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REVIEW

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Extracellular PDI in thrombosis and vascular injury

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Abstract

Protein disulfide isomerase (PDI) catalyzes the reduction, oxidation, and isomerization of disulfide bonds. Although initially discovered as an endoplasmic reticulum (ER)-residing protein, PDI has been demonstrated to play critical roles on cell surfaces and in the extracellular milieu under different pathophysiological settings. During thrombosis extracellular PDI regulates both platelet activation and coagulation, while during vascular injury PDI modulates proinflammatory neutrophil recruitment and the homeostasis of vascular cells. The identification of PDI substrates using mass spectrometry-based techniques such as mechanism-based kinetic trapping and differential cysteine alkylation has significantly advanced our understanding of the mechanisms whereby extracellular PDI regulates these pathophysiological processes. PDI may reduce or oxidize allosteric disulfide bonds and change the function of adhesive receptors, coagulation-related plasma proteins and signaling molecules that are important during thrombosis and vascular injury responses. The catalytic cysteines of PDI can also be post-translationally modified to enable PDI to transmit redox active species. This review aims to summarize the most recent advances about the roles of extracellular PDI in thrombosis and vascular injury and their mechanisms. With the discovery of novel PDI inhibitors, this body of knowledge will provide novel opportunities to develop strategies for the treatment of thrombotic and vascular diseases.

Keywords Protein disulfide isomerase, Disulfide bonds, Thrombosis, Vascular injury

Introduction

Disulfide bonds maintain protein structure and functions in many pathophysiological conditions. Protein disulfide isomerase (PDI), a prototypical member of the thiol isomerase family, is a multi-functional enzyme critical for protein folding and redox homeostasis within the endoplasmic reticulum (ER). By catalyzing the formation, isomerization, and reduction of disulfide bonds, PDI ensures proper tertiary structure of nascent polypeptides. It can also serve as a chaperone to facilitate protein folding. PDI has four domains and an *x* linker arranged in the order of *a-b-b′-x-a′* (Fig. 1). The *a* and *a'* domains contain catalytic motifs CGHC (Cys-Gly-His-Cys), whose sequences are similar to thioredoxin (TRX), while the *b* and *b'* domains are catalytically inactive, with tertiary structure also similar to TRX [1]. The

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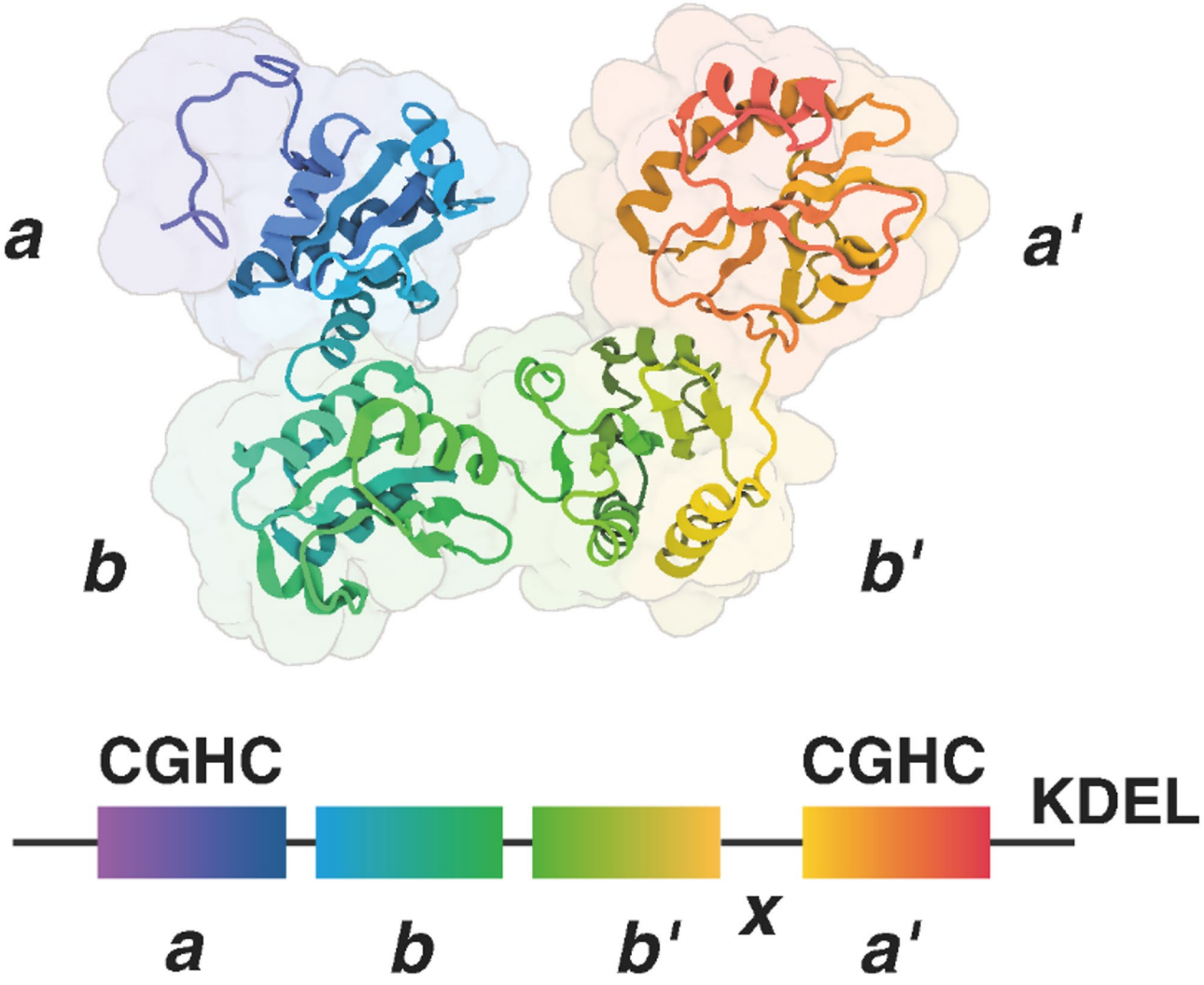

