UPPGRADER: a bioinformatics-based novel E3 ligase discovery platform

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Introduction

In Target Protein Degradation (TPD) field, conventional E3 ligases utilized in the clinic are limited to CRBN and VHL, which opens the need for exploration of novel E3 ligases for the advancement of TPD. To address this, we have developed a proprietary platform, UPPGRADER, for systematic discovery of novel E3 ligases based on -omics data including single cell RNA sequencing (scRNA-seq) and whole genome sequencing (WGS), in conjunction with proteomics data and bioinformatics tools. UPPGRADER enables systematic analyses on alterations occurred in all genomic areas including E3 ligase genes and potential target genes.

For example, analyzing Pan-Cancer Analysis of Whole Genomes (PCAWG) data identified amino acid changes into Cysteine or Lysine, which can be targeted by covalent binders with cancer specificity. Through such analysis, we discovered several novel E3 ligases, including but not limited to FBXW7, with the categorization of the findings based on specific cancer types.

At the single cell expression level, UPPGRADER provides the capability to assess gene expression levels of E3 ligases and target proteins by proprietary measurements based on gene detection rate. This system also allows assessment of E3 ligase-Target gene co-expression per single cell. In colorectal cancer, UPPGRADER has shown that the co-expression of E3 ligase FBXW7 and well-known colorectal cancer target (KRAS) increases from 1% (4 out of 334) in normal epithelial cells to 17% (2,967 out of 17,458) in cancerous epithelial cells, highlighting a significant change in their simultaneous expression patterns during cancer progression.

UPPGRADER also identified UP1013 which showed high co-expression level with KRAS gene alongside universal expression properties and high cancer cell dependencies. For UP1013, we developed novel small molecule binders (nM binding affinity) with bioavailability > 20% in animal pharmacokinetics. Bifunctional degraders utilizing these binders displayed BRD4 degradation in more than 20 cell lines with double to triple digit nM DC50 and DC90 values. Other targets (AURKA, CRBN and others) were potently degraded with anticipated mechanism of action (assessed by E3 ligase KO cell line and proteasomal inhibitors).

To conclude, UPPGRADER is a comprehensive bioinformatics tool for discovering novel E3 ligases with biological validations. Through recent explosion of scRNA-seq and WGS datasets in different diseases and mechanism-based identification of additional molecular targets, UPPGRADER is poised to advance the field of TPD.

UPPGRADER employs -omics to discover promising E3 ligases to develop next generation bifunctional degraders

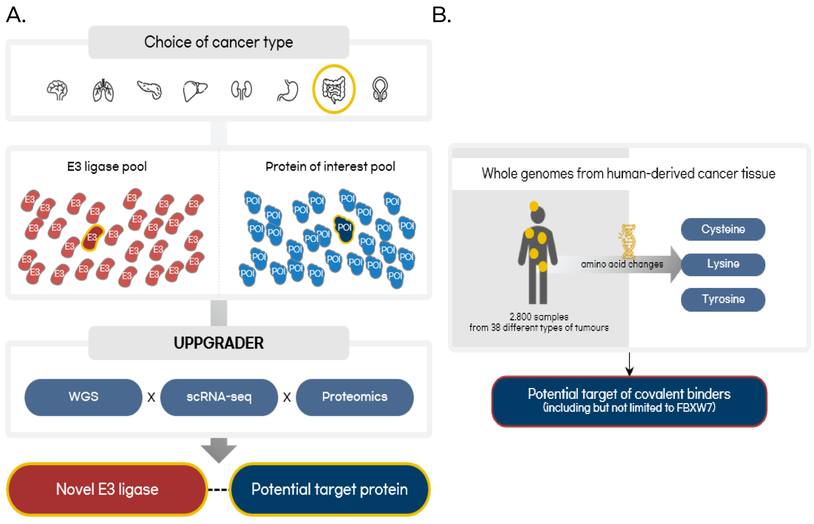

Figure 1. UPPGRADER platform employs a bioinformatics approach to identify the best pair of E3 ligases and target protein for bifunctional degrader development, and evaluates mutation profiling of proteins in cancer tissues to assess potential druggable sites for covalent approach. (A) Schematic of the UPPGRADER platform for the discovery of novel E3 ligases and potential target protein with -omics data. Choice of cancer type is initially selected, and relevant omics data are analyzed to visualize expression pattern of novel E3 ligase and potential target protein. VHL and CRBN E3 ligases are analyzed in parallel for direct comparison with novel E3 ligase (B) Elucidation of potential target proteins' amino acid changes in human-derived cancer tissues through whole genome studies to identify potential druggable sites with chemoproteomics approach targeting cysteine, lysine and tyrosine residues.

