Platelets are megakaryocyte-derived nuclear-free fragments that participate in cardiovascular diseases including acute myocardial infarction, ischemic stroke, hypertension, and atherosclerosis. At the endothelium damage site, platelets interact with sub-endothelial matrix proteins such as glycoprotein VI/Fc receptor γ-chain (GPVI/FcRγ), G protein-coupled receptor/phospholipase Cγ(β) (GPCR/PLCγ(β)), Rho/RhoK and integrin. The activation of these signaling pathways triggers intracellular calcium increase and causes platelet adhesion, aggregation, granule release and finally thrombus formation. Some endogenous platelet modulators reported to negatively regulate this process are: (1) platelet surface inhibitory receptors: carcinoembryonic antigen cell adhesion molecule 1, 2 (CEACAM 1, 2), platelet endothelial cell adhesion molecule-1 (PECAM-1), and G6b-B; (2) nuclear receptors: retinoic X receptor (RXRs), glucocorticoid receptor (GR), peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs); (3) intracellular adaptor proteins: CLP36, paxillin, downstream of tyrosine kinase (Dok), c-Casitas B-lineage lymphoma (c-Cbl), protein kinase Cδ (PKCδ), glycogen synthase kinase(GSK)-3β, phospholipase D2 (PLD2), peroxiredoxin II (PrxII), T-cell ubiquitin ligand-2 (TULA-2); (4) extracellular modulators released from platelet granules: adapter protein disabled-2 (DAB2) and diadenosine 5,5-P1, P2-diphosphate (Ap2A). The discovery of biological or endogenous modulators of platelet function is regarded as a potential therapeutic target for thrombotic disease. This review highlights the recent findings on the endogenous negative regulatory molecules released from platelets and their impact on platelet thrombus formation.

Key Words: Platelet, Adhesion molecules, Nuclear receptor, Adaptor proteins, Thrombosis.
activation\(^6\). However, when the integrity of the vascular endothelium is compromised by blood vessel damage or vascular disease, roles of NO and PGI\(_2\) on platelet activation are weakened or even suppressed. So NO and PGI\(_2\) are not included in our consideration for this purpose, meanwhile some exogenous chemicals are also excluded. Platelet surface inhibitory receptors containing platelet endothelial cell adhesion molecule-1 (PECAM-1), carcinoembryonic antigen cell adhesion molecule 1, 2 (CEACAM 1, 2) and G6b-B have been studied for decades. Furthermore, nuclear receptors and intracellular adaptor proteins, such as LIM domain family, downstream of tyrosine kinase (Dok) and c-Casitas B-lineage lymphoma (c-Cbl), are involved in assembling downstream signaling complexes after platelet activation by agonists especially collagen and collagen-related peptide (CRP) upon binding to GPVI-Fc\(\gamma\) chain. Some signal molecules, such as protein kinase C\(\delta\) (PKC\(\delta\)), glycogen synthase kinase (GSK)-3\(\beta\), (GSK3\(\beta\)) and phospholipase D2 (PLD2) also facilitate platelet inactivation. Besides, once activated, platelets release active molecules from its granular stores into the blood, of which adapter protein disabled-2 (DAB2) and diadenosine 5,5-P\(_1\),P\(_2\)-diphosphate (AP2A) are typically known to negatively modulate platelet function. Therefore, in this review we highlight several important negative modulators of platelet function during platelet activation.

