SCP4: A Small Nuclear Phosphatase Having a Big Effect on FoxOs in Gluconeogenesis

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Hyperglycemia has detrimental effects on normal organ functions in people with diabetes, chiefly contributing to the development of diabetic complications in both central and peripheral organs (1). Hyperglycemia, a hallmark of diabetes, results from dysregulation of both glucose intake/production and uptake/utilization. In type 1 diabetes, due to pancreatic β-cell death and insulin deficiency, glucose uptake and disposal decrease significantly while endogenous glucose production increases. In type 2 diabetes, due to insulin resistance and impaired insulin secretion, glucose homeostasis is similarly dysregulated. With regard to the endogenous glucose production, there are two metabolic processes—gluconeogenesis and glycogenolysis. The liver is the major organ responsible for both processes (2). Thus, the control of hepatic glucose production is highly relevant to the treatment of both type 1 and type 2 diabetes.

Hepatic gluconeogenesis is regulated at multiple levels, including expression of key gluconeogenic genes, enzymatic activity, and substrate flux. Phosphoenolpyruvate carboxylase (PEPCK) and glucose-6-phosphatase (G6Pase) are two of the key enzymes that are involved in gluconeogenesis. Expression of these two genes is reciprocally controlled by insulin and glucagon (3). Upon insulin binding to insulin receptor (IR), the IR is activated and initiates the insulin signaling cascade through insulin receptor substrates (IRS), phosphoinositide 3-kinase (PI3K), 3-phosphoinositide–dependent protein kinase 1 (PDK1), and AKT/PKB kinase (4). Multiple metabolic processes are regulated by the AKT kinases, including gluconeogenesis and synthesis of glycogen, lipid, and protein (5). Forkhead box O transcription factors (FoxOs) are key regulators for hepatic gluconeogenesis (6–8). There are four FoxO genes in mammals—FoxO1/3/4/6 (9). The transcriptional activity of FoxOs is tightly controlled by protein phosphorylation and dephosphorylation. AKT is a major kinase that potently inhibits the activity of FoxOs by phosphorylating the well-conserved sites in FoxOs: FoxO1-T24/S256/S319, FoxO3-T32/S253/S315, FoxO4-T32/S197/S262, and FoxO6-T26/S184 (5). Upon phosphorylation of FoxOs by AKT, the phosphorylated FoxOs can bind 14-3-3 adaptor proteins and are subsequently translocated to the cytoplasm (10).

In contrast to abundant studies of kinases for FoxOs, much less is known about phosphatases for this group of transcription factors. Protein phosphatase 2A (PP2A) and dual specificity phosphatase 6/mitogen-activated protein kinase phosphatase 3 (DUSP6/MKP3) have been implicated in the dephosphorylation of FoxO1 in the cytosol (11,12). However, it is unclear whether PP2A or MKP3 plays a significant role in the dephosphorylation of FoxOs in the nucleus. In this issue of *Diabetes*, Cao et al. (13) identified a new FoxO1/3a phosphatase—CTD (C-terminal domain of RNA polymerase II) small phosphatase-like 2 (CTDSPL2), also known as small CTD phosphatase 4 (SCP4)—in the nucleus. This is a significant advancement of our understanding of the activation of the FoxO transcription by a nuclear phosphatase. This study was initiated from a screen of a 40-member phosphatase library using FoxO1 and FoxO3a as substrates. SCP4 turned out to be a top hit. Serial in vitro and in vivo experiments verified that FoxO1/3a are bona fide substrates of SCP4, including in vitro dephosphorylation assays using purified enzymes, substrates, and phosphatase-inactive mutants. Interestingly, FoxO1–p-Ser^{256} and FoxO3a–p-Ser^{253} are preferred dephosphorylation sites by SCP4. As expected, SCP4 modulates subcellular localization of FoxO1/3a according to their phosphorylation status. Overexpression of wild-type SCP4 but not phosphatase-inactive mutants increases nuclear localization of FoxO1/3a. Furthermore, SCP4 enhances the transcriptional activity of FoxOs, with a focus on regulation of the PEPCK and G6PC genes and hepatic glucose production. The physiological data from the SCP4-deficient mice (created by gene trap) have provided strong evidence to support the role of SCP4 in glucose homeostasis as the SCP4-deficient pups die within 24 h after birth due to hypoglycemia. Glucose supplementation extends the life span of the mutant pups by about 20 h. At molecular levels, SCP4

