Hoxa9–Meis1 or Aml1-Eto9a. The choice of models was interesting as both MLL-ENL KI and Hoxa9–Meis1 are likely to directly activate HIF-1α through MEIS1 expression, whereas Aml1-Eto9a is not known to signal through HIF-1α. They studied the oncogenes in wild-type cells with Hif-1α alleles or where Hif-1α alleles could be conditionally deleted after engraftment. In all 3 models, the results were clear; there was no dependence on HIF-1α for leukemia initiation, propagation, and leukemia-initiating cell self-renewal in transplantation assays. If anything, the onset of leukemia was accelerated in cells deleted for Hif-1α in the Hoxa9–Meis1 model and when mice were secondarily transplanted with Aml1-Eto9a transduced leukemic cells. One obvious caveat is that compensation by HIF-2α may have obscured a physiologic role for HIF-1α. Although that may be the case, the data do suggest that simply targeting HIF-1α may not be sufficient. Studying AML initiation and propagation in cells with both Hif-1α and Hif-2α conditional alleles would address this question.

So where does this leave the field? Although there are still important mechanistic questions about the role of HIF and adaptation to hypoxia by normal stem/early progenitor cells, the bulk of evidence supports a critical role for HIF function in this area. Clearly, more work needs to be done to define any differential functional effects of HIF-1α and HIF-2α between humans and mice. In AML and other hematologic malignancies, the situation is likely to be more complex. The role of HIF (and specifically HIF-α subunits) may depend on a number of parameters. For example, the nature of oncogenic drivers (genetic and epigenetic) is likely to dictate genome integrity and genome robustness. One could hypothesize that loss of HIF function in some malignancies (and AML in particular) may make tumor initiating and propagating cells more vulnerable to genotoxic stress just like their normal hemopoietic stem/early progenitor counterparts, whereas this may not be true in cells with an altered TP53 function. Oncogenic drivers are also likely to influence self-renewal, the need (or lack of) for quiescence, and optimal metabolism for leukemia initiating and propagating cells. Taken together, this is likely to determine the nature of optimal niches and thus the requirement for HIF function. If these hypotheses are shown to be correct, it would also suggest that HIF requirement will not only vary between patients, but also within a patient at different stages of the disease. Thus, the data from Valasco-Hernandez et al should give pause for more thought and an opportunity to probe more deeply into the interaction between hypoxic adaption and function of cell populations that initiate and propagate AML and other cancers.

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● ● ● **THROMBOSIS & HEMOSTASIS**

New checkpoint of the coagulant phenotype

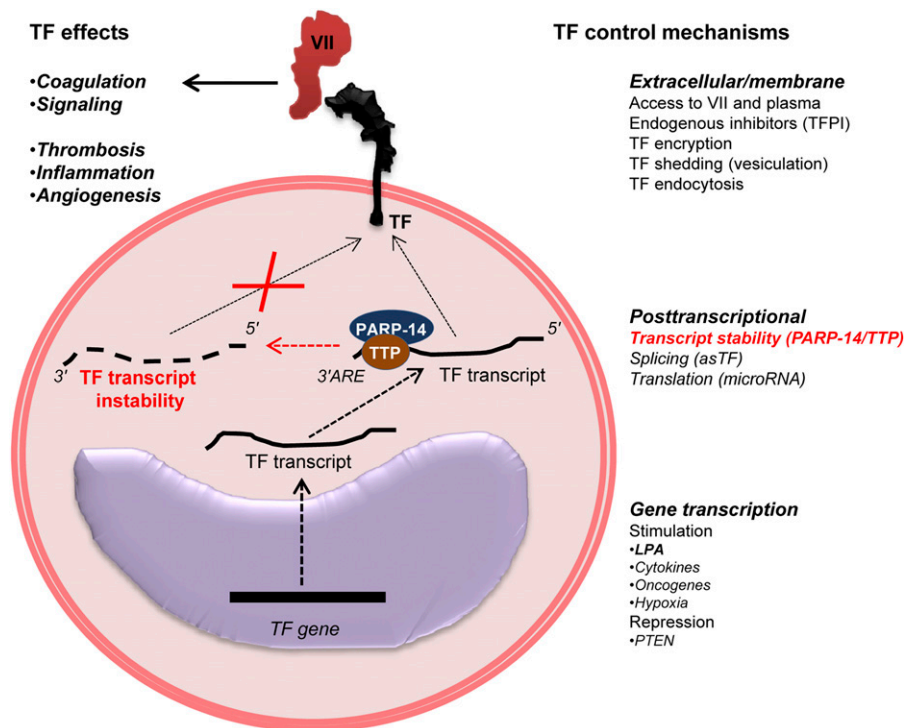Janusz Rak   THE RESEARCH INSTITUTE OF THE McGILL UNIVERSITY HEALTH CENTRE

