Developing proteolysis targeting chimera (PROTACs) for amyloid precursor protein modulation

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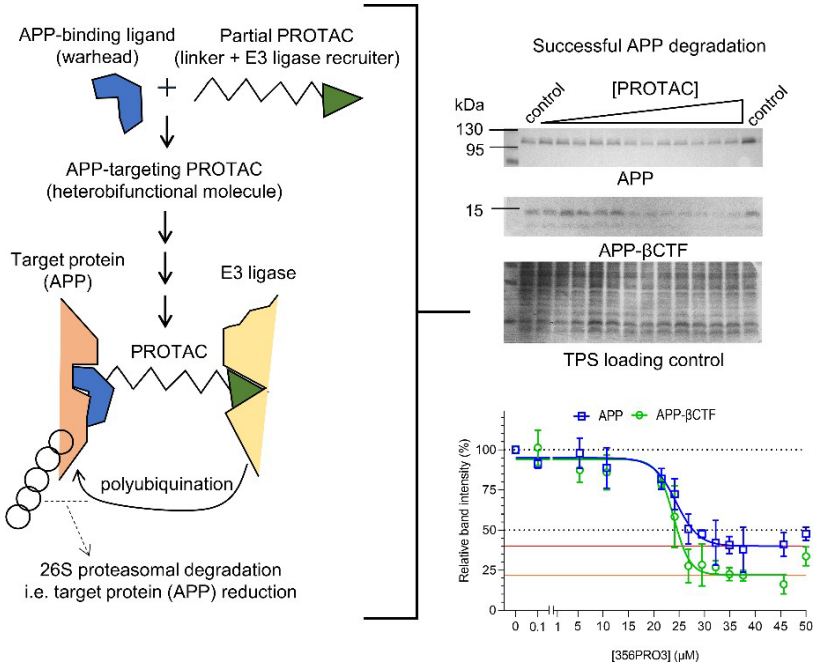

The identified amyloid precursor protein (APP)-binding ligands were conjugated to a series of partial PROTACs to produce a library of APP-targeting PROTACs with the ability to promote proximity between APP and the recruited E3 ligase. This induced polyubiquitination and subsequent 26S proteasomal degradation of APP (left panel). Successful degradation of both APP (blue line) and its βC-terminal fragment (APP-βCTF, green line) with increasing PROTAC concentration as determined by quantitative western blot (n = 3). Red line, degradation max for APP; orange line, degradation max for βCTF (right panel).

Introduction: The amyloid precursor protein (APP) and its βC-terminal fragment (βCTF) have been implicated in several human diseases including Alzheimer's disease, bipolar disorder, Parkinson disease, brain injury, strokes, diabetes, and obesity. Therefore, the development of molecules that canmodulate the level of APP would have significant clinical applications.

Methodology: We use in silico and in vitro techniques (thermal shift assay and cellular thermal shift assay) to identify novel APP-binding ligands. Using these ligands, we subsequently develop a library of bifunctional molecules called proteolysis targeting chimeras (PROTACs) to screen for degradation by recruiting the ubiquitin proteasome system. Quantitative western blot, in two cell lines, was employed to determine the degradability of the developed APP-targeting PROTACs.

Results: We identify five novel APP-binding ligands with the ability to induce a significant shift in thermalmelting temperature of ≥ 2 °C in all cases. After PROTAC conjugation, we observed no cytotoxicity under the conditions tested. We further report successful degradation of both APP and the βCTF by five of the seven PROTACs developed and identify our most optimal degrader, **356PROTAC3**, with a DC_{50} of 24.4 ± 0.7 μM for APP and 23.9 ± 0.5 μM for the βCTF and a DC_{max} of 60.0 ± 2.6 % for APP and 78.0 ± 2.7 % for the βCTF, in our APP-overexpressing cell line. We ensured that **356PROTAC3** had the ability to degrade endogenous APP with a DC_{50} of 35.3 ± 0.4 μM and DC_{max} of 84.0 ± 6.1 % and degradation was proteasome dependent, where the PROTAC effect was abolished in the presenceof proteasomal inhibitor, MG132.

Discussion and Conclusion: The functional PROTACs produced here possess significant potential to be further optimised into therapeutic agents for the treatment of APP-associated diseases.

Keywords: Amyloid precursor protein (APP), induced protein degradation, PROTACs, thermal shift assay, cellular thermal shift assay.