Check for updates

ensure efficient resolution of R-loops. These include RNA processing and export pathways that direct RNA away from the DNA template, RNA-modifying enzymes, topoisomerases, helicases, and nucleases with RNase H activity, which degrade RNA within an RNA-DNA hybrid.

PIWIL4 is an RBP belonging to the Argonaute family.[5] Along with the other PIWI family members, it is predominantly expressed in germ cells in the testis. The canonical function of PIWI proteins is the PIWI-interacting RNA (piRNA)-directed silencing of transposable elements, which would otherwise threaten the genomic integrity of germ cells.[6] However, evidence is beginning to emerge that suggests PIWI proteins may play roles outside of the germ cell and in some cases in a piRNA-independent manner.[7] The study reported in this issue of *Blood* begins with the surprising observation that PIWIL4 is expressed at strikingly high levels in AML cells and leukemic stem cells (LSCs). Indeed, AML oncogenes were able to drive increased PIWIL4 expression in hematopoietic stem and progenitor cells (HSPCs). Moreover, the authors show how AML and LSCs depend upon PIWIL4 for survival, engraftment, and propagation. In contrast, nonmalignant HSPCs showed normal function after PIWIL4 depletion. This dispensability for normal hematopoiesis is consistent with a previous mouse model,[8] confirming a cancer-specific dependency upon PIWIL4. These intriguing findings prompt the question, why is PIWIL4, a germ cell-associated RBP, so important to malignant myeloid cells? Photoactivatable ribonucleoside-enhanced cross-linking and immunoprecipitation experiments showed minimal association with piRNA, leading the authors to conclude that PIWIL4 must be acting independently from its canonical role in the PIWI pathway. Instead, through a series of carefully designed experiments, they elucidate how AML and LSCs coopt the RNase H catalytic activity of PIWIL4 to resolve R-loops. Interestingly, PIWIL4-dependent R-loop resolution was most enriched at the loci of genes essential for AML growth and LSC maintenance. PIWIL4 depletion in AML cells led to accumulation of R-loops, which in turn resulted in stalled transcription of AML and LSC signature genes, replicative stress, DNA damage, and loss of cellular fitness. These detrimental effects were specific to AML cells and were potentiated by pharmacological inhibition of ATR, highlighting a potential approach for AML-specific therapeutic targeting.

The findings of this study are important and provocative and raise new questions. First, the study uncovers a novel moonlighting function for PIWIL4 in the resolution of R-loops and maintenance of genomic integrity. At the same time, the limited rescue with RNase H leaves open the possibility that AML dependence on PIWIL4 may involve other functions beyond R-loop resolution. It also leaves open the question as to whether the R-loop-resolving function of PIWIL4 is unique to AML or might be shared with other cancer types. Second, the study highlights the importance of the complex interplay between RNA and RBPs in the regulation of hematopoiesis and hematopoietic malignancy and an emerging theme that RBPs frequently moonlight beyond a single canonical function.[9] Finally, the requirement to resolve R-loops reveals a specific vulnerability of AML cells and LSCs that appears not to be shared with normal HSPCs. Precisely why this requirement should be so specific to AML cells is yet to be fully elucidated. However, this question and the ways this cancer-specific vulnerability might be exploited as a therapeutic strategy to target AML without damaging normal myeloid precursors will be the subject of exciting future research.

