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Review

Lactadherin: An unappreciated haemostasis regulator and potential therapeutic agent

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ABSTRACT

Lactadherin is a small (53–66 kDa) multifunctional glycoprotein belonging to the secreted extracellular matrix protein family. It has a multi-domain structure and is involved in many biological and physiological processes, including phagocytosis, angiogenesis, atherosclerosis, tissue remodeling, and haemostasis regulation. Lactadherin binds phosphatidylserine (PS)-enriched cell surfaces in a receptor-independent manner. Interaction between lactadherin and PS is crucial for regulation of blood coagulation processes. This review summarizes recent knowledge on the possible role of lactadherin in haemostasis control, emphasizing the great significance of the interaction between lactadherin and PS expressed on activated platelets and extracellular vesicles. The possible role of lactadherin as a therapeutic target and biomarker is also discussed.

1. Introduction

1.1. Haemostasis principle

Haemostasis is a complex and tightly controlled physiological process which maintains blood in the fluid state under normal circumstances, or stops bleeding after an injury. The main components involved in haemostasis regulation are: platelets, the vessel wall, plasma coagulation factors, and a fibrinolytic system [1,2]. To preserve haemostasis, maintenance of an appropriate balance between procoagulant and anticoagulant plasma activity is very important. The traditional model of haemostasis divides this process into two stages - primary and secondary haemostasis. Primary haemostasis refers to vasoconstriction, platelet aggregation and adhesion in the site of injury, while secondary haemostasis refers to the formation of a stable fibrin clot generated by the coagulation cascade [3]. Nevertheless, the key element of a proper functioning blood coagulation system is the interaction of platelet extracellular matrix (ECM) adhesion receptors with ECM proteins originating from different cells lining central or peripheral organs such as the liver, vessel walls, smooth muscles, or even platelets [4]. The main components within the ECM that interact directly with platelets are laminin, fibronectin, vitronectin, and collagen. Among these proteins, one specific glycoprotein released upon macrophage activation – *lactadherin* – seems to play a regulatory role in haemostasis *via* the initiation and propagation of coagulation processes.

This review summarizes recent knowledge on the role of *lactadherin* in haemostasis regulation, emphasizing a great significance for the interaction between *lactadherin* and phospholipids (PLs), especially phosphatidylserine (PS), involved in this process.

1.2. Decisive role of platelets in haemostasis

Platelets are very lipid-rich cell-derived blood elements [5]. As a result of their small size (1–2 μm), their plasma membrane surface is relatively large in relation to their volume and they are enriched in various haemostatic elements including surface glycoproteins such as CD36, CD41, CD42a, and integrins (α2β1, α5β1, α6β1, αIIbβ3 (IIbIIIa - CD49b/CD61), αvβ3), which helps in intercellular trafficking and adhesion [6,7]. Platelet adhesion, activation, and forthcoming aggregation after injury are crucial stages in primary haemostasis. Platelets play a decisive role in the maintenance of haemostasis by adhesion to damaged endothelium in order to participate in clot formation [8].

Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; APL, acute promyelocytic leukemia; Del-1, developmental endothelial locus-1; ECM, extracellular matrix; EGF, endothelial growth factor; EVs, extracellular vesicles; FA, fatty acid; LDL, low density lipoprotein; MFG-E8, milk fat globule-epidermal growth factor 8; MPs, microparticles; P2Y1/P2Y12, G protein-coupled purinergic signaling receptors; PAR1/4, protease-activated receptors 1/4; PE, phosphatidylethanolamine; PL, phospholipid; PMVs, platelet-derived microvesicles; PS, phosphatidylserine; RBC, red blood cell; RGD, arginine-glycine-aspartic acid; SED1, secreted protein containing EGF-like repeats and discoidin/F5/8 complement domains; SLE, systemic lupus erythematosus; SP, sphingolipid; ST, steroid; TF, tissue factor; vWF, von Willebrand Factor

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According to our canonical knowledge about the circulation system, primary haemostasis is initiated by injured endothelium leading to exposure of the above mentioned procoagulant ECM proteins (laminin, collagens, fibronectin, and vitronectin). Additionally, endothelial cells secrete von Willebrand Factor (vWF) which facilitates platelet aggregation.

