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REVIEW

Glycobiology of platelet–endothelial cell interactions

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Under normal conditions, platelets do not interact with blood vessel walls; however, upon activation, platelets firmly attach to endothelial cells. Communication between platelets and endothelial cells during the normal or activated state takes place at multiple levels. Cross-talk may occur over a distance via transient interactions or through receptor-mediated cell–cell adhesion. Platelets may release or transfer substances that affect endothelial cell function and *vice versa*. Excessive dialogue between platelets and the endothelium exists in several disease states as a causative factor and/or as a consequence of the disease process. Glycans are covalent assemblies of sugars that exist in either free form or in covalent complexes with proteins or lipids. Among other functions, glycans confer stability to the proteins to which they are attached, play key roles in signal transduction and control cell development and differentiation. Glycans not only influence the structure and function of hemostatic molecules but are also increasingly recognized as key molecules regulating platelet-endothelial interactions. The present review outlines the current knowledge regarding glycan-mediated interactions between platelets and endothelial cells and their role in physiopathological processes.

Keywords: angiogenesis / endothelial cells / glycobiology / inflammation / platelets

Introduction

Although platelets are widely recognized as having a critical role in primary hemostasis and thrombosis, increasing experimental and clinical evidence has also identified these enucleated cells as relevant modulators of other physiopathological processes, such as tissue repair, wound remodeling, antimicrobial host defense, atherosclerosis, sepsis, lung diseases, arthritis and cancer (Leslie 2010; Katz et al. 2011; Semple et al. 2011; Etulain et al. 2012; Etulain et al. 2013).

It is well known that in normal conditions, platelets circulate without interacting with the intima of the endothelial cells lining the vessel wall, and that platelets will only firmly adhere to the adhesive proteins exposed on the subendothelial matrix after endothelial injury, allowing thrombus formation (Cines et al. 1998). However, during the last decade, substantial experimental and clinical data have revealed that during inflammatory states, even in the absence of any apparent morphological damage, platelets can bind to the intact endothelium, partly because the physiological inhibitory mechanisms are impaired and partly because new adhesion molecules are expressed on the surfaces of activated platelets and endothelial cells (Chen and Lopez 2005). During the sequential steps of the adhesion process, platelets become activated by rolling over activated endothelium or subendothelium and secrete from their alpha granules, an arsenal of potent inflammatory and angiogenic molecules. In the adjoining endothelial cells, the platelet-secretory mediators alter the chemotactic, adhesive and photolytic properties of the endothelium, further promoting the switch to an angiogenic, inflammatory and thrombotic endothelial phenotype (Etulain and Schattner 2012; Etulain et al. 2013; Rondina et al. 2013).

The interaction between both cell types is primarily mediated by glycoproteins and glycans. While platelet-associated glycoproteins that participate in platelet–endothelial cell interactions have been extensively studied (van Gils et al. 2009), the impact of individual glycans in this process is poorly understood. Here, we discuss the most important recent advances in glycan-mediated interactions between platelets and endothelial cells and their role in physiopathological processes.

Glycan-binding proteins expressed on platelets and endothelial cells

Glycans can mediate a wide variety of biological processes by virtue of their mass, shape, charge and other physical properties. However, many biological roles of glycans involve their specific interactions with glycan-binding proteins (GBPs) or lectins. Most lectins are members of families with defined carbohydrate-recognition domains (CRDs) that apparently evolved from shared ancestral genes, often retaining specific features of the primary amino acid sequence or three-dimensional structure. In contrast, protein interactions with sulfated glycosaminoglycans (GAGs) seem to involve surface clusters of positively charged amino acids that line up with internal regions of extended anionic GAG chains (Varki et al. 2009).

Lectins

The following five main groups of animal lectins are distinguished based on their structural alignments: (i) C-type lectins, (ii) I-type lectins, (iii) S-type lectins (galectins), (iv) pentraxins and (v)

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P-type lectins (Kaltner and Gabius 2001). Platelet–endothelial cell interactions are mediated by some lectins from the C-, I- and S-type groups.

