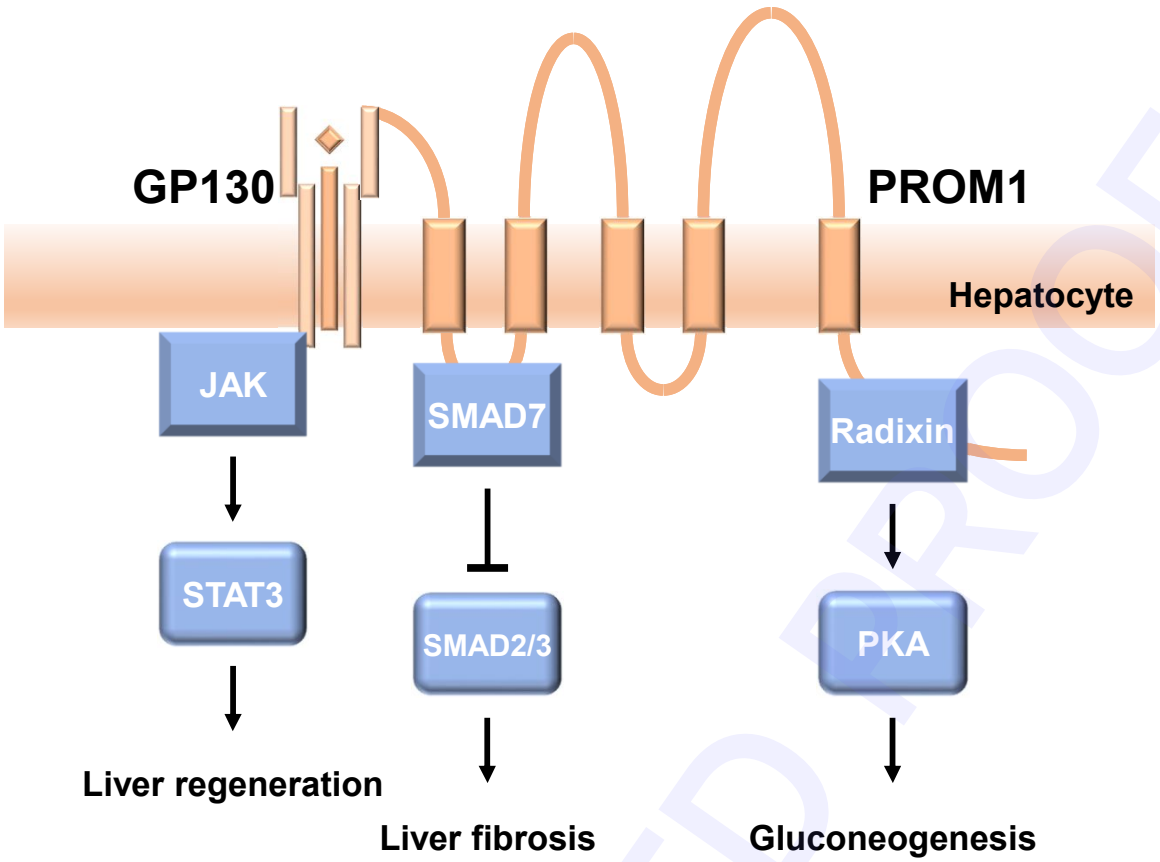

Figure 2

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

Table 1. Mouse models used in PROM1 research.