Degradation from within

By Lev Osherovich, Senior Writer

Cornell University researchers have devised a way to selectively destroy intracellular proteins of interest using ubiquibodies—engineered molecules coupled to an enzyme that marks targets for degradation by the proteasome.[1] The technique could be useful for screening the effects of knocking down targets that cannot be readily hit by siRNA.

Team leader Matthew DeLisa, a professor of engineering at Cornell, said that ubiquibodies are a type of intracellular antibody—a transgenic antibody fragment expressed within a cell to block the activity of a target protein.[2]

"We had long been working on intracellular antibodies, which are single-chain fragments expressed inside the cytosol. These have found some use in interfering with the targets they bind to," said DeLisa.

The problem is that intracellular antibodies have been hard to work with because of the difficulty in achieving the high levels of protein expression needed to inactivate targets.

With intracellular antibodies, "you have to get expression levels at one-to-one stoichiometry toward your target, so their activity depends critically on the expression level," said DeLisa. "To address the problem of expression, we thought about arming intracellular antibodies with a mechanism for clearance of the target. We have now achieved this by conjugating them to proteins that are targeted for intracellular degradation."

DeLisa's new technique uses antibody-derived designer binding proteins to first bind their targets and then tag them with ubiquitin. Ubiquitin is a small protein that directs proteins toward the proteasome.

As the target protein undergoes degradation, the ubiquibody falls off and moves on to find and bind the next target molecule.

"With this system, one ubiquibody can cause the degradation of many copies of its target, reducing the need for high expression," said DeLisa.

Designer destruction

DeLisa's team created ubiquibodies from engineered gene fusions encoded by a viral vector.

One half of each ubiquibody is a minimized portion of a type of E3 ubiquitin ligase called CHIP (STIP1 homology and U-box containing protein 1; STUB1) (*see* **Figure 1, "Ubiquibodies"**). CHIP performs the last in a series of enzymatic steps that leads to ubiquitination of targets.

The target specificity of ubiquibodies comes from the other half of the fusion—a target-binding domain that is derived from a class of engineered intracellular proteins with antibody-like binding specificity.

As proof of principle, the team made ubiquibodies against two bacterial proteins—*Escherichia coli* β-galactosidase and *E. coli* maltose binding protein.

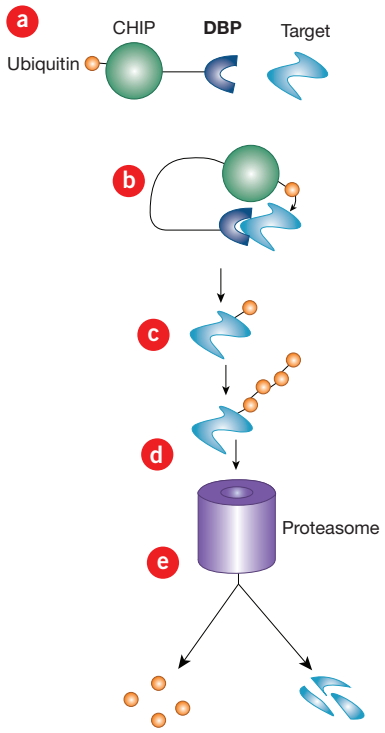

Figure 1. Ubiquibodies. Portnoff *et al.* have demonstrated a strategy for targeted destruction of intracellular proteins using engineered intracellular antibodies that deliver ubiquitin to a target protein. Ubiquitin is a small protein that tags intracellular proteins for degradation by the proteasome, a cellular garbage disposal system.

The team constructed a transgene encoding the E3 ubiquitin ligase domain of STIP1 homology and U-box containing protein 1 (STUB1; CHIP) fused to a mAb-derived designer binding protein (DBP) that targets a model substrate (**a**). The resulting fusion protein is called a ubiquibody.

In cell culture, DBP trapped a model target protein (**b**), and the CHIP domain of the fusion protein added ubiquitin to the captive target (**c**), triggering the addition of further ubiquitin molecules (**d**) and degradation of the target by the proteasome (**e**).

