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Phosphatidylinositol 3-monophosphate: A novel actor in thrombopoiesis and thrombosis

Colin Valet PhD[1] | Marie Levade PhD[1] | Marie Bellio[1] | Manuella Caux[1] | Bernard Payrastre PhD[1,2] | Sonia Severin PhD[1]

[1]Inserm U1048 and Paul Sabatier University, Institute of Cardiovascular and Metabolic Diseases, Toulouse, France

[2]Hematology Laboratory, Toulouse University Hospital, Toulouse, France

Correspondence
Sonia Severin, Inserm U1048 and Toulouse 3 University, I2MC, 31432 Toulouse Cedex 4, France.
Email: sonia.severin@inserm.fr

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Abstract

Phosphoinositides are lipid second messengers regulating in time and place the formation of protein complexes involved in the control of intracellular signaling, vesicular trafficking, and cytoskeleton/membrane dynamics. One of these lipids, phosphatidylinositol 3 monophosphate (PtdIns3P), is present in small amounts in mammalian cells and is involved in the control of endocytic/endosomal trafficking and in autophagy. Its metabolism is finely regulated by specific kinases and phosphatases including class II phosphoinositide 3-kinases (PI3KC2s) and the class III PI3K, Vps34. Recently, PtdIns3P has emerged as an important regulator of megakaryocyte/platelet structure and functions. Here, we summarize the current knowledge in the role of different pools of PtdIns3P regulated by class II and III PI3Ks in platelet production and thrombosis. Potential new antithrombotic therapeutic perspectives based on the use of inhibitors targeting specifically PtdIns3P-metabolizing enzymes will also be discussed. Finally, we provide report of new research in this area presented at the International Society of Thrombosis and Haemostasis 2019 Annual Congress.

KEYWORDS

megakaryocytes, phosphatidylinositol 3 monophosphate, phosphoinositide 3-kinases, platelets, thrombosis

Essentials

- The housekeeping pool of phosphatidylinositol 3 monophosphate (PtdIns3P) regulated by phosphoinositide 3-kinase α controls platelet membrane structure and remodeling.
- The Vps34-dependent PtdIns3P pool controls granule biogenesis and platelet production in megakaryocytes.
- The Vps34-dependent inducible pool of PtdIns3P regulates platelet secretion and thrombus growth under shear stress.
- Are PtdIns3P metabolizing enzymes potential targets in cardiovascular diseases?

1 | INTRODUCTION

Phosphoinositides (PIs) are glycerophospholipids composed of 2 fatty acids (predominantly but not exclusively stearic acid [C18:0] in position 1 and arachidonic acid [C20:4] in position 2) attached to a glycerol backbone linked to an inositol ring that can be phosphorylated at the position 3, 4 and/or 5 (as exemplified in Figure 1A for phosphatidylinositol 3 monophosphate [PtdIns3P]). These inositol lipids are involved in the spatiotemporal regulation of signaling pathways, membrane/cytoskeleton remodeling, and intracellular trafficking by finely and reversibly organizing protein complexes at appropriate location and time through interaction with specific protein domains (as PH, PX, or FYVE domains).[1–3] Their metabolism is finely tuned by kinases, phosphatases, and phospholipases that can rapidly synthesize and degrade the different PIs in discrete membrane domains. Importantly, mutations of several of these enzymes are responsible for several human diseases,[3,4] showing a critical organismal role for PIs.

Among PIs, the D3-PIs are produced by phosphoinositide 3-kinases (PI3Ks) through the phosphorylation of the 3 position of the inositol ring of phosphatidylinositol (PtdIns), phosphatidylinositol 4 monophosphate (PtdIns4P) and phosphatidylinositol (4,5) bisphosphate (PtdIns(4,5)P_2) to generate, respectively, PtdIns3P, phosphatidylinositol (3,4) bisphosphate (PtdIns(3,4)P_2). and phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P_3). PtdIns(3,4,5)P_3 is the best characterized D3-PI in platelets. It is a typical lipid second messenger that is rapidly and transiently produced mainly by the isoform β of class I PI3K following platelet activation through G protein–coupled receptor– and immunoreceptor tyrosine-based activation motif/tyrosine kinase–dependent pathways. PtdIns(3,4,5)P_3 plays a pivotal role in platelet signaling, by recruiting and activating PH domain–containing proteins including the well-known protein kinase Akt as well as Btk; the cytohesins 1, 2, and 3; the Ras GTPase-activating protein (RASA) 2 and 3, dual adapter for phosphotyrosine and 3-phosphotyrosine and 3-phosphoinositide (DAPP1) as well as Arf6 guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs).[5] The transient PtdIns(3,4,5)P_3 production is followed by an accumulation of PtdIns(3,4)P_2 largely due to the action of the 5-phosphatase Src homology 2 domain containing inositol polyphosphate 5-phosphatase 1 (SHIP1) on PtdIns(3,4,5)P_3 that is dependent of the $\alpha_{IIb}\beta_3$ integrin engagement [6,7]. PtdIns(3,4)P_2 interactome has been recently analyzed in platelets by a proteomic approach, which identified, in addition to known interactors such as Akt, novel PtdIns(3,4)P_2 effectors including the pleckstrin homology domain–containing family A member 1 and 2 (also known as TAPP1 and 2), Arf-GAP with dual PH domain-containing protein 1, DAPP1, myotubularin-related protein 5, RASA3 and GRB2-associated binding protein 3. Through interacting with a wide range of proteins, PtdIns(3,4)P_2 is involved in platelet signaling, protein trafficking, and cytoskeleton organization.[8] Here, we review the role, regulation, and effectors of PtdIns3P in platelets and megakaryocytes (MKs), as this lipid has recently been the focus of several laboratories.

