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Review Article

The role of phosphoinositide 3-kinase β in thrombosis

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Abstract: Pathological thrombus can lead to a variety of cardiovascular diseases. Current antithrombotic therapies are limited by their deleterious effects on hemostasis, which lead to bleeding complications. Ideal antithrombotic therapies would selectively target events that are crucial for pathological thrombus formation without affecting hemostasis. Phosphoinositide 3-kinase β (PI3Kβ) is involved in platelet activation signaling pathways in response to high shear stress. Antithrombotic drugs targeting PI3Kβ may selectively inhibit thrombus resulting from high shear stress instead of disrupting normal hemostasis. Therefore, it is worth studying the role of PI3Kβ in thrombosis. This article provides a brief introduction to PI3Kβ and reviews the relationship between PI3Kβ and thrombosis and summarizes the signaling pathways through which PI3Kβ induces platelet aggregation and thrombus formation. We also discuss the potential of PI3Kβ for therapeutic targeting and provide personal opinions on new antithrombotic drugs and therapies targeting PI3Kβ.

Keywords: PI3K, antithrombotic target, thrombosis, cardiovascular diseases, shear stress

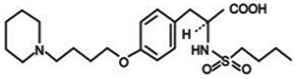
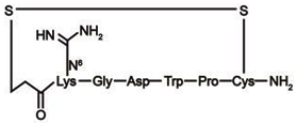
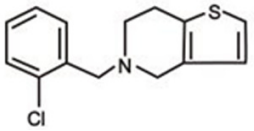
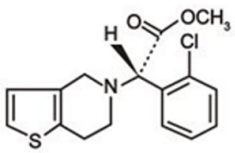
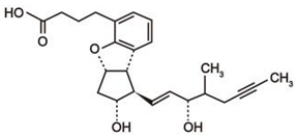
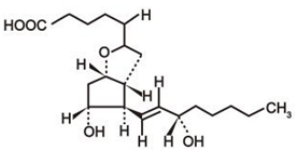
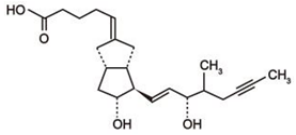
Introduction

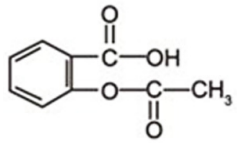
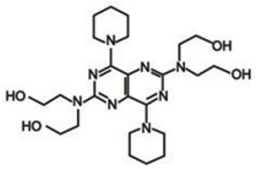
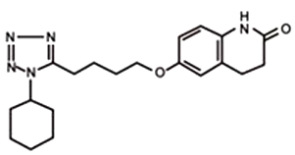
In mammals hemostasis is the main defense system that protects the integrity of the circulatory system. Damage to vessel walls activates the hemostatic pathway; however, strong activation of the hemostatic pathway that exceeds the normal regulatory counterbalance maintained by anticoagulant factors leads to thrombosis [1]. Thrombus causes a variety of cardiovascular diseases such as myocardial infarction, intermittent claudication, pulmonary embolism and cerebral thrombosis. Platelet aggregation is the key process in thrombosis. All clinical antiplatelet agents target one or more major steps in the platelet activation process, ultimately downregulating the adhesive function of integrin αIIbβ3 (GPIIbIIIa), and the antiplatelet targets include adhesion receptors, platelet agonist receptors and platelet signaling pathways (**Table 1**). Nevertheless, current antithrombotic therapies are limited by their deleterious effects on hemostasis, which lead to bleeding complications [2]. Ideal antithrombotic therapies would selectively inhibit the pathological thrombus formation without affecting hemostasis. Arterial thrombus typically forms under the conditions of high blood flow [1]. One strategy for developing antithrombotic therapies that can attenuate the platelet thrombus formation without inducing bleeding complications is to target the platelets in response to high shear stress. Recently, researchers have demonstrated that the β isoform of phosphoinositide 3-kinase (PI3K) plays a crucial role in the signaling pathway that promotes the shear activation of platelets; therefore, inhibition of PI3Kβ is considered a novel antithrombotic strategy that overcomes the limitations of current antithrombotic strategies. This article gives a brief introduction to PI3Kβ, reviews the relationship between PI3Kβ and thrombosis and summarizes the signaling pathways through which PI3Kβ induces the platelet aggregation and thrombus formation. We also discuss the potential of PI3Kβ and provide personal opinions on new antithrombotic drugs and therapies targeting PI3Kβ.

