PROTAC Technology for Novel Drug Discovery

Webinar Presenters:
Dr Emelyne Diers, University of Dundee
Understanding The Ternary Complex: On The Road To Better Degraders
Cyrille S Kounde, Imperial College London
Expanding The Horizon Of Targeted Protein Degradation
Dr Induka Abeysena, SP Genevac
Eliminating Bottlenecks In Protac Synthesis – Focus On Evaporation

Cell homeostasis depends on accurate control of protein synthesis and degradation. Dysfunction of these processes can be damaging to the body leading to life threatening diseases, such as abnormal cell proliferation in cancer. Targeted inhibition of abnormal proteins and their pathways has been a successful therapy for certain conditions, but a novel group of small molecules has demonstrated an alternative approach to removing these proteins from the body. Proteolysis Targeting Chimeras (PROTACs) are small heterobifunctional molecules that target selected proteins of interest (POI) and degrade them by hijacking the body's own natural disposal system. PROTACs (Fig 1) were initially discovered about 20 years ago and since then have been investigated as an additional modality to the drug discovery toolbox. Studies have suggested that lower concentrations of PROTACs are needed to be administered compared to current drugs to achieve the therapeutic effect, promising fewer adverse effects and reducing the possibility of drug resistance.

In cancer, many PROTACs target have been developed to eliminate the protein responsible for uncontrolled or abnormal growth, such as BCR-ABL which is found in certain types of leukemia or human epidermal growth factor 2 which is associated with breast cancer, but it is only recently that the first PROTAC, ARV-110 (Arvinas, USA) has entered phase I clinical trials for metastatic castration-resistant prostate cancer. At the same time, there has been an explosion of scientific publications, some of which offer the potential to target the "undruggable" proteome, which comprises of about 85% of human proteins.

Recently, Biopharma Group and SP Scientific Products hosted a webinar with three key opinion leaders discussing the design of PROTACs and the evaporation challenges relating to their synthesis. This tech note summarizes the presentations.

Molecular Mechanism Of PROTACs

PROTACs consist of three components: an E3 ligase ligand, a ligand for the POI and a linker that joins them together. The E3 ligand and POI ligand bind to their respective targets to form a ternary complex. Once bound, the E3 and POI are in close proximity, triggering the E3 to transfer multiple ubiquitin molecules to the POI which is then recognized by the proteosome as part of the ubiquitin-proteosome system (UPS) and finally leading to degradation of the POI. This differs from the mechanism of many of the inhibition drugs on the market that bind to the POI and inhibit it but do not remove it from the body.

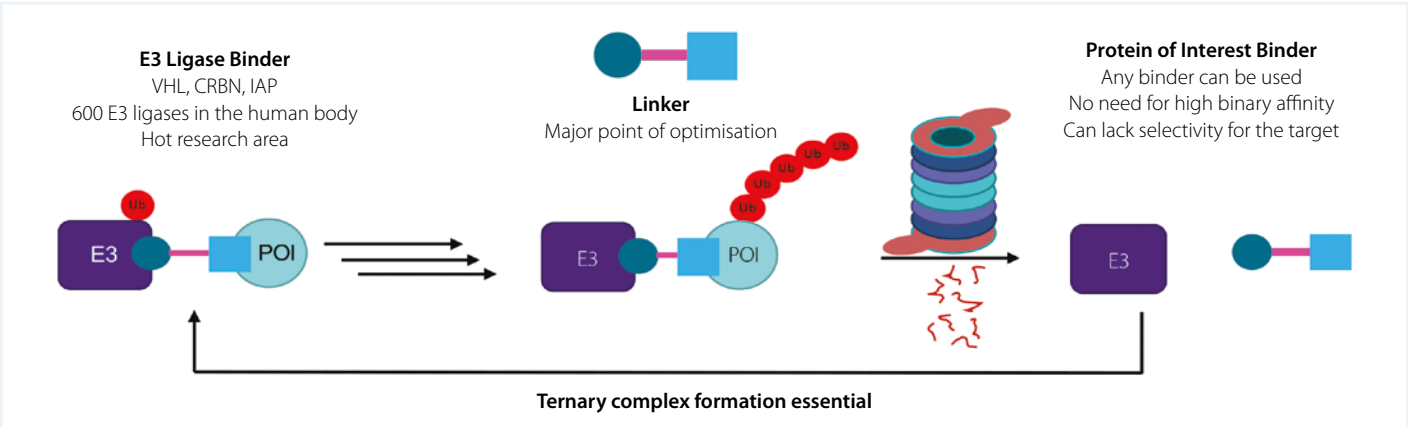

Figure 1: *Mechanism of action*

Understanding The Ternary Complex: On The Road To Better Degraders

The design of PROTACs is largely empirical and commonly involves screening of sizable, small molecule libraries. A rational design approach could relieve the burden of screening large libraries and result in a selection of more specific small molecules. Dr Emelyne Diers from Prof Ciulli's group at University of Dundee, UK described her work in collaboration with Boehringer Ingelheim on the importance of the ternary complex in the rational design of a PROTAC.