Fig. 1 The domain structures of PDI. The three-dimensional structure of reduced PDI was downloaded from the Protein Data Bank (PDB) database (PDB ID: 4EKZ). PDI has four domains with the *b'* for substrate binding

CGHC motifs confer the oxidoreductase and isomerase activities on PDI, while the *b′* domain has a hydrophobic region that is composed of Leu242, Leu244, Phe258, Ile272, and is mainly responsible for substrate binding [2–5]. The *x* linker has 19 amino acids that are essential for conformational alteration that allows for substrate access [6, 7]. The C-terminus is highly acidic and contains a KDEL (Lys-Asp-Glu-Leu) motif, which is important for its retention in the ER [8–10]. Notably, the C-terminus of PDI is critical to stabilize its chaperone activity, and oxidation of *a′* domain promotes the exposure of *b'* domain therefore facilitating the chaperone activity [10, 11].

Emerging evidence shows that PDI is also functional on cell surfaces and in the extracellular milieu. This unique pool of PDI has been detected in many different cell types such as platelets, endothelial cells, neutrophils, vascular smooth muscle cells, etc. [12]. Extracellular PDI has been implicated in various pathophysiological conditions, including thrombosis, cancer progression, immune and inflammatory responses, vascular homeostasis and erythrocyte physiology. The exact functions of PDI, whether it behaves as a thiol reductase, oxidase, isomerase or chaperone, depend on the distinct substrates and the pathophysiological scenarios. In general, the most well-characterized activity of extracellular PDI is thiol reductase, especially in platelets, neutrophils and tumor cells [12–15], while the oxidase activity was reported particularly in vascular smooth muscle cells (VSMCs) [16, 17]. The pathophysiological roles of cell surface and extracellular PDI have been extensively reviewed in our previous publication [12]. The recent advances in the development of PDI inhibitors have been reviewed elsewhere [18]. In this review, we focus on the roles of extracellular PDI in thrombosis and vascular injury, aiming to provide an overview of recent advances on the mechanisms whereby

extracellular PDI derived from platelets and neutrophils modulates these diseases.

