Podoplanin requires sialylated O-glycans for stable expression on lymphatic endothelial cells and for interaction with platelets

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Key Points

- Sialylated O-glycans protects PDNP from proteolytic degradation.
- Sialylated core 1 O-glycans on lymphatic endothelial cells (LECs) are required for interacting with platelets.

O-glycosylation of podoplanin (PDNP) on lymphatic endothelial cells is critical for the separation of blood and lymphatic systems by interacting with platelet C-type lectin-like receptor 2 during development. However, how O-glycosylation controls endothelial PDNP function and expression remains unclear. In this study, we report that core 1 O-glycan–deficient or desialylated PDNP was highly susceptible to proteolytic degradation by various proteases, including metalloproteinases (MMP)-2/9. We found that the lymph contained activated MMP-2/9 and incubation of the lymph reduced surface levels of PDNP on core 1 O-glycan–deficient endothelial cells, but not on wild-type ECs. The lymph from mice with sepsis induced by cecal ligation and puncture, which contained bacteria-derived sialidase, reduced PDNP levels on wild-type ECs. The MMP inhibitor, GM6001, rescued these reductions. Additionally, GM6001 treatment rescued the reduction of PDNP level on lymphatic endothelial cells in mice lacking endothelial core 1 O-glycan or cecal ligation and puncture-treated mice. Furthermore, core 1 O-glycan–deficient or desialylated PDNP impaired platelet interaction under physiological flow. These data indicate that sialylated O-glycans of PDNP are essential for platelet adhesion and prevent PDNP from proteolytic degradation primarily mediated by MMPs in the lymph. (Blood. 2014;124(24):3656-3665)

Introduction

Podoplanin (PDNP), a type 1 transmembrane mucin-type O-glycoprotein, was initially discovered as a platelet aggregation-inducing glycoprotein expressed on tumor cells. Subsequent studies have shown that it is expressed on several cell types, including lymphatic endothelial cells (LECs). PDNP on LECs is critical for the initiation and maintenance of an independent lymphatic vascular system.

PDNP consists of 172 amino acids in mice and 163 amino acids in humans. It has an extracellular domain, a transmembrane domain, and a short cytoplasmic domain. A striking feature of the extracellular domain of PDNP is a high content of serine and threonine residues that can potentially be attached by mucin-type O-glycans (also known as O-N-acetylgalactosamine [GalNAc] glycans or simply O-glycans for this study). O-glycosylation is a common form of posttranslational modification of membrane and secreted proteins. It occurs in the Golgi apparatus via sequential reactions catalyzed by specific glycosyltransferases. The core of all mucin-type O-glycans is serine/threonine-linked GalNAc, also known as the Tn antigen, which is normally not exposed due to further modification in forming distinct subtypes of O-glycans. Among them, core 1 O-glycans are a predominant form. Core 1 O-glycans are synthesized by adding galactose in β3 linkage to Tn antigen, which is catalyzed solely by the T-synthase (core 1 synthase, C1galt1). Core 1 structure can be further branched to form extended core 1 or core 2 structures, or can be modified by adding sialic acids. These glycans are known as core 1-derived O-glycans.

The molecular weight of the core PDNP polypeptide is about 17 kDa; however, PDNP isolated from different cell types has an apparent molecular weight ranging from 37 kDa to 41 kDa, suggesting extensive, heterogeneous O-glycosylation. In our previous study, mice lacking endothelial core 1 O-glycans display impaired PDNP expression that is required for the development and maintenance of an independent lymphatic vascular system. PDNP binding to the platelet C-type lectin-like receptor 2 (CLEC-2) induces platelet aggregation that seals initial blood-lymphatic vascular connections during embryonic development. Sialylcore 1 O-glycans on PDNP are required for interacting with CLEC-2 using Chinese hamster ovary cells (CHO) or synthetic glycopeptides. However, CHO cells lack core 2 and extended core 1 O-glycans. Therefore, whether PDNP on endothelial cells (ECs) requires similar O-glycosylation to interact with CLEC-2 is unclear. Additionally, how O-glycosylation regulates function and expression of PDNP on LECs remains to be addressed.
In this study, we show that core 1 O-glycan–deficient or desialylated PDPN on LECs is highly susceptible to proteolytic degradation, suggesting the importance of O-glycosylation for controlling stability on EC surfaces. Notably, matrix metalloproteinases (MMPs) in the lymph are essential for the proteolytic degradation of core 1 O-glycan–deficient or desialylated PDPN. Furthermore, core 1 O-glycan–deficient or desialylated PDPN on LECs exhibits impaired interactions with platelets under flow. Our results provide strong evidence that sialylated O-glycans control the expression and function of endothelial PDPN.