2 | GENERAL FUNCTION OF PTDINS3P

PtdIns3P is present at low levels in eukaryotic cells, mostly generated by phosphorylation of PtdIns in position 3 of the inositol ring by class II and class III PI3Ks. Of note, PtdIns3P can also be produced through dephosphorylation of PtdIns(3,4)P_2 by 4-phosphatases (such as INPP4) and of PtdIns(3,5)P_2 by a 5-phosphatase called FIG4 (or Sac3). Inversely, phosphatidylinositol 3 monophosphate 5-kinase, MTMs, and type II phosphatidylinositol 3 monophosphate 4-kinase metabolize PtdIns3P (Figure 1B). PtdIns3P is mainly located in intracellular compartments, particularly in early endosomes and in autophagosomes.[9,10] PtdIns3P has also been described at the inner or the outer leaflet of the plasma membrane.[11,12] At specific cell locations, through the recruitment of effectors bearing the PtdIns3P-interacting domains (FYVE or PX domains), PtdIns3P is involved in several cell processes. The first main role concerns the regulation of endosomal trafficking: (1) endosomal membrane dynamics and protein sorting through early endosome antigen 1 (EEA1) and hepatocyte growth factor–regulated tyrosine kinase substrate (HRS) for instance, (2) vesicle recycling to the plasma membrane and to the trans-Golgi via the sorting nexin protein family, and (3) vesicular sorting to lysosomal compartments. The second main role is in the initiation of autophagy (a catabolic process that involves

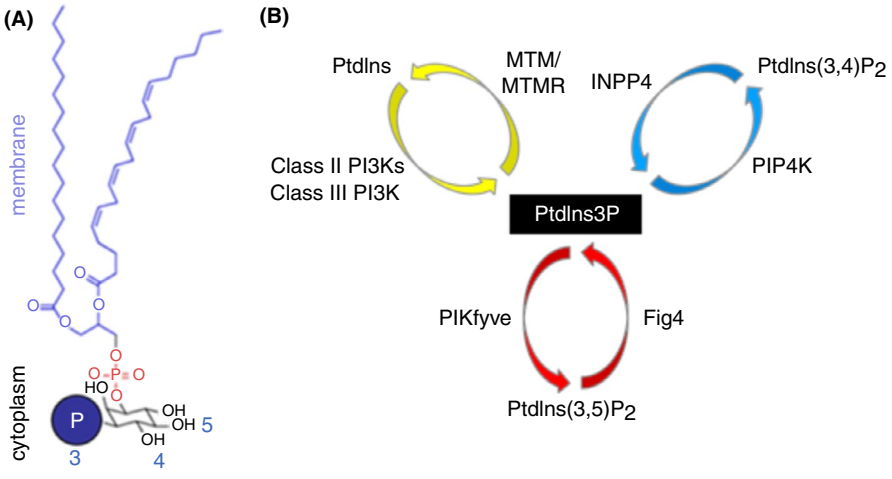

FIGURE 1 Illustration of PtdIns3P structure (A) and metabolism (B). FIG4: Phosphatidylinositol 3,5-bisphosphate 5-phosphatase; INPP4: Inositol polyphosphate 4-phosphatase type I; MTM: myotubularin; MTMR: myotubularin-related phosphoinositide phosphatase; PI3K: phosphoinositide 3-kinase; PIKfyve: phosphatidylinositol 3 monophosphate 5-kinase; PIP4K: phosphatidylinositol 3 monophosphate 4-kinase

