SIRT5 regulation of proteins involved in metabolism

December 10 2013

The Sirtuin family of protein deacylases has received considerable attention in recent years due to its links to longevity, diabetes, cancer, and metabolic regulation. In a new study published in the Dec. 3rd 2013 issue of *Cell Metabolism*, Buck Institute researchers have now identified widespread regulation of proteins involved in metabolism by the mitochondrial sirtuin, SIRT5. Using a novel quantitative proteomic method developed at the Buck Institute, the Gibson lab in collaboration with Eric Verdin's group at the Gladstone Institute was able to identify hundreds of proteins in the mitochondria that undergo modification by lysine succinylation and its subsequent regulation by SIRT5. These findings have widespread implications for understanding metabolic function in both normal and disease states.

"Before you can study a process you first have to understand who the players are," says Bradford Gibson, PhD, Professor and Director of Chemistry and Mass Spectrometry at the Buck Institute. SIRT5 is found within mitochondria, a cellular organelle primarily responsible for energy production and homeostasis, which is present in virtually all cells in our body. By quantifying the differences between mice lacking the SIRT5 gene and control animals, Gibson and his colleagues discovered that SIRT5 selectively removes specific sites of succinyl modifications in over 140 different proteins that are involved in essential metabolic pathways, including fatty acid-oxidation, oxidative phosphorylation, and ketone body production. Matthew Rardin, a postdoctoral fellow in the Gibson's lab and a lead author of the study explains, "Within mitochondria there is widespread succinylation across multiple proteins

and pathways, and SIRT5 appears to be the only enzyme within mitochondria that is responsible for the regulation of this structural modification."

"We have found that lysine succinylation can have huge effects on enzyme activity," adds Gibson. Succinylation involves the transfer of a four-carbon negatively charged succinyl group to the primary amine of lysine residues, one of 20 amino acids found in all proteins. Under physiological conditions, this modification reverses the charge state of typically positively charged lysine moieties to a structure that now has a negative charge.

"When proteins become hypersuccinylated we see a disruption of metabolic pathways, including a buildup of fatty acids in the liver and a decrease in ketone body production," says Rardin. The researchers showed, for example, that HMGCS2 – a rate-limiting enzyme in ketone body production important for energy production during fasting – has at least 15 sites of succinlyation. Moreover, they demonstrated that succinylation of specific lysine residues near the substrate binding pocket on HMGCS2 abolishes enzyme activity. Therefore, the role of SIRT5 appears to be the removal of these succinyl modifications and restoration of enzyme activity at HMGCS2 as well as other mitochondrial enzymes. "While we still don't know all the implications that lysine succinylation has for mitochondrial function, we have assembled a large list of proteins whose succinylation state appears to be highly regulated by SIRT5," said Gibson. "This list of proteins and sites will be an extremely valuable resource for scientists to examine how these structural alterations are affecting many critical metabolic pathways in normal and pathological conditions."

This work was part of a larger continuing investigation by this same group to better understand the role of sirtuins in mitochondrial biology and metabolism. In work published earlier this year (Rardin et al., *Proc*

Natl Acad Sci, 2013), these scientists examined the activity of the related sirtuin, SIRT3, which regulates lysine acetylation in mitochondria. In contrast to succinylation, which carries a negative charge, acetylation of lysine residues only neutralizes the positive state of the lysine residue.

Taken together, these two papers highlight a vast amount of "cross talk" between lysine acetylation and succinylation, and Gibson and his colleagues believe that SIRT3 and SIRT5 are fine-tuning multiple metabolic pathways through the selective regulation of these two modifications. Many of the same proteins are being regulated by both SIRT3 and SIRT5, often at the same sites. This suggests that these modifications are likely to have important functional affects, similar to those this group demonstrated for HMGCS2. "We were surprised by the extent of crosstalk, especially as previous work had suggested little substrate overlap between these two sirtuins", says Gibson.

These researchers were quick to point out that this is still early days for assessing the functional role of both SIRT3 and SIRT5. "At some point we hope that the resources we have assembled here will help us to better understand the role of sirtuins in disease processes ranging from neurodegeneration to diabetes, especially where mitochondria are known to play a key role," adds Gibson. "This is just the beginning."

More information: Rardin M.J., *He W., Nishida Y., Newman J.C., Carrico C., Danielson S.R., Guo A., Gut P., Sahu A.K., Li B., Uppala R., Fitch M., Riff T., Zhu L., Zhou J., Mulhern D., Stevens R.D., Ilkayeva O.R., Newgard C.B., Jacobson M.P., Hellerstein M., Goetzman E.S., Gibson B.W., and Verdin E. SIRT5 regulates the lysine mitochondrial succinylome and metabolic networks. *Cell Metabolism*. 2013 Dec 3;18(6):920-933.

Rardin M.J., Newman J.C., Held J.M., Cusack M.P., Sorensen D.J., Li B., Schilling B., Mooney S.D., Kahn C.R., Verdin E., and Gibson B.W.

Label-free quantitative proteomics of the lysine acetylome in mitochondria identifies substrates of SIRT3 in metabolic pathways. *Proceedings of the National Academy of Sciences*. 2013. April 16;110(16):6601-6606.