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FAP finds FGF21 easy to digest

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Abstract

Fibroblast growth factor 21 (FGF21) is an endocrine hormone that regulates carbohydrate and lipid metabolism. In humans, circulating FGF21 is inactivated by proteolytic cleavage of its C-terminus, thereby preventing signalling through a receptor complex. The mechanism for this cleavage event and the factors contributing to the post-translational regulation of FGF21 activity has previously been unknown. In a recent issue of the *Biochemical Journal*, Zhen et al. have identified fibroblast activation protein (FAP) as the endopeptidase responsible for this site-specific cleavage of human FGF21 (hFGF21), and propose that inhibition of FAP may be a therapeutic strategy to increase endogenous levels of active FGF21.

Keywords

dipeptidyl peptidase IV (DPP-IV); fibroblast activation protein (FAP); fibroblast growth factor 21 (FGF21); proteolytic cleavage

Fibroblast growth factor 21 (FGF21) is an endocrine hormone that is a critical regulator of energy homoeostasis and a potential therapeutic target for treating diabetes and obesity. Pharmacological administration of FGF21 to diabetic and obese animal models markedly improves insulin sensitivity and causes weight loss [1]. Administration of FGF21 analogues to obese humans also improves plasma metabolic profiles [2,3] and significantly increases weight loss [2]. Therefore, considerable effort has been employed to identify the mechanism of FGF21 action and to identify ways to increase its therapeutic efficacy to treat diabetes and obesity.

Circulating levels of FGF21 are produced primarily by the liver, but may also be produced from other tissues during stress [1]. FGF21 plays a critical role in a number of physiological

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processes including the adaptive fasting response [4,5], enhancement of insulin sensitivity during refeeding and overfeeding [6], and the regulation of macronutrient preference [7]. Upon entering circulation, FGF21 functions by signalling to specific tissues that express both a traditional FGF receptor, fibroblast growth factor receptor 1 (FGFR1), and the co-receptor β-Klotho. Although FGFR1 has a broad tissue expression pattern, β-Klotho is expressed in a limited number of metabolic tissues and provides specificity for FGF21 signalling [8]. FGF21 interacts with FGFR1 through its N-terminus and with β-Klotho through its C-terminus. This C-terminal region of FGF21 is essential to activate the receptor complex to initiate signalling [9,10].

Much less is known, however, about the mechanisms regulating FGF21 stability and activity in circulation. The half-life of exogenously administered FGF21 is short, being approximately 0.4–2 h depending on the species and the method of delivery [11-13]. Previous work with mice and monkeys revealed that full-length human FGF21 (hFGF21; 181 amino acids) is rapidly cleaved on its C-terminus between Pro^{171} and Ser^{172} [14,15]. Studies with truncated FGF21 proteins determined that deletion of these ten residues from the C-terminus of FGF21 disrupts its ability to interact with β-Klotho [9,10], thereby eliminating its ability to effectively signal to target tissues. Importantly, the incorporation of site-specific mutations around Pro^{171} eliminates the proteolytic processing of FGF21 at this site and markedly extends protein half-life [15]. These data suggest that a specific protease may be responsible for the proteolytic processing of FGF21, which, if identified, could potentially be targeted to increase endogenous levels of active FGF21.

In this issue of the *Biochemical Journal*, Zhen et al. [16] have identified fibroblast activation protein (FAP) as the protease responsible for the inactivation of circulating FGF21 through the C-terminal cleavage at Pro^{171}. FAP is a serine protease, a member of a larger superfamily of serine hydrolases (>200 genes in humans) that contains a number of enzymes relevant to metabolic disease, including proprotein convertase subtilisin/kexin type 9 (PCSK9) and dipeptidyl peptidase IV (DPP-IV) [17]. Like DPP-IV, FAP is a prolyl peptidase, and by sequence homology is most closely related to DPP-IV [18]. Using MS, the authors identified all of the sites of hFGF21 processing and reported that hFGF21 is cleaved after three proline residues at positions 2, 4 and 171 [16], consistent with previous reports [15,19]. Whereas cleavage at Pro^{171} inactivates FGF21, cleavage at Pro^{2} and Pro^{4} was shown previously not to impair FGF21 function [9,10]. To identify the protease(s) responsible for the proteolytic processing of FGF21, the authors noted that all cleavage events occurred after proline residues, suggesting prolyl peptidases may be involved. Using inhibitors for DPP-IV, the authors then discovered that DPP-IV was responsible for the cleavage of FGF21 at Pro^{2} and Pro^{4}, but not Pro^{171} both *in vitro* and *in vivo*. Three additional prolyl peptidases were then obtained and evaluated on their ability to cleave FGF21. Notably, FAP, but not prolyl endopeptidase [PrEP, also called prolyl oligopeptidase (PrOP)] or lysosomal Pro^{X} carboxypeptidase (PrCP), rapidly cleaved hFGF21 at Pro^{171} [16].

