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HIGHLIGHT

TransTACs: Transforming antibodies into targeted protein degraders

KEY WORDS

Targeted protein degradation;
Membrane and extracellular proteins;
Transferrin receptor-targeting chimeras;
Protein homeostasis regulation;
Tumor therapy

Over the past two decades, proteolysis-targeting chimeras (PROTACs) have gradually evolved from chemical biology tools into potential clinical candidates, marking a significant advancement in our ability to modulate protein homeostasis for therapeutic purposes[1]. Unlike conventional targeted therapies, which primarily utilize a target occupancy-driven approach, protein homeostasis modulation strategies intervene through event-driven mechanisms, demonstrating distinct clinical potential for undruggable or mutation-prone targets compared to traditional small molecule drugs[2]. However, most existing PROTACs target intracellular soluble proteins, while there are relatively few successful cases involving membrane proteins and extracellular proteins, which also play crucial roles in disease pathogenesis.

In the past three years, various studies have begun exploring the feasibility of regulating membrane protein and extracellular protein homeostasis through degradation pathways, achieving notable progress. Strategies include lysosome-targeting chimeras (LYTACs) and molecular degraders of extracellular proteins (MoDEs) based on cation-independent mannose 6-phosphate receptor (CI-M6PR) and asialoglycoprotein receptor (ASGPR)[3,4], cytokine receptor-targeting chimeras (KineTAC) based on chemokines and their receptors[5], antibody-based PROTACs (AbTAC) and proteolysis-targeting antibodies (PROTAB) based on transmembrane E3 ligases[6,7], and recently reported folate receptor-targeting chimeras (FRTAC)[8].

In September 2024, Zhang et al.[9] from Dana-Farber Cancer Institute and Harvard Medical School published a paper in Nature introducing a novel membrane protein degradation technology based on transferrin (TF)—transferrin receptor-targeting chimeras (TransTAC, Fig. 1). Initially, TransTAC was developed as a degrader for chimeric antigen receptors (CARs) to control the activity of CAR-T cells. Early explorations on anti-CD19 CAR revealed a meaningful structure-activity relationship, indicating that bivalent TransTACs promote the internalization of the protein of interest (POI) much more effectively than monovalent TransTACs, likely due to the homodimer format of human transferrin receptor 1 (TfR1). However, further research revealed suboptimal POI degradation post-internalization due to recycling endosome entry facilitated by TF-TfR1 binding. Zhang et al.[9] innovatively introduced tandem cathepsin-sensitive linkers (Gly−Phe−Leu−Gly−Glu−Val−Arg, GFLG-EVR) and substituted TfR1's natural ligand (TF) with a synthetic ScFv (H7), enhancing degradation by reducing POI recycling. The resulting CAR TransTACv1.0 effectively suppressed the activation of Jurkat and primary human CAR-T cells, as well as IFN-γ secretion, with a low half-maximal inhibitory concentration (IC_{50}) of 0.4 nmol/L, enabling reversible regulation of CAR-T cell-mediated tumor-killing activity.

Furthermore, the TransTACv1.0 platform was used to construct various heterobifunctional antibodies targeting membrane proteins, achieving efficient lysosome-dependent degradation (>80 %) of several membrane proteins, including epidermal growth factor receptor (EGFR), programmed death-ligand 1 (PD-L1), and CD20. Notably, EGFR TransTAC showed efficacy in several tyrosine kinase inhibitor (TKI)-resistant non-small cell lung cancer (NSCLC) cell lines and one EGFR Del19/T790M patient-derived cell line (DFCI243). In co-culture models of PC9 Del19/T790M/C797S and HFF1, EGFR TransTAC demonstrated effective killing of tumor cells with safety towards normal cells, significantly outperforming TKI-

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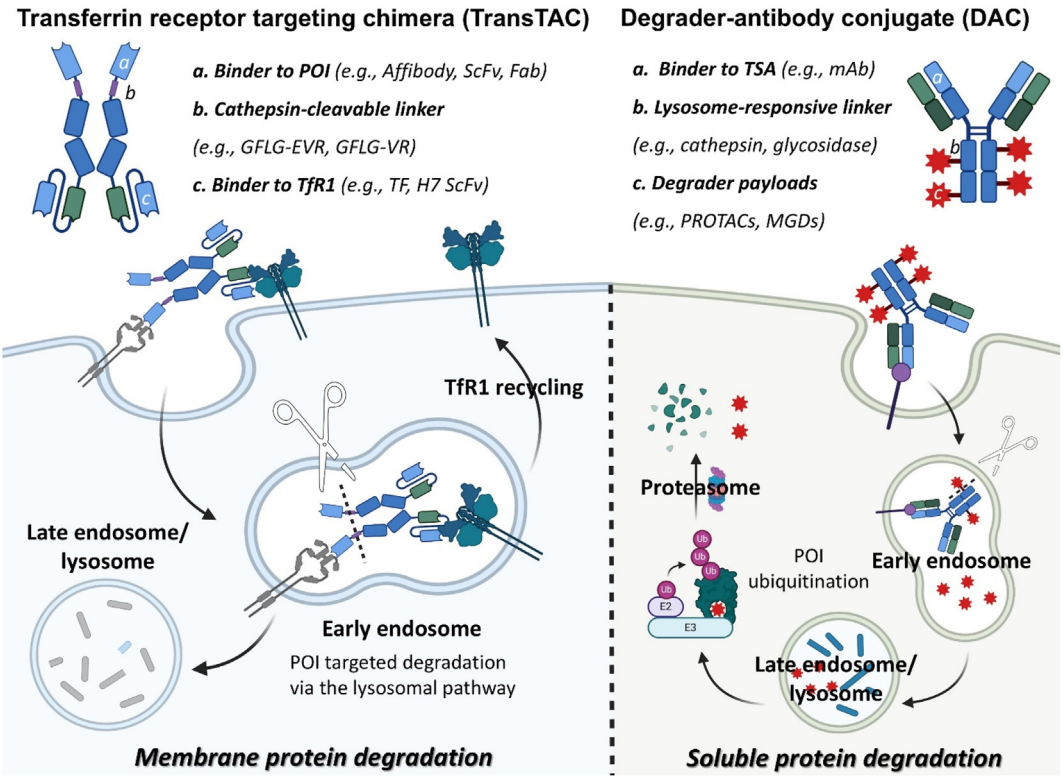

