Editorial

TRPing out Platelet Calcium

TRPM7 (Transient Receptor Potential Melastatin-Like 7) Modulates Calcium Mobilization and Platelet Function via Phospholipase C Interactions

Anh T.P. Ngo, Owen J.T. McCarty, Joseph E. Aslan

Originally identified as coagulation factor IV, calcium (Ca^{2+}) is now well established as a key cofactor in the formation of the tenase and prothrombinase complexes on the extracellular surfaces of activated platelets to ultimately mediate fibrin generation and hemostasis.[1] Similarly, Ca^{2+} has long been known to serve an important intracellular role in orchestrating the cell biological responses of platelets in hemostatic plug formation.[2–5] During the past several decades, as biochemical efforts have identified and refined roles for Ca^{2+} as an essential second messenger in virtually all cells,[3] complementary studies of platelets have similarly detailed how spatiotemporal changes in intracellular Ca^{2+} levels regulate platelet granule secretion, cytoskeletal dynamics, aggregation, and other cell biological outputs underlying platelet physiology. On a general mechanistic level, changes in intracellular Ca^{2+} concentrations that trigger the cellular responses driving platelet function are solicited downstream of a variety of receptors that differentially activate phospholipase C (PLC) family members, resulting in inositol-1,4,5-trisphosphate (IP_3) production and IP_3R (IP_3 receptor)-mediated release of Ca^{2+} from intracellular stores (Figure).[4,6–9]

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Given the critical importance of intracellular Ca^{2+} concentration to platelet function, diverse endeavors have aimed to understand and target the myriad of molecular processes regulating and driven by Ca^{2+} dynamics. In addition to mobilization from internal stores after IP_3 generation, extracellular Ca^{2+} also enters platelets via store- and receptor-operated calcium entry routes involving more recently described players such as Orai1, STIM1 (stromal interaction molecule 1), and transient receptor potential (TRP) family channels.[3] In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Gotru et al[10] now uncover a novel mechanism by which the TRP subfamily member TRPM7 (transient receptor potential melastatin-like 7) modulates PLC phosphorylation and intracellular calcium mobilization to effect store-operated calcium entry and platelet function. By taking advantage of a transgenic mouse model with a loss-of-function mutation in the cytosolic TRPM7 C-terminal serine/threonine kinase domain (*Trpm7*$^{R/R}$),[11] this study specifies a role for the TRPM7 kinase domain—rather than its constitutive Mg^{2+} and Ca^{2+} channel activity—in modulating the phosphorylation and activation of PLC family members and consequently intracellular calcium responses and platelet functional processes underlying both hemostasis and thrombosis (Figure).

Earlier work from Stritt et al[12] suggested a general role for TRPM7 in regulating platelet function through pathways controlling megakaryocyte and platelet cytoskeletal dynamics. In the current study, Gotru et al[10] now more specifically show altered signaling responses in *Trpm7*$^{R/R}$ platelets, including a delay in phosphorylation of Syk (spleen tyrosine kinase), LAT (linker for activation of T-cells), PLCγ2, and PKCε (protein kinase C) in response to the platelet GPVI (glycoprotein VI) receptor agonist collagen-related peptide. Similar effects are also found to be associated with rhodocytin→CLEC-2 (C-type lectin-like receptor 2)→PLCγ2 as well as thrombin→PAR (protease-activated receptor)→PLCβ3 signaling axes. To investigate the physiological consequences associated with these alterations in signaling kinetics in platelets, Gotru et al also analyze many platelet phenotypes and functional responses in *Trpm7*$^{R/R}$ mice, revealing defects in granule secretion, integrin activation, and platelet aggregation in vitro. In association with these altered platelet responses, *Trpm7*$^{R/R}$ mice display prolonged tail bleeding times and significantly less vessel occlusion in response to arteriole injury compared with wild-type counterparts, supporting roles for the TRPM7 kinase domain in modulating platelet function in vivo. Perhaps most remarkably, antithrombotic effects of mutating the TRPM7 kinase domain seem to be mediated through marrow-derived cells, especially platelets, because wild-type chimeric mice transplanted with bone marrow from *Trpm7*$^{R/R}$ mice, or thrombocytopenic wild-type mice transfused with platelets from *Trpm7*$^{R/R}$ animals, were similarly protected from infarct progression and showed overall improved neurological and motor function outcomes relative to matched controls after transient middle cerebral artery occlusion to model ischemic stroke.