Structure and function of PI3Kβ

PI3K isoforms are categorized into three classes (class I, II and III) based on structural characteristics, regulatory mechanisms and substrate

Table 1. Features and targets of existing antithrombotic drugs

Targets	Drugs	Molecular structures or formulas	Advantages	Disadvatage	References
Adhesion receptor					
GPIIbIIIa	Tirofiban		Has a good therapeutic effect with percutaneous coronary, and lower rates of thrombocytopenia compared with abciximab.	Long-term use associated with a high risk of nuisance bleeding.	[41]
	Abciximab	$C_{2101}H_{3229}N_{551}O_{673}S_{15}$	Powerful antithrombotic effect undergoing percutaneous coronary interventions.	Has a remarkable adverse effects of thrombocytopenia.	[42]
	Eptifibatide		Has a good therapeutic effect undergoing percutaneous coronary interventions.	Bleeding risks of eptifibatide in specific patient populations.	[43]
Platelet agonist receptors					
$P2Y_{12}$	Ticlopidine		Ticlopidine is more effective and safe in patients having undergone coronary and femoral bypass surgery than clopidogrel.	Causes side effect such as lupus erythematosus in elderly men.	[44]
	Clopidogrel		Well-tolerated oral drugs. Bleeding risk low as monotherapy.	Relatively weak antithrombotic effect. Increased bleeding risk with combination therapy. Significantly more expensive than aspirin.	[45]
Prostacyclin receptor	Beraprost		Beraprost is as efficacious as ticlopidine in the treatment of patients with peripheral arterial disease. A well-tolerated agent.	Main adverse events include headache, hot flushes, diarrhoea and nausea.	[46]
	Epoprostenol		Epoprostenol is an effective and potent treatment in pulmonary arterial hypertension and has greatly improved survival, exercise capacity, Pulmonary arterial hypertension symptoms, pulmonary hemodynamics and disease progression.	A main disadvantage is that it can only be delivered through a continuous intravenous pump infusion.	[47]
	Iloprost		Iloprost improves the patient's quality of life on the overall considering their favorable effect on pulmonary hemodynamics, symptoms reduction and exercise tolerance.	Larger, long-term clinical trials are needed to solidify the role for therapeutic effect of iloprost.	[48]

Platelet signaling pathways					
Cyclooxygen-ase	Aspirin		Well-tolerated oral drug. Highly cost effetcive. Bleeding risk low as monotherapy.	Weak antithrombotic action with limited efficacy.	[49]
Phosphodiest-erase	Dipyrid-amole		No increased bleeding risk when combined with aspirin.	Limited efficacy as monotherapy. Side effects such as headache, hypotension, blood pressure lability and gastrointestinal irritation.	[50]
	Cilostazol		Safe, well-tolerated.	Weak antithrombotic action.	[51]

