Original Article

MOLECULAR METABOLISM

FGF21 mimetic antibody stimulates UCP1-independent brown fat thermogenesis via FGFR1/βKlotho complex in non-adipocytes

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ABSTRACT

Objective: Fibroblast Growth Factor 21 (FGF21) is a potent stimulator of brown fat thermogenesis that improves insulin sensitivity, ameliorates hepatosteatosis, and induces weight loss by engaging the receptor complex comprised of Fibroblast Growth Factor Receptor 1 (FGFR1) and the requisite coreceptor βKlotho. Previously, recombinant antibody proteins that activate the FGFR1/βKlotho complex were proposed to act as an FGF21-mimetic; however, in vivo action of these engineered proteins has not been well studied.

Methods: We investigated the mechanism by which anti-FGFR1/βKlotho bispecific antibody (bFKB1) stimulates thermogenesis in UCP1-expressing brown adipocytes using genetically engineered mice. Anti-FGFR1 agonist antibody was also used to achieve brown adipose tissue restricted activation in transgenic mice.

Results: Studies with global *Ucp1*-deficient mice and adipose-specific *Fgfr1* deficient mice demonstrated that bFKB1 acts on targets distal to adipocytes and indirectly stimulates brown adipose thermogenesis in a UCP1-independent manner. Using a newly developed transgenic system, we also show that brown adipose tissue restricted activation of a transgenic FGFR1 expressed under the control of *Ucp1* promoter does not stimulate energy expenditure. Finally, consistent with its action as a FGF21 mimetic, bFBK1 suppresses intake of saccharin-containing food and alcohol containing water in mice.

Conclusions: Collectively, we propose that FGFR1/βKlotho targeted therapy indeed mimics the action of FGF21 in vivo and stimulates UCP1-independent brown fat thermogenesis through receptors outside of adipocytes and likely in the nervous system.

Keywords FGF21; Therapeutic antibody; Agonist antibody; Thermogenesis; Brown adipose tissue

1. INTRODUCTION

Obesity is recognized as a leading cause for the development of chronic diseases such as type 2 diabetes mellitus, non-alcoholic steatohepatitis (NASH), cardiovascular disease, and various forms of cancer [1–3]. Under the condition of continual excess calorie consumption, white adipose tissues (WAT) accumulate lipids beyond its innate storage capacity leading to low-grade inflammation, endoplasmic reticulum stress, fibrosis, and insulin resistance [4]. In obese individuals, ectopic lipids also accumulate in non-adipose organs such as liver and skeletal muscle, exacerbating hyperinsulinemia and dyslipidemia, the hallmarks of the metabolic syndrome [5–7]. Therapeutic agents that correct energy imbalance and improve insulin sensitivity by promoting energy expenditure (EE) without significant adverse effects may present a novel approach to treating obesity and its comorbidities. The recent re-discovery of functional thermogenic brown adipose tissues (BAT) in adult humans has led to the examination of this metabolic tissue as a potential pharmacological target for the treatment of obesity [8–12]. Similar to WAT, BAT consists mainly of adipocytes; however, unlike WAT, which acts as a repository for energy storage, BAT serves as an endogenous heating element through combustion of macronutrients [13]. The expression of the inner-mitochondrial protein uncoupling protein 1 (UCP1) defines thermogenic brown adipocytes by its ability to dissipate heat by uncoupling proton transport from ATP synthesis [14]. The stimulation of human BAT activity by mild cold exposure correlates with an increase in the whole body resting metabolic rates, indicating a functional significance

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Abbreviations: AUC, area under the curve; BAT, brown adipose tissue; ECD, extracellular domain; EE, energy expenditure; eWAT, epididymal white adipose tissue; FDG, fluorodeoxyglucose; FGF21, Fibroblast Growth Factor 21; FGFR1, Fibroblast Growth Factor Receptor 1; HFD, high-fat diet; iBAT, interscapular brown adipose tissue; HMW, high molecular weight; ingWAT, inguinal white adipose tissue; KLB, βKlotho; MAPK, mitogen-activated protein kinase; NASH, non-alcoholic steatohepatitis; UCP1, uncoupling protein 1; WAT, white adipose tissue

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[15]. In adult humans, BAT can be found most prominently in the supraclavicular area but is also found in other regions in the upper body [10,11]. In rodents, BAT can be found throughout the body, but most prominently in the interscapular region (interscapular BAT or iBAT) [16].

Fibroblast Growth Factor 21 (FGF21) is an endocrine member of the FGF super family that has been identified as a regulator of BAT thermogenesis and nutrient metabolism [17,18]. Recombinant FGF21 protein and its modified forms exhibit potent anti-obesity and insulin-sensitizing effects associated with increased BAT thermogenesis in rodents [19,20]. Certain aspects of the anti-obese and insulin sensitizing effects of these molecules have also been observed in non-human primates and humans [21–25]. FGF21 functions as a ligand for the receptor complexes consisting of FGFR and βKlotho (KLB) [26]. While FGF21 is capable of activating three FGFR isoforms (1c, 2c, and 3c) in tandem with KLB [27], FGFR1c appears to play the predominant role in mediating its metabolic effects in obese mice. First, *aP2*-CRE-mediated *Fgfr1* gene deletion almost completely abolishes the metabolic effects of recombinant FGF21 [28,29]. Second, the engineered humanized effector-less bispecific antibody bFKB1 selectively binds and activates the FGFR1/KLB co-receptor complex and largely mimics the action of recombinant FGF21 in mice [30]. In addition, bFKB1 and a different FGFR1/KLB agonist antibody induced weight loss in non-human primates [29,30]. Thus, FGFR1 activation is necessary and sufficient for the metabolic action of FGF21, although FGFR2 and FGFR3 have not yet be ruled out in contributing to the action of FGF21. Both long-acting FGF21 analogs and agonist FGFR1/KLB antibodies have been or are currently being investigated in clinical trials in Type 2 diabetics and obese individuals (e.g., ClinicalTrials.gov, NCT02413372, NCT02538874, NCT02593331, NCT02708576, NCT03060538).