Their group have taken a multidisciplinary approach to designing PROTACs. The process starts by studying the chemistry of the three components that make up a PROTAC – the E3 ligase ligand (e.g. Von Hippel Lindau (VHL)), the POI ligand and the linker that binds them together to create an optimal ternary complex. As a novel approach, there are less rules or standards of development therefore a selection of candidates is chosen for subsequent studies. Biophysical assays determine the cooperativity and stability of the selected molecules and crystal structures visualize the possible interactions between the components in them. The selected structures can then be tested in cell lines to assess how the ternary complex influences the efficacy of the degrader. Although all PROTACs form ternary complexes, not all ternary complexes show degradation in biological assays.

Crystal structures have proved to be beneficial in designing better PROTACs. The first reported ternary crystal structure was that of MZ1, a bromodomain-containing protein 4 (BRD4) selective degrader. MZ1 brings the transcription factor, BRD4 and VHL (the E3 ligase ligand) in a highly cooperative complex which is stable, long-lived and demonstrates a fast rate of degradation of BRD4[1]. BRD4 has been the target of many inhibition drug candidates due to its role in enhancing the expression of factors critical for the pathogenesis of cancer. Some of the current BRD4 degraders have shown more efficient anti-cancer activities than inhibition alone, suggesting MZ1 may be a good candidate for cancer therapy.

Another example of the importance of studying the crystal structure when designing a PROTAC is that of ACBI1, a small molecule based on VHL and SMARCA binders. SMARCA is part of the BAF chromatin remodeling complex that is mutated in at least 20% cancers. In leukemia, cancer cell growth is thought to be dependent on SMARCA4 but has proved to be difficult to treat with inhibition drugs and considered undruggable. In a recent study, a small molecule PROTAC (PROTAC1) was identified from library screening and the degradation activity further improved by high-resolution ternary complex crystal structure analysis[2]. ACBI1 was found to be a potent degrader of SMARCA2, SMARCA4 and PBRM1 and induced apoptosis in cancer cell lines.

It is evident from this work that a clear understanding of the ternary complex is fundamental. These studies demonstrate that a structure-based design approach to ternary complex formation is also beneficial in creating optimal PROTACs that can even target previously undruggable targets.

Expanding The Horizon Of Targeted Protein Degradation

PROTACs have the potential to regulate protein levels in time and space controlling when and where a protein is degraded. For example, you may want to degrade the protein at a specific time in the cell cycle or in a specific part of the body but not in other organs. The ability to turn a drug on and off could have dramatic consequences for a drug's safety profile. In Mr Cyrille Kounde's presentation, he describes the work that he and his colleagues in Prof Ed Tate's group have undertaken at Imperial College London, UK creating PROTACs with conditional targeted degradation mechanisms. In one case, proteolysis is induced by light, an external stimulus that is non-invasive and fast. Another approach delivers the PROTAC via an engineered antibody-conjugate.

Light Activated Degradation

Using light as a precision tool has been demonstrated in medicine as photodynamic therapy (PDT) for conditions, such as head and neck cancer and skin disorders. The duration and intensity of light can be controlled easily so that the drug is delivered quickly and precisely without invasion into the body. Applying this to the delivery of PROTACs requires an inactive complex to be designed that only forms an active ternary complex that induces degradation when exposed to light. No UPS engineering or protein modification is required, making this an attractive option for a conditional PROTAC molecule.

Mr Kounde and his colleagues designed a light activated PROTAC targeting the BRD4 transcription factor. The inactive form includes a caging group attached to the E3 ligase ligand which is removed by exposure to 365 nm UV light for 1 minute. When compared with an active non-conditional PROTAC2 in HeLa cells, this conditional PROTAC3 behaved with comparable kinetics and in a similar dose dependent manner when activated by light[3]. In addition, PROTAC3 also demonstrated inhibition of cell proliferation over time after irradiation. Future work will explore the activity of this conditional PROTAC on various cancer cell lines.

Antibody Targeted Degradation

An alternative strategy to treat cancer without affecting other cells is to target the POI using a monoclonal antibody (mAb) system. There are 79 mAbs approved by the United States Food and Drug Administration (FDA), 30 of which have been approved for cancer. Therefore, it would make sense to design a PROTAC antibody-conjugate based on one of these approved mAbs.

