Original article

MOLECULAR METABOLISM

Fibroblast activation protein (FAP) as a novel metabolic target

Miguel Angel Sánchez-Garrido [1,2,3], Kirk M. Habegger [4], Christoffer Clemmensen [1,2,3], Cassie Holleman [4], Timo D. Müller [1,2,3], Diego Perez-Tilve [5], Pengyun Li [7], Archita S. Agrawal [7], Brian Finan [1,2,3], Daniel J. Drucker [6], Matthias H. Tschöp [1,2,3], Richard D. DiMarchi [7,**], Alexei Kharitonenkov [7,*]

ABSTRACT

Objective: Fibroblast activation protein (FAP) is a serine protease belonging to a S9B prolyl oligopeptidase subfamily. This enzyme has been implicated in cancer development and recently reported to regulate degradation of FGF21, a potent metabolic hormone. Using a known FAP inhibitor, talabostat (TB), we explored the impact of FAP inhibition on metabolic regulation in mice.

Methods: To address this question we evaluated the pharmacology of TB in various mouse models including those deficient in FGF21, GLP1 and GIP signaling. We also studied the ability of FAP to process FGF21 *in vitro* and TB to block FAP enzymatic activity.

Results: TB administration to diet-induced obese (DIO) animals led to profound decreases in body weight, reduced food consumption and adiposity, increased energy expenditure, improved glucose tolerance and insulin sensitivity, and lowered cholesterol levels. Total and intact plasma FGF21 were observed to be elevated in TB-treated DIO mice but not lean animals where the metabolic impact of TB was significantly attenuated. Furthermore, and in stark contrast to naïve DIO mice, the administration of TB to obese FGF21 knockout animals demonstrated no appreciable effect on body weight or any other measures of metabolism. In support of these results we observed no enzymatic degradation of human FGF21 at either end of the protein when FAP was inhibited *in vitro* by TB.

Conclusions: We conclude that pharmacological inhibition of FAP enhances levels of FGF21 in obese mice to provide robust metabolic benefits not observed in lean animals, thus validating this enzyme as a novel drug target for the treatment of obesity and diabetes.

Keywords FAP; FGF21; DPP4; Metabolic regulation; Obesity; Diabetes

1. INTRODUCTION

Fibroblast activation protein (also known as FAPα or seprase) is a serine oligopeptidase that was originally identified in 1986 as an inducible cell surface glycoprotein F19 [1] and renamed FAP in 1994 based upon its abundance in activated fibroblasts [2]. Subsequently, FAP expression was observed on a variety of cell surfaces in adult tissues [3] and also detected in plasma in a soluble form that lacks the transmembrane domain [4]. Structurally, FAP belongs to the same di-peptidyl protease (DPP) subfamily as DPP4 that also includes DPP2, DPP8, and DPP9 [5]. The enzymatically active form of FAP is purported to be a 170 kD homodimer. FAP is a post-proline dipeptidase but unlike other members of the DPP4 family can also function as an endopeptidase. As such, FAP degrades gelatin and type 1 collagen, as well as several neuropeptides, including B-type natriuretic peptide, peptide YY and substance P [6]. The enrichment of FAP in fibroblasts associated with epithelial malignancies led to the hypothesis that this enzyme may promote oncogenesis [7] via remodeling of the cancer cell microenvironment [8]. Inhibition of FAP impedes tumor progression in various animal models [9,10], but only limited efficacy was observed in cancer patients treated with FAP inhibitors [11,12].

DPP4 serves as a protease that has been successfully inhibited to clinically lower blood glucose. It shares 48% sequence identity with FAP [13]. However, the role of FAP in metabolic regulation has not been studied but some of the known substrates of this enzyme suggest a potential connection. Recently, neuropeptide Y was reported as a physiological substrate of FAP linking it to liver disease [14]. Fibroblast Growth Factor 21 (FGF21), a powerful metabolic regulator in animals and man [15], has also recently been demonstrated to be inactivated by FAP cleavage [16–18]. But the most resounding evidence for FAP involvement in metabolic regulation derives from genetics and the reported phenotype of FAP deficient mice. On normal chow, these animals are indistinguishable from their wild type littermates [19].

[1]Institute for Diabetes and Obesity (IDO), Helmholtz Diabetes Center, Munich, 85748, Germany [2]Division of Metabolic Diseases, Department of Medicine, Technische Universität München, Munich, 85748, Germany [3]German Center for Diabetes Research (DZD), Neuherberg, 85764, Germany [4]Department of Medicine Endocrinology, Diabetes & Metabolism, University of Alabama at Birmingham, Birmingham, AL, 35294, United States [5]Division of Endocrinology, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, 45237, United States [6]Lunenfeld Tanenbaum Research Institute, Mt. Sinai Hospital, University of Toronto, 600 University Ave, TCP5-1004 Mailbox 39, Toronto, Ontario, M5G 1X5, Canada [7]Department of Chemistry, Indiana University, Bloomington, IN, 46405, United States

*Corresponding author. E-mail: alexkhar@indiana.edu (A. Kharitonenkov).

**Corresponding author E-mail: rdimarch@indiana.edu (R.D. DiMarchi).

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However, fed a high fat diet (HFD) they remain lean, glucose tolerant and insulin sensitive. The resistance to diet-induced obesity prompted by the targeted ablation of FAP is comparable in magnitude to what is observed in DPP4 null mice [20]. It thus seems plausible that medicinal inhibition of FAP enzymatic activity might improve metabolism, but this prospect has yet to be explored.

We have pursued the role of FAP in metabolic regulation in mice by application of a well-established oral FAP inhibitor, named talabostat (or PT-100) [21]. We observed in DIO mice that TB robustly lowers body weight, blood glucose, plasma insulin and total cholesterol while improving glucose tolerance and insulin sensitivity. In contrast, these effects are much diminished in lean mice. We also found that TB elevates FGF21 levels in plasma of DIO but not in lean mice. Furthermore, the metabolic efficacy of TB is not observed in animals with tamoxifen-induced deletion of FGF21. We also confirmed that FGF21 is an FAP substrate *in vitro*, acting alone to process the terminal ends of the human protein but inhibited by TB. Mouse FGF21 was observed to be selectively cleaved by FAP at the N-terminus, consistent with previously reported results [17].

Finally, and given that TB may inhibit other DPP4-like enzymes [21,22] we also evaluated the role of incretins in propagating TB in vivo effects. Importantly, and in contrast to FGF21 elevation, no apparent change in total and intact GLP1 plasma levels was observed in TB-treated DIO mice. Furthermore, TB was fully efficacious in GIP-R null mice. Its metabolic effects, however, were partially attenuated in GLP1-R knockout animals suggestive that in addition to FGF21 action GLP1 signaling fractionally contributes to TB in vivo pharmacology.

Collectively, our results indicate FAP modulates FGF21 biology, and that inhibition of this enzyme alone or in combination with DPP4 represents a novel approach to treat metabolic disease.

2. MATERIALS AND METHODS

2.1. Reagents

Talabostat was obtained from Wuhan Golden Wing Industry & Trade Co., Ltd. All other reagents were purchased from Sigma unless otherwise stated.

2.2. FGF21 expression and purification

The human FGF21 gene sequence was optimized based on *E. coli* preferred codons and was synthesized by Integrated DNA Technologies. LIC-SUMO vector was used as an expression vector, which was modified from pet21b (Novagen) by inserting yeast small ubiquitin-like modifier (SUMO) sequence after 6XHis tag and the deletion of MCS region. Amplified LIC-Sumo vector and FGF21 gene fragment were mixed together, treated using In-Fusion HD EcoDry Cloning Plus systems kit and transfected into stellar *E. coli* competent cells (both Clontech). Positive clones were selected on ampicillin/LB agar plate with single colonies picked to amplify plasmids with QIA prep miniprep kit (Qiagen). Plasmid inserts were confirmed by sequencing. Plasmids containing correct sequence were denoted as LIC-SUMO-hFGF21 and used to express FGF21 protein. To do so, the expression vector was transfected into OrigamiB (DE3) *E. coli* cells (Novagen). Transformants were grown in Luria–Bertani (LB) medium containing 50 μg/ml of ampicillin, 25 μg/ml of kanamycin and 5 μg/ml tetracycline at 37 °C until OD_{600nm} of 0.8–1 was reached. At that point the culture temperature was decreased to 25 °C, and isopropyl-D-thiogalactoside (IPTG) at 0.2 mM was added to induce expression. Cells were cultured overnight, and then harvested by centrifugation.