Materials and methods

Mice and cells

Mice lacking EC and hematopoietic core (EHC) 1 O-glycans (C1galt1fl/fl; Tie2Cre) and platelet-specific Clec2−/− mice were described previously.8,13 Mice with doxycycline-inducible global deficiency of core 1 O-glycans (inducible C1galt1fl/fl) were generated by crossing C1galt1fl/fl mice with Rosa26-rtTA, tetO-Cre Tg mice (The Jackson Laboratory). Primary LECs were isolated from different tissues as described in “Results.” Some of the LECs were used for culture. Stable PDPN-eGFP-expressed ECs are established by transfection of immortalized wild-type (WT) or C1galt1fl/fl ECs8 with DNA construct of mouse PDPN tagged with a C-terminal eGFP (PDPN-eGFP). CHO cells were cotransfected with complementary DNA constructs encoding PDPN-eGFP and glycosyltransferases. All mouse experiments were performed in compliance with protocols approved by the Institutional Animal Care and Use Committee of the Oklahoma Medical Research Foundation.

Platelet adhesion under flow

WT or Clec2−/− platelet adhesion on ECs or CHO cells expressing PDPN-eGFP were performed under flow.16

Lymph or protease treatment

PDPN-eGFP transfected WT or C1galt1fl/fl ECs were incubated with 200 μL of mouse lymph or buffer containing proteases.

Intraperitoneal (IP) injection of MMP inhibitor

IP injections of GM6001 were performed. Primary LECs isolated from mesentery and cryosections of ileum were used for staining to examine Tn, Lyve-1, and PDPN expression.

Cecal ligation and puncture (CLP) sepsis model

CLP was performed with or without IP injection of sialidase inhibitor or GM6001. The lymph and mesentery were collected for EC treatment and flow cytometry.

More details of materials and methods used are described in the supplemental Methods, available on the Blood Web site.

Statistics

The unpaired Student t test was used to determine P values as indicated in the figures.

Results

Lack of core 1 O-glycosylation reduces PDPN on LECs in vivo

We report here that mice lacking core 1 O-glycans (EHC C1galt1−/−) exhibited reduced levels of PDPN in lymphatic microvessels.8 To determine whether the reduction of PDPN occurred on larger collecting lymphatic vessels, we performed immunostaining on lymphatic vessels in embryonic day (E17) mesentery. We first probed the Prox1-positive LECs of the collecting lymphatic vessels of EHC C1galt1−/− mesenteries with mAb to Tn antigen (Figure 1A).8,9 Anti-Tn mAb stained C1galt1−/− but not WT LECs, indicating efficient excision of the C1galt1 gene. Desialylation did not appreciably affect the intensity of anti-Tn staining (data not shown), indicating that most exposed Tn is not sialylated. Immunofluorescent images revealed that the collecting lymphatic vessels of EHC C1galt1−/− mesenteries had reduced PDPN levels relative to WT collecting lymphatic vessels. Using primary LECs, which were defined as CD31+/Lyve-1+ cells (supplemental Figure 1A), flow cytometric analyses showed that the surface level of PDPN on mesenteric LECs from EHC C1galt1−/− mice was reduced compared with that from WT mice (Figure 1B). Reduced surface levels of PDPN were also found on isolated primary LECs from collecting lymphatic vessels in 3-week-old and 8-week-old EHC C1galt1−/− mesentery (supplemental Figure 1B), on isolated primary LECs from Pl1 (supplemental Figure 1C), or in cryosections of lymphatic microvessels of 8-week-old EHC C1galt1−/− small intestine (supplemental Figure 1D). These results indicate that loss of core 1 O-glycans causes defective expression of PDPN in different types of C1galt1−/− lymphatic vessels.