Platelet adhesion is the result of direct interaction between platelet receptors $\alpha_2\beta_1$ or GPVI and collagen, this process is preceded by the formation of the GP1b-IX-V/vWF complex which then interacts with collagen. The interaction between platelet surface receptors and endothelium matrix proteins leads to the next stage, platelet activation, which induces a change in their shape to a more amorphous form with long protrusions. Finally, activated platelets release their content in the degranulation process. It has been proposed that, platelets release four types of granules through their canalicular system: (1) α-granules with adhesive proteins (platelet factor 4, vWF, fibrinogen, fibronectin, factor V, factor XI, protein S, and PAI-1); (2) dense δ-granules (nucleotides, bioactive amines – serotonin, and Ca^{2+}); and the lesser known (3) λ-granules and (4) γ-granules similar to lysosomes with hydrolytic enzymes [9,10]. The platelet changes during the activation process are induced by numerous agonists such as thrombin, ADP, collagen, or thromboxane A2, which interact with specific receptors on the platelet plasma membrane (Table 1, Fig. 1).

1.3. Platelet lipids as a component of the coagulation system

Platelet activation is related to changes in lipid membrane asymmetry. The lipid fraction of platelets contains common PLs, sphingolipids (SPs), steroids (STs), prenol lipids, and minor structural derivatives (positional isomers and hydrocarbon saturates of fatty acids (FA)), among which, phosphatidylethanolamine (PE) and phosphatidylserine (PS) are most the abundant [11,12]. PS is a negatively-charged aminophospholipid belonging to the family of glycerophospholipids. It represents between 2 and 10% of all lipids in nucleated mammalian cells [13]. Under normal conditions, PS is located preferentially in the cytosolic inner membrane leaflet. After platelet activation, PS is distributed from the inner space and exposed on the platelet membrane surface to facilitate platelet procoagulant activity (Fig. 1) [14]. Platelet PS exposure is necessary, but not sufficient for coagulation promotion and amplification [13,15]. Exposure of surface PS during the platelet aggregation process promotes activity of the coagulation pathway by forming electrostatic and hydrophobic interactions involved in the binding site formation for a number of vitamin K-dependent coagulation factors including activated enzymatic factors IX (IXa), X (Xa), non-enzymatic activated cofactors V (Va), VIII (VIIIa), and prothrombin [16,17]. This PS-binding has consequential biochemical and clinical implications including local increase in coagulation factors, restriction of the 2-D mobility of clotting proteins and, more essentially, induction of conformational changes upon binding [18]. What is more, PS stimulates the assembly of *prothrombinase* and *tenase* complexes - crucial activators of the coagulation cascade which accelerate fibrin formation [19,20].

Table 1
Major platelet receptors and their ligands.

Receptor	Ligand	Receptor type
GPIb-V-IX (CD42)	vWF	Glycoprotein
PAR1, PAR4	Thrombin	G protein-coupled receptor
P2Y12, P2Y1	ADP	G protein-coupled receptor
GPIIb-IIIa ($\alpha_{IIb}\beta_3$)	Fibrinogen, Laminin, vWF	Integrin
GPVI	Collagen	Glycoprotein
GPIa-IIa ($\alpha_2\beta_1$)	Collagen/laminins	Integrin
GPIV (CD36)	Thrombospondin 1, LDL	Glycoprotein
TPα, TPβ	Thromboxane 2	G protein-coupled receptor

Abbreviations: ADP – adenine triphosphate; CD36 – platelet glycoprotein 4 (GPIV); GPIb-V-IX – platelet glycoprotein receptor; GPVI – glycoprotein VI; LDL – low density lipoprotein; PAR1/4 – protease-activated receptors 1/4; P2Y1, P2Y12 – purinergic signaling receptors; TPα – thromboxane receptor α, TPβ – thromboxane receptor β; vWF – von Willebrand Factor (according to Heemskerk et al. [43]).