Extracellular PDI in thrombosis

Extracellular PDI in platelets

PDI is secreted and relocates to cell surface with other thiol isomerases upon platelet activation [19, 20]. Although the route of PDI externalization is not fully understood, it has been shown that extracellular PDI directly interacts with β3 integrin on the platelet surface following vascular injury [21]. Notably, this interaction only occurs with active conformation of β3 integrin. Wang et al. reported that the *b′* domain of PDI binds to integrin β3 on the surface of activated platelets, while the *a* and *a′* domains optimize the binding [22]. Although the extracellular fraction of PDI accounts for less than 10% of total PDI in activated platelets [21], it plays important roles in platelet activation and thrombus formation. In a murine model of thrombosis induced by laser injury, blocking extracellular PDI by a monoclonal antibody or PDI inhibitor impeded both platelet thrombus formation and fibrin generation [23]. In addition, mice with platelet-specific deletion of PDI exhibited defects in platelet accumulation, but displayed intact initial adhesion of platelets and normal fibrin generation, a phenomenon attributed to PDI released from endothelial cells [24, 25]. Further, the CGHC motif in the *a'* domain instead of the *a* domain is important for the role of PDI during thrombosis [26].

It has long been proposed that extracellular PDI directly modulates disulfide exchange on platelet integrins and thus induces conformational change and integrin activation [27, 28]. This premise is mainly based on the observation that the activation of platelet integrins such as αIIbβ3 is associated with exposure of free sulhydryl groups in the extracellular region [29, 30], and pharmacological inhibition or genetic deletion of PDI blocks platelet activities such as adhesion and aggregation attributed to integrins [24, 31]. Although site-directed mutagenesis studies have identified potential functional disulfide bonds that are important for integrin conformation, e.g., Cys560-Cys583 in αIIbβ3 and Cys523-Cys544 in αvβ3 [32], there is currently no evidence that extracellular PDI directly targets these disulfides. In addition to the oxidoreductase activity, the redox inactive PDI mutant was shown to regulate integrin-mediated cell adhesion, indicating PDI may also serve as a chaperone for conformation change of integrins [33]. Another example of substrates of PDI in platelets is glycoprotein Ibα (GPIbα). GPIbα and PDI reside closely on platelet surface, and a PDI-blocking antibody reduces GPIbα activation and thus impedes the interaction between platelets and von Willebrand factor (vWF) [19]. Li et al. using mass spectrometry and platelet-specific knockout mice showed that extracellular PDI cleaves two allosteric disulfide bonds (Cys4-Cys17 and Cys209-Cys248) in GPIbα to control its ligand binding activity and platelet-neutrophil interaction [34].

In addition to the above adhesive receptors on platelet surface, it has also been shown that multiple components in platelet α-granules such as vitronectin and thrombospondin-1 (TSP-1) are substrates of extracellular PDI during thrombosis. Using mechanism-based kinetic trapping strategy, a method involving PDI mutants that form stable disulfide-linked complexes with its substrates allowing for mass spectrometric identification, Bowley et al. showed that PDI *via* its CGHC motifs formed disulfide bond(s) with vitronectin and TSP-1 in platelet-rich plasma [35]. Reduction of 2 disulfide bonds of vitronectin (Cys137-Cys161 and Cys274-Cys453) by PDI induces the exposure of cryptic hemopexin-like domains and therefore promotes vitronectin binding to β3 integrins and thrombus formation [35]. Earlier studies have shown that redox modification of disulfides on TSP-1 by PDI promotes the exposure of adhesive motif and alters its adhesive activities [36]. It was also shown that PDI promotes the interaction of thrombin-antithrombin with TSP-1 [37] and vitronectin [38], respectively, by catalyzing the formation inter-molecular disulfide bonds. More recently, the study by Khan et al. indicated that PDI uses thrombin-antithrombin complex as a template to promote its interaction with substrates and influence blood coagulation rates [39]. Nevertheless, the in vivo physiological relevance to thrombosis and the biochemical details of PDI-mediated disulfide reactions in these studies remain elusive. The proposed substrates and possible disulfide bond(s) targeted by PDI during thrombosis are listed in Table 1.

The activity of extracellular PDI is determined by multiple factors including the redox potential in the extracellular milieu, the interplay between intracellular and extracellular redox couples, the kinetics of the redox reactions, the enzymatic modulation by other oxidoreductases, and the posttranslational modification [12, 40]. Post-translational modification of cysteines in the catalytic CGHC motifs provides a mechanism whereby extracellular redox stress influences the activity of PDI and therefore thrombus formation [12]. It has been reported that the catalytic cysteines could be modified by S-nitrosylation [41], S-glutathionylation [42], and lipid aldehydation [43]. Notably, S-nitrosylation of PDI in response to extracellular reactive nitrogen species such as S-nitrosothiols confers the role of S-transnitrosylase on cell-surface PDI which transmits nitric oxide into the cytosol to inhibit platelet activation and thrombus formation [44]. Recently, Yang et al. reported that PDI could be S-sulfenylated in response to oxidative stress during atherosclerosis, inflammation, and aging [45]. S-sulfenylation of PDI in the *a* domain promotes its oxidase activity

