Two opposite therapeutic redox-modulating strategies for the treatment of CLL. (A) Using antioxidant to neutralize ROS, CLL cells produce and release ROS into the microenvironment and ROS are dangerous to T cells. Antioxidant can neutralize ROS and prevent ROS-mediated damage to T cells. (B) Rapid induction of ROS. Blocking the mETC causes rapid ROS production by mitochondria and this could induce apoptotic cell death of CLL cells.

our previous finding that leukemic cells meet high energy demand by increasing mETC activity, rather than increasing aerobic glycolysis.[6] The superoxide radical is generated by mitochondrial complex I and III as an incompletely reduced by-product. ROS production is often induced when mETC is uncoupled with ATP synthesis.[7] Interestingly, this study found that the mitochondria in CLL cells are in a perfectly coupled condition.

To connect ROS production and mitochondrial biogenesis, Jitschin et al manipulated redox status by treating CLL cells with antioxidants NAC or MitoQ and found that expression of both TFAM and HO-1 was reduced after treatment with an antioxidant. Interestingly, inhibition of HO-1 by SnPP significantly reduced TFAM expression. Therefore, the authors drew an indirect interconnection between ROS production, cellular adaption to ROS, and mitochondrial biogenesis. Finally, PK11195, a benzodiazepine derivate inhibitor for F1F0-ATPase, the complex V of mETC, was used to block oxidative phosphorylation. PK11195-mediated overproduction of superoxide selectively induced apoptosis in CLL B cells, leaving T cells and normal B cells unaffected.

Therefore, the authors propose 2 opposite therapeutic redox-manipulating strategies for

CLL (see figure). First, treatment of CLL cells using antioxidants could neutralize endogenous ROS and protect the immune system. Second, induction of ROS overproduction by targeting mETC could selectively kill CLL cells. Thus, mitochondria, the source of dangerous power in CLL, need to be controlled in favor of the immune system and can be used as a killer within the CLL cells. This study improves our understanding that leukemic cells circulating in a normoxic condition rely on mitochondrial oxidative phosphorylation for energy supply. This is distinct from solid tumor cells which

grow in the hypoxia condition with dysfunctional mitochondria and therefore mainly rely on anaerobic glycolysis. Tumor cells produce higher levels of ROS by dysfunctional mitochondria. The causes of ROS overproduction by CLL mitochondria pose challenging questions. It is not yet clear how increased ROS levels are generated by functional coupled mETC and whether this is merely due to increased mitochondrial biogenesis in CLL cells.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

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● ● ● **PHAGOCYTES, GRANULOCYTES, & MYELOPOIESIS**

Comment on Starnes et al, page 2703

# Podosomes are disrupted in PAPA syndrome

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In this issue of *Blood*, Starnes et al describe a novel proline-serine-threonine phosphatase-interacting protein 1 (PSTPIP1) mutation that is responsible for pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome. This helps us understand how PSTPIP1 regulates podosome and filopodia formation and migration of macrophages.[1]

PAPA syndrome, first described in 1997, is a rare autosomal dominant disease in which patients show a combination of severe arthritis, skin ulcerations, and acne from an early age. All the lesions are sterile because of excessive autoinflammation involving leukocytes. Arthritis usually begins during early childhood after minor trauma or sporadically. In young adults, joint symptoms tend to decrease, and cutaneous symptoms become more active.[2]

PAPA syndrome is the result of mutations in the *PSTPIP1* gene. PSTPIP1 is an adaptor protein highly expressed in hematopoietic cells that links the plasma membrane to the cytoskeleton. It contains both an F-BAR domain, which has been shown to bind the protein-tyrosine phosphatase (PTP) peptide sequence rich in proline (P), glutamic acid (E), serine (S), and threonine (T) (PTP-PEST), a partner of various cytoskeleton and cell adhesion molecules, and an SRC homology 3 (SH3) domain, which interacts with several proteins, such as the inflammasome regulator pyrin, the tyrosine kinase c-Abl and, critically for the Starnes et al study,

with the hematopoietic lineage-restricted Wiskott-Aldrich syndrome protein (WASP) (see figure). WASP is an activator of the actin nucleating complex actin-related protein (Arp)-2 and Arp3, and the scaffold activity of PSTPIP1 makes it possible for PTP-PEST to dephosphorylate WASP.[2]

The PAPA mutations originally identified in *PSTPIP1*, A230T and E250Q, were located in the F-BAR domain. They abolish the interaction of PSTPIP1 with PTP-PEST, leading to hyperphosphorylation of PSTPIP1, which was proposed to cause activation of the inflammasome and release of interleukin-1β (IL-1β).[2] A previous study from Cortesio et al[3] reported that, in patients with those mutations, there was an apparent defect in macrophage migration. This involved defective macrophage chemotaxis to macrophage colony-stimulating factor, an impaired migration in Matrigel invasion chambers, a decreased ability to degrade the extracellular matrix (ECM) protein gelatin, and a significant defect in podosome formation.[3]