In this issue of *Blood*, Iqbal et al shed new light on how the procoagulant potential of monocytes/macrophages is controlled by the hitherto unsuspected mechanism modulating the fate of tissue factor (TF) messenger (m)RNA.[1]

Monocytes, macrophages, and their precursors are cellular mavericks programmed to travel in blood and across tissue barriers to sites of infection, inflammation, injury, and repair.[2] This property requires a precise, timely, and localized expression of different functional aptitudes. A startling example of this is the ability of monocytes to enter the circulating blood while effectively "managing" their relationship with the coagulation system.[2]

Contact with blood can be risky. Monocytes possess the intrinsic potential to activate clotting through expression of TF, the cell surface receptor for the coagulation factor (F)VII/VIIa and potent trigger of the coagulation cascade.[3] If monocytes were to express active TF in an unscheduled or exuberant manner, the consequences could be catastrophic, resulting in uncontrolled intravascular activation of clotting processes, as observed in sepsis.[4]

The remarkable feature of the hemostatic system is its ability to maintain the systemic liquidity of the circulating blood while being able to locally "solidify" blood components to plug up the site of a vascular injury by clots composed of fibrin and platelets. This is accomplished, in part, by the physical separation of latent clotting factors (zymogens) and their potential activators, such as procoagulant surfaces of extracellular matrix and TF expressed by cells outside of the

TF effects

- *Coagulation*
- *Signaling*

- *Thrombosis*
- *Inflammation*
- *Angiogenesis*

TF control mechanisms

Extracellular/membrane
Access to VII and plasma
Endogenous inhibitors (TFPI)
TF encryption
TF shedding (vesiculation)
TF endocytosis

Posttranscriptional
Transcript stability (PARP-14/TTP)
Splicing (asTF)
Translation (microRNA)

Gene transcription
Stimulation
- *LPA*
- *Cytokines*
- *Oncogenes*
- *Hypoxia*
Repression
- *PTEN*

Control mechanisms restricting TF activity in the novel posttranslational role of the poly(adenosine 5′-diphosphate [ADP]-ribose)-polymerase (PARP)-14/tristetraprolin (TTP) pathway. Several mechanisms control TF expression, procoagulant activity, and signaling in various cell types.[9] The study of Iqbal et al[1] documents a novel posttranscriptional mechanism of TF regulation through selective destabilization of the TF transcript. In this setting, PARP-14 forms ternary complexes containing 3′ AU-rich element (ARE) sequences of the TF mRNA and a regulatory protein, TTP, resulting in accelerated decay of the TF transcript. Obliteration of PARP-14 expression leads to upregulation of TF in macrophages and to exacerbated experimental thrombosis in mice.[4] LPA, lipopolysaccharide; PTEN, phosphatase and tensin homolog, tumor suppressor; TFPI, TF pathway inhibitor.

vascular lumen.[3] Cells that must come into contact with the circulating blood either shut down their TF transcription (endothelial cells), or control TF activity through a repertoire of plausible but still poorly understood mechanisms, including downregulation of TF gene expression, intracellular retention, conformational inactivation (encryption), presence of inhibitors (TF pathway inhibitor), alternative splicing, and other events that may prevent the contact between TF and FVIIa (see figure).