Cell pellets were re-suspended in lysis buffer (20 mM Tris, pH 8.0, 300 mM NaCl, 10 mM imidazole), and sonicated. Lysates were centrifuged at 15,000 rpm for 30 min, and supernatant was loaded onto Ni-NTA column (Qiagen) that were pre-equilibrated with lysis buffer. The column was then washed with 20 mM Tris, pH 8.0, 300 mM NaCl, 20 mM imidazole buffer, and SUMO-FGF21 fusion protein was eluted from the affinity matrix using the buffer containing 20 mM Tris, pH 8.0, 300 mM NaCl, and 500 mM imidazole. SUMO protease was added to the eluate and incubated at 4 °C overnight. The buffer in the digested sample was changed to 20 mM Tris, pH 8.0, and 10% glycerol buffer using Sephadex G-25 desalting column (GE Healthcare), and the protein was purified by Q-Sepharose column (GE Healthcare). The FGF21 protein was eluted from the column using a 50–300 mM NaCl gradient. The fractions were analyzed by SDS-PAGE, and those containing pure FGF21 were collectively pooled. Endotoxin was removed from all FGF21 protein preparations using ToxinEraser™ Endotoxin Removal Kit (GenScript). FGF21 protein that was used in these studies was >95% pure and had endotoxin levels of <0.5 IU/mg of protein or lower.

2.3. Western blotting

Western blots were performed to analyze the ability of FAP to cleave FGF21 at both N- and C-termini. To this end, 25 μM FGF21 protein was incubated with 125 nM FAP enzyme (R&D Systems) at 37 °C for the prescribed times (0, 30, 60 or 90 min) in the presence or absence of 10 μM of Talabostat. The reaction was stopped by addition of Laemmli buffer (Bio-Rad) and heated at 95 °C for 10 min. Samples were subjected to electrophoresis using Mini-PROTEAN TGX 8–16% gel (Bio-Rad) and electro transferred to nitrocellulose membrane (Bio-Rad). The membrane was blocked for 30 min with Membrane blocking solution (Invitrogen) and subsequently probed with respective antibody at room temperature for 4 h. Antibodies used were total, and anti N- and C-terminus specific (Eagle Biosciences). Membranes were incubated overnight with respective HRP conjugated secondary antibody (R&D Systems) at 4 °C and washed with TBST 5 times for 20 min each. The membrane was dipped in Clarity ECL solution (Bio-Rad) and imaged by exposing the blot to an X-ray film.

2.4. Liquid chromatography–mass spectrometry (LC–MS)

In order to check whether mouse FGF21 is susceptible to FAP cleavage, 25 μM mouse FGF21 protein was incubated with or without 125 nM recombinant human FAP (R&D systems) at 37 °C. The protein samples were analyzed by LC–MS (Agilent 1260 Infinity coupled with Agilent 6120 Quadrupole mass spectrometer). 10 μg of protein was analyzed by RP C8 column (kinetex, 2.6 μm, 75 × 4.6 mm) with a linear gradient from 10% aqueous acetonitrile (0.05% TFA) to 80% aqueous acetonitrile (0.05% TFA) over 10 min at a flow rate of 1.0 ml/min.

2.5. Animals and diets

2.5.1. Lean and DIO mice

Eight-week old male C57BL/6J mice (Jackson Laboratories) were given *ad libitum* access to either a chow diet (lean mice), or a high-fat high-sugar diet (HFD, DIO mice) containing 58% kcal from fat (D12331; Research Diets). The mice were maintained at 22 °C on a 12-h light–dark cycle with free access to water. All animals were maintained on such conditions for a minimum of 3 months prior to initiation of the pharmacological studies. Before the beginning of the studies, mice were randomized into treatment groups according to body weight and body composition. All injections and tests were performed during the light cycle with a group size of $n = 8$.

2.5.2. FGF21^{del}, GLP1-R and GIP-R knockout mice

Male FGF21 floxed and tamoxifen inducible *GtRosaER*-Cre mice were obtained from Jackson Laboratories (stocks #022361 and #008463, respectively). Mice were fed a standard chow diet from weaning and then switched to HFD at 10–12 weeks of age. After 10 weeks of HFD lead-in, all mice received three sequential tamoxifen injections (intraperitoneal); 1 mg dose at a concentration of 10 mg/ml in corn oil (Sigma–Aldrich) separated by 48 h each. FGF21-deficiency was induced only in mice containing both floxed and Cre alleles (FGF21$^{\Delta/\Delta}$), while animals with only floxed and Cre alleles served as littermate controls. Pharmacological experiments using these FGF21^{del} mice were performed 2 weeks after the last tamoxifen administration and during the light cycle.

GIP-R null mice were purchased from RIKEN.

GLP1-R knockout mice were a gift from Dan J. Drucker's laboratory.

2.6. Ethical approvals

All animal studies were approved by and performed according to the guidelines of the Institutional Animal Care and Use Committee of the University of Alabama (Birmingham, USA), University of Cincinnati (Cincinnati, USA) and the Helmholtz Zentrum Munchen (Munich, Germany).

2.7. Pharmacological and metabolic studies

2.7.1. Acute TB studies

For the determination of the acute effects of TB on basal glucose levels and food consumption, DIO mice were treated with single doses of the FAP inhibitor. TB was dissolved in sterile phosphate buffered saline (PBS) containing 0.5% 2-hydroxyethyl cellulose (HEC, Sigma Aldrich) and administered at 0.04, 0.2, 1 and 5 mg/kg body weight doses via oral gavage. Tail blood glucose concentrations were determined using a glucometer (TheraSense FreeStyle) before (0) and 1, 3, 6 and 24 h after TB administration. Food intake was also monitored 24 h upon single TB injection.

2.7.2. Chronic TB studies

TB dissolved in sterile phosphate buffered saline (PBS) containing 0.5% 2-hydroxyethyl cellulose (HEC, Sigma Aldrich) was administered via daily oral gavage during the first hours of the light cycle at the indicated doses. Body weight and food intake were recorded every day after the first compound administration. Body composition (fat and lean mass) was measured the day before study initiation and at the end of the experiment by using a nuclear magnetic resonance spectroscopy (EchoMRI). Assessment of energy expenditure and home-cage activity was performed using an indirect calorimetry system (TSE Systems). Following 48 h of adaptation, O_2 consumption and CO_2 production were measured every 10 min for a total of 40 h to determine the respiratory quotient and energy expenditure. Home-cage locomotor activity was determined using a multidimensional infrared light beam system integrated in the calorimetry system. All studies were performed with a group size of $n = 8$.

2.7.3. Glucose and insulin tolerance tests

For the determination of glucose tolerance following chronic treatment, mice were subjected to a glucose tolerance test on day 7 of treatment and 24 h after the last TB dose. Mice were fasted for 6 h, and subsequently they were injected intraperitoneally with 1.5 g glucose per kg body weight for DIO mice and orally with 2 g glucose per kg body weight for FGF21^{del} mice (20% w/v D-glucose (Sigma–Aldrich) in 0.9% w/v saline). Glucose levels were measured in blood collected from the tail veins before (0) and at 15, 30, 60 and 120 min post injection. Insulin sensitivity was assessed in 6 h-fasted mice following an ip injection of 0.75 U of insulin (Actrapid) per kg body weight. Blood glucose levels were measured before (0) and at 15, 30, 60 and 120 min after insulin administration. All glucose concentrations were measured using a handheld glucometer (TheraSense FreeStyle).

