Involvement of SIK2/TORC2 signaling cascade in the regulation of insulin-induced PGC-1α and UCP-1 gene expression in brown adipocytes

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Muraoka M, Fukushima A, Viengchareun S, Lombès M, Kishi F, Miyaucci A, Kanematsu M, Doi J, Kajimura J, Nakai R, Uebi T, Okamoto M, Takemori H. Involvement of SIK2/TORC2 signaling cascade in the regulation of insulin-induced PGC-1α and UCP-1 gene expression in brown adipocytes. Am J Physiol Endocrinol Metab 296: E1430 –E1439, 2009. First published April 7, 2009; doi:10.1152/ajpendo.00024.2009.—Salt-inducible kinase 2 (SIK2) was identified as an isoform of regulated CREB activity (TORC2). Phosphorylation at Ser587 mediated gene expression by phosphorylating the coactivator transducer of cAMP-response element-binding protein (CREB) and represses cAMP-response element-binding protein (C/EBP) gene expression(s) for enzyme catalyzing carnitine palmitoyltransferase-1 (CPT-1), and for thermogenesis, e.g., PGC-1α and uncoupling protein-1 (UCP-1), and for thermogenesis, e.g., PGC-1α and uncoupling protein-1 (UCP-1), are also induced by the cAMP-PKA cascade (10, 64). The activation of cAMP-response element (CRE)-binding protein (CREB) is essential for adipocyte differentiation, especially in the early stage, by inducing C/EBPβ (33, 51). The inhibition of CREB in 3T3-L1 white adipocytes by overexpression of a dominant-negative CREB results in cell death because of a reduction in AKT expression (52). In BAT, CREB regulates thermogenic gene expression in cooperation with other factors, such as activating transcription factor 2 (ATF2), C/EBPα/β, and nuclear receptors (8, 10, 11, 17, 39, 46, 61).

Salt-inducible kinase 2 (SIK2) was identified as an isoform of the SIK family and was found to be highly expressed in mice (29) and human (20) adipocytes. SIK family kinases belong to the AMP-activated protein kinase (AMPK) family and negatively regulate CREB activity by phosphorylating the CREB-specific coactivator transducer of regulated CREB activity (TORC) (13, 31, 55). When PKA activates CREB, it simultaneously inactivates SIK2 by means of phosphorylation at prososome proliferator-activated receptor-γ (PGC-1α) was identified as a factor induced by cold stimulation in BAT and was found to perform essential functions in the regulation of mitochondrial biogenesis (49, 50). It has been suggested that impairment of signaling cascades involved in the regulation of PGC-1α gene expression and protein activation causes obesity and insulin resistance in human and animal models (12, 14, 21, 23, 24, 37, 54, 58).

The cAMP cascade is important for the regulation of adipocyte functions. When adipocytes are stimulated by β3-adrenergic signaling, cAMP-dependent protein kinase A (PKA) is first activated by cAMP and then phosphorylates hormone-sensitive lipase and perilipin A, a lipid droplet-associated protein (2, 9). The other adipose-enriched lipase, adipose triglyceride lipase, is also activated by the phosphorylation of perilipin A (4, 43). These PKA-dependent phosphorylation cascades initiate lipolysis and triglyceride catabolism.

Gene expression(s) for enzyme catalyzing β-oxidation, e.g., carnitine palmitoyltransferase-1 (CPT-1), and for thermogenesis, e.g., PGC-1α and uncoupling protein-1 (UCP-1), are also induced by the cAMP-PKA cascade (10, 64). cAMP signaling is eventually blunted by its own feedback mechanisms, such as induction of phosphodiesterase (PDE) 4/8 (19, 41). Insulin signaling apparently acts as an adversarial cascade for cAMP in white adipocytes, e.g., by activating PDEs (15, 66), whereas it has been found to upregulate UCP-1 gene expression in brown adipocytes via insulin receptor substrate-1/phosphatidylinositol 3-kinase (PI 3-kinase)-dependent v-AKT murine thymoma viral oncogene homolog (AKT) signaling (60).

The activation of cAMP-response element (CRE)-binding protein (CREB) is essential for adipocyte differentiation, especially in the early stage, by inducing C/EBPβ (33, 51). The inhibition of CREB in 3T3-L1 white adipocytes by overexpression of a dominant-negative CREB results in cell death because of a reduction in AKT expression (52). In BAT, CREB regulates thermogenic gene expression in cooperation with other factors, such as activating transcription factor 2 (ATF2), C/EBPα/β, and nuclear receptors (8, 10, 11, 17, 39, 46, 61).