cytoplasmic component degradation by lysosomes) by recruiting double FYVE–containing protein 1 and WD-repeat protein interacting with phosphoinositide proteins.[9,10,13] PtdIns3P is also involved in insulin and glucose transporter 4 exocytosis, in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation by recruiting one of its component (p40phox) and in cytokinesis as it recruits the protein FYVE-HUNDRED to cytokinetic bridges (for the final stage of cell division).[14–16] A mass spectrometry proteomic study in colon cancer cells allowed to identify a total of 681 proteins able to directly or indirectly interact with PtdIns3P, with only 69 proteins bearing the known PtdIns3P-binding domains.[17] Table 1 illustrates putative PtdIns3P-binding proteins expressed in MKs and platelets.

Quantifying changes in PtdIns3P levels in mammalian cells remains challenging, as its cellular level is low. Different approaches have been developed to quantify the whole amount of PtdIns3P in cells, including a [^{32}Pi] or [^{3}H]-*myo*-inositol metabolic labeling followed by high-performance liquid chromatography analysis[18] and a specific PtdIns3P mass assay.[19,20] The spatiotemporal organization of PtdIns3P pools in different cell compartments have been largely studied by imaging approaches using protein domains that specifically bind PtdIns3P, such as the 2xFYVE or the 2xPX domains. These domains can either be used as fluorescent-tagged recombinant proteins on fixed and permeabilized cells or be overexpressed as fluorescent-tagged fusion proteins in live cells. However, caution must be taken, as overexpressing these PI-binding domains blocks the endogenous effect of PtdIns3P by competing with target proteins and interfering with downstream signaling. Another approach is using antibodies specifically directed against PtdIns3P on fixed and permeabilized cells, under cautioned fixation and permeabilization procedures knowing that lipids cannot be fixed.[21] It is now accepted that the maintenance of a basal housekeeping pool of PtdIns3P is essential for cellular homeostasis, particularly in the control of intracellular trafficking. A massive increased level of this lipid has been described in cells infected by the pathogen *Plasmodium falciparum*.[22] Modest increases have been reported in mammalian cells following insulin[11,23] or lysophosphatidic acid stimulation.[24] In platelets, PtdIns3P basal level (1%-2% of PtdIns4P) increases by 1.5- to 2.5-fold following thrombin or collagen-related peptide (CRP) stimulation.[19,25–27] The mechanisms by which PtdIns3P-metabolizing enzymes regulate the spatiotemporal regulation of PtdIns3P in platelets start to emerge.

TABLE 1 A nonexhaustive list of potential PtdIns3P-binding proteins present in platelets

PtdIns3P-binding proteins	PtdIns3P-binding domain
Early endosome antigen 1	FYVE
Phosphatidylinositol 3 monophosphate 4-kinase	
Synaptotagmin-like protein 4	
Hepatocyte growth factor–regulated tyrosine kinase substrate	
FYVE and coiled-coil domain-containing protein 1	
RUN and FYVE domain-containing protein 1	
WD repeat and FYVE domain-containing protein 1	
Pleckstrin homology domain-containing family F member 2	
FYVE RhoGEF and PH domain–containing protein 3	
Sorting-nexin proteins 1, 2, 3, 5, 6, 12	PX
Class II PI3K C2 α and β	
Phospholipases D1 and D2	
Nicotinamide adenine dinucleotide phosphate oxidase 1	
Talins 1 and 2	Unknown domain
Spectrin	
Filamin	
Myosin-9	
Dynamin-2	
Glycogen synthase kinase 3β	
Neural Wiskott-Aldrich syndrome protein	
Focal adhesion kinase	

3 | PI3KC2α-DEPENDENT PTDINS3P POOL IS ESSENTIAL FOR MEMBRANE MORPHOLOGY AND DYNAMICS IN MEGAKARYOCYTES AND PLATELETS

In 1998, a study from Zhang et al[27] started to suggest a potential role for class II PI3Ks in platelets. It was just recently, with the development of several mouse models invalidated for the different isoforms of class II PI3Ks, that J. Hamilton laboratory and our group highlighted the involvement of this class of PI3Ks in MKs and platelets.[25,28] Class II PI3K family is composed of 3 isoforms in humans: PI3KC2α, C2β, and C2γ. PI3KC2α and PI3KC2β are ubiquitously expressed, whereas PI3KC2γ is mainly present in exocrine glands. These enzymes are able to phosphorylate both PtdIns and PtdIns4P at the position 3 of the inositol ring to respectively produce PtdIns3P and PtdIns(3,4)P_2. Class II PI3Ks have 2 characteristic C2 domains, a catalytic domain, a Ras-binding domain, and a specific phosphoinositide-binding PX domain. Recent reviews detailed the broad actions of PI3KC2α and PI3KC2β in several cellular functions with their major implications in vesicular trafficking.[29,30]

