Identification of novel VPS4A inhibitors for the treatment of VPS4B-deleted cancers

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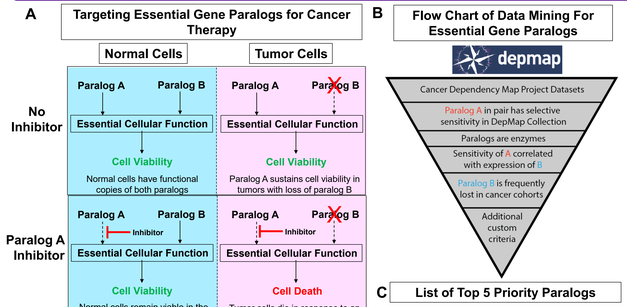
Abstract

Synthetic lethality occurs when a single gene alteration is compatible with cell viability, but an additional co-occurring genetic alteration leads to cell death. In the context of cancer therapy, synthetic lethality can occur through the inhibition of a target that is selectively essential to tumors harboring a specific genetic alteration. Gene paralog pairs represent one promising class of synthetic lethal cancer targets, wherein the function of one paralog is lost in tumor cells, rendering them dependent on the remaining paralog to carry out an essential cellular process. To identify essential gene paralog pairs as starting points for drug discovery programs, we mined publicly available CRISPR genetic loss-of-function data and associated molecular datasets collected across a diverse panel of cancer cell lines. We first identified pairs of gene paralogs where one paralog was essential in a subset of cell lines, and then filtered these genes based on function, known literature, enrichment in specific lineages and integration of external datasets. These efforts identified VPS4A as a synthetic lethal target in cancers harboring copy number loss of VPS4B. VPS4A and VPS4B are highly homologous AAA ATPases that carry out multiple essential cellular processes including nuclear membrane remodeling and endosomal membrane biogenesis. VPS4B loss occurs as a passenger deletion during loss of the tumor suppressors SMAD2 and SMAD4. Loss of VPS4B creates a genetic dependency on VPS4A to drive essential VPS4-dependent processes. VPS4B deletion occurs at a frequency of up to 3% in multiple solid tumor types including esophageal, head and neck, pancreatic and colorectal cancers. To further explore the potential of VPS4A as a therapeutic target in VPS4B-deleted tumors, we first validated the synthetic lethal relationship between VPS4A/B using isogenic cell line pairs. HCT116 cells with an engineered homozygous loss of VPS4B, but not wild-type HCT116 cells, showed profound cell kill in response to genetic silencing of VPS4A. Moreover, simultaneous siRNA-mediated knockdown of VPS4A and VPS4B resulted in cell death across a panel of cancer cell lines (e.g. H1975, Panc0403), while knockdown of either gene alone was compatible with cell viability. Encouraged by these results, we profiled several previously reported small-molecule inhibitors of VPS4A (e.g. DBeQ and MSC1094308) in a suite of biochemical assays. Notably, these molecules were inactive against VPS4A. We have discovered a novel series of VPS4A inhibitors and are advancing this inhibitor series through lead optimization. Potent, selective, and pharmacologically active VPS4A inhibitors are expected to be well tolerated and have strong single-agent activity in tumors bearing VPS4B homozygous deletions.

Computational Identification of Synthetic Lethal Gene Paralogs



Paralog A (Selective Dependency)	Paralog B (Biomarker)	Pearson's Correlation	TCGA Homozygous Deletion Frequency (%)
Redacted	Redacted	0.45	0.78
VPS4A	**VPS4B**	**0.44**	**1.48**
Redacted	Redacted	0.35	2.83
SMARCA4	SMARCA2	0.34	1.51
VPS4B	**VPS4A**	**0.31**	**0.78**

Figure 1. (A) Conceptual framework for targeting essential gene paralogs for cancer therapy. Tumor cells can have loss-of-function mutations to one paralog, rendering them dependent on the remaining paralog to sustain cell viability. **(B)** Bioinformatics pipeline for identification of synthetic lethal gene paralog pairs. CRISPR knockout data from ~1700 cancer cells lines from the Cancer Dependency Map (DepMap) was utilized as the starting point. **(C)** List of top 5 high-priority essential gene paralogs where Paralog A represents a potential drug target and Paralog B represents the corresponding biomarker of dependency. We would like to acknowledge our collaborators at the Broad DepMap Consortium for access to the latest functional genomic datasets.