1.4. Platelet-derived microvesicles and their role in the coagulation processes

Platelet activation leads to their degranulation and release of nano- and micro-sized extracellular vesicles (EVs) [21]. These EVs, usually called platelet-derived microvesicles (PMVs), present PS on their surface to allow them to contribute to procoagulant activity [22]. The PMVs are shed from the platelet surface membrane, in a process that involves cytoskeleton reorganization, membrane budding, and finally changes in lipid membrane asymmetry, resulting in external exposure of PS [23–25]. Increased PMVs formation is very often associated with pathology and is postulated to be a recognized mechanism of a hypercoagulation state [23,26,27]. In their study, Van der Zee et al. demonstrated that subpopulations of CD63- and P-selectin-positive PMVs reflect platelet activation and their concentrations are higher in patients with myocardial infarction and peripheral arterial disease [28]. Zhao et al. showed increased exposure of PS on blood cells and microparticles (MPs) which may contribute to enhanced procoagulant activity in patients with internal carotid artery stenosis who have undergone carotid artery stenting [29]. In cancer patients, levels of PS-positive platelets and PMVs were increased significantly in stage III/IV colon cancer patients, leading to the conclusion that PS-positive platelets and MPs contribute to hypercoagulability and represent a potential therapeutic target to prevent thrombosis [30]. Additionally, PMVs have a high procoagulant potential related to the PS presence on their surface and they promote the competence of tissue factor (TF) which accelerates the formation of coagulation complexes [28,31–33].

Owing to their specific properties including small size, molecular composition, and electrostatic charge, PMVs create an additional catalytic surface not only for blood coagulation factors but also for other proteins involved in coagulation and fibrinolysis processes. It is known that PMVs display surface receptors involved in haemostasis maintenance and control, including integrins (GPIIb-IIIa – αIIbβ3; GPIa-IIa – α2β1), glycoproteins (GPIb-V-IX; CD36), selectins (P-selectin), receptors for coagulation factors (VIII), and anticoagulant plasma proteins (protein S) (Table 1) [34–37]. Selectins, which are present in the α-granules of platelets and the Weibel-Palade bodies of endothelial cells can be transferred *via* MPs even to distant locations, and they have a crucial role in thrombus formation. It has been demonstrated that platelet origin selectin (P-selectin) induces the expression of TF on monocytes, mediates the binding of platelets to monocytes and neutrophils, and it is involved in inflammation, wound healing, and immune response [38–42].

2. Lactadherin – structure and biological function

Lactadherin also known as MFG-E8 (Milk Fat Globule-Epidermal Growth Factor 8) or SED1 (secreted protein containing EGF-like repeats and discoidin/F5/8 complement domains) belongs to the family of secreted ECM glycoproteins. In most species, *lactadherin* occurs as two splice variants: ~53 kDa and ~66 kDa that includes an *O*-glycosylated proline/threonine rich sequence [44]. The smaller variant is present in the mammary glands of adolescent female animals as well as other tissues and organs (sweat glands, bile ducts) and body fluids (serum, urine, cerebrospinal fluid). It can be expressed and released by activated macrophages, epithelial cells, immature dendritic cells, pancreatocytes, and keratinocytes [45–47]. The larger variant of the *lactadherin* globule is secreted into milk by mammary epithelial cells of humans, cows, or mice and it is most abundant in the fraction of milk-fat-globule membranes [44,48].

The protein sequence of bovine *lactadherin* contains 427 amino acids and occurs in two glycosylation variants: PAS-6 (52 kDa) and PAS-7