The role of PI3KC2α in MKs and platelets has only emerged in the past few years with the generation of mouse models displaying a deletion or a partial inactivation of the protein. The [^{32}Pi] metabolic labeling showed that neither PI3KC2α nor PI3KC2β has a major role in PtdIns(3,4)P_2 production in platelets, contrary to what has been shown in the regulation of endocytosis and mammalian target of rapamycin complex 1.[31,32] This is not surprising, as in platelets a large part of PtdIns(3,4)P_2 comes from the metabolization of PtdIns(3,4,5)P_3 by the 5-phosphatase SHIP1.[7] However, a PtdIns3P mass assay highlighted that PI3KC2α controls the level of PtdIns3P in platelets, particularly the housekeeping pool of PtdIns3P, but is not involved

in the regulation of the agonist-inducible pool.[25] In other cell types, the pool of PtdIns3P generated by PI3KC2α has also been recently reported in the context of Rab11 regulation and primary cilium function.[33,34] Interestingly, invalidating PI3KC2α in mice highlighted an unsuspected role for PI3KC2α in platelet membrane morphology and remodeling with consequences on platelet thrombotic capacities. Despite a normal platelet count, platelets from mice deleted or partially inactivated for PI3KC2α exhibit an enlarged open canalicular system (OCS) and a tortuous/invaginated plasma membrane. Structural membrane alterations were also observed in PI3KC2α-invalidated MKs. These defects in platelet ultrastructure are associated with abnormal membrane biophysical properties with a reduced elasticity, shown by atomic force microscopy, and defective dynamics, highlighted by a decreased tether and filopodia formation.[25,28,35] This platelet membrane phenotype was not due to a modification of membrane lipid composition, as shown by sensitive lipidomic methods coupling liquid chromatography and mass spectrometry, or to an affected signaling downstream of major platelet receptors.[25,35] Interestingly, we showed in platelets with a partial inactivation of PI3KC2α that the recruitment and/or stabilization of several proteins of the membrane skeleton (including spectrin and myosin) as well as proteins linking this membrane skeleton to the plasma membrane (eg, glycoprotein [GP] Ib, GPIIb, filamin, and moesin) are affected. These data strongly suggest that the PI3KC2α-dependent pool of PtdIns3P plays an important role in the integrity of platelet membrane skeleton.[25] The spectrin-based membrane skeleton is critical for platelet membrane properties and has a strong influence on membrane protein composition and structure.[36,37] Overall, these outcomes show an involvement of PI3KC2α and its housekeeping PtdIns3P pool in platelet membrane structure/remodeling by regulating the integrity of the membrane skeleton (Figure 2). A significant enrichment of the so-called barbell-shaped proplatelets, an intermediate stage of platelet production hardly detectable in the circulation (<1% of total platelets) in normal situations,[38] was detected in the bloodstream of mice displaying a partial inactivation of PI3KC2α.[25] This is consistent with the role of PI3KC2α in membrane skeleton integrity as membrane skeleton plays a crucial role in barbell-shaped proplatelet division to produce 2 mature platelets[37,38] (Figure 2). How this PI3KC2α-dependent housekeeping pool of PtdIns3P can regulate membrane skeleton integrity remains to be investigated. It is thus tempting to speculate that this pool of PtdIns3P is involved in the recruitment of membrane skeleton-containing proteins by directly interacting with PtdIns3P, as shown, for example, for spectrin, myosin, and filamin.[17] Also, PtdIns3P is known to regulate endosomal trafficking, which may indirectly contribute to the adequate localization of membrane skeleton proteins. This strongly instigates further studies for a better understanding of the link between membrane skeleton and the housekeeping pool of PtdIns3P in platelets. Platelets displaying a decreased PI3KC2α activity or protein expression, leading to an abnormal membrane morphology and remodeling, exhibit dysfunctions in their activation. Mountford et al[28] have shown the formation of large and unstable thrombi in the absence of platelet PI3KC2α in vivo with no major impact on the tail bleeding time. In our kinase-inactivated PI3KC2α mouse model, we observed a decreased thrombus growth by using ex vivo whole blood microfluidic assays and a delayed arterial thrombus formation in vivo without affecting the tail bleeding time[25] (Figure 2). These discrepancies may be explained by the use of different mouse models: a total deletion of the protein in contrast to a partial protein inactivation. In the latter case, the model mimics the use of an inhibitor and may limit the emergence of compensatory mechanisms by other PI3Ks that could happen in deficient mouse models. Also, the kinase-inactivated