**Inhibitory Receptors on the Surface of Platelets**

At the vascular injury site, the sub-endothelial matrix such as collagen is exposed and resting platelets are immediately activated to interact with the collagen. This process is called early platelet activation\(^6\). The interaction of platelets and collagen is mediated by integrin \(\alpha_2\beta_1\) and the GPVI/Fc\(\gamma\)-chain complex\(^8\), whereas platelet activation by laminin is mediated by the related integrin \(\alpha_6\beta_1\), and similarly requires signaling via GPVI/Fc\(\gamma\) \(\gamma\)-chain. Hence, GPVI/Fc\(\gamma\) \(\gamma\)-chain signal largely dominates collagen-mediated platelet response. At least five immunoreceptors tyrosine-based inhibitory motif (ITIM)-bearing receptors namely recruit protein tyrosine phosphatases SHP-1 and SHP-2\(^9\) to down-modulate platelet-collagen interactions PECAM-1, CEACAM-1/2, G6B, TREM-like transcript 1 (TLT1) which are expressed on platelets via Src homology-2 (SH2) domain\(^10\)\(^15\). Although individually these receptors exert moderate inhibition of collagen or thrombin signaling in platelets, collectively they regulate the cell-contact signaling events involved in the initiation of platelet thrombus formation to limit occlusive pathological thrombi. In contrast to other ITIM receptors, TLT1 is enhanced rather than inhibited Fc\(\gamma R\) I-mediated calcium signaling in rat basophilic leukemia cells\(^16\).

**CEACAM Super Family**

**CEACAM1**

CEACAM1 is expressed on the surface and in intracellular pools of resting murine and human platelets\(^8\), which has two isoforms: long ITIM isoform (CEACAM1-L; 70-73 amino acids) and short ITIM-less isoform (CEACAM1-S; 10-12 amino acids)\(^7\). CEACAM1-L protein has two ITIMs that bind SHP-1 and SHP-2 to dephosphorylate Src family kinases (SFKs). Inactive SFKs cannot phosphorylate collagen-mediated GPVI/Fc\(\gamma\) \(\gamma\)-chain downstream signals. Therefore, the CEACAM1 inhibits platelet activation. As demonstrated, CEACAM1\(^-/-\) mice were more susceptible to thrombosis, and CEACAM1\(^-/-\) platelets displayed enhanced type I collagen and CRP-mediated platelet aggregation, adhesion and dense granule secretion\(^8\).

**CEACAM2**

CEACAM2 negatively regulate collagen-mediated GPVI/Fc\(\gamma\)-chain and the C-type lectin-like receptor 2 (CLEC-2) signaling\(^15\). Compared with wild-type platelets, CEACAM2\(^-/-\) platelets had elevated platelet aggregation, adhesion, and dense granule release. Furthermore, thrombi formed in CEACAM2\(^-/-\) mice were larger and more stable than in wild-type controls in vivo. Unlike CEACAM1, CEACAM2 can negatively regulate both collagen GPVI and CLEC-2 ITAM signaling pathways in platelets\(^15\). In a physiological arterial shear flow analysis in vitro, CEACAM2 defect increased the surface coverage of platelets on immobilized type I collagen, but not thrombin or PAR-4 agonist peptide. This platelet aggregation reaction was different from that of CEACAM1\(^-/-\) platelets\(^5\). CEACAM1\(^-/-\) platelets had comparable amplitude and slope of thrombin-mediated aggregation response compared with wild-type platelets\(^8\). These data demonstrate that platelet responses are orchestrated by both CEACAM1 and CEACAM2 through the GPVI-Fc\(\gamma\)-chain-integrin \(\alpha_2\beta_1\). However, both CEACAM1 and CEACAM2 have no effect on G-protein-coupled re-
receptor (GPCR) signaling. Despite these findings, recent observations have revealed controversial questions that CEACAM1 and CEACAM2 were negative modulators in GPVI/FcRγ-chain signaling on platelet activation. CEACAM1/2 is essential for normal integrin αIIbβ3-mediated platelet function. Disruption of mouse Ceacam1/2 induced integrin αIIbβ3-mediated functional defects\textsuperscript{18,19}. In resting platelets, CEACAM1/2 is constitutively associated with integrin αIIbβ3. CEACAM1\textsuperscript{-/-} mice had prolonged tail-bleeding time and high blood loss, which was rescued by supplementation with CEACAM1/2 platelets\textsuperscript{18}. CEACAM1/2\textsuperscript{-/-} platelets exhibited kinetic defects in platelet fibrinogen spreading and impaired retraction of the fibrin clot \textit{in vitro}. However, CEACAM1/2\textsuperscript{-/-} platelets displayed normal “inside-out” signaling properties\textsuperscript{19}. Therefore, CEACAM1/2 is essential for normal integrin αIIbβ3-mediated platelet function.

