News & views

Chemical biology

Protein degraders extend their reach

Claire Whitworth & Alessio Ciulli

Molecules have previously been made that induce protein destruction inside cells. A new class of molecule now induces the degradation of membrane and extracellular proteins — opening up avenues for drug discovery. **See p.291**

Most drugs act by binding to a specific site in a target protein to block or modulate the protein's function. The activity of many proteins, however, cannot be altered in this way. An emerging class of drug instead brings proteins into proximity with other molecules, which then alter protein function in unconventional ways[1–3]. One such approach uses drug molecules called protein degraders, which promote the tagging of proteins with ubiquitin, another small protein. Tagged proteins are then broken down into small peptide molecules by the cell's proteasome machinery. But because the ubiquitin-mediated degradation pathway occurs inside the cell, protein degraders developed so far attack mainly intracellular targets. On page 291, Banik *et al.*[4] now report a different mechanism that opens up extracellular and membrane-bound proteins for targeted degradation.

The authors report protein degraders that they call lysosome-targeting chimaeras (LYTACs), which are bifunctional (they have two binding regions; Fig. 1). One end carries an oligoglycopeptide group that binds to a transmembrane receptor (the cation-independent mannose-6-phosphate receptor; CI-M6PR) at the cell surface. The other end carries either an antibody or a small molecule that binds to the protein targeted for destruction. These two regions are joined by a chemical linker.

The formation of a trimeric CI-M6PR–LYTAC–target complex at the plasma membrane directs the complex for destruction by protease enzymes in membrane-enclosed organelles called lysosomes. LYTACs are conceptually related, but complementary, to proteolysis-targeting chimaeras[5] (PROTACs) — another bifunctional class of protein degrader that mainly targets intracellular proteins by recruiting them to E3 ligases (the enzymes that tag proteins with ubiquitin).

Banik *et al.* began by making LYTACs of varying size and linker composition, and which used a small molecule called biotin as the protein-binding component — biotin binds with exceptionally high affinity to avidin proteins. The authors observed that these LYTACs rapidly shuttled an extracellular fluorescent avidin protein to intracellular lysosomes in a way that required engagement with CI-M6PR. When the authors replaced biotin with an antibody that recognizes apolipoprotein E4 (a protein implicated in neurodegenerative diseases), this protein was also internalized and degraded by lysosomes. LYTACs can, therefore, repurpose antibodies from their normal immune function to direct extracellular proteins for lysosomal degradation.

Next, Banik *et al.* investigated whether LYTACs could induce the degradation of membrane proteins that are targets for drug discovery. In several cancer cell lines, LYTACs did indeed induce the internalization and lysosomal degradation of the epidermal growth factor receptor (EGFR) — a membrane protein that drives cell proliferation by activating a signalling pathway. Depletion of EGFR levels by LYTACs in the cancer cell lines reduced signal activation downstream of EGFR, compared with the amount observed when EGFRs were blocked by antibodies alone. This result confirms a previously reported[5] advantage of using target degradation in therapeutic applications, rather than target blocking.

Similar outcomes were observed with LYTACs for other single-pass transmembrane proteins (proteins that span the cell membrane only once), including programmed

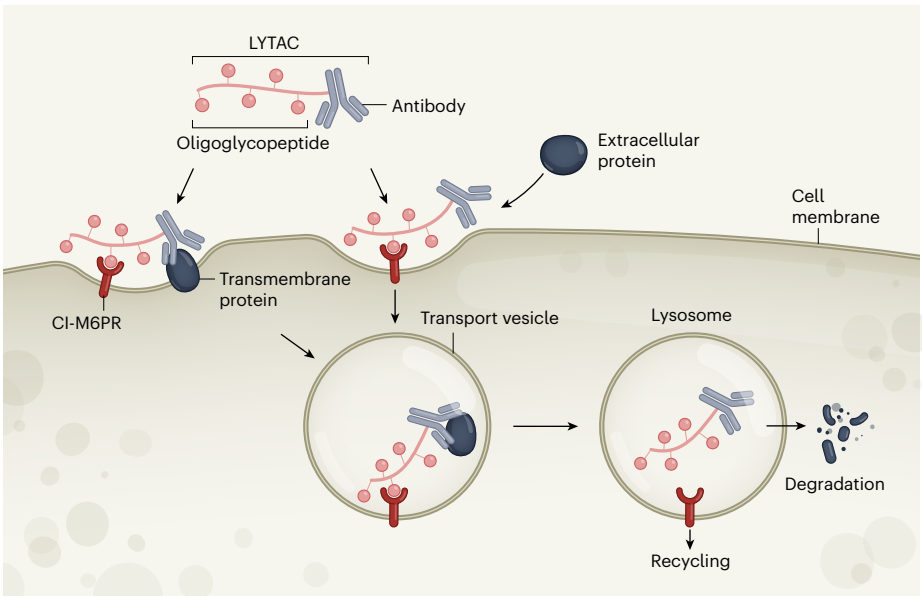

Figure 1 | Mechanism of action of lysosome-targeting chimaeras (LYTACs). Banik *et al.*[4] report LYTAC molecules, which consist of an oligoglycopeptide group (which binds to a cell-surface receptor, CI-M6PR) and an antibody that binds to a specific transmembrane or extracellular protein. The antibody can also be replaced by a small protein-binding molecule (not shown). On simultaneously binding to both CI-M6PR and the target protein, the resulting complex is engulfed by the cell membrane, which forms a transport vesicle. This carries the complex to a lysosome (an organelle that contains protein-degrading enzymes). The protein is degraded and the receptor is recycled; it remains to be seen whether the LYTAC is also degraded. LYTACs are potentially useful for therapeutic applications.

