PI3K recruits serine/threonine kinase AKT to the plasma membrane where it is activated by a variety of activators such as the mammalian target of rapamycin, integrin-linked kinase, and 3-phosphoinositide-dependent protein kinase 1. PI3K activity in AKT activation is counterbalanced by PTEN. Although the roles of PI3K and AKT are well recognized in regulating platelet activation, the function of PTEN has not been studied. Weng and colleagues, for the first time, provide convincing evidence about the negative regulatory role of PTEN in collagen-induced platelet activation. They show that PTEN regulates collagen signaling in both a PI3K/AKT-dependent and -independent manner.

Because nonspecific ablation of PTEN is embryonically lethal, Weng and colleagues used inducible and hematopoietic tissue–specific deleted mice and found that PTEN suppresses collagen-induced platelet activation. PTEN deficiency significantly reduced bleeding time and increased responsiveness of platelets to collagen. Deficiency of PTEN enhanced collagen-induced activation of AKT. Surprisingly, inhibition of PI3K prevented the aggregation of wild-type platelets, but not PTEN-deficient platelets, suggesting that PTEN may regulate collagen-induced signaling through PI3K/AKT in both a dependent and independent manner. As depicted in the figure, PI3K converts PIP2 to PIP3, which activates AKT, whereas PTEN opposes this process. One would therefore predict that PTEN deletion would increase AKT activation. As expected, PTEN-deficient platelets show enhanced AKT activation. Furthermore, PTEN helps replenish the pool of PIP2, which is important for granular secretion. Thus, decreased secretion due to reduced PIP2 availability was expected in PTEN-deficient platelets. Contrary to the prediction, collagen-induced granular secretion is increased in the absence of PTEN. Furthermore, inhibition of PI3K in wild-type platelets abrogates collagen-induced AKT activation and platelet aggregation, suggesting that collagen-induced platelet activation is entirely PI3K/AKT-dependent.

Surprisingly, in PTEN-deficient platelets, inhibition of PI3K failed to inhibit collagen-induced aggregation. These results suggest that, in addition to dephosphorylation of PI3P, PTEN exerts an inhibitory effect on granular secretion and/or integrin activation, which is PI3K/AKT independent. One possibility is that PTEN may dephosphorylate signaling proteins downstream of protein kinase C (PKC) during granular secretion and integrin activation. This is conceivable, considering the protein phosphatase activity of PTEN. In addition to being a phosphatidylinositol-3-phosphatase, PTEN is also a dual specificity protein phosphatase. It is also well established that PTEN is a tumor suppressor. PTEN's role in tumor suppression has been extended beyond simply down-regulation of the PI3K/AKT pathway. It has been shown to regulate phospholipase D and PLC. Furthermore, genetically modified mice confirm that mutation in PTEN results in tumorogenesis; however, activated AKT transgenic lines do not develop tumors, suggesting that AKT-independent mechanisms contribute to PTEN tumorigenesis.

Another exciting possibility is that deletion of PTEN together with inhibition of PI3K may alter PIP2 levels or its interaction with downstream signaling proteins. For example, PIP2 has been shown to regulate talin, a key activator of integrin α5β3. Further experimentation will be needed to identify the PI3K/AKT-independent inhibitory mechanism of collagen-induced platelet activation by PTEN. Nevertheless, the finding reported by Weng and colleagues not only identifies PTEN as a novel regulator of collagen signaling, but also opens new avenues for investigation of its role beyond PI2P production in platelets.

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

REFERENCES

Iron, bone, and marrow

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Osteoporosis is a common problem in disorders characterized by iron overload, such as the thalassemias and hereditary hemochromatosis. The exact role of iron in the development of osteoporosis in these disorders is not established. In this issue of Blood, Tsay et al1 suggest that increased bone resorption caused by increased iron-associative oxidative stress may be a significant pathogenetic mechanism.

