Erythropoietin induces bone marrow and plasma fibroblast growth factor 23 during acute kidney injury

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It is accepted that osteoblasts/osteocytes are the major source for circulating fibroblast growth factor 23 (FGF23). However, erythropoietic cells of bone marrow also express FGF23. The modulation of FGF23 expression in bone marrow and potential contribution to circulating FGF23 has not been well studied. Moreover, recent studies show that plasma FGF23 may increase early during acute kidney injury (AKI). Erythropoietin, a kidney-derived hormone that targets erythropoietic cells, increases in AKI. Here we tested whether an acute increase of plasma erythropoietin induces FGF23 expression in erythropoietic cells of bone marrow thereby contributing to the increase of circulating FGF23 in AKI. We found that erythroid progenitor cells of bone marrow express FGF23. Erythropoietin increased FGF23 expression *in vivo* and in bone marrow cell cultures *via* the homodimeric erythropoietin receptor. In experimental AKI secondary to hemorrhagic shock or sepsis in rodents, there was a rapid increase of plasma erythropoietin, and an induction of bone marrow FGF23 expression together with a rapid increase of circulating FGF23. Blockade of the erythropoietin receptor fully prevented the induction of bone marrow FGF23 and partially suppressed the increase of circulating FGF23. Finally, there was an early increase of both circulating FGF23 and erythropoietin in a cohort of patients with severe sepsis who developed AKI within 48 hours of admission. Thus, increases in plasma erythropoietin and erythropoietin receptor activation are mechanisms implicated in the increase of plasma FGF23 in AKI.

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Fibroblast growth factor 23 (FGF23) is a hormone that promotes excretion of phosphate and decreases the production of 1,25-dihydroxyvitamin D in the kidney.[1,2] Phosphaturic actions of FGF23 depend on the binding to specific receptors in target tissues (FGF receptors [FGFRs] 1–4), with the formation of protein complexes between FGFRs, FGF23, and cofactor protein Klotho.[3–5] It is accepted that osseous tissue is the major source for circulating FGF23[2,6] and is secreted by osteoblasts/osteocytes.[7,8] In addition, previous data showed that bone marrow (BM) expresses FGF23. Transgenic mice expressing enhanced green fluorescent protein under the control of the *Fgf23* gene promoter showed fluorescence in cells surrounding BM sinusoids,[9] and BM erythroid cells presented FGF23 mRNA.[10] Moreover, recent studies deleting the FGF23 gene in early osteoblasts/osteocytes[11] showed that nonosseous tissues contribute to circulating FGF23.[11] However, the role of erythroid progenitor cells (EPCs) in acute changes of FGF23 has not been studied.

Acute kidney injury (AKI) is a syndrome caused by an acute decline of renal function and is associated with poor outcomes.[12–15] The incidence of AKI in hospitalized adults is $\sim$21%,[12,16] increasing >40% in patients admitted to intensive care units (ICUs).[17] Sepsis is the most common cause of AKI.[18] Circulating FGF23 increased in ICU patients with AKI[19–22] and after cardiac surgery.[21,23,24] The mechanisms that cause the increase of FGF23 in AKI are not well understood, but seem to be independent of parathyroid hormone, 1,25-dihydroxyvitamin D, and phosphate.[23,25] Recent studies

indicate that decreased renal clearance of FGF23 and/or increased production by unidentified sources modulated by renal factors[25] may participate. We postulate that BM EPCs, modulated by erythropoietin (EPO), may be a relevant source of FGF23. Under physiological conditions, EPO promotes survival, proliferation, and differentiation of EPCs in BM (burst-forming units erythroid, colony-forming units erythroid [CFU-E]) via the homodimeric EPO receptor (EPOR).[26] In addition to the expression of EPOR and FGF23 mRNA, EPCs present fibroblast growth factor receptors and Klotho mRNA.[10] FGF23 modulates the action of EPO in BM cells[10]; also recent data suggest that EPO stimulates FGF23,[27,28] implying the existence of a physiological link between FGF23 and EPO. Interestingly, circulating EPO increases in ICU patients with AKI,[29,30] suggesting that EPO targets EPC during AKI. However, the EPO effects on BM or circulating FGF23 and its potential role as an inducer of FGF23 in AKI have not been explored.

We hypothesized that an acute increase in plasma EPO induces FGF23 expression in BM erythropoietic cells, contributing to increased circulating FGF23 in AKI. We characterized FGF23 expression in BM EPCs and the effects of EPO in BM expression of FGF23 and plasma FGF23. We studied the source of FGF23 and the role of EPO as a modulator of FGF23 in experimental AKI. To evaluate whether EPO contributes to the rapid increase in FGF23 in AKI patients, we measured plasma FGF23 and EPO in a cohort of septic ICU patients. Our results show that EPO induces FGF23 expression in BM cells via EPOR, and an acute increase in circulating EPO increases plasma FGF23 in AKI.