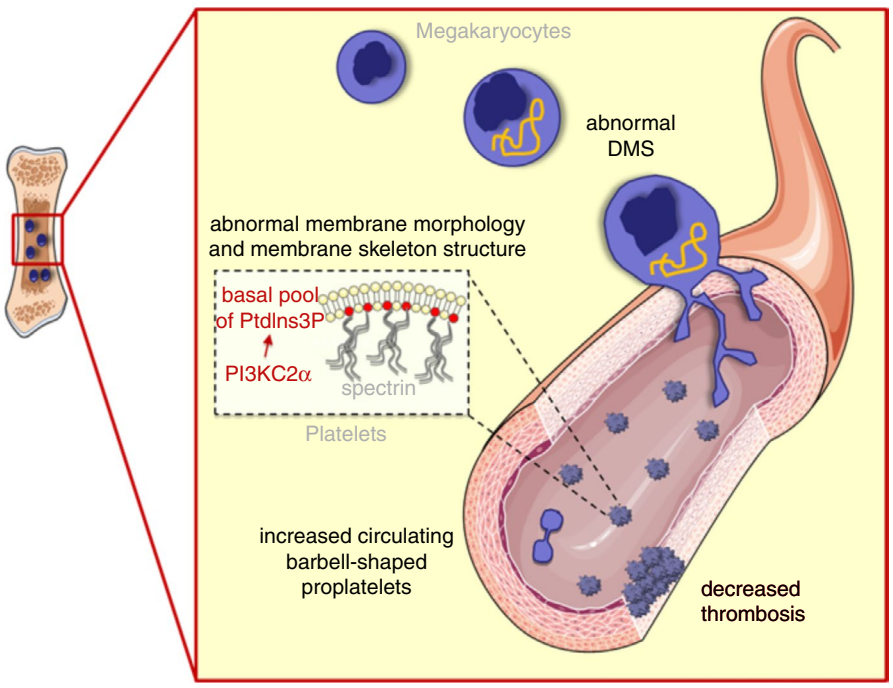

FIGURE 2 The class II PI3K PI3KC2α and its housekeeping pool of phosphatidylinositol 3 monophosphate (PtdIns3P) in megakaryocyte and platelet membrane morphology and thrombosis. Class II PI3K PI3KC2α, by regulating a basal pool of PI3P and organizing the spectrin-rich membrane skeleton, is important for maintaining normal platelet membrane morphology/remodeling that is important for platelet thrombotic capacities under shear stress conditions

PI3KC2α mouse model spares the loss of a potential scaffolding role of the PI3KC2α. Overall, these 2 recent studies show that by disturbing the PI3KC2α-dependent housekeeping PtdIns3P pool, platelet thrombotic functions are affected.

Consistent with the fact that PtdIns3P and PtdIns(3,4)P_2 production in basal or stimulated platelets appears normal when PI3KC2β is invalidated in platelets, PI3KC2β-deficient mice have a normal platelet count, no obvious morphological and functional platelet defects, and a normal bleeding time.[28] J. Hamilton laboratory has recently shown that PI3KC2α and PI3KC2β have nonredundant roles in platelets, as none of these isoforms are able to compensate the role of each other. In the context of double deficiency of class II PI3Ks, Vps34 expression increases and might be able to partly compensate the combined deficiency of class II PI3Ks.[39]

4 | VPS34-DEPENDENT PTDINS3P POOL IS MAJOR FOR PLATELET PRODUCTION AND GRANULE BIOGENESIS IN MEGAKARYOCYTES AND FOR PLATELET ACTIVATION

Vps34, the single isoform of class III PI3K, is the most ancient form of PI3Ks conserved from yeast to human. Ubiquitously expressed, Vps34 is composed of a C2 domain, a helical domain, and a kinase domain. Its regulatory subunit, Vps15, also known as PIK3R4, contains an N-terminal myristoylated kinase–like domain, a helical domain containing a series of internal huntingtin, elongation factor 3, the PR65/A subunit of protein phosphatase 2A, and target of rapamycin repeats and a C-terminal WD40 domain that forms a β-propeller structure. Vps34 is part of 2 tetrameric complexes that share a core composed of Vps34, Vps15, and Beclin 1 associated to either ATG14 for complex I or ultraviolet radiation–associated gene protein for complex II.[40] By producing different PtdIns3P pools from PtdIns, Vps34 is mainly involved in endosomal trafficking[40,41] and in autophagy.[13]