Table 1 Proposed substrates and possible allosteric disulfide bonds targeted by extracellular PDI in thrombosis

Target	Possible Disulfides	Effect on substrate	References
β3	Cys560-Cys583 (αIIbβ3, αvβ3), Cys523-Cys544 (αvβ3)	Promote integrin activation	[108]
GPIbα	Cys4-Cys17, Cys209-Cys248	Increase ligand binding affinity	[34]
Vitronectin	Cys137-Cys161, Cys274-Cys453	Increase ligand binding affinity	[35, 38]
Thrombospondin-1	ND*	Increase the adhesive and binding activity	[36, 38]
Thrombin-antithrombin complex	ND*	Promote the binding with PDI substrates and influence coagulation rates	[39]
TF	Cys186-Cys209	Promote decryption	[109]
FXI	Cys362-Cys482	Enhance enzyme activation	[68]
FV	ND*	Enhance enzyme activation	[69]
HRG	Cys306-Cys309	Increase ligand binding affinity	[71]
	Cys390-Cys434		
	Cys409-Cys410		

*ND, not determined

and platelet aggregation, thus leading to oxidized low-density lipoprotein (oxLDL)-augmented thrombus formation. Further, the activity of PDI on the platelet surface is also subjected to regulation by another oxidoreductase. Wang et al. showed that endoplasmic reticulum (ER) oxidoreductase-1α (Ero1α) oxidizes PDI on the platelet surface and the Ero1α/PDI system oxidizes reduced glutathione (GSH), therefore establishing a redox potential in the extracellular milieu that is optimal for platelet aggregation [46]. However, a more recent study by Jha et al. [47] using platelet-specific Ero1α-deficient mice showed that Ero1α catalyzes the rearrangement of allosteric disulfide bonds on Ca^{2+} signaling molecules and regulates their functions and therefore intracellular Ca^{2+} mobilization in platelets. The study suggests that it is the intracellular Ero1α that is critical for platelet activation and thrombus formation, and the mechanism is independent of extracellular PDI. The reason for the different observations is currently unknown but may be attributed to the different concentrations of recombinant oxidoreductases employed between the two studies [47].

In addition to PDI, multiple members of the thiol isomerase family including ERp5 [48–50], ERp57 [51–53], ERp72 [54, 55], ERp46 [56], ERp18 [57, 58], ERp29 [59], TMX1 [60–62], TMX4 [63], etc., have been identified as positive or negative regulators of platelet activity and thrombus formation. It is possible that these thiol isomerases have different spectra of substrates, but with partial overlapping, during thrombus formation. The nature of complexity and multiplicity of the roles of thiol isomerases may provide a mechanism of "redundancy" in the regulation of thrombus formation, and offer a mechanistic explanation whereby PDI inhibitors reduce thrombus formation without increasing bleeding risks [64, 65].

Extracellular PDI in coagulation system

As a critical part of thrombus formation, activation of the coagulation system is also regulated by extracellular PDI. Several members of the coagulation cascade including tissue factor (TF), factor XI (FXI) and factor V (FV) have been proposed as potential substrates of PDI during thrombosis. Although it remains a subject of debate, the role of PDI was implicated in the encryption and decryption of TF. PDI was proposed to catalyze the reduction/oxidation of a single allosteric disulfide bond Cys186-Cys209 [66]. In line with this hypothesis, peripheral blood mononuclear cells (PBMCs) with PDI deficiency exhibited decreased thrombin generation in the plasma in a TF-dependent manner [67]. It was also reported that PDI targets the Cys362-Cys482 disulfide bond of FXI to regulate its activation. The reduced form of FXI can be more efficiently activated by thrombin and FXIIa in the coagulation cascade [68]. Stopa et al. showed that PDI forms disulfide bond(s) with FV and promotes the activation of FV in platelets. Inhibition of PDI suppresses FVa production and therefore reduces platelet-dependent thrombin generation [69]. Other mechanisms whereby PDI modulates coagulation reactions have also been proposed. It was reported that PDI regulates the exposure of phosphatidylserine on endothelial cells, which is a critical step for the initiation of coagulation cascade [70]. Khan et al. showed that PDI binds to thrombin-antithrombin complex to regulate the activity of antithrombin and other PDI substrates, and thus blood coagulation [39]. More recently, using mechanism-based kinetic trapping experiment [71], it was shown that histidine-rich glycoprotein (HRG) is a substrate of PDI during thrombosis. HRG is a multifaceted plasma protein. In the context of blood coagulation, PDI has multiple binding partners including heparin, FXIIa, and heparan sulfate on endothelial cells [72]. HRG binds to heparin/heparan sulfate