Salt-inducible kinase 2 (SIK2) was identified as an isoform of the SIK family and was found to be highly expressed in mice (29) and human (20) adipocytes. SIK family kinases belong to the AMP-activated protein kinase (AMPK) family and negatively regulate CREB activity by phosphorylating the CREB-specific coactivator transducer of regulated CREB activity (TORC) (13, 31, 55).
Ser\(^{587}\) in the COOH-terminal domain (29), which reduces the TORC phosphorylation activity of SIK2 (35, 59).

Impairment of SIK2 signaling in the liver, e.g., by knockdown with RNAi and via hyperphosphorylation at Ser\(^{587}\) by PKA, enhances dephosphorylation of TORC2 followed by upregulation of PGC-1\(\alpha\) gene expression, which in turn results in enhanced gluconeogenesis (38, 57). In contrast, phosphorylation of SIK2 at Ser\(^{587}\) by AKT is found to be upregulated TORC2 phosphorylation activity when insulin signaling was activated during refeeding (18). In addition to liver, TORC2 was found to be a potent regulator of the PGC-1\(\alpha\) promoter in muscle (63), although involvement of SIK cascades in CREB regulation in muscle cells remains unclear cells.

SIK2 expression is quickly induced by differentiation stimuli in 3T3-L1 preadipocytes, especially by glucocorticoids, and its high level is maintained in mature adipocytes (29). db/db obese mice express a higher level of SIK2 protein in the white adipose tissue (WAT) than control mice, suggesting that SIK2 in adipocytes may be implicated in obesity. However, overexpression or knockdown analyses of SIK2 in 3T3-L1 adipocytes provided evidence that SIK2 can downregulate expression of various lipogenic genes, such as fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and stearoyl-CoA desaturase 1 (SCD1), when adipocytes are stimulated with nutritional depletion or AMPK agonists (20). This finding seems to be inconsistent with the level of SIK2 protein observed in obese mice.

In the study presented here, we focus on SIK2 functions in the BAT. Results obtained with T37i brown adipocytes, mice with diet-induced obesity, and transgenic mice suggested that insulin inhibits SIK2 activity by enhancing phosphorylation at Ser\(^{587}\), which results in accelerated dephosphorylation of TORC2. The dephosphorylation of TORC2 mat then induce expression of PGC-1\(\alpha\) and UCP-1 genes.

MATERIALS AND METHODS

Chemicals and reagents. Insulin, forskolin, isobutyl methylxanthine, triiodothyronine, and culture media (DMEM and DMEM-F-12) were purchased from Sigma Aldrich (St. Louis, MO), and insulin, triiodothyronine, and culture media (DMEM and DMEM-F-12) supplemented with FCS and triiodothyronine, treated with or without insulin for 30 min, and fixed with 1 ml of formaldehyde/PBS by directly adding it to the medium. After being washed with PBS, cells were harvested and collected by centrifugation at 2,000 rpm for 5 min. Cell pellets were lysed with 150 µl of lysis buffer [1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 µg/ml aprotinin], and DNA was broken by sonication. The resultant solution was diluted with a 10-fold volume of ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl, 1 mM PMSF, and 1 µg/ml aprotinin), and small aliquots were kept as input controls. To avoid nonspecific binding of DNA to a complex of protein A-Sepharose/IgG, the protein A-Sepharose (35 µl) and the IgG (10 µg) were premixed with 50 µg sonicated salmon sperm DNA and 150
μg BSA for 1 h and then recovered by centrifugation. After overnight incubation with protein A-Sepharose/IgG, precipitates were washed with wash buffer(s) (0.1% SDS, 1% Triton X-100, 2 mM EDTA, and 20 mM Tris·HCl, pH 8.1 supplemented with 150 mM NaCl and 500 mM NaCl), LiCl buffer (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, and 10 mM Tris·HCl, pH 8.1), and Tris-EDTA (TE) buffer. The precipitated DNA was eluted with 100 μl of elution buffer (10 mM dithiothreitol, 1% SDS, and 0.1 M NaHCO₃). After addition of 4 ml of NaCl, the solution was incubated at 65°C for 6 h, after which DNA was recovered with a spin column for plasmid purification (Promega, Madison, WI). The proximal region of the PGC-1α promoter was amplified by PCR with the primers 5'-TAAAGCTTACCTCAGGACAGA/5'-AAAAGTAGCCTGG-GCTGTCATCAG. Similarly, the CRE region of the UCP-1 promoter was amplified with 5'-TCTCTGGCCATAATCAGGAAC/5'-CAGGTTCTCAAGAAGCTGAC.