RESULTS

FGF23 is synthesized in EPCs

We analysed FGF23 expression in BM from mouse tibia and other FGF23-expressing tissues (e.g., osseous tissue, spleen, liver, thymus). To evaluate potential contamination of BM samples with osseous tissue, we measured Col1a1 mRNA, a bone-specific transcript. We found almost undetectable levels in BM (Figure 1a). FGF23 mRNA was abundant in BM and osseous tissue (Figure 1b). The tissue with the highest amount of protein was BM ($P < 0.05$ vs. other tissues [Figure 1c and d]).

EPO promotes erythropoiesis in BM by acting on EPCs via the homodimeric EPO receptor (EPOR).[26] We analyzed FGF23 expression in BM hematopoietic stem and progenitor cells. We separated hematopoietic stem and progenitor cells from BM cells that expressed lineage antigens (Lin^+), specific to terminally differentiated blood cells (CD3, CD11b, CD19, CD45R/B220, Ly6G/C/Gr-1, and TER119). Hematopoietic stem and progenitor cells included hematopoietic stem cells, burst-forming units erythroid, CFU-E and proerythroblasts, and a small fraction of myeloid and lymphoid cells without significant expression of lineage antigens. Hematopoietic stem and progenitor cells showed >4-fold higher amount of FGF23 mRNA compared with whole BM (Figure 1e) and high EPOR expression (Figure 1f). Moreover, FGF23 and EPOR mRNA was barely detectable in Lin^+ cells. Consistently, human hematopoietic cells differentiated from umbilical cord stem cells *in vitro* (Supplementary Figure S1)[31] presented the greatest amount of mRNA at burst-forming units erythroid/CFU-E stage (day 3), associated with EPOR mRNA (Figure 1g and h). The amount of FGF23 mRNA decreased at day 6, whereas EPOR mRNA increased to the highest levels expected for CFU-E and proerythroblasts. These data indicated that FGF23 is transiently expressed during early erythropoiesis.

EPO upregulated FGF23 via EPOR in BM

EPO actions in target tissue are mediated via homodimeric EPOR and/or heterodimeric EPOR/CD131 receptors. Therefore, we tested whether these receptors modulated FGF23 mRNA *ex vivo*. Mice BM cells were incubated with recombinant human (rh) EPO (rhEPO) for 2 hours. The proto-oncogene serine/threonine-protein kinase-1 (Pim1) mRNA transcript upregulated by EPO *via* EPOR served as a positive control.[32] As expected, rhEPO increased Pim1 mRNA, an effect prevented by co-incubation with an EPOR antagonist (EMP9). In contrast, a specific EPOR/CD131 heterodimeric receptor agonist (ARA290) did not modify Pim1 mRNA (Figure 2a). Also, rhEPO increased FGF23 mRNA (Figure 2b) and FGF23 concentration in culture medium (Figure 2c), whereas ARA290 had no effect. The induction of FGF23 by rhEPO was completely blocked by EMP9 (Figure 2b and c). These results showed that BM cells secreted FGF23 and that activation of the homodimeric EPO receptor increased FGF23 expression and secretion.

To test the effect of EPO on FGF23 expression *in vivo*, mice were injected with a single dose of rhEPO, and intact FGF23 ($[iFGF23]_P$) and C-terminal FGF23 ($[cFGF23]_P$) in plasma were measured. We found that both $[iFGF23]_P$ and $[cFGF23]_P$ significantly increased at 4 hours post-injection (Figure 2d and e). rhEPO increased Pim1 mRNA levels in BM by ~1.5-fold (Figure 2f) and FGF23 mRNA levels by ~8-fold (Figure 2g) in BM, whereas in osseous tissue, it only increased ~2-fold compared with vehicle-injected mice (Figure 2h). No significant changes were detected in serum phosphate and calcium (Figure 2i and j). These data showed that EPO is a positive modulator of BM FGF23 transcript and circulating FGF23.

Hemorrhagic shock increased circulating EPO, BM FGF23 mRNA, and circulating FGF23

Hemorrhagic shock (HS) caused by severe blood loss induces global ischemia, a rapid increase in plasma EPO,[33,34] and AKI.[35–38] Thus, we evaluated whether HS increased FGF23 in rats with mean arterial pressure–controlled HS for 90 minutes,[37] followed by resuscitation with crystalloid solution (Figure 3a). HS decreased the glomerular filtration rate (GFR) at 2 and 24 hours after HS (Figure 3b and c). HS rats presented a decreased hematocrit at 2 and 24 hours (Figure 3d) and increased blood urea nitrogen at 24 hours (Figure 3e). Plasma EPO showed a rapid increase at 2 hours, which reached >54-fold at 24 hours (Figure 3f).

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