Letter to the Editor

Letters to the Editor will be published, if suitable, as space permits. They should not exceed 1000 words (typed double-spaced) in length and may be subject to editing or abridgment.

Letter by Sonkar et al Regarding Article, "Class III PI3K Positively Regulates Platelet Activation and Thrombosis via PI(3)P-Directed Function of NADPH Oxidase"

To the Editor:

We read with great interest the recent article by Liu et al[1] on the role of phosphoinositide 3-kinase (PI3K) in platelet activation and thrombosis. Liu et al demonstrated that mice lacking platelet-specific VPS34, a PI3K isoform, exhibit diminished platelet activation and aggregation and are protected from accelerated thrombosis. This phenotype was associated with loss of translocation of the p40phox cytoplasmic subunit of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase to the plasma membrane, suggesting that VPS34 may regulate platelet reactive oxygen species (ROS) generation and subsequent platelet activation by mediating assembly of a functional NADPH oxidase. The authors did not directly measure platelet NADPH oxidase activity, however, which raises the possibility that VPS34 may influence platelet ROS generation and aggregation through a NADPH oxidase-independent pathway.

The role of NADPH oxidase in ROS-dependent platelet activation and aggregation is controversial.[2–4] Platelets are known to express NADPH oxidases,[1–8] which assemble from cytoplasmic subunits (p47phox, p67phox, p40phox, and Rac1/2) and membrane subunits (Nox2 and p22phox).[9] Several studies have demonstrated that apocynin, a nonspecific inhibitor of NADPH oxidase subunit assembly, prevents platelet ROS generation and aggregation,[6,10] and Delaney et al[4] recently reported that deficiency of Nox2 within platelets leads to decreased superoxide generation and aggregation. However, other studies have questioned the importance of NADPH oxidase in platelet ROS production, activation, and aggregation. For example, Dharmarajah et al[2] observed that platelets isolated from mice lacking NADPH oxidase subunits such as Nox2 or p47Phox exhibited aggregation responses to thrombin and collagen that were similar to platelets from wild-type mice. Additionally, they showed that apocynin-inhibited platelet aggregation to a similar extent in platelets from wild-type and NADPH oxidase–deficient mice.[2] Similar results were obtained by Walsh et al,[3] who found that platelets from Nox2-deficient mice were not impaired in platelet ROS generation, activation, or aggregation responses. The explanation for these discrepant findings in murine models is not yet understood, but it is noteworthy that platelets from human patients with chronic granulomatous disease (a genetic disorder characterized by deficiency of Nox2) also exhibit normal aggregation responses.[11,12] In light of these observations, it is possible that loss of VPS34 affects platelet ROS generation and aggregation responses through pathways that are independent from NADPH oxidase assembly.

As noted by Liu et al, both VPS34 and NADPH oxidases may represent attractive targets for antithrombotic therapeutics. Therefore, it will be important in future studies to define the specific downstream pathways of VPS34 signaling leading to platelet activation, perhaps by using genetic or pharmacological approaches to inhibit VPS34 in platelets from mice deficient in Nox2 and other NADPH oxidase subunits.

Sources of Funding

This study was supported in part by National Institutes of Health grants AG049784 to S. Dayal.

Disclosures

None.

Vijay K. Sonkar
Steven R. Lentz
Sanjana Dayal
Department of Internal Medicine
University of Iowa Carver College of Medicine
Iowa City

