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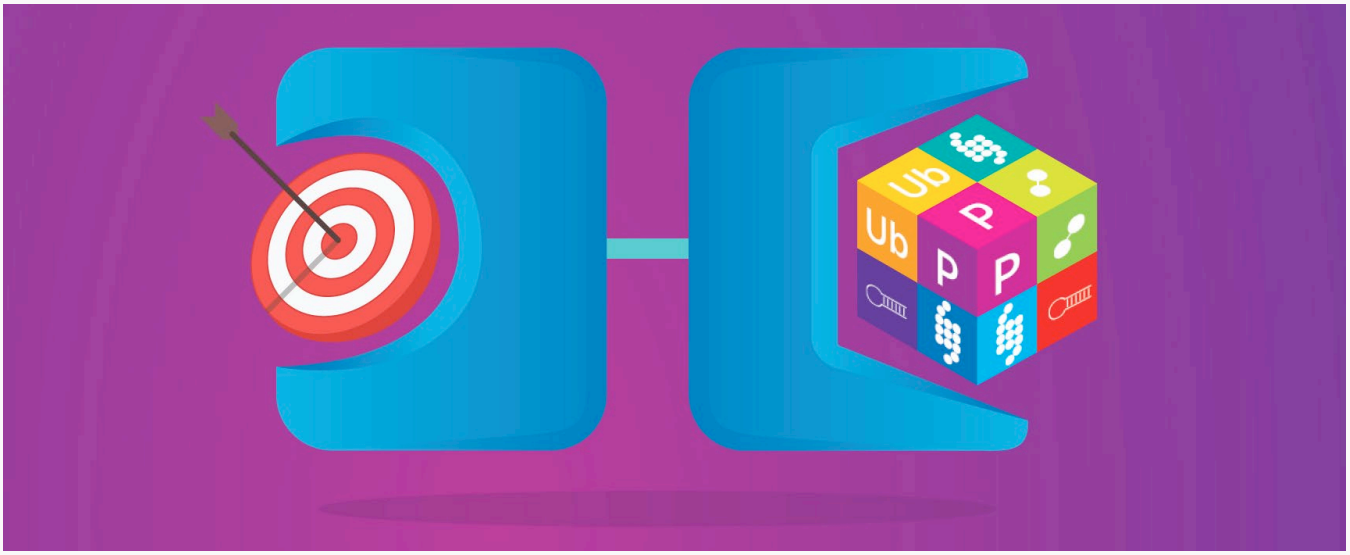

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TOOLS & TECHNIQUES

Beyond PROTACs and the proteasome: broadening the TAC toolbox

BY KAREN TKACH TUZMAN, ASSOCIATE EDITOR

The idea of sending disease-driving proteins to the cell's garbage can is only the first act in a field that now centers around targeted degradation. In the sequel, researchers are extending the principal to other types of cellular machinery, with iterations that promise access to an even broader range of targets, and to enzyme functions beyond protein degradation.

These next-generation targeting chimeras, or "TACs," are opening to door to therapies that activate, deactivate or protect targets of interest. But the first translational challenges are likely to be getting the right bioavailability and kinetics.

At least seven companies have launched in the last eight years to develop therapeutics based on targeted protein degraders -- a class of molecules introduced in the early 2000s by Yale University's Craig Crews, who dubbed them PROTACs (proteolytic targeting chimeras). The approach offers a way to block the activity of intracellular proteins whose structure, localization or resistance mutations make them difficult to target by conventional small molecules or mAbs.

Each of these companies is following a similar blueprint: bind a protein of interest and bring it to a ubiquitin ligase, which then tags it for degradation by the proteasome. The first clinical data came in October from Arvinas Inc. (NASDAQ:ARVN), which was founded by Crews in 2012; the company showed its two lead candidates were orally bioavailable and produced no serious AEs, and plans to release its first efficacy data in 1H20 (see "Arvinas Clears Key Hurdle in Clinic").

But targeted degraders are just one type of compound that brings two molecules close enough to interact. This concept, often referred to as chemically induced proximity, has been around since the early 1990s, and may finally be taking off broadly.