Although pre-clinical and clinical studies suggest that FGF21 analogs or FGFR1/KLB-targeting antibodies may become an effective therapy for obesity related disorders via the ability to improve whole body energy metabolism, the mechanism by which FGFR1/KLB activation leads to BAT thermogenesis remains elusive. Historically the UCP1 protein has been identified as the primary conduit for generating non-shivering thermogenesis through its ability to produce heat from futile cycling [13,14]. Previously, three groups independently tested the activity of FGF21 in *Ucp1* deficient (KO) mice and suggested that the metabolic improvement by FGF21 is independent of UCP1, although the results regarding the requirement of UCP1 in increasing EE showed disagreements [31–33]. Additionally, the tissues that mediate the FGFR1/KLB receptor response are unclear. Much of the early evidence implicated adipose tissue as the central mediator of this effect. The metabolic benefits of FGF21 were absent in lipodystrophic mice [34,35]. Furthermore, it was found that deletion of the *Klb* gene in adipocytes utilizing the *aP2*-CRE system blocks the acute insulin sensitizing effects of FGF21 [36]. Similarly, two independent studies with *aP2*-CRE-mediated *Fgfr1* gene deletion reported the loss of elevated EE and the beneficial metabolic effects associated with the pharmacological administration of FGF21 [28,29]. This conclusion was consistent with the tissue expression pattern of *Fgfr1* and *Klb* mRNA that are co-expressed at high levels in both white and brown adipose tissues [27,37]. Likewise, treatment of cultured primary subcutaneous adipocytes by FGF21 or bFKB1 increases *Ucp1* mRNA expression, supporting the model of direct adipocyte-mediated action [30,38]. At the same time, *Klb* mRNA can also be detected in distinct regions of the brain [39], and selective deletion of the gene in the *Camk2a*-CRE background abolishes the metabolic action of FGF21 [40]. Collectively, these studies suggested that the receptors expressed in adipocytes and specific neurons in the brain are both necessary for the anti-obese and anti-diabetic actions of FGF21.

In the present study, we employ loss-of-function and gain-of-function genetic analyses in mice to address the mechanism and the site of action of FGFR1/KLB agonists using the anti-FGFR1/KLB antibody bFKB1 as a model. We demonstrate that *Ucp1* is not essential for bFKB1 to stimulate BAT thermogenesis in obese mice. Using adipocyte-selective *Fgfr1* gene knockout and a newly developed system to achieve cell-type specific activation of FGFR1 signaling with an agonist antibody, we also find that activation of FGFR1 in brown adipocyte is neither necessary nor sufficient for BAT stimulated EE. Finally, we show that bFKB1, similar to FGF21, alters sweet and alcohol preference, suggesting the role of the nervous system in the action of this antibody. Taken together, our data offers evidence that bFKB1 indeed acts as an FGF21 mimetic protein whose in vivo metabolic activity originates outside of the adipocytes.

2. MATERIALS AND METHODS

2.1. Recombinant proteins

The generation of bFKB1 and 14B6 was described previously [30]. bFKB1 has the human IgG1 backbone with the knob-hole and the effector-attenuating NG modifications. 14B6 has the mouse IgG2a backbone with the effector-attenuating DANG modifications [41]. Recombinant FGFR1 extracellular domain (ECD) proteins were produced either in *E. Coli* or CHO cells and purified using conventional methods.

2.2. Animal studies

All animal studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH) (NIH Publication 8523, revised 1985). The Institutional Animal Care and Use Committee (IACUC) at Genentech reviewed and approved all animal protocols. All the mice were maintained in a pathogen-free animal facility under standard 12 h light/12 h dark cycle at 21 °C with access to normal chow (Labdiet 5010) or high-fat diet (HFD; Harlan Teklad TD.06414, 58.4% calories from fat) and water *ad libitum* unless otherwise indicated. All the mice used for intervention studies were around 2–6 months old and were randomized into groups based on body weight, blood glucose levels, and/or specific parameters being examined at the pre-dose state. All antibody doses administered were at a concentration of 10 mg/kg and delivered via intraperitoneal injection.

2.3. Mouse strains

Ucp1 KO mice in C57BL/6J background [42] or the control wild type (WT) mice were bred at The Jackson Laboratory and housed at 30 °C throughout the study after transferring to the Genentech mouse facility. Adipose-specific *Fgfr1* CKO mice were generated by crossing the exon 4 floxed *Fgfr1* CKO mice [43] with *Adipoq*-CRE mice [44,45], both obtained from The Jackson Laboratory. To generate *Ucp1-hmFGFR1c* transgenic mice, a plasmid encoding chimeric FGFR1c (D1 domain from human FGFR1 and the rest from mouse FGFR1) was constructed, linearized, and used for pronuclear injection. This chimeric construct contains 8.4 kb mouse *Ucp1* upstream sequence, a human/mouse chimeric cDNA followed by an SV40 pA signal and 4.3 kb downstream *Ucp1* genomic region (mouse exons 3–6) [46]. A line that selectively expresses *hmFGFR1c* in iBAT was selected by qPCR using human *FGFR1* specific primers (5′-aaccaaaccccgtagctccatat-3′ and 5′-gtcccactggaagggcat-3′).