C-type

C-type lectins are Ca^{2+}-dependent cell adhesion molecules that share primary and secondary structural homology in their CRDs. Some of these proteins are secreted, and others are transmembrane proteins (Varki et al. 2009).

P-selectin. P-selectin is the main C-type lectin involved in the interactions between platelets, the vessel wall and leukocytes. P-selectin is stored in the alpha granules of platelets and in the Weibel–Palade bodies of endothelial cells (McEver 2001; Vandendries et al. 2004). Following activation, P-selectin is rapidly translocated to the cell surface. Both membrane and soluble forms are present in platelets and endothelial cells (Johnston et al. 1989). The membrane form of P-selectin, expressed on stimulated endothelial cells and activated platelets, functions as a cell adhesion molecule mediating leukocyte binding to endothelial cells and platelet rosetting on leukocytes–endothelial cells (Frenette et al. 2000; Wagner and Frenette 2008; Etulain and Schattner 2012). The major counter-receptor for P-selectin on neutrophils and monocytes, the sialomucin P-selectin glycoprotein ligand-1 (PSGL-1), is constitutively expressed on the platelet membrane but at a concentration 25–100 times lower than on the leukocyte cell surface (Frenette et al. 2000). The observation that platelet interactions with activated endothelium were only partially reduced by a blockade of platelet PSGL-1 pointed to the existence of other platelet ligands. Similar to PGSL-1, platelet glycoprotein Ibα of the glycoprotein Ib-IX-V complex contains a region with three sulfated tyrosine residues at its amino terminus that are necessary for P-selectin recognition, and it was shown that this protein mediates the initial tethering and rolling of non-activated platelets on activated endothelium (Romo et al. 1999). More recently, Merten et al. reported that platelets express sulfatides (sulfated glycosphingolipids that have been shown to bind P-selectin) and serve as ligands for P-selectin (Merten and Thiagarajan 2001). Although they observed that a sulfatide antagonist inhibits platelet adhesion to P-selectin-coated surfaces, the role of sulfatides in the platelet–endothelium interaction has not yet been determined.

Endothelial cells also express PSGL-1 and the glycoprotein Ibα, which mediate tethering and firm adhesion of activated platelets (P-selectin-expressing cells) to the endothelium (Beacham et al. 1999; Tan et al. 1999; da Costa Martins et al. 2007). Other P-selectin ligands present on endothelial cells are glycosylation-dependent cell adhesion molecule-1 (Koenig et al. 1997) and endoglycan (a member of the CD34 family of sialomucins) (Kerr et al. 2008); the expression of these molecules could also mediate the interaction of the endothelium with platelet P-selectin.

P-selectin supports a true rolling motion of the platelets in small venules where shear is high; in vessels with less shear, other cell adhesion molecules, such as von Willebrand factor (vWF), are major players in the platelet–endothelial interaction (Andre et al. 2000).

In the inflammatory focus, a carpet of platelets forms within seconds after the expression of P-selectin. Platelets translocate slowly downstream, but in the absence of additional stimulation, they disengage from the vessel wall in a few minutes and return to circulation (Wagner and Frenette 2008).

