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New way to control protein activity could lead to cancer therapies

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Investigators at the Stanford University School of Medicine have found a way to quickly and reversibly fine-tune the activity of individual proteins in cells and living mammals, providing a powerful new laboratory tool for identifying — more precisely than ever before — the functions of different proteins.

The new technique also could help to speed the development of therapies in which cancer-fighting proteins are selectively delivered to tumors.

The procedure, described in a Nature Medicine paper to be published online Sept. 28, appears to be broadly applicable to efforts to understand the biological roles of all kinds of proteins, including those that are secreted by cells. This category includes many potent intercellular signaling proteins that can influence the immune system, for example by attracting its attention to an existing tumor.

"We have yet to find a protein the system doesn't work with," said senior author Steve Thorne, PhD, an assistant professor at the University of Pittsburgh who was involved in the work while a research associate at Stanford. The work was conducted under the direction of Chris Contag, PhD, associate professor of pediatrics, of radiology and of microbiology and immunology; and Tom Wandless, PhD, assistant professor of chemical and systems biology.

This technique, which was tested in mice, involves pairing specially bioengineered proteins with a drug, aptly named Shield-1, that prevents

the proteins from being degraded.

This approach stands in contrast to current ways of learning about proteins' functions, which are largely based on impeding a cell's production of the protein. Unfortunately, that cellular process can be slow and cumbersome, meaning that scientists get a sluggish response to such manipulations. In addition, current methods to perturb protein function are either irreversible — once a protein's production is knocked out, it can't be turned back on — or difficult to execute.

The new technique, instead, influences the level of speed with which the protein is broken down—a much faster process than its production. Moreover, it is reversible and works like a dimmer switch for an overhead light. The rate of a protein's degradation — and, thus, the level of its biological activity — can be increased or decreased by supplying more or less of Shield-1, permitting scientists to study the biological effects of slightly increasing or diminishing a protein's activity inside a cell over short time frames: for example, during a particular period in an organism's development.

The Stanford team succeeded in controlling levels of proteins by a relatively simple method pioneered by Wandless and his then-graduate student, Laura Banaszynski, PhD. They created special, bioengineered versions of several different proteins, in each case altering the protein by adding a small extra piece that didn't interfere with its biological function, but flagged it for rapid degradation. This degradation can be halted in its tracks, however, by Shield-1, which binds to the bioengineered protein, shielding it from destruction by the cell's breakdown machinery. The drug thus can enhance the bioengineered protein's intracellular concentration and activity; withdrawing the drug has the opposite effect.

"The process is tunable, and fast. As soon as you remove the drug, you

affect the degradation time of the protein," said Mark Sellmyer, a graduate student at the School of Medicine, who shares lead authorship of the study with Banaszynski.

The degradation-vulnerable bioengineered proteins were each produced by attaching the gene coding for a protein to another DNA sequence coding for the small extra piece that flags the protein for rapid degradation. The scientists then inserted the altered gene into a virus capable of infecting cells and introducing the altered gene into the cells' genomes.

In experiments demonstrating for the first time that the new technique can be used to effectively regulate a physiologically active protein in live mice, cultured tumor cells were grafted under the skin of immunologically impaired mice. As expected, the mice developed numerous tumors. The investigators had altered these cultured tumor cells so that they produced a degradation-prone bioengineered version of the protein IL-2 that, when secreted by cells, sends potent signals drawing the immune system's attention to those cells. When these altered tumor cells were grafted subcutaneously in the absence of Shield-1, the tumors grew just as before.

But if the tumor cells were first pretreated with Shield-1 they secreted IL-2, preventing any initial tumor growth. If Shield-1 was withheld at first and then administered to the mice five days after the grafts, tumors that had developed in those first few days regressed. By day 14, the tumors were gone.

Another set of experiments employed a mutant virus that had been previously developed by Thorne as a cancer therapy. The investigators inserted the gene for a bioengineered, degradation-prone form of a cell-killing protein into the specialized virus. They then administered it intravenously to live, tumor-bearing mice. When no Shield-1 was

provided, the tumor growth was only slightly diminished. But if Shield-1 was supplied three days after infection, when the virus had established a solid foothold in the tumors but been cleared from normal cells, tumors were completely eradicated in 90 percent of the mice. Meanwhile, normal cells were spared the substance's lethal effects.

Source: Stanford University Medical Center