TARGETED PROTEIN DEGRADATION

Protein degradation reaches further out

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Although the development of efficient and selective small-molecule inhibitors and monoclonal antibodies has improved over the years, 'undruggable' proteins can still elude these established drug modalities. Targeted protein degradation with proteolysis-targeting chimaeras (PROTACs) has emerged as an attractive route to target these difficult proteins. However, this approach is limited to proteins that have intracellular domains, meaning that ~40% of the proteome is out of scope. Now, Bertozzi and colleagues have pushed the PROTAC concept further by harnessing the endosome machinery that traffics extracellular proteins to the lysosomes for degradation.

Protein degradation is a normal process of protein renewal and quality control within the cell, and PROTACs work by harnessing the ubiquitin–proteasome degradation system. They are heterobifunctional small molecules that bind to a target protein and an E3 ligase, which results in ubiquitination and subsequent degradation of the target protein by the proteasome. This system, however, only works inside the cell.

So, Bertozzi and colleagues decided to explore other degradation systems that could work outside the cell, such as the lysosomal trafficking system. In a similar way to PROTACs, the approach consists of conjugates — lysosome-targeting chimaeras (LYTACs) — that bind both the extracellular domain of a target protein and a cell surface lysosome-shuttling receptor as a vehicle for delivering the targeted protein to the lysosome.

The authors used the cation-independent mannose 6-phosphate receptor (CI-M6PR, also known as M6PR) as the vehicle lysosome-shuttling receptor. "We took inspiration from the literature on lysosomal enzyme replacement therapies, in which recombinant enzymes bearing the mannose 6-phosphate mark on their N-glycans are taken up by cells and delivered to lysosomes via the M6PR system," explains Bertozzi. "Learnings from that field, particularly with regard to the design of synthetic sugar analogues that bind M6PR, provided us with a foundation to work from when designing LYTACs," she adds.

On the basis of these studies, the authors chose *N*-carboxyanhydride (NCA)-derived glycopolypeptides bearing multiple serine-*O*-M6P (M6Pn) residues as an optimal agonist for M6PR. Then, they assessed the ability of biotinylated poly(M6Pn) polypeptides to bind protein targets and transport them for degradation by measuring the uptake of NeutrAvidin-647 (NA-647) and Alexa Fluor-647 (AF64) — to which biotin binds — in several cell lines.

Next, the authors evaluated whether conjugation of a poly(M6Pn)-bearing glycopolypeptide to an antibody would reprogramme the antibody to direct extracellular proteins — such as membrane receptors — to the lysosome. To that end, they generated a LYTAC antibody that would bind EGFR by conjugating the EGFR-blocking antibody cetuximab (ctx) to M6Pn glycopolypeptides (ctx-M6Pn Ab-2). HeLa cells treated with ctx as control showed low levels of receptor phosphorylation, but degradation of EGFR with ctx-M6Pn Ab-2 reduced phosphorylation even further, showing the advantages of receptor degradation. They also tried a non-specific conjugated antibody (LYTAC Ab-1) — in which an anti-mouse IgG was conjugated to M6Pn glycopolypeptides — against transferrin receptor 1 (CD71), as well as a LYTAC antibody for PDL1 (anti-PD-L1-M6Pn LYTAC). Both LYTAC antibodies effectively reduced levels of expression of the corresponding receptors (80% and 33% reduction, respectively), compared with conventional antibodies that target these receptors.

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"These findings expand the spectrum of targets for which a degradation strategy can be therapeutically relevant," says Bertozzi. "Also, as their mechanism of action does not require passage across cell membranes, LYTACs can be constructed from small or large molecule building blocks, increasing the representation of biologics as potential therapeutic degraders."

The LYTACs described in this study take advantage of M6PR, but the authors argue that other shuttling receptors could be co-opted, which can be helpful in the event of resistance when targeting oncoproteins. The different ways to modulate LYTACs will offer the opportunity to target a wide range of proteins, not just receptor tyrosine kinases, but also protein aggregates and immune complexes, or circulating antibodies in autoimmune disorders. The potential of this strategy is being explored by a new startup company, Lycia Therapeutics, which has licensed the technology from Stanford University.

M. Teresa Villanueva

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