Iron-Deficiency Anemia Modulates Erythropoiesis-Bone Crosstalk and Drives Bone Loss

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INTRODUCTION: Bone is a dynamic organ that regulates its formation and resorption through bi-directional communication with multiple tissues, organs, and microenvironments via hormonal, inflammatory, and mechanical stimuli. Over a billion people worldwide suffer from disorders of red blood cell formation (erythropoiesis) in the bone marrow, such as iron-deficiency anemia, sickle cell disease, and thalassemia, where skeletal complications are frequent. However, the mechanistic link between altered iron metabolism, erythropoiesis, and bone integrity remains incompletely understood. Our previous work suggests that erythroid precursor cells serve as key regulators of the bone marrow microenvironment, directly implicating them in bone homeostasis and repair [1]. Iron homeostasis is essential for erythropoiesis, enabling hemoglobin synthesis and the full maturation of erythroid cells to reticulocytes and erythrocytes in the peripheral blood. In this study, we investigated how iron metabolism mediates the crosstalk between erythroid precursors and bone tissue. Specifically, we asked whether iron-deficiency anemia triggers bone loss in skeletally mature mice. To address this, we utilized an inducible, intestine-specific ferroportin (Fpn) knockout mouse model ($Fpn^{\Delta IE}$), which leads to iron-deficiency anemia by blocking dietary iron absorption via targeted deletion of Fpn, the principal iron exporter [2]. Furthermore, we assessed whether pharmacological intervention with a dual kinase inhibitor Saracatinib targeting ALK2, a BMP type I receptor involved in iron-related pathways, could reverse these effects.

METHODS: Transgenic mice expressing a tamoxifen inducible, intestinal epithelium specific Cre recombinase ($Vil^{CreERT2}$) were used in combination with mice homozygous floxed mice for ferroportin ($Fpn^{fl/fl}$) to generate $Vil^{CreERT2}$; $Fpn^{fl/fl}$ mice. Tamoxifen (100 mg/kg IP, 3 days) was administered at 6 weeks of age. Both $Fpn^{\Delta IE}$ and control $Fpn^{fl/fl}$ mice were aged to 24 weeks, then treated with Saracatinib (5 mg/kg BW/day) or vehicle by oral gavage for 7 days (n≥5/group, with both sexes included and sex-matched across groups). All procedures involving mice were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by an IACUC (Protocol no.: PRO00011805). Anemia was characterized by automated hematology from peripheral blood (Cell Blood Count - CBC, reticulocyte counting via thiazole orange flow cytometry). Bone marrow cellularity and erythroid lineage distribution were quantified using multiparametric flow cytometry, with two antibody panels targeting early (c-Kit, Sca-1, CD150, CD41, CD16/32, CD105) and late (CD71, TER119, CD44) erythroid populations. Cells were analyzed using a MACSQuant10 flow cytometer and FlowJo v.10 software. Trabecular and cortical bone parameters were evaluated by nanoCT (GE Phoenix Nanotom M) and analyzed using DragonFly 3D software (V2024.1.0.1627). Immunofluorescence staining was performed on cryosectioned femurs stained for CD31 (endothelial cells), CD71, and Ter119 (both erythroid cells). Mann-Whitney test was used for statistical comparison with $^*P<0.05$.

RESULTS: $Fpn^{\Delta IE}$ mice exhibited a pronounced anemic phenotype, with significantly reduced levels of red blood cells (RBC), hemoglobin (HGB), hematocrit, and reticulocytes in peripheral blood (Fig. 1a). Among $Fpn^{\Delta IE}$ animals, two developed mild/moderate anemia (HGB > 9 g/dL), while three were severely anemic (HGB < 9 g/dL); subsequent analyses focused on the latter group. Multiparametric flow cytometry of total bone marrow revealed notable alterations in erythroid lineage populations, with an increase in early progenitors and a marked elevation in the proerythroblast ($Ter119^{lo}$ $CD71^{+}$) subpopulation, accompanied by a reduction in late-stage erythroblasts ($Ter119^{+}$ $CD71^{-}$) in severely anemic mice. Immunofluorescence staining confirmed the increased abundance of $CD71^{+}$ erythroid precursors and revealed pronounced alterations in the vascularized bone marrow niche (Fig. 1b). Three-dimensional nanoCT analysis demonstrated significant trabecular bone loss, as indicated by reductions in bone volume fraction (BV/TV) and bone mineral density (BMD; Fig. 1c, d), as well as by increases in trabecular separation and number. Cortical bone parameters, including cortical thickness and cortical area/total area, were also significantly diminished. These combined findings establish that $Fpn^{\Delta IE}$ mice recapitulate severe iron-deficiency anemia and experience a concomitant deterioration in bone architecture, suggesting a mechanistic link between iron metabolism, erythropoiesis, and bone homeostasis. Femur length remained unchanged across all groups, consistent with an inducible knockout model and excluding developmental confounding. Remarkably, a one-week course of Saracatinib treatment effectively reversed both the anemic phenotype and skeletal deficits resulting from intestinal ferroportin deletion (Fig. 1e).

