Genetic and pharmacological disruption of the HBS1L/PELO complex impairs mRNA homeostasis and leads to in vivo tumor regressions in FOCAD-deleted cancers

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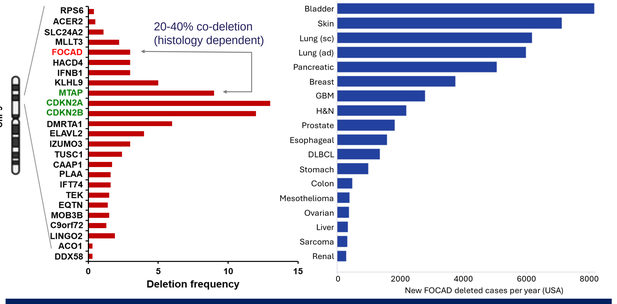
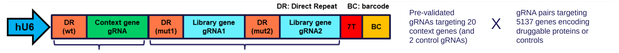
Abstract

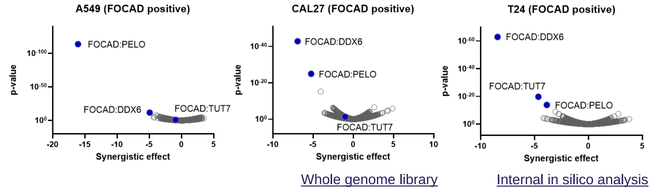
Loss of tumor suppressor genes in cancer often results in the co-deletion of nearby genes around the chromosomal locus, creating potential collateral damage contexts for targeted therapy. One such example is the loss of *MTAP*, which is frequently co-deleted with the tumor suppressor genes *CDKN2A/B* and creates a synthetic lethal dependency on PRMT5 and susceptibility to MTA-cooperative PRMT5 inhibitors. To identify additional novel drug targets for patients with chromosome 9p21 loss, we first conducted unbiased, combinatorial CRISPR screens evaluating the pairwise knockout of 'context' genes lost on Ch9p21 against a focused library of ~5,000 'target' genes. We identified loss of *FOCAD*, the gene encoding the SKI complex interacting protein that facilitates exonuclease activity, as synthetic lethal with loss of PELO, a ribosome rescue factor. To expand on this genetic interaction, we conducted genome-wide CRISPR screens in *FOCAD* isogenic cell lines discovering both PELO and its binding partner HBS1L as synthetic lethal targets in FOCAD-null cells. In *FOCAD*-deleted xenografts, knockout of HBS1L eliminates tumor growth and results in protein loss of PELO, likely through destabilization of the HBS1L/PELO protein complex. The dependency of FOCAD-null cells on HBS1L/PELO could be rescued by exogenous cDNA expression of *FOCAD*, and conversely, knockout of *FOCAD* in WT cells rendered them dependent on HBS1L, demonstrating the robustness of this interaction and validating the HBS1L/PELO protein complex as a promising target for drug discovery. Through cellular phenotypic screens and a medicinal chemistry campaign, we identified pharmacological disruptors of the HBS1L/PELO complex that selectively kill *FOCAD*-deleted, but not *FOCAD*-proficient, cells across diverse models. Oral dosing of mice harboring *FOCAD*-deleted xenografts with our leading compound led to dose-dependent tumor regression and modulation of PD biomarkers. Mechanistically, HBS1L and PELO form a complex responsible for resolving stalled elongation complexes during protein translation. We propose a model in which loss of FOCAD (and/or other SKI complex members) leads to accumulation of aberrant mRNAs on which ribosomes become stalled, leading to hyperdependence on HBS1L/PELO for ribosome rescue. This model is supported by the observation of translational arrest and activation of the unfolded protein response upon HBS1L/PELO inactivation. Clinically, *FOCAD* loss occurs in ~30% of all *MTAP* deleted tumors, representing ~3% of all solid tumors (including ~7% of NSCLC). Our lead molecule provides preclinical validation for targeting the HBS1L/PELO complex as a novel strategy for the potential treatment of a large population of patients with *FOCAD*-deleted tumors.