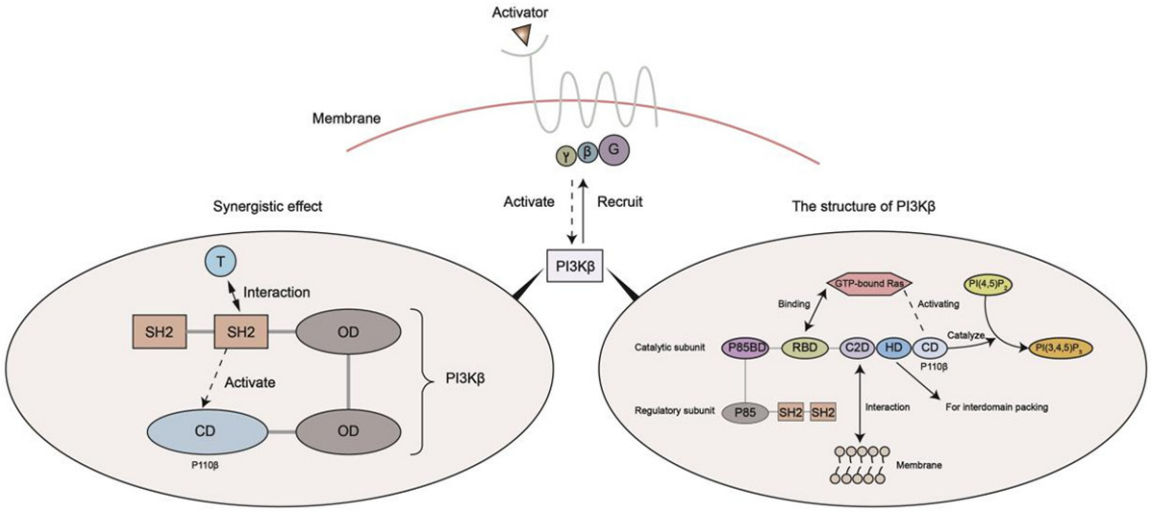


Figure 1. The activation of PI3Kβ. Each domain of PI3Kβ has specific function: P85BD is for combination with the regulatory subunit. RBD can bind to GTP-bound Ras to activate CD, and then CD phosphorylate $PI(4,5)P_2$ to generate $PI(3,4,5)P_3$; C2D has an interaction with membrane; and HD is central to the interdomain packing interacting with the CD, the RBD and the C2D. PI3Kβ is activated synergistically by tyrosine phosphorylation sequence and Gβ/γ subunits of heterotrimeric G proteins. Upon activation of the G protein, PI3Kβ is recruited to the membrane interacting with its substrate. On the other hand, tyrosine phosphorylation sequence interacts with SH2 domains to activate the CD. Abbreviation: P85BD, P85 binding domain; RBD, Ras-binding domain; C2D, C2 domain; HD, helical domain; CD, catalytic domain; SH2, Src homology 2; T, tyrosine phosphorylation sequence; OD, other domains; PI3K, Phosphoinositide 3-kinase; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate.

preferences, and class I PI3Ks are divided into two subclasses (class IA and IB) according to the regulatory subunit of the isoform. PI3Kβ is a class IA PI3Ks isoform that phosphorylates phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) to generate $PI(3,4,5)P_3$ which is downstream of the activation of many cell surface receptors [3]. Recently, researchers have confirmed that individual class I PI3K isoforms have specific cellular and physiological functions implicated in oncogenesis, innate and adaptive immunity, various inflammatory diseases and thrombosis.

Molecular structure of PI3Kβ

PI3Kβ is a heterodimeric protein comprised of a catalytic subunit and a regulatory subunit. The catalytic subunit p110β contains a Ras-binding domain (RBD), a C2 domain (C2D), a helical domain (HD) and a catalytic domain (CD). The regulatory subunit p85 contains two Src homology 2 (SH2) domains [4]. All of these domains have specific regulatory functions (**Figure 1**). The RBD can bind to GTP-bound Ras to activate the catalytic subunit [5]. The C2D may interact with membranes. The HD is key for interdomain interactions among the CD, the RBD and the C2D [6]. The function of the CD is phosphorylating $PI(4,5)P_2$ to generate the lipid second messenger $PI(3,4,5)P_3$. The SH2 domains can recognize tyrosine phosphorylated peptides to activate PI3Kβ [7]. Classical and present studies have revealed that PI3Kβ can be activated not only by receptor tyrosine kinases, but also the Gβ/γ subunits of heterotrimeric G proteins or both [8]. Upon activation of the immunoreceptor tyrosine-based activation motif (ITAM) and heterotrimeric G-protein-coupled receptors (GPCRs), PI3Kβ is recruited to the plasma membrane, where its substrates are located, leading to $PI(3,4,5)P_3$ production (**Figure 1**).