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deficiency elevates FoxO phosphorylation and decreases expression of the PEPCK and G6PC genes in the livers of SCP4 mutant mice. Interestingly, starvation induces the SCP4 gene in mouse liver, revealing a connection to the starvation process of which gluconeogenesis is a part.

Identification of SCP4 as a key regulator of FoxOs in hepatic gluconeogenesis advances our understanding of glucose homeostasis. The study by Cao et al. (13) elegantly demonstrates the biochemical nature of the SCP4-FoxO1/3a interaction as phosphatase and substrates. The cellular and mouse data also support a strong role of SCP4 in hepatic gluconeogenesis. The neonatal lethality in the SCP4-deficient mice is very striking. Nevertheless, some cautions should be exercised when one interprets the findings from this work. First, as the SCP4 mutant mice are systemically deficient in SCP4, the lethality phenotype cannot be solely attributed to hepatic gluconeogenesis and the SCP4 function in other metabolically critical organs should be examined and characterized. Previously, it has been shown that SCP4 is also involved in globin gene expression, CTD-Ser^5 dephosphorylation, bone morphogenetic protein signaling and mesenchymal differentiation, and muscle wasting in chronic kidney disease (14–17). Moreover, a conditional knockout animal model is needed to characterize the tissue-specific function of SCP4. Second, as glucose homeostasis is a balance between input (intake and synthesis) and output (utilization and storage), in addition to gluconeogenesis examined in the current study, glucose uptake, glycolysis, glycogenolysis, and citric acid cycle in the liver, skeletal muscle, adipose tissue, and brain should be analyzed as well. It is known that FoxOs also suppress expression of glycolytic genes, including glucokinase and pyruvate kinase (18,19). Third, it is rather surprising that autophagy is not changed in the liver of SCP4-deficient mice. It has been previously reported that FoxOs induce a number of autophagy-related genes (ATG), including ATG5, ATG14, and MAP1LC3B (20). An alternative assay such as autophagy flux might be used to validate the finding. Last, whether SCP4 impacts other known FoxO phosphatases, such as PP2A and MKP3, should be addressed in a future study as well.

In summary, identification of SCP4 as a key nuclear phosphatase for FoxO transcription factors should help us better understand the dynamics of checks and balances of these critical mediators in the insulin signaling pathway

