Innovative screening strategy swiftly uncovers new drug candidates, new biology

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Scientists at The Scripps Research Institute (TSRI) have demonstrated a drug-discovery strategy with a double payoff—it enables the rapid selection of chemical compounds that have a desired effect on cells and also highlights how the compounds work.

To illustrate the power of the innovative technique, the TSRI researchers used it to identify a compound that shows promise for treating obesity-linked diabetes. At the same time, they were able to identify the fat-cell enzyme that the compound inhibits—an enzyme that has not yet been a focus of diabetes drug development.

"This integrated strategy we've developed has the potential to accelerate the discovery of important biological pathways and may lead to faster development of new drugs for multiple diseases," said TSRI Associate Professor Enrique Saez.

Saez and his colleague Benjamin F. Cravatt, chair of TSRI's Department of Chemical Physiology, were the senior authors of the new study, which is reported December 22, 2013, in an advance online issue of *Nature Chemical Biology*.

Facilitating Drug Discovery

The new strategy has great potential to streamline drug discovery, a process whose importance to human health can hardly be

overemphasized.

Typically, pharmaceutical scientists start the discovery process by "screening" large libraries of chemical compounds in search of one or a few that might treat disease. The dominant strategy of recent decades has been to screen compounds for a specific activity against a known target, for example, inhibiting the function of a certain enzyme thought to be critical for the disease in question. A key advantage of this "target-based" screening is that it uses biochemical tests that can be done relatively simply in a test-tube—or rather, in a large array of tiny test tubes via automated, rapid screening systems that sort through hundreds of thousands of different compounds.

Target-based screening has enabled scientists to discover many useful new drugs, but some wonder whether this basic discovery strategy has already taken all the "low hanging fruit." In recent years, compounds selected with target-based in vitro tests have seemed to be failing increasingly often when tested in the more realistic biological environments of cells and animals.

An older strategy, "phenotypic" screening, avoids much of this problem by testing compounds for their ability to produce a desired effect directly on living cells. Unfortunately, such cell-based tests often leave open the question of how a useful compound works. "If you don't know what its relevant molecular target is, then developing that compound into a drug—optimizing its potency, its selectivity, its half-life in the bloodstream and so on—is going to be difficult," said Saez.

Identifying the molecular targets of compounds selected by phenotypic screens is typically burdensome and time-consuming. But in their new study, Saez, Cravatt and their colleagues were able to speed up the process dramatically. Indeed, their combined phenotypic screening and target-identification approach enabled them to quickly discover,

characterize and carry out preclinical tests of a potential new drug for obesity-linked diabetes: a complex metabolic disorder that affects 347 million people worldwide.

A New Diabetes Drug Candidate, Plus Insights into the Disease

The strategy makes use of the increasing availability of special libraries of related compounds that act as inhibitors of entire enzyme classes. In this case, the researchers used a set of compounds, recently synthesized by Cravatt's laboratory, that tend to inhibit serine hydrolases—a vast enzyme family whose members participate in most biological processes in mammals.

The scientists started with a phenotypic screen, testing their library of compounds for the ability to make young fat cells mature faster and store more fat. Better fat storage means that less fat leaks from fat cells into the liver, muscles and pancreas—a process that frequently occurs with obesity, often interfering with insulin signaling enough to bring on diabetes.

The screen quickly yielded several compounds that had a strong effect in promoting fat-cell fat storage. The researchers then used a method called "activity-based profiling" to identify the fat-cell serine hydrolases that the compounds inhibited most strongly. One of the most potent compounds, WWL113, turned out to work principally by inhibiting Ces3, a serine hydrolase enzyme that scientists have not studied in the context of obesity or diabetes.

The researchers quickly demonstrated WWL113's effectiveness in two different mouse models of obesity-linked diabetes—one in which the mice are genetically programmed to become obese and diabetic, and

another in which normal mice are made obese and diabetic with a high-fat diet. "The treated animals showed resistance to weight gain—they were not putting on as much weight as the controls," said Saez. "Their blood biochemistry also was getting normalized; their glucose, triglyceride and cholesterol levels were coming down towards normal levels."

In these mouse tests, WWL113—without any optimization for use as a drug—performed about as well as the FDA-approved diabetes treatment rosiglitazone (Avandia). Notably, the new compound lacked one of the side effects that drugs in rosiglitazone's class have in mice: the toxic accumulation of lipids in the liver.

"Our compound clears lipids from the diabetic mouse liver, whereas rosiglitazone has the opposite effect," said Saez.

To explore the relevance of these results to humans, the TSRI team worked with collaborating researchers in Australia to test fat samples from obese humans and diabetics. The tests confirmed that the human version of Ces3 also is unusually active in such patients. This suggests that an inhibitor may also work as a diabetes treatment in people.

Saez and his colleagues will next focus on using the new screening strategy to uncover more biological pathways that could yield new mechanisms to develop potential therapies.

More information: Integrated phenotypic and activity-based profiling links Ces3 to obesity and diabetes, dx.doi.org/10.1038/nchembio.1429

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