to displace antithrombin, thus exerting prothrombotic effect. HRG also binds to FXIIa to inhibit the intrinsic coagulation, thus exerting antithrombotic effect. The study by Lv et al. showed that PDI cleaves three allosteric disulfide bonds, Cys306-Cys309, Cys390-Cys434 and Cys409-Cys410, in HRG. Reduction of HRG by PDI promotes the exposure of the histidine-rich region, and therefore enhances HRG's binding to endothelial heparan sulfate and FXIIa. Physiologically, such redox modification of HRG by PDI allows for rapid initiation of blood coagulation by inhibiting antithrombin, and prevents excessive blood clotting by inhibiting the intrinsic pathway. Therefore, the PDI-HRG pathway represents a novel mechanism whereby blood coagulation is fine-tuned to differentiate thrombosis and hemostasis [71].

It is now clear that extracellular PDI has a broad spectrum of substrates in platelets and the coagulation system. While the overall effect of PDI is to positively contribute to thrombosis, the exact effects of PDI on platelet activation, blood coagulation and kinetics of thrombus formation depend on the distinct substrates, which are further subjected to in vivo temporal and spatial regulation. In the "master switch" hypothesis proposed by Furie et al., the secretion of PDI along with other thiol isomerase family members following vascular injury serves as a hub of initiation signaling that unleashes the functions of many vascular and plasma components whose functions are "clamped" in an inactive state by functional disulfide bonds under normal condition to prevent untoward blood clotting [73]. Although the overall working model whereby PDI and other thiol isomerases orchestrate thrombosis and hemostasis remains elusive, growing pieces of observations regarding PDI interactions with individual substrates are now accumulating to build up a more comprehensive scenario.

Extracellular PDI in vascular injury responses

Extracellular PDI in neutrophils

The recruitment of peripheral neutrophils to inflamed endothelium is a critical contributor to neutrophil-mediated vasculopathy. The heterotypic interactions between neutrophils and endothelial cells are mediated by pairs of adhesive receptors. The tethering/rolling of neutrophils is mediated by interactions between selectins and their ligands [74]. The firm adhesion and crawling of neutrophils are mediated by integrins, mainly β2 integrins including αLβ2 (LFA-1) and αMβ2 (Mac-1), and their counter receptors on endothelial cells such as intercellular adhesion molecule-1 (ICAM-1) [75]. Consistent with the hypothesis on the roles of PDI in integrin activation [27, 28], PDI has been reported to regulate Mac-1 activity. Hahm et al. showed that PDI modulates the sulfhydryl exposure of αM subunit in lipid rafts and the ligand-binding activity of Mac-1 but not LFA-1 on neutrophil surface [15]. Notably, this study did not identify a conformation change of Mac-1 induced by PDI, which, instead influences the clustering of Mac-1, which is a critical step for the increase of ligand-binding activity of integrins during neutrophil activation [15, 76]. A recent study by Dupuy et al. showed that PDI cleaves the Cys224-Cys264 disulfide bond in β2 subunit. Breakage of this disulfide bond in the βI domain changes the conformation of β2 and allosterically alters the exposure of an αI epitope, leading to a lower binding affinity of Mac-1 integrin. Functionally, PDI-induced shift in the binding affinity of Mac-1 releases neutrophils from endothelial cells at the trailing edge and promotes its directional movement under shear [14]. It is noteworthy that, in addition of PDI, thiol isomerase ERp72 has also been shown to regulate Mac-1 functions. A recent study by Li et al. demonstrates that ERp72, also in the lipid rafts, cleaves the Cys654-Cys711 disulfide bond at the interface between the thigh and genu domains of αM subunit. Breakage of this bond induces the shift of αM to an open extended conformation with increased binding affinity with ligand ICAM-1, and promotes neutrophil crawling and adhesion [77]. Other mechanisms involving PDI that indirectly modulates leukocyte recruitment have also been reported. For instance, the activity of a disintegrin and metalloprotease-17 (ADAM17), which regulates the shedding of L-selectin, an adhesive molecule important for neutrophil rolling, is regulated by PDI-mediated disulfide isomerization [78]. Extracellular PDI directly binds to the membrane-proximal domain of ADAM17 on neutrophil surface to prevent its dimerization and substrate recognition, thus blocking ADAM17-mediated cleavage of L-selectin and promoting neutrophil tethering and rolling [79, 80].

