The critical platforms of PROTAC validation

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Abstract

In recent years, proteolysis targeting chimeric (PROTAC) technology has become an effective endogenous protein degradation tool in drug discovery. As a class of heterobifunctional small molecules, PROTACs recruit an E3 ubiquitin ligase to a given substrate protein, resulting in its targeted degradation through the endogenous ubiquitin-proteasome system (UPS). PROTAC consists of three chemical elements: a ligand that binds to a target protein, a ligand that binds to an E3 ubiquitin ligase, and a linker that connects the two ligands. Compared with traditional drug development, PROTAC technology possesses unique advantages including overcoming drug resistance and targeting the “undruggable”.

Here we generated a kind of PROTAC target, like KRAS, BRD4, ESR1, associated cell lines to accelerate the discovery of new protein degradation drugs and the target protein degradation therapeutics by an optimized insertion system. In the beginning, we developed a system of different tag targeted cell lines, which can be easily used to introduce HiBiT, Flag, GFP, Halo Tag, etc. to target protein by CRISPaint or CRISPR Cas9 mediated HDR system. We also developed an arbitrary point mutation system based on the optimized PE3 mutation system, which can be used to randomly modify any point mutation to simulate disease models *in vitro*. During this process, we optimized a series of validation system, like Targeted Protein Degradation (TPD), High-throughput flow cytometry (HTFC) and In-Cell Western (ICW) to shorten the detection time and improve detection stability. It is worth mentioning that we also developed a HiBiT and GFP dual-signal system, which can be easily to validate the target protein degradation and cell viability. We hope that our detection system can speed up the development of new drugs and contribute to anti-tumor therapy.

Method

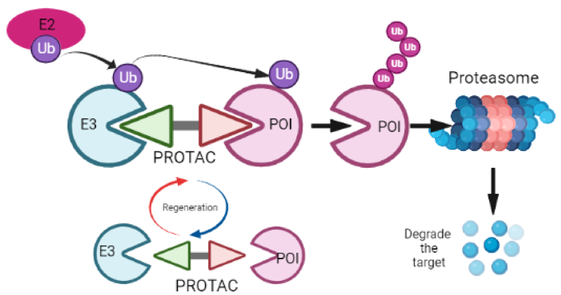

Fig 1. Schematic of the PROTAC technology

- Protein-based evaluation
  - point of interesting (POI) engagement
  - Ternary complex formation
  - POI degradation (In-Cell Western)
- Cell line-based evaluation
  - POI degradation
  - POI engagement
  - Ternary complex formation
  - POI ubiquitination
  - Downstream functional assays

Fig 2. Strategy of the PROTAC validation

Results

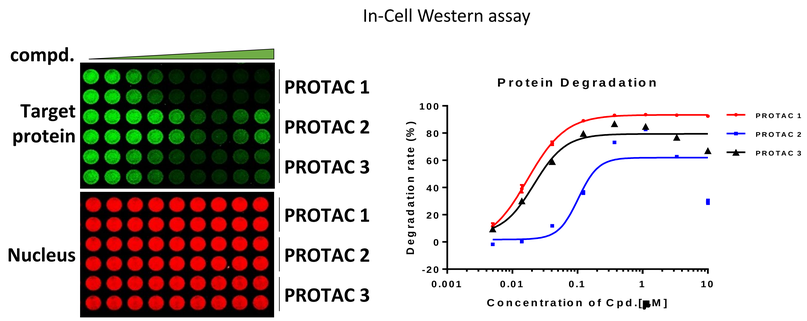

Fig 3. Protein-based assay for PROTAC screening (ICW assay)

Parental cells were treated with different compounds of PROTACs at serial diluted concentration. The cell viability was detected by in cell western assay. PROTACs can induce cell death through target protein degradation at indicated concentration.