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

REFERENCES

1. Bamezai S, Pulikkottil AJ, Yadav T, et al. A noncanonical enzymatic function of PIWIL4 maintains genomic integrity and leukemic growth in AML. *Blood*. 2023;142(1):90-105.
2. Brickner JR, Garzon JL, Cimprich KA. Walking a tightrope: the complex balancing act of R-loops in genome stability. *Mol Cell*. 2022; 82(12):2267-2297.
3. Crossley MP, Bocek M, Cimprich KA. R-loops as cellular regulators and genomic threats. *Mol Cell*. 2019;73(3):398-411.
4. Kotsantis P, Petermann E, Boulton SJ. Mechanisms of oncogene-induced replication stress: jigsaw falling into place. *Cancer Discov*. 2018;8(5):537-555.
5. Sasaki T, Shiohama A, Minoshima S, Shimizu N. Identification of eight members of the Argonaute family in the human genome. *Genomics*. 2003;82(3):323-330.
6. Siomi MC, Sato K, Pezic D, Aravin AA. PIWI-interacting small RNAs: the vanguard of genome defence. *Nat Rev Mol Cell Biol*. 2011; 12(4):246-258.
7. Dong P, Xiong Y, Konno Y, et al. Critical roles of PIWIL1 in human tumors: expression, functions, mechanisms, and potential clinical implications. *Front Cell Dev Biol*. 2021;9:656993.
8. Nolde MJ, Cheng EC, Guo S, Lin H. PIWI genes are dispensable for normal hematopoiesis in mice. *PLoS One*. 2013;8(8): e71950.
9. Hodson DJ, Screen M, Turner M. RNA-binding proteins in hematopoiesis and hematological malignancy. *Blood*. 2019;133(22):2365-2373.

https://doi.org/10.1182/blood.2023020672

© 2023 by The American Society of Hematology

RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Courbon et al, page 106

Matryoshka hormones

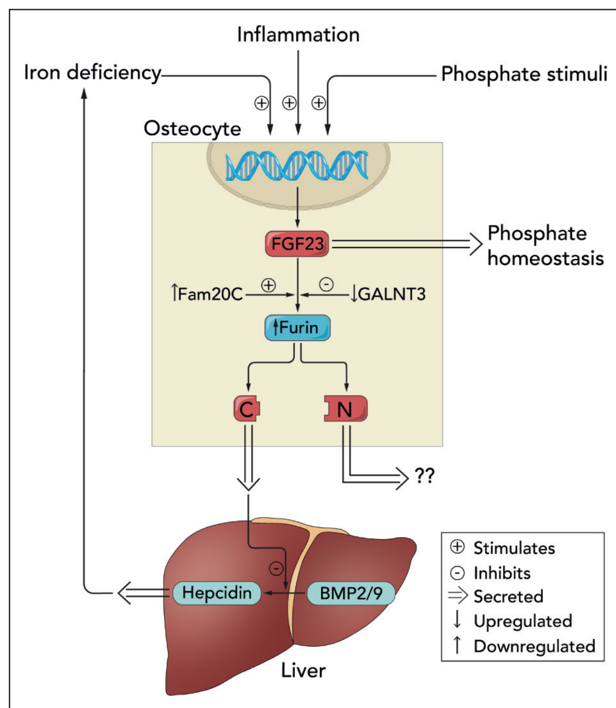
Myles Wolf | Duke University School of Medicine

In this issue of *Blood*, Courbon et al demonstrate that inflammation stimulates production of C-terminal fibroblast growth factor 23 (cFGF23) fragments that downregulate hepcidin and mitigate iron deficiency.[1]

Why would a cell engage in an energy-intensive and a seemingly wasteful exercise of producing a peptide hormone only to immediately degrade it intracellularly and secrete the resulting fragments into circulation? And why do inflammation and iron deficiency recruit such a process to produce large amounts of fragments of a phosphate-regulating hormone?

FGF23 is a bone-derived peptide hormone that regulates mineral homeostasis via multiple negative endocrine feedback loops.[2] FGF23 production is stimulated by increases in 1,25-dihydroxyvitamin D (1,25D), parathyroid hormone (PTH), serum calcium, and kidney-derived glycerol-3-phosphate, which is the effector of phosphate sensing by the proximal tubule

Downloaded from http://ashpublications.net/blood/article-pdf/142/1/7/2067165/blood_bld-2023-020770-c-main.pdf by guest on 03 June 2024



Regulation of iron or phosphate homeostasis by C-terminal vs full-length FGF23. Inflammation, iron deficiency, and phosphate-related stimuli activate *FGF23* transcription in osteocytes. Newly translated FGF23 protein is either glycosylated by GALNT3 and secreted into circulation to regulate phosphate homeostasis or phosphorylated by Fam20C and cleaved by furin. Courbon et al demonstrate that inflammation also increases expression of Fam20C and Furin and decreases GALNT3 expression. C-terminal FGF23 fragments are secreted by osteocytes and travel to the liver to antagonize BMP-mediated increases in hepcidin production. Presumably, N-terminal FGF23 fragments are also secreted, but their potential biological functions are currently unknown. Professional illustration by Somersault18:24.