References

1. Liu Y, Hu M, Luo D, Yue M, Wang S, Chen X, Zhou Y, Wang Y, Cai Y, Hu X, Ke Y, Yang Z, Hu H. Class III PI3K positively regulates platelet activation and thrombosis via PI(3)P-directed function of NADPH oxidase. *Arterioscler Thromb Vasc Biol*. 2017;37:2075–2086. doi: 10.1161/ATVBAHA.117.309751.
2. Dharmarajah J, Arthur JF, Sobey CG, Drummond GR. The anti-platelet effects of apocynin in mice are not mediated by inhibition of NADPH oxidase activity. *Naunyn Schmiedebergs Arch Pharmacol*. 2010;382:377–384. doi: 10.1007/s00210-010-0552-3.
3. Walsh TG, Berndt MC, Carrim N, Cowman J, Kenny D, Metharom P. The role of Nox1 and Nox2 in GPVI-dependent platelet activation and thrombus formation. *Redox Biol*. 2014;2:178–186. doi: 10.1016/j.redox.2013.12.023.
4. Delaney MK, Kim K, Estevez B, Xu Z, Stojanovic-Terpo A, Shen B, Ushio-Fukai M, Cho J, Du X. Differential roles of the NADPH-oxidase 1 and 2 in platelet activation and thrombosis. *Arterioscler Thromb Vasc Biol*. 2016;36:846–854. doi: 10.1161/ATVBAHA.116.307308.
5. Seno T, Inoue N, Gao D, Okuda M, Sumi Y, Matsui K, Yamada S, Hirata KI, Kawashima S, Tawa R, Imajoh-Ohmi S, Sakurai H, Yokoyama M. Involvement of NADH/NADPH oxidase in human platelet ROS production. *Thromb Res*. 2001;103:399–409.
6. Chlopicki S, Olszanecki R, Janiszewski M, Laurindo FR, Panz T, Miedzobrodzki J. Functional role of NADPH oxidase in activation of platelets. *Antioxid Redox Signal*. 2004;6:691–698. doi: 10.1089/1523086041361640.
7. Dayal S, Wilson KM, Motto DG, Miller FJ Jr, Chauhan AK, Lentz SR. Hydrogen peroxide promotes aging-related platelet hyperactivation and thrombosis. *Circulation*. 2013;127:1308–1316. doi: 10.1161/CIRCULATIONAHA.112.000966.
8. McCarty OJ, Larson MK, Auger JM, Kalia N, Atkinson BT, Pearce AC, Ruf S, Henderson RB, Tybulewicz VL, Machesky LM, Watson SP. Rac1 is essential for platelet lamellipodia formation and aggregate stability under flow. *J Biol Chem*. 2005;280:39474–39484. doi: 10.1074/jbc.M504672200.
9. Lassègue B, Griendling KK. NADPH oxidases: functions and pathologies in the vasculature. *Arterioscler Thromb Vasc Biol*. 2010;30:653–661. doi: 10.1161/ATVBAHA.108.181610.
10. Begonja AJ, Gambaryan S, Geiger J, Aktas B, Pozgajova M, Nieswandt B, Walter U. Platelet NAD(P)H-oxidase-generated ROS production regulates alphaIIbbeta3-integrin activation independent of the NO/cGMP pathway. *Blood*. 2005;106:2757–2760. doi: 10.1182/blood-2005-03-1047.
11. Pignatelli P, Carnevale R, Di Santo S, Bartimoccia S, Sanguigni V, Lenti L, Finocchi A, Mendolicchio L, Soresina AR, Plebani A, Violi F. Inherited human gp91phox deficiency is associated with impaired isoprostane formation and platelet dysfunction. *Arterioscler Thromb Vasc Biol*. 2011;31:423–434. doi: 10.1161/ATVBAHA.110.217885.
12. Pignatelli P, Sanguigni V, Lenti L, Ferro D, Finocchi A, Rossi P, Violi F. gp91phox-dependent expression of platelet CD40 ligand. *Circulation*. 2004;110:1326–1329. doi: 10.1161/01.CIR.0000134963.77201.55.

(*Arterioscler Thromb Vasc Biol*. 2018;38:e25. DOI: 10.1161/ATVBAHA.117.310686.)

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Letter by Sonkar et al Regarding Article, "Class III PI3K Positively Regulates Platelet Activation and Thrombosis via PI(3)P-Directed Function of NADPH Oxidase"

Vijay K. Sonkar, Steven R. Lentz and Sanjana Dayal

Arterioscler Thromb Vasc Biol. 2018;38:e25
doi: 10.1161/ATVBAHA.117.310686
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636