AMP-activated kinase is a regulator of fibroblast growth factor 23 production

Philipp Glosse[1], Martina Feger[1], Kerim Mutig[2], Hong Chen[3], Frank Hirche[1], Ahmed Abdallah Hasan[4], Mohamed M.S. Gaballa[4], Berthold Hocher[4], Florian Lang[3] and Michael Föller[1]

[1]Department of Agricultural and Nutritional Sciences, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany; [2]Department of Anatomy, Charité-Universitätsmedizin Berlin, Berlin, Germany; [3]Department of Physiology I, Eberhard-Karls University of Tübingen, Tübingen, Germany; and [4]Department of Nutritional Sciences, University of Potsdam, Potsdam, Germany

Fibroblast growth factor 23 (FGF23) is a proteohormone regulating renal phosphate transport and vitamin D metabolism as well as inducing left heart hypertrophy. FGF23-deficient mice suffer from severe tissue calcification, accelerated aging and a myriad of aging-associated diseases. Bone cells produce FGF23 upon store-operated calcium ion entry (SOCE) through the calcium selective ion channel Orai1. AMP-activated kinase (AMPK) is a powerful energy sensor helping cells survive states of energy deficiency, and AMPK down-regulates Orai1. Here we investigated the role of AMPK in FGF23 production. *Fgf23* gene transcription was analyzed by qRT-PCR and SOCE by fluorescence optics in UMR106 osteoblast-like cells while the serum FGF23 concentration and phosphate metabolism were assessed in AMPKα1-knockout and wild-type mice. The AMPK activator, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) down-regulated, whereas the AMPK inhibitor, dorsomorphin dihydrochloride (compound C) and AMPK gene silencing induced *Fgf23* transcription. AICAR decreased membrane abundance of Orai1 and SOCE. SOCE inhibitors lowered *Fgf23* gene expression induced by AMPK inhibition. AMPKα1-knockout mice had a higher serum FGF23 concentration compared to wild-type mice. Thus, AMPK participates in the regulation of FGF23 production *in vitro* and *in vivo*. The inhibitory effect of AMPK on FGF23 production is at least in part mediated by Orai1-involving SOCE.

Kidney International (2018) ■, ■–■; https://doi.org/10.1016/j.kint.2018.03.006

KEYWORDS: calcium; FGF23; Klotho; Orai1; phosphate; parathyroid hormone

Correspondence: *Michael Föller, Martin-Luther-Universität Halle-Wittenberg, Institut für Agrar- und Ernährungswissenschaften, Von-Danckelmann-Platz 2, D-06120 Halle (Saale), Germany. E-mail: michael.foeller@landw.uni-halle.de*

Received 28 July 2017; revised 1 March 2018; accepted 8 March 2018

Fibroblast growth factor 23 (FGF23) is a protein mainly produced by osteocytes in bone. Being a classical hormone, FGF23 acts on target organs to which it travels via the bloodstream.[1,2] An important target is the kidney where FGF23 inhibits phosphate transporter NaPi-IIa and *Cyp27b1* expression, the gene coding the key enzyme for the synthesis of calcitriol or $1,25(OH)_2D_3$, the active form of vitamin D.[2–5] Thus, FGF23 lowers the plasma phosphate and calcitriol concentration. FGF23 also acts on the heart, inducing hypertrophy of the left ventricle.[6] The renal receptor for FGF23 depends on the protein Klotho as a coreceptor,[2,7] whereas the cardiac receptor does not.[6] Membrane-bound Klotho has an extracellular domain that can be cleaved off, yielding soluble Klotho. Soluble Klotho can be found in the blood, in urine, and in cerebrospinal fluid and exerts hormone-like effects.[8]

The significance of FGF23 and Klotho goes far beyond the regulation of phosphate metabolism. FGF23- or Klotho-deficient mice have a very short life span and exhibit a wide range of aging-associated features that are also typical of aging humans including muscle, skin, neuronal, metabolic, and cardiovascular abnormalities.[9,10] Massive calcification can be found in most tissues and organs of FGF23- or Klotho-deficient mice and is the result of deranged phosphate metabolism. Consequently, a low vitamin D or low phosphate diet attenuates or even prevents calcification, premature aging, and early death of these mice.[8]

The production of FGF23 by bone cells is regulated by parathyroid hormone (PTH),[11] $1,25(OH)_2D_3$,[12] the iron status,[13] dietary phosphate,[14] and inflammation.[13,15] Proinflammatory cytokines such as tumor necrosis factor α or interleukin-1 up-regulate FGF23.[16] Moreover, the inflammatory transcription factor complex nuclear factor κB is also involved in the formation of FGF23: nuclear factor κB up-regulates Ca^{2+} release-activated Ca^{2+} channel Orai1/stromal interaction molecule 1 (STIM1).[17] Orai1/STIM1–mediated store-operated calcium entry (SOCE) induces the transcription of the *Fgf23* gene.[17] Polycystic kidneys are another source of FGF23 production.[18]

Apart from its role as a hormone, FGF23 has gained broad attention as a disease biomarker.[19] In particular, in chronic kidney disease (CKD),[20] an elevated FGF23 plasma concentration has been observed early before onset of hyperparathyroidism and hyperphosphatemia, typical sequelae of

CKD.[21,22] An elevation of the plasma FGF23 concentration is also observed in cardiovascular, metabolic, and inflammatory diseases,[19] and for several of those diseases, a positive correlation between severity of the disorder and plasma FGF23 has been established. Therefore, FGF23 is under investigation to uncover its suitability as a sensitive biomarker for several acute and chronic clinical conditions.[19]