Because the density of P-selectin on platelets after their release from the platelet's alpha granules is much higher than on the endothelium (Linden and Jackson 2010), leukocytes are easily recruited to the adherent activated platelets. They roll on the platelets and, after activation, transmigrate. In addition, circulating P-selectin-expressing platelets avidly bind to PSGL-1 on leukocytes, forming mixed platelet–leukocyte aggregates that can be attracted to the endothelium directly or via the monolayer of activated platelets (Evangelista et al. 1996; Lam et al. 2011). Thus, platelets may facilitate leukocyte recruitment to inflamed or injured vessel walls in different ways. The interaction of platelets, endothelial cells and leukocytes through P-selectin and its counter-receptors results in their reciprocal activation. Signaling via the P-selectin/PSGL-1 axis stimulates pro-inflammatory cytokine secretion (e.g., tumor necrosis factor (TNF) alpha, monocyte chemoattractant protein (MCP)-1 and interleukin (IL)-1) by monocytes and neutrophils, and activated platelets release from their alpha granules a plethora of pro-thrombotic and -inflammatory molecules that activate the endothelium and leukocytes (Wagner and Frenette 2008; Etulain and Schattner 2012). Interestingly, distinct conditions of the inflammatory microenvironment, such as acidosis, not only increase platelet P-selectin and the interaction with leukocytes and the endothelium (Etulain et al. 2012) but also strengthen P-selectin/PSGL-1 binding (Cao et al. 2013). This amplification loop of activation between platelets, endothelial cells and leukocytes is critical for an adequate inflammatory response. However, in the absence of counter-regulatory systems, these cellular responses promote a persistent vascular inflammation that contributes to the pathogenesis of chronic inflammatory diseases, such as atherosclerosis, arthritis, lung and bowel diseases and diabetes (Etulain and Schattner 2012). Because P-selectin is a pivotal molecule in the inflammatory response, P-selectin is a strong candidate target for developing novel therapeutic strategies to treat inflammatory-related diseases.

Other than inflammation, P-selectin-mediated interactions also have a relevant role in thrombosis and hemostasis by stimulating the formation of pro-coagulant microparticles (Falati et al. 2003; Hrachovinova et al. 2003; Myers et al. 2003). While this phenomenon could be beneficial because it has been shown to correct hemostasis in a mouse model of hemophilia (Hrachovinova et al. 2003), this process could also promote thrombotic events (Myers et al. 2003).

Thrombosis mediated by P-selectin is not limited to the interactions between platelets, endothelium and leukocytes; P-selectin also binds to sialylated, fucosylated carbohydrates on the surface of tumor cells mediating the heterotypic aggregation between cancer cells, platelets and the endothelium. This phenomenon not only promotes cancer-related thrombosis, or Trousseau syndrome (Wahrenbrock et al. 2003; Shao et al. 2011), but it has a relevant role in promoting metastasis and tumor growth (Kozlowski et al. 2011; Qi et al. 2014). Circulating mixed aggregates of platelets and tumor cells contribute to metastasis by promoting stable adhesion to the endothelium and/or the transmigration of tumor cells outside of the vasculature, a process mediated by the cross-linking of P-selectin and their counter-receptors in endothelial

cells and platelets (Boucharaba et al. 2004; Bendas and Borsig 2012). Interestingly, heparin, which is the most common anti-coagulant and antithrombotic drug used for the prevention and treatment of cancer-associated thromboembolism, has recently been shown to exhibit anti-metastatic and anti-inflammatory activities that are linked to the inhibition of P-selectin-mediated cellular interactions (Shao et al. 2011).

C-type lectin-like receptor-2. In addition to their known protective function in hemostasis, platelets are also required for the separation of the nascent lymphatic vasculature from blood vessels during embryonic lymphangiogenesis. This phenomenon is mediated by the C-type lectin (CLEC)-like receptor-2 signaling of platelets in response to the surface *O*-*N*-glycosylated transmembrane protein podoplanin, expressed on the endothelial cells of lymphatic vessels (Carramolino et al. 2010; Kim and Koh 2010). Initial studies demonstrated that podoplanin activates CLEC-2, which triggers platelet aggregation and the release of granule contents, such as transforming growth factor beta family proteins that inhibit migration, proliferation and tube formation of lymphatic endothelial cells, allowing blood and lymphatic vessel separation (Bertozzi et al. 2010; Uhrin et al. 2010; Finney et al. 2012; Osada et al. 2012). Interestingly, this platelet activity appears not to be restricted to embryonic stages because a more recent study showed that platelet-mediated thrombus formation through podoplanin-CLEC-2 signaling proteins Syk and SLP-76 at the lymphovenous valve is a newly recognized form of hemostasis that functions to safeguard the lymphatic vascular network throughout life (Hess et al. 2014).