In their study, Tsay et al examined the effect of iron excess on bone using an iron-overloaded mouse model treated by weekly iron dextran injections for a period of 2 months. Iron overload had a pronounced dose-dependent effect. Iron-overloaded mice had increased reactive oxygen species (ROS) and elevated serum tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) concentrations. Exposure of the osteoclast-like RAW cell line to ferric iron resulted in increased TNF-α production. Th euchanges observed in vivo were associated with increased bone resorption (see figure), increased oxidative stress, and evidence of systemic inflammation. Significantly, they were prevented by treatment with the antioxidant N-acetyl-L-cysteine, supporting the hypothesis that oxidative stress is involved in their pathogenesis. Osteoblast...
Treatment with an antioxidant prevents the bone loss induced by iron overload. Micro-CT images of placebo, iron dextran, and iron dextran + NAC animals. Trabecular bone at proximal femur and cortical bone at mid-diaphysis femur (image is Figure 4B from Tsay et al.).

function was unaltered. In view of these observations, the authors conclude that iron overload in mice results in increased bone resorption, oxidative stress, and inflammation, leading to changes in bone microarchitecture and other properties.

The association of osteoporosis with iron overload in general, and in thalassemia in particular, is poorly understood. The associated vastly expanded erythropoietic mass in thalassemia may by themselves cause abnormalities in bone architecture. Ascorbate deficiency is caused by increased oxidative breakdown of ascorbic acid catalyzed by iron, leading to scurvy and the associated bone defects. However, the unaltered collagen synthesis found in the present study suggests that ascorbate levels have not decreased to the point of inhibiting hydroxylation of key proline residues in collagen, a rate-limiting step in collagen synthesis.

The relation between iron overload and increased oxidative stress is well documented. It is also known that cytokines such as TNF-α and IL-6 mediate bone loss primarily by increasing bone resorption. Tsay et al propose a model wherein iron excess leads to increased oxidative stress: oxidative stress in turn induces inflammatory changes in a dose–dependent fashion, then mediates bone loss through changes in bone remodeling. However, the evidence linking increased oxidative stress to increased cytokine production, and the role of iron in cytokine-mediated bone loss, is at present unclear. In fact, in hereditary hemochromatosis, cytokine production is impaired. Conversely, increased cytokine production in sickle cell disease compared with thalassemia is not related to the degree of iron overload, but to the underlying unique pathology of sickle cell disease.

The study by Tsay et al addresses a significant problem encountered in chronic iron overload that so far has not received sufficient attention and its pathogenesis is poorly understood. The study is particularly strong in its methodology, offering carefully collected data supporting the claim that the bone defect associated with iron overload is caused by increased osteoclastic activity. The clear prevention of iron-associated bone abnormalities by the antioxidant N-acetylcysteine is strong evidence supporting the role of ROS in the observed bone abnormalities. By contrast, the experiments involving inflammatory cytokines are less convincing. This is not because of the correlations observed, but the lack of evidence supporting a mechanistic cause-and-effect relation.

Despite these caveats and the need for further studies to validate these concepts in the clinical settings, the above observations may have significant implications for the management of clinical complications encountered in patients with chronic iron overload.

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**REFERENCES**


**RED CELLS & IRON**

Comment on Moyer et al, page 2590

**“Capping”: necessary for graduation**

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In this issue of *Blood*, Moyer and colleagues show that deficiency of tropomodulin, an actin filament capping protein in red cell membranes, leads to mild spherocytic anemia, thus identifying yet another molecular defect that can lead to inherited red cell membrane disorders. Whereas nucleated cells have an actin-based cytoskeleton, non-nucleated mammalian red cells have a unique membrane-associated spectrin-actin-based skeletal network. The 2-dimensional skeletal network (see figure) is quasi-hexagonal with long, flexible spectrin connecting strands and short actin filaments at vertices serving as cores for assembly of junctional complexes composed of protein 4.1R, adducin, dematin, tropomyosin, and tropomodulin. This network, in conjunction with its coupling to the membrane by

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