Loss of core 1 O-glycans occurs during embryonic development in EHC C1galt1−/− mice. To determine whether postnatal loss of core 1 O-glycosylation impairs PDPN expression, we used inducible C1galt1−/− mice. Six weeks after a doxycycline diet, Tn antigen was detected in the intestine of inducible C1galt1−/− mice but not in WT littermates (supplemental Figure 1E). Lyve-1–positive submucosal lymphatic vessels of the WT intestine expressed a high level of PDPN (Figure 1C, left). In contrast, the level of PDPN was diminished in those of the inducible C1galt1−/− intestine. Flow cytometric analyses showed reduced PDPN levels on primary LECs from the inducible C1galt1−/− mesentery compared with that from WT littermates (Figure 1C, right). These data indicate that reduced PDPN levels in lymphatic vessels of EHC C1galt1−/− mice occur during both developmental and postnatal stages.

EHC C1galt1−/− mice exhibit disorganized and blood-filled lymphatic vessels.8 To determine whether these lymphatic vascular abnormalities contribute to defective PDPN expression, we examined PDPN levels on LECs isolated from mice lacking CLEC-2 (Clec2−/−), which exhibit the same lymphatic vascular abnormalities.12,13 PDPN expression on LECs from Clec2−/− mice was equivalent to that from WT mice, indicating that lymphatic abnormalities do not cause the reduced PDPN expression (Figure 1D). WT and C1galt1−/− LECs expressed a similar amount of PDPN messenger RNA, suggesting that lack of core 1 O-glycans does not affect the transcription (supplemental Figure 2). Collectively, these data indicate that lack of core 1 O-glycans causes a reduction of PDPN in C1galt1−/− lymphatic vessels.

Lack of core 1 O-glycosylation does not affect intracellular trafficking or protein turnover of PDPN

O-glycan deficiency may result in defective synthesis, impaired intracellular trafficking, or increased degradation of glycoproteins.8,17-24 To determine how the lack of core 1 O-glycan resulted in reduced expression of PDPN, we isolated primary skin ECs enriched in LECs from WT or EHC C1galt1−/− mice,8 and then cultured the isolated cells for 5 days to increase cell numbers for analysis (referring to cultured primary LECs). Most of these cells were CD31 and Lyve-1

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positive, consistent with their LEC phenotype (supplemental Figure 3). Anti-Tn antibody stained C1galt1−/− but not WT cultured primary LECs (Figure 2A). Immunofluorescent staining of permeabilized cells revealed similar levels of PDPN inside the WT and C1galt1−/− cultured primary LECs, indicating that the lack of O-glycans does not affect the intracellular localization of PDPN.

Next, we compared the stability of PDPN using PDPN-eGFP transfected WT or C1galt1−/− EC lines.5 Immortalized WT ECs expressed endogenous PDPN, but C1galt1−/− ECs had lost their endogenous PDPN at the messenger RNA level (supplemental Figure 4A). After transfection with PDPN-eGFP, PDPN expressed on the WT and C1galt1−/− EC surfaces was detected similarly by Syrian hamster anti-mouse PDPN monoclonal antibody (mAb, clone 8.1.1), indicating that the antibody binds to PDPN in an independent manner (supplemental Figure 4B). Both ECs expressed endothelial (CD31) and lymphatic (Lyve-1) markers. Tn antigen was expressed in C1galt1−/− ECs, indicating the loss of core 1 O-glycans (supplemental Figure 4C). The WT ECs expressed endogenous PDPN (38 kDa) and PDPN-eGFP (64 kDa), whereas the C1galt1−/− ECs only expressed an underglycosylated form of the transfected PDPN-eGFP (52 kDa) due to lack of the core 1 O-glycan (Figure 2B). PDPN protein synthesis was inhibited by cycloheximide, and the decay of PDPN over time was determined by immunoblot analysis. WT and C1galt1−/− ECs were lysed, and then PDPN in cell lysates was immunoprecipitated with anti-PDPN 8.1.1-conjugated agarose beads. The beads were treated with trypsin or buffer control. Immunoblots using the eluted proteins demonstrated that the control nontransfected WT ECs expressed only endogenous 38 kDa PDPN, whereas the control transfected C1galt1−/− ECs expressed 52 kDa PDPN-eGFP. Trypsin treatment resulted in a partial degradation of WT PDPN, whereas it degraded all core 1 O-glycan–deficient PDPN, as no intact PDPN was detected after treatment. Further analyses indicated that core 1 O-glycan–deficient PDPN was also highly susceptible to degradation by another serine protease, elastase (Figure 3A-B), but not by thrombin, plasmin, or cathepsin-G (supplemental Figure 5B).