I-type

Intercellular adhesion molecule-1. The I-type lectins are GBPs that belong to the immunoglobulin superfamily (Angata and Brinkman-Van der Linden 2002). Intercellular adhesion molecule-1 (ICAM-1) is the main I-type lectin involved in the interaction between platelets and the endothelium. This lectin is expressed on the surface of activated endothelial cells and interacts with platelet $\alpha_{IIb}\beta_3$ integrin in a fibrinogen-dependent bridging mechanism (Bombeli et al. 1998). Moreover, activated platelets promote endothelial ICAM-1 gene transcription and expression through the secretion of several molecules, including platelet-activating factor, MCP-1, IL-1β, macrophage inflammatory protein alpha and the molecule TNFSF 14, which belongs to the TNF superfamily (Schmid et al. 1995; Gawaz et al. 1998; Cha et al. 2000; Celik et al. 2007). ICAM-1 plays a crucial role in the adhesion and migration of leukocytes to the sites of inflammation, and this uncontrolled process is involved in the pathogenesis of several diseases, including autoimmune, proliferative and cardiovascular diseases (Lawson and Wolf 2009). In this context, endothelial ICAM-1 up-regulation mediated by platelets represents one of the mechanisms by which platelets contribute to inflammation.

In contrast to these data, very recent elegant experiments conducted by Gidlof et al. showed that platelets decrease ICAM-1 expression through the shedding of microRNAs (miRNAs). Moreover, they demonstrated that endothelial cells take up functional platelet miRNAs that then regulate endothelial gene expression (Gidlof et al. 2013). These results indicated a role for platelet-derived miRNA in limiting the effects of pro-inflammatory mediators. Further investigation is needed to better understand the role of platelet-derived miRNA in endothelial cell biology.

The platelet–endothelial cell adhesion molecule-1. Platelet–endothelial cell adhesion molecule-1 (PECAM-1), also known as CD31, is another I-type lectin expressed on the endothelium, platelets and leukocytes (Woodfin et al. 2007). Although PECAM-1 is generally considered the principal ligand for PECAM-1 (homophilic interaction), $\alpha_v\beta_3$ integrin, which is expressed on platelets and endothelial cells (Cheresh et al. 1989) has also been described as a putative PECAM-1 ligand (heterophilic interaction) (Buckley et al. 1996), suggesting that reciprocal hetero and/or homophilic interactions between platelets and endothelial cells could be mediated by these molecules. However, few studies have addressed this issue, and the data obtained are controversial. While in vitro studies have shown that PECAM-1 blockade does not exert a significant effect on platelet adhesion to the endothelium (Bombeli et al. 1998), intravital microscopy studies in mice have demonstrated that PECAM-1 is an important modulator of platelet adhesion–aggregation at sites of minor endothelial damage (intact monolayer) in brain arterioles (Rosenblum et al. 1996). Thus, the functional relevance of PECAM-1 on platelet–endothelium interactions is still poorly understood and remains an interesting area of investigation.

S-type (galectins)

Recent findings highlighted the emergence of galectins in the control of platelet–endothelial cell interactions. Galectins constitute a family of animal lectins that bind to β-galactoside residues expressed in a wide variety of cells and tissues, and galectins play an important role in various cellular mechanisms, including cell signaling, proliferation, migration, apoptosis and mRNA splicing (Liu and Rabinovich 2010). Galectins are also associated with different pathologic processes including cancer progression (Liu and Rabinovich 2005; Rabinovich and Croci 2012).

