Original article

MOLECULAR METABOLISM

Skeletal muscle salt inducible kinase 1 promotes insulin resistance in obesity

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ABSTRACT

Objective: Insulin resistance causes type 2 diabetes mellitus and hyperglycemia due to excessive hepatic glucose production and inadequate peripheral glucose uptake. Our objectives were to test the hypothesis that the proposed CREB/CRTC2 inhibitor salt inducible kinase 1 (SIK1) contributes to whole body glucose homeostasis *in vivo* by regulating hepatic transcription of gluconeogenic genes and also to identify novel SIK1 actions on glucose metabolism.

Methods: We created conditional (floxed) SIK1-knockout mice and studied glucose metabolism in animals with global, liver, adipose or skeletal muscle *Sik1* deletion. We examined cAMP-dependent regulation of SIK1 and the consequences of SIK1 depletion on primary mouse hepatocytes. We probed metabolic phenotypes in tissue-specific SIK1 knockout mice fed high fat diet through hyperinsulinemic-euglycemic clamps and biochemical analysis of insulin signaling.

Results: SIK1 knockout mice are viable and largely normoglycemic on chow diet. On high fat diet, global SIK1 knockout animals are strikingly protected from glucose intolerance, with both increased plasma insulin and enhanced peripheral insulin sensitivity. Surprisingly, liver SIK1 is not required for regulation of CRTC2 and gluconeogenesis, despite contributions of SIK1 to hepatocyte CRTC2 and gluconeogenesis regulation *ex vivo*. *Sik1* mRNA accumulates in skeletal muscle of obese high fat diet-fed mice, and knockout of SIK1 in skeletal muscle, but not liver or adipose tissue, improves insulin sensitivity and muscle glucose uptake on high fat diet.

Conclusions: SIK1 is dispensable for glycemic control on chow diet. SIK1 promotes insulin resistance on high fat diet by a cell-autonomous mechanism in skeletal muscle. Our study establishes SIK1 as a promising therapeutic target to improve skeletal muscle insulin sensitivity in obese individuals without deleterious effects on hepatic glucose production.

Keywords SIK1; Salt inducible kinase; CRTC; Gluconeogenesis; Insulin resistance; CREB

1. INTRODUCTION

Maintenance of glucose homeostasis is fundamental to mammalian survival. Multiple molecular mechanisms driven by endocrine hormones contribute to appropriate timing of glucose liberation and synthesis when food is scarce and glucose uptake and storage when food is plentiful. The liver is the primary source of endogenous glucose production during fasting, when the catabolic hormone glucagon stimulates hepatic glycogen breakdown as well as gluconeogenesis by activating transcription of rate-limiting gluconeogenic enzymes and transcriptional regulators including *Pepck, G6pase* and *Pgc1α* [1]. After feeding, insulin stimulates glucose uptake and storage primarily

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Abbreviations: AKT, protein kinase B; AMPK, AMP-activated protein kinase; BAT, brown adipose tissue; cAMP, cyclic adenosine monophosphate; CHX, cycloheximide; CREB, cAMP response element-binding protein; CRTC, CREB regulated transcription coactivator; $EndoR_a$, endogenous rate of glucose appearance; FGF21, fibroblast growth factor 21; FOXO1, forkhead box protein O1; FSK, forskolin; G6pase, glucose 6-phosphatase; GDR, glucose disposal rate; GIR, glucose infusion rate; Glgn, glucagon; GTT, glucose tolerance test; Glut, glucose transporter; HDAC, histone deacetylase; HFD, high fat diet; HSP, heat shock protein; IBMX, 3-isobutyl-1-methylxantine; ITT, insulin tolerance test; Pepck, phosphoenolpyruvate carboxykinase; Pgc, peroxisome proliferator-activated receptor gamma coactivator; PTT, pyruvate tolerance test; SIK, salt inducible kinase; WAT, white adipose tissue

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in skeletal muscle and suppresses hepatic glucose production [2]. When animals become obese and insulin resistant, blood glucose becomes poorly controlled due to the combination of excess hepatic glucagon action and multi-tissue insulin resistance. The increasing prevalence of pre-diabetes and type 2 diabetes has resulted in an urgent need to identify new therapeutic strategies to improve insulin sensitivity.