To determine whether core 1 O-glycan–deficient PDPN is susceptible to other types of proteases, we examined cysteine protease calpain-2 and matrix MMP-2/9. Our results indicated

**Core 1 O-glycans protect PDPN from proteolytic degradation**

To test whether core 1 O-glycan–deficient PDPN is sensitive to proteolytic degradation, we first treated the PDPN-transfected ECs described above with serine protease trypsin. Flow cytometry demonstrated that trypsin treatment significantly reduced the PDPN level on the surfaces of C1galt1−/− but not WT ECs. This reduction was rescued by trypsin-like serine protease inhibitor benzamidine, illustrating its specificity (Figure 3A). The WT or C1galt1−/− ECs with buffer control or trypsin treatment were also lysed for immunoblot analyses (Figure 3B). Control WT EC lysates exhibited two major bands when probed with anti-PDPN (8.1.1). Although 8.1.1 detected no obvious degraded PDPN, anti-GFP antibody recognized a 30-kDa band from lysates of trypsin-treated WT ECs, suggesting that trypsin generated a minor cleaved product of WT PDPN under the conditions tested in this study. Control core 1 O-glycan–deficient PDPN from the C1galt1−/− ECs was blotted around 52 kDa with 8.1.1, whereas 8.1.1 did not detect PDPN from the trypsin-treated C1galt1−/− ECs. These results suggest that trypsin degraded the core 1 O-glycan–deficient PDPN and eliminated epitopes recognized by the 8.1.1 antibody. Consistent with this, anti-GFP antibody detected several fragments of core 1 O-glycan–deficient PDPN after trypsin treatment. To confirm these results, we used a complementary cell surface biotinylation method (supplemental Figure 5A). Surface-biotinylated nontransfected WT ECs or transfected stable C1galt1−/− ECs were lysed, and then PDPN in cell lysates was immunoprecipitated with anti-PDPN 8.1.1-conjugated agarose beads. The beads were treated with trypsin or buffer control. Immunoblots using the eluted proteins demonstrated that the control nontransfected WT ECs expressed only endogenous 38 kDa PDPN, whereas the control transfected C1galt1−/− ECs expressed 52 kDa PDPN-eGFP. Trypsin treatment resulted in a partial degradation of WT PDPN, whereas it degraded all core 1 O-glycan–deficient PDPN, as no intact PDPN was detected after treatment. Further analyses indicated that core 1 O-glycan–deficient PDPN was also highly susceptible to degradation by another serine protease, elastase (Figure 3A-B), but not by thrombin, plasmin, or cathepsin-G (supplemental Figure 5B).
that calpain-2 and MMP-2/9 reduced PDPN levels on the C1galt1−/− but not the WT ECs (Figure 3A). EDTA treatment rescued these reductions because these enzymes are divalent cation-dependent. Additionally, the MMP inhibitor GM6001 rescued PDPN expression following MMP-2/MMP-9 treatment. These experiments demonstrated the specificity of these proteolytic degradations. Further immunoblot analyses with anti-PDPN and anti-GFP indicated that MMP-2/9 degraded core 1 O-glycan–deficient PDPN, whereas WT PDPN remained largely intact (Figure 3B). Similar results were seen with calpain-2 treatment. These results are consistent with analyses showing that the extracellular domain of PDPN has predicted cleavage sites for trypsin, elastase, calpain-2, and MMP-2/9, but not for thrombin or cathepsin-G (Figure 3C).

Interestingly, most of the predicted protease cleavage sites were located in close proximity to the potential O-glycosylation sites of PDPN. These data suggest that a key function of O-glycosylation is to protect glycoproteins from proteolytic degradation.

**MMMPs are responsible for the degradation of core 1 O-glycan–deficient PDPN**

To determine if MMPs are involved in degrading core 1 O-glycan–deficient PDPN in vivo, we tested lymph isolated from WT mice because PDPN on LEC surfaces was present in the fluid. Lymph significantly reduced the PDPN level on the C1galt1−/− but not on the WT ECs (Figure 4A), which was prevented in the presence of a protease inhibitor cocktail, demonstrating that the reduction of PDPN by lymph is protease-dependent. Importantly, while GM6001 or EDTA completely rescued the reduction of PDPN on the lymph-treated C1galt1−/− ECs, benzamidine or phenylmethylsulfonyl fluoride (PMSF) rescued PDPN to a lesser degree (supplemental Figure 6). GM6001 did not block the activity of trypsin, elastase, or calpain-2 (supplemental Figure 7), thereby supporting its specificity. MMP-2/9, mainly activated MMP-2, was detected in WT lymph by gelatin zymography (Figure 4B), but not in the conditioned medium of cultured ECs (supplemental Figure 8). Collectively, these data demonstrate that matrix MMPs in the lymph not derived from PDPN-expressing ECs, contribute to the degradation of core 1 O-glycan–deficient PDPN on C1galt1−/− LECs.

