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Targeted protein degradation by PROTACs☆

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ABSTRACT

Targeted protein degradation using the PROTAC technology is emerging as a novel therapeutic method to address diseases driven by the aberrant expression of a disease-causing protein. PROTAC molecules are bifunctional small molecules that simultaneously bind a target protein and an E3-ubiquitin ligase, thus causing ubiquitination and degradation of the target protein by the proteasome. Like small molecules, PROTAC molecules possess good tissue distribution and the ability to target intracellular proteins. Herein, we highlight the advantages of protein degradation using PROTACs, and provide specific examples where degradation offers therapeutic benefit over classical enzyme inhibition. Foremost, PROTACs can degrade proteins regardless of their function. This includes the currently "undruggable" proteome, which comprises approximately 85% of all human proteins. Other beneficial aspects of protein degradation include the ability to target overexpressed and mutated proteins, as well as the potential to demonstrate prolonged pharmacodynamics effect beyond drug exposure. Lastly, due to their catalytic nature and the pre-requisite ubiquitination step, an exquisitely potent molecules with a high degree of degradation selectivity can be designed. Impressive preclinical in vitro and in vivo PROTAC data have been published, and these data have propelled the development of clinically viable PROTACs. With the molecular weight falling in the 700–1000 Da range, the delivery and bioavailability of PROTACs remain the largest hurdles on the way to the clinic. Solving these issues and demonstrating proof of concept clinical data will be the focus of many labs over the next few years.

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1. Introduction

Over the past few decades several novel pharmacological approaches have emerged to target disease. The classical small molecule inhibitor paradigm is now complemented by being able to block extracellular signalling with monoclonal antibodies and by degrading target mRNA with RNA interference approaches. The major advantage of antibody therapies stems from their very high binding affinity to their targets and their prolonged pharmacokinetic profile due to endosomal FcRn-IgG recycling of the antibody. A primary therapeutic avenue for

Abbreviations: AR, Androgen Receptor; IV, intravenous; PROTAC, PROteolysis TArgeting Chimera; SC, subcutaneous; TPD, Targeted Protein Degradation; VHL, Von Hippel-Lindau Tumor Suppressor, E3 Ubiquitin Protein Ligase.

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antibodies takes advantage of their ability to block extracellular protein:protein or protein:ligand interactions. The challenges that are difficult to overcome with antibodies, however, include their inability to cross cell membranes, need for parenteral delivery and high "cost of goods". RNA interfering molecules, when formulated and conjugated properly, can be delivered to cross the cell membrane. RNAi can achieve high potencies to their targets and, given their catalytic nature, the siRNA molecules often demonstrate prolonged durability of target mRNA knockdown. The catalytic nature of RNAi also affords them efficacy at low exposures because each siRNA molecule can degrade many mRNA transcripts. The deficiencies of the current generation of RNAi therapeutics include their lack of oral bioavailability, poor PK and limited tissue distribution. Hence, the majority of current RNAi therapies target liver diseases (Bobbin & Rossi, 2016). Yet, both antibody and RNAi approaches can target proteins beyond the enzymatic inhibition and modulating the ligand binding domain, both of which have been the mainstay for the small molecule therapies (Table 1).

A desirable feature would be to combine several aspects of the small molecule, antibody and RNAi modalities. That is, ideally one would have a molecule that has the ability to also target intracellular proteins, including the un-druggable proteome, possesses high selectivity and oral bioavailability, distributes well into various tissues, including possibly the central nervous system (CNS), and exhibits catalytic mode of action allowing low exposures to be efficacious (Crews, 2010).

A novel approach that has the potential to achieve most of these goals is Targeted Protein Degradation (TPD) (Deshaies, 2015). Unlike mRNA degradation with siRNA, here the outcome is the degradation of the disease associated proteins. There are a few examples of targeted protein degradation with simple small molecules. The observation that Estrogen Receptor alpha (ERα) antagonist fulvestrant leads to proteasomal degradation of the receptor has been supported by observations that similar ligand-mediated degradation of target proteins can occur with IAP, RARα, AR and others (Feltham et al., 2011; Gustafson et al., 2015; Preisler-Mashek, Solodin, Stark, Tyriver, & Alarid, 2002). However, TPD with simple small molecues is rare and, in fact, the opposite is also observed frequently. For instance, Brd4 and Mcl1 ligands can lead to large increases in their target proteins (Leverson et al., 2015; Lu et al., 2015). Thus, ligand-mediated destabilization of the target protein is wrought with great uncertainties and often the micromolar potencies needed for this effect are not therapeutically achievable.

A more predictable method of TPD employs PROTAC (PROteolysis TArgeting Chimeras) technology. PROTAC is a heterobifunctional molecule that simultaneously binds E3 ubiquitin ligase and the target protein, thus driving the exposed lysines on the target protein to be ubiquitinated by the E3-ubiquitin ligase complex (Toure & Crews, 2016). Upon poly-ubiquitination of the target protein, it is recognized by the cap 19S domain of the proteasome and catalytically digested into amino acids and small peptides (Fig. 1).