2.7.4. Blood parameters measurements

Blood was collected at the indicated times from tail veins or after euthanasia using EDTA-coated microvette tubes (Sarstedt), immediately placed on ice, centrifuged at 5,000 g and 4 °C for 10 min, and plasma was stored at −80 °C until analyzed. Plasma levels of insulin were quantified by using an ultrasensitive ELISA assay (ALPCO Diagnostics). Triglycerides and cholesterol levels in the collected fractions were determined by colorimetric assay (Thermo Fischer Scientific). Total and intact plasma FGF21 and GLP1 levels were measured using ELISA kits (R&D Systems (#MF2100), Eagle Biosciences (#F2131-K01), and EMD Millipore, respectively).

2.8. Statistical analyses

Statistical analyses were performed on data distributed in a normal pattern using one- or two-way ANOVA followed by Tukey post hoc multiple comparison analysis to determine statistical significance among treatment groups. Energy expenditure was analyzed using ANCOVA, with body weight as covariate as previously reported [23]. All results are presented as mean ± SEM, and $P < 0.05$ was considered significant. Group size estimations were based upon a power calculation to minimally yield an 80% chance to detect a significant difference in body weight of $P < 0.05$.

3. RESULTS

To assess the metabolic consequences of FAP inhibition, we initially administered TB to DIO mice via oral gavage at a dose of 5 or 25 mg/kg. Each treatment induced approximately 8% body weight reduction within three days of administration (Figure 1A). Lowering of plasma glucose was also observed, even though the animals at the beginning of treatment were only mildly hyperglycemic (Figure 1B). The maximal effect on glucose was reached already after one day of dosing with animals exhibiting essentially normal glycemia through the two additional days of treatment (Figure 1B). TB administration also induced a profound reduction in food consumption, (data not shown). The absence of any apparent difference in the magnitude of change in body weight and blood glucose at either TB dose, as well as a significant attenuation of food intake suggested that these two initial doses were at the upper end of pharmacological testing and likely unnecessarily high.

Consequently, a dose titration (0.04, 0.2, 1 and 5 mg/kg) was conducted to measure time course of glucose lowering following a single dose of TB in comparably obese mice. The maximal effect on plasma glucose was observed at 6 h after oral gavage of TB, and it was not dose-dependent in these first hours. After 24 h, glucose lowering was enhanced at the 5 mg/kg dose, remained unchanged at 1 mg/kg, and trended back to baseline at the two lower doses (Figure 1C). Importantly, there was no significant effect of TB on food consumption at the two lowest doses and only a trend at 1 mg/kg (Figure 1D) but the highest tested TB dose dramatically reduced food intake in DIO mice when monitored within the first 24 h following initial administration (Figure 1D). This result comes consistent with the impressive pharmacological effects initially observed (Figure 1A and B). Collectively,

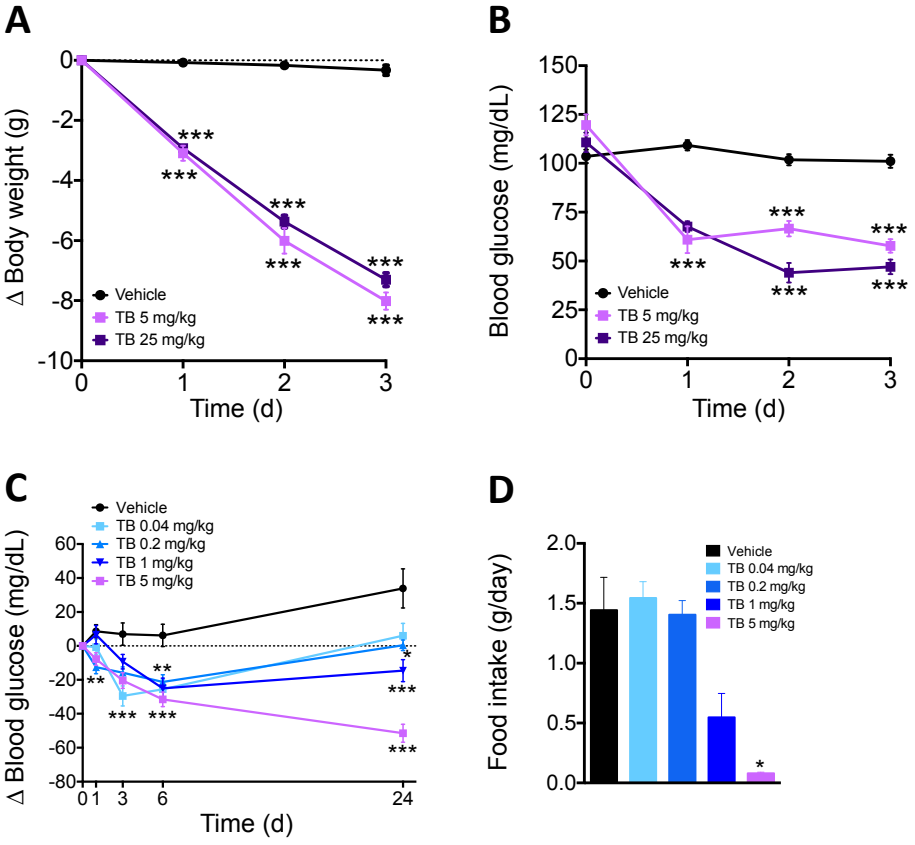

Figure 1: Metabolic consequences of FAP inhibition at high TB doses and acute TB metabolic effects in DIO mice. (**A**–**B**) Effects on (**A**) body weight change and (**B**) *ad libitum*-fed blood glucose of male DIO mice treated daily with vehicle or TB (5 mg/kg and 25 mg/kg) for 3 days. (**C**–**D**) Effects on (**C**) *ad libitum*-fed blood glucose and (**D**) food intake of male DIO mice acutely treated with vehicle or TB (0.04, 0.2, 1 and 5 mg/kg). Data are presented as mean ± SEM; n = 8; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, determined by ANOVA comparing effects following compound administration to vehicle treatment. ANOVA was followed by Tukey *post hoc* multiple comparison analysis to determine statistical significance.

our findings suggested that TB doses of less than 1 mg/kg could improve glucose control without changing food intake, and that higher doses might safely modulate body weight as there was no apparent toxicity throughout this dose range.

To more fully establish the therapeutic index of TB in DIO mice a more extended study employing a daily dose range of 0.03–1 mg/kg was conducted. Within the range of 0.03–0.3 mg/kg, the administration of FAP inhibitor for 16 days induced a subtle reduction in body weight of 3–5% (Figure 2A), with minimal effect on food intake (Figure 2B). There was also a trend to lowered adiposity, without affecting lean mass (Figure 2C). At a 1 mg/kg TB dose, animals lost 15% body weight and ~12% of their adipose tissue (Figure 2A and C). Importantly, this highest TB dose did not affect lean mass (Figure 2C), while food consumption was significantly reduced to less than half of vehicle treated mice (Figure 2B).

The effect of chronic TB administration on glucose handling was assessed through an intraperitoneal glucose tolerance test (ipGTT) after chronic treatment had terminated. The ipGTT was performed at two doses of TB (0.3 and 1 mg/kg) that had delivered appreciable and differing effects on systemic metabolism. The high TB dose markedly improved glucose tolerance when compared to vehicle, and the lower dose was intermediate (Figure 2D). Glucose AUC through 120 min post glucose challenge was proportionally reduced in TB-treated mice in a dose-dependent manner (Figure 2E). Of particular importance, TB also lowered insulin level by half with the effect reaching a nadir at the lowest dose of 0.03 mg/kg (Figure 2F). This finding was indicative of TB-driven improvements in total body insulin sensitivity. To validate this point an insulin tolerance test was conducted at day 7 of treatment. A significantly enhanced sensitivity to insulin action was observed (Figure 2G), that was consistent with the improved glycemic control recorded earlier (Figure 2D and E). Of final note, a dose-dependent reduction in total cholesterol was demonstrated after 7 days of TB therapy (Figure 2H), without any apparent effect on triglycerides (data not shown).