Although FAP expression in healthy tissue is low [20], the authors reported that FAP also circulates in an active form in human and mouse plasma. Plasma FAP concentrations in normal, overweight and obese individuals varied approximately 5-fold between subjects

with no correlation between FAP concentration and body mass index (BMI) being observed [16]. Despite the variation in plasma FAP levels, plasma FAP protein concentration correlated with prolyl endopeptidase activity, suggesting that circulating FAP is active. By using either a FAP-specific inhibitor or FAP immunodepletion from human plasma, the authors determined that proteolysis of hFGF21 at Pro^{171}, but not Pro^{2} and Pro^{4}, was completely abrogated when FAP activity is lost. In addition, whereas hFGF21 is effectively cleaved at Pro^{171} when spiked into plasma from wild-type mice, this effect was completely abolished when using plasma from FAP knockout mice [16]. Recently, another group, Dunshee et al. [21], supported these findings by demonstrating that recombinant FAP cleaves FGF21 at Pro^{171}, that pharmacological inhibition of FAP prevents C-terminal cleavage of FGF21, and that genetic deletion of FAP in mice eliminates hFGF21 cleavage. In addition, Dunshee et al. [21] extended these findings by demonstrating the importance of FAP-mediated cleavage of FGF21 *in vivo* by administering a FAP-specific inhibitor to cynomolgus monkeys. Pharmacological inhibition of FAP in monkeys increased the level of intact endogenous FGF21 more than 3-fold. Together, these data demonstrate that FAP is responsible for the regulation of circulating FGF21 activity *in vivo*.

These two reports raise several interesting points regarding studies involving FGF21. First, it is well documented that plasma FGF21 levels are paradoxically elevated in insulin resistant and obese human and animal models [1]. Although tissues may also be resistant to FGF21 under these conditions, these data reinforce the concept that a significant percentage of total circulating FGF21 is not active [22]. Therefore, observed elevations in human plasma FGF21 levels, typically determined by ELISAs recognizing total FGF21 protein levels, do not necessarily reflect the amount of active FGF21 present in the circulation. To accurately assess FGF21 function, both total and active FGF21 levels should be measured in models of disease or in human studies attributing beneficial effects to increased FGF21 levels. However, Dunshee et al. [21] found that two ELISAs commonly used to assess total FGF21 levels actually detect intact FGF21 with better efficiency than the C-terminally truncated form, suggesting these ELISAs may underestimate total levels of FGF21 in circulation. Therefore, the development of ELISAs which can accurately quantify and discriminate total compared with active FGF21 are needed.

Secondly, both studies also determined that, whereas hFGF21 is cleaved and processed by FAP, mouse FGF21 is not [16,21]. Since FAP has a stringent Gly-Pro sequence requirement [18,23], and since mouse FGF21 has a glutamate residue at the corresponding glycine residue at position 170, mouse FGF21 is resistant to FAP cleavage [16,21]. Therefore, caution should be taken when comparing the physiological regulation of FGF21 in rodent studies to human physiology as the half-lives of intact, endogenous FGF21 may differ. And finally, as noted by Zhen et al. [16],FAP and DPP-IV are closely related and share similarity in their active site. Inhibition of DPP-IV has therapeutic benefits by reducing glucagon-like peptide-1 (GLP-1) degradation and since FAP could be inhibited to reduce FGF21 inactivation, a dual DPP-IV/FAP inhibitor could be utilized or developed to treat metabolic disease. Interestingly, the approved DPP-IV inhibitor linagliptin also inhibits FAP activity (IC_{50} = 89 nM for FAP and IC_{50} = 1 nM for DPP-IV) [24], suggesting that higher doses of linagliptin, which appear to be safely tolerated [25], could be utilized to simultaneously elevate circulating active GLP-1 and FGF21 with a single orally available small molecule.

The work by Zhen et al. [16] and Dunshee et al. [21] represent a key step in the development of small-molecule compounds to increase circulating levels of active FGF21, offering a potential therapeutic alternative to recombinant protein injection to augment FGF21 activity *in vivo*.

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Abbreviations:

DPP-IV	dipeptidyl peptidase IV
FAP	fibroblast activation protein
FGF21	fibroblast growth factor 21
FGFR1	fibroblast growth factor receptor 1
GLP-1	glucagon-like peptide-1
hFGF21	human FGF21

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