The role of Vps34 was only recently described in MKs and platelets by using newly developed specific inhibitors (IN1 and SAR406)[42,43] as well as mouse models targeting Vps34 specifically in the MK/platelet lineage.[26,44] In MKs, Vps34 contributes to 40% of the PtdIns3P production and is essential for the production of functional platelets. Decreased Vps34-dependent PtdIns3P pool in MKs results in an abnormal endocytic/endosomal trafficking with an accumulation of large early endosomes responsible for a defective late endosome/lysosome and recycling trafficking.[26] Platelet granules are formed in MKs through a still elusive intracellular trafficking and sorting process. α and dense granules are produced from vesicle budding originating from both the trans-Golgi network (endogenous secretory pathway) and the plasma membrane through early endosomes (endocytic pathway). These vesicles will either fuse with existing granules or be directed to multivesicular bodies, which are primordial sorting compartments for granule biogenesis.[45–47] In a very schematic way, vacuolar protein-sorting 33B (Vps33B)/16B (Vps16B) control α granule membrane biogenesis and Neurobeachin-like 2 (NBEAL2) regulates granule loading. Gray platelet syndrome derives from mutational loss of *NBEAL2* gene function, whereas arthrogryposis, renal dysfunction, and cholestasis (ARC) syndrome results from loss of function mutations of the *VPS33B* or *VPS16* genes. Other rare platelet syndromes are associated with granule formation defects, such as Paris-Trousseau syndrome, which originates from a friend leukemia integration 1 transcription factor (FLI1) mutation responsible for the presence of rare but very large α granules and the lack of dense granules in platelets.[46,48] Hermansky-Pudlak syndrome resulting from *BLOC*, *HPS*, and *AP3* gene mutations, and Chediak-Higashi syndrome from a mutation in the *LYST* gene are hereditary pathologies with platelet dense granule biogenesis defects.[45] In Vps34-deficient MKs, granule biogenesis is disturbed, which leads to fewer but larger α granules and less dense granules in Vps34-depleted platelets.[26] Vps34 appears to be a new actor involved in α and dense granule biogenesis. This is an important new information to take into account for a more comprehensive understanding of the molecular mechanisms involved in all steps of granule biology. In the bone marrow, MKs release platelets in the bloodstream after being able to reach the vascular niche. A defective directional migration capacity of Vps34-invalidated MKs leads to an ectopic and premature platelet release in the bone marrow and thus a decreased platelet count in the circulation.[26] As directed cell migration is a mechanism finely regulated by intracellular trafficking and includes the cell capacities to sense, polarize, and move toward the chemoattractant,[49] one can speculate that the defective vesicular trafficking in MKs displaying a decreased production of the Vps34-dependent PtdIns3P is responsible for the aberrant MK migration and the premature platelet release in the bone marrow outside the sinusoids (Figure 3). Further investigation is needed to precisely characterize the localization of the Vps34-dependent PtdIns3P pool in MKs and to decipher how it controls vesicular trafficking and granule biogenesis.

In platelets, the production of a stimulation-dependent PtdIns3P pool induced by GPVI agonists (CRP or convulxin) or a G protein–coupled receptor agonist (thrombin) is mainly controlled by Vps34 and is associated with a significant increase in Vps34 lipid-kinase activity.[28] Vps34 is weakly involved (around 10%) in the production of the housekeeping PtdIns3P pool in platelets, in contrast to what has been shown in several cell types.[26,44] The role of the Vps34-dependent inducible pool of PtdIns3P has been recently characterized in thrombosis and hemostasis by Liu et al[44] and our group.[26] Whereas primary hemostasis is not affected by Vps34 deletion in MKs and platelets, thrombotic capacities of Vps34-deficient platelets (analyzed ex vivo by perfusing whole blood under arterial shear rate through a collagen matrix or in vivo following carotid $FeCl_3$ lesion) were significantly affected[26,44] (Figure 3). It is noteworthy that this functional platelet defect when Vps34 is deleted is observed under shear stress conditions, whereas in in vitro stirring condition Vps34-deficient platelet aggregation is sparse following agonist stimulation. Mechanistically, a dysregulation of the spatiotemporal regulation of platelet secretion, a decreased NADPH oxidase –dependent reactive oxygen species generation and a dampened mammalian target of rapamycin signaling in Vps34-deficient platelets