The expression of galectins has been reported in endothelial cells of different sources and origin and appears to be confined to four family members: galectin-1, -3, -8 and -9 (Lotan et al. 1994; Baum et al. 1995; Imaizumi et al. 2002; Thijssen et al. 2008; Delgado et al. 2011). Although low levels of galectin-2, -4 and -12 mRNA have also been detected in cultured endothelial cells, the corresponding proteins were not (Thijssen et al. 2008). The expression of endothelial galectins and their translocation to the endothelial cell surface are increased by several stimuli, including cytokines, lipoproteins, lipopolysaccharides, viruses and the interaction of neutrophils with vessel walls (Thijssen et al. 2013). In addition, most endothelial galectins are up-regulated in the activated tumor endothelium, and this feature correlates with tumor progression, tumor aggressiveness and the acquisition of a metastatic phenotype (Thijssen et al. 2008; Thijssen et al. 2010; Rabinovich and Croci 2012; Croci et al. 2014; Heusschen et al. 2014). Induction of angiogenesis by galectin-1, -3 and -8 represents one of the strategies that tumor cells use to grow and invade new tissues. Specifically, the galectin-1-*N*-glycan axis can link tumor hypoxia to vascularization (Croci et al. 2012) and can promote vascular endothelial growth factor (VEGF)-like signals in tumors that are refractory to anti-VEGF therapy (Croci et al. 2014). The knockdown of

endogenous endothelial galectin expression can hamper endothelial cell functions including their proliferation and migration (Thijssen et al. 2010; Delgado et al. 2011). Whether there is an interaction between endogenous and exogenous galectins that affects endothelial function remains unclear.

Human platelets, similar to almost all vascular cells, express substantial levels of galectin-1 and -8 (Pacienza et al. 2008; Romaniuk et al. 2010). Human platelets express the two splice variants of galectin-8. Moreover, whereas galectin-8, similar to P-selectin, is absent from the surface of resting platelets, it is expressed on the outer membrane of thrombin-stimulated platelets (Romaniuk et al. 2010). In contrast, galectin-1 is not expressed on the platelet surface upon activation (Schattner et al. unpublished observation). The differences between these galectins remain unclear. Interestingly, the addition of lactose or thiodigalactoside, both competitive inhibitors of galectin binding, moderately decreases the aggregation induced by classical agonists, suggesting that platelet-derived galectins might enhance or amplify platelet activation mediated by classical agonists (Pacienza et al. 2008; Romaniuk et al. 2010).

Exogenous soluble and immobilized galectin-1 and -8 bind to the major platelet membrane proteins $\alpha_{IIb}\beta_3$ integrin and the glycoprotein Ibα, respectively. The interactions of galectin-1 and -8 with their receptors result in platelet functional responses, such as adhesion, aggregation and granule secretion (Pacienza et al. 2008; Romaniuk et al. 2010, 2012). Interestingly, we found that whereas galectin -1, -3 and -8 trigger VEGF release, only galectin-8 induces endostatin secretion (Etulain et al. 2014). These observations suggest that despite the similarities in structure and primary sequence, different galectins exert distinct and specific functions, a concept not only true for platelet regulation but also for other processes in which galectins are involved (Thijssen and Griffioen 2014).

Given the described effects of galectin-1 and -8 in platelet physiology, the exposure of these endogenous lectins in the subendothelium or in activated endothelial cells is expected to trigger platelet adhesion, platelet spreading and thrombus formation. In this context, we have recently demonstrated that galectin-8 activates endothelial cells and promotes platelet adhesion, suggesting that galectin-8 might orchestrate the interaction between platelets and endothelial cells (Cattaneo et al. 2014). However, galectin-1 and -3 have been shown to co-localize with vWF in endothelial cells and remain associated following secretion. Moreover, studies of both galectins in knock-out mice suggest that the association of galectin-1 and -3 with vWF from the endothelial cell surface could be relevant not only in the control of platelet adhesion and thrombus formation but also in other mechanisms of platelet–endothelium interactions mediated by galectins (Saint-Lu et al. 2012).

Platelets are known to contribute to tumor progression and metastasis through the formation of mixed-cell aggregates between tumor cells expressing mucins and platelets expressing P-selectins (Shao et al. 2011). Accordingly, the interaction between tumor cells expressing high levels of galectins and platelets could represent another molecular mechanism through which both platelets and galectins promote the metastatic cascade. Interestingly, and in contrast to mucin-P-selectin interactions that require the expression of P-selectin on the activated platelet surface, the interaction of tumor cells with platelets via galectins would not require the presence of activated platelets because galectins bind to glycoproteins constitutively expressed on the platelet membrane (Romaniuk et al. 2010, 2012). Furthermore, the overexpression of galectins in tumor vessels could represent a trigger for platelet activation, allowing the release of the contents of alpha granules, such as growth factors, which can promote tumor progression and angiogenesis. In this sense, we have recently shown that despite the selective release of VEGF or endostatin mentioned above, supernatants from platelets stimulated with galectin-1, -3 and -8 promote endothelial cell proliferation and tubule-like formation through the release of pro-angiogenic factors, indicating that the direct angiogenic activity of these galectins could be amplified by the release of pro-angiogenic factors from platelets in the tumor microenvironment (Etulain et al. 2014).