The 5′–adenosine monophosphate (AMP)–activated protein kinase (AMPK; Enzyme Commission 2.7.11.31) is a serine and threonine protein kinase expressed in all organs and tissues.[23–25] As its name suggests, it is activated by an increase in the cellular AMP level indicating a lack of adenosine triphosphate, the main cellular energy substrate.[23–25] AMPK is a heterotrimer consisting of an α (α1 or α2), β (β1 or β2), and γ (γ1, γ2, or γ3) subunit.[23] The α subunit is catalytically active, whereas the γ subunit binds AMP.[23–25] In addition to binding AMP, the activation of AMPK requires the phosphorylation of the protein, which is accomplished by the tumor suppressor liver kinase B1, another serine and threonine kinase, or by calcium-calmodulin–dependent protein kinase 2, a Ca^{2+}-activated protein kinase. Thus, at least in some cells, Ca^{2+} influx also triggers activation of AMPK.[23–25]

Taken together, AMPK acts as a cellular energy sensor, which protects the cell against energy deficiency.[23–25] It induces energy-providing cellular pathways (e.g., fatty acid oxidation, glycolysis) and inhibits energy-consuming processes (e.g., protein synthesis, lipogenesis).[23–25] AMPK also regulates membrane transport including glucose transporter 4,[26] sodium-dependent glucose transporter 1,[27] or phosphate transporter NaPi-IIa.[28] In addition, AMPK controls ion channels including the epithelial Na^+ channel[29–31] or big potassium K^+ channel.[32] Importantly, AMPK has also been demonstrated to down-regulate Ca^{2+} release-activated Ca^{2+} channel Orai1, which accomplishes SOCE.[33] SOCE is relevant for a broad range of cellular functions including cell proliferation, migration, and differentiation in many cell types.[34,35] Recently, the production of FGF23 has been shown to be dependent on SOCE through Orai1.[17]

In view of the regulation of Orai1 by AMPK and Orai1-dependent formation of FGF23, we investigated whether AMPK is relevant for the production of FGF23.

RESULTS

AMPK down-regulates *Fgf23* expression in UMR106 cells

First, we analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) whether UMR106 osteoblast-like cells express AMPK subunits. As demonstrated in Figure 1a, PCR products specific for *Ampkα1*, *Ampkα2*, *Ampkβ1*, and *Ampkγ1* could readily be detected. Weaker bands for *Ampkβ2* and *Ampkγ2* were observed (Figure 1a). Western blotting confirmed these results at the protein level (Figure 1b). Thus, UMR106 osteoblast-like cells express functional AMPK. Next, we explored in UMR106 cells whether AMPK activity influences FGF23 production. We incubated UMR106 cells with or without AMPK activator 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) and inhibitor compound C and determined *Fgf23* transcripts. AICAR decreased whereas compound C increased *Fgf23* gene expression (Figure 1c). Similar to pharmacological inhibition, the joint small, interfering RNA (siRNA)-mediated silencing of *Ampkα1* and *Ampkα2* genes resulted in a significant increase in *Fgf23* gene expression (Figure 1d). Silencing was effective as specific siRNA reduced *Ampkα1* transcript levels by 62% ± 7% ($n = 7$; $P < 0.001$) relative to nonsense siRNA. Hence, AMPK is a regulator of FGF23 down-regulating the production of this hormone.

The inhibition of Orai1-mediated SOCE participates in the AMPK effect on FGF23 formation

The synthesis of FGF23 is driven by SOCE involving Ca^{2+} release-activated Ca^{2+} channel Orai1 in UMR106 cells.[17] Orai1-mediated SOCE has been demonstrated to be inhibited by AMPK.[33] To test whether AMPK activity influences SOCE in UMR106 cells, we determined intracellular Ca^{2+} by measuring Fura-2-dependent fluorescence. SOCE was estimated from the increase in Ca^{2+}-dependent fluorescence following readdition of Ca^{2+} to the extracellular fluid of UMR106 cells that had been treated with the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) inhibitor thapsigargin in Ca^{2+}-free solution before. Thapsigargin depletes intracellular Ca^{2+} stores, enabling SOCE. According to Figure 2a and c, activation of AMPK with AICAR resulted in reduced SOCE, pointing to an inhibitory effect of AMPK on SOCE in UMR106 cells. Interestingly, also the thapsigargin-induced increase in intracellular Ca^{2+} was moderately, but significantly reduced by AICAR (Figure 2b).

AMPK regulates various ion channels by influencing their membrane abundance. Because Orai1 is relevant for SOCE in UMR106 cells, we investigated whether AMPK activity altered Orai1 expression or membrane insertion or both. As depicted in Figure 2d, AMPK activation with AICAR did not change *Orai1* transcript levels in UMR106 cells. However, AICAR significantly reduced the membrane abundance of Orai1 (Figure 2e). These results suggest that AMPK activity reduced the insertion of Orai1 in the cell membrane of UMR106 cells. Our next series of experiments explored whether the inhibitory effect of AMPK on Orai1-mediated SOCE is required for AMPK to down-regulate *Fgf23* gene expression. Inhibition of AMPK with compound C again elevated *Fgf23* transcript levels (Figure 2f). This effect was significantly and almost completely abolished by SOCE inhibitors 2-aminoethoxydiphenyl borate or MRS 1845 (*N*-Propylargylnitrendipine) or by specific Orai1 inhibitor AnCoA4 (Figure 2f). Thus, the inhibitory effect of AMPK on FGF23 production is dependent on Orai1-mediated SOCE.

FGF23 production is enhanced in AMPKα1-deficient mice

We sought to test whether the regulation of FGF23 by AMPK is also relevant *in vivo*. To this end, we analyzed AMPKα1-deficient mice ($ampk^{-/-}$) and compared them with wild-type mice ($ampk^{+/+}$). First, we determined the serum