death ligand 1 (PD-L1), which helps cancer cells to evade the immune system. The next step will be to establish whether LYTACs can also induce the degradation of multi-pass proteins that span the membrane several times, such as the ubiquitous G-protein-coupled receptors and proteins that transport materials across membranes (ion channels and solute-carrier proteins, for example). If so, it will be interesting to compare the performance of LYTACs, which would bind to the extracellular domains of such proteins, with that of PROTACs, which can bind to the intracellular domains of these proteins (as was recently demonstrated[6] for solute-carrier proteins).

As with any new drug modality, there is scope for improvement. For example, Banik and colleagues' first PD-L1-targeting LYTACs produced only partial degradation of the protein, which the authors attributed to low expression of CI-M6PR in the cell lines used. When the authors made a second type of LYTAC that incorporated a more potent PD-L1 antibody, degradation increased, albeit in cells that expressed greater levels of CI-M6PR than did the original cell lines. This shows that low abundance of the lysosome-shuttling receptor hijacked by the LYTAC (in this case, CI-M6PR) can reduce the effectiveness of these degraders. Similarly, the loss of core components of E3 ligases is a common mechanism by which cells become resistant to PROTACs[7]. Lysosome-shuttling receptors other than CI-M6PR could be used by LYTACs as alternatives, should resistance emerge. Degraders that target cell-type-specific receptors might also have improved safety profiles compared with conventional small-molecule therapeutics, which are not always cell-type selective.

What sets PROTACs and LYTACs apart from conventional drugs is their mode of action. For example, after a PROTAC has brought about the destruction of a target protein, the PROTAC is released and can induce further cycles of ubiquitin tagging and degradation, thereby acting as a catalyst at low concentrations[1,5]. Mechanistic studies are now warranted to determine whether LYTACs also work catalytically.

Another aspect of the mode of action of both PROTACs and LYTACs is that they bring two proteins together, to form a trimeric complex. A general feature of such processes is the hook effect, whereby trimer formation, and thereby the associated biological activity, decreases at high drug concentrations. This is because dimeric complexes generally form preferentially at high drug concentrations – an undesirable effect that can be alleviated by ensuring that all three components interact in such a way that trimer formation is more favourable than is dimer formation[1].

Kinetics also matters for protein degraders. For example, stable and long-lived trimeric complexes that involve PROTACs accelerate target degradation, improving drug potency and selectivity[8]. It will be crucial to understand how the complexes formed by LYTACs can be optimized to improve degradation activity.

PROTACs and LYTACs are larger molecules than conventional drugs. As a result of their size, PROTACs often do not permeate well through biological membranes, which can make them less potent drugs than the biologically active groups they contain. Size should be less of a problem for LYTACs because they do not need to cross the cell membrane, although they would still need to pass through biological barriers to combat diseases of the central nervous system. The development of lysosomal degraders that are smaller and less polar than LYTACs – and therefore more able to pass through membranes – will be eagerly anticipated. Small 'glue' molecules that bind to E3 ligases can already do the same job as PROTACs[9].

Targeted protein degradation is a promising therapeutic strategy, and the first PROTACs are currently in clinical trials[10]. LYTACs will need to play catch-up, but they have earned their place as a tool poised to expand the range of proteins that can be degraded. Their development as therapies will require an understanding of their behaviour in the human body – their pharmacokinetics, toxicity, and how they are metabolized, distributed and excreted, for example. It can be challenging to optimize the biological behaviour of molecules that incorporate large groups, such as antibodies and oligoglycopeptides, during drug discovery, but this problem can be overcome by further engineering the structures of these groups[11]. Banik and colleagues' new approach to degradation therefore warrants an all-hands-on deck approach.

Scientists working in drug discovery will eagerly await the development of LYTACs and the emergence of other methods for the drug-induced degradation of proteins[12]. Is no protein beyond the reach of degraders?

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Ecology

Rethinking extinctions that arise from habitat loss

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Does the loss of species through habitat decline follow the same pattern whether the area lost is part of a large or a small habitat? An analysis sheds light on this long-running debate, with its implications for conservation strategies. **See p.238**

Understanding how habitat size affects the abundance of all the species living in a community provides ecological insights and is valuable for developing strategies to boost biodiversity. On page 238, Chase *et al.*[1] report results that might help to settle a long-running debate about the relationship between the area of a habitat and the diversity of species it can host.

Land transformation by human activity is a major component of global change. The loss of natural habitats reduces the local diversity and abundance of species[2], and has been implicated in more than one-third of animal extinctions worldwide between 1600 and 1992 (ref. 3). A report from the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services estimates that currently more than half a million species – about 9% of all terrestrial species – might lack the amount of habitat needed for their long-term survival[4]. Moreover, their disappearance would compromise many key ecosystem services, such as pollination or the control of pests or disease-causing agents.

The effect of habitat loss on biodiversity has