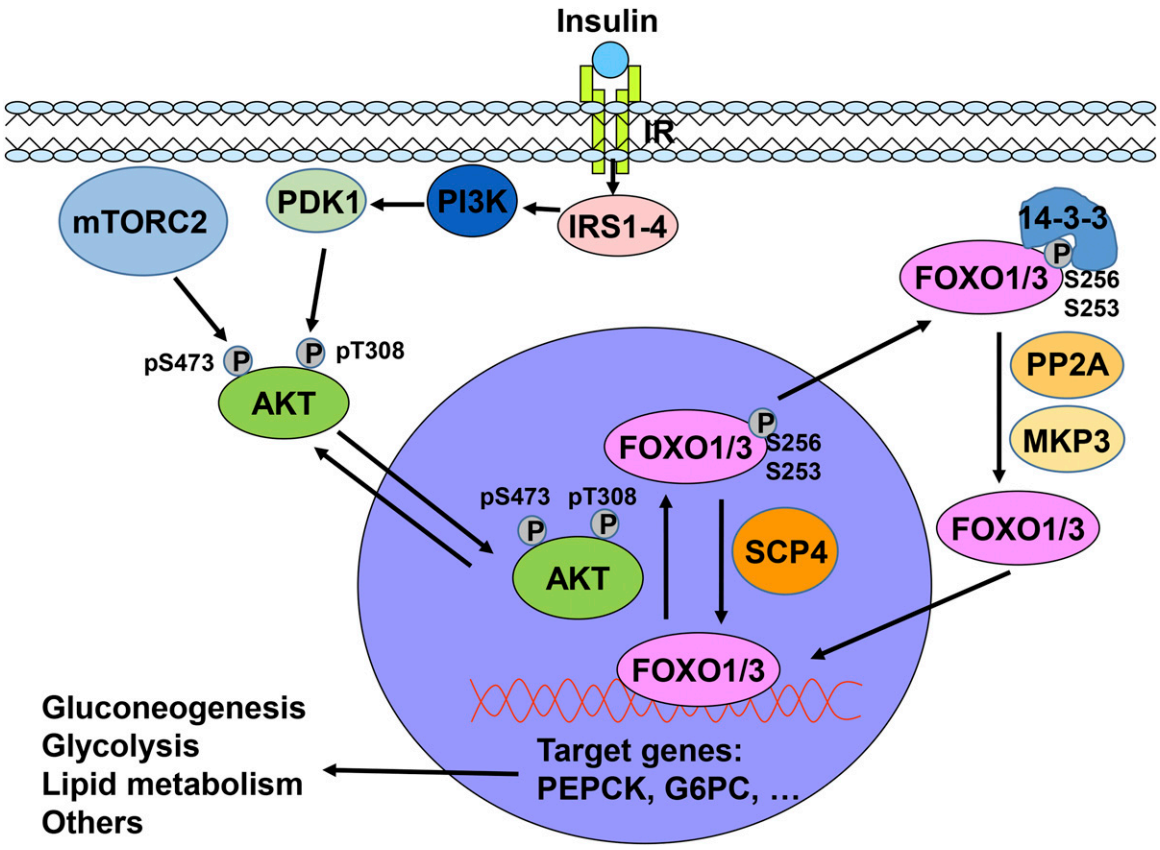

Figure 1—Regulation of the FoxO1/3 activity by phosphorylation. FoxO transcription factors, including FoxO1/3, are tightly regulated by phosphorylation and dephosphorylation events. Upon insulin stimulation, AKT is activated by the upstream signaling pathway IR → IRS → PI3K → PDK1/mTORC2. The activated AKT kinase regulates numerous target proteins including FoxO1 and FoxO3a through phosphorylation. The Akt phosphorylation of FoxOs, especially at Ser^{256} (FoxO1) and Ser^{253} (FoxO3a), is inhibitory. Phosphorylated FoxOs are subject to binding with 14-3-3 scaffold proteins and nuclear exclusion. When SCP4 has access to phosphorylated FoxO1/3a (Ser^{256}/Ser^{253}) that are not bound by 14-3-3, FoxO1/3a can be dephosphorylated and reactivated in the nucleus. As a result, hepatic gluconeogenic genes, including PEPCK and G6PC, are induced. It is also expected that other FoxO-regulated processes are activated by SCP4 as well.

(Fig. 1). Whether SCP4 can be used as a drug target for diabetes therapeutics awaits comprehensive understanding of the role of SCP4 in pathophysiology. Is the expression or activity of SCP4 changed in metabolically active tissues like liver, skeletal muscle, adipose tissue, and pancreatic islets? Will inhibition or knockdown of SCP4 improve hyperglycemia or diabetes as a whole? How does SCP4 regulate lipid homeostasis? Detailed characterization of relevant animal models and molecular profiling of the SCP4 interactome should provide much needed insights into the potential of targeting SCP4 for diabetes treatment.

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