of the kidney.[2,3] Closing the feedback loops, each of these stimuli induce secretion of full-length FGF23 by bone, which travels to the kidney to stimulate renal phosphate excretion, reduce 1,25D concentrations, and effectuate downstream changes in calcium and PTH.[2]

Inflammation and iron deficiency also activate FGF23 production, but these stimuli couple increases in *FGF23* transcription to increased intracellular cleavage of newly translated FGF23 into C-terminal and N-terminal fragments (see figure).[4] This coordinated activation of FGF23 production and cleavage results in no change in full-length FGF23 levels but markedly increases circulating concentrations of FGF23 fragments that have no effect on phosphate homeostasis.[4] The discovery of this peculiar phenomenon was facilitated by the serendipitous development of C-terminal FGF23 (cFGF23) assays that detect full-length FGF23 *and* its C-terminal fragments.[5] This assay complements intact FGF23 assays (iFGF23) that exclusively detect the full-length hormone. Since iFGF23 levels and thus serum phosphate are normal in patients with inflammation and iron deficiency, markedly elevated concentrations of cFGF23 fragments that are detected by cFGF23 assays are the only clinical footprint of excessive FGF23 production and cleavage.[6]

In this issue of *Blood*, Courbon et al elegantly address why inflammation activates the FGF23 production-cleavage cycle in bone. The authors confirm that increased transcription and intracellular cleavage of FGF23 by furin in osteocytes is the source of the excess C-terminal FGF23 fragments that are produced in response to inflammation (see figure). Although bone marrow stromal cells were another major source of basal FGF23 production, inflammation did not alter their FGF23 production. To elucidate potential actions of C-terminal FGF23 fragments, the authors investigated the effects of inflammation on hepcidin production in a series of mouse models with deficient or excessive production of C-terminal FGF23 fragments. Compared with wild-type mice, inflammation-induced iron deficiency was exacerbated by further increases in hepcidin levels in mice that were unable to augment production of C-terminal FGF23 fragments due to osteocyte-specific deletion of FGF23 or furin. Conversely, injection or transgenic overexpression of C-terminal FGF23 fragments in inflamed mice decreased hepcidin levels and mitigated iron deficiency.

The authors confirmed that the effects on hepcidin were specific to C-terminal FGF23 fragments vs full-length FGF23 by demonstrating no changes in hepcidin or iron stores in a mouse model with isolated increases in full-length FGF23. As the underlying molecular mechanism, the authors reported that C-terminal FGF23 fragments antagonize bone morphogenic protein (BMP) 2/9-driven hepcidin production (see figure), perhaps by preventing BMP2/9 from binding to BMP receptors. In summary, breaking open full-length, phosphate-regulating FGF23 reveals a smaller, iron-regulating C-terminal fragment. One parent peptide, 2 separate hormones governing 2 distinct pathways.

Competing posttranslational modifications determine whether nascent FGF23 is cleaved or secreted intact. Glycosylation of FGF23 by GALNT3 protects FGF23 from cleavage by furin and results in secretion of full-length hormone, whereas phosphorylation by Fam20C earmarks FGF23 for cleavage.[4] Courbon et al found that inflammation rapidly increases expression of *Fgf23*, *Fam20C*, and *Furin* while rapidly decreasing *Galnt3* expression (see figure). Although the authors did not report on the molecular mechanism of how inflammation orchestrates this program of gene expression that culminates in increased production of FGF23 fragments, prior studies suggest that stabilizing or increasing expression of hypoxia-inducible factor (HIF) 1α may play a central role.[7] Prolyl hydroxylase inhibitors potentiate the effects of HIF1α and are known to decrease hepcidin.[8] Whether increased production of C-terminal FGF23 fragments contributes to hepcidin suppression by factors that stabilize HIF1α is worthy of additional research.

