Oxygen's Dark Side

We know that fruits and vegetables, green tea, and even wine are healthy for us because they are full of antioxidants. But what do they actually do once we eat them? It turns out that many of the antioxidant compounds found in these health foods activate a pathway in our cells that protects us from cellular damage caused by reactive oxygen species (ROS) that are a normal byproduct of oxygen metabolism. In times of environmental stress, levels of ROS increase, damaging our proteins and DNA, and, sometimes, causing disease. The stress protection pathway is regulated by a protein called Nrf2 that activates over 600 genes that ameliorate the damage caused by ROS. Due to its central role in this important protective process, Nrf2 has been the focus of efforts to find drugs that can treat diseases caused by environmental and oxidative stress. A research team screened more than 250,000 compounds to identify small molecules that activate the Nrf2 pathway. Their work, conducted via x-ray diffraction studies at the LRL-CAT 31-ID-D beamline at the APS, provides new hope for the development of drugs for the treatment of a wide array of diseases including cancer, Alzheimer's disease, cystic fibrosis, asthma, and chronic kidney disease in type 2 diabetes.

Nrf2 is a transcription factor that regulates the expression of genes in the nucleus of the cell. However, it spends most of its time outside of the nucleus in a complex with the Keap1 protein. Keap1 maintains Nrf2 in an inactive state until ROS are detected. If there is no need for activation, Keap1 sends the Nrf2 to the cellular trash removal system, the proteasome, for degradation. If ROS are present, they are able to react irreversibly with cysteine amino acids in the Keap1 molecule and this leads to release of Nrf2 so it can enter the nucleus and activate genes to neutralize the ROS threat.

A number of molecules have been identified that mimic this activation of Nrf2 but, because they also react irreversibly with cysteines (a semi-essential amino acid), these drugs can react with other proteins and this results in off-target interactions that can cause toxic side effects for patients. Therefore, the research team sought to identify small molecules that activate Nrf2 through a new mechanism.

In order to find new compounds that might inhibit the Nrf2/Keap1 interaction, the researchers from Biogen Idec, NoValiX (France), and Evotec AG (Germany), Merrimack Pharmaceuticals, the Celgene Corporation, and Keimyung University (South Korea) screened 267,551 compounds from a chemical library. For their test assay, they measured whether the candidate compounds could inhibit the interaction between the small pieces of Nrf2 and Keap1 that had previously been identified as essential for their interaction. After library screening, 18 possible compounds were identified that fell into two main chemical subclasses, and two promising representatives, compounds 15 and 16, were further investigated.

Compound 15 was co-crystallized with the Keap1 domain and found to bind in a 2:1 stoichiometry (Fig. 1). Also, as they had hoped, the structure revealed that the two molecules of compound 15 interacted through reversible hydrophobic and electrostatic interactions, not through covalent binding to Keap1 reactive cysteines.

Compound 16, which inhibited the Nrf2/Keap1 interaction more strongly in the test assay, was also the only compound found to activate Nrf2 in a cell-based assay. This suggests that, with some molecular tinkering, compound 16 could become an effective drug candidate. Also encouraging was the fact that the crystal structure of the Keap1 domain with compound 16 showed that the interaction was mediated in a manner that involved hydrophobic and electrostatic interactions, but did not involve covalent binding to the active Keap1 cysteine amino acids. The structure also showed that they bound in a 1:1 stoichiometry and identified features of the interaction that explained its improved performance compared to compound 15 and suggested design strategies to improve its inhibitory activity further.

These results are an important first step in identification of the molecular features of chemical inhibitors that might be used to treat a number of conditions where failure to protect cellular components from the damaging effects of oxidative stress is an important factor in the disease. The researchers hope that what they have learned from small molecules like compound16 can be used to develop a specific, effective, and safe Nrf2-activating drug.