Although Gotru et al have astutely elucidated platelet-associated roles for the TRPM7 kinase domain in complete and proper platelet function, many intriguing questions remain on how TRPM7 impacts platelet physiology in hemostasis and thrombosis. For instance, it is not clear how mutation of this serine/threonine kinase domain translates into a delay in the tyrosine phosphorylation of receptor-proximal components of platelet-signaling systems, especially as physiological substrates of TRPM7 are limited and have not yet

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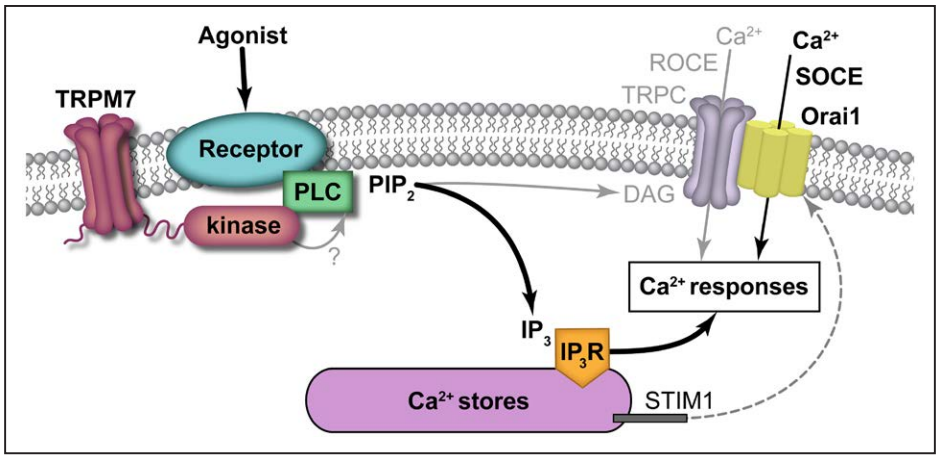

Figure. TRPM7 (transient receptor potential melastatin-like 7) kinase domain interacts with phospholipase C (PLC) family members to regulate intracellular Ca^{2+} responses and platelet function (model). Platelet activation downstream of GPVI (glycoprotein VI), CLEC-2 (C-type lectin-like receptor 2), PARs (protease-activated receptors), and other receptors results in intracellular signaling events that upregulate PLC (PLCγ2 and PLCβ3) phosphorylation and activity to drive the metabolism of phosphatidylinositol 4,5-bisphosphate (PIP_2) on the cytosolic face of the platelet plasma membrane to produce inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Rather than serving as a constitutively active Mg^{2+}/Ca^{2+} channel or regulating DAG-mediated receptor-operated calcium entry (ROCE) through TRP channels (TRPC) and other associated processes, this study supports a model whereby the TRPM7 kinase domain interacts with PLC family members to modulate PLC phosphorylation and activation, IP_3 production, intracellular Ca^{2+} mobilization, and STIM1-Orai1–mediated store-operated calcium entry (SOCE), ultimately modulating intracellular Ca^{2+} concentrations and associated Ca^{2+} responses underlying platelet function.[10] Illustration provided by Inky Mouse Studios ©2018—all rights reserved.

been demonstrated in platelets. Furthermore, these findings remain to be placed into the context of the former studies by Stritt et al,[12] which made use of megakaryocyte/platelet lineage-specific deletions of TRPM7 that resulted in altered cytoskeletal phenotypes. Along these lines, as platelets from *Trpm7*$^{R/R}$ mice exhibit delayed PKC phosphorylation kinetics, many other signaling processes around the platelet cytoskeleton may be disrupted, especially given the spatial and temporal link between intracellular calcium mobilization and PKC activation in regulating Rho GTPases and platelet function.[13,14] Finally, from a translational perspective, the rather unique TRPM7 kinase domain may represent a specialized therapeutic or biomarker target; however, validation of the relevance of TRPM7 within human platelet biology remains to be shown. Regardless, whether TRPM7 serves as a kinase, a molecular scaffold, an effector, or some combination thereof, Gotru et al provide a rich contribution to the ever-evolving and increasingly important understanding of mechanisms regulating intracellular Ca^{2+} dynamics in health and disease in platelet physiology and beyond.

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