Three recent reports link FAP activity to proteolytic degradation of FGF21 [16–18]. Consequently, we measured the plasma levels of this metabolic regulator and GLP1, as TB can also inhibit DPP4 [21]. Of note, after chronic administration TB induced a sizable dose-dependent elevation in FGF21 levels, with the effect on intact protein reaching a maximum increase of six-fold at the 0.3 mg/kg dose (Figure 2I). These results indicate that FAP inhibition in DIO mice promotes elevation of FGF21 circulating concentration by altering some combination of biosynthesis and metabolic clearance. In contrast to the effect on FGF21 there was relatively little to no modulation of total or intact GLP1 plasma levels (Supplemental Figure 1), suggesting the latter to be of little consequence to the observed pharmacology of TB.

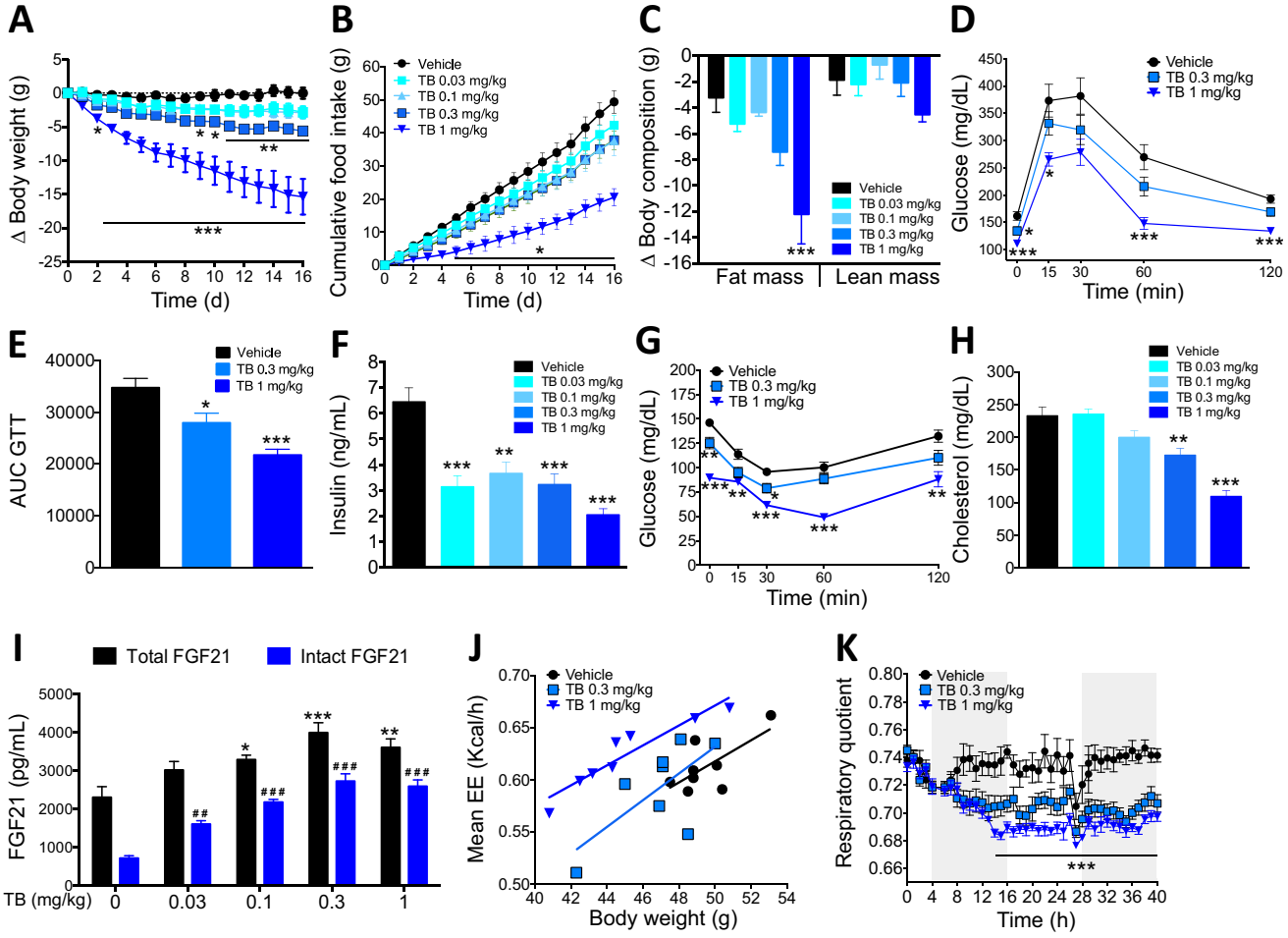

Figure 2: Chronic FAP inhibition by TB induces metabolic and glycemic benefits and enhances plasma half-life of FGF21 in DIO mice. (**A**–**K**) Effects on (**A**) body weight change, (**B**) food intake, (**C**) body composition change, (**D**–**E**) intraperitoneal glucose tolerance, (**F**) plasma insulin, (**G**) insulin tolerance, (**H**) plasma cholesterol, (**I**) total and intact plasma FGF21, (**J**) body weight-corrected energy expenditure and (**K**) real-time respiratory quotient of male DIO mice treated daily with vehicle or TB (0.03, 0.1, 0.3 and 1 mg/kg) for 16 days. In **D**, **E**, **G**, **J** and **K**, only mice treated with vehicle, 0.3 and 1 mg/kg/day were analyzed. The glucose (**D**) and insulin (**G**) tolerance tests were performed in different cohorts of animals at day 7 of treatment. In **K**, shaded regions represent time during the dark cycle of light. Data are presented as mean ± SEM; n = 8; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, determined by ANOVA comparing effects following compound administration to vehicle treatment. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$, determined by ANOVA comparing effect following compound administration to vehicle (intact FGF21 value) treatment. In both comparisons, ANOVA was followed by Tukey *post hoc* multiple comparison analysis to determine statistical significance. Energy expenditure data were analyzed using ANCOVA, with body weight, fat mass and lean mass as covariates. $P = 0.002$ when compared the highest TB dose (1 mg/kg) to vehicle group. $P = 0.003$ when comparing vehicle and both TB doses.

In light of these outcomes, and in the context of the well-known role of FGF21 to regulate energy homeostasis [24,25], we investigated whether the weight-lowering effects of TB pharmacology might impose changes in energy expenditure. Supportive of this expectation, energy expenditure was observed to be elevated in mice administered the 1 mg/kg dose of TB, relative to vehicle-treated mice (Figure 2J). Consistent with the decreased fat mass (Figure 2C), TB treatment also lowered respiratory quotient versus the vehicle (Figure 2K) without any change in locomotor activity (Supplemental Figure 2A and B) while oxygen consumption was increased (Supplemental Figure 2C). This implies that FAP inhibition alters nutrient partitioning as a result of enhanced fat oxidation.

