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**FOXtrotting with PUMILIOs**

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In this issue of *Blood*, Naudin et al investigated the functional relevance of PUMILIO 1 and 2 (PUM1 and PUM2), 2 highly conserved RNA-binding proteins (RBPs) and members of the PUM and FBF (PUF) family of posttranscriptional regulators, in early hematopoiesis and acute myeloid leukemia (AML).1

Previous work by the Vigon and Lauret groups linked PUM upregulation to HOXB4/C4 and 4/Notch signaling in hematopoietic stem/progenitor cells (HSPCs).2,3 Here, Naudin et al show that in both human and mouse models, knockdown (KD) of PUM1 and PUM2 is detrimental to HSPC expansion, leading to exit from the cell cycle and resulting in apoptosis in vitro. Engraftment and hematopoietic reconstitution were severely impaired in PUM1 KD or PUM2 KD human and mouse HSPCs, although homing was unaffected.1

PUM proteins primarily exert posttranscriptional repression. They bind to the PUM-binding elements (PBEs) in the 3′ untranslated region (3′UTR) of their target messenger RNAs (mRNAs) causing direct and indirect disruption of translation, for example, as part of a repressor complex such as NANOS4 or in conjunction with microRNAs.5 The PUF proteins in human and Drosophila likely control ~7% to 11% of their respective transcriptomes.5 Human PUM1 and PUM2 have unique as well as common targets,6 likely with various degrees of cooperation in diverse tissues and developmental stages. For example, putative PUM1 and PUM2 targets have been analyzed in tumor cell lines, indicating that these proteins bind a large set of transcripts with many overlapping putative targets.6

Using a proteomics approach, Naudin et al identified FOXP1 as a downstream target of PUM1 and PUM2, where, surprisingly, reduction of PUM1 or PUM2 levels led to a decrease in FOXP1 levels. PUM1 and PUM2 directly bind 2 PBEs located in the 3′UTR of the *FOXP1* mRNA, albeit the mechanism of increased FOXP1 protein is as yet unknown. Reduction in *FOXP1* mRNA levels in PUM1 KD and PUM2 KD in AML cell lines, however, might indicate a stabilizing or protective effect by PUMs on the *FOXP1* transcript. PUM proteins have also been shown to promote posttranscriptional activation by interacting with the polyadenylation machinery and triggering translational activation of the target mRNA, as previously shown for oocyte maturation in *Xenopus*.7 Therefore, the ability of PUFs to modulate translation in a spatiotemporal fashion also makes them versatile regulators of hematopoietic development.

Knockdown of FOXP1 phenotypically mirrored the knockdown of PUM1 and PUM2 whereas overexpression of FOXP1 in PUM1 KD or PUM2 KD HSPCs partially restored normal function. In this context, FOXP1 reduces the levels of the cell cycle inhibitor proteins, p21CIP1 and p27KIP1, possibly through transcriptional repression, maintaining and promoting HSPC expansion properties (see figure). Previous works have linked FOXP1 to B-cell development and lymphoid malignancies as well as to neurogenesis.8,9

A major finding of Naudin et al is the necessity of PUM1 and PUM2 as well as FOXP1 for primary human AML.1 There could be great potential in exploring how crucial PUM1, PUM2, and FOXP1 are in various World Health Organization (WHO) AML subgroups. Based on the expression pattern of PUM1 and PUM2 in HSPCs, it is conceivable that leukemias with more “stemness” would show a higher degree of dependence on these proteins. The significant sensitivity of normal HSPCs to reduced levels of PUM1 and PUM2 could limit the efficacy of these proteins as therapeutic targets in AML. However, it is probable that certain subgroups of AML with high degrees of addiction to PUMs and FOXP1 function are especially susceptible to direct modulation as well as indirect therapies targeting their downstream pathways. Given the importance of PUMs in various somatic and germ line stem cells,10 it is yet to be determined if direct targeting is the best therapeutic strategy.

In conclusion, Naudin and colleagues have for the first time linked the PUM RBPs to hematopoietic stem cell physiology and pathophysiology which promotes the expression of FOXP1 leading to the modulation of cell cycle inhibition and maintenance of both HSPC function and leukemic growth.

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**Diagram:**

**FOXP1** mRNA

3′UTR

Ribosome

PUM1 and PUM2

**FOXP1**

p21CIP1

p27KIP1

Cell cycle

Elevated FOXP1 levels lead to reduction in p21 and p27 protein levels promoting cell cycle progression.
incorporated into the thrombus. 

This narrowed zone created a locale where platelets spend signifi-
cantly more time in contact with the growing thrombus, increasing the probability of their binding and incorporation into the clot. Thus, elevated hematocrit, perhaps unexpectedly, was shown to enhance thrombotic risk by increasing the number of platelets incorporated into the thrombus.

The prevalence of primary erythrocytosis in the United States has been reported to be 44 to 57 per 100 000. 

The natural history of PV is complicated by thromboembolic events; patients with myeloproliferative disorders suffer from both arterial and venous thrombosis, which accounts for 40% of the mortality associated with these disorders. 

However, the etiology of this high thrombotic rate has remained unclear. Recent advances attributed the thrombotic risk to blood hyperviscosity, as well as platelet and leukocyte dysfunction. 

However, one of the paradoxical findings in this disorder is that an elevation in platelet count is not correlated with the risk of thrombosis; to date, no study has demonstrated a correlation between the absolute platelet number or platelet functionality and the risk of thrombosis in PV or other myeloproliferative disorders. In the European Collaboration on Low-Dose Aspirin in Polycythemia Vera study, platelet count thresholds could not predict the risk of thrombosis, making this parameter unlikely to be useful for risk stratification.

Instead, elevated hematocrit and leukocytosis have been linked to platelet activation via cathepsin G and CD62P (P-selectin). 

In addition, the cyto-reduction-PV trial clearly demonstrated that the risk of thrombosis in PV was highly correlated with elevated hematocrit. 

In this multicenter trial, patients with a high hematocrit carried a four times higher thrombotic risk than those in the lower hematocrit group. The importance of these parameters is best emphasized by the fact that the cornerstone of treatment for PV is to use therapeutic phlebotomy to keep the hematocrit at a level <45%. 

In the article by Walton et al, a novel mechanism by which elevated hematocrit can lead to increased thrombosis risk has now been elucidated. The heightened hematocrit, which is the cornerstone of PV, results in redistribution of the blood cells within the circulatory bed. The RBCs push the platelets closer to the vessel wall, increasing the probability of adhesion and activation via von Willebrand factor and collagen, ultimately resulting in thrombotic manifestations. This novel finding is consistent with the etiology of thrombosis in PV, where an elevated platelet count does not explain thrombotic risk, and may finally provide an explanation for the mechanism of thrombosis in these patients.

Arterial clots are dependent on platelets for their propagation; therefore, the article by Walton et al clearly establishes a mode by which elevated hematocrit increases the risk of arterial thrombotic events. However, patients with an elevated hematocrit can present with both arterial and venous thrombosis. 

Therefore, what remains to be understood is whether similar principles apply to the formation of venous thrombi, which are not platelet-dependent but instead largely RBC- and fibrin-rich. Although the increased risk of venous thrombosis has also been tied to elevated hematocrit, it has been proposed that
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