Targeted protein degradation as a new paradigm in drug discovery. Is there a role for peptides?

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During the last century, drug discovery has largely focused on finding enzyme inhibitors, agonists and antagonists of different receptors and ion channel blockers. Most of these drugs, making up the largest part of the medicine arsenal currently available in the market, target biomolecules with well-defined pockets able to bind small molecules.

A recent alternative approach to expand the search space for new medicines is targeting protein-protein interactions (PPI).[1] The first PPI-targeting cancer drug was launched a few years ago, and several more candidates are already under clinical development.[1] In this challenging strategy, peptides are commonly used as starting prototypes to validate the target and to facilitate a subsequent search for peptidomimetics. Previous contributions to this section have dealt with this strategy.[2] Both of the above approaches rely on the use of high affinity compounds, and in achieving high drug concentrations to trigger and sustain the anticipated pharmacological activity. Such exposure to high drug levels underlies the increasing awareness of the risk of off-target side effects, including unwanted increased target expression. Other lines of attack, such as antibodies, the CRISPR/Cas9-mediated gene knockouts, and RNAi approaches have also met with limitations.

As a radically different paradigm in drug discovery, targeted protein degradation is already raising tremendous interest and investment from pharmaceutical and biotech companies seeking to bring first-in-class medicines to patients, especially for the so called "undruggable" proteins. To this end, two main approaches can be distinguished: a) protein-targeting chimeric molecules (PROTACs), consisting on a ligase-recruiting moiety linked to a ligand of the target protein, promoting the proximity-induced ubiquitination of the target protein and its degradation by the proteasome;[3,4] and b) hydrophobic tagging (HyT), based on recruitment of the degradation machinery by a bulky hydrophobic ligand (ubiquitin-like) linked to a compound that specifically recognizes the target protein, to finally elicit direct proteasomal degradation.[5] In both cases, the strategy requires the preparation of bifunctional molecules bearing, on the one hand, a ligand of the target protein and, on the other, a moiety capable of recruiting the cell's own machinery to initiate protein degradation (Figure 1).

Compared to clasical strategies, induced protein degradation offers several advantages: a) low concentration of the bifunctional molecule is required, because of its catalytic mode of action to initiate the degradation cascade; b) broad applicability across cells and *in vivo* systems; c) novel and improved pharmacology, by targeting the "undruggable" proteome or by completely silencing proteins with multiple functions.

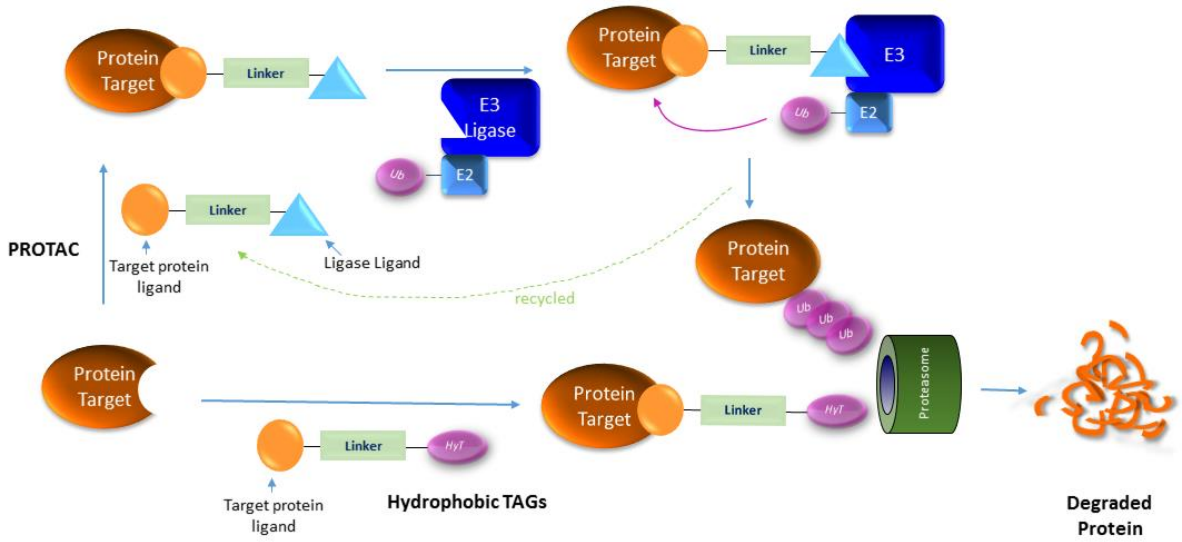

Figure 1. Schematic representation of induced protein degradation strategies

Although most PROTACs examples refer to small-molecule derivatives,[6] here I will comment on some representative examples containing peptides/peptide derivatives either as protein target ligands, linkers or ligase ligands, or a combination of them.

The group of M.C. Crew, one of the pioneers and most active in this field, described hybrid molecule **1** (Figure 2), as an inactive PROTAC that upon specific phosphorylation by TrkA is able to recruit fibroblast grow factor receptor substrate 2α, to be ubiquinated and degraded, which at the end resulted in blockade of neuronal differentiation.[7] This peptide conjugate contains an *N*-terminal peptide sequence, corresponding to the TrkA *trans*-autophosphorylation site, two consecutive aminohexanoic acid molecules forming an amide-type linker, followed by the heptapeptide binding sequence of the E3 ubiquitin ligase von Hippel Lindau protein, and a *C*-terminal polyR (CPP) to facilitate cell penetration. Interestingly, this approach could be used for the selective inhibition of particular signaling cascades, through the degradation of downstream-signaling proteins instead of targeting directly the corresponding kinase.