Arvinas Inc. and **GlaxoSmithKline plc** are independently developing proteolysis-targeting chimeric molecules, or PROTACs, that recruit a different E3 ubiquitin ligase to target disease-linked proteins. PROTAC compounds from Arvinas are in lead optimization to treat cancer, and GSK has PROTACs in lead discovery for undisclosed indications.

In bacterial cell lysates, the two ubiquibodies bound their targets and caused them to become ubiquitinated.

In mammalian cells expressing the two bacterial targets, concurrent expression of matching ubiquibodies led to ubiquitination and degradation of the target proteins.

Results were reported in *The Journal of Biological Chemistry*.

Post-translational knockdown

DeLisa thinks that the best application for the technology is in target validation studies focused on proteins that are hard to hit with gene knockdown methods.

"There are things that we can go after that wouldn't be possible with RNA-targeting methods," said DeLisa. "RNAi is a sledgehammer that goes after everything so the protein doesn't even get made, whereas we can go after protein isoforms."

"You could imagine isoform-specific ubiquibodies that eliminate the phosphorylated form of a protein but leave the unphosphorylated form alone," he added.

DeLisa's study "is a clever combinatorial strategy to utilize antibodies to recruit things to the ligase. By swapping out the ligase's binding site, you can get the ligase to bind whatever you want," said Timothy Shannon, CEO of **Arvinas Inc.**

Arvinas is developing small molecules to promote targeted protein degradation.

Shannon said that ubiquibodies could be rapidly adapted to hit a variety of intracellular targets thanks to their recombinant, modular design. However, he said that more work is needed to demonstrate the technology's potential for hitting mammalian cell proteins implicated in disease.

"I'd like to see if this is scalable for a broad array of targets," said Shannon.

DeLisa said that his next steps are to optimize delivery methods for the transgenic construct that encodes ubiquibodies and test whether ubiquibodies could be delivered from outside the cell.

He and study coauthor Jeffrey Varner, an associate professor of engineering at Cornell, cofounded **Ubiquizyme Inc.** to develop screening technology based on the technique. Ubiquizyme is in the process of licensing pending patents on the technology from Cornell.

DeLisa said that the company's initial focus is to develop research tools, but the eventual goal is to develop therapeutics.

PROTACs vs. ubiquibodies

Ubiquibodies are a biologic-based counterpart to proteolysis-targeting chimeric molecules (PROTACs), a class of small molecules developed in the laboratory of Craig Crews.[3] Crews is a professor of chemistry, pharmacology, and molecular, cellular and developmental biology at **Yale University**.

PROTACs work by bridging target proteins to a different E3 ubiquitin ligase called von Hippel-Lindau tumor suppressor (vHL) that also causes ubiquitination and degradation.

In 2012, Yale licensed patents on PROTACs to **GlaxoSmithKline plc** to pursue undisclosed cancer targets. Last year, Arvinas licensed PROTAC patents to pursue targets not covered by GSK's license. Arvinas has PROTACs in lead optimization for undisclosed cancer indications, and GSK has PROTACs in lead discovery for undisclosed indications.

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—Matthew DeLisa, Cornell University

Raymond Deshaies, a professor of biology at the **California Institute of Technology**, said that ubiquibodies and PROTACs will likely serve two distinct roles, with the former being used for research and the latter for therapeutics.

The PROTAC technique "has potential as a therapeutic approach because it is based on small molecules. However, it is likely to be far more difficult to make custom-designed PROTACs that target specific proteins," said Deshaies. "In general, it should be easier to get antibody mimetics that bind with high specificity and affinity to a target than it is to get a small molecule with the same properties."

Deshaies said that the biggest challenge for ubiquibody-based therapeutics is the difficulty of delivering the bulky proteins directly into the cytoplasm. In contrast, small molecule PROTACs have better odds of getting into the cell.

"I don't see PROTACs as being a practical approach for the development of research tools, but they could be a feasible approach for the development of therapeutics," he added. "Ubiquibodies are the inverse."

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COMPANIES AND INSTITUTIONS MENTIONED

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