Sirtuin 1 as a key player of 'metabolic memory'

The term, 'metabolic memory', was first advocated by the authors of the Diabetes Control and Complications Trial/Epidemiology of the Diabetes Interventions and Complications Study (DCCT/EDIC) and is well known for the benefits of achieving tight glycemic control on the progression of macrovascular events in diabetic patients in the early stage of disease, which might not be immediately seen, but becomes more evident with longer follow up. In other words, 'metabolic memory' suggests that memory of early glycemic control is retained in vascular endothelial cells. One of the plausible hypotheses to account for the potential mechanisms of memorizing a bad glycemic environment involves an excess of mitochondrial reactive oxygen species (ROS) in response to hyperglycemia in endothelial cells as an initiating factor of the pathogenesis of diabetic vascular complications. Overproduction of mitochondrial ROS in endothelial cells then results in inactivation of glyceraldehyde-3-phosphate dehydrogenase by poly (ADPribose) polymerase (PARP) activation and subsequent adenosine diphosphate ribosylation. However, the mechanism by which hyperglycemia induces the overproduction of mitochondrial ROS remains elusive.

Silent mating type information regulation 2 homolog (Sirtuin) 1 (SIRT1), the mammalian homolog of yeast silent information regulator 2 (Sir2), is a nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase protein critically involved in a broad range of cellular processes, including stress responses, cellular metabolism and aging through interactions with targets, such as the histones H3 and H4, p53, nuclear factor-kappa B (NF-κB), peroxisome proliferator-activated receptor γ (PPAR-γ) coactivator (PGC)-1α and forkhead box protein O (FOXO)s[1]. It was shown that Sir2 levels increase with nutrient deprivation, and that caloric restriction does not extend lifespan in Sir2/SIRT1-deficient *Drosophila*, yeast or mice. Furthermore, overexpression of Sir2 in *Drosophila* increases lifespan, which is not, however, further influenced by caloric restriction. In mammals, SIRT1 expression and activity are regulated by nutrient availability, and modulate the adaptive response to caloric restriction. Recent reports suggesting that SIRT1 regulates levels of ROS[2] prompted Zheng *et al.* to investigate if SIRT1 plays a role in the pathogenesis of the metabolic memory phenomenon[3]. The study by Zheng *et al.* shed new light on the strategy of SIRT1-based therapies for not only day-to-day glycemic control, but also the prevention of diabetic vascular complications in a long term by modifying 'metabolic memory', accumulated in vascular endothelial cells, via intervention through SIRT1 activity.

Growing evidence has shown that SIRT1 expression is suppressed in obesity and type 2 diabetes, and SIRT1 activation or increase in SIRT1 expression stimulates hepatic fatty acid oxidation, increases circulating adiponectin levels and suppresses inflammatory process (Figure 1)[4].

In the liver, SIRT1 mediates gluconeogenesis by deacetylating FOXO1 and PGC-1α, thereby increasing transcription of their gluconeogenic target genes and inhibiting the expression of glycolytic genes. Signal transducers and activator of transcription (STAT) 3-mediated repression of gluconeogenesis is also inhibited by SIRT1 through deacetylation of STAT3. In rodents, it was shown that reduction of hepatic SIRT1 gene expression resulted in lower expression of gluconeogenic genes (phosphoenolpyruvate carboxykinase [PEPCK], fructose-1,6-bisphosphatase [FBPase] and glucose 6-phosphatase [G6Pase]) as a result of increased STAT3, FOXO1 and PGC-1α acetylation, and mild hypoglycemia, whereas overexpression of SIRT1 resulted in mild hyperglycemia. These animals showed increased whole-body insulin sensitivity, attributable to an increased hepatic responsiveness to insulin.

Hepatic deletion of SIRT1 also reduced lipid synthesis, which subsequently lowered fasting plasma glucose and fed insulin concentrations, thereby improving high-fat diet-induced glucose intolerance. Regulation of hepatic lipid metabolism by SIRT1 is also known to be crucial in hepatic steatosis and hepatic insulin sensitivity. SIRT1 has been shown to regulate hepatic lipid metabolism through the activation of 5′ adenosine monophosphate-activated protein kinase (AMPK) and PGC-1α. Overexpression of SIRT1 led to an increase in phospholylated AMPK levels in cultured HepG2 cells and prevented hepatic lipid accumulation in these cells. In contrast, knockdown of SIRT1 increased hepatic fatty acid and cholesterol levels in association with enhanced PGC-1α acetylation. Mouse models of SIRT1 overexpression, in which AMPK, PGC-1α and peroxisome proliferator-activated receptor-α (PPAR-α) activities were enhanced, were protected from high-fat diet-induced steatosis. Increased adiponectin levels, induced by SIRT1 overexpression, activates AMPK, thereby improving hepatic insulin sensitivity and preventing lipid deposition. Furthermore, it was also reported recently that SIRT1 might reduce hepatic lipogenesis by deacylating and inhibiting the sterol regulatory element-binding protein (SREBP)-1c activity.

