New method may accelerate drug discovery for difficult diseases like Parkinson's

July 13 2009

Whitehead Institute scientists have developed a rapid, inexpensive drug-screening method that could be used to target diseases that until now have stymied drug developers, such as Parkinson's disease. This technique uses baker's yeast to synthesize and screen the molecules, cutting target discovery and preliminary testing time to a matter of weeks.

The current drug discovery process is arduous, requiring identification of potential drug targets, synthesis of large collections of molecular compounds that might interact effectively with an identified target, screening of compounds with expensive assays and robotics, and defining the compounds' structures largely through trial and error. At the end of this months-long process, a large team of chemists and biologists usually deem only 1% or fewer of the compounds worthy of further testing in living cells.

A novel method, demonstrated by Whitehead scientists and described in the July 13 issue of *Nature* Chemical Biology, uses baker's yeast cells to perform most of the same work in a matter of weeks, with the added benefit that the testing is all done in living cells. At the core of this approach are extremely small proteins, called cyclic peptides, which are capable of targeting the protein-protein interactions found in almost every cellular process. Most current drugs act by wedging themselves into small pockets on the surfaces of target proteins. However, these traditional drugs are unable to adhere to smooth, flat protein surfaces, rendering the drugs ineffective for inhibiting the key interactions among

proteins that occur at these surfaces. Cyclic peptides have the ability to bind where traditional drugs cannot, allowing for the identification of previously overlooked targets to fight disease.

"We're getting at a chemical space that is very underexplored by traditional drug development and screening," says Joshua Kritzer, author of the *Nature Chemical Biology* paper and a postdoctoral researcher in Whitehead Member Susan Lindquist's lab.

"I think it's a very exciting method," says Lindquist, who is also a professor of biology at MIT and a Howard Hughes Medical Institute Investigator. "It provides much greater diversity in the chemical compounds you can study because you can screen millions of compounds in the same go."

Adapting previous work by the Benkovic lab at Pennsylvania State University, Kritzer created a vast "library" of cyclic peptides containing various amino acid combinations. He then inserted the cyclic peptides into cells of a well-established yeast model of Parkinson's disease that was created in the Lindquist lab.

Parkinson's disease is a neurodegenerative disorder characterized by tremors, muscle rigidity, and slowed movements. In the neural cells of Parkinson's patients' brains, researchers have noted Lewy bodies, abnormal aggregates primarily composed of the protein alpha-synuclein. There is currently no cure for the disease, and current Parkinson's therapies only address disease symptoms. In the Lindquist yeast model, the cells exhibit many of the hallmarks of cells in Parkinson's disease patients' brains, including death due to toxic overproduction of alpha-synuclein.

Once the cyclic peptides were inserted into the model yeast cells, Kritzer switched the yeast into Parkinson's mode and waited to see which yeast

cells survived. Of the approximately 5 million yeast cells that were inserted with a cyclic peptide, Kritzer ended up with only two cyclic peptides able to rescue the cells from death.

After sequencing them, Kritzer found that both effective cyclic peptides needed only the first four amino acids to work and those amino acids had a common motif (cysteine - any amino acid - a hydrophobic amino acid - cysteine). This particular four-amino-acid motif is very similar to some important biochemical structures, including molecules that oxidize or reduce other molecules and molecules that bind to metals.

Interestingly, there are already links between Parkinson's and the metal manganese. Overexposure to the metal manganese can lead to parkinsonism, a Parkinson's disease-like syndrome. Also, earlier work conducted by Aaron Gitler and Melissa Geddie in the Lindquist lab found that the normal version of the gene PARK9, which can be mutated in Parkinson's disease patients, protects cells from toxic levels of manganese.

With these possible modes of action in mind, Kritzer and colleagues are now trying to figure out how the new cyclic peptides work. Using the Lindquist yeast model and a worm model of Parkinson's disease from the Caldwell lab at the University of Alabama, they confirmed that the effective cyclic peptides have the same potency as natural genes that regulate Parkinson's related cellular processes, but intercept the disease's progress at a later point. This demonstrates that these cyclic peptides act at a point in the disease process that had not been targeted by other, more traditional approaches.

According to Kritzer, who will be starting this September as an Assistant Professor of Chemistry at Tufts University, a next step in this line of research will be to determine precisely how the effective cyclic peptides affect Parkinson's disease cells - by changing reduction or oxidation

within the cell, binding to metal molecules, or perhaps another mechanism. In addition, more potent structures may be possible, so the cyclic peptides' known structure can be used as a starting point for more libraries which may produce even more effective versions.

Lindquist also says the technique is not limited to just yeast or just Parkinson's disease. "There's absolutely no reason we couldn't apply the same process to mammalian cells. And it should be applicable to all sorts of diseases that are modeled in yeast," she says. "In fact, that's some of the stuff we've started doing with this technique."

More information: "Rapid selection of cyclic peptides that reduce alpha-synuclein toxicity in yeast and animal models," *Nature Chemical Biology*, July 13, 2009

Source: Whitehead Institute for Biomedical Research (news : web)