Figure 1 Mechanism of TransTAC targeting membrane protein degradation and the comprehensive landscape for tumor-targeted protein homeostasis regulation. (Created with BioRender.com).

targeted therapies and chemotherapy. Additionally, researchers characterized the safety and efficacy of EGFR TransTAC in mice, showing favorable pharmacokinetics, safety, tissue distribution, and antitumor efficacy in an *in vivo* environment. Remarkably, TransTAC may benefit from TfR1-mediated recycling, providing a longer plasma half-life than Fc-fusion proteins (Affibody-Fc), thus supporting its POI-targeting degradation and antitumor activity.

Through the TfR1-mediated lysosomal pathway, TransTACs effectively transform antibodies into targeted protein degraders. Unlike previous lysosome-targeting membrane protein degradation strategies, Zhang et al.[9] ingeniously employed cathepsin-cleavable linkers to regulate the internalization of lysosome-targeting receptors (LTRs) and POIs, thereby maintaining the number of LTRs on the cell membrane and achieving efficient degradation of POIs. As an iteration and extension of membrane protein degradation strategies, TransTAC prompts our further consideration of another novel targeted degradation approach that also frequently utilizes cathepsin-cleavable linkers—degrader-antibody conjugates (DACs). Similarly depending on the tumor-targeting ability of biomolecules and the degradation activity following linker cleavage (Fig. 1), DACs combine antibodies with highly potent PROTACs or molecular glues for intracellular soluble protein degradation, showing significant clinical potential with several molecules entering Phase I trials[10]. In the future, intervention strategies targeting membrane proteins and extracellular proteins such as TransTAC will be expected to collaborate with DACs to construct a comprehensive landscape of tumor-targeted protein homeostasis regulation.

Compared to the previously reported lysosome-targeting protein degradation strategies, from the perspective of drug modality, TransTACs are similar to KineTACs in that both are bispecific antibodies (BsAbs), while LYTACs, MoDEs and FRTACs are classified as conjugates. Notably, although both TransTACs and KineTACs have undergone systematic protein engineering optimizations, they adopt different formats. TransTACs tend to utilize a 2+2 format, whereas KineTACs employ a 1+1 format, potentially due to differences related to LTRs. Regarding degradation kinetics, all the aforementioned strategies have demonstrated optimal D_{max} values exceeding 75 % and nanomolar-level DC_{50} across various proteins of interest, highlighting the potential of these approaches. Interestingly, unlike other strategies, TransTACs did not validate the degradation potential for extracellular soluble proteins during its discovery process, which may need to be considered for subsequent platform applicability expansion. In terms of drug metabolism and pharmacokinetics (DMPK), most strategies based on conjugation negatively impact the pharmacokinetic of biomacromolecules. This is especially evident in LYTACs with high GalNAc-to-antibody ratios, where rapid clearance may result from liver targeting and further metabolism, highlighting the need to balance degradation efficacy and circulation stability by adjusting conjugation ratios. In contrast, KineTACs and TransTACs benefit from the BsAb format, exhibiting excellent PK properties and providing long circulation for sustained pharmacological effects.

Overall, leveraging the upregulation of TfR1 expression due to the iron demands of tumor cells, TransTACv1.0's 2+2 BsAb format enables dual enrichment at the tumor site by targeting both antigens and LTRs, thus providing a novel approach for regulating membrane protein homeostasis. Its tumor-targeting capability, high degradation efficiency, modularity, and superior

pharmacokinetic properties make it broadly applicable in cancer and other biological fields. Future exploration of extracellular protein targets and alternative endosomal dissociation mechanisms to enable degrader recycling will provide further opportunities for TransTAC development. Given the robust development of the BsAbs in recent years and the clinical progress of TfR1-targeted delivery platforms represented by Denali Therapeutics, the druggability of TransTAC that combines these two aspects is promising. Selecting target proteins prone to pathogenic/resistant mutations or with scaffold functions would broaden the clinical scope for this strategy. Once suitable proteins of interest are identified and undergo systematic optimization and thorough efficacy and safety evaluations, TransTAC is poised to demonstrate its further potential as a therapeutic agent.

Author contributions

Yu Guo: Writing - original draft, Conceptualization. Jinxin Che: Writing - review & editing, Supervision. Xiaowu Dong: Supervision, Writing - review & editing, Conceptualization.

Conflicts of interest

The authors declare no conflicts of interest.

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