UPPGRDAER discovered FBXW7 and UP1013 which is highly co-expressed with target protein KRAS in colorectal cancer cells

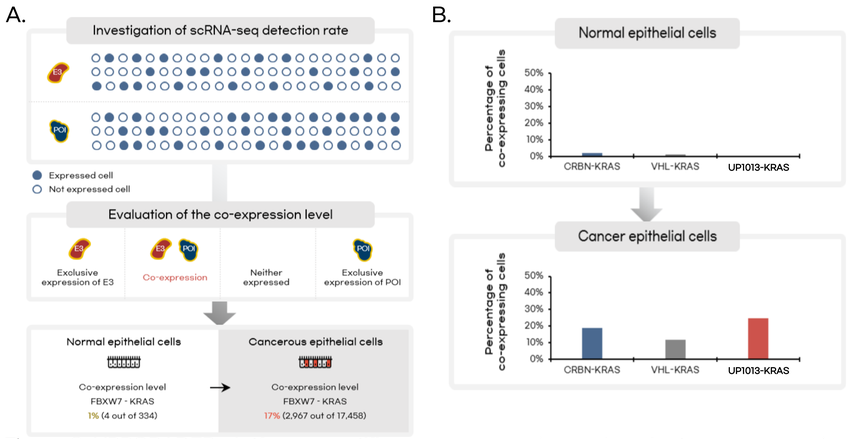

Figure 2. UPPGRADER platform quantifies gene expression and evaluates co-expression dynamics between E3 ligases and potential target proteins at the single cell RNA-seq level. (A) Expression levels of E3 ligase FBXW7 and target protein KRAS have been analyzed at the single cell level to quantify co-expression level. Co-expression of FBXW7 and KRAS increased from 1% (4 out of 334) in normal epithelial cells to 17% (2,967 out of 17,458) in cancerous epithelial cells (B) Co-expression dynamics between E3 ligases and KRAS at the single cell RNA-seq level. The number of normal epithelial cells used is 344, and number of cancer epithelial cells is 17,458. Co-expression of UP1013 and KRAS was the robust compared to CRBN and VHL.

UPPGRADER discovered UP1013 which is enriched universally with high cancer cell dependencies

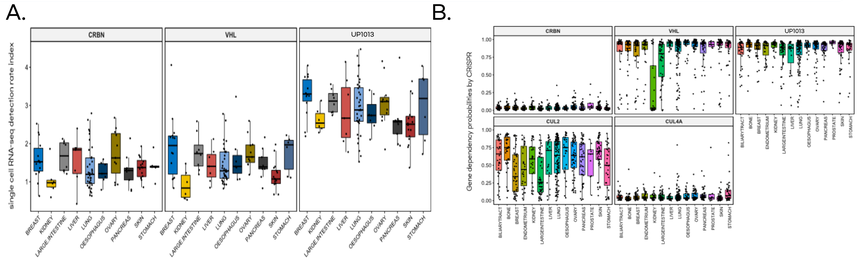

Figure 3. UPPGRADER platform identified UP1013 which is universally expressed and essential gene for cancer cell survival. (A) Expression levels of UP1013 along with CRBN and VHL have been analyzed at the single cell level across 10 cancer types from 120 cancer cell lines. UP1013 is widely and more universally expressed compared to CRBN and VHL (B) CRISPR knock out data of 14 cancer types from 621 cancer cell suggest UP1013 is essential for cancer cell survival. UP1013-based degraders would have lower risk of potential PROTAC drug resistance caused by acquired mutation from the E3 ligase component.

UP1013 is PROTACtable and applicable for TPD approach

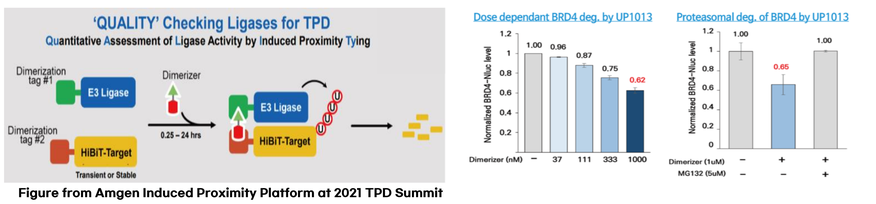

Figure 4. UP1013 is capable of degrading target protein BDR4 through QUALITY (Dimer-tag) assay. To confirm PROTAC ability of UP1013, we employed dimer-tag assay by artificially bringing target protein BRD4 in close proximity to UP1013 for degradation. Addition of dimerizer degraded BRD4 dose-dependently and co-treatment with proteasome inhibitor, MG132, fully rescued BRD4 degradation suggesting proteasomal mediated degradation.