Physiological and pathological functions of PI3Kβ

PI3Kβ has various physiological and pathological functions and participates in endothelial cell migration, cell growth and proliferation, metabolism, oncogenesis and thrombosis. Its functions are related to cell growth and proliferation and metabolism, which are kinase-independent functions, while its functions related oncogenesis and thrombosis are kinase-dependent functions. PI3Kβ is required for normal

induction of migration by sphingosine-1-phosphate. Rac1 is a major mediator downstream of PI3Kβ, and PI3Kβ mediates migration through Akt in a manner that is independent of Rac1 and endothelial NO synthesis [9]. Mouse mutants expressing a catalytically inactive p110β can survive to adulthood but exhibit growth retardation and mild insulin resistance with age [10]. Consistent with these findings, ablation of p110β in the livers of mice leads to impaired insulin sensitivity and glucose homeostasis, and deletion of p110β from mouse embryonic fibroblasts retards cell proliferation [11]. These events are independent of Akt-phosphorylation, indicating that they are kinase-independent functions of PI3Kβ, while the induction of oncogenesis and thrombosis by PI3Kβ is related to Akt-phosphorylation. Deletion of p110β in the prostate in animals impedes tumorigenesis and concomitantly decreases Akt phosphorylation [12]. The role of PI3Kβ in thrombosis which is the main part of this review is also kinase-dependent and is discussed below.

PI3Kβ as a novel antithrombotic target

Evidence for PI3Kβ as an antithrombotic target

Platelet activation is a vital step in thrombosis. Platelets are exposed to a diverse range of activating stimuli including adhesive proteins such as fibrillar collagens, von Willebrand factor (vWf) and fibronectin, and soluble agonists such as thrombin, adenosine diphosphate (ADP), thromboxane A_2 (TXA_2) and serotonin when the vessel is injured. In addition, platelet adhesion to the exposed collagen in the damaged vessel wall leads to platelet activation through the complex immunoglobulin family receptor-dependent glycoprotein VI (GPVI), signal transduction pathway [13]. Karen Gilio et al. found that PI3Kβ is required for normal GPVI-dependent platelet Ca^{2+} signaling in platelets and thrombus formation, and that PI3Kβ inhibitors can selectively suppress downstream targets of the GPVI signaling pathway [14]. Valérie Martin et al. also demonstrated that PI3Kβ plays a critical role in platelet activation through ITAM, the GPVI-Fc receptor γ-chain complex, and the production of $PI(3,4,5)P_3$ and the activation of protein kinase B/Akt which participates in platelet activation and is strongly inhibited in p110β null platelets [15]. Therefore, PI3Kβ is important for platelet activation.

The signaling pathways that induce platelet activation ultimately converge to activate platelet integrin αIIbβ3 and thus enhance the adhesive and signaling functions of αIIbβ3. Integrin αIIbβ3 mediates platelet-platelet and platelet-vascular wall adhesion interactions. Shaun P Jackson et al. identified a key role for PI3Kβ in regulating the formation and stability of integrin αIIbβ3 adhesion bonds and found that PI3Kβ specific inhibitors can prevent stable integrin αIIbβ3 adhesion contacts and platelet thrombus formation [16]. Judith M. E. M. Cosemans et al. found that PI3Kβ is crucial for the stable aggregation of platelets and that blocking PI3Kβ delays initial thrombosis and reduces individual platelet-platelet contact [17]. Valérie Martin et al. and Simone M. Schoenwaelder et al. suggested that PI3Kβ is involved in enhancing the affinity of integrin αIIbβ3 adhesion bonds and that platelets do not adhere well to fibrinogen in PI3Kβ null platelets, resulting in inhibition of platelet aggregation [18]. Ilaria Canobbio et al. provided genetic evidence for a crucial and selective role of PI3Kβ in signaling through GPVI and integrin αIIbβ3 [19]. These findings indicate that PI3Kβ is a potential target for antithrombotic therapies.

The signaling pathway PI3Kβ is involved in for platelet activation

The signal transduction pathways that PI3Kβ participates include the $P2Y_{12}$ receptor, the thrombin receptor and GPVI dependent signaling pathways (**Figure 2**). Studying the signaling pathways involved in platelets is useful for gaining of further insight into the mechanisms and the theoretical basis of PI3Kβ as a potential antithrombotic target.