**PECAM-1**

PECAM-1 has been shown to be expressed on platelets and hematopoietic cells\textsuperscript{20,21}. The PECAM-1 protein contains a 574 amino acid extracellular domain, a 19 amino acid transmembrane domain, and a cytoplasmic domain. In addition, PECAM-1 also possesses two ITIMs, which recruits Src homology 2 (SH2) domain-containing proteins such as protein tyrosine phosphatase 2 (SHP-2), during inflammatory response\textsuperscript{22}. Following platelets adhesion to immobilized laminin, PECAM-1 is tyrosine phosphorylated on its cytoplasmic ITIM-motifs. At the same time, the GPVI-FcRγ-chain signaling is activated. Both of these processes lead to the activation of integrin α6β1. However, laminin-induced tyrosine phosphorylation of PECAM-1, Syk and the FcR-γ chain was observed after 15-30 min while PECAM-1 inhibited thrombus formation \textit{in vivo} 5-10 min after vascular injury\textsuperscript{7}. Stimulation of the PECAM-1 signaling at high concentrations of agonists (collagen, CRP and convulins) reduces platelet aggregation\textsuperscript{23}. Consequently, this leads to decrease in platelet secretion, calcium mobilization, inositol phosphates production, and total protein tyrosine phosphorylation. Consistent with the inhibition of PECAM-1 \textit{in vitro}, PECAM-1-deficient platelets exhibited hyper-reactivity to collagen or CRP. In addition, platelet aggregation was enhanced at low agonist concentrations and the threshold for the release of serotonin or ATP from dense granules was reduced. This led to increased thrombus formation \textit{in vitro} on immobilized Type I collagen under arterial flow conditions\textsuperscript{11,12}. Taken together, these observations indicate that the three types of adhesion molecules present on the platelet surface and intracellular pools (CEACAM1/2 and PECAM-1), once bound to their corresponding ligands, result in the inhibition of platelet adhesion and aggregation and release particles by their ITIMs (Figure 1).

**Figure 1.** The platelet surface inhibitory receptors PECAM-1, G6b-B and CEACAM-1 inhibit integrin inside-out signaling. These three receptors contain ITIM/ITSM in the cytoplasmic tails, which bind the SH2 domain containing non-transmembrane PTPs, SHP-1 and SHP-2. Then SHP-1/2 dephosphorylates src family kinases, which prevent SFKs from activating integrin αIIbβ3. The conformation change from inactive to active provides binding sites for fibrinogen or vWF factor through RGD domains.
**Endogenous negative modulators of platelet function**

**G6b-B**

G6b also belongs to the immunoglobulin superfamily and has two ITIMs, which undergo alternative splicing to produce G6b-A and G6b-B isoforms. Tyrosine phosphorylation of GPVI-FcRγ-chain and CLEC-2 is critical for the interaction of G6b with SHP-1 and SHP. G6b-B is closely related to platelet production and function. G6b-B gene knockout in mice was associated with a bleeding tendency. Megakaryocytes in G6b-B-deficient mice showed enhanced metalloproteinase production, which led to increased hydrolysis of cell-surface receptors, including GPVI and GPIbα. In addition, G6b-B-deficient megakaryocytes displayed reduced integrin-mediated functions and defective formation of proplatelets. Thus, G6b-B is a major inhibitory receptor regulating megakaryocyte activation and platelet production.