To evaluate the contribution of elevated plasma FGF21 on the metabolic effects of FAP inhibition, we treated FGF21 deficient mice ($FGF21^{del}$) and their wild type littermates with TB. The existence of FGF21-deficiency in $FGF21^{del}$ mice was confirmed in *ad libitum* and fasting conditions (Supplemental Figure 3). In this study we used a single TB dose of 0.3 mg/kg as it had been validated as efficacious in our earlier experiments (Figure 2) and devoid of a food intake effect observed with TB treatment at the higher doses (Figure 2B). Through 7 days of TB treatment, wild type mice lost ~3 g of body weight (Figure 3A) mainly via reductions in fat mass (Figure 3B) representing a nearly 10% reduction, which slightly exceeds prior observations in conventional DIO mice (Figure 2A). In contrast, TB failed to reduce body weight and adiposity in $FGF21^{del}$ mice (Figure 3A and B). No TB effect on lean mass was observed in either strain of mice (Figure 3B).

Fasting glucose levels were elevated in $FGF21^{del}$ mice relative to wild type littermates, which is consistent with the known physiology of FGF21 to regulate glucose homeostasis in rodents [26,27]. Interestingly, TB induced a trend ($P = 0.06$) to lower fasted blood glucose in wild type mice (Figure 3C), and this effect was absent in $FGF21^{del}$ animals (Figure 3C). Furthermore, when wild type TB-treated mice were subjected to an oral GTT on day 7 of study, they showed clear

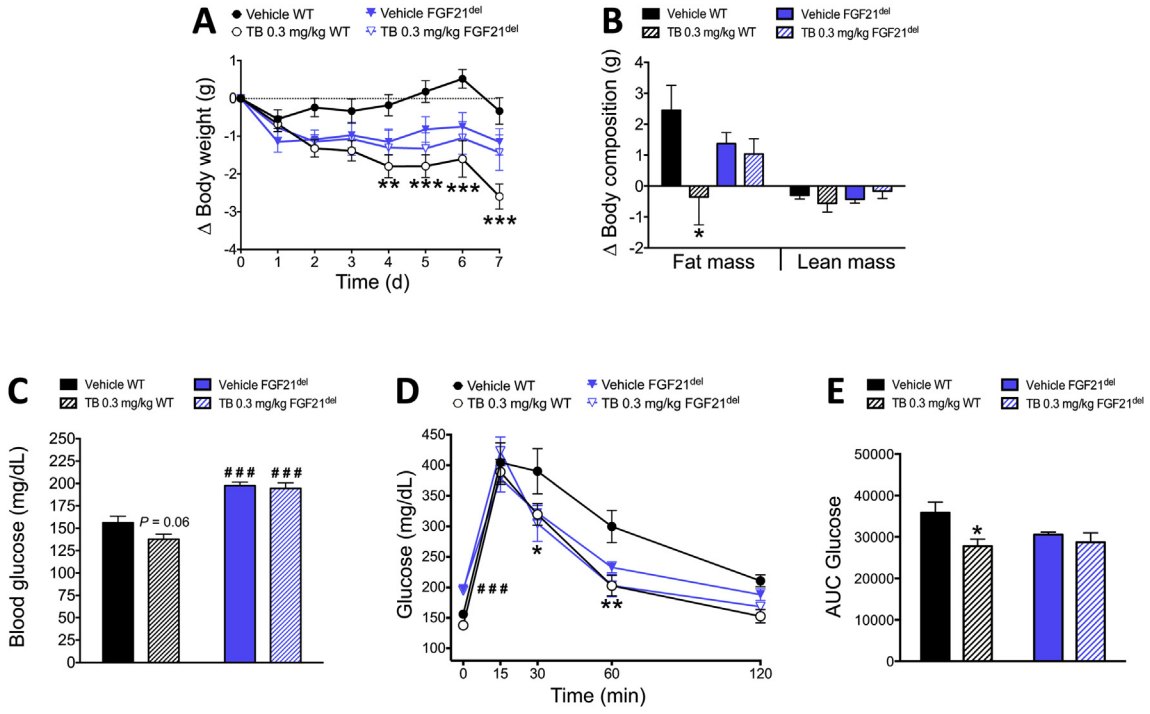

Figure 3: The metabolic and glycemic benefits of FAP inhibition are blunted in FGF21^{del} mice. (**A**—**E**) Effects on (**A**) body weight change, (B) body composition, (**C**) fasting blood glucose and (**D**—**E**) oral glucose tolerance of male FGF21^{del} mice treated daily with vehicle or TB (0.3 mg/kg) for 7 days. Data are presented as mean ± SEM; n = 8; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, determined by ANOVA comparing effects following compound administration to vehicle treatment. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$, determined by ANOVA comparing effect of FGF21 ablation to wild type mice. In both comparisons, ANOVA was followed by Tukey *post hoc* multiple comparison analysis to determine statistical significance.

improvements in glucose disposal compared to vehicle-treated mice (Figure 3D—E). Collectively, the pharmacology associated with FAP inhibition on body weight, fasting glucose, and glucose tolerance was selectively absent in FGF21^{del} mice relative to their genetically unaltered littermates (Figure 3).

We also evaluated TB pharmacology in GIP-R and GLP1-R knockout mice at the same 0.3 mg/kg daily dose. Through 6 days of administration the metabolic effects of TB were fully retained in DIO animals with GIP-R ablation. Partial attenuation of TB efficacy to reduce body weight and food intake, but not plasma glucose was observed in GLP1-R null vs. wild type mice (Supplemental Figures 4 and 5). This latter result was larger than predicted by the recorded changes in intact GLP1 and suggested that the changes recorded are contributing a meaningful, but fractional contribution to the observed pharmacology. While the magnitude of weight loss in DIO mice was encouraging, it also raised the concern as to whether TB could induce unwanted reduction in lean mass in non-obese animals. To address this uncertainty the inhibitor in a dose range of 0.03—3 mg/kg was administered to lean mice of average weight 19 g for 12 days. At all doses tested, TB induced just a subtle reduction (0.5—1.5 g) in body weight (Figure 4A). This minor effect was also transient and observed only within the first 5 days of administration at 0.3, 1 and 3 mg/kg doses, with body weights returning with sustained administration to that of vehicle-treated mice. Noteworthy, in lean mice TB had no significant effect on food intake (Figure 4B), or body composition (Figure 4C), and did not induce hypoglycemia (data not shown). It should be noted that the TB dose range in this study at the high end exceeded doses that were efficacious in DIO animals (Figure 2). Additionally, there was no TB-dependent upregulation of plasma FGF21 levels in lean animals (Figure 4D), as observed in DIO mice (Figure 2I).

Our data in GIP-R and GLP1 null mice coupled with the lack of TB effects in FGF21-deficient animals (Figure 3, Supplemental Figures 4 and 5) was suggestive that TB pharmacology is to a large degree dependent on FAP inhibition and its consequent effect on circulating FGF21 levels. To further this conclusion an enzymatic assay was used to confirm that FAP can digest human FGF21 at both the N- and C-termini, and that TB prevented such processing (Figure 4E). Furthermore, consistent with the work of others [16—18] spiking of human FGF21 into mouse and human sera also led to FGF21 N- and C-terminal degradation, with FAP inhibition once again blocked the proteolytic processing (data not shown).

Finally, we subjected mouse FGF21 to an enzymatic FAP assay. While FAP does not cleave this protein at the C-terminus due to G170E substitution in the rodent sequence [16—18], approximately 40% of the N-terminal tetrapeptide (YPIP) in mouse FGF21 was clipped by FAP after 3 h incubation confirming a prior report [17]

4. DISCUSSION

FGF21 is a potent regulator of energy homeostasis in animals and in man. Pre-clinically FGF21 pharmacology provides multiple benefits which include glucose lowering, amelioration of obesity via reduced adiposity, normalization of lipids and improved longevity [15]. When tested in diabetic patients FGF21-based analogs profoundly improve dyslipidemia, insulin resistance and lower body weight [28—30].