$P2Y_{12}$ is a G protein-coupled seven transmembrane purinergic receptor expressed on platelets, and ADP, it is the physiological agonist of $P2Y_{12}$, it can predominantly exert its physiological effects on platelets via the $P2Y_{12}$ dependent signaling pathway [20]. Activation of the $P2Y_{12}$ receptor leads to a cascade of intracellular events involving calcium mobilization, granule release, TXA_2 generation and αIIbβ3 activation. Secreted ADP and generated TXA_2 act as positive-feedback mediators and activate greater platelet responses to aid in a stable hemostatic plug formation [21]. These phenomena are key steps in platelet aggregation and stabilization.

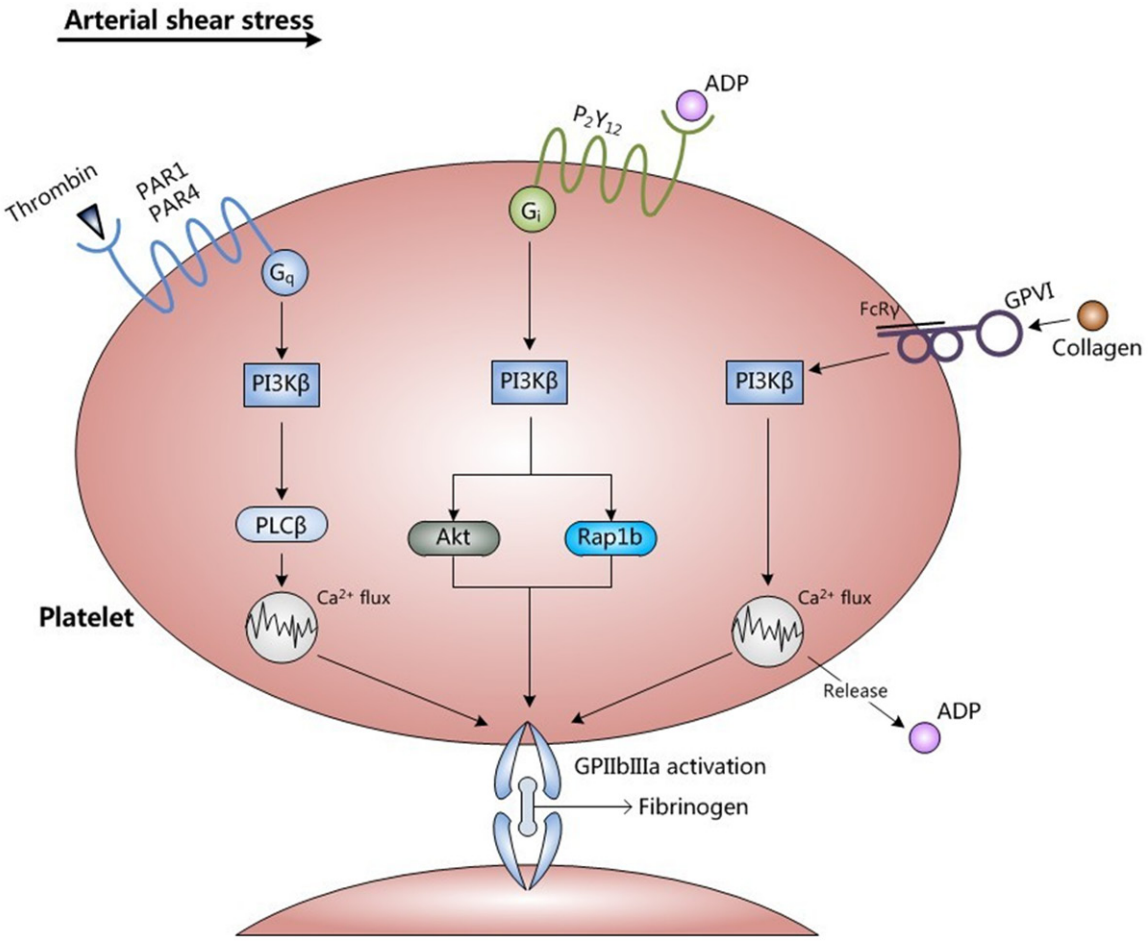


Figure 2. The signaling processes PI3Kβ involved in. PI3Kβ participates in the $P2Y_{12}$ receptor, the thrombin receptor and GPVI dependent signaling pathways to activate platelet.