Podosomes are F-actin–rich cone structures surrounded by a ring of adhesion

proteins and proteins linking integrins to the cytoskeleton[4] that degrade the ECM. They form at the ventral membrane of only a few cell types (macrophages, immature dendritic cells, and osteoclasts). Altered formation of podosomes has been shown to translate into impaired cell adhesion and ECM degradation, defective chemotaxis, and reduced migration in three-dimensional (3D) environments in vitro and in vivo.[4,5]

In this issue of *Blood*, a novel mutation in the *PSTPIP1* gene is described in a patient with PAPA syndrome.[1] The R405C mutation is the first mutation described in the SH3 domain of PSTPIP1. It is distant from the binding domain of PTP-PEST, which normally interacts with PSTPIP1. The patient's macrophages were found to have very few F-actin dots that did not colocalize with vinculin, a hallmark of podosomes. Instead, they showed prominent filopodia and a highly active matrix-degrading activity, and they were more chemotactic and invasive compared with control cells.

The functional consequences of that mutation were studied in PSTPIP1-depleted THP-1 macrophages expressing the mutant protein. Like the patient's cells, THP-1 cells expressing PSTPIP1-R405C also exhibited disrupted podosomes and prominent formation of filopodia. Interestingly, WASP activity was enhanced in these cells, which correlated with an impaired interaction of WASP with PSTPIP1-R405C. Thus PSTPIP1-R405C causes WASP relocation away from PTP-PEST. However, WASP hyperphosphorylation could not be detected, suggesting additional regulation mechanisms.

WASP-deficient macrophages do not form podosomes, but constitutively active WASP leads to podosome disassembly.[4] The phosphomimetic active form of WASP has varying degrees of impact on podosome formation, depending on the cell type studied.[6,7] Introduction of constitutively active CDC42, an activator of WASP, triggers podosome disruption and filopodia formation.[4] Taken together, those studies[4,6,7] suggest that WASP activity has to be finely tuned to maintain normal podosome assembly and dynamics. This equilibrium is clearly disturbed in macrophages from the R405C patient. It will be interesting to find out how the A230T or E250Q mutations alter WASP activity. In addition to WASP binding, it will be interesting to explore the binding of the other
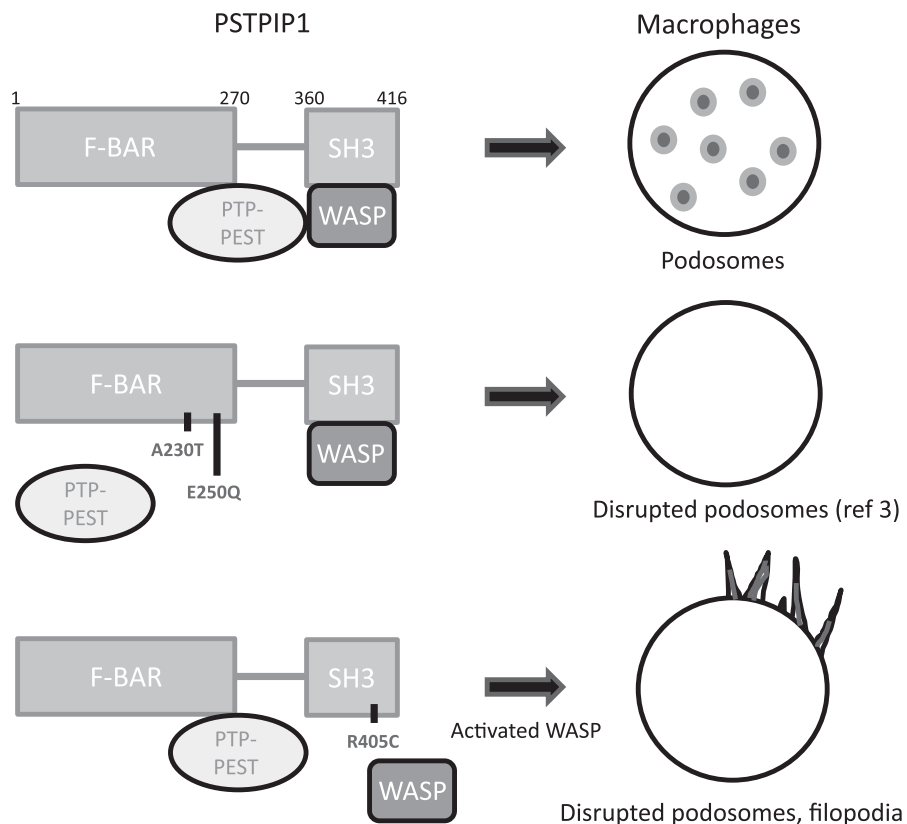


PSTPIP1 interacts with PTP-PEST and WASP. In PAPA patients, two mutations in the F-BAR domain impair the interaction with PTP-PEST. In the article by Starnes et al, a novel mutation was identified in the SH3 domain that affects WASP binding. The consequences of these mutations on macrophage podosomes and filopodia are illustrated.

proteins that interact with the SH3 domain of PSTPIP1 to create a broader picture of the general consequences of the R405C mutation.