**Real-time PCR analyses.** To examine mRNA expression level in tissues and cultured adipocytes, total RNA was used as the template of the RT reaction with the Transcript TM Kit (Roche Diagnostic, Basel, Switzerland), and the resultant cDNA was used for real-time PCR analyses with the Platinum SyBR Green qPCR Superscript (Invitrogen) supplemented with 10% dimethyl sulfoxide. Real-time PCR analyses were performed by using the MyiQ real-time PCR system (Bio-Rad Laboratories, Hercules, CA). Primers used in this study are listed in Supplementary Figs. 1–5 (Supplemental data for this article can be found on the American Journal of Physiology: Endocrinology and Metabolism website.). Each amplicon was cloned into the pGEM-T easy vector (Promega) and used as the standard. Amplification was performed at 90°C for 20 s, 58°C for 20 s, and 72°C for 20 s.

**Statistical analysis.** Data are presented as the means and SD. Statistical differences between mean values were determined with the two-tailed unpaired Student’s t-test. A P value <0.05 was considered statistically significant.

**RESULTS**

Insulin signaling induces phosphorylation of SIK2 at Ser⁵⁸⁷ in T37i brown adipocytes. We first examined CRE activity in T37i brown adipocytes by using a reporter system consisting of the CRE-luciferase gene. As shown in Fig. 1A, a twofold induction of CRE-luciferase activity was observed after insulin treatment, and the induction was suppressed by overexpression of wild-type SIK2. Suppression by the SIK2 mutant S587A, which constitutively phosphorylated TORC, thus representing

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**Fig. 1. Insulin signaling induces phosphorylation of salt-inducible kinase 2 (SIK2) at Ser⁵⁸⁷.**

A: SIK2 expression vectors [wild type (WT), S587A, K49M, and S358A] and its empty vector [(-): pTarget, 200 ng] were transformed into T37i adipocytes (day 3 after differentiation) with the cAMP-response element (CRE)-luciferase (Luc) reporter or its empty reporter (pTAI, 400 ng) and the Renilla-Luc internal reporter (60 ng). After 24 h incubation without insulin, the cells were treated with insulin (1 μg/ml) for 6 h and then harvested for reporter assays. Firefly luciferase activities were normalized with Renilla luciferase activities, and expressed as fold of the CRE-less empty reporter activities. Data are shown as means and SD (n = 3 experiments). *P < 0.05 and **P < 0.01, NS, not significant. B: T37i adipocytes (day 3 after differentiation) were infected with adenoviruses for lacZ, SIK2 (S587A) and A-cAMP-response element-binding protein (CREB) (at multiplicity of infection = 1,000), and, after 48 h, the cells were treated with insulin (1 μg/ml) for 3 h and then harvested for RNA extraction (n = 3; *P < 0.05 and **P < 0.01 compared with lacZ-transfected cells). PGC-1α, peroxisome proliferator-activated receptor-coactivator-1α; UCP-1, uncoupling protein-1. C: chromatin-immunoprecipitation assay (ChIP) analyses of the proximal promoter regions of PGC-1α and UCP-1 genes. T37i cells were treated with insulin (1 μg/ml) for 1 h and then fixed with glutaraldehyde for ChIP analyses. The antibody against hexokinase not located in the nucleus was used as control IgG. TORC, transducer of regulated CREB activity. D: level of phospho-SIK2 at Ser⁵⁸⁷ after insulin treatment. T37i adipocytes were treated with insulin (1 μg/ml) for the indicated periods and then harvested for immunoprecipitation (IP) using the SIK2 or TORC2 antibody followed by Western blot (WB) analyses. Cell lysate was used for the detection of CREB, v-AKT murine thymoma viral oncogene homologue (AKT), and PGC-1α. Shown are representative sets from triplicate experiments. Signals for pSIK2 (pS587) and SIK2 were quantified by using the Quantity One software (Bio-Rad), and the ratio pSIK2-SIK2 at 0 min was normalized to 1. Means of fold enhancement and SD are indicated. The level of total phosphorylation of TORC2 was evaluated by band position in SDS-PAGE.
a dominant-positive of SIK2, was more efficient than the wild type, whereas the kinase-defective SIK2 mutant K49M failed to suppress induction. The SIK2 mutant S358A, which had been found to be inactive in liver, was as effective as the wild-type SIK2 to impair the insulin-induced CRE reporter activity, suggesting that Ser\textsuperscript{358} might not be essential for the regulation of CRE-dependent transcription in brown adipocytes. Similar results were obtained when forskolin (cAMP agonist) was used as a CRE stimulant (Supplementary Fig. 1A).