**Nuclear Receptors**

**RXRs**

Retinoid X receptors (RXRs) consist of three nuclear receptor isoforms (α, β, and γ) that are activated by 9-cis-retinoic acid (9cRA). 9cRA inhibit ADP-induced platelet aggregation, but its isomer all-trans-retinoic acid, which is not an activator of RXRs, has no effect on platelet aggregation. This demonstrates that RXRs have a pharmacological activity that inhibits the positive feedback of additional ADP and TXA2 produced by the activated platelets. ADP and TXA2 receptors in platelets link predominantly to Gq, Gi, and Gq/G12/13 G proteins. When incubated with 9cRA, RXRs rapidly binds Gq in a ligand-dependent manner and inhibits Gq-induced Rac activation and intracellular calcium release. Since RXRs ligands are involved in the anti-atherosclerotic effects associated with inhibition of platelet activation, RXRs ligands have significant effects in reducing the progression of atherosclerosis in apoE knockout mice (Figure 2).

**PPARs**

Peroxisome proliferator-activated receptors (PPARs) is a family of three nuclear receptor isoforms (α, β/δ, and γ) that modulate transcription of the target genes. Rosiglitazone and prostaglandin, 15-deoxy-D12,14-prostaglandin J2 (15d-PGJ2), are synthetic ligands for PPARγ that have clinical use. Rosiglitazone and 15d-PGJ2 inhibited collagen-stimulated platelet aggregation and thrombus formation under arterial flow conditions, and this effect was mediated, at least in part, through binding to PPARγ in platelets. Moreover, PPARγ ligands inhibit tyrosine phosphorylation levels of multiple components of the GPVI signaling pathway. Besides, PPARγ ligands have been reported to inhibit platelet aggregation in response to ADP accompanied by a reduction in markers of platelet activation such as P-selectin...
exposure, TXA₂ synthesis and sCD40L release⁴⁷. In conclusion, these findings indicate that PPAR ligands inhibit platelet activation through attenuation of GPVI and GPCR signals.

**LXRs**

Liver X receptors (LXRs) is another member of the nuclear receptor superfamily, whose natural ligands, oxysterols, are cholesterol derivatives⁴⁸. LXRs maintain cholesterol homeostasis by regulating the transcription of genes associated with lipid metabolism, such as cytochrome P4507 alpha-hydroxylase 1 (Cyp7a1) and apolipoprotein E (ApoE)⁴⁹. LXRs ligands, GW3965 and T0901317, modulate a series of agonist-stimulated non-genomic platelet aggregation by acting on LXR-β⁵⁰. These data illustrate that GW3965 caused LXR to associate with the signaling components proximal to the collagen receptor, GPVI, suggesting a potential mechanism of LXRs action in platelets that leads to diminish platelet responses. These results indicate that GW3965 has antithrombotic effects and reduce the size and stability of thrombi⁵¹. These atheroprotective effects of GW3965, together with its novel anti-platelet/thrombotic effects, indicate that LXRs is a potential target for prevention of athero-thrombotic disease⁵¹.

**Intracellular Adaptor Proteins**

### LIM-Domain Family Members

Several LIM-domain family members (such as CLP36 and Paxillin) communicate with platelet activation. The CLP36 is an adaptor protein containing PDZ domain at the N-terminus, a short linker region, and the LIM-domain at the C-terminus⁵². Paxillin on the other hand, contains two conserved structural domains, the N-terminus and C-terminus, which consists of...
four LIM-domains\textsuperscript{53}. Leupaxin and Hic-5 have been identified as variants of paxillin on murine platelets\textsuperscript{54}. However, only Hic-5 has been shown to be predominantly expressed in human platelets\textsuperscript{55}.

**CLP36**

CLP36, the only PDZ-LIM family member expressed in platelets, negatively regulated platelets granule secretion, aggregation, integrin activation, and thrombus formation\textsuperscript{56}. CLP36-deficient platelets were sensitive to the agonists of CRP, collagen and convulxin rather than ADP and thrombin. This was associated with hyperphosphorylation of GPVI downstream signaling proteins and enhanced Ca\textsuperscript{2+} mobilization, granule secretion, and integrin activation. Moreover, GPVI-induced tyrosine phosphorylation of PLC\textgamma2, Src family kinases (including Yes, Fyn, Lyn), and FcR\gamma chain, as well as inositol-1,4,5-trisphosphate production, and Ca\textsuperscript{2+} mobilization were increased in CLP36-deficient function platelets\textsuperscript{56}.

