New method opens door to development of many new medicines

June 15 2016

Scientists at The Scripps Research Institute (TSRI) have developed a powerful new method for finding drug candidates that bind to specific proteins.

The new method, reported in this week's issue of *Nature*, is a significant advance because it can be applied to a large set of proteins at once, even to the thousands of distinct proteins directly in their native cellular environment. The TSRI researchers demonstrated the technique to find "ligands" (binding partners) for many proteins previously thought to bind poorly to small molecules that can be used to determine the functions of their protein targets and can serve as starting compounds for the development of drugs.

Among the newly discovered ligands are selective inhibitors of two caspase enzymes, which have key roles in multiple diseases but have largely eluded efforts to target them with drugs.

"Our data suggest that the human proteome is much more broadly targetable with small molecules than has been previously appreciated," said principal investigator Benjamin F. Cravatt, chair of the Department of Chemical Physiology and member of the Dorris Neuroscience Center and Skaggs Institute for Chemical Biology at TSRI. "That opens up new possibilities for developing scientific probes and ultimately drugs."

Putting Fragments to Use

Researchers have long sought better ways to identify small-molecule ligands for human proteins and to determine which proteins in our cells are inherently "ligandable." Biologists typically have used complex computer algorithms to predict whether a given class of proteins can bind adequately to small molecules. Pharmaceutical researchers often won't even try to develop drugs to target proteins that are thought unligandable. Several large protein classes are believed to be in this category, and so far only about 600 of the roughly 20,000 human proteins have been targeted successfully with FDA-approved drugs.

"There really hasn't been a way to determine empirically, rather than theoretically, what fraction of the human proteome can be targeted by small molecules," Cravatt said.

The new method is partly based on an approach known as fragment-based ligand discovery, which uses candidate ligand molecules about half the size of the small molecules in pill-based drugs. For initial screens to find potential ligands, such "fragment" molecules are much more efficient than drug-sized molecules, requiring much smaller libraries of compounds for a thorough search.

Cravatt's team attached candidate fragment molecules to a class of other molecules that, when they can get close enough, react strongly with cysteine amino-acids on proteins, locking the ligands to the proteins with strong "covalent" bonds.

"You still need an affinity-based interaction, but the covalent bonding event provides a significant boost in potency," said Keriann M. Backus, a research associate in the Cravatt laboratory who is first author of the study with TSRI Professional Scientific Collaborator Bruno Correia.

The scientists devised a screening system in which they can apply these covalent-bonding fragment molecules one by one to entire collections of

proteins expressed in human cells. The method can even be used with intact, living cells in a culture dish. The system allows researchers to detect and identify which small-molecule fragments have bound covalently to which proteins in the samples and which specific sites on the proteins are responsible for binding.

Applying a small library of cysteine-reactive fragments to the proteins found in two types of human cancer cells, the scientists found that the fragments successfully "liganded" more than 750 different cysteines found on more than 600 distinct proteins—which equated to more than 20 percent of all the proteins assayed in the samples.

Many of these proteins belonged to protein classes, such as transcription factors, that have been considered virtually unligandable, and therefore "undruggable." In fact, about 85 percent of the newly liganded proteins are not listed in a standard database of proteins with known small-molecule ligands.

"This experiment has effectively expanded what we think of as the ligandable proteome," said Backus.

Potential to Develop Probes and Drugs

The researchers were able to confirm the accuracy of the system, for example, by showing that it can identify the known protein targets of a covalent-binding anti-cancer drug, ibrutinib.

The team also demonstrated that some of the protein-binding ligand molecules identified with the system have strong biological activity—and thus have the potential to be developed into scientific probes or drugs. Newly discovered ligands for the enzymes IDH1 and IDH2, for example, turned out to block the activity of the normal versions of the enzymes as well as the mutant versions implicated in

many cancers.

In a final set of experiments, the team showed that one of its identified ligands inhibits the activities of caspase-8 and caspase-10, two enzymes that help switch on the cellular self-destruct process known as apoptosis. An excess of apoptosis is thought to contribute to neurodegenerative conditions such as Alzheimer's and Huntington's disease, as well as the brain damage occurring in the aftermath of strokes and other brain injuries. By contrast, a lack of apoptosis in certain cells is believed to contribute to cancers and autoimmune diseases. However, scientists don't understand enough about the roles of these enzymes, since they have been largely unable to find small molecules that selectively inhibit specific caspases.

In this case, Cravatt's team found that their initially identified anti-caspase ligand works by binding the precursor forms of caspase-8 and -10. They chemically modified the ligand into one that selectively binds just the precursor of caspase-8, and, using their two ligands as probes, were able to discover new details of how caspase-8 and -10 promote apoptosis in human T-cells.

"In essence, we demonstrated that our new platform works and that we can use the ligands it identifies to do useful biology," said Backus.

Backus and other members of the Cravatt laboratory are now doing several further studies: optimizing many of the newly identified ligands into probes for exploring protein functions; making a more comprehensive catalogue of cysteine-containing proteins that are ligandable; and expanding the method to target other amino-acids besides cysteine.

"We will be occupied for some time following up on this development," said Cravatt.

More information: Proteome-wide covalent ligand discovery in native biological systems, *Nature*, DOI: 10.1038/nature18002

Provided by The Scripps Research Institute