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Commentary

Are SIRT1 activators another indirect method to increase AMPK for beneficial effects on aging and the metabolic syndrome?

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The discovery that caloric restriction enhances lifespan and that sirtuins 1 (Sirt1) may mediate these effects ushered in a renaissance of research into the development of novel small molecule activators targeting this highly conserved family of deacetylases (Sinclair and Guarente, 2014). Subsequent findings that the polyphenol resveratrol, a substance enriched in the skin of red and purple fruits and most notably red wine, was a potent Sirt1 activator created further excitement around developing more specific agonists towards this new molecular target for anti-aging therapies (Sinclair and Guarente, 2014). It has now been over a decade since the first synthesis and characterization of potent, novel, small molecule, Sirt1 activating compounds (STACs) and their reported ability to improve glucose tolerance and insulin sensitivity in rodent models of obesity and type 2 diabetes (T2D) (Sinclair and Guarente, 2014). Since this time, STACs (*i.e.*, SRT501, SRT1720, SRT2104) have been shown to improve lipid profiles, glucose tolerance, lifespan and healthspan in rodents (Mitchell et al., 2014). Clinical trials with these activators for a variety of conditions are currently underway or completed (*i.e.* NCT00938275, NCT00933062, NCT00964340, NCT01018017, NCT01018628, NCT00933530, NCT00937326).

However, the development of STACs for clinical use has not been without controversy, and a major question regarding their use has centered on their specificity for Sirt1. These concerns were initially raised when the utility of the fluorescent cell-free assays used to identify direct Sirt1 activation was questioned (Sinclair and Guarente, 2014). Further questions were raised by findings that Sirt1 was dispensable for the beneficial metabolic effects of resveratrol, and that these activities were mediated by adenosine monophosphate (AMP)-activated protein kinase (AMPK) in some (Park et al., 2012; Um et al., 2010), but not all studies (Price et al., 2012). Like Sirt1, AMPK is a highly conserved protein that has evolved to sense cellular energy status. Activation of AMPK in response to alterations in adenine nucleotides leads to the inhibition of energy consuming and actuating energy producing processes. Activation of AMPK holds exciting potential as a therapeutic target, as evidenced by the clinical effectiveness of metformin and salsalate, which activate AMPK indirectly by inhibiting mitochondrial function (Smith and Steinberg, in press). This potential has sparked significant interest and progress in developing new, direct AMPK activators. Notably, AMPK activation produces many of the same effects as those observed with STACs, such as improved glucose homeostasis (Smith and Steinberg, in press), increased mitochondrial biogenesis (Smith and Steinberg, in press) and, at least in the case of metformin, improved healthspan and lifespan (Martin-Montalvo et al., 2013).

In the April 2017 issue of *EBioMedicine*, Park et al. (2017) present evidence that, similar to resveratrol, the beneficial metabolic effects of STACs may also require AMPK. The authors show that AMPK activation by SRT1720 does not require Sirt1 in C2C12 muscle cells and Sirt1 knockout (KO) animals. Assays in mouse embryonic fibroblasts (MEFs) *in vitro* or in skeletal muscle of muscle-specific Sirt1 KO mice *in vivo* indicate that SRT1720 competes with cyclic AMP (cAMP); this competition inhibits recombinant cAMP-degrading phosphodiesterases *in vitro*. Using pharmacological inhibitors and siRNA gene manipulation in C2C12 and HeLa cells, the authors find that SRT1720-mediated activation of AMPK occurs through cAMP-Epac1 signaling involving the release of sarcoplasmic reticulum calcium and subsequent activation of calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ) and protein kinase A (PKA). This mechanism of action is very similar to that previously demonstrated for resveratrol by the same authors (Park et al., 2012).

Interestingly, the authors find using MEFs lacking AMPKα1/α2 or Sirt1 that AMPK appears to be the predominant regulator of mitochondrial biogenesis. Importantly and consistent with changes in mitochondrial biogenesis, the authors show that in the absence of AMPKα2 (the primary AMPK subunit expressed in skeletal muscle) SRT1720-induced improvements in glucose tolerance and insulin sensitivity are markedly blunted. Collectively, these data suggest that SRT1720 activates AMPK independently of SIRT1 and that AMPK is essential for the beneficial metabolic effects of this compound on mitochondrial biogenesis and glucose homeostasis.

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Despite these interesting findings several limitations should be noted. The *in vivo* mouse experiments conducted here use a germline whole body deletion of the AMPK α2 isoform and a muscle-specific Sirt1 deletion, which leaves room for compensatory activity of AMPK α1 or parallel pathways that do not reflect normal biology. Specifically, AMPK α2 KO mice have elevated catecholamines (which would increase cAMP) due to hyperactivation of the autonomic nervous system, and this could be an important factor contributing to *in vivo* insulin resistance and hypoinsulinemia (Viollet et al., 2003). In addition, the importance of CaMKKβ in regulating AMPK activity in muscle has been questioned given that liver kinase B1 (LKB1) accounts for nearly all detectable AMPK activity (Sakamoto et al., 2005). Lastly, experiments assessing glucose tolerance and insulin sensitivity were not performed in Sirt1 KO mice to rule out potential co-dependency of AMPK and Sirt1 *in vivo*. Considering the limitations noted above, experiments using inducible muscle-specific CaMKKβ, AMPK and Sirt 1 null mice are still required to clarify the inter-relationship/contribution of AMPK and Sirt1 to the physiological effects of STACs.

In conclusion, the study by Park and colleagues (Park et al., 2017) suggest it may be important to re-evaluate whether activation of AMPK is required for the metabolic benefits of STACs. Given the recent development of more specific AMPK activators (Smith and Steinberg, in press), these data raise the question of whether therapeutic interventions indirectly targeting Sirt1 *via* multifaceted upstream signaling is the best approach, or whether direct activation of AMPK would be a more efficient means to elicit beneficial metabolic effects. Lastly, given the widespread clinical use of metformin and salsalate, which are safe, well-tolerated and effective activators of AMPK, it is currently unclear whether STACs may provide additional therapeutic benefit for improving insulin resistance and healthspan. However, given the STACs' distinct mechanism of action, investigating whether there may additive or synergistic benefits by combining them with direct and indirect AMPK activators will be an interesting area of future study.

Disclosures

The authors declare no conflicts of interests.

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