Metformin, metabolic stress, and mitochondria. Focus on “A novel inverse relationship between metformin-triggered AMPK-SIRT1 signaling and p53 protein abundance in high glucose-exposed HepG2 cells”

Mary C. Sugden and Mark J. Holness
Centre for Diabetes, Blizard Institute, St Bartholomew’s and the Royal London School of Medicine and Dentistry, Queen Mary, University of London, London, United Kingdom

THE P53 PROTEIN, BEST KNOWN as a tumor suppressor, participates in responses to cellular stresses, including DNA damage and hypoxia, but also determines metabolic substrate disposition. In cancer, p53 represses glycolysis while promoting mitochondrial oxidative phosphorylation via effects on gene transcription (3). Peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1), a member of a family of transcriptional coactivators that regulate the capacity for oxidative metabolism, interacts with and functions as a p53 transcriptional activator (17). PGC-1α-p53 interaction is promoted by transient glucose starvation (17). However, hepatic p53 protein abundance increases under conditions of nutrient excess (20).

AMP-activated protein kinase (AMPK) is also a cellular stress sensor. Its activity increases during glucose scarcity. ATP deprivation (usually due to impaired nutrient or oxygen delivery) and the antidiabetic drug metformin both activate AMPK (8, 18). AMPK activation suppresses anabolic processes (glycogen, protein and lipid synthesis) and gluconeogenesis (an energy-requiring process) to conserve ATP, while increasing mitochondrial carbohydrate and lipid oxidation to increase ATP production via the Krebs cycle and oxidative phosphorylation (Fig. 1). AMPK can cooperate with the fuel/NAD+-dependent sirtuin class of histone/protein deacetylases (SIRTs). Caloric restriction increases SIRT1 activity (9); conversely, both AMPK and SIRT1 are downregulated in the liver under conditions of nutrient excess [e.g., hyperglycemia, high-fat diet, obesity (4, 5)]. Nelson et al. (11) in this issue of American Journal of Physiology-Cell Physiology present novel data clarifying the mechanism of metformin action and highlight new insights into the interplay between p53, AMPK, and SIRT1 by investigating increased hepatic p53 protein expression and acetylation status in response to high glucose-induced changes in AMPK and SIRT1.

Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia partly through a mismatch between insulin resistance and insulin secretion. Insulin resistance impairs insulin-stimulated glucose uptake and suppression of hepatic glucose production (HGP). Antidiabetic effects of metformin are attributed to improved insulin (or insulin-like) actions, including decreased HGP. Metformin is believed to inhibit complex I of the electron transport chain (6), lowering the cellular energy charge ([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP]) (Fig. 1). Although AMPK and its upstream kinase LKB1 may not be obligatory for metformin-induced decreases in HGP in mice (7), the metformin-induced lowering of energy charge is predicted to activate AMPK (21) (Fig. 1).

NADH generated via oxidation enters complex I of the electron transport chain to contribute to generation of the mitochondrial proton gradient necessary for ATP production. During oxidative phosphorylation, both reactive oxygen species (ROS) and ATP are generated (Fig. 1). Excess ROS impair respiratory chain function and cause mitochondrial and nuclear DNA damage. Various cellular stressors, including oxidative stress, can trigger p53 accumulation (1) (Fig. 1). Furthermore, p53 is deacetylated and repressed upon DNA damage or oxidative stress (10, 19).

Nelson and colleagues (11) employed HepG2 hepatoma cells exposed to high (25 mM) glucose as a metabolic stressor. Exposure to high, compared with low (5 mM), glucose increased ROS production. Metformin increased AMPK activity at both low glucose (LG) and high glucose (HG) and decreased ROS production in response to HG. With HG, AMPK knockdown increased ROS production and partially blunted metformin’s effect on ROS production, suggesting that part, but not all, of metformin’s action is linked to AMPK activation rather than to direct inhibition of complex I and lowering of the cellular energy charge. Metformin also dose-dependently decreased p53 protein expression, suggesting that p53 expression in HepG2 cells under HG conditions is a consequence of oxidative stress. Metformin-induced decreases in p53 protein abundance with HG were dependent on AMPK activation: overexpression of a dominant negative AMPK [adenovirus (Ad)-DN-AMPK] in HepG2 cells both increased p53 abundance under basal conditions and prevented metformin-induced decreases in p53 protein abundance (Fig. 1). Furthermore, the decrease in p53 abundance caused by metformin was abolished by inhibition of murine double minute 2 (MDM2), a ubiquitin ligase that mediates p53 degradation.

To investigate potential reciprocity, the authors used nutlin-3 (an inhibitor of p53 degradation) to increase its expression. However, nutlin-3 did not affect ROS, implying that, although oxidative stress increases p53 protein expression, its expression alone does not affect ROS generation. While this might suggest that upregulation of p53 protein expression is peripheral to alleviation of oxidative stress induced by HG, the possibility remains that regulation of p53 activity (e.g., by posttranslational modifications or by binding proteins affecting its transcriptional activity) could be involved.