Disruption of any of these barriers, either through physical (injury) or functional (infection) insults, brings the "critical masses" of TF and FVIIa into proximity, resulting in the explosive but usually self-contained chain of coagulation events that result in thrombin activation and formation of hemostatic clots.[3] Notably, TF/FVIIa complexes also possess the ability to generate intracellular signals that "inform" TF-expressing cells about their contact with the clotting blood. This process activates cellular programs required

for tissue "cleansing" through inflammation and for their repair involving angiogenesis and wound closure.[5] Indeed, the coagulation system has evolved over 450 million years to initially act as a primordial mechanism to trap and contain infectious agents[6] and has since retained its links to inflammation and wound healing in higher organisms.[2]

Not surprisingly, macrophages/monocytes, the cellular "masterminds" of inflammation, are also the main source of TF in the circulating blood.[4] TF is expressed after macrophage activation through contact with infectious agents and their products such as bacterial lipopolysaccharide (LPS), as well as chemokines and cytokines.[2] This exposure leads to mobilization of the TF coagulant potential, both on the cellular surfaces and through emission of microparticles, fragments of the plasma membrane (also known as extracellular vesicles) that could transfer TF to platelets and endothelial cells.[4] These responses leading to formation of clots physically protect

the damaged and infected tissue[2] and make profound biological sense, but only when they occur in a localized and timely manner. How do macrophages "know" what is the right time, order, and circumstance to express active TF?

Due to the destructive potential of blood vessel perturbations, many powerful vascular regulators, including TF, vascular endothelial growth factor, and several others,[7] must be precisely titrated by their expressing cells through several levels of molecular control (see figure). Accordingly, the regulation of *TF/F3* (coagulation factor 3 gene) transcription has been extensively studied,[3] but relatively little is known about the posttranscriptional processing of TF mRNA, with the possible exception of a limited number of studies exploring the role of microRNA in cancer cells.[8,9] This gap is surprising, because the availability of the functional mRNA is the important step in control of the levels of bioactive TF protein on the cell surface.

In this regard, the findings of Iqbal et al[1] represent a significant breakthrough. While investigating the little-known molecular member of the PARP family of enzymes known as PARP-14,[10] these authors noted a marked upregulation of TF mRNA in *Parp-14*^{−/−} mice, especially in their bone marrow–derived macrophages treated with bacterial LPS. Thus, PARP-14 must represent a new control element acting at the level of TF transcript. Indeed, the study reveals that PARP-14 binds to the 3′ untranslated region of the TF mRNA through the AU (sequence)-rich element. In so doing, PARP-14 forms a complex with TTP, another posttranscriptional regulatory protein, and this event selectively accelerates the decay of the TF mRNA. This mechanism cooperates with the effects of microRNA binding sites, collectively resulting in reduced TF expression and procoagulant activity.

This is an unsuspected finding, and one that is both mechanistically fascinating and potentially important. It is fascinating because PARP-14 decouples TF regulation from other TTP targets such as tumor necrosis factor-α mRNA and thereby provides a "timing device" for delivery of different functional components of the macrophage activation program (coagulant and inflammatory). This finding is also potentially important because in *Parp-14*^{−/−} mice,

the experimental thrombosis is exacerbated in vivo due to excessive production of TF.

This study raises several novel questions that may impact how the procoagulant potential of macrophages (possibly other cells) is understood and confronted in the clinic. How is the PARP-14/TTP pathway regulated in various procoagulant settings such as sepsis, venous thromboembolism, or cancer? Is PARP-14 deregulation a part of disease pathogenesis? What is the impact of these processes on TF signaling, angiogenesis, and other functions? If the PARP-14 pathway can be therapeutically modulated, then when, how, and to what end would this be desirable? While we await answers, we may take a moment to marvel about the intricacies of hemostatic mechanisms and the power of the science able to unravel them.

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