Second, we examined mRNA levels of endogenous candidates for TORC2 targets in brown adipocytes. As shown in Fig. 1B, insulin induced PGC-1\textalpha{} and UCP-1 mRNA in T3\textgamma{}i adipocytes, and this induction was inhibited by overexpression of SIK2 (S587A) and dominant-negative CREB (A-CREB), both of which were shown to be able to inhibit TORC2 (13). To confirm whether insulin could form a CREB-TORC complex on the promoters of PGC-1\textalpha{} and UCP-1 genes, we performed chromatin immunoprecipitation experiments targeting the proximal region of these promoters that contained CREs. Insulin recruited pCREB and TORCs on the PGC-1\textalpha{} and UCP-1 promoters (Fig. 1C), suggesting that the CREB-TORC complex might directly upregulate transcription of PGC-1\textalpha{} and UCP-1 genes in response to insulin.

Third, we examined phosphorylation levels of SIK2 and TORC2 in insulin-treated T3\textgamma{}i adipocytes. Insulin slowly elevated the level of SIK2 phosphorylation at Ser\textsuperscript{587} in conjunction with the dephosphorylation of TORC2 (Fig. 1D), while, in contrast, the phosphorylation of AKT or CREB occurred rapidly after the insulin treatment. An increase in the protein level of PGC-1\textalpha{} was observed 1 h after the stimulation. Experiments using various inhibitors against kinases that had been shown to modulate CREB activities in insulin signaling suggested that, although PI 3-kinase and mitogen/extracellular signal-regulated kinase (MEK) might be important for the expression of PGC-1\textalpha{} and UCP-1 genes (Supplementary Fig. 1B), the insulin-induced phosphorylation of SIK2 and dephosphorylation of TORC2 may occur in a PI 3-kinase- or MEK-1-independent manner (Supplementary Fig. 1C). Put together, these results indicate that SIK2-TORC cascades may play an important role in the regulation of insulin-dependent expression of PGC-1\textalpha{} and UCP-1 genes in brown adipocytes.

**Negative correlation between diet-induced obesity and SIK2-phosphorylation at Ser\textsuperscript{587} in BAT.** Next, we investigated the relationship between the levels of phosphorylation of SIK2 and TORC2 in vivo. Twenty male mice from a close colony of C57BL/6J were fed with a high-fat diet for 2 mo and separated into three categories following their body weight (Fig. 2A). Although the level of blood insulin was higher in the obese group than that in the lean group (Fig. 2B), levels of PGC-1\textalpha{} and UCP-1 gene expression in BAT were lower in the obese group (Fig. 2C), which correlated with a low level of SIK2 phosphorylation at Ser\textsuperscript{587} (Fig. 2D). The protein level of TORC2 was higher in mice in the obese than in the lean group, but the phosphorylation level of TORC2 was also high in the obese group. These results for T3\textgamma{}i brown adipocytes and mice with diet-induced obesity suggest that the phospho/dephospho balance of SIK2 and TORC2 might be involved in the regulation of insulin-induced PGC-1\textalpha{} and UCP-1 gene expression and prompted us to further examine the involvement by overexpressing SIK2 in mice.