Extracellular PDI in vascular cells

Extracellular PDI derived from vascular cells has been implicated in vascular injury responses such as post-injury remodeling and aortic dissection. Surface and extracellular PDI promotes expansive remodeling and mechano-response of vessel wall via cytoskeletal rearrangement induced by β1 integrin, whose activity is subjected to redox regulation by PDI on the surface of VSMCs [16, 17]. It was reported that recombinant PDI influences the in vitro maturation of transforming growth factor-β (TGF-β), a cytokine that modulates vascular homeostasis [81, 82]. From this perspective extracellular PDI may also influence vascular remodeling by controlling TGF-β signaling although the in vivo relevance is unclear. It was recently shown that overexpression of PDI protects against vascular rupture in murine models of aortic aneurysm and dissection. The mechanism is likely to be mediated by the extracellular pool of PDI in VSMCs since the phenotype is independent of ER stress,

Table 2 Proposed substrates and possible allosteric disulfide bonds targeted by extracellular PDI during vascular injury

Target	Cell type	Possible Disulfide(s)	Effect on substrate	References
β2	Neutrophils	Cys224-Cys264	Promote Mac-1 disengagement from ligand	[14]
αM	Neutrophils	Cys654-Cys711 (targeted by ERp72)	Increase integrin binding affinity	[15, 77]
ADAM17	Lymphocytes	ND*	Prevent its dimerization and reduce enzymatic cleavage	[78, 79]
β1	VSMCs	ND*	Promote integrin activation	[16, 17]
SLC3A2, LAMC1	Endothelial cells	ND*	Promote their secretion	[84]
Nox1	VSMCs	Cys196	Operate in the cytosol Upregulate its transcription Promote its assembly	[85, 87, 88]

*ND, not determined

a contributor to VSMC loss that is sensitive to intracellular PDI level in the ER [83]. Further, Gaspar et al. recently showed that surface and extracellular PDI in endothelial cells modulates platelet adhesion on endothelium under hyperglycemic condition. Mechanistically, the effect is mediated by PDI-dependent secretion of solute carrier family 3 member 2 (SLC3A2) and laminin subunit gamma 1 (LAMC1), two adhesion-related proteins which potentially affect endothelial membrane biophysics [84]. Notably, the effects of PDI on vascular injury responses also involve the contribution from the intracellular pool of PDI. Several studies have demonstrated critical roles of PDI in the regulation of NADPH oxidase 1 (Nox1), an important signaling molecule generating reactive oxygen species (ROS) and controlling the phenotype of VSMCs during vascular diseases [85]. PDI upregulates the transcription of Nox1 by promoting the translocation of Activating Transcription Factor 1 (ATF1) to the nucleus in VSMCs [86]. PDI also regulates the assembly and thus the activation of Nox1 by directly forming an inter-molecular disulfide bond between Cys400 of PDI and Cys196 of the $p47^{phox}$ subunit [87]. Fernandes et al. showed that PDI regulates the phenotype of VSMCs by controlling the expression of Nox1 and Nox4, which supports the phenotype of proliferation and differentiation, respectively [88]. Further, Pescatore et al. reported that PDI is required for the activation of small GTPase such as Rac1 and therefore the migration of VSMCs [89]. In addition to the findings regarding PDI and Noxes in vascular cells, Gaspar et al. reported that PDI interacts with Nox1 in platelets downstream of glycoprotein VI (GPVI) receptor. PDI and Nox1 together contribute to GPVI signaling and platelet aggregation [90]. In line with this finding, PDI was shown to regulate intracellular ROS production and ROS-thromboxane A_2 signaling pathway during GPVI-mediated platelet activation [91]. PDI also participates the release of platelet-derived extracellular vesicles [92]. It was also shown in leukocytes that PDI supports Nox activity by serving as a redox-dependent complex organizer in the cytosol *via* association with $p47^{phox}$ subunit [93]. The proposed substrates and possible disulfide bond(s) targeted by extracellular PDI during vascular injury responses are listed in Table 2.