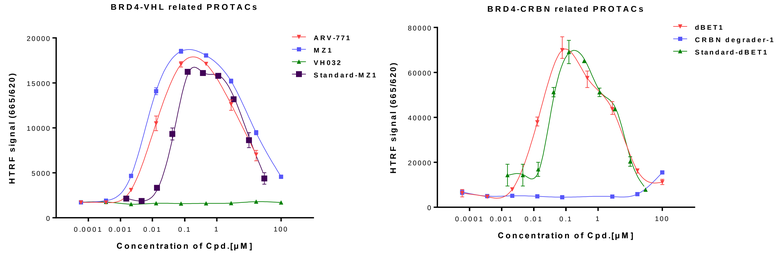

Fig 4. BRD4/E3 PROTAC BINDING assay (Ternary complex formation assay)

The HTRF BRD4/CRBN PROTAC Binding Assay is designed to measure the ternary complex formation between BRD4, PROTAC degrader and E3 proteins. As shown in Figure, the interaction between Tag1-BRD4 and Tag2-CRBN is detected by using anti-Tag1-Terbium (HTRF donor) and anti-Tag2-d2 (HTRF acceptor). Different negative and positive PROTACs were used in the assay.

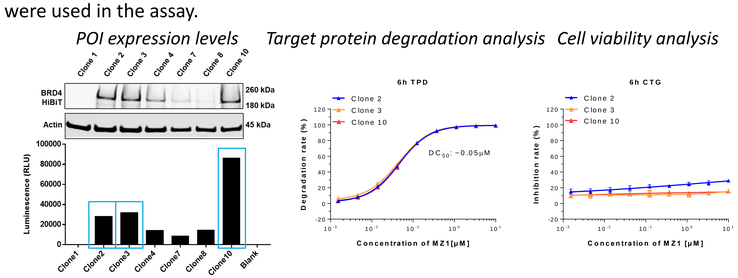

Fig 5. *In vitro* assay for endogenously tagged HiBiT-BRD4 in A549 cell line

MZ1 (BRD4 PROTAC) induced target protein degradation (left), while did not show significant inhibition on cell proliferation (right) after 6 hrs’ incubation.

POI expression levels *Target protein degradation analysis* *Cell viability analysis*

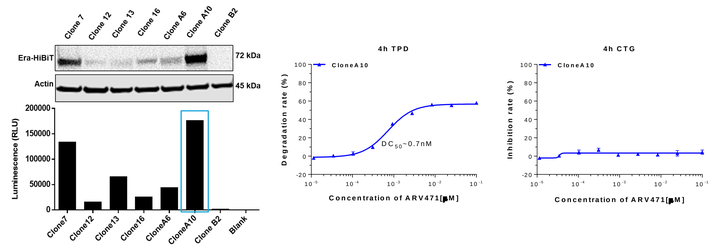

Fig 6. *In vitro* assay for endogenously tagged HiBiT-ESR1 in MCF7 cell line

PROTAC (ARV471) induced target protein degradation (left), while did not show inhibition on cell proliferation (right) after 4hrs’ incubation.

POI expression levels *Target protein degradation analysis* *Cell viability analysis*

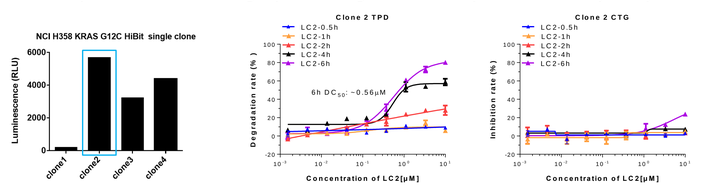

Fig 7. *In vitro* assay for endogenously tagged HiBiT-KRAS^{G12C} in NCI-H358 cell line

PROTAC (LC2) induced target protein degradation in a time-dependent manner (left), while did not show obvious inhibition on cell proliferation (right) at various time points post treatment.

Summary

- We have successfully established a series of *in vitro* Protein-based assays for PROTACs screening.
- We also have successfully generated a series of cell lines based on different POI.
- Furthermore, we have also developed a series of cell line-based validation assays.

Reference

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