**Paxillin**

Paxillin, another LIM-domain family member, is localized in platelets\textsuperscript{57}. Paxillin-\textsuperscript{-/-} platelets were slightly enlarged and had elevated integrin αIIbβ3 activation following stimulation of both GPVI and GPCRs, which was different to CLP36-\textsuperscript{-/-} platelets\textsuperscript{58}. Thromboxane A2 biosynthesis and the release of α and dense granules following platelet adhesion and thrombus formation in vivo were also augmented in paxillin-\textsuperscript{-/-} mice. Hic-5-deficient mice exhibited prolonged tail bleeding times. In addition, thrombin rather than convulxin, U46619, and ADP slightly impaired the activation of integrin αIIbβ3\textsuperscript{58}. It is not yet known whether these platelet responses are similar in human (Figure 2).

**Dok**

Dok adaptor proteins are expressed on both human platelets (Dok-1, Dok-2 and Dok-3) and mouse platelets (Dok-1 and Dok-2)\textsuperscript{59-61}. Dok-1 is a negative modulator of platelet integrin αIIbβ3 that involved in the ‘outside-out’ signaling\textsuperscript{61}. It was shown to enhance platelet spreading on fibrinogen and clot retraction, increase phosphorylation of PLC\textgamma2, and accelerate arterial thrombosis in Dok-1-deficient mice, but did not affect the ‘inside-out’ signaling, which displayed normal aggregation, P-selectin expression and soluble fibrinogen binding\textsuperscript{62}.

**c-Cbl**

c-Cbl is a universally expressed adaptor protein that function in hematopoietic cells\textsuperscript{63}. It can be phosphorylated at tyrosine residues and interact with proteins that contain the SH2 domain\textsuperscript{64,65}. C-Cbl is phosphorylated in activated human platelets, whereas its phosphorylation is decreased in Fyn- and Lyn-deficient platelets\textsuperscript{65}. In c-Cbl mutant platelets, both CRP and thrombin-induced platelet aggregation in the GpVI signal pathway were increased\textsuperscript{66}. This confirms that c-Cbl is an important negative modulator of the GpVI signaling in regulating platelet activation (Figure 3).

**Inhibitory Molecules Involved in the Platelet Activation Pathway**

**PKC\textgammabeta**

The protein kinase C (PKC) family plays a role in the regulation of exocytosis and cell adhesion\textsuperscript{67,68}. Based on the previous research, various PKC isoforms have been shown to act as positive regulators of platelet cytoskeleton reorganization and aggregation\textsuperscript{69}. However, Pula et al\textsuperscript{70} proposed that PKC\textgammabeta may have a negative role in platelet filopodia formation, actin polymerization, and platelet aggregation. Using PKC-selective inhibitor, they showed that the vasodilator-stimulated phosphoprotein (VASP), a marker of platelet skeleton organization, was increased\textsuperscript{70}. Moreover, PKC\textgammabeta has also been shown to negatively influence platelet formation in vitro\textsuperscript{71}. Hence, PKC\textgammabeta plays a crucial role in the formation and activation of platelets, as well as in stabilization of thrombus.