PDI inhibitors for future therapeutics

Given the critical roles of extracellular PDI in thrombosis and vascular injury responses, PDI inhibitors bear significant interest in the treatment of these diseases. In addition to the earlier nonspecific inhibitors such as bacitracin, which targets the catalytic domains of PDI, recent progress has led to the discovery of series of novel PDI inhibitors such as synthetic molecules PACMA31 [94], Bepristats [95, 96], LOC14 [97], ML359 [98], compounds containing 2-trifluoromethyl acrylamide [99], and those molecules from natural products such as rutin [96, 100], isoquercetin [64], myricetin [101], tannic acid [102], and piericone A [65]. In addition, zafirlukast, a cysteinyl leukotriene receptor antagonist that is used to treat asthma, and its analogues were identified to be inhibitors of a broad spectrum of thiol isomerases including PDI, ERp57 and ERp72 [103, 104]. ADTM, a derivative from natural products, also showed inhibitory effects on multiple thiol isomerases including PDI, ERp5, ERp57 and ERp72 but with highest efficacy against ERp57 [105]. Yang et al. identified a class of galloylated polyphenols that inhibit multiple thiol isomerases [106]. Notably, many of these PDI inhibitors exhibit potent antithrombotic potential in animal models and isoquercetin has been shown to reduce thrombotic risks without causing major bleeding events in cancer patients in a phase II clinical trial [64]. Further investigation should be guaranteed not only for the development of novel inhibitors but also for the evaluation of their potential in future therapeutics in different clinical scenarios.

Conclusions

In summary, extracellular PDI plays critical roles in thrombosis and vascular injury responses. In the context of thrombus formation, PDI regulates both platelets and coagulation. PDI modulates the activity of adhesive receptors and α-granule components secreted onto the platelet surface. Modification of the catalytic cysteines of surface PDI not only influences its enzymatic activity,

but also enables its role to inward transmit redox active species. PDI also modulates the activities of coagulation-related plasma proteins to affect the kinetics of blood coagulation. In the context of vascular injury, PDI influences both the inflammatory insult to the vessel, e.g., the recruitment of neutrophils, and the post-injury responses such as vascular remodeling. On the neutrophil surface PDI controls the functions of integrins and selectins during chemotaxis. In vascular cells such as VSMCs, surface PDI, together with the intracellular pool of PDI, regulates cytoskeletal rearrangement, cell survival, membrane biophysics and phenotype switch between proliferation and differentiation. In line with this progress in the investigations of PDI functions, mass spectrometry-based techniques including mechanism-based kinetic trapping and differential cysteine alkylation have led to the identification of PDI substrates and the allosteric disulfide bonds it targets [35, 77, 107]. Future studies are needed to determine the pathophysiological consequences of PDI-catalyzed modification of these substrates. With significant advances in the development of novel PDI inhibitors [18, 65], unveiling these mechanism(s) mediated by surface and extracellular PDI may provide novel therapeutic strategies for the treatment of thrombotic and vascular diseases.

Abbreviations

ADAM17	ADAM metallopeptidase domain 17
ATF1	Activating Transcription Factor 1
CGHC	Cys-Gly-His-Cys
ER	Endoplasmic reticulum
Ero1α	Endoplasmic reticulum oxidoreductase-1α
FV	Factor V
FXI	Factor XI
GPIbα	Glycoprotein Ibα
GPVI	Glycoprotein VI
GSH	Glutathione
HRG	Histidine-rich glycoprotein
ICAM-1	Intercellular adhesion molecule-1
KDEL	Lys-Asp-Glu-Leu
LAMC1	Laminin subunit gamma 1
LFA-1	Lymphocyte function-associated antigen 1
Mac-1	Macrophage-1 antigen
Nox1	NADPH oxidase 1
oxLDL	Oxidized low-density lipoprotein
PBMCs	Peripheral blood mononuclear cells
PDB	Protein data bank
PDI	Protein disulfide isomerase
ROS	Reactive oxygen species
SLC3A2	Solute carrier family 3 member 2
TF	Tissue factor
TGF-β	Transforming growth factor-β
TRX	Thioredoxin
TSP-1	Thrombospondin-1
VSMCs	Vascular smooth muscle cells
vWF	von Willebrand factor

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Author contributions
C.F. conceived the original idea and supervised the writing. J.W. wrote and revised the manuscript. P.J.H., X.X., and C.F. provided critical feedback. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent to publish
Not applicable.

Competing interests
The authors declare no competing interests.

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