Shear activation of platelets requires the cooperation between the adhesive and signaling functions of integrin GPIb and GPIIbIIIa and signals amplified via the $P2Y_{12}$ signaling pathway [22]. Thrombi are intrinsically dynamic structures that require continuous paracrine release of ADP and persistent PI3K signaling via PI3Ks to prevent dissociation of the thrombus from CPVI-vWf/collagen interaction, and PI3Kβ is needed to stabilize growing thrombi and prevent their dissolution via continuous $P2Y_{12}$ pathway signaling [17]. Stimulation of the $P2Y_{12}$ receptor can result in G protein-mediated activation of PI3Ks, and then induce the activation of Akt and Rap1b which regulate the adhesive function of integrins. PI3Kβ is the dominant PI3K isoform that regulating G_i-dependent integrin αIIbβ3 activation through the catalytic modulation of Rap 1b or Akt activation in ADP-stimulated platelets. PI3Kβ is required for ADP-induced Akt phosphorylation, is partially required for Rap1b activation [19], and can also mediate ADP-induced TXA_2 generation by regulating extracellular-signal-regulated kinase phosphorylation [23].

Thrombin is a key enzyme in the final step of the blood coagulation cascade, as it cleaves fibrinogen to form fibrin. Moreover, thrombin acts as the most potent platelet activator through two GPCRs, the proteinase-activated receptor (PAR) 1 and PAR4 [24]. PAR1 and PAR4 function through proteolysis of the external N-terminal sequence, and results in the exposure of a new N-terminus that can act as a ligand for the receptor [25]. Both PAR1 and PAR4 couple to phospholiase Cβ (PLCβ) which hydrolyses $PI(4,5)P_2$ to generate inositol-3-phosphate for calcium transfer and diacylglycerol (DAG) for protein kinase C (PKC) activa-

tion. PAR1 initiates the initial platelet aggregation in response to thrombin, while PAR4 may contribute to the later stability of platelet aggregates [26]. PI3Kβ appears to be involved in maintaining irreversible platelet aggregation and the maintenance of integrin αIIbβ3 activation in response to PAR1-activating peptide, and PAR4 and PI3Kβ act in parallel to maintain thrombin-induced integrin αIIbβ3 activation and platelet aggregation through PKC activation and an increase in intracellular calcium [27]. However, more evidence for the role of the p110β isoform in PAR1 and PAR4 dependent pathways is needed, and the mechanisms by which PI3Kβ interacts with PAR1 and PAR4 remains to be elucidated. It is possible that upon activation of PI3Kβ, $PI(3,4,5)P_3$ can be generated to induce the production of the second messenger inositol-3-phosphate and activation of integrin αIIbβ3 through PKB/Akt phosphorylation by PLCβ.

vWf and collagen are important for initiating vascular clot formation. The interactions between the platelet-surface receptors, GPIb-IX-V and GPVI, and between vWf and collagen lead to platelet aggregation. GPVI which is a member of the immunoglobulin superfamily nonconvalently binds with the ITAM-FcRγ chain and has a dominant position in platelet adhesion and thrombosis [28]. The phosphorylation of tyrosine in the FcRγ chain can cause a series downstream events: $PI(3,4,5)P_3$ production, plasma Ca^{2+} mobilization, integrin αIIbβ3 activation, ADP loading of or ATP secretion by platelet granules, and exposure of phosphatidylserine (PS) to the platelet surface exposure to ensure coagulation [29, 30]. PI3Kβ participates in the process of $PI(3,4,5)P_3$ and Ca^{2+} signal generation, indicating that PI3Kβ plays a necessary role in PLCγ2 activation to transduce GPVI dependent signals. Moreover, PI3Kβ deficiency can inhibit Ca^{2+} movement and PS exposure, thus reducing platelet aggregation [31].