Future studies should also address how this complex system responds to competing stimuli, for example, when inflammation and iron deficiency demand production of C-terminal FGF23 fragments at the same time that high-phosphate diet calls for full-length FGF23. Likewise, future studies should

Downloaded from http://ashpublications.net/blood/article-pdf/142/1/7/2067165/blood_bld-2023-020770-c-main.pdf by guest on 03 June 2024

investigate how administration of intravenous ferric carboxymaltose converts patients with iron deficiency anemia from a state of high C-terminal FGF23 fragments to a state of high full-length FGF23 that often causes severe hypophosphatemia.[6,9] Based on the work by Courbon et al, it seems likely that ferric carboxymaltose somehow reduces FGF23 cleavage by short-circuiting the cellular controls that dictate the relative production of full-length vs FGF23 fragments.

Chronic kidney disease is another state of reduced FGF23 cleavage in which circulating concentrations of full-length FGF23 progressively increase while C-terminal fragments gradually decline.[2,4] In this setting, reduced FGF23 cleavage is considered to be an adaptive mechanism for osteocytes to augment production of full-length FGF23 to maintain normal serum phosphate in the face of severe reductions in kidney function. However, the data presented by Courbon et al suggest that this process might also be maladaptive if reduced production of C-terminal FGF23 fragments contributes to worsening anemia of kidney disease by failing to suppress hepcidin.

The new data from Courbon et al will have far-reaching effects on our understanding of iron and phosphate homeostasis and anemia of chronic disease, including in patients with chronic kidney disease. They also suggest potential therapeutic applications. For example, perhaps exogenous cFGF23 fragments or small molecules that harness their ability to inhibit BMP-mediated production of hepcidin could become novel candidates to improve the lives of patients with anemia of chronic disease.

Conflict-of-interest disclosure: M.W. has equity interests in Akebia, Unicycive, and Walden and has served as a consultant for Bayer, Enyo, Jnana, Kissei, Launch, Pharmacosmos, Reata, and Torii. ■

REFERENCES

1. Courbon G, Thomas JJ, Martinez-Calle M, et al. Bone-derived C-terminal FGF23 cleaved peptides increase iron availability in acute inflammation. *Blood*. 2023;142(1):106-118.

2. Musgrove J, Wolf M. Regulation and effects of FGF23 in chronic kidney disease. *Annu Rev Physiol*. 2020;82:365-390.

3. Zhou W, Simic P, Zhou IY, et al. Kidney glycolysis serves as a mammalian phosphate sensor that maintains phosphate homeostasis. *J Clin Invest*. 2023;133(8):e164610.

4. Edmonston D, Wolf M. FGF23 at the crossroads of phosphate, iron economy and erythropoiesis. *Nat Rev Nephrol*. 2020;16(1):7-19.

5. Jonsson KB, Zahradnik R, Larsson T, et al. Fibroblast growth factor 23 in oncogenic osteomalacia and X-linked hypophosphatemia. *N Engl J Med*. 2003;348(17):1656-1663.

6. Wolf M, Koch TA, Bregman DB. Effects of iron deficiency anemia and its treatment on fibroblast growth factor 23 and phosphate homeostasis in women. *J Bone Miner Res*. 2013;28(8):1793-1803.

7. David V, Martin A, Isakova T, et al. Inflammation and functional iron deficiency regulate fibroblast growth factor 23 production. *Kidney Int*. 2016;89(1):135-146.

8. Noonan ML, Ni P, Agoro R, et al. The HIF-PHI BAY 85-3934 (Molidustat) improves anemia and is associated with reduced levels of circulating FGF23 in a CKD mouse model. *J Bone Miner Res*. 2021;36(6):1117-1130.

9. Wolf M, Rubin J, Achebe M, et al. Effects of iron isomaltoside vs ferric carboxymaltose on hypophosphatemia in iron-deficiency anemia: two randomized clinical trials. *JAMA*. 2020;323(5):432-443.

https://doi.org/10.1182/blood.2023020770

© 2023 by The American Society of Hematology

Downloaded from http://ashpublications.net/blood/article-pdf/142/1/7/2067165/blood_bld-2023-020770-c-main.pdf by guest on 03 June 2024