SENSORS AND PROBES

Light-responsive enzyme inhibitors

A hybrid of a photo-responsive ligand and a small-molecule inhibitor enables optical control of epigenetic mechanisms.

A common interest in epigenetics brought together Harvard scientist Stephen Haggarty, from the Department of Neurology, and Ralph Mazitschek, a chemical biologist at the Center for Systems Biology at Massachusetts General Hospital. Inspired by the use of optogenetics in neuroscience, Mazitschek envisioned a chemical probe that could inhibit a histone-modifying enzyme in response to light. At first, Haggarty was skeptical that this would be feasible in living cells, but he agreed that *if* such an inhibitor could be created, its potential would be transformative. It would provide precise temporal and spatial control for epigenetic mechanisms.

Mazitschek and his team focused on inhibitors of human histone deacetylases (HDACs), enzymes that remove acetyl groups from lysine on histones and thereby, in general, silence gene expression. Several HDAC inhibitors have already been approved by the US Food and Drug Administration, and others are currently in clinical trials.

In contrast to those drugs, which are always active while in an organism, Mazitschek sought to develop a compound that is active only after exposure to light.

The team designed hybrid compounds, combining a photo-switchable ligand and HDAC inhibitors in one molecule. Two features proved essential for a successful reagent: very rapid self-deactivation of any unbound molecule in the absence of light, and a long residency time of the inhibitor once engaged with its target. This strategy obviates the need for repeated exposure to light while providing tight spatiotemporal control. "It was the marriage of chemistry and knowledge of pharmacology that allows these molecules to be exploited in cool ways," says Mazitschek about the probes, which the team referred to as COMET, for 'chemo-optical modulation of epigenetically regulated transcription'.

Mazitschek is confident that, in addition to HDAC inhibitors, they can design inhibitors, and potentially activators, of histone methyltransferases, demethylases and even—leaving the realm of epigenetics—kinases and G-protein-coupled receptors.

When the teams wanted to use COMET probes in multiple parallel experiments to construct a dose-response curve and correlate the degree of enzyme inhibition with the amount of light delivered, they ran into unexpected problems. Mazitschek recalls searching papers describing optogenetic tools for tunable light sources and being dismayed at the findings. "They cost upward of $20,000," he recalls; "for a crazy idea, that is a lot of money." Having performed high-throughput experiments in the past, he was also not excited about being able to do only one or two experiments at a time. Instead, he built an array using LEDs (light-emitting diodes) in which the amount of light delivered to each well in a 96-well plate can be regulated with readily available microcontrollers. The device, which cost around $100 to build, can easily be programmed and fits into a tissue culture incubator, enabling long-term experiments.

Although the device is easy to use, Haggarty remembers their learning curve for figuring out how to administer the correct amount of light and avoid phototoxicity.

The 96-well array allowed them to test many different settings, and after a few iterations they identified optimal conditions to strongly inhibit HDACs, resulting in characteristic gene expression signatures only in the cells exposed to light.

Currently, Haggarty's team is using the compounds in postmitotic mouse and human neuronal cell cultures to regulate the epigenome, and Mazitschek is expanding the number of compounds. Both researchers also envision a clinical application down the line for the treatment of tumors that either are easily accessible by light or harbor an implanted LED. The drug would be delivered systemically but would be activated only in the tumor by light exposure. "Previously you could only control the pharmacokinetics of a compound by changing its structure or delivery," says Haggarty. "Here you have another handle to control activity. This opens up ways of spatial and temporal controls that were not there before."

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RESEARCH PAPERS

Reis, S.A. *et al.* Light-controlled modulation of gene expression by chemical optoepigenetic probes. *Nat. Chem. Biol.* http://dx.doi.org/10.1038/nchembio.2042 (2016).