Identification and Validation of Predictive Biomarker of EZH1/2 Dual Inhibitor, HM97662, through Bioinformatics Analysis

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Introduction

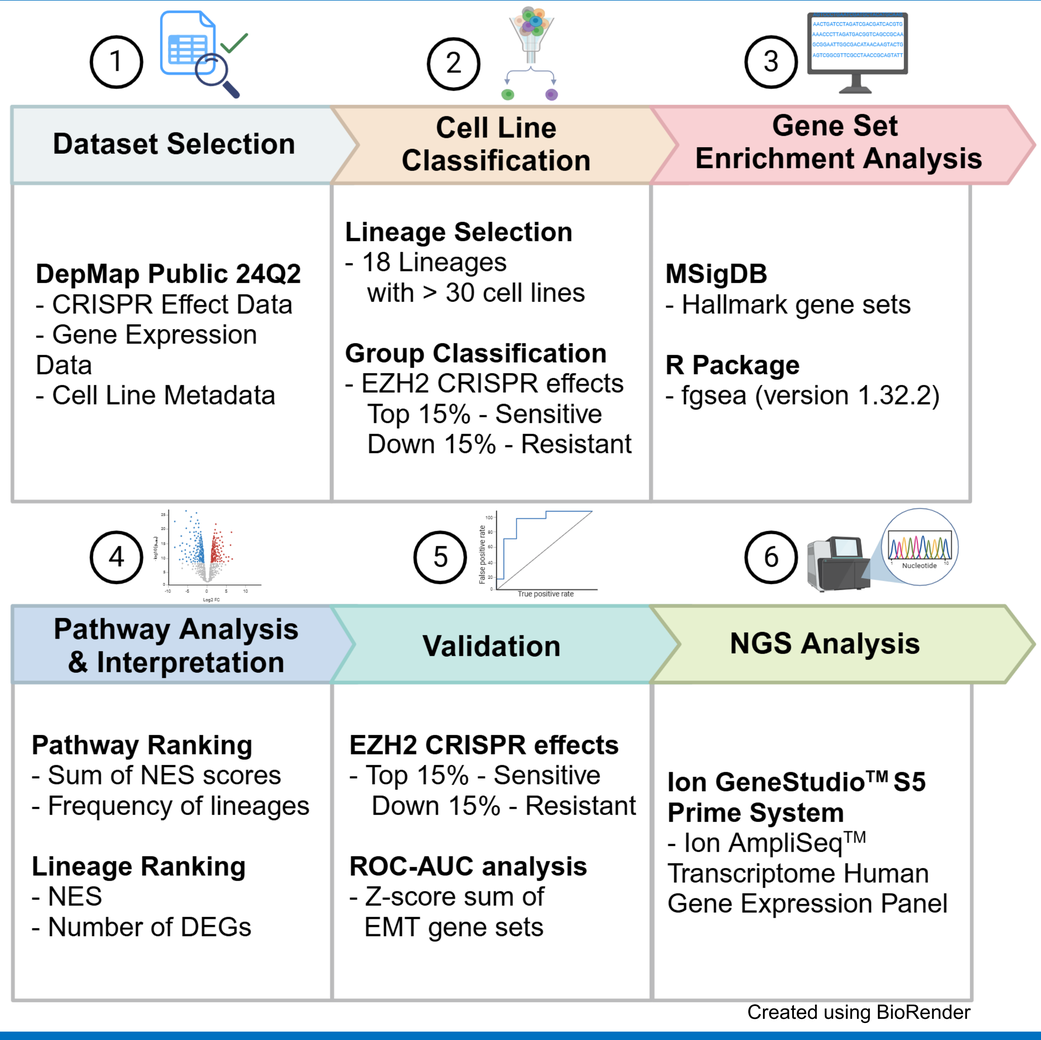
Enhancer of zeste homolog 2 (EZH2) is an enzymatic subunit of polycomb repressive complex 2 (PRC2) which leads to transcriptional repression by trimethylation of lysine 27 on histone 3 (H3K27me3)[1]. It was reported that EZH2 is a key factor in cancer development, progression, and metastasis across solid and hematologic cancers[2].

Loss-of-function mutations in SWI/SNF family members such as ARID1A are being used as biomarkers predicting sensitivity to EZH2 inhibitors[3]. However, identification of additional biomarkers that drive tumor dependence on EZH1 and EZH2 could expand the range of eligible patients and tumor types for EZH(1)2-targeted therapies.

Here, we propose bioinformatics workflow for identifying novel predictive biomarker of EZH(1)2 inhibition using publicly available repositories. Additionally, we demonstrate its effectiveness in predicting the response of our HM97662, an EZH1/2 dual inhibitor, by integrating wet-lab experiments with NGS-based pharmacogenomic analysis.