Drug developers and academics are using the same logic to subject therapeutic targets to other enzymatic reactions, including

protein phosphorylation, dephosphorylation, deubiquitination and lysosomal degradation. The explorations extend beyond proteins, with modifications that can trigger degradation of RNA by nucleases, and cleavage of carbohydrate residues via sialidase (see Figure: "There's a TAC for That").

The approach could theoretically be applied to any enzymatic reaction, including the more than 200 known post-translational modifications. Creating broader sets of compounds that direct enzymes to therapeutic targets could vastly expand the functionalities available to drug developers.

A new technology from Bertozzi's lab has made extracellular proteins accessible for targeted degradation as well. Dubbed lysosome targeting chimera (LYTAC), the platform was described in a chemRxiv preprint last March, and is the driving force behind Lycia.

LYTACs link mAbs against extracellular protein targets to a glycopeptide that binds mannose-6-phosphate receptors on plasma membranes. The complex is then pulled into the endocytic pathway, leading to target degradation by the enzymes and harsh conditions in the endosome and lysosome.

"IT HINTS AT A WHOLE WORLD OF MODALITIES WHERE A SMALL MOLECULE CAN TRANSACT A GAIN OF FUNCTION."

CAROLYN BERTOZZI, STANFORD UNIVERSITY

"It hints at a whole world of modalities where a small molecule can transact a gain of function," said Stanford University professor Carolyn Bertozzi. Bertozzi is the scientific founder of Lycia Therapeutics Inc. and Palleon Pharmaceuticals Inc., which are developing targeted degraders of extracellular proteins and sialylated glycans, respectively.

Close enough for comfort

The approach can be accomplished with molecules that join two independently discovered ligands via a linker, or with single molecules, known as "molecular glues," that are capable of binding both the enzyme and its substrate.

The breadth of opportunities lies in the range of functions enzymes perform in cells, which offer a multiple ways of controlling therapeutic targets.

"Right now a lot of modalities focus on blocking to antagonize or agonize a certain function, but enzymes are a great modality for intervening in biology," said Palleon CSO Li Peng told BioCentury.

A 2018 study from Crews' lab made the case that targeted degradation offers more potent and durable functional blockade than classical inhibitors, in part because it kills all aspects of a protein's function, including its role as a scaffold. The paper also showed that PROTACs were capable of degrading transmembrane receptors, in addition to intracellular proteins.

Children of PROTACs

Research studies are starting to show preclinical proof-of-concept for various forms of targeted post-translational modification.

A December study in the *Journal of Medicinal Chemistry* from the Genentech Inc. unit of Roche (SIX:ROG; OTCQX:RHHBY) showed the strategy could be used to control the phosphorylation status of key targets.

The paper described phosphatase recruiting chimeras (PhoRCs) that brought a phosphatase close enough to a target receptor to cause dephosphorylation, offering a potentially more potent alternative to kinase inhibition, according to the authors.

In one line of cell-based experiments, the authors linked the serine/threonine phosphatase PP1 to a small molecule AKT inhibitor, and showed the PhoRC caused PP1-dependent AKT dephosphorylation.

In another line of experiments, Genentech demonstrated it could divert PP1 to another target, the tyrosine kinase EGFR, overcoming the natural amino acid substrate selectivity of the phosphatase. The company did not return requests for comment in time for publication.

Amit Choudhary, a professor at the Broad Institute of MIT and Harvard, is doing the reverse -- developing compounds that induce phosphorylation by bringing kinases close to targets of interest. A study describing the technology is under review.