Humans express seven SIRTs (SIRT1–SIRT7) with distinct functions. SIRT1 counters cellular senescence and augments lifespan (9). Nutrient restriction increases SIRT1 expression;
**Transport chain; Ac, acetylation. ROS, reactive oxygen species; T2DM, type 2 diabetes mellitus; ETC, electron transport chain.**

Nelson et al. (11) found restriction in model organisms is accompanied by increased steatosis (14). The increased lifespan associated with caloric pressing mice are protected from high-fat diet-induced hepatic conversely, high-fat feeding suppresses it (4). Sirt1-overexpressing mice are protected from high-fat diet-induced hepatic steatosis (14). The increased lifespan associated with caloric restriction in model organisms is accompanied by increased mitochondrial density and respiration. Nelson et al. (11) found that metformin treatment of HG-exposed HepG2 cells increases SIRT1 activity (assessed by NH2-terminal histone H3 acetylation [Ac-H3]) and decreased hepatocyte triglyceride accumulation. While metformin did not increase SIRT1 activity in LG conditions - which may indicate that SIRT1 activation at HG is linked to changes in oxidative status and ROS production - its effect to increase SIRT1 activity at HG was attenuated by Ad-DN-AMPK. This indicates that the metformin-induced increases in SIRT1 activity (as assessed by Ac-H3) is mediated by increased AMPK activity (Fig. 1). The study raises the questions of the links between increased SIRT1 activity and p53 protein abundance and the obligatory participation of SIRT1 in regulating p53 protein abundance. Supporting a role for SIRT1, the metformin-promoted decrease in p53 abundance was abolished by shRNA-mediated knockdown of SIRT1. Conversely, overexpression of p53 (treatment with nutlin-3) decreased SIRT1 gene expression, suggesting that p53 may interfere with the SIRT1 promoter. As a downstream indicator of altered metabolic flux, metformin’s action to lower hepatocyte triglyceride was attenuated by p53 overexpression and lowered SIRT1 gene expression, while the SIRT activator SRT2183 reversed these effects. To complicate matters further, p53 is deacetylated (inactivated) by SIRT1. SIRT1-knockout mice show widespread p53 activation (2). Consistent with SIRT1 activation in response to metformin at high glucose, metformin increased p53 deacetylation at a SIRT1-targeted site (Lys382). However, SIRT1 knockdown did not affect Lys382 acetylation of p53. Furthermore, pharmacological SIRT1 activation by SRT2183 did not alter p53 acetylation or histone H3 acetylation. These results may indicate that metformin affects p53 Lys382 acetylation independently of SIRT1, that there is redundancy in the protein deacetylases affecting p53 acetylation status, or that other factors are involved [e.g., PGC-1α, whose activation induces mitochondrial biogenesis, triggers mitochondrial proliferation and increases mitochondrial function (18) and also suppresses the transcription of glycolytic genes (15)].

One consequence of impaired SIRT1 function is impaired posttranslational deacetylation and activation of PGC-1α (12, 15). Deacetylation (inactivation) of p53 by SIRT1 has been linked to de-repression of PGC-1α expression. Telomere dysfunction in mice null for either telomerase reverse transcriptase or telomerase RNA component genes (16) is associated with p53 activation, impaired mitochondrial function, reduced ATP generation, and increased ROS production attributed to deactivation of PGC-1α and PGC-1β, with direct suppression of PGC-1 genes. PGC-1α may complement SIRT1 since it opposes p53 acetylation within the DNA binding domain (Lys120) (17), i.e., a site distinct from that targeted by SIRT1 (Fig. 1). Thus multisite acetylation status of p53 may affect metabolic stress-triggered changes in gene expression.

In summary, the authors demonstrate that AMPK activation is required for SIRT activation and suppression of p53 expression by metformin in HepG2 cells (11). These findings suggest that reciprocal interactions exist between AMPK/SIRT1 activities and p53 protein abundance under conditions of nutrient excess which complement interactions in nutrient deprivation. However, a number of questions arise. Both SIRT1 and p53 augment oxidative metabolism, but metformin appears to activate SIRT1 and suppress p53 expression. What are the potential roles of other SIRTs, most notably SIRT3, in fine-tuning mitochondrial oxidative processes? Intriguingly, SIRT1 is linked to telomere biology and protection from DNA damage (13), which is consistent with the inverse relationship between SIRT1 and p53 in the current study. Importantly, if altered p53 protein abundance is accompanied by altered p53 transcriptional activity and modulation of glycolytic/oxidative flux—as inferred from changes in triglyceride accumulation—and occur in vivo in the normal liver as well as in HepG2 cells, the results could have implications for the pathogenesis and treatment of T2DM and other metabolic diseases with impaired mitochondrial function.

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**DISCLOSURES**

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**AUTHOR CONTRIBUTIONS**

M.C.S. drafted the manuscript; M.C.S. and M.J.H. edited and revised the manuscript; M.C.S. and M.J.H. approved the final version of the manuscript; M.J.H. prepared the figure.
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