The pharmacodynamic action of FGF21 is purportedly shortened by its proteolytic cleavage in plasma [31] that renders the protein inactive

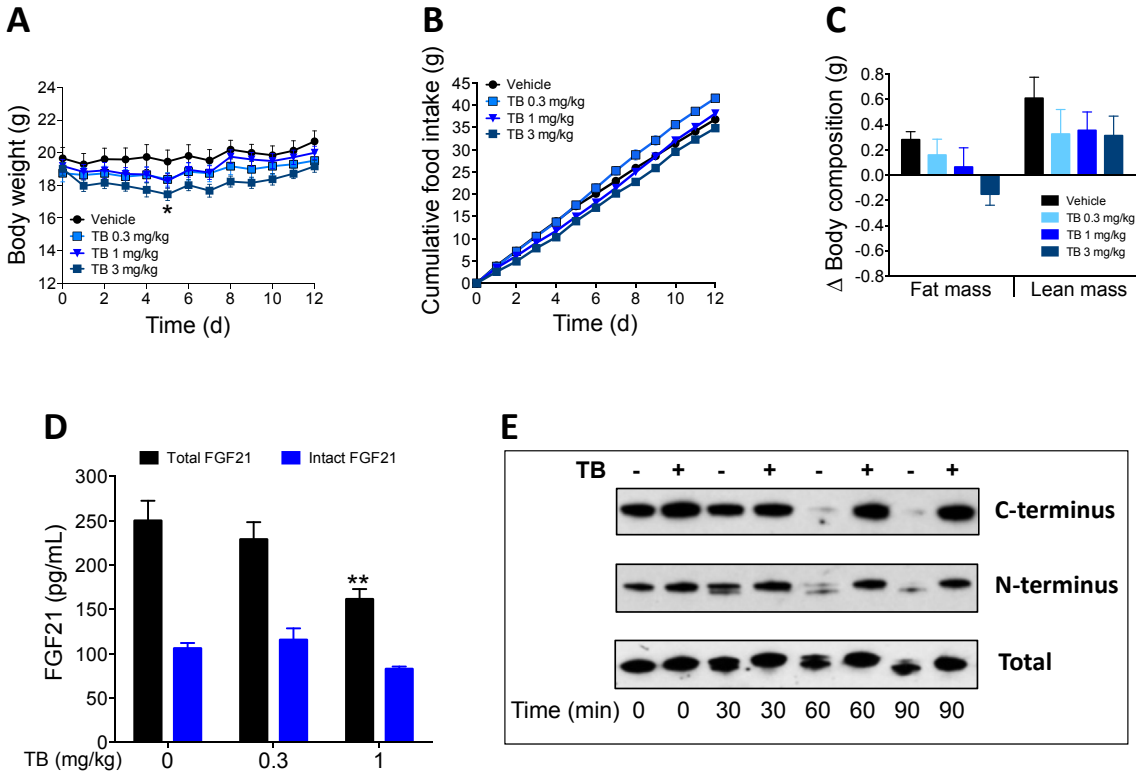

Figure 4: Chronic FAP inhibition by TB does not have any metabolic influence and does not alter plasma half-life of FGF21 in lean mice. (**A**–**D**) Effects on (**A**) body weight, (**B**) food intake, (**C**) body composition change and (**D**) total and intact plasma FGF21 of male lean mice treated daily with vehicle or TB (0.3, 1 and 3 mg/kg) for 12 days. (**E**) Effect of recombinant FAP on FGF21 cleavage *in vitro*. hFGF21 was incubated with recombinant human FAP in the presence or absence of TB for the indicated times in PBS buffer. The products of the enzymatic reaction were resolved on SDS-PAGE and detected with total, N- and C-terminal specific FGF21 antibodies in Western blots. Data are presented as mean ± SEM; n = 8; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$, determined by ANOVA comparing effects following compound administration to vehicle treatment. ANOVA was followed by Tukey *post hoc* multiple comparison analysis to determine statistical significance.

[32,33]. In the absence of knowing which enzymes are responsible for its inactivation, protein structural optimization has served to stabilize FGF21 analogs [34,35].

The metabolic phenotype of FAP null mice when fed a high fat diet [20] resembles that of FGF21 transgenic animals [36], and as such recruited our attention to FAP as a potential candidate to inactivate FGF21. FAP is a classical N-terminal di-peptidase that can also function as a post-proline endopeptidase [6]. N-terminal HPIP [37] and post-proline cleavages (P171) [31] are signature degradation points in human FGF21, and as such our interest in FAP as a candidate protease was deeply enriched.

To evaluate the impact of pharmacological FAP inactivation on metabolism, we used a previously described small molecule FAP inhibitor, talabostat (TB). It should be mentioned that this small molecule is not selective to FAP and may also inhibit other DPP4 peptidases [21,22]. As DPP4 action is closely linked to incretin biology [13,38,39], we have profiled TB effects in the animal models of GLP1 and GIP signaling deficiency to complement experimentation in naïve and FGF21 null mice. Finally, and of important note, TB was pursued earlier as an anti-cancer treatment and was found to be sufficiently safe to support repeat dose human clinical trials [40]. In that regard, we believe it was doubly appealing to test TB as it provides an accelerated route for its use in an investigational human study.

Oral daily dosing of TB within the dose range of 0.1–1 mg/kg range led to profound and dose-dependent lowering of body weight, blood glucose and total cholesterol in DIO mice. These systemic metabolic improvements were correlated with enhancements of glucose tolerance and insulin sensitivity by direct measurements. An increase in energy expenditure without incidence of hypoglycemia was noted. The profound TB pharmacology observed in obese mice was considerably attenuated in lean animals, with only minimal effects on body weight, food intake and blood glucose at doses exceeding efficacious ones used in DIO mice. Importantly, these pharmacological observations are consistent with the phenotypes of FAP null and FGF21 transgenic mice when studied on chow vs. high fat diet, as well as lean and obese mice administered FGF21 [20,36].

To further establish a connection between FAP and FGF21 we examined FGF21 circulating concentration in TB-treated animals. TB therapy elevates plasma FGF21 to therapeutically relevant levels in obese mice [24,41] with the effect being more evident when the native form of FGF21 is measured. The presence of truncated forms of FGF21 in mouse plasma and the ability of TB to change the ratio between the levels of total and intact protein is worthy to mention in light of "FGF21 resistance" in obese animals [42]. Finally, and consistent with the attenuated pharmacology there was however no apparent increase in FGF21 with TB treatment in lean mice, suggesting other downstream mediators associated with elevated body

weight that may influence FGF21 biology. To note, no apparent modulation of either total or active GLP1 plasma levels was observed in this study, but this needs to be considered in the context of pharmacology in GLP1-R KO mice.

The increase in FGF21 expression and plasma levels can be induced by multiple stimuli [43], and our results demonstrating selective FGF21 elevation with TB in DIO animals relative to lean mice is a correlative observation deserving additional mechanistic attention [44]. Nonetheless, to evaluate this observation we tested comparable FAP inhibition in FGF21^{del} mice. Noticeably, no TB effect on body weight, blood glucose and glucose tolerance was observed in FGF21-deficient mice. This further strengthens the argument that the metabolic improvements of FAP inhibition are causally connected to FGF21 action.

In the course of this work three independent reports demonstrated that FAP can degrade FGF21 *in vitro* [16–18]. To confirm these observations, we evaluated the ability of FAP to cleave human and mouse FGF21 in a cell-free enzymatic assay. Using commercially available FGF21 antibodies we found that human FGF21 is clipped at both N- and C-termini when co-incubated with FAP enzyme, and this effect is totally blocked by TB. Similar results were obtained in adding human FGF21 to mouse and human serum, consistent with observations made in the recent reports [16–18]. Thus, FAP cleaves the N-terminal HPIP sequence and ten C-terminal amino acids following Pro171 in human FGF21, with the latter truncation known to impair FGF21 activity [32,33]. This enzyme, however, can only clip YPIP N-terminal sequence in mouse FGF21. Overall, our enzymatic data are consistent with the recent findings of others [16–18] and establish FAP as a unique protease to cleave FGF21.