**Generation of SIK2 transgenic mice.** To generate SIK2 transgenic mice, the adipose-specific promoter aP2 and the SIK2 mutant S587A were used. The functionality of this transgene (Fig. 3A) was tested by a transient reporter assay
Fig. 3. Generation of SIK2 (S587A) transgenic (Tg) mice. A: diagram of adipocyte fatty acid-binding protein 2 (aP2)-SIK2 (S587A) construct. B: CRE activity is suppressed by SIK2 (S587A) induced from the aP2 promoter in both white and brown adipocytes, but not in preadipocytes. Constructs for the aP2 promoter alone and aP2-SIK2 (S587A) (200 ng) were transformed into 3T3-L1 or T37i preadipocytes (growing) or adipocytes (day 3 after differentiation) with the CRE-Luc reporter (fluc) (400 ng) and the Renilla-Luc internal reporter (rluc) (60 ng). After 24 h, cells were treated with or without 20 μM forskolin for 6 h and then harvested for reporter assays. Data are shown as means and SD (n = 3). C: Northern blot analyses of white adipose tissue [WAT (W)] and BAT (B) of Tg mice. *Degraded bands of endogenous SIK2 mRNA. C, control. D: identification of the transcript derived by means of Pst I digestion from the transgene after RT-PCR of RNA from the liver, muscle, WAT, and BAT of Tg mice (line 15). 36B4, PCR product from an internal control RNA. The level of mRNA in macrophages was also examined (bottom). E: Western blot analyses of WAT and BAT of transgenic mice, line 15, and their littermate control mice. SIK2 protein and TORC2 protein were precipitated with specific antibodies (IP) from cell homogenates and then separated on SDS-PAGE followed by Western blot analyses.

with the CRE-luciferase vector in pre- or mature adipocytes. The construct had no or negligible effect on forskolin-induced CRE activity in undifferentiated white (3T3-L1) and brown (T37i) preadipocytes, but it could efficiently suppress the CRE activity in differentiated cells (Fig. 3B).

Five lines of mice with the transgene in their genome were obtained, and three of them expressed the mRNA for the mutant SIK2 at detectable levels. However, high-level expression was observed only in BAT, but not in WAT (Fig. 3C and Supplemental Fig. 2). This BAT-preferred expression was also observed when a dominant-negative FOXO1-transgene was expressed via the aP2 promoter in another recently established transgenic model (44). Preliminary experiments with small populations of mice [weight gain (Supplementary Fig. 3) and gene expressions in male and in female mice (Supplementary Fig. 4)] suggested that, under our conditions, male mice from line 15 were the most helpful model to investigate SIK2 signaling cascades in the BAT.

Tissue-specific expression of the transgene was confirmed by RT-PCR analyses, which enabled us to identify SIK2 (S587A) transcripts by Pst I digestion. As shown in Fig. 3D, transgenic mice (line 15) expressed the transgene exclusively in BAT. Macrophages, another cell type expressing aP2, did not express recombinant or endogenous SIK2.

An increase in the protein level of SIK2 in BAT was detected (Fig. 3E) but was not as marked as in mRNA levels. No or little difference in phosho-TORC2 levels was observed in WAT between control and transgenic mice. The level of phospho-TORC2 in BAT of control mice was lower than that in WAT, whereas the level in transgenic mice was restored to a level similar to that in WAT, suggesting that the recombinant SIK2 (S587A) expressed in BAT may have enhanced the level of phospho-TORC2.

Male SIK2 (S587A) transgenic mice show accelerated weight gain during high-fat feeding. Transgenic mice showed accelerated weight gain after high-fat-diet feeding (60% calories), whereas no changes were observed when the mice were fed with a chow diet (Fig. 4A). There was no difference in the amount of food intake between transgenic and control groups. Although statistical significance was not obtained, the measurements of tissue weight (Fig. 4B) and the results of CT analyses (Supplemental Fig. 5) suggested that the accelerated weight gain might be related to the passive gain of WAT. On the other hand, an analysis of BAT showed triglyceride accumulation in BAT of transgenic mice (Fig. 4C). Similar inconsistency of weight gains between whole body and WAT was recently reported in mice with a disrupted UCP-1 gene (22).

Rectal temperature indicated that the regulation of high-fat-induced thermogenesis may have been impaired in the transgenic mice (Fig. 4D) because it showed an apparent negative correlation with the level of blood insulin (Fig. 4E). In addition, transgenic mice presented with glucose intolerance (Fig. 4F) and a lowered sensitivity to insulin (Fig. 4G).

Finally, we examined mRNA levels of lipogenic genes and PGC-1α and UCP-1 in BAT. The expression of lipogenic genes, such as FAS and SCD-1, were downregulated in the BAT of transgenic mice, suggesting that impaired regulation of lipogenic gene expression is not responsible for enhanced accumulation of fat in BAT (Fig. 5A). Similarly, the expression of the thermogenic genes PGC-1α and UCP-1 was downregulated (Fig. 5B).

