ALS SCIENCE HIGHLIGHT

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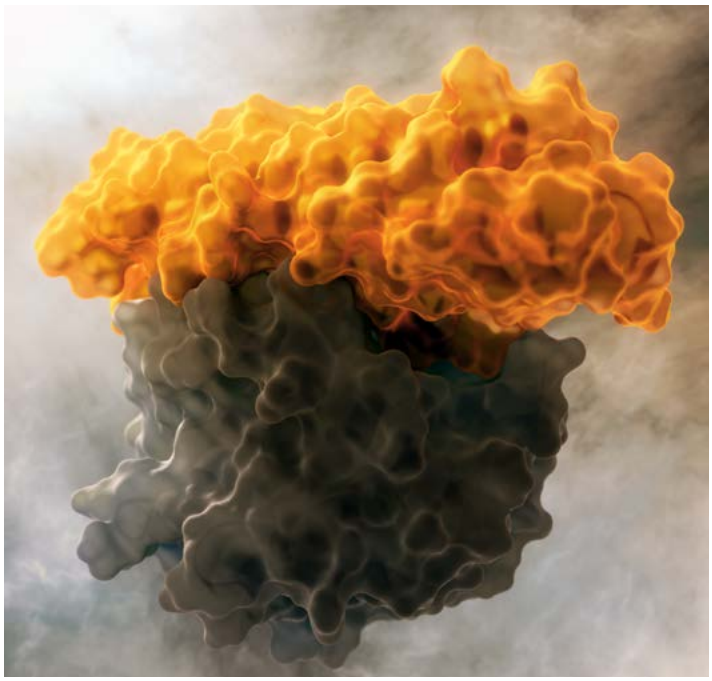
Designer Proteins Target Epstein-Barr-Virus-Associated Cancer

Immortality is not a good thing for cells, and in fact, cells will destroy themselves in a process called apoptosis when they are a danger to other cells. For instance, when a cell is infected by a virus it becomes an unwilling factory for the virus, which uses the cell machinery to produce ever more copies of itself. Eventually, if the cell doesn't die, it will spew all those new viruses into the bloodstream. The process of apoptosis spares other cells this same fate.

Some viruses plan for this, and produce proteins to stop apoptosis, like creating "zombie cells" which are not truly alive, but exist only to infect others. The Epstein-Barr virus (EBV) is one of those zombie-cell producing viruses. Another negative outcome of stopping apoptosis is that keeping cells alive beyond their healthy point can contribute to the development of cancer. The EBV was one of the first viruses to be identified in association with human cancer, but it is not the only one.

Keeping cells alive requires a delicate balancing act between proteins that promote apoptosis (executioners) and proteins that inhibit it (survivalists), as well as other proteins that inhibit the executioners, and still other proteins that enhance the survivalists. EBV produces a protein called BHFR1 to sequester and inhibit the executioners, leading to cell survival even when the cell is infected.



The crystal structure of Epstein-Barr Viral BHRF1 (black) bound to a designed protein inhibitor (gold). Rendering by Vikram Mulligan.

The researchers in this study wanted to design a protein to inhibit the inhibitor (BHFR1). The idea was to design a protein to recognize and bind to BHFR1, in order to stop the EBV from halting apoptosis. The first step was determining the specific sequence of amino acids on BHFR1 that binds to the executioner protein. This "folding nucleus" was then grafted into a larger helix bundle, which served as the scaffold. The trick was to

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Why Cells Die

Cells go through a natural process called apoptosis when they are damaged or can no longer function properly. By taking themselves out of commission they reduce the danger to the organism when, for instance, they are infected with a virus. But this process of apoptosis is not advantageous for a virus, which infects a cell and then forces the cell to make more copies of itself, as is the case with the Epstein-Barr virus (EBV). The EBV actually produces inhibitor proteins to counteract the process of apoptosis, keeping the host cell alive long enough to infect other cells. The scientists in this study used a new process of de-novo protein design to computationally build a novel protein that would bind to and inhibit the inhibitor. The method started with grafting the binding region into a three or four-helix scaffolding bundle, and then through mutation of residues both in the graft region and the scaffolding, computationally determined the best proteins for the job. The top candidates were expressed and purified in "real life," then tested in animal models, and further characterized using crystallography to see how and why the proteins worked. The method was successful in determining a new protein inhibitor of EBV, and so can now we used to design proteins to fight other infectious agents and cancer.

use sequences similar to naturally occurring proteins, which have the correct number of helix bundles, and then mutate a section of one of the helices into the target sequence. Residues around the graft site were also mutated such as to minimize the energy of the binding between BHFR1 and the designer proteins, and these turned out to be critical in maximizing the affinity of BHFR1 to the designed protein. The "directed evolution" of the scaffolding structure itself contributed largely to the stability of the graft-target interaction. The researchers designed thousands of such proteins and analyzed them for stability and projected binding affinity, and then selected less than a hundred of the top proteins for further analysis. A small number of the top proteins were expressed and purified from E. Coli, and further binding tests selected two proteins that bound to the BHFR1 with acceptable affinities. The top candidate was crystallized bound to BHFR1, and the structure solved at Beamline 5.0.2, showing exactly how the designed protein matched to reality; the small loop areas which were not predicted by the computational modeling were an important part of the feedback process, because they inform future computational designs. Finally, the top protein was tested against infection and it held up well: the designed inhibitor not only triggered apoptosis in EBV cancer cell lines in the lab, but also when delivered with a carrier system in live animals, suppressed tumor growth in the animals and extended survival rate.

The design of proteins "de novo" is not a new field, but until recently designer proteins were rarely also functional. In this study, new design approaches were defined and then used successfully to develop a potential inhibitor to the EBV. The study shows not just how to help defeat EBV, but also opens up a whole new way to design proteins against viruses and ultimately, cancer.