To determine if MMPs are involved in degrading core 1 O-glycan–deficient PDPN in vivo, P7 EHC C1galt1−/− or WT mice were treated for 6 days with daily IP injections of GM6001 or vehicle control, and then sacrificed at P14. GM6001 did not cause any significant physiological changes in the treated mice, as GM6001- and vehicle-treated EHC C1galt1−/− mice exhibited comparable peripheral blood cell counts and growth curves (supplemental Table 1; supplemental Figure 9). Additionally, GM6001- and vehicle-treated EHC C1galt1−/− mice expressed similar levels of Tn antigen (Figure 4C), indicating equivalent efficiency of C1galt1 deletion. Immunofluorescent staining of cryosections of intestines showed increased PDPN levels in lymphatic vessels from GM6001-treated but not vehicle-treated EHC C1galt1−/− mice. Furthermore, flow cytometry demonstrated that PDPN surface levels on isolated mesenteric LECs from GM6001-treated EHC C1galt1−/− mice was significantly higher than that on LECs of vehicle-treated EHC C1galt1−/− mice (Figure 4D). These data indicate that MMPs are responsible
for proteolytic degradation of PDPN on LECs in EHC C1galt1<sup>−/−</sup> mice.

**Sialylated O-glycans protect PDPN from proteolysis in the CLP sepsis model**

Sialic acids are the common capping structure of O-glycans. Using the WT and C1galt1<sup>−/−</sup> ECs with similar levels of PDPN (Figure 5A) that was not altered by sialidase treatment, the glycan profile showed that limax flavus agglutinin (LFA) (to sialic acids) and maackia amurensis lectin II (MAL-II) (to α2,3 sialylated glycans), but not peanut agglutinin (PNA) (to desialylated core 1 O-glycans), sambucus nigra agglutinin (to α2,6 sialylated GalNAc-Ser/Thr), helix pomatia agglutinin (HPA) (to Tn antigen), and ricinus communis agglutinin (RCA120) (to LacNAc), interacted strongly with WT PDPN (Figure 5B). Sialidase treatment decreased reactivity of WT PDPN with LFA and MAL-II, and increased that with PNA and RCA120. These results indicate that WT PDPN is modified by α2,3 sialylated core 1 and core 1-derived O-glycans (supplemental Figure 10). In contrast, core 1 O-glycan–deficient PDPN with or without sialidase treatment reacted only with HPA, indicating that core 1 O-glycan–deficient PDPN presents terminal GalNAc without detectable sialylation. These data suggest that lack of sialylation or core 1 O-glycosylation, or both in combination, may contribute to the increased PDPN degradation on C1galt1<sup>−/−</sup> LECs. To test this, we pretreated the WT ECs with sialidases and then incubated them with MMP-2/9 or trypsin (Figure 5C and supplemental Figure 11). Surface level of PDPN on sialidase-treated WT ECs was reduced by MMP-2/9 or trypsin, but not as much as on the C1galt1<sup>−/−</sup> ECs. These reductions were rescued with GM6001 or benzamidine. This result supports that lack of sialylation is sufficient to cause significant degradation of PDPN.

To determine the pathological relevance of this finding, we used a CLP sepsis model, which has increased sialidase activity in serum.26 Firstly, lymph was collected from CLP or sham mice. Consistent with the published data, increased sialidase activity was detected in the CLP lymph, which was blocked by the sialidase inhibitor but not in the sham lymph (Figure 5D). The CLP lymph
reduced PDPN levels on WT ECs, which was blocked by GM6001 (Figure 5E). A combination of PMSF with GM6001 or sialidase inhibitor alone blocked this PDPN reduction more effectively. Furthermore, we found that the levels of PDPN on LECs from CLP mice were significantly reduced compared with that on LECs from sham mice (Figure 5F). This reduction was rescued by an IP injection of GM6001 or a sialidase inhibitor. These data indicate that sialylation of O-glycans is important in preventing PDPN from proteolytic degradation in a sepsis model.