Bearing in mind that galectins are involved in the pathogenesis of several processes, including inflammatory and infectious diseases, cardiovascular events and cancer progression (Rubinstein et al. 2004; Thijssen et al. 2008; Al-Ansari et al. 2009; Norling et al. 2009; Liu and Rabinovich 2010; Rabinovich and Croci 2012) and that platelet–endothelium interactions are key players in the development of these diseases (Katz et al. 2011; Etulain and Schattner 2012; Etulain et al. 2013), understanding the effect of galectins in the physiology of both cell types may contribute to the design of novel therapeutic approaches for treating these diseases.

The platelet–endothelium interactions mediated by lectins are schematized in Figure 1.

Glycosaminoglycan-binding proteins

GAGs are long, unbranched polysaccharides consisting of a repeating disaccharide unit. Based on core disaccharide structures,

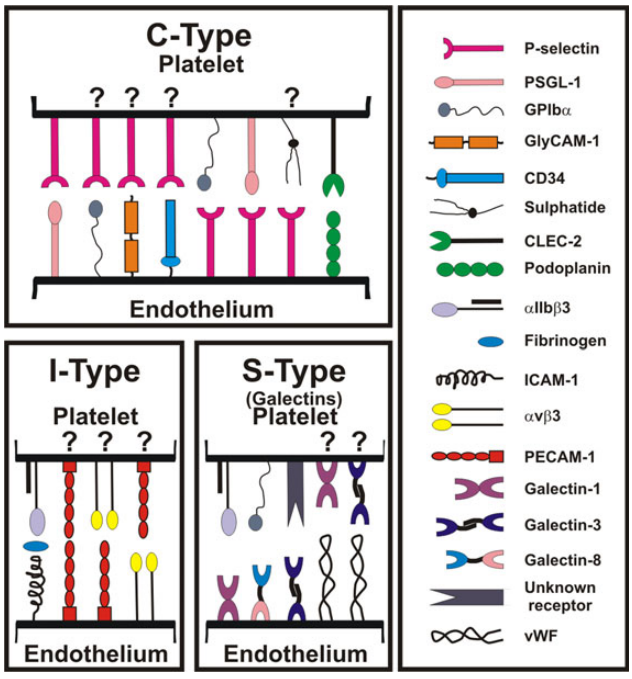

Fig. 1. Platelet–endothelial interactions mediated by lectins.

GAGs are classified into three groups: (i) heparin/heparan sulfate (HS), (ii) chondroitin/dermatan sulfate and (iii) keratan sulfate (Varki et al. 2009). Of particular relevance to cell transport, HS is strongly expressed by vascular endothelial cells and is found throughout basement membranes. Endothelial HS has long been thought to have a role in maintaining blood vessel patency through the presentation of its non-thrombogenic surface to the circulating blood. This effect is known to depend on the high net negative charge of the HS molecule. Under normal conditions, the endothelium does not support the adhesion of platelets or inflammatory cells to itself, and it is possible that this lack of non-specific adhesion is also an effect of the HS-derived charge, given that circulating cells also express this molecule. The role of GAG-binding proteins in platelet–endothelium interactions is largely associated with chemokines, cytokines and growth factors released from platelet alpha granules. The nature of these interactions rests on the characteristic physicochemical properties of these molecules, e.g., a positive charge and high affinity for the negatively charged GAGs, enabling their retention on the surface of endothelial cells and the presentation to their cognate receptors even in the presence of shear forces (Baltus et al. 2005; Varki et al. 2009).

