Burning the fat

By Lev Osherovich, Senior Writer

A team led by researchers at the **University of California, San Francisco** has identified sirtuin 3 as a critical regulator of fatty acid oxidation, a process by which the body burns fat.[1] The findings have been licensed to the **Sirtris Pharmaceuticals Inc.** unit of **GlaxoSmithKline plc**, which already has a discovery-stage program aimed at activating the enzyme to treat metabolic syndrome, obesity and type 2 diabetes. However, it could be challenging to pharmacologically activate the hard-to-reach mitochondrial protein *in vivo*.

Sirtuins are a family of protein deacetylases that have been linked to metabolic regulation.[2] For example, sirtuin 1 (SIRT1), a homolog of SIRT3 found mostly in the nucleus, regulates a range of metabolic pathways and is the target of Sirtris' SRT2104. The small molecule SIRT1 activator is in Phase II trials for type 2 diabetes.

The new mouse study, conducted by a team led by researchers at the **Gladstone Institute of Virology and Immunology** and UCSF, now strengthens the case for pursuing SIRT3 by establishing the enzyme's normal function.[1] The researchers found that SIRT3 becomes activated to ramp up fatty acid metabolism when sugar levels are low.

SIRT3's control of fatty acid oxidation thus appears to complement the role of SIRT1 in controlling sugar use.

"Fatty acid oxidation is a hugely important metabolic pathway, and there are a lot of conditions associated with its dysfunction, including metabolic syndrome," said Eric Verdin, senior investigator at Gladstone and professor of medicine at UCSF. "SIRT3 is a protective factor against metabolic syndrome."

Verdin also sits on Sirtris' scientific advisory board.

> **"Sirtuin pathways are affected by a lot of things and there are a lot of ways to modulate those pathways. Let's mine around those pathways."**
> *—Peter DiStefano, Elixir Pharmaceuticals Inc.*

Fuel to the fire

Previous studies of *Sirt3* knockout mice hinted at a role for the enzyme in acetylating mitochondrial proteins,[3] but the relevance of this process to metabolism wasn't clear because the mice were generally healthy.

Verdin's team first noted that Sirt3 levels and mitochondrial protein deacetylation activity rose when they withheld food from wild-type mice. Food deprivation triggers the burning of stored-up fat as an alternative fuel source.

The researchers then measured mitochondrial activity and metabolism of fasting *Sirt3* knockouts. Compared with wild-type controls, the mutant mice had lower fatty acid oxidation activity and accumulated high levels of fatty acids containing long chains of carbons, but not short- or medium-chain fatty acid derivatives, in their livers and blood plasma.

Those data suggest that in the absence of Sirt3, fasting still triggers release of fat from adipocyte reservoirs, but the mice have a defect in fatty acid oxidation and are unable to fully break down lipids for energy.

Consistent with this idea, *Sirt3* knockouts showed other indicators of fatty acid oxidation deficiency. The mutant mice, as compared to wild-type controls, couldn't keep their ATP and blood sugar levels elevated and showed cold intolerance when deprived of food.

The data were published in *Nature*.

Verdin's findings suggest that SIRT3 is needed to obtain energy from fatty acids in times of stress and predict that stimulating SIRT3 would ramp up the use of fat as a fuel, thus aiding weight loss.

"It's thought that increasing fat metabolism would be useful for metabolic disease," said Michael Sack, principal investigator at the **National Heart, Lung, and Blood Institute**. "In both type 2 diabetes and obesity, fatty acid oxidation is down, so you would want to activate SIRT3."

"This paper gives a good rationale for why you want to target this enzyme," said George Vlasuk, president of Sirtris.

"SIRT3 is attractive as a target," agreed Peter DiStefano, CSO of **Elixir Pharmaceuticals Inc.**

Elixir and partner **Siena Biotech S.p.A.** are developing SEN0014196 (EX-527), an oral SIRT1 inhibitor that is in Phase I testing to treat Huntington's disease (HD).

According to Sack, SIRT3 most likely controls an emergency backup system for fatty acid metabolism, because the knockout mice were healthy when well fed. SIRT3 appears to respond to metabolic stress like fasting, cold and exercise to ramp up fatty acid oxidation. As an example, he noted that "high-intensity training increases SIRT3 in muscle, paralleling the increase in oxidative capacity in response to greater oxygen utilization."

If SIRT3 is indeed required to burn fat, overfed mice should be more susceptible to obesity and diabetes. Sack said he would like to see what happens to *Sirt3* knockouts fed a diet that overloads the homeostatic control of fat metabolism.

Verdin said such experiments are underway.

Target practice

The question now is whether SIRT3 activity can be boosted to aid weight loss or to correct the energy imbalance in metabolic syndrome and type 2 diabetes.

Verdin said his team is "making a *Sirt3* transgenic to see if you're better off having a little more of it." However, he noted that "you can't necessarily equate overexpression with activation" and that using a small molecule approach to activate Sirt3 in a mouse model would be better.

Sirtris has experience activating sirtuins with small molecules, but SIRT3 poses extra challenges because of its complex regulation and mitochondrial localization.

"Targeting SIRT3 isn't going to be easy," said Verdin. The real challenge, he told *SciBX*, lies in "how to get the drug into the mitochondria."

He said Sirtris now has an *in vitro* assay for SIRT3 activity and a rational design strategy informed by a recently published crystal structure of the enzyme.[4]

DiStefano noted that screening for selective SIRT3 agonists requires "great caution" because previous screens for small molecule sirtuin activators have yielded false positives.

Instead, DiStefano thinks it would be more fruitful to pursue the downstream targets of SIRT3 or the acetylase enzymes that perform the opposite function of SIRT3 in the mitochondria. He cited a recent report identifying a large number of proteins that become acetylated in response to metabolic changes[5] as a place to look at alternative ways to target the SIRT3 pathway.

"Sirtuin pathways are affected by a lot of things and there are a lot of ways to modulate those pathways," said DiStefano. "Let's mine around those pathways."

Indeed, Verdin's team has found that one mitochondrial protein involved in fatty acid metabolism—acyl–coenzyme A dehydrogenase, long chain (ACADL; LCAD)—becomes hyperacetylated in *Sirt3* knockout mice compared with in wild-type controls. Although this hyperacetylation reduced LCAD activity, it's unknown whether changing the activity of LCAD alone would have a desirable effect on metabolic disease.

Verdin noted that his team has identified at least one more undisclosed target of SIRT3 besides LCAD. He said Gladstone and UCSF have filed several patents in connection to their discoveries, "some of which are licensed to Sirtris, others of which are available for licensing."

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