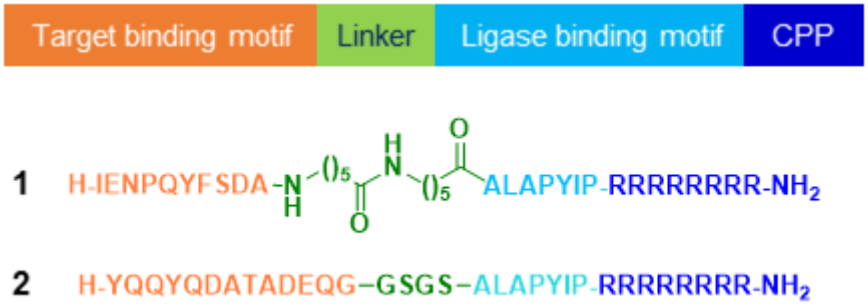

Figure 2. Selected examples of peptide-based PROTACs

A similar strategy, but using the all-peptide PROTAC **2**, has been exploited by the group of Y.-M. Li to induce the specific knockdown of endogenous Tau protein through the ubiquitin-proteasome degradation.[8] In this case, the chimeric molecule is composed of a Tau binding motif, a tetrapeptide (GSGS) linker, the sequence to recruit E3 ligase and again the polyR CPP. This multifunctional PROTAC was able to interact with Tau, increased its ubiquitination through promoting the interaction with VHL E3 ligase, leading to lower Tau levels and reduced Ab toxicity in an AD mouse model. The knockdown of Tau protein was also achieved by using a closely related PROTAC, in which the E3 ligase recruiting segment (ALAPYIP) has been changed by the LDEPTGEYL peptide sequence, designed to join Keap1 protein, a substrate adaptor protein for ubiquitinE3 ligase.[9]

Several recent examples describe the use of 4-hydroxyproline-containing dipeptide derivatives as ligase binding moieties in small-molecule PROTACs (Figure 3). Thus, compound **3**, described by the group of J.E. Bradner,[10] combines a potent and selective inhibitor of the TRIM24 bromodomain to a Hyp-based dipeptide responsible for the VHL E3 ubiquitin ligase recruitment. This heterobifunctional molecule elicited the potent and selective degradation of TRIM24, important in acute leukemia, showing enhanced anti-proliferative responses compared to direct bromodomain inhibition. Likewise, related molecules, composed of good BET bromodomain inhibitors, a polyethylene glycol linker, and the same Hyp-containing dipeptide derivative as the VHL ligand, behaved as potent target degraders and displayed antiproliferative activities in acute myeloid leukemia cell lines.[11] Hyp-derived protein degrader **4**, discovered through a systematic inspection of linker length and ligand affinities for TBK1 kinase, induced a potent and selective degradation of this kinase and was an important tool to evaluate the implication of TBK1 as a target in mutant K-Ras cancer cells.[12]

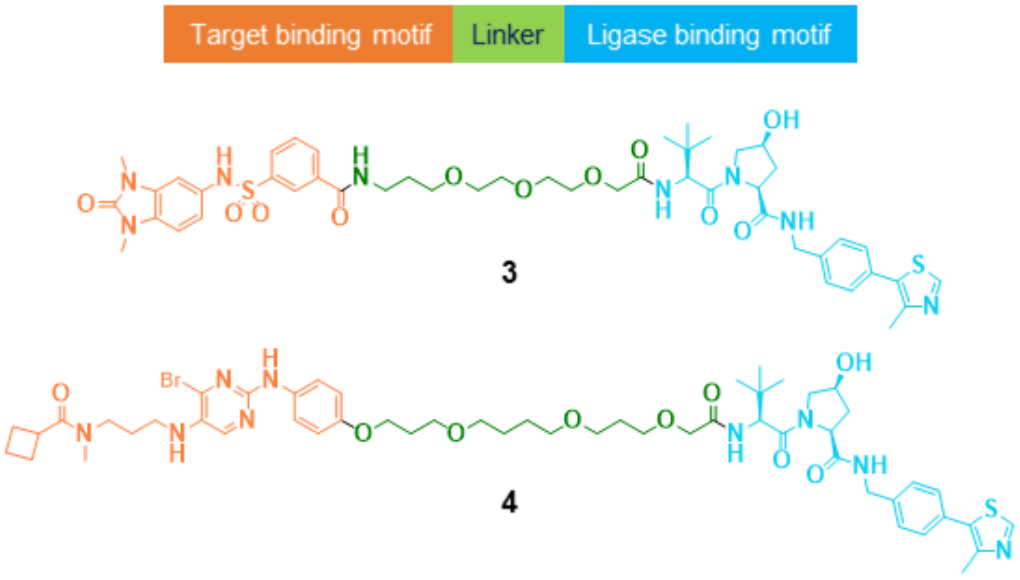

Figure 3. Selected examples of Hyp-based PROTACs

Considering the high number of bioactive peptides already known, their highly diverse protein targets, and the scarce examples of peptide-based PROTACs described to date in the literature, we can envisage an important contribution of peptides to study this exciting strategy about the manipulation of disease-relevant proteins. In addition, while some interesting examples of small-molecule conjugated to hydrophobic tags (HyT) have been described to induce protein degradation, to the best of our knowledge, this approach has not been applied yet to bioactive peptides. Therefore, the potential of peptide-derived PROTACs and peptide-HyT in the emerging paradigm of induced protein degradation remains mostly unexplored.

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