UP1013-based degraders demonstrate potent degradation in multiple cancer cells and compare favorably to CRBN-based degraders

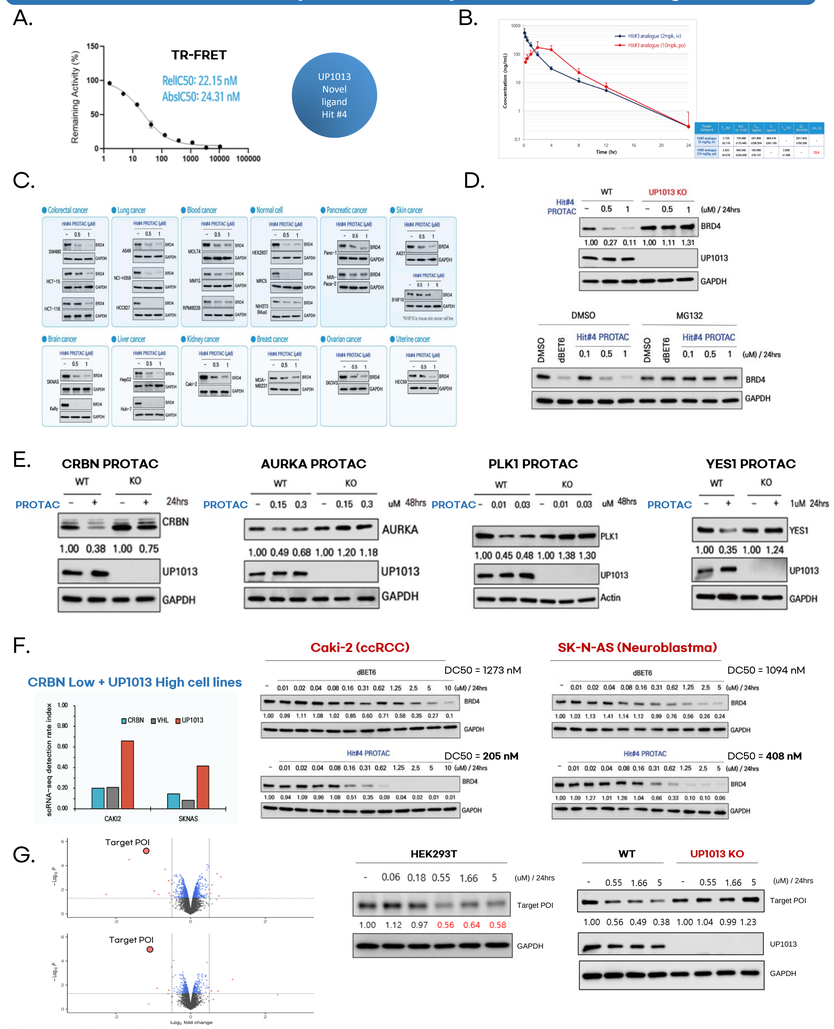

Figure 5. UP1013-based degraders with potential to develop next generation bifunctional degraders

(A) Identification of UP1013 hit binders through DEL screening.

(B) Pharmacokinetic profile of UP1013.

(C) Degradation of BRD4 across 24 cell lines with UP1013-based BRD4 degrader.

(D) MoA studies of UP1013-based BRD4 degrader validated with UP1013 knock out cell line and proteasome inhibitor, MG132.

(E) Degradation of protein targets other than BRD4 suggesting UP1013 can be widely applicable to develop diverse bifunctional degraders.

(F) Superiority of UP1013-based BRD4 degrader in Caki-2 and SK-N-AS cell lines with low CRBN expression.

(G) Characterization of UP1013 as a potential molecular glue degrader, demonstrating its capability for targeted protein degradation

Summary

- UPPTHERA has used its proprietary bioinformatics platform, UPPGRADER, to identify E3 ligases based on the expression pattern to develop next generation bifunctional degraders.
- UP1013 identified showed robust co-expression with KRAS compared to CRBN, VHL, and FBXW7. UP1013 is more universally expressed compared to CRBN and VHL with high cancer cell dependencies.
- UP1013-based degraders have been successfully developed and compare favorably to CRBN-based degraders.
- On-going efforts to develop next generation bifunctional degrader undisclosed target.
- Seeking a partner to fully unleash the potential of our UP1013 in TPD.