Platelets release several GAG-binding proteins that promote vessel formation, including VEGF, fibroblast growth factor (FGF), matrix metalloproteinases, platelet-derived growth factor and angiopoietin-1, among others (Etulain et al. 2013). It has been extensively demonstrated that heparin–heparan sulfate glycosaminoglycan (HS-GAG) in vessel walls not only interacts with these growth factors inducing sequestration but also that HS-GAGs bind to the endothelial receptors of these proteins, interfering with the signaling pathways that they trigger (Lever and Page 2002; Sasisekharan et al. 2002; Varki et al. 2009).

Platelets also release anti-angiogenic molecules, including thrombospondin-1, endostatin, angiostatin, tissue inhibitor of metalloproteinases-1 and -4 and plasminogen activator inhibitor-1 (Etulain et al. 2013). These molecules act by several mechanisms, including the inhibition of endothelial proliferation and migration, interference with the mitogenic effects of pro-angiogenic molecules and the induction of endothelial cell apoptosis (Maione et al. 1990; Schnaper et al. 1993; Roberts 1996; Jurasz et al. 2006; Aidoudi and Bikfalvi 2010). Binding of these anti-angiogenic molecules to their high-affinity sites on endothelial cells is modulated by the presence of GAGs (Petitou et al. 1999; Karumanchi et al. 2001; Lever and Page 2002; Sasisekharan et al. 2002; Rein et al. 2011). Most studies regarding the anti-angiogenic role of platelets have focused on the release of endostatin. The interaction of this molecule with endothelial cells critically depends on GAGs, and two models have been proposed for this process. The first model posits that GAGs cooperate in the binding of endostatin to its receptor expressed on the endothelial cell surface. The second model involves the presence of two receptors: one for endostatin and another for GAG. In this model, GAGs could act as co-receptors by binding endostatin and presenting it to its high-affinity receptors, triggering intracellular signals (Karumanchi et al. 2001).

Considering the ability of these pro- and anti-angiogenic molecules to bind to GAGs, heparin and related molecules are able to both inhibit pro-angiogenic molecules' activities and also enhance the anti-angiogenic effect mediated by other substances. For this reason, HS-GAGs are considered therapeutic tools to inhibit angiogenesis-related diseases including cancer (Lever and Page 2002; Sasisekharan et al. 2002; Casu et al. 2010).

Other than growth factors, platelet alpha granules also contain numerous cytokines and chemokines that can be released or expressed on the cell surface after platelet activation caused by platelets rolling over inflamed endothelium (Karshovska et al. 2013). All chemokines tested thus far have positively charged domains. GAGs present on the endothelial cell surface or extracellular matrix bind to and modify the activities of these cytokines, chemokines and their receptors (Taylor and Gallo 2006).

The most well-understood relationship between platelet-derived chemokines and endothelial GAGs involves platelet factor-4 (PF-4). PF-4 has two important functions in the vasculature. It has a pro-atherogenic role and an anti-angiogenic effect. When platelets are activated and PF-4 is released, PF-4 tightly binds to endothelial GAGs and promotes not only the recruitment of circulating monocytes towards the inflamed endothelium but also the migration of the monocytes towards the intima where they are transformed into inflammatory macrophages (Scheuerer al. 2000; Sachais et al. 2007). Interestingly, and unlike other platelet-derived chemokines that promote angiogenesis (Karshovska et al. 2013), PF-4 exerts anti-angiogenic activity through several mechanisms, including direct growth factor binding, activation of the CXCR3B chemokine receptor, interaction with the lipoprotein-related protein-1, and direct binding to integrins or proteoheparan sulfates (Aidoudi and Bikfalvi 2010). These different mechanisms may operate in parallel or in cooperation depending on the site or the type of vessel that is undergoing angiogenesis. Specifically, PF-4 not only binds to FGF and VEGF but also competes with the HS-receptor binding sites, disrupting or preventing them from interacting with their signaling receptors (Gengrinovitch et al. 1995; Perollet et al. 1998; Chadderton and Stringer 2003). The manipulation of this mechanism may prove useful for clinical intervention to modulate angiogenesis.