Collectively, these results suggested that SIRT1 expression/activation increases with fasting to stimulate gene expression related to fatty acid oxidation and gluconeogenesis, and to reduce lipogenic gene expression. The beneficial effects of SIRT1 on lipid metabolism are enhanced under the high-caloric conditions, leading to improvement in hepatic insulin sensitivity.

Although SIRT1 inhibits adipogenesis by inhibiting PPAR-γ activity, SIRT1-mediated deacetylation of FOXO1

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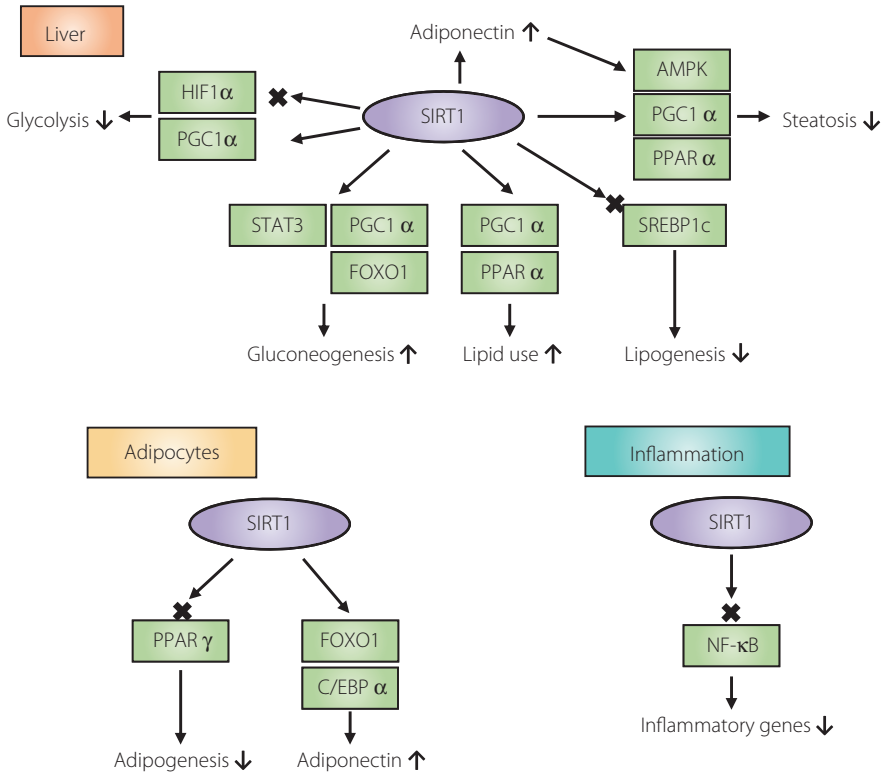

Figure 1 | Sirtuin 1 (SIRT1)-mediated metabolic responses. In the liver, SIRT1 regulates hepatic glucose production through peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), forkhead box protein O1 (FOXO1), and signal transducers and activator of transcription (STAT3). SIRT1 decreases glycolysis through PGC-1α and hypoxia-inducible factor-1α (HIF-1α), and suppresses lipogenesis through deacylating and inhibiting sterol-response element-binding protein-1c (SREBP-1c). In addition, SIRT1 protects against high-fat diet-induced steatosis by enhancing AMPK, PGC-1α, and peroxisome proliferator-activated receptor-α (PPAR-α). In adipose tissue, SIRT1 inhibits adipogenesis by repressing PPAR-γ, but stimulated adiponectin transcription by deacetylatiing FOXO1, and enhanced association of FOXO1 with CCAAT-enhancer-binding protein- α (C/EBP-α). SIRT1 has also been shown to deacetylate nuclear factor-kappa B (NF-κB), silencing the expression of inflammatory genes.

stimulated adiponectin transcription through enhanced association of FOXO1 with CCAAT-enhancer-binding protein-α (C/EBP-α) in differentiated 3T3-L1 adipocytes. Whole-body overexpression of SIRT1 in mice resulted in increments of circulating adiponectin levels, whereas adipocyte-specific knockdown of SIRT1 caused a large decrease in plasma adiponectin concentrations. Mice overexpressing SIRT1 were protected from high-fat diet-induced glucose intolerance, which was associated with reduced voluntary locomotor activity and oxygen consumption. In contrast, $SIRT1^{-/-}$ mice, which were 30–50% smaller than the wild type, consumed similar amounts of food as wild-type mice, which was driven by large increases in whole-body oxygen consumption. Taken together, these results show that SIRT1 is an important player in the switch from the *ad libitum*-fed to the calorie-restricted condition in the liver and adipose tissue.