Furthermore, the study by Starnes et al provides new insight into the role of PSTPIP1 as a regulator of the transition between podosomes and filopodia on 2D substrates. While there are examples of podosome alterations that correlate with inhibited ECM degradation,[5,8] here, in the absence of podosomes, the delivery of proteases appeared to be transferred to filopodia with an even more efficient matrix-degrading activity in PSTPIP1-R405C cells than in control macrophages. This suggests that filopodia can substitute for podosomes to degrade ECM proteins. Migration in 3D dense matrices such as Matrigel requires ECM degradation for macrophages to create paths.[9] In these environments, macrophages form cell protrusions called 3D podosomes at the tip of which podosome proteins accumulate along with F-actin and matrix proteolytic activity.[9,10] The study by Starnes et al provides a very important observation because it shows that the ability of this patient's cells to migrate in dense matrices is highly efficient despite podosome disruption. It will be interesting to characterize whether filopodia-bearing cells in 2D do form 3D podosome structures in dense matrices.

Mutations in *PSTPIP1* that disrupt the interaction with PTP-PEST result in impaired chemotaxis and 3D migration of macrophages, whereas the R405C mutation triggers an opposite phenotype, which would be expected to lead to different clinical outcomes. Indeed, the patient with the R405C mutation showed aggressive pyoderma gangrenosum but no arthritis, whereas arthritis is usually very prominent in patients carrying A230T or E250Q mutations. Those observations suggest that different mechanisms are involved in the two aspects of this autoinflammatory syndrome. Thus, extension of our knowledge about PAPA syndrome mutations may eventually help to adapt therapies. In line with this, therapies targeting IL-1β lack efficacy in some patients[2] and the study by Starnes et al may help us understand why.

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

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● ● ● **PLATELETS & THROMBOPOIESIS**

# Eliminate dark side from antiplatelet therapy

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In this issue of *Blood*, Stefanini and colleagues report a new way to regulate platelet function. Mice expressing a talin-1 mutant (W359A) that disrupts its binding to the NPxY motif in β3 were protected from experimental thrombosis without undermining hemostasis by decelerating αIIbβ3 activation.[1]

In addition to their essential role in hemostasis, platelets play a crucial role in a pathological thrombus formation, particularly within atherosclerotic arteries subjected to high shear stress.[2] As an initial step in thrombogenesis (or hemostasis), platelets adhere to altered vascular surfaces or exposed subendothelial matrices, then become activated and aggregate each other to grow thrombus (or platelet plug) volume. These processes are primarily mediated by platelet surface glycoproteins: GPIb-IX-V, integrin $\alpha_2\beta_1$ (also known as GPIa-IIa), GPVI, and integrin αIIbβ3 (GPIIb-IIIa).[3] Integrins are a family of αβ heterodimeric adhesion receptors that mediate cellular attachment to the extracellular matrix and cell cohesion.[4] Among integrins, platelet αIIbβ3 is a prototypic non-I domain integrin and plays an essential role in platelet aggregation and thrombus growth as a physiological receptor for fibrinogen and von Willebrand factor, as evidenced by the clinical features of congenital bleeding disorder—Glanzmann thrombasthenia.[3] After exposure to subendothelial matrix including collagen and von Willebrand factor and/or several mediators (agonists) including adenosine 5′-diphosphate (ADP), thromboxane $A_2$, and thrombin, platelets become activated and a series of intracellular signaling events ("inside-out" signaling) that rapidly induce a high-affinity state of αIIbβ3 from its low-affinity state for soluble ligands (αIIbβ3 activation) are generated. In fact, αIIbβ3 activation is indispensable for platelet aggregation, and specific binding of the cytoskeletal proteins, talin and kindlin-3, to the cytoplasmic tail of the β3 subunit is the final common step in αIIbβ3 activation.[5] Although αIIbβ3 represents a rationale target for antithrombotic therapy, strong inhibition of αIIbβ3 increased bleeding complication. This is also true in the case of $P2Y_{12}$ inhibitors.[6]

Investigators are still searching for the "magic bullet" that selectively targets pathological thrombus formation without