VPS4A KO is Synthetic Lethal with VPS4B CN-Loss in DepMap

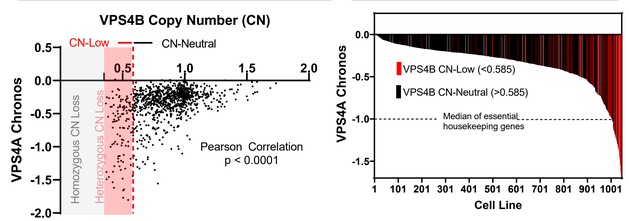


Figure 2. (A) Correlation between response to VPS4A CRISPR knockout (Chronos score) and VPS4B copy number (CN) across ~1000 human cancer cell lines of the Cancer Dependency Map (DepMap) database. A lower Chronos score indicates a stronger dependency on the gene for cell viability. CN is expressed as $\log_2$(relative to ploidy +1): 0.35 < CN < 0.585 indicates heterozygous CN loss. CN < 0.35 is consistent with homozygous CN loss. **(B)** Waterfall plot of VPS4A Chronos score. **(C)** Ideogram of Chromosome 18 showing relative locations of VPS4B with tumor suppressors SMAD2 and SMAD4 and oncogene BCL2. **(D)** Volcano plot showing the strongest gene dependencies in VPS4B copy number-low cell lines (CN < 0.585) relative to VPS4B copy number neutral cell lines (CN > 0.585).

VPS4B KO Creates a Synthetic Lethality Dependency on VPS4A

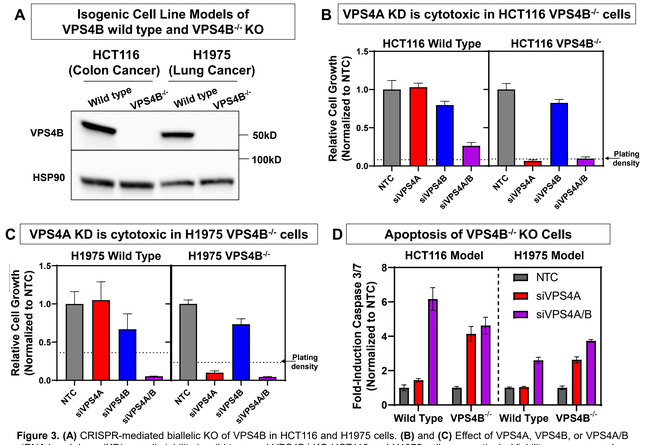


Figure 3. (A) CRISPR-mediated biallelic KO of VPS4B in HCT116 and H1975 cells. **(B)** and **(C)** Effect of VPS4A, VPS4B, or VPS4A/B siRNA knockdown (KD) on cell viability in wild type and VPS4B-/- KO HCT116 and H1975 cells, respectively. Viability was assessed on day 6 by Cell Titer Glo. Data are normalized to a non-targeting control siRNA (NTC). **(D)** Induction of apoptosis following treatment with the indicated siRNA in isogenic cell line pairs. Caspase activation was assessed on day 3 post-transfection.

Heterozygous VPS4B-Deleted Cells Are Dependent on VPS4A

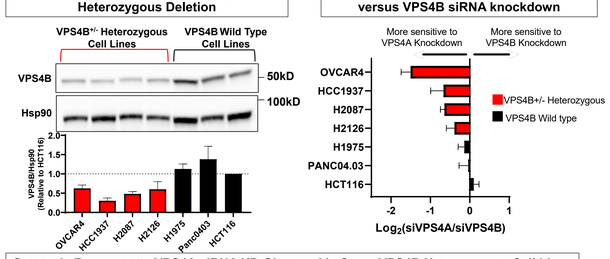


Figure 4. (A) Western blot of cancer cell lines with a reported VPS4B copy number (CN) loss (DepMap dataset). CN-low cells lines are consistent with a heterozygous loss of VPS4B. **(B)** Response of VPS4B heterozygous cell lines to VPS4A siRNA knockdown relative to response to VPS4B siRNA knockdown assessed by cell viability using CellTiter-Glo after 6 days. **(C)** Representative examples of the response to VPS4A, VPS4B or double VPS4A/B siRNA knockdown relative to non-targeting controls (NTC). The dashed line indicates the cell plating density.

Loss of VPS4 Perturbs Membrane and Nuclear Morphology

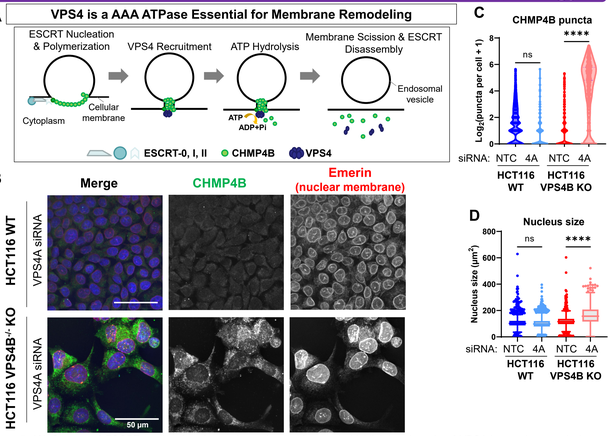
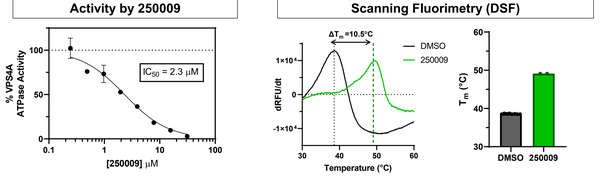