**GSK-3\beta**

Glycogen synthase kinase (GSK3\beta) is a serine/threonine kinase regulated by Akt phosphorylation on ser 9\textsuperscript{72}. It has been shown to possess anti-melanogenic effects of Aster spathulifolius and to suppress metastasis of hepatocellular carcinoma cells\textsuperscript{73}. ADP and thrombin regulate platelet function through the GPCR complex and phosphoinositide 3-kinase (PI3K)/Akt pathways\textsuperscript{74}. In PI3K and Akt deficient mice, platelet adhesion and aggregation were reduced, suggesting that PI3K and Akt are positive regulators of platelet function\textsuperscript{75}. Considering that the kinase GSK3\beta is a downstream effector of Akt, whether it is a positive or negative regulator of platelet function is not clear. However, Akt negatively regulated the GSK3\beta activity by phosphorylating GSK3\beta...
The intracellular inhibitory signal molecules mainly aim at GPVI-FcR γ and GPCR signaling Pathway. The deficiency of CLP36, TULA2, c-Cbl and PrxII universally improve the phosphorylation of GPVI downstream signaling proteins and calcium release (green arrow), while Paxillin defect both affect GPVI and GPCR signaling pathway (green arrow and red arrow). PKC and PI3K/Akt positively regulate integrin activation; however, PKCδ and GSK3β (PI3K/Akt downstream) defect negatively regulate integrin activation.

**PLD2**

Phospholipase D (PLD) catalyzes the hydrolysis of phosphatidylcholine into phosphatidic acid (PA) and choline, which participates in platelet activation\(^7\). The PLD has two isoforms, PLD1 and PLD2. PLD1 has low basal activity and is activated by PKC and small GTPases (adenosine diphosphate (ADP)-ribosylation factor (ARF) and Rho family)\(^7\). On the other hand, PLD2 has high basal activity and is activated by a variety of agonists such as collagen, thrombin, and the TXA2-mimetic U46619\(^7\). Lack of PLD1 in platelets impaired integrin αIIbβ3 activation and shear dependent thrombus formation. This led to protection against arterial thrombosis and ischemic brain infarction while degranulation and aggregation were unaffected\(^8\). PLD inhibitor, 5-fluoro-2-indolyl des-chlorohalopemide (FIPI), enhanced dense granule secretion and aggregation on human and mouse wild-type and PLD1\(^−/−\) platelets following stimulation with high concentrations of agonists that activate Gα12/13 and Gq signaling in platelets\(^8\). It seems that PLD1 and PLD2 have different platelet functions in vivo. The enhanced aggregation and secretion in FIPI treatment of PLD1\(^−/−\) platelets closely related to the high basal activity of PLD2. Hence, PLD2 might be a potential negative regulator of platelet sensitivity\(^8\).

**PrxII**

Peroxiredoxin II (PrxII) is a cellular peroxidase that breaks down endogenous hydrogen peroxide (H\(_2\)O\(_2\)) produced upon platelet-derived growth factor or epidermal growth factor activation\(^9\). H\(_2\)O\(_2\) functions to promote GPVI signaling activation. PrxII deficiency enhanced GPVI signaling pathway during platelet activation, which correlated with the accumulation of H\(_2\)O\(_2\) in the body. Reduction of H\(_2\)O\(_2\) increases the oxidative inactivation of SHP-2, which results in en-
Enhanced tyrosine phosphorylation of key components of the GPVI signaling cascade. The PrxII/SHP-2-mediated inhibition of platelet activation appears to occur in lipid rafts. PrxII-deficient platelets showed a marked increase in adhesion and aggregation activity in vitro. The anti-thrombotic activity of PrxII was also verified in arterial injury in vivo model. Hence, PrxII down-regulates the GPVI signaling and thrombosis and is recognized as a potential target for anti-platelet and anti-thrombotic therapy.

**TULA-2**

T-cell ubiquitin ligand-2 (TULA-2), is a histidine tyrosine phosphatase, that occurs as the sole family member of TULA in platelets. It is encoded by the ubiquitin associated and SH3 domain-containing protein B (UBASH3B) gene. TULA-2 dephosphorylated the spleen tyrosine kinase (Syk). Ablation of TULA-2 resulted in hyperphosphorylation of Syk and its downstream effector phospholipase C-γ2 as well as enhanced GPVI-mediated platelet responses. Therefore, TULA-2 functions as a negative regulator of GPVI signaling in platelets. Not only does it negatively regulate murine platelet activation via glycoprotein VI (GPVI)/Fc receptor γ-chain (FcγR), but also negatively modulates FcγRIIA, which is the sole IgG-receptor present in human platelets. Interference of endogenous murine mmu-miR-148a-3p with locked nucleic acids (anti-miR-148a-3p LNA), upregulated platelet TULA-2. This leads to hypophosphorylation of Syk and protection of thrombocytopenia (HIT), a disorder characterized by low platelet count and thrombosis.