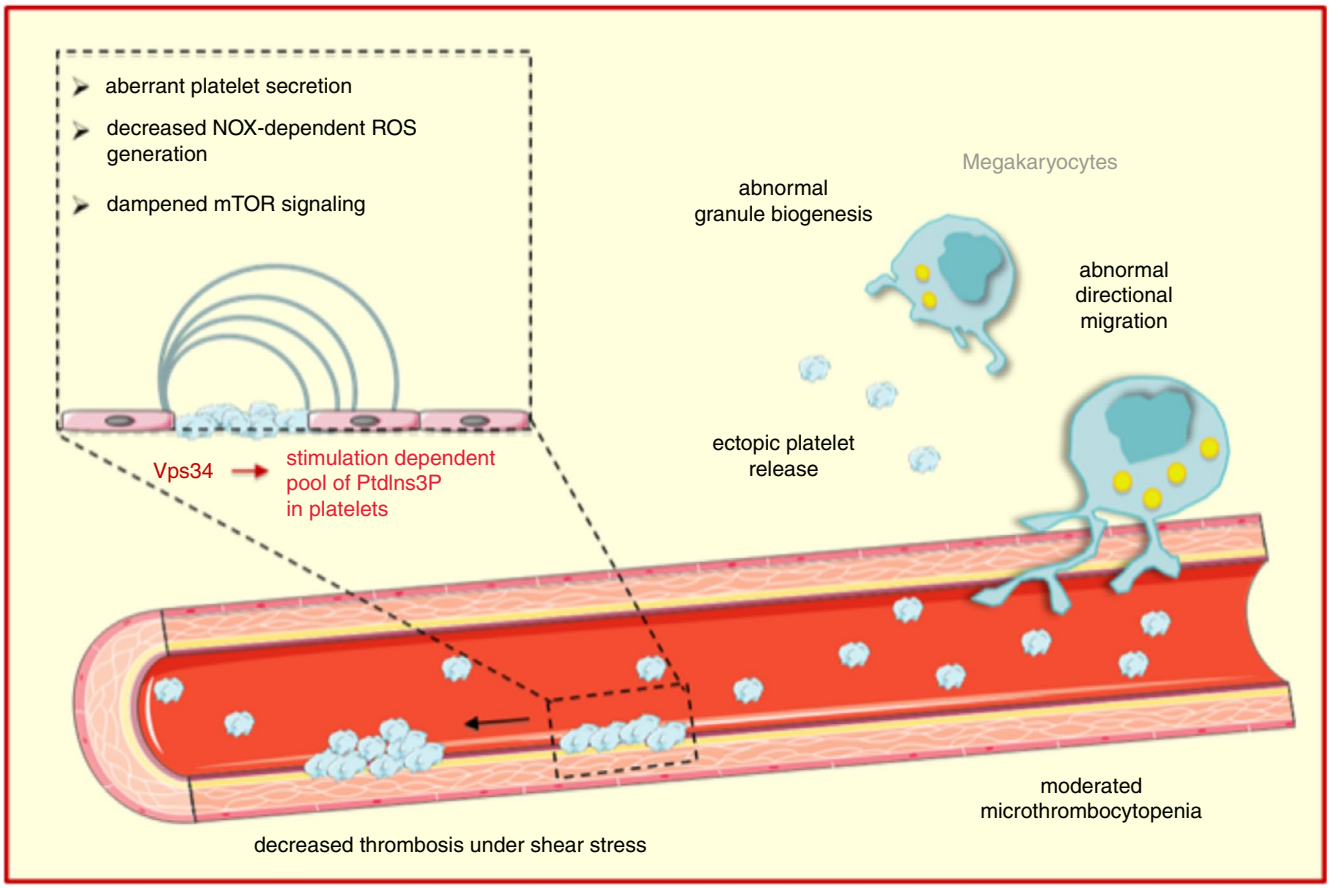

FIGURE 3 The class III PI3K Vps34 regulates specific pools of phosphatidylinositol 3 monophosphate (PtdIns3P) that have different implications in platelet production and activation. The class III PI3K Vps34, by regulating a specific pool of PtdIns3P that controls endocytic/endosomal trafficking, granule biogenesis, and directional migration in megakaryocytes, maintains normal platelet production (platelet granule content and circulating platelet count and size). In platelets, Vps34 regulates a stimulation-dependent pool of PtdIns3P involved in the control of arterial thrombus growth under shear stress by regulating platelet secretion, NADPH oxidase–dependent reactive oxygen species generation and mammalian target of rapamycin signaling

may be the causative factors of their defective thrombotic capacities.[26,44] Also, Liu et al[44] showed that the defect of Vps34-deficient platelets in thrombosis is independent of its role in autophagy. Further studies are now required to identify the different effectors of PtdIns3P involved in these processes.