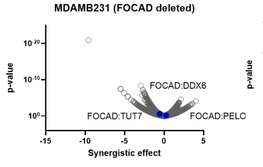
Unbiased CRISPR screens identify FOCAD as synthetic lethal with the HBS1L/PELO complex

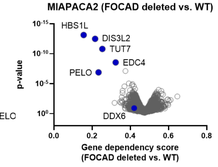


Combinatorial CRISPR with sub-genome library

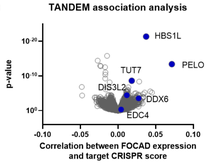


Whole genome library

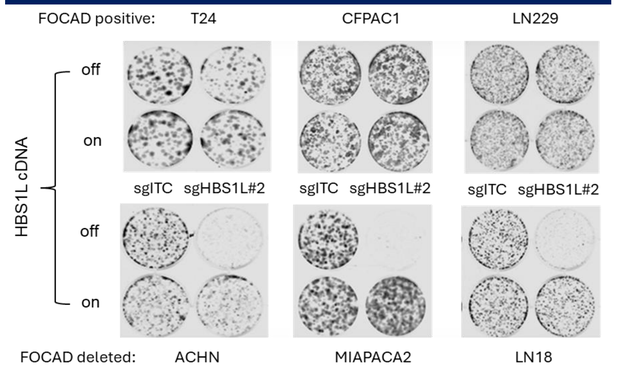


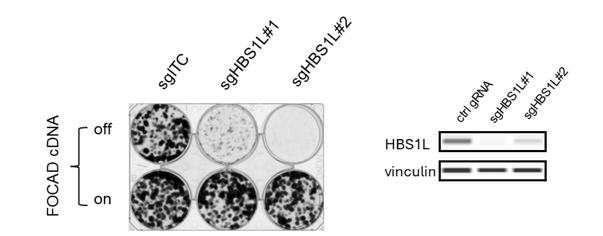


Internal in silico analysis

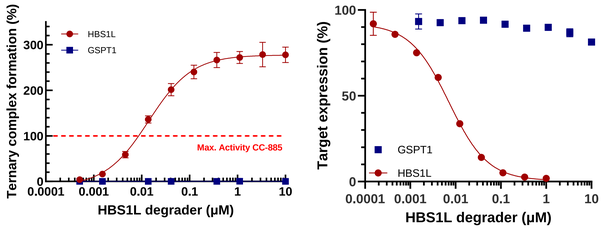


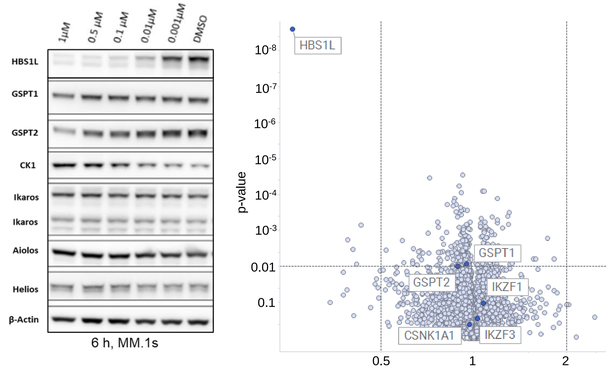
Genetic ablation of HBS1L is lethal in FOCAD-deleted cell lines



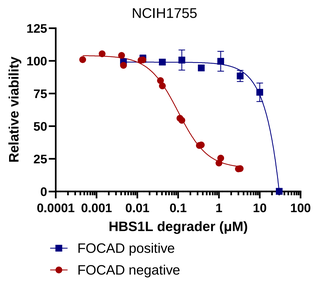


A novel molecular glue degrader selectively targets HBS1L for degradation

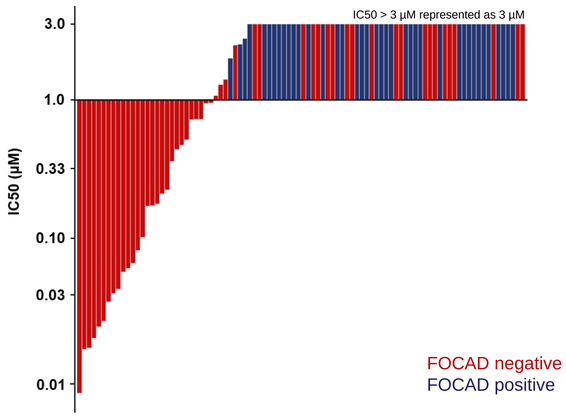




HBS1L molecular glue degrader is selectively lethal in FOCAD-inactivated cancel cell lines