**Extracellular Modulators Released from Platelet Granules**

**DAB2**

Adapter protein disabled-2 (DAB2) contains three domains: the N-terminal phosphotyrosine binding (PTB) domain, the aspartic-acid-proline-phenylalanine (DPF) motif, and the C-terminal proline-rich region. DAB2 is a modulator released from platelet granules in a PKC-dependent manner during platelet activation. DAB2 negatively regulate the interaction of fibrinogen with integrin αIIbβ3 located in the extracellular region of the platelet surface. Biochemical and mutational analysis revealed that the DAB2 contains the Arg-Gly-Asp (RGD) motif (with amino acid residues 64-66). The binding of fibrinogen to integrin αIIbβ3 occurs through the RGD motif (amino acid residues 171-464). Therefore, the RGD motif of DAB2 competitively occupies the binding region on the integrin αIIbβ3, thereby preventing integrin and fibrinogen binding, which forms the basis of DAB2 inhibition of platelet aggregation.

**Ap2A**

There are three types of new growth-promoting extracellular mediators in platelet secretory granules: diadenosine 5,5-P1,P2-diphosphate (Ap2A), adenosine guanosine diphosphate (Ap2G), and diguanosine diphosphate (Gp2G). The ADP-ribosyl cyclase (CD38) enzyme synthesizes Ap2A and its two isomers, P18 and P24 from cyclic ADP-ribose and adenine (Ade). The Ap2As are characterized by an unusual N-glycosidic bond between one adenine and the ribose (C1-N1 in P18 and C1-N3 in P24). Furthermore, it was shown that CD38 was expressed on the human platelet. Presently, it has been proven that Ap2A influences platelet aggregation. Additionally, Ap2A and its isomers were expressed in resting human platelets and were released during thrombin-induced platelet activation. They are also involved in the inhibition of platelets aggregation, which indicates a feedback mechanism that limits platelet aggregation through a generation of adenylic dinucleotides. Ap2A, P18, and P24 bind to the purinergic receptor P2Y11, thereby increasing intracellular cAMP concentration and production of nitric oxide. These results suggest a role for Ap2A, P18, and P24 as negative endogenous regulators of platelet aggregation (Figure 4).

**Conclusions**

Small molecules and chemically designed drugs have been widely used to treat cardiovascular diseases and malignant tumors. However, limited experiments are carried out in humans and the detrimental effects of long-term use of these drugs have greatly hindered their application. Therefore, the endogenous regulatory molecules present an excellent choice to research. This paper demonstrates a clear role of endogenous negative modulators of platelets. Furthermore, we explore their therapeutic potential especially for their use in thrombosis and atherosclerosis. Further research is advocated to advance and exploit their value as prospective anti-platelet and anticoagulant drugs.
Acknowledgements
The author is grateful to the National Natural Science Foundation of China (No: 81402918) and the Natural Science Foundation of Jiangsu Province (No: BK20140228) for its assistance, and the support of the China Postdoctoral Science Foundation Committee (No: 2016M591924).

Conflict of Interest
The Authors declare that they have no conflict of interests.

References
Endogenous negative modulators of platelet function


45) Sokolowska M, Kawolski ML, Pawliczak R. Peroxisome proliferator-activated receptors-gamma (PPAR-gamma) and their role in immunoregulation and inflammation control. Postepy Hig Med Dosw (Online) 2005; 59: 472-484.


74) Hwang GY, Choung SY. Anti-melanogenic effects of Aster staphylicfolius extract in UVB-exposed C57BL/6J mice and B16F10 melanoma cells through the regulation of MAPK/ERK and AKT/PRAS40. J Pharm Pharmacol 2016; 68: 4035-4044.