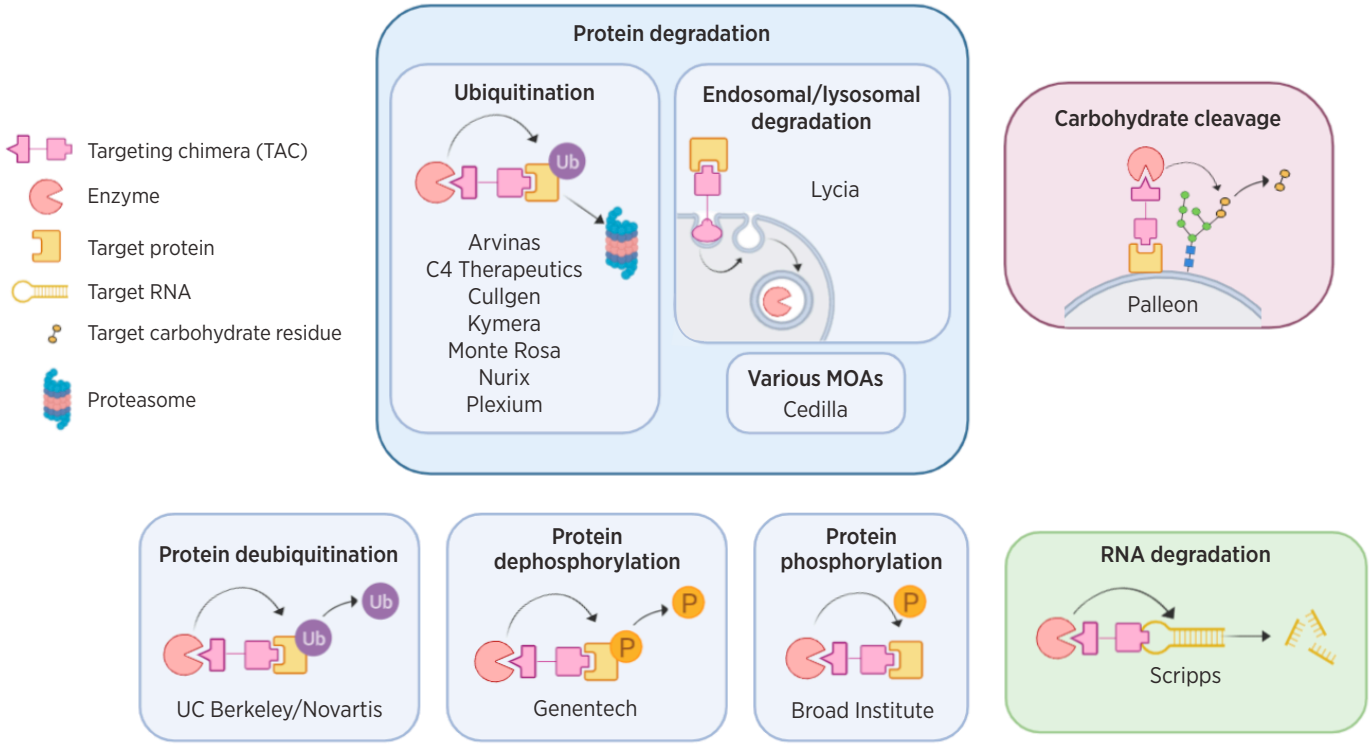
Choudhary told BioCentury the technology can add phosphate groups to not only to targets that routinely use them for signaling, but also to

There's a tac for that

Companies and academics are developing a wide array of targeting chimeras (TACs) that simultaneously bind a molecular target and an enzyme that can modify the target. The technologies are an extension of the idea behind PROTACs (proteolysis targeting chimeras), the first in the class of targeted protein degraders, which capitalize on ubiquitination. The expanding toolbox should give drug developers access to a wider range of therapeutic targets and enable them to manipulate the targets in new ways.

These TACs can either be heterobifunctional -- consisting of two independently discovered ligands linked together -- or monofunctional "molecular glues" -- single molecules capable of binding both the target and the enzyme. The diagram depicts the former.

The strategies shown in the top row are being deployed in company pipelines, while the strategies in the bottom row are still at the research stage.



those lacking natural phosphorylation sites. "I think we can now induce phosphorylation on any protein of interest inside cells."

Researchers at the University of California Berkeley lab of Daniel Nomura are developing compounds that perform the opposite function of PROTACs -- preventing target proteins from being degraded. The concept relies on deubiquitinases, enzymes that prevent proteins from being sent to the proteasome by removing their ubiquitin tags. Deubiquitinases are themselves targets of company programs in cancer and neurology (see "Golden State of DUBs").

Nomura said one "obvious" application of his lab's deubiquitinase-targeting chimera (DUBTAC) platform is stopping the degradation of tumor suppressors like p53.

His team is investigating the approach in collaboration with Novartis AG (NYSE:NVS; SIX:NOVN). In 2017, the partners launched the Novartis-Berkeley Center for Proteomics and Chemistry Technologies to discover therapies against difficult-to-drug targets. The DUBTAC technology is not yet published.