Type 2 deiodinase (Dio2), an enzyme producing active triiodothyronine, and type 3 deiodinase (Dio3), an inactivating enzyme, are important for adaptive thermogenesis, which is accompanied by the enhanced expression of PGC-1α and UCP-1 genes (16, 28). Expression of Dio2 and Dio3 gene in
BAT is regulated by multiple stimuli, including cAMP. The levels of Dio2 and Dio3 genes were higher in high-fat-fed mice than those in chow-diet-fed mice, but no significant difference was observed between control and transgenic mice (Fig. 5).

The protein level of PGC-1α/β reflected its mRNA level (Fig. 5D), indicating that impaired induction of PGC-1α and UCP-1 gene expression might be one of causes of the accelerated weight gain observed in transgenic mice and that SIK2 may perform important functions in the regulation of PGC-1α and UCP-1 gene expression in BAT in response to insulin.

**DISCUSSION**

PGC-1α and UCP-1 gene expression in the BAT is regulated by a number of factors, such as ATF2, c/EBPα/β, and nuclear receptors, and is controlled by a variety of hormones (8, 10, 11, 17, 39, 46, 61). CREB is also reported to play an important role in this regulation. The proximal region of the PGC-1α promoter to which CREB binds has been found to contain the target site of the PRDM16-PGC-1α complex. Competitive occupancy by CREB and c-Jun on the CRE in the...
UCP-1 promoter also influences the level of cAMP response of this promoter (64), suggesting that CREB and its interactive partners may be roles for the regulation of PGC-1α and UCP-1 gene expression in BAT.

SIK2 was isolated by using its homology with SIK1 and was found to be expressed abundantly in adipose tissues (29). SIK family kinases share the ability to suppress CREB activity by phosphoinactivating the CREB-specific coactivator TORC (34, 55). SIK family kinases belong to an AMPK kinase family that senses energy stresses (27), whereas AMPK was observed to phosphorylate TORC2 in liver (38). The phosphorylation of TORC2 by SIK1, SIK2, and AMPK reduces the rate of gluconeogenesis during periods of fasting, refeeding, and energy depletion, respectively.

Recently, mice with a disrupted gene for MARK2/Par-1b, another kinase in the AMPK family, were found to be lean, insulin hypersensitive, and resistant to high-fat-diet-induced weight gain, due to enhanced glucose uptake and thermogenesis in BAT (30). MARK2 can induce the nuclear export of TORC2 by phosphorylating at Ser275 (32), which suggests involvement of CREB-TORC2 cascades in the regulation of thermogenesis in BAT.

Because PGC-1α and UCP-1 are abundant in brown adipocytes and are essential for thermogenesis, we decided to use the brown adipocyte T37i to examine the importance of SIK2-TORC2 in insulin signaling in brown adipocytes. The T37i cell line was established from an hibernoma of a SV40 T-antigen transgenic mouse controlled by the proximal promoter of mineralocorticoid receptor gene (65). Insulin, thyroxin, or thiazoline derivatives differentiate T37i preadipocytes into adipocytes. The fully differentiated T37i brown adipocytes respond to insulin, β-adrenergic stimuli, prolactin, retinoic acid, and steroids and express adipocyte markers leptin and resistin (5), as well as brown adipocyte markers, e.g., PGC-1α and UCP-1 (44, 61, 62). Results obtained with the T37i cells suggested that SIK2 was able to inhibit the action of TORC2. Insulin might have inactivated SIK2 by enhancing the level of phosphorylation at Ser587, which resulted in a recruitment of CREB-TORC2 complex on the PGC-1α and UCP-1 promoters.

Because it has been suggested that phosphorylation-dependent modification of SIK2-TORC2 cascades is implicated in the regulation of body weight in mice with diet-induced obesity, we used the adipose-specific promoter aP2 to generate SIK (S587A) transgenic mice. Although the transgenic mice expressed the mutant SIK2 only in BAT, male mice showed diet-induced obesity accompanied by low rectal temperature. The mRNA levels for PGC-1α and UCP-1 were also lower in BAT of transgenic mice than in control mice.