Figure 5. (A) Cartoon of ESCRT-mediated endosomal membrane biogenesis highlighting the role of VPS4 in membrane scission. VPS4 is also involved in nuclear membrane remodeling, cytokinesis, and virus shedding. **(B)** Immunofluorescence microscopy of HCT116 wild type and VPS4B-/- KO cells following 3 days of siRNA-mediated knockdown of VPS4A. Emerin is a marker of nuclear membrane. **(C)** and **(D)** Quantitation of images from part (B). Non-targeting siRNA control (NTC).

A Novel Series of ATP-Competitive VPS4 Inhibitors

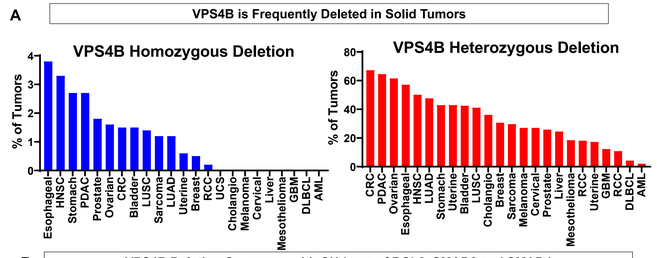


C

Compound	Reference	IC_{50} VPS4A Biochemical Assay (μM)		DSF Assay (ΔT_M °C)	IC_{50} VPS4B Biochemical Assay (μM) Low ATP
		Low ATP	High ATP		
250009	Internally discovered	2.3	6.8	10.5	1.3
DBeQ	DOI: 10.1038/nchembio.1313	>40	>40	-1.2	N.D.
MSC1094308	DOI: 10.1002/anie.201711429	29.0	9.2	0.3	N.D.

Figure 6. (A) Inhibition of VPS4A ATPase activity by 250009. **(B)** Differential scanning fluorimetry (DSF) assay of VPS4A in the absence and presence of 250009. **(C)** VPS4 inhibitory activity of 250009 in the presence of low (0.125 mM) or high (1 mM) ATP compared with previously reported VPS4 inhibitors. The activity of previously reported VPS4 inhibitors could not be validated in our assays.

VPS4B is Frequently Lost in Human Tumors



B

TCGA PanCancer Database (N = 10,967)		VPS4B Homozygous Deletion (N = 102)			VPS4B Heterozygous Deletion (N = 3545)	
		Co-occuring alteration (%)	χ^2 Analysis		Co-occuring alteration (%)	χ^2 Analysis
BCL2	Homozygous Deletion	98%	p < 0.0001	Heterozygous Deletion	99%	p < 0.0001
SMAD4		59%	p < 0.0001		95%	p < 0.0001
SMAD2		27%	p < 0.0001		92%	p < 0.0001

Figure 7. (A) The frequency of homozygous **(left)** and heterozygous **(right)** deletion of VPS4B by tumor type from TCGA PanCancer Atlas studies (10,967 samples). **(B)** Chi-square analysis was performed to assess the degree of co-occurrence of VPS4B CN alterations with homozygous or heterozygous deletions of tumor suppressors and/or oncogenes in close proximity to VPS4B on chromosome 18q (BCL2, SMAD2 and SMAD4).

Conclusions

- **Computational mining of the Cancer Dependency Map database identified VPS4A/VPS4B as one of several essential gene paralog pairs that represent potential therapeutic targets.**
- **Knockdown of VPS4A was synthetic lethal in cells with engineered VPS4B biallelic knockout and synthetic lethal in some cells with naturally-occurring VPS4B heterozygous CN-loss.**
- **Loss of VPS4 led to disruption of endosomal membrane structures, nuclear deformation, caspase activation and cell death.**
- **VPS4B is a common passenger deletion across multiple solid tumor types (up to 3.8% frequency of homozygous deletion, up to 67% frequency of heterozygous deletion) and these tumors are expected to be highly sensitive to a VPS4A inhibitor.**
- **Tumors with VPS4B CN-loss can be identified using commercial NGS panels using BCL2, SMAD2 or SMAD4 as surrogates.**
- **We identified a series of novel VPS4 inhibitors with on-target, ATP-competitive inhibitory activity and are advancing this series to improve potency, isoform selectivity and drug-like properties.**
- **VPS4A represents a validated target for precision oncology in patients with tumors harboring CN-loss of VPS4B.**
- **Future target validation work will also explore the dependency of VPS4B in VPS4A-deleted cell lines and tumor models.**