Approaches to the development of antithrombotic therapies targeting PI3Kβ

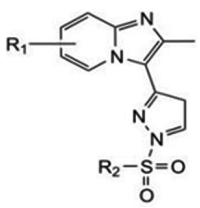
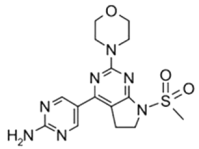
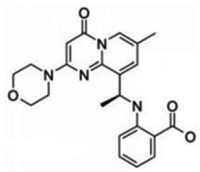
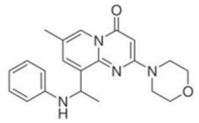
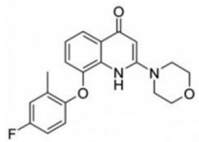
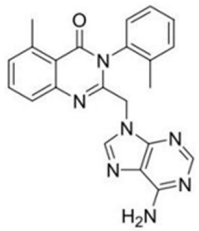
Recently a large number of PI3K inhibitors have emerged in academia and industry (**Table 2**), but there are no inhibitors specific for PI3Kβ that are used in the clinic. Methods based on molecular structure can be used to design and develop highly selective PI3Kβ inhibitors. Because different kinases have ATP-binding pockets of the similar structures, most inhibitors are ATP-competitive inhibitors, however, it is difficult to design selectivity for ATP-competitive inhibitors that are selective [32]. Inhibitors that target the induced-fit hydrophobic pocket adjacent to the ATP-binding site do not directly bind ATP and therefore may be achieved with higher cellular potency and selectivity; nevertheless, the most selective inhibitors are ATP-noncompetitive inhibitors that bind exclusively to sites outside the ATP-binding site [33]. Therefore, PI3Kβ inhibitors can be developed as ATP-noncompetitive inhibitors to achieve higher selectivity.

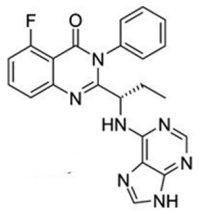
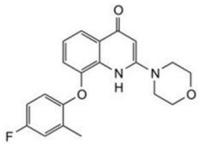
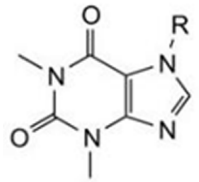
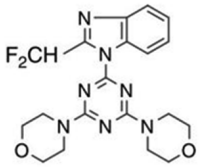
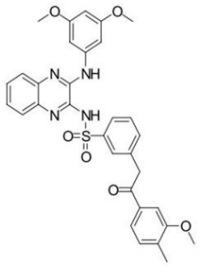
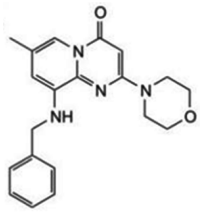
There are a variety of useful tools for PI3Kβ inhibitor development, such as the X-ray technology for crystal structure studies [34], molecular docking technologies [35], molecular dynamics simulation [36], fragment-based approaches [37], and chemical probes and so on [38]. These methods have been used to identify new inhibitors of other kinases and PI3K isoforms, and they are also ideal and appropriate tools for developing novel PI3Kβ inhibitors. Molecular modeling has indicated that increased PI3Kβ specificity is caused by the interaction between Lys782-Asp923 and Asp862, which creates a unique pocket in PI3Kβ, and the selectivity of inhibitors of PI3Kβ can be enhanced by improving the interactions between the unique residues (Lys782, Asp923 and Asp862) at the selectivity pocket [39].