The cAMP response element binding protein (CREB) directly stimulates expression of hepatic gluconeogenesis genes and is required to maintain fasting blood glucose levels in mice [3]. cAMP-regulated transcriptional co-activator 2 (CRTC2) is a key component of activated CREB complexes on *Pepck, G6pase* and *Pgc1α* promoters and contributes to the hepatic fasting gluconeogenic response [4–7]. In insulin resistant rodents, in which hepatic CREB/CRTC activity becomes elevated [8], acute knock-down of CREB or CRTC2 in liver normalizes blood glucose [9,10]. Global CRTC2 knockout mice fed high fat diet have improved insulin sensitivity, possibly secondary to reduced hepatic glucose production [6]. CRTC subcellular localization and subsequently activity are regulated by AMPK-related kinases. In particular, salt-inducible kinases (SIK1-3) directly phosphorylate CRTCs on 14-3-3 binding sites and thereby cause cytosolic retention [11]. In addition to SIKs, MARK2 and AMPK itself also phosphorylate CRTCs on the same sites [4,12,13].

Among CRTC kinases, SIK1 is unique in that it is a direct CREB target gene in liver, skeletal muscle, adrenal cortical cells and neurons [14]. SIK1 is therefore thought to constitute an intrinsic inhibitory feedback circuit to efficiently stop CREB target gene expression after the original stimulus ends, such as after re-feeding when glucagon and catecholamine signaling decline. Indeed, adenoviral knockdown of *Sik1* in liver was sufficient to increase CREB activity and blood glucose levels [4]. However, in obese *db/db* mice, *Sik1* mRNA increases in several tissues including liver [15], yet hepatic CRTC2 activity remains abnormally high [8]. It is therefore clear that, at least in some settings, SIK1 is not required to regulate CRTC2. Indeed, a recently reported global SIK1 knockout mouse strain did not exhibit hyperglycemia but rather improved insulin secretion. SIK1 was found to catalyze activating phosphorylation of PDE4D in beta cells; SIK1 knockout increased intracellular cAMP in beta cells and potentiated glucose-stimulated insulin secretion [16]. However, these studies were conducted on global SIK1 knockout mice, so tissue specific effects may not have been apparent.

Sik1 is broadly expressed, indicating that it may have multiple roles in physiology. We previously showed that in skeletal muscle, SIK1 maintains MEF2 activity by catalyzing inhibitory phosphorylation on class II HDAC kinases [17]. This pathway is also operant during myoblast differentiation, when SIK1 accumulates by transcriptional and post-translational mechanisms [18]. In adult muscle, *Sik1* expression is acutely induced by strenuous exercise training [19], as well as by over-nutrition (obese *db/db*) [15] and acute muscle injury [20]. However, nothing is known about how skeletal muscle SIK1 contributes to metabolic homeostasis or how SIK1 may exert distinct functions in other metabolic tissues because to date no conditional *Sik1* knockout model has been available.

To analyze cell-autonomous and cell non-autonomous roles of *Sik1* in glucose homeostasis *in vivo*, we generated conditional *Sik1* knockout mice lacking exons encoding the catalytic kinase domain. Here we show that genetic deletion of *Sik1* in all tissues does not result in hyperglycemia or increased hepatic gluconeogenesis *in vivo,* but rather a marked improvement in glucose tolerance, peripheral insulin sensitivity and skeletal muscle glucose uptake on high fat diet. Liver *Sik1* deletion alone did not de-repress gluconeogenesis, despite the fact that isolated hepatocytes lacking SIK1 showed elevated transcription of gluconeogenic genes and glucose output. *Sik1* mRNA is elevated in skeletal muscle of HFD-fed mice, and skeletal muscle-specific SIK1-KO mice, but not liver or adipose tissue SIK1-KO, have enhanced insulin sensitivity after HFD feeding. We therefore identify skeletal muscle as the site of SIK1 action required for development of full insulin resistance in obesity and provide the first evidence that SIK1 is a promising therapeutic target to improve peripheral insulin sensitivity in obese individuals.