The broader targeting strategies are also being developed against non-protein substrates, including glycoproteins and RNA.

Palleon's EAGLE platform fuses mAbs against targets on tumor cell surfaces to a recombinant sialylase enzyme that removes immunosuppressive sialic acid residues from sugars on tumor cell surfaces (see "SIGLECs: Next Generation Checkpoints").

CEO and founder Jim Broderick said targeted delivery of an exogenous enzyme required overcoming protein engineering and manufacturing challenges. But he thinks the advantage over the alternative used by most TACs -- targeted recruitment of endogenous enzymes -- is not having to rely on cellular machinery. "You're actually controlling what you're trying to do directly," he said.

On the RNA front, Scripps Research Professor Matthew Disney is developing ribonuclease targeting chimeras (RIBOTACs) that trigger nuclease-mediated degradation of specific RNAs (see "Disney Uncovers RNA-Binding Small Molecule for TNBC").

"YOU'RE STILL ALMOST CERTAINLY GOING TO BE DEALING WITH LARGER-THAN-AVERAGE SMALL MOLECULES."

IAN TAYLOR, ARVINAS

Disney said that despite the name, RIBOTACs were more inspired by antisense oligos, which trigger RNA degradation via RNAse H, than by PROTACs. Still, he thinks RIBOTACs and PROTACs are both examples of using chemistry to trigger the cell's quality control machinery.

MIT's Choudhary believes researchers should not limit the technology's applications to replicating natural functions.

"We will have tunnel vision if we just say, we're only going to aim for biologically relevant modifications," Chouhary said. "We should not ignore the unnatural post-translational modifications and their potential utility in therapeutic development."

Evolving therapies

The expansion of the technology spans academic and industry labs. However, going from prototypes to therapeutic products will require tackling the same issues that slowed translation of targeted protein degraders.

"There's a lot of activity now in induced proximity strategies, beyond protein degradation," said Arvinas CSO Ian Taylor. "It's similar to the early days of PROTACs, though hopefully it won't take 18 years for some of these technologies."

One key hurdle will be PK, particularly for oral delivery (see "Down the Hatch").

"You're still almost certainly going to be dealing with larger-than-average small molecules, and making those into drug-like molecules that can be orally bioavailable will still be a challenge," he said. "As people study how we did it, there can be learnings from that, and hopefully that will speed up getting them into the clinic."

He said another challenge for enzyme-targeting chimeras of any kind is to "outrun the synthesis rate" of its substrate. "The timescale of the event you're countering really matters."

Versant Ventures' Clare Ozawa said the firm is closely watching for advances in other targeting chimera technologies, such as RNA degraders. "These are all areas we're quite interested in and thinking proactively about."

Versant has been one of the most active players at seeding companies developing targeted protein degraders, and the first to take the paradigm beyond the proteasome.

Bertozzi's Lycia is Versant's third play in targeted degradation; the firm also invested in Vividion Therapeutics Inc., which uses its chemoproteomics platform to find ligands to incorporate into targeted protein degraders, and stealth company Monte Rosa Therapeutics Inc., which identifies targeted protein degraders via its phenotypic screening platform.

At least one other VC has disclosed multiple portfolio companies in the space. The Column Group has backed two targeted protein degradation companies with ubiquitination-based platforms: Plexium Inc. and Nurix Therapeutics Inc.

Taylor said Arvinas is sticking with PROTACs for now. But he thinks the company will contribute to the development of these newer fields as its patents become public.

The basic science of new enzyme-targeting strategies is still being worked out.

Crews' lab last month retracted a May publication in *ACS Central Science* describing endosome targeting chimeras (ENDTACs) that induced endolysosomal degradation of extracellular proteins by linking one ligand that binds the cell surface GPCR CXCR7 to a second, covalent ligand for a target protein. Arvinas declined to comment on the retraction, and Crews did not return requests for comment.

TARGETS

AKT (AKT1; PKB; PKBA) - Protein kinase B
CXCR7 - CXC chemokine receptor 7
EGFR (ErbB1; HER1) - Epidermal growth factor receptor
p53 (TP53)
PP1 - Protein phosphatase 1 (PPP1)

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