The initial proof of concept work for PROTACs was published 15 years ago when a chimeric molecule that simultaneously binds MetAP2 protein and the E3 ubiquitin ligase SCF-β-TRCP was shown to lead to polyubquitination of MetAP2 (Sakamoto et al., 2001). While a small molecule ligand was readily available for MetAP2, at the time there were no known small molecule ligands for E3 ligases. Thus, the study employed a 10-aa phosphopeptide known to bind β-TRCP. These data were encouraging but they also highlighted the need to find more drug-like ligands for E3 ligases that could be incorporated into a PROTAC. Fortuitously, in early 2001, when the first peptidic PROTAC paper was published, two papers described the binding mode of HIFα peptide to E3 VHL (Ivan et al., 2001; Jaakkola et al., 2001). It was known that VHL mediates the degradation of HIFα, and now it was shown that there is a specific proline P564 hydroxylation on HIFα that needs to occur prior to binding to VHL. Borrowing from the SCF-β-TRCP idea, short hydroxyproline peptides were incorporated into a peptidic PROTAC to recruit VHL E3 ligase and these PROTACs were demonstrated to lead to degradation of FKBP12 and AR (Schneekloth et al., 2004). The peptidic nature of the HIFα moiety in the PROTAC limits their use in vivo, yet the non-ionic nature of the hydroxyproline core suggested that drug-like E3 ligase ligands were achievable. Concomitantly, the publication of Nutlins as MDM2 ligands prompted the testing of incorporating these ligands as an E3-recruiting moiety in a PROTAC setting. Unfortunately, the potencies of these PROTACs were only modest (Schneekloth, Pucheault, Tae, & Crews, 2008).

A series of publications in 2012 demonstrated the development of small molecule inhibitors of the HIFα and VHL interaction (Buckley, Gustafson, et al., 2012; Buckley, Van Molle, et al., 2012). These ligands retain the central hydroxyproline residue, but the peptidic nature of the HIFα is diminished and the molecular weight reduced to ~400 Da. With the available crystal structure of VHL, the K_d of these compounds was driven below 1 μM and, importantly, the molecule's properties (e.g. PSA, logP, HBD) became more drug-like. A series of publications on small molecule VHL-based PROTACs in 2015 was the culmination of more than 10 years of work in the field (Bondeson et al., 2015; Buckley et al., 2015). Alongside VHL, the recognition that thalidomide interacts with the E3 ligase Cereblon prompted several groups to build PROTACs using thalidomide analogs as E3 recruiting moieties (Lu et al., 2015; Winter et al., 2015). Thus far, both VHL- and Cereblon-based PROTACs have been discovered, validated, and published. Since PROTACs can be made to behave like traditional small molecules, yet their mode of action affords one to achieve new therapeutic functions, there is also commercial interest in converting PROTACs into marketed drugs, as evidenced by the founding of Arvinas in 2013 and C4 Therapeutics in 2016 (Fig. 2).

This review will focus on what makes the PROTAC approach unique compared to other therapeutic interventions. Foremost, we will emphasize the benefits of TPD over the classical inhibitor paradigm. We will also discuss the critical issues PROTACs need to overcome in order to fully realize the promise of TPD in humans.

Table 1
The features of therapeutic modalities.

	Small molecule	Monoclonal antibody	siRNA	TPD
Intracellular targets	Yes	No	Yes	Yes
Systemic delivery	Yes	Yes	No	Yes
Tissue penetration	Yes	Poor	Poor	Yes
Targets proteins with scaffolding function	No	Yes	Yes	Yes
Eliminates pathogenic proteins	No	No	Yes	Yes
Oral bioavailability	Yes	No	No	Yes
Ease of developing high potency and selectivity	Poor	Yes	Yes	Yes
Catalytic MOA	No	No	Yes	Yes
Route of delivery	PO/IV/SC	IV/SC	IV/SC	PO/IV/SC

2. Why protein degradation?

We will discuss six reasons why PROTAC mediated TPD is different than, and often superior to, current treatment modalities.

2.1. The ability to target the undruggable proteome

Currently, the FDA has approved agents against about 400 human proteins (Rask-Andersen, Almén, & Schiöth, 2011). More than 90% of them fall into the category of enzymes, transporters, GPCRs, CD markers, voltage gated ion channels and nuclear receptors. These target classes are attractive drug targets, but they are also fairly easily druggable by current methodologies. The numbers vary but it has been estimated that there are about 3000 disease causing genes, suggesting that the current therapies can target only 13% (400 out of 3000 genes) of the therapeutic proteome (http://www.omim.org/statistics/geneMap). Thus, about 85% of proteins associated with disease

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