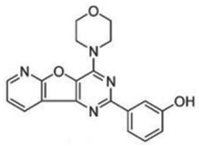
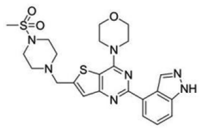
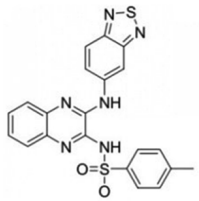
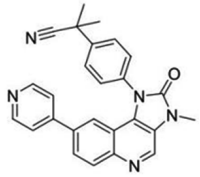
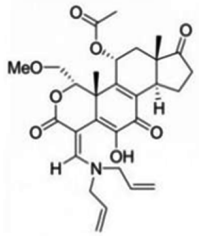
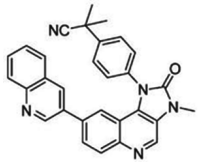
Another method for improving PI3Kβ inhibitors is modifying and optimizing existing PI3K inhibitors. This method has been applied to many other selective PI3K inhibitors. For example, modifications of wortmannin, a classical PI3K inhibitor usually used to specifically inhibit the PI3K isoform, that open the furan ring at the active C20 position yields compounds that not only extend its half-life but also increase its selectivity for particular PI3K isoforms [40]. By modifying and optimizing efficient PI3Kβ inhibitors, the target PI3Kβ inhibitors with high potency and selectivity can be generated; selectivity for PI3Kβ over PI3Kα can be achieved by combining 2-aminoprymidine with the pyridyl sulfonamide moiety [39].

We are building a chemical library of currently used PI3K inhibitors that do not have high β isoform specificity but have been identified as

Table 2. A part of current inhibitors of PI3Ks

Inhibitors	Molecular structures	Selective PI3K isoforms	Research phase	Potential activities	Reference
Imidazopyridine derivatives (R_1 = halogen, R_2 = aromatic)		α	-	Anticancer	[52]
CH5132799		α	-	Antitumor	[53]
AZD-6482		β	Phase I clinical trials	Antithrombosis	[54]
TGX221		β	-	Antithrombosis	[55]
TGX155		β	Phase I clinical trials	Antithrombosis	[54]
IC87114		δ	Clinical trials	Antitumor	[56]

CAL-101		δ	Clinical trials	Antitumor	[57]
Quinazolinones (IC87114)		δ	-	Inhibition of acute myeloid leukemia cell proliferation and anti-inflammation	[58]
Caffeine (R = Me) or theophylline (R = H)		δ	-	Blockage of insulin transportation of glucose transport	[59]
ZSTK474		δ	-	Antitumor	[60]
XL765		γ	Clinical trials	Antitumor	[61]
Pyridopyrimidine		α β	-	Antithrombosis	[62]

PI-103		α β	-	Anti-leukemia	[63]
GDC-0941		α δ	Phase I clinical trials	Antitumor	[64]
XL147		α δ γ	Clinical trials	Antitumor	[65]
BEZ235		α δ γ	Clinical trials	Antitumor	[66]
PX-866		α δ γ	Early clinical trials	Antitumor	[67]
NVP-BEZ235		-	Phase I/II clinical trials	Anticancer	[68]

more efficient candidates with those that induce less side-effects, and building a chemical library containing these chemical agents can provide a platform for antithrombotic drug screening. The inclusion of PI3K inhibitors that have antitumor functions with few side effects in the chemical library allows its antithrombotic effect to be evaluated without worrying about its chemical toxicity.

Summary and prospects

PI3Kβ can activate platelets via many receptor-dependent signaling pathways, and the studies based on these signaling pathways can give an explicit explanation for the mechanisms by which PI3Kβ is induced in thrombosis. However, many questions remain to be answered. The mechanisms by which heterotrimeric G proteins and tyrosine kinases coordinate their action remain unknown; how $P2Y_{12}/G_i$ impacts on PI3Kβ activation in cooperation with different agonists is still unclear; and the mechanism of activation of PI3Kβ downstream of αIIbβ3 is still not elucidated. More research on the functions of PI3Kβ in other cells are needed to ensure that the antithrombotic drugs that target PI3Kβ do not have undesirable effects on the regular physiological functions of other cells.

There are currently no selective PI3Kβ inhibitors used in clinic. Approaches for designing and developing of PI3Kβ inhibitors are based on the molecular structure of PI3Kβ or the modification and optimization of existing PI3K inhibitors via screening of chemical compounds with high selectivity and potency.

Although research on the role of PI3Kβ in thrombosis is still in the very early stages, PI3Kβ which induces platelet activation in response to the arterial shear stress is an ideal target. Antithrombotic therapies targeting PI3Kβ can selectively inhibit shear activation of pathological thrombi without inducing harmful bleeding complications. Therapies targeting PI3K have potential value for antithrombotic treatment.

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Disclosure of conflict of interest

None.

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