The TORC phosphorylation activity of SIK is negatively regulated by phosphorylation in their COOH-terminal regulatory domains (at Ser587 in the case of SIK2). PKA has been identified as the kinase responsible for SIK phosphorylation (59). Although we could not identify the second kinase responsible for the SIK2 phosphorylation at Ser587 in insulin signaling, the phosphorylation of SIK2 at Ser587 may be important for the regulation of TORC activity in insulin signaling in BAT.
AKT phosphorylates SIK2 at Ser^358 in liver when insulin signaling is initiated (63). This phosphorylation enhances SIK2 kinase activity and induces phosphorylation-dependent ubiquitination of TORC2, which terminates gluconeogenesis. We also examined the effect of Ser^358Ala substitution for SIK2 in insulin-induced PGC-1α and UCP-1 gene expression. However, no effect on the expression(s) or on CRE reporter activities was observed in T371 adipocytes, suggesting that Ser^358 might not be essential for TORC2 regulation in BAT.

Because overexpression of SIK2 in cultured adipocytes was shown to downregulate forskolin-dependent expression of the CRE reporter (29), we examined whether β-signaling was impaired in transgenic mice. Unexpectedly, transgenic mice treated with the β3-agonist CL316243 showed accelerated weight loss that was accompanied by enhanced expression of the mRNAs of PGC-1α and UCP-1 (unpublished observation). The fact that expression of another CREB target gene, NR4A2, and β3-receptor were upregulated in BAT of transgenic mice (unpublished observation) suggested SIK2 in BAT may perform other functions in cAMP signaling.

Thyroid hormone is a stimulatory effector of thermogenesis in BAT, and Dio2 converts the precursor thyroxine into the active triiodothyronine. Expression of the Dio2 gene is upregulated by multiple stimuli, including cAMP, and a CRE in the proximal promoter is important for the regulation of the Dio2 gene (3). In addition to CREB, the complex of c-Jun/c-fos is also recruited on the Dio2 CRE to exert epidermal growth factor-dependent transcriptional activity (7). Recently, TORC1 was found to activate c-Jun/c-fos, which is independent of CREB (6). Although both the expressions of the PGC-1α gene (Supplementary Fig. 1) and the Dio2 gene (7) were sensitive to the mitogen-activated protein kinase inhibitor PD-98059, only PGC-1α gene expression was suppressed in SIK2-transgenic mice (Fig. 5, B and C), suggesting that SIK2 may not regulate all genes that contain CREs or TORC-sensitive elements.

The question of whether SIK2 can regulate expression of other genes in adipocytes has been answered by studies using overexpression and RNAi techniques, which have shown that SIK2 is activated by nutritional depletion and suppresses expression of lipogenic genes, such as FAS, ACC2, and SCD1, in 3T3-L1 white adipocytes (20). Similar suppression, except for ACC2, was observed in BAT of SIK2 transgenic mice (Fig. 5A).

ACC2 knockout mice show a low level of malonyl-CoA in the muscles (1), and because malonyl-CoA inhibits fatty acid transport in mitochondria by blocking the action of CPT-1 (42), ACC2 knockout mice are characterized by accelerated β-oxidation of fatty acids and resistance against high-fat feeding. SCD1 knockout mice also present with resistance to high-fat feeding because of enhanced mitochondrial biogenesis (40, 47). ACC2 expression in BAT of SIK2 transgenic mice in our study did not change, but a significant suppression of FAS expression was observed. Under these conditions, malonyl-CoA may have accumulated and thus inhibited β-oxidation of fatty acids in mitochondria. These findings and hypotheses suggest that SIK2 transgenic mice may be different from mice with disrupted lipogenic genes.

In contrast, lipogenesis is coupled with lipolysis in muscles (45). The activation of AMPK and peroxisome proliferator-activated receptor-δ by treating mice with small compounds induces expressions of not only lipolytic genes (UCP-1, UCP-3, CPT-1b, and hormone-sensitive lipase) but also lipogenic genes (FAS, SCD1, and acetoacetyl-CoA synthetase) in muscles, and this is then accompanied by PGC-1α gene expression. It has also been reported that some regulators for cell lineage and functions of BAT were shared by those of muscles (56), which suggests that downregulation of FAS and SCD1 gene expression in the BAT of SIK2 transgenic mice may result in a reduction in the size of lipid pools for lipolysis. However, it is also true that triglyceride content was higher in the BAT of SIK2 transgenic mice than in that of control mice (Fig. 4C). Further studies are thus needed to identify more molecules in SIK2 cascades that may contribute to the regulation of uptake, synthesis, store, release, and consumption of lipids in adipose tissues.

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DISCLOSURES

M. Muraoa, F. Kishi, and A. Mukushima are researchers at ProteinExpress Co., Ltd.

REFERENCES