Extracellular platelet glycosylation of target molecules

Glycosyltransferases catalyze glycosidic bond formation using sugar donors containing a nucleoside phosphate or a lipid phosphate leaving group. Most of these enzymes are Golgi-located membrane-bound proteins. Sizable pools of freely circulating glycosyltransferases are also found in the blood however, and despite the potential of these extrinsic enzymes to remodel glycans on distal cell surfaces, their physiologic role remained elusive mainly because an extracellular source of sugar donor substrate was unknown. Recent studies by Wandall et al. showed that upon thrombin-induced activation, platelets not only release a reservoir of glycosyltransferases but also sugar nucleotides to the extracellular space (Wandall et al. 2012). These novel findings were further confirmed and extended by Lee et al. who demonstrated that at physiologic pH and platelet densities conditions, activated platelets can efficiently supply particles containing the sialic acid donor substrate to functionally drive extracellular glycosylation by extrinsic ST6Gal-1 sialyltransferase, one of the prominent blood borne glycosyltransferases. Specifically, they showed that activated platelets can

support extrinsic α2,6 sialylation of hematopoietic progenitor cells (Lee et al. 2014).

Collectively, these fascinating discoveries reveal that although anucleated platelets lack an organized glycosylation machinery, they are a rich source of glycosyltransferases and contain a sufficient pool of sugar nucleotides that support glycosylation reactions. How this effect influences platelet–endothelial interactions remains to be explored.

Conclusions

Although the roles of platelets and endothelial glycoproteins in hemostasis and thrombosis have been studied for several years, the impact of GBPs has not been well elucidated. However, with the discovery of P-selectin in platelets and endothelial cells and given the major role P-selectin plays in several physiopathological processes, including inflammation, thrombosis and cancer, during the last decade, several research groups have focused their attention on the glycobiology of platelets, leukocytes and the vessel wall. In this review, we have provided fresh insights into the relevant role of lectins, GAG-binding proteins and GAGs in the interaction between platelets and endothelial cells and their effects on health and disease. In particular, the expression of galectins in the vascular system and on platelets has suggested that these molecules are not only key elements in the regulation of the immune response and inflammation but also in hemostasis and thrombosis. Several questions remain to be answered: how do glycan changes affect the pathophysiology of platelets and endothelial cells? Because the endothelial cells lining the vessels are highly heterogeneous, is the glycosylation pattern similar in all vascular beds? If yes, does the glycosylation pattern influence the sensitivity or the phenotype of the inflammatory response or the metastatic cascade mediated by platelets? Is the glycosylation pattern similar in young and in old platelets? Finally, how does the local release of the recently described platelet glycosyltransferases influence the glycosylation of endothelial extracellular acceptor molecules? Although the glycobiology of platelet interaction with endothelial cells is a fast-growing field, evidence remains circumstantial and further studies, particularly in vivo, of the pathogenic mechanisms that contribute to thrombosis, chronic inflammatory diseases and cancer progression through platelet–endothelial glycan-associated structures will help us to design better therapeutic strategies to combat these diseases.

Authors' contributions

J. E. contributed to the literature review and interpretation, the article concept and design and the drafting of the article; M. S. contributed to the drafting of the article, providing a critical and substantive review of the intellectual content, and approved the final published version.

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Conflict of interest statement

None declared.

Abbreviations

CLEC, C-type lectin; CRD, carbohydrate-recognition domain; FGF, fibroblast growth factor; Gal, galectin; GAG, glycosaminoglycan; GBP, glycan-binding protein; HS, heparin–heparan sulfate; HS-GAG, heparin–heparan sulfate glycosaminoglycan; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; MCP, monocyte chemotactic protein; miRNAs, microRNAs; PECAM-1, platelet–endothelial cell adhesion molecule-1; PF-4, platelet-derived factor-4; PSGL-1, P-selectin glycoprotein ligand-1; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.

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