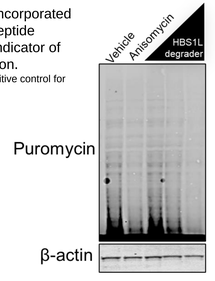


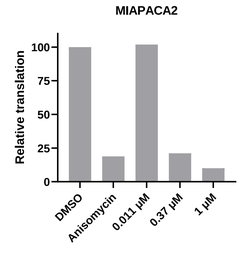
Cell line	FOCAD null IC50 (μM)	FOCAD positive IC50 (μM)	Selectivity
NCIH1755	0.103	>10	>100X
MIAPACA2	0.152	~10	~65X
HAP1	0.120	>10	>80X
HeLa	0.102	>10	>100X
MDAMB231	0.090	>10	>100X

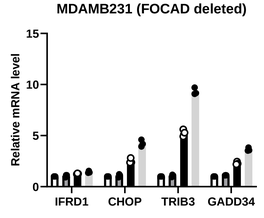


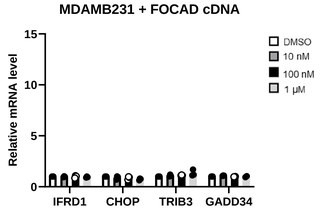
HBS1L degradation leads to translational arrest and activation of the unfolded protein response in FOCAD-inactivated cancer cells

Puromycin is incorporated into growing peptide chains as an indicator of active translation.
Anisomycin is a positive control for translational arrest.

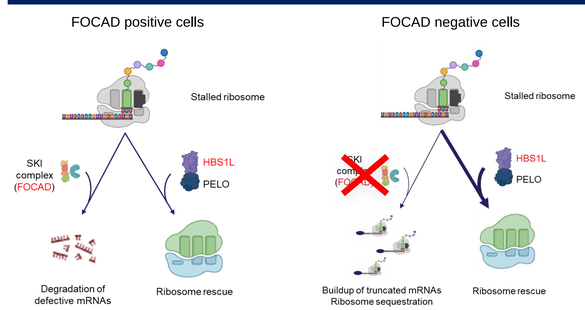




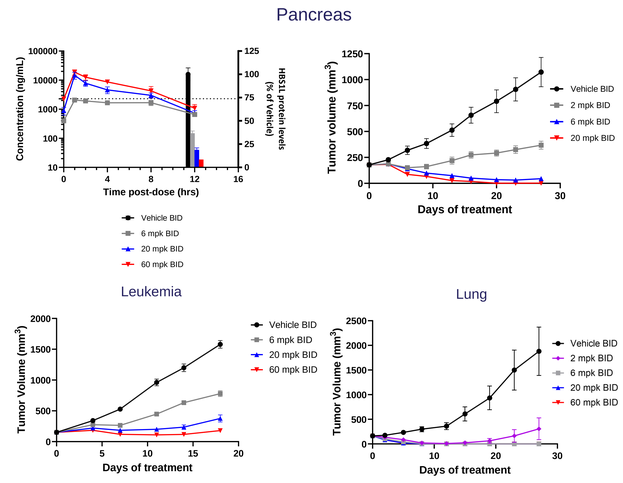




Proposed mechanism: Cells lacking FOCAD are hyperdependent on HBS1L for ribosome recycling



Oral dosing of HBS1L molecular glue degrader shows PK/PD correlation and leads to tumor regressions in FOCAD null xenograft models



Summary

- CRISPR screens identify the HBS1L/PELO complex as a synthetic lethal target for the treatment of ch9p21 deleted tumors with *FOCAD* loss
- Genetic studies confirm that *FOCAD*-null cancer cells require HBS1L for ribosome recycling, with *HBS1L* knockout causing lethality in *FOCAD* negative cells, but not *FOCAD* positive cells
- Pharmacological degradation of HBS1L with a novel, potent, and selective molecular glue (i) activates the unfolded protein response specifically in *FOCAD* negative cancer cells, and (ii) leads to tumor regressions in multiple FOCAD-deleted xenograft models
- A development candidate has been nominated for IND enabling studies

Acknowledgements

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