Endothelial PDPN requires sialylated core 1 O-glycans to interact with platelets under flow

Previous studies using transfected CHO cells show that sialylated core 1 O-glycans on PDPN are important for interacting with platelet CLEC-2.7,14 Unlike ECs, CHO cells do not express extended core 1 or core 2 O-glycans. Lympathic PDPN interacts with platelet CLEC-2 to regulate lymphatic functions, likely under venous flow conditions.8,27 Therefore, it remains to be determined whether sialylated core 1 O-glycans are required for endothelial PDPN to bind to platelet CLEC-2 under physiological flow. Flow chamber data revealed stable adhesion and aggregation of WT but not Clec-2−/− platelets on the WT ECs under shear stresses similar to the venous flow conditions.12,28 Sialidase treatment markedly reduced this platelet adhesion (Figure 6A). In contrast, only a few platelets adhered on the C1galt1−/− ECs with or without sialidase treatment at 0.25 dyn/cm² of shear stress. These data suggest that sialylated core 1 and/or core 1-derived O-glycans are important for endothelial PDPN to interact with platelets.

To determine whether extended core 1 or core 2 O-glycans are important for PDPN function (supplemental Figure 10), we co-transfected CHO cells with complementary DNAs encoding PDPN-eGFP and empty vector (PDPN/core 1), PDPN-eGFP and core2GlcNAcT (PDPN/core 2), or PDPN-eGFP and core1GlcNAcT (PDPN/extended core 1). These CHO cells express similar levels of PDPN (Figure 6B). Glycan analysis using lectins as probes (supplemental Figure 10) showed that LFA and MAL-II interacted with PDPN/core 1, PDPN/core 2, and PDPN/extended core 1 (Figure 6C), which was abolished by sialidase treatment. In contrast, PNA bound to all forms of desialylated PDPN, and RCA120 only reacted with desialylated PDPN/core 2 and PDPN/extended core 1 but not PDPN/core 1. These results support that PDPN is primarily modified by...
The protective role of O-glycosylation against proteolysis of modified proteins has been previously demonstrated in vitro. However, the nature of the proteases and whether this function is important in vivo have not been elucidated. Our results show that core 1 O-glycan-deficient PDPN is susceptible to different types of proteases that have predicted cleavage sites in the extracellular domain of PDPN, such as serine protease, trypsin and elastase, cysteine protease calpain-2, and MMP2/9. Interestingly, most of the predicted protease cleavage sites are located in close proximity to the potential O-glycosylation sites of PDPN (Figure 3C). Mucin-type O-glycoproteins typically have a rod conformation. The first α-linked GalNAc is essential for the extended conformation. Because the α-GalNAc remains attached to the peptide backbone of PDPN in the absence of core 1 O-glycosylation, it is unlikely that the increased proteolytic degradation of core 1-deficient PDPN is due to its conformational changes that favor the accessibility of proteases to their cleavage sites.

Negatively charged sialic acids are common capping structures in O-glycans and are reported to be important for protecting...
The lymph of humans and rodents contains proteases, including MMP-2/9, and serine proteases thrombin, plasmin, and factor X. Therefore, although our in vitro and in vivo analyses showed that MMPs are a major form of proteases in degrading core 1 O-glycan–deficient or desialylated PDPN, other proteases such as serine proteases, may also be involved in the regulation of PDPN levels.

PDPN induces platelet aggregation by interacting with CLEC-2 on platelets. Previous studies using transfected CHO cells and the glioblastoma cell line demonstrated that sialylated core 1 O-glycans on PDPN is required for platelet aggregation. Our results, using Lyve-1–positive ECs show that sialylated core 1 structure is present on WT PDPN, consistent with published data. Platelets adhered and aggregated on WT, but not on core 1 O-glycan–deficient PDPN, under shears. Our studies further demonstrate that extended core 1 or core 2 O-glycans are not required for PDPN to interact with platelet CLEC-2. These results support that sialylated core 1 O-glycan on endothelial PDPN is sufficient to mediate stable platelet adhesion and aggregation under shears.

Recently, PDPN-mediated platelet activation has been found to be critical in maintaining the integrity of high endothelial venules in the lymph node and for lymphovenous hemostasis in the adult mouse. Therefore, O-glycosylation in regulating the function and expression of PDPN is important not only for the development of an independent lymphatic vascular system, but also for the maintenance of the established blood-lymphatic vascular systems throughout life. Abnormal glycosylation of PDPN may contribute to
pathogenesis of diseases such as sepsis, as demonstrated in the CLP model.

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Authorship


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