Scientists find 'outlier' enzymes, potential new targets to treat diabetes, inflammation

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The team included (left to right) Matthew Kolar, Siddhesh Kamat, Enrique Saez, Armand Cognetta, Alan Saghatelian, William Parsons and Ben Cravatt (not pictured). Credit: Photo courtesy of The Scripps Research Institute.

A team led by scientists at The Scripps Research Institute (TSRI) and the Salk Institute for Biological Studies has discovered two enzymes that appear to play a role in metabolism and inflammation—and might someday be targeted with drugs to treat type 2 diabetes and inflammatory disorders.

The discovery is unusual because the enzymes do not bear a resemblance—in their structures or amino-acid sequences—to any known class of enzymes. The team of scientists nevertheless identified them as "outlier" members of the serine/threonine hydrolase class, using newer techniques that detect biochemical activity.

"A huge fraction of the human 'proteome' remains uncharacterized, and this paper shows how chemical approaches can be used to uncover proteins of a given functionality that have eluded classification based on sequence or predicted structure," said co-senior author Benjamin F. Cravatt, chair of TSRI's Department of Chemical Physiology.

"In this study, we found two genes that control levels of lipids with anti-diabetic and anti-inflammatory activity, suggesting exciting targets for diabetes and inflammatory diseases," said co-senior author Alan Saghatelian, who holds the Dr. Frederik Paulsen Chair at the Salk Institute.

Into the Unknown

The study, which appears as a *Nature Chemical Biology* Advance Online Publication on March 28, 2016, began as an effort in the Cravatt laboratory to discover and characterize new serine/threonine hydrolases using fluorophosphonate (FP) probes—molecules that selectively bind and, in effect, label the active sites of these enzymes.

Pulling FP-binding proteins out of the entire proteome of test cells and

identifying them using mass spectrometry techniques, the team matched nearly all to known hydrolases. The major outlier was a protein called androgen-induced gene 1 protein (AIG1). The only other one was a distant cousin in terms of sequence, a protein called ADTRP.

"Neither of these proteins had been characterized as an enzyme; in fact, there had been little functional characterization of them at all," said William H. Parsons, a research associate in the Cravatt laboratory who was co-first author of the study.

Experiments on AIG1 and ADTRP revealed that they do their enzymatic work in a unique way. "It looks like they have an active site that is novel—it had never been described in the literature," said Parsons.

Initial tests with panels of different enzyme inhibitors showed that AIG1 and ADTRP are moderately inhibited by inhibitors of lipases—enzymes that break down fats and other lipids. But on what specific lipids do these newly discovered outlier enzymes normally work?

Regulators of FAHFAs

At the Salk Institute, the Saghatelian laboratory was investigating a class of lipids it had discovered in 2014. Known as fatty acid esters of hydroxy fatty acids (FAHFAs), these molecules showed strong therapeutic potential. Saghatelian and his colleagues had found that boosting the levels of one key FAHFA lipid normalizes glucose levels in diabetic mice and also reduces inflammation.

"Ben's lab was screening panels of lipids to find the ones that their new enzymes work on," said Saghatelian, who is a former research associate in the Cravatt laboratory. "We suggested they throw FAHFAs in there—and these turned out to be very good substrates."

The Cravatt laboratory soon developed powerful inhibitors of the newly discovered enzymes, and the two labs began working together, using the inhibitors and genetic techniques to explore the enzymes' functions in vitro and in cultured cells. Co-first author Matthew J. Kolar, an MD-PhD student, performed most of the experiments in the Saghatelian lab.

The team concluded that AIG1 and ADTRP, at least in the cell types tested, appear to work mainly to break down FAHFAs and not any other major class of lipid.

In principle, inhibitors of AIG1 and ADTRP could be developed into FAHFA-boosting therapies. "Our prediction," said Saghatelian, "is that if FAHFAs do what we think they're doing, then using an enzyme inhibitor to block their degradation would make FAHFA levels go up and should thus reduce inflammation as well as improve glucose levels and insulin sensitivity."

The two labs are now collaborating on further studies of the new enzymes—and the potential benefits of inhibiting them—in mouse models of diabetes, inflammation and autoimmune disease.

"One of the neat things this study shows," said Cravatt, "is that even for enzyme classes as well studied as the hydrolases, there may still be hidden members that, presumably by convergent evolution, arrived at that basic enzyme mechanism despite sharing no sequence or structural homology."

More information: AIG1 and ADTRP are atypical integral membrane hydrolases that degrade bioactive FAHFAs, *Nature Chemical Biology*, DOI: 10.1038/nchembio.2051

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