The mechanism behind TB-induced elevation in plasma FGF21 levels in obese mice is not known but worthy of a follow-up examination. While FGF21 is considered to be a starvation hormone [45–47], the maximal FGF21 level was achieved at 0.3 mg/kg dose at which TB does not affect food intake in mice. Thus, it is sufficiently clear that the increase in FGF21 in our study is not related to undernutrition of such mice. Yet whether this effect is primarily driven by increased FGF21 production, inhibition of clearance, or some combination of the two remains an uncertainty. Given that C-terminal truncation greatly diminishes FGF21 interaction with its co-receptor KLB [32] it is plausible that FAP inhibition may interfere with receptor-mediated clearance. However, it should be noted that the impact of C-terminal truncation alone on half-life in circulation is rather subtle [18]. Also, it should be mentioned that mouse FGF21 is protected from cleavage at the C-terminus due to G170E mutation in rodents [16–18], and yet we observed an elevation in endogenous FGF21 levels in obese mice (Figure 2I). Therefore it is reasonable to suspect that FAP-induced N-terminal clipping of FGF21 may constitute a primary signal affecting clearance of this protein in mice.

While TB is a non-selective FAP inhibitor [21,22] the pharmacological effects of this small molecule are clearly not a simple function of DPP4-inhibition. In our study, TB affects metabolic parameters in wild-type DIO mice in an acute and chronic manner, and when pharmacological doses of 1 mg/kg or below are administered. In contrast, pharmacology of selective DPP4 inhibitors, such as sitagliptin, is complex in rodents and observed only at suprapharmacologic doses delivered for several weeks to an elaborate animal model [39], or a glucose tolerance test is required [13]. As opposed to FGF21, no apparent TB-dependent change in levels of GLP1, a natural DPP4 substrate, was observed in our report. Furthermore, TB pharmacology was also fully retained in GIP-R null mice, and lost in FGF21^{del} animals. Nevertheless, a measurable attenuation of TB effects in GLP1-R knockout animals toward body weight and food intake requires further evaluation and indeed is suggestive of an opportunity to simultaneously target FAP and DPP4 using combinational therapy or a "dual action" small molecule.

In summary, we demonstrate here that the FAP inhibitor, talabostat, as a single agent, provides body weight reduction, glucose control, insulin sensitization, cholesterol lowering, increased energy expenditure associated with elevated plasma FGF21 levels. The absence of any effect on glucose or weight reduction in FGF21-deficient mice supports our hypothesis that FGF21 is a central mediator of the efficacy delivered by TB therapy. All of these effects are substantially attenuated in lean mice suggesting the ability to selectively inhibit FAP without overt toxicity. Furthermore, the fact that TB has been used in human clinical study provides an accelerated path for translating these observations to a meaningful clinical experiment. We conclude that FAP inhibition offers appreciable promise as a novel therapeutic target for the treatment of abnormalities associated with Type 2 Diabetes and the metabolic syndrome. The success in inhibiting DPP4 provides precedent to this approach and conceivably could be used in combination with FAP inhibitors, or potentially as single molecules that serve to selectively inhibit precisely these two enzymes.

AUTHOR CONTRIBUTIONS

MASG, KMH, CC, CH, DPT performed all in vivo studies. PL expressed and purified FGF21, ASA and AK performed *in vitro* studies and FGF21/GLP1 ELISA measurements. DJD provided GLP1-R null mice. BF, TDM, MHT, RDD and AK analyzed the data. MASG, KMH, DPT, BF, RDD and AK wrote and edited the script. MHT, RDD and AK conceptualized and designed the studies.

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CONFLICT OF INTEREST

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2016.07.003.

REFERENCES

[1] Rettig, W.J., Chesa, P.G., Beresford, H.R., Feickert, H.J., Jennings, M.T., Cohen, J., et al., 1986. Differential expression of cell surface antigens and glial fibrillary acidic protein in human astrocytoma subsets. Cancer Research 46: 6406–6412.

[2] Rettig, W.J., Su, S.L., Fortunato, S.R., Scanlan, M.J., Raj, B.K., Garin-Chesa, P., et al., 1994. Fibroblast activation protein: purification, epitope mapping and induction by growth factors. International Journal of Cancer Journal International du Cancer 58:385–392.

[3] Keane, F.M., Yao, T.W., Seelk, S., Gall, M.G., Chowdhury, S., Poplawski, S.E., et al., 2013. Quantitation of fibroblast activation protein (FAP)-specific protease activity in mouse, baboon and human fluids and organs. FEBS Open Bio 4:43–54.

[4] Lee, K.N., Jackson, K.W., Christiansen, V.J., Lee, C.S., Chun, J.G., McKee, P.A., 2006. Antiplasmin-cleaving enzyme is a soluble form of fibroblast activation protein. Blood 107:1397–1404.

[5] Liu, R., Li, H., Liu, L., Yu, J., Ren, X., 2012. Fibroblast activation protein: a potential therapeutic target in cancer. Cancer Biology & Therapy 13:123–129.

[6] Keane, F.M., Nadvi, N.A., Yao, T.W., Gorrell, M.D., 2011. Neuropeptide Y, B-type natriuretic peptide, substance P and peptide YY are novel substrates of fibroblast activation protein-alpha. The FEBS Journal 278:1316–1332.

[7] Garin-Chesa, P., Old, L.J., Rettig, W.J., 1990. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. Proceedings of the National Academy of Sciences of the United States of America 87:7235–7239.

[8] Brennen, W.N., Isaacs, J.T., Denmeade, S.R., 2012. Rationale behind targeting fibroblast activation protein-expressing carcinoma-associated fibroblasts as a novel chemotherapeutic strategy. Molecular Cancer Therapeutics 11:257–266.

[9] Cheng, J.D., Valianou, M., Canutescu, A.A., Jaffe, E.K., Lee, H.O., Wang, H., et al., 2005. Abrogation of fibroblast activation protein enzymatic activity attenuates tumor growth. Molecular Cancer Therapeutics 4:351–360.

[10] Adams, S., Miller, G.T., Jesson, M.I., Watanabe, T., Jones, B., Wallner, B.P., 2004. PT-100, a small molecule dipeptidyl peptidase inhibitor, has potent antitumor effects and augments antibody-mediated cytotoxicity via a novel immune mechanism. Cancer Research 64:5471–5480.

[11] Narra, K., Mullins, S.R., Lee, H.O., Strzemkowski-Brun, B., Magalong, K., Christiansen, V.J., et al., 2007. Phase II trial of single agent Val-boroPro (Talabostat) inhibiting Fibroblast Activation Protein in patients with metastatic colorectal cancer. Cancer Biology & Therapy 6:1691–1699.

[12] Hamson, E.J., Keane, F.M., Tholen, S., Schilling, O., Gorrell, M.D., 2014. Understanding fibroblast activation protein (FAP): substrates, activities, expression and targeting for cancer therapy. Proteomics Clinical Applications 8:454–463.

[13] Mulvihill, E.E., Drucker, D.J., 2014. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. Endocrine Reviews 35: 992–1019.

[14] Wong, P.F., Gall, M.G., Bachovchin, W.W., McCaughan, G.W., Keane, F.M., Gorrell, M.D., 2016. Neuropeptide Y is a physiological substrate of fibroblast activation protein: enzyme kinetics in blood plasma and expression of Y2R and Y5R in human liver cirrhosis and hepatocellular carcinoma. Peptides 75: 80–95.

[15] Kharitonenkov, A., Adams, A.C., 2014. Inventing new medicines: the FGF21 story. Molecular Metabolism 3:221–229.

[16] Dunshee, D.R., Bainbridge, T.W., Kljavin, N.M., Zavala-Solorio, J., Schroeder, A.C., Chan, R., et al., 2016. Fibroblast activation protein cleaves and inactivates fibroblast growth factor 21. The Journal of Biological Chemistry.

