RESEARCH HIGHLIGHT

Beyond targeted protein degradation: LD-ATTECs clear cellular lipid droplets

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Cell Research (2021) 31:945–946; <https://doi.org/10.1038/s41422-021-00546-1>

AuTophagy-Tethering Compounds (ATTECs) are a novel class of bifunctional molecules proposed to hijack the autophagosomal pathway for the degradation of potentially any cellular components in addition to proteins. Targeting stored fats in cells with LD-ATTECs suggests a novel approach to combating diseases implicating accumulation of lipid droplets, such as obesity, cardiovascular diseases, or cancer.

With two new drug candidates advancing in phase II clinical trials, PROteolysis TARgeting Chimeras (PROTACs) offer a conceptually new way to approach therapeutic challenges.^{1,2} The now well-established concept that endogenous degradation pathways can be hijacked to remove a target protein rather than merely altering its function has dramatically widened the range of potentially druggable proteins. A PROTAC is a synthetic heterobifunctional molecule that links a ligand for a target protein to a ligand for an E3 ligase, a class of proteins responsible for recognition and ubiquitin tagging of proteins for degradation. As such, PROTACs bring together these two proteins within the cellular environment in a transient ternary complex that leads to polyubiquitination of the target which is then recognized and degraded by the proteasome machinery. More recently, it has been proposed that the autophagosomal pathway may be hijacked by AUTACs (autophagy-targeting chimeras), which exploit an alternative ubiquitin-dependent protein tagging strategy to direct a target protein for degradation via autophagocytosis.³

While these novel therapeutic modalities have the potential to transform the drug discovery field, it is becoming clear that they present limited scope, being able to target only proteins that can be effectively polyubiquitinated. Hence, further modalities may be needed to address more complex targets, such as organelles and non-proteinaceous components, by targeted degradation. To overcome this limitation, the Lu group has pioneered the concept of AuTophagy-Tethering Compounds (ATTECs).^{4–7} ATTECs directly recruit LC3, a lipidated protein that is found on the membranes of autophagosomes, and thus direct a target of interest for autophagocytosis without the need of a tag for recognition. Autophagosomes are endogenous vesicles that clear cytoplasmic components, including not only proteins but also organelles and microorganisms. Autophagocytosis is accomplished by fusion of these vesicles to lysosomes, leading to degradation of the autophagosome content into simpler components, which are eventually recycled within the cell.

In a previous proof-of-concept study, the Lu group identified bispecific molecules that could cause selective degradation of

mutant Huntingtin (mHTT), the protein that causes Huntington's disease, but not wild-type HTT. These compounds act as molecular glues, bringing together mHTT and autophagosomal LC3, thus resulting in ubiquitin-independent selective mHTT protein degradation.⁴ In this work, the authors already speculated on the use of such compounds for the generation of bifunctional molecules, in a similar fashion to PROTACs, to recruit further targets to LC3 for degradation.

Now, in an attempt to broaden the potential of ATTECs, Fu and colleagues have developed a new class of molecules, named LD-ATTECs, that efficiently clear lipid droplets (LDs) in cells (Fig. 1a).⁷ LDs are organelles for the intracellular storage and release of lipids, which comprise a neutral lipid core enclosed by a phospholipid monolayer membrane embedding specific proteins.⁸ Being primarily composed of lipids, LDs could not be targeted by PROTACs or AUTACs. Nonetheless, LDs can accumulate in various pathological settings, such as obesity, cardiovascular diseases,⁹ and also cancer,¹⁰ therefore specific degradation of these organelles could accelerate our understanding of these diseases, as well as provide unprecedented therapeutic strategies. To achieve degradation of LDs, bifunctional molecules were synthesized by linking Sudan dyes, which have high affinity for non-polar lipids, to the previously reported LC3 ligands (Fig. 1b).⁴ These probes induced ternary complex formation between triacylglycerol, a major component of the LD core, and LC3 in vitro, hence holding the potential to induce proximity between LDs and autophagosomes in cells. In fact, when tested in cellular systems, LD-ATTECs caused autophagy-dependent clearance of both LDs induced by oleic acid in fibroblasts and also endogenous LDs in differentiated adipocytes. The observed phenotype could be reverted by LC3 knockout, further supporting the proposed mode of action. Furthermore, other lipid-containing membranes were not affected by the treatment, likely due to the polar nature of membrane lipid composition, which is not targeted by the Sudan dye ligand. Proteomics data also showed that LD-ATTECs induced no significant changes in the proteome beyond LD-associated proteins, demonstrating that impressive selectivity for the lipid target can be achieved despite the reliance on non-specific hydrophobic interactions for target engagement.

When tested in in vivo mouse models of metabolic disorders, such as obesity, LD-ATTEC treatment correlated with a significant reduction in body weight accompanied by reduced levels of circulating lipids, which reverted to values typical of the healthy

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Fig. 1 Autophagosomal degradation of lipid droplets mediated by LD-ATTECs. **a** LD-ATTECs form ternary complexes between non-polar lipids contained in the lipid droplets (yellow) and LC3 proteins (purple), which get encapsulated by forming autophagosomes (blue). Degradation is mediated by fusion to lysosomes (pink), resulting in simpler components which can be recycled by the cell. **b** Chemical structures of LD-ATTEC 1 (left) and LD-ATTEC 4 (right), reported by Fu et al.⁷ Created with BioRender.com.

mice. These findings were confirmed by lipidomics studies, showing lowered percentages of non-polar fatty acid in the tissues of mice treated with LD-ATTECs. Direct targeting of pathological excess fat stored in cells as LDs represents an interesting approach to combating obesity, a disease with a dire need for treatments with new mechanisms of action.¹¹ Significant hurdles remain before LD-ATTECs could enter the clinic, however, with alternative lipid targeting warheads required that bypass the well-known carcinogenic properties of the Sudan dyes while retaining their LD selectivity. Nevertheless, as a proof of concept, LD-ATTECs have highlighted the potential of novel heterobifunctional molecules such as ATTECs to offer paradigm shifts in design of therapeutics for intractable diseases.

In this study, Fu and colleagues validate the first LD-ATTECs, firmly establishing the ATTEC bifunctional modality as a rising star in the field of targeted degradation. ATTECs open the stage for degradation of non-proteinaceous cellular components, such as other macromolecules and organelles. Meanwhile, emerging bifunctional molecules are being studied for the targeted induction of different cellular processes, such as post-translational modifications.¹ These studies are rapidly generating myriad tools that will change the way biological processes are studied and reveal a major new direction for novel therapeutic modalities.

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COMPETING INTERESTS

EWT is a director and shareholder at Myricx Pharma Ltd; all other authors declare no competing interests.

ADDITIONAL INFORMATION

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