— Sandy Field

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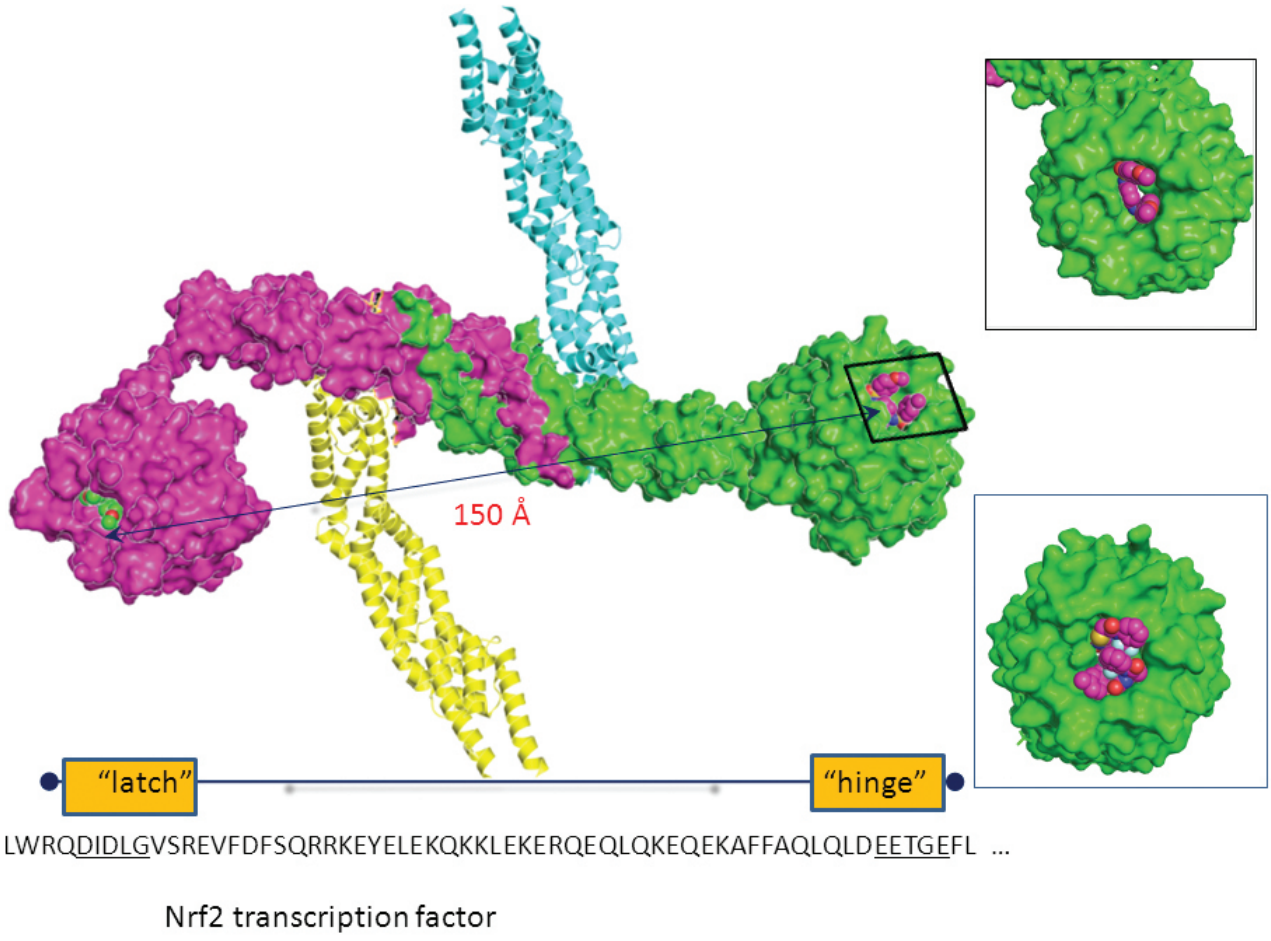

Fig. 1. Model of how compounds 15 and 16 inhibit Nrf2/Keap1 interaction. The full length Keap1 dimer (magenta and green) bound to proteasomal targeting proteins (yellow and blue ribbons) is shown. The insets show how compounds 16 (top) and 15 (bottom) are thought to bind to the Keap1 dimer at each end to block the binding of the "hinge" and "latch" peptide regions within the Nrf2 transcription factor (sequence shown at bottom). The predicted distance between the Keap1 domains (150 Å) matches the distance between the Nrf2 hinge and latch regions. Figure was made using Pymol (The PyMOL Molecular Graphics System, Version 1.7 Schrödinger, LLC.)