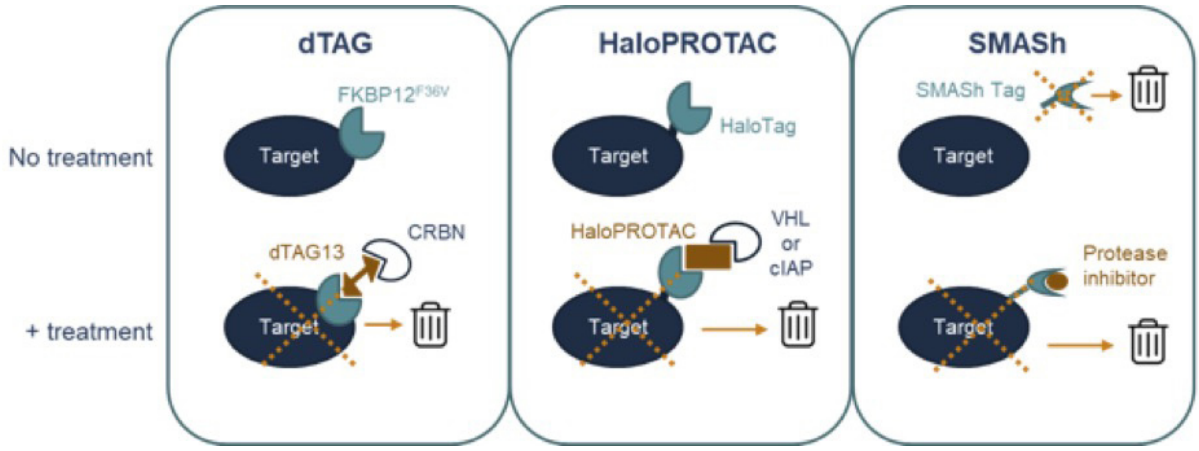
Degradation Tag Systems: a good way to SMASh your target

A commentary 13/04/2021

Drug development is a long and sinuous road that is broadly known to present many obstacles, together responsible for the attrition rate. One important key to identify a potential failing drug in the earliest stages of development is the use of optimized and well-designed models, from target validation to toxicity studies. This is especially true when the target protein does not have a (known) direct ligand. Degradation tags represent valid solutions to this issue, as they selectively control the expression of target proteins using specific ligands. The systems using such approach for protein degradation include dTAG, HaloPROTAC, and SMASh.[1,2]

The dTAG degradation system requires the fusion of the target protein to a mutant FKBP12 protein (FKBP12F36V). This has been achieved using CRISPR/Cas9 Knockin or via lentiviral infection.[3] Upon treatment with dTAG13, which serves as an adapter between the fusion protein and cereblon (CRBN) E3 ligase, FKBP12F36V is ubiquitylated thus directing the target protein to the proteasome for degradation.[3,4]



The HaloPROTAC system induces ubiquitylation and degradation of HaloTag7 fusion proteins by exploiting von Hippel–Lindau (VHL) or cellular inhibitor of apoptosis protein 1 (cIAP) E3 ubiquitin ligases.[5–7] Notably, this method has been used to degrade endogenous HaloTag fusion proteins in vivo.[8]

Finally, the SMASh system consists of fusing the target protein to the SMASh tag that includes a degron, hepatitis C virus (HCV) NS3 protease and its cleavage site. This method requires only one genetically encoded modification, i.e., the addition of the SMASh tag to the sequence of the protein of interest. In the presence of a protease inhibitor, the SMASh tag stays fused to the protein of interest inducing all newly synthetized proteins to be rapidly degraded; in its absence, the SMASh tag self-cleaves, yielding to a protein with minimal modification. Interestingly, the SMASh system presents advantages over both dTAG and HaloPROTAC. First, the inducing drug does not serve as an adapter for the recruitment of an E3 ligase as the SMASh tag includes a degron. Also, the “expressed form” of the protein of interest, i.e., without treatment, is released from the SMASh tag and thus closer to its native form, whereas in the dTAG and HaloPROTAC systems they are fused to their respective tags.

The three approaches have been successfully used to degrade a number of nuclear, cytoplasmic, single and multi-pass transmembrane proteins, opening a new era for drug discovery and development, including in cancer immunotherapy.[9]

genOway recently developed two MC38 cell lines, invalidated for mouse Pd-l1 and overexpressing a SMASh-tagged form of human PD-L1: MC38-SMASh-hPD-L1 and MC38-hPD-L1-SMASh[(a)]. These cells express the SMASh tag at the N-terminal or C-terminal domain of hPD-L1, respectively. We have already validated both lines, confirming the drug-inducible regulation of human PD-L1 expression and demonstrating the efficacy of the SMASh system to induce reversible degradation of a protein of interest in a cellular model.

See also:
Targeted Protein Degradation: New Promises for "Undruggable" Disease
https://www.genoway.com/commentaries/targeted-protein-degradation.htm

References:

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(a) https://www.genoway.com/services/customized-cell/inducible-knockdown.htm#smash