DISCUSSION: Our preliminary findings suggest a link between intestinal iron transport, erythropoietic activity, and bone homeostasis. By investigating how erythroid precursor cells act as pivotal mediators within the bone-hematopoietic niche and respond dynamically to systemic iron metabolism, we explore new facets of cross-organ communication essential for skeletal maintenance. Especially, the directionality and causality within this relationship remain unresolved. For example, it is unclear whether iron deficiency compromises bone health primarily through direct deprivation of iron in bone cells, or via impaired/inefficient erythropoiesis and subsequent inflammatory changes in the marrow niche. While our current model suggests a potential causal axis, further studies employing cell type-specific iron deprivation strategies will be necessary to delineate these mechanisms definitively. Importantly, our data suggests that pharmacological manipulation of iron availability, specifically at the intersection of erythropoiesis and bone biology, may offer novel therapeutic avenues for anemia-associated bone loss and fragility. Notably, inhibition of ALK2 (a BMP type I receptor) rescued systemic effects of iron deficiency but also led to rapid and marked improvement in skeletal microarchitecture, a synergy seldom observed in adult bones. Collectively, these results reveal a potentially rapid and reciprocal crosstalk between iron metabolism, erythropoiesis, and skeletal integrity.

SIGNIFICANCE/CLINICAL RELEVANCE: Taken together, our results suggest the existence of a link between iron metabolism, erythropoiesis, and bone homeostasis, likely governed by as-yet-undiscovered regulatory pathways. Elucidating these mechanisms and identifying dual-action therapeutic targets has the potential to transform clinical management of disorders affecting both blood and bone.

REFERENCES: [1] Lang et al. bioRvix 2025, [2] Schwartz et al. JBC 2019
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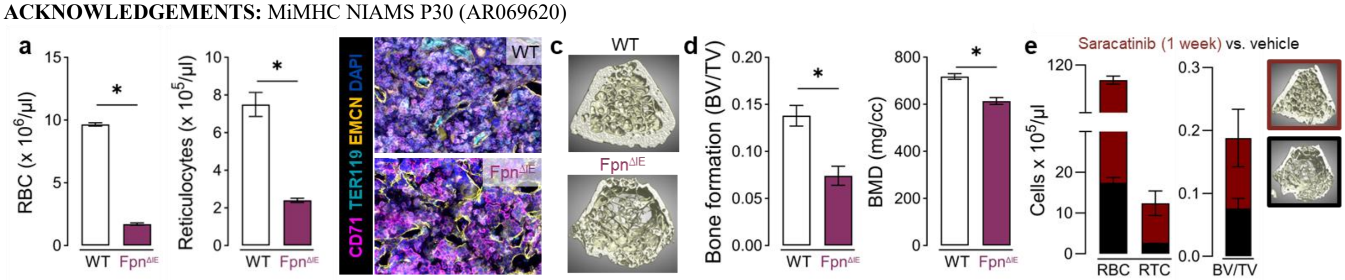

Fig. 1: Hematological changes and bone loss in an iron-deficiency anemia mouse model. (**a**) Hematological analysis reveals markedly reduced red blood cells (RBC) and reticulocytes in the peripheral blood following intestinal-specific ferroportin deletion at 6 weeks of age. (**b**) Immunofluorescence staining of bone marrow showing CD71 (erythroid precursors), Ter119 (mature erythroid cells), and Emcn (vasculature). (**c**) 3D microCT reconstructions highlight substantial trabecular bone loss in 24-week-old $Fpn^{\Delta IE}$ mice. (**d**) Quantification of bone volume fraction (BV/TV) and bone mineral density (BMD). (**e**) One-week treatment with Saracatinib rapidly reversed both hematologic and skeletal deficits in $Fpn^{\Delta IE}$ mice. Black data indicate anemic mice; brown indicates mice after Saracatinib treatment. RTC, reticulocytes.