The HER2 mAb-PROTAC3 caged conjugate was constructed by Mr Kounde and colleagues to target and degrade BRD4[4]. Using cell lines that expressed BRD4 and either expressed or lacked expression of HER2, the HER2 mAb-PROTAC3 was only cleaved in the HER2+ cell lines which led to HER2-dependent BRD4 degradation. Evidence of the internalization of the PROTAC was confirmed using a fluorescent lysosome-targeting antibody.

Both multifunctional PROTACs degrade proteins with higher precision than a non-conditional PROTAC suggesting a future in this targeted approach to cancer therapy.

Eliminating Bottlenecks In PROTAC Synthesis – Focus On Evaporation

As described earlier, a multidisciplinary approach between chemistry and biology is required for small molecule or peptide-based PROTAC synthesis. We have seen that understanding the ternary PROTAC complex will help to rationally design the small molecule libraries which will ultimately reduce time and money. However, in the early stages of this technology, the process is still time consuming with numerous evaporation steps that cannot be eliminated causing bottlenecks in the generation of PROTACs and a slower development time for drug discovery.

The third presentation by Induka Abeysena, Portfolio Manager at SP Genevac, UK discussed how to overcome the evaporation limitations in PROTAC synthesis to produce a high-quality final product.

Evaporation steps that occur during the small molecule synthesis include: initial PROTAC complex synthesis, as cleavage or deprotection groups are removed; pre- and post-purification when concentrating the unprocessed mixture or the combined high performance liquid chromatography (HPLC) fractions; and post-reformatting where the finished PROTAC molecule is transformed into the desired format for transportation. These processes are crude involving heat, vacuums and centrifugation, together with a range of different solvents making it difficult to retain the quality and integrity of the final product. In some cases, the heat applied to a sample to speed up the evaporation is not well controlled and can damage the compound. It is also possible that compounds dry at different rates causing heterogeneity in the quality of evaporation. Another challenge in the evaporation process is the risk of contamination between samples when drying mixed solvent with different boiling points and physical properties. These samples are often close together showering the neighboring samples with unwanted solvents or another sample. Finally, each drying step takes time, and this is dependent on volume size - the larger the volume to be evaporated, the slower the evaporation rates. Increasing time also leads to higher labor costs.

Development of technology in commercial scale evaporators has improved many of these limitations. SP Scientific Products manufacture centrifugal evaporators for parallel sample evaporation to fit many different needs. The SP Genevac HT Series 3i and EZ-2 personal solvent evaporators are ideal for medium to high-throughput sample processing as they can be pre-programmed to dry many samples at once, retaining consistency in all samples and enabling unattended operation. They are compatible with many different solvents in various vessel types facilitating the synthesis of a wide range of small molecule and peptide based PROTAC compounds. These systems can also freeze dry samples especially those that are difficult to dry.

The SampleGuard and DriPure® proprietary technologies enable these features by controlling the sample temperature and eliminating sample bumping to avoid overheating, sample damage, compound sublimation and sample contamination. For the purposes of crystal production, the EXALT technology has been designed to permit a wide range of solvents to be evaporated at same time at slow rates for polymorph screening and structure characterization.

Although these evaporators are mainly used on the benchtop, they are also capable of being used for potent compounds, such as Antibody Drug Conjugates (ADCs) that require safe handling in confined conditions eg by installing the drying system in a glove box.

In addition to the parallel sample evaporator line, a recent addition to the SP Genevac evaporator portfolio includes the Ecodyst® series, evolutionary single-sample evaporators with a built-in intelligent condenser. These will assist the medicinal chemist working with individual samples to work more efficiently due to the fast evaporation rates and lack of maintenance required. Also, the need for consumables, such as dry ice and anti-freeze is unnecessary.

Conclusion

In the last three years, interest in PROTACs has grown exponentially with more than 150 scientific publications on PROTAC research found on PubMed in 2020, compared to 30 in 2017. Many of these are targeting cancer, but also neurodegenerative diseases and beyond. The furthest in clinical trials are ARV-110 for prostate cancer and ARV-471 for breast cancer (Arvinas, USA). As with all cancer drugs, being able to target the POI without affecting other proteins is the holy grail. Conditional PROTACs that can be switched on at a specific time and place could be a step towards this ideal.

A multidisciplinary approach is necessary to design and create the optimal PROTAC examining the chemical interactions but also the manufacturing process and biological activity in vivo. The presentations in this webinar provide an overview to some of the areas that are important to consider when going through this process including optimal equipment to overcome operational bottlenecks.

Although there is potential for PROTACs to be powerful standalone therapeutic drugs, targeted protein degradation could be a beneficial additional modality to the drug discovery toolbox to work alongside other drugs on the market.

References

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