5 | OTHERS POOLS OF PTDINS3P

Of note, wortmannin, known to inhibit all isoforms of class I PI3Ks but also the class III PI3K Vps34, abrogates PtdIns3P production following thrombin stimulation in platelets.[19] This may suggest that in addition to Vps34, class I PI3Ks may also contribute to the production of the inducible pool of PtdIns3P. Further investigation is needed to analyze this point.

No role for other PtdIns3P-producing enzymes such as the 4-phosphatases (INPP4) and 5-phosphatase (FIG4) has yet been described in MKs and platelets. Whether particular pools of PtdIns3P are regulated by these phosphatases in these cells remains to be investigated.

6 | CLASS II AND III PI3KS TARGETING: CLINICAL IMPLICATION AND APPLICATIONS IN HEMOSTASIS/THROMBOSIS

Unraveling that Vps34 and its PtdIns3P pool is important in granule biogenesis and secretion makes it possible to uncover novel molecular actors to implement our knowledge in this field. The inherited disorders of platelet granules (storage pool diseases [SPDs]) are a heterogeneous collection of rare bleeding disorders with symptoms ranging from mild to life-threatening conditions such as ARC syndrome, Hermansky-Pudlak syndrome, Chediak-Higashi syndrome, or gray platelet syndrome.[45,46,50] Despite advances in the understanding of the etiology of these granule defects (secretion and abnormalities of granules quantity or loading), the underlying molecular mechanisms still remain to be better characterized. Increasing our scarce knowledge in the mechanisms and molecular players underlying SPDs should help in the diagnosis of patients with bleeding disorders due to granule defects and put forward new therapeutic strategies. Bleeding disorders have also been observed in Budd-Chiari and May-Heggling syndromes, where platelets present OCS/

membrane abnormalities.[51] It is important to improve our knowledge in how PI3KC2α regulates membrane structure for a better understanding of the etiology of syndromes related to defective platelet OCS/membrane.

Metabolic disorders (including obesity and type 2 diabetes) are powerful and prevalent predictors of cardiovascular events, a leading cause of mortality and morbidity in industrialized countries. It is now well documented that platelet hyperreactivity plays a pivotal role in the pathogenesis of atherothrombosis in patients affected by metabolic disorders.[52,53] Novel strategies in the prevention/treatment of atherothrombosis in metabolic disorders are needed, as resistance to antiplatelet drugs is often observed in these diseases. Targeting PI3KC2s and Vps34 appears to be promising in metabolic diseases by modulating insulin sensitivity.[23,54–56] Even though it is still premature to propose PtdIns3P-metabolizing enzymes as potential new targets for the development of antithrombotic drugs in the pathogenesis of metabolic disorders, inhibiting these kinases might have a direct impact on metabolic organs per se and concomitantly on atherothrombosis by counteracting platelet activity without increasing the bleeding risk. The recent progress made in the development of specific class II and III PI3K inhibitors provides hope for novel therapeutic strategies in the context of metabolic syndromes.[42,43,57,58]

7 | CONCLUSION

This review highlights the importance of PtdIns3P metabolism in part regulated by class II and III PI3Ks in platelet production and functions. Emerging evidence shows that different pools of PtdIns3P regulated by specific enzymes play distinct roles. For instance, PI3KC2α regulates a basal housekeeping pool of PtdIns3P that controls platelet membrane structuration and remodeling. Vps34-dependent PtdIns3P pool controls MK vesicular trafficking and granule biogenesis. Vps34 is also involved in the control of platelet secretion and signaling. Many exciting questions remain on the localization and dynamics of the different pools of PtdIns3P as well as their specific effectors and roles in MKs and platelets. How PtdIns3P acts on membrane remodeling and platelet granule biology will be an important issue, knowing that several bleeding syndromes are associated to platelet granule or OCS/platelet membrane abnormalities.

8 | ISTH MELBOURNE REPORT

Moon and colleagues[59] presented an interesting perspective on targeting PI3KC2α as an antithrombotic therapy through the development of a first-generation PI3KC2α inhibitor. This inhibitor reproduces, in human platelets, the membrane structural effects observed in platelets from PI3KC2α-invalidated mouse models. Also, mice treated with this inhibitor were protected against in vivo induced thrombosis with no adverse effects on bleeding. Therefore, the generation of PI3KC2α inhibitors is of interest for their antithrombotic therapeutic benefit and appears to improve the safety of current antithrombotic therapies.

RELATIONSHIP DISCLOSURE

The authors have no conflicts of interest to report.

AUTHOR CONTRIBUTIONS

CV and SS wrote the manuscript. ML, MB, MC, and BP revised the manuscript.

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