Recently, it is generally believed that low-grade inflammation is associated with obesity, insulin resistance and type 2 diabetes although the contribution of this association with the pathogenesis of these diseases still remains unclear in many ways. Recent studies suggest the possibility that SIRT1 is also involved in the etiology of the low-grade inflammatory state, associated with obesity and insulin resistance. SIRT1 has been shown to deacetylate NF-κB p65 by reducing its ability to induce target gene expressions and histones to silence the expression of inflammatory genes. The genomic sequence flanking SirT1 in mice and humans contains several NF-κB binding elements, suggesting that the expression of SIRT1 is regulated by NF-κB activation.

Animals with altered levels of SIRT1 expression or activity were also shown to exhibit inflammatory phenotypes. The $SIRT1^{-/-}$ mouse develops an autoimmune condition, showing the hepatic and renal accumulation of immunoglobulin; and the dysregulation of inflammatory processes is also observed in tissue-specific deletion of SIRT1. Liver-specific ablation of SIRT1 resulted in elevated expression of components of the NF-κB signaling pathway, accompanied by increased expression of cytokines. Thus, it is plausible that the reduced SIRT1 activity observed in obesity, insulin resistance and type 2 diabetes might also contribute to pathogenesis, including non-alcoholic steatohepatitis (NASH).

The aforementioned stockpiling of the knowledge about the beneficial roles of SIRT1 in obesity, insulin resistance and type 2 diabetes now raises intense attention to the possibility of SIRT1-based therapies through activating SIRT1 for the treatment of these diseases[5]. In addition, Zheng *et al.* have shown that the dysfunction of the liver kinase B1 (LKB1)–AMPK–ROS pathway resulted in a cellular metabolic memory of high glucose. They showed that hyperglycemia led to PARP activation, which in turn have downregulated SIRT1, and that small interfering ribonucleic acid-mediated SIRT1 ablation in bovine retinal capillary endothelial cells had increased sensitivity to hyperglycemic stress. In contrast, SIRT1 overexpression or activation by metformin inhibited PARP through the upregulation of LKB1–AMPK activity, by which an increased activity of mitochondrial ROS-mediated glyceraldehyde-3-phosphate dehydrogenase induced by hyperglycemia was inhibited, ultimately suppressing the inflammatory gene, NF-κB, and the proapoptotic gene, B-cell lymphoma (BCL)-2-associated X protein (Bax), induced by hyperglycemia (Figure 2).

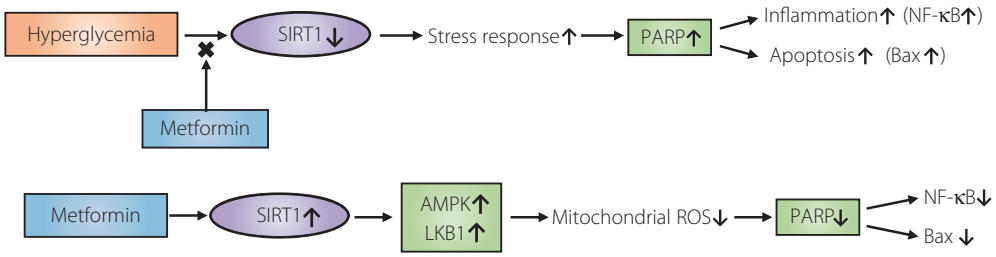

Figure 2 | Sirtuin 1 (SIRT1)-mediated metabolic memory of hyperglycemia. Zheng *et al.* showed that SIRT1 overexpression or activation by metformin upregulated liver kinase B1 (LKB1)/5′ adenosine monophosphate-activated protein kinase (AMPK) activity, which inhibited hyperglycemia-induced mitochondrial reactive oxygen species (ROS) activity, ultimately suppressing the inflammatory and the proapoptotic genes. Bax, B-cell lymphoma (BCL)-2-associated X protein; NF-κB, nuclear factor-kappa B; PARP, poly (adenosine diphosphate-ribose) polymerase.

They also showed that metformin suppressed the cellular metabolic 'memory' of hyperglycemia stress resulting from the suppression of ROS–PARP signaling in the diabetic retinas, which was linked to the upregulation of the SIRT1–LKB1–AMPK pathway.

These results suggested the crucial role of SIRT1 in the control of inflammation and apoptosis through LKB1–AMPK-dependent pathways, and SIRT1 expression and activation were inversely correlated with PARP, an upstream gene of the inflammatory and apoptotic pathways. They suggested that obviously SIRT1 can be a potential therapeutic target for treatments of insulin resistance and type 2 diabetes, and the use of metformin in such treatments aimed at reducing cellular metabolic memory of high glucose should provide some additional benefits for microvascular and macrovascular complications beyond its antihyperglycemic activity.

It goes without saying that worldwide expansion of metabolic disease in recent years necessitates the need for new therapeutic strategies, and the engineering of sirtuin-activating compounds is obviously one of the strongest candidates on an eligible list. Nevertheless, despite the expansion of the knowledge about SIRT1 functions in metabolic control brought about by a decade of intense work by many groups, we are still waiting for it to become a viable therapy for the treatment of type 2 diabetes. We hope that the aforementioned and future studies will provide additional insights into the functions of this valuable enzyme that opens new avenues for the treatment of metabolic diseases.

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