2. METHODS

2.1. Mice

Generation of *Sik1* conditional KO mice (MGI accession nos. 5648544, 5648545, 5648836) and crosses to generate tissue-specific lines are described in Supplementary Material and Supplementary Table T1. Male animals aged 8–30 weeks were used for metabolism studies. Knockout mice were backcrossed 3–7 generations to C57Bl6/J, at which point they were 94–99% C57Bl6/J based on SNP mapping (Charles River MaxBax Mouse 384 SNP panel). Animals were housed at 22 °C in individually ventilated cages with a 12 h light/dark cycle (9 AM–9PM) with free access to water and irradiated chow diet (LabDiet 5053). Animals were fasted in cages with synthetic bedding at 5 PM (overnight) or 9 AM (6 h). *Ad lib* blood samples were collected between 9 AM and 2 PM from conscious mice by tail or submandibular bleed. Blood glucose was tested with a glucometer (OneTouch Ultra). Animals were fed 60% high fat diet (HFD, TestDiet 58G9) for up to 20 weeks. Glucose and pyruvate tolerance tests (GTT, PTT) were performed on overnight fasted mice, 1–1.5 g/kg glucose or 2 g/kg sodium pyruvate IP. For insulin tolerance tests (ITT), mice were fasted for 6 h followed by IP insulin (HumulinR®) injection (0.75–1 U/kg). For acute insulin signaling studies, mice were fasted for 4–6 h, injected IP with 0.75 (chow diet) or 1.5 (HFD) U/kg insulin and euthanized 15 min later. Recombinant AAV (AAV2/8-TBG-GFP or AAV2/8-TBG-Cre, 1.5×10^{11} vector genomes per animal, Penn Vector Core) was injected into the tail vein of 8–10 week old mice $\geq$7 days prior to testing. Body composition was determined by ECHO-MRI. Hyperinsulinemic-euglycemic clamps were performed in a similar manner to prior studies [21] on overnight fasted, unrestrained HFD-fed mice using a continuous infusion of regular insulin (Humulin R, 8 mU/min/kg body weight). Cold glucose (25%) was infused at a variable rate throughout the experiment to clamp blood glucose levels between 100 and 140 mg/dL (see also Supplementary Material). HPLC purified [3-^{3}H]glucose was infused as previously described to determine glucose fluxes [21]. For glucose uptake during clamps, a 10 μCi bolus of [1-^{14}C]2-deoxyglucose was injected 45 min before the end of the clamp, with blood samples collected at intervals over 45 min. Skeletal muscle glucose uptake during the clamp was calculated from the plasma [^{14}C]2-deoxyglucose profile fitted with a double exponential curve and the tissue content of [^{14}C]2-deoxyglucose-6-phosphate. Alternatively, ^{18}F-labelled 2-deoxyglucose (Cyclotope, Houston TX) was injected (0.1 nmol, ~0.4 mg/kg) into the tail vein of anesthetized mice fed HFD for 18 weeks followed immediately by an IP injection of insulin (0.75 mg/kg). 30 min later, animals were euthanized and tissues were collected for ^{18}F quantification in a gamma counter, with data reported as % injected dose/g tissue weight.

2.2. Primary cells

Primary hepatocytes were prepared from anesthetized mice by hepatic perfusion with type IV collagenase (Sigma, C5138, 120 U/mL) as described [22]. Where indicated, hepatocytes were infected with