[17] Zhen, E.Y., Jin, Z., Ackermann, B.L., Thomas, M.K., Gutierrez, J.A., 2015. Circulating FGF21 proteolytic processing mediated by fibroblast activation protein. The Biochemical Journal.

[18] Coppage, A.L., Heard, K.R., DiMare, M.T., Liu, Y., Wu, W., Lai, J.H., et al., 2016. Human FGF-21 is a substrate of fibroblast activation protein. PLoS One 11:e0151269.

[19] Niedermeyer, J., Kriz, M., Hilberg, F., Garin-Chesa, P., Bamberger, U., Lenter, M.C., et al., 2000. Targeted disruption of mouse fibroblast activation protein. Molecular and Cellular Biology 20:1089–1094.

[20] Gorrell M, Song S, Wang X. Novel metabolic disease therapy. US20120053222; 2012.

[21] Ryabtsova, O., Jansen, K., Van Goethem, S., Joossens, J., Cheng, J.D., Lambeir, A.M., et al., 2012. Acylated Gly-(2-cyano)pyrrolidines as inhibitors of fibroblast activation protein (FAP) and the issue of FAP/prolyl oligopeptidase (PREP)-selectivity. Bioorganic & Medicinal Chemistry Letters 22:3412–3417.

[22] Chen, S.J., Jiaang, W.T., 2011. Current advances and therapeutic potential of agents targeting dipeptidyl peptidases-IV, -II, 8/9 and fibroblast activation protein. Current Topics in Medicinal Chemistry 11:1447–1463.

[23] Tschop, M.H., Speakman, J.R., Arch, J.R., Auwerx, J., Bruning, J.C., Chan, L., et al., 2012. A guide to analysis of mouse energy metabolism. Nature Methods 9:57–63.

[24] Coskun, T., Bina, H.A., Schneider, M.A., Dunbar, J.D., Hu, C.C., Chen, Y., et al., 2008. Fibroblast growth factor 21 corrects obesity in mice. Endocrinology 149: 6018–6027.

[25] Xu, J., Lloyd, D.J., Hale, C., Stanislaus, S., Chen, M., Sivits, G., et al., 2009. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. Diabetes 58:250–259.

[26] Badman, M.K., Koester, A., Flier, J.S., Kharitonenkov, A., Maratos-Flier, E., 2009. Fibroblast growth factor 21-deficient mice demonstrate impaired adaptation to ketosis. Endocrinology 150:4931–4940.

[27] Assini, J.M., Mulvihill, E.E., Burke, A.C., Sutherland, B.G., Telford, D.E., Chhoker, S.S., et al., 2015. Naringenin prevents obesity, hepatic steatosis, and glucose intolerance in male mice independent of fibroblast growth factor 21. Endocrinology 156:2087–2102.

[28] Gaich, G., Chien, J.Y., Fu, H., Glass, L.C., Deeg, M.A., Holland, W.L., et al., 2013. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metabolism 18:333–340.

[29] Dong, J.Q., Rossulek, M., Somayaji, V.R., Baltrukonis, D., Liang, Y., Hudson, K., et al., 2015. Pharmacokinetics and pharmacodynamics of PF-05231023, a novel long-acting FGF21 mimetic, in a first-in-human study. British Journal of Clinical Pharmacology.

[30] Talukdar, S., Zhou, Y., Li, D., Rossulek, M., Dong, J., Somayaji, V., et al., 2016. A long-acting FGF21 molecule, PF-05231023, decreases body weight and improves lipid profile in non-human primates and Type 2 diabetic subjects. Cell Metabolism 23:427–440.

[31] Hager, T., Spahr, C., Xu, J., Salimi-Moosavi, H., Hall, M., 2013. Differential enzyme-linked immunosorbent assay and ligand-binding mass spectrometry for analysis of biotransformation of protein therapeutics: application to various FGF21 modalities. Analytical Chemistry 85:2731–2738.

[32] Micanovic, R., Raches, D.W., Dunbar, J.D., Driver, D.A., Bina, H.A., Dickinson, C.D., et al., 2009. Different roles of N- and C- termini in the functional activity of FGF21. Journal of Cellular Physiology 219:227–234.

[33] Yie, J., Hecht, R., Patel, J., Stevens, J., Wang, W., Hawkins, N., et al., 2009. FGF21 N- and C-termini play different roles in receptor interaction and activation. FEBS Letters 583:19–24.

[34] Hecht, R., Li, Y.S., Sun, J., Belouski, E., Hall, M., Hager, T., et al., 2012. Rationale-based engineering of a potent long-acting FGF21 analog for the treatment of type 2 diabetes. PLoS One 7:e49345.

[35] Weng, Y., Chabot, J.R., Bernardo, B., Yan, Q., Zhu, Y., Brenner, M.B., et al., 2015. Pharmacokinetics (PK), pharmacodynamics (PD) and integrated PK/PD modeling of a novel long acting FGF21 clinical candidate PF-05231023 in diet-induced obese and leptin-deficient obese mice. PLoS One 10:e0119104.

[36] Kharitonenkov, A., Shiyanova, T.L., Koester, A., Ford, A.M., Micanovic, R., Galbreath, E.J., et al., 2005. FGF-21 as a novel metabolic regulator. The Journal of Clinical Investigation 115:1627–1635.

[37] Kharitonenkov, A., Beals, J.M., Micanovic, R., Strifler, B.A., Rathnachalam, R., Wroblewski, V.J., et al., 2013. Rational design of a fibroblast growth factor 21-based clinical candidate, LY2405319. PLoS One 8:e58575.

[38] Xu, J., Wei, L., Mathvink, R., Edmondson, S.D., Mastracchio, A., Eiermann, G.J., et al., 2006. Discovery of potent, selective, and orally bioavailable pyridone-based dipeptidyl peptidase-4 inhibitors. Bioorganic & Medicinal Chemistry Letters 16:1346–1349.

[39] Mu, J., Woods, J., Zhou, Y.P., Roy, R.S., Li, Z., Zycband, E., et al., 2006. Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves

pancreatic beta-cell mass and function in a rodent model of type 2 diabetes. Diabetes 55:1695—1704.

[40] Cunningham, C.C., 2007. Talabostat. Expert Opinion on Investigational Drugs 16:1459—1465.

[41] Xu, J., Stanislaus, S., Chinookoswong, N., Lau, Y.Y., Hager, T., Patel, J., et al., 2009. Acute glucose-lowering and insulin-sensitizing action of FGF21 in insulin-resistant mouse models—association with liver and adipose tissue effects. American Journal of Physiology Endocrinology and Metabolism 297: E1105—E1114.

[42] Fisher, F.M., Chui, P.C., Antonellis, P.J., Bina, H.A., Kharitonenkov, A., Flier, J.S., et al., 2010. Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. Diabetes 59:2781—2789.

[43] Adams, A.C., Kharitonenkov, A., 2012. FGF21: the center of a transcriptional nexus in metabolic regulation. Current Diabetes Reviews 8:285—293.

[44] Habegger, K.M., Stemmer, K., Cheng, C., Muller, T.D., Heppner, K.M., Ottaway, N., et al., 2013. Fibroblast growth factor 21 mediates specific glucagon actions. Diabetes 62:1453—1463.

[45] Inagaki, T., Dutchak, P., Zhao, G., Ding, X., Gautron, L., Parameswara, V., et al., 2007. Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metabolism 5:415—425.

[46] Badman, M.K., Pissios, P., Kennedy, A.R., Koukos, G., Flier, J.S., Maratos-Flier, E., 2007. Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metabolism 5:426—437.

[47] Lundasen, T., Hunt, M.C., Nilsson, L.M., Sanyal, S., Angelin, B., Alexson, S.E., et al., 2007. PPARalpha is a key regulator of hepatic FGF21. Biochemical and Biophysical Research Communications 360:437—440.