The public EZH2 CRISPR screen data in DepMap were used to identify and classify cancer cell lines of 18 lineages into sensitive and resistant groups. We conducted gene expression data analysis with Gene Set Enrichment Analysis (GSEA) which is a robust and biologically meaningful approach for biomarker discovery[4]. To measure the performance of the biomarker in solid cancers such as lung, breast, ovary, and esophagus cancer, ROC and AUC curve analysis was applied to public and internal data.

Analysis Workflow



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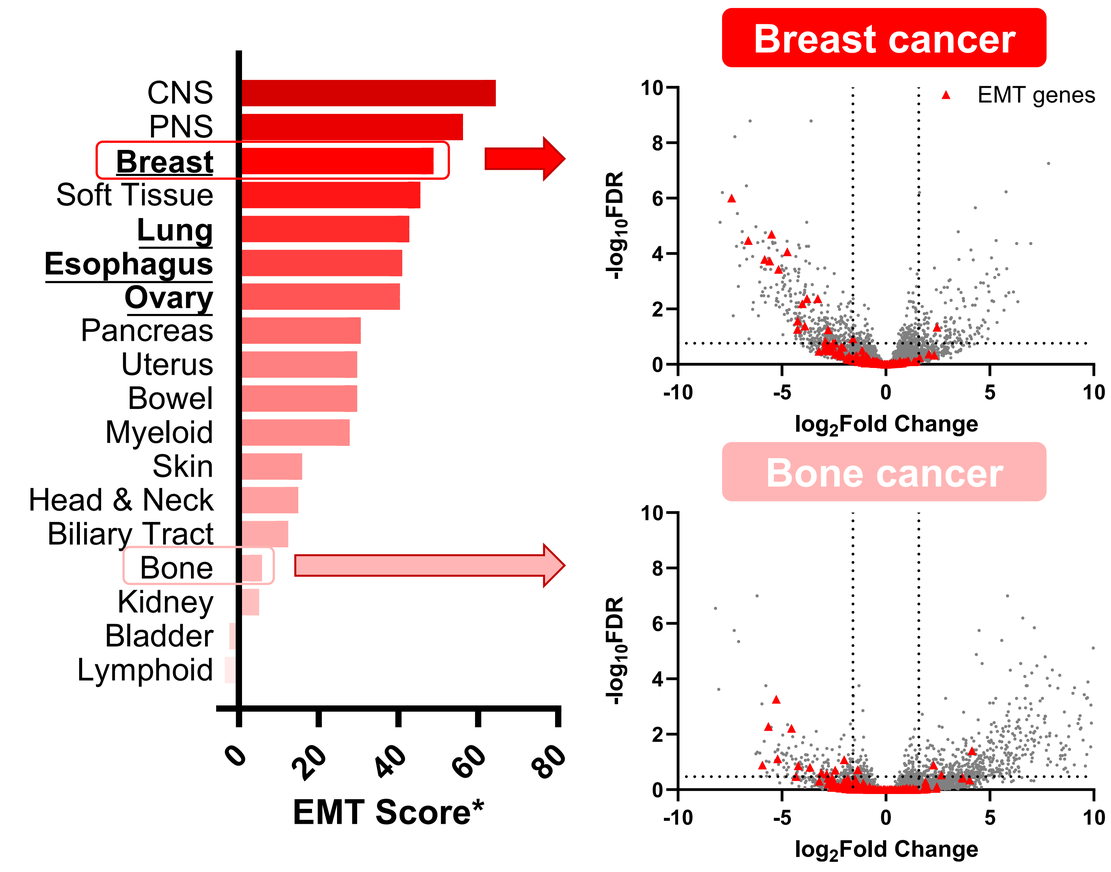
Results

(A) Top 5 significantly downregulated pathways of DEGs

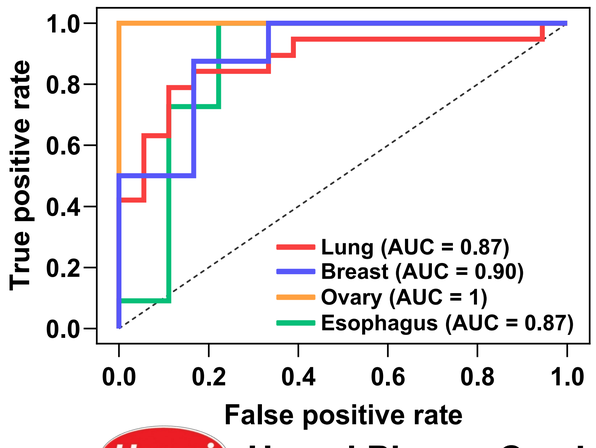
HALLMARK Pathway (Sensitive vs. Resistant)	Total NES	Frequency
EPITHELIAL_MESENCHYMAL_TRANSITION	**-32.2**	**18**
TNFA_SIGNALING_VIA_NFKB	-25.3	17
COMPLEMENT	-22.9	14
INFLAMMATORY_RESPONSE	-21.7	15
ALLOGRAFT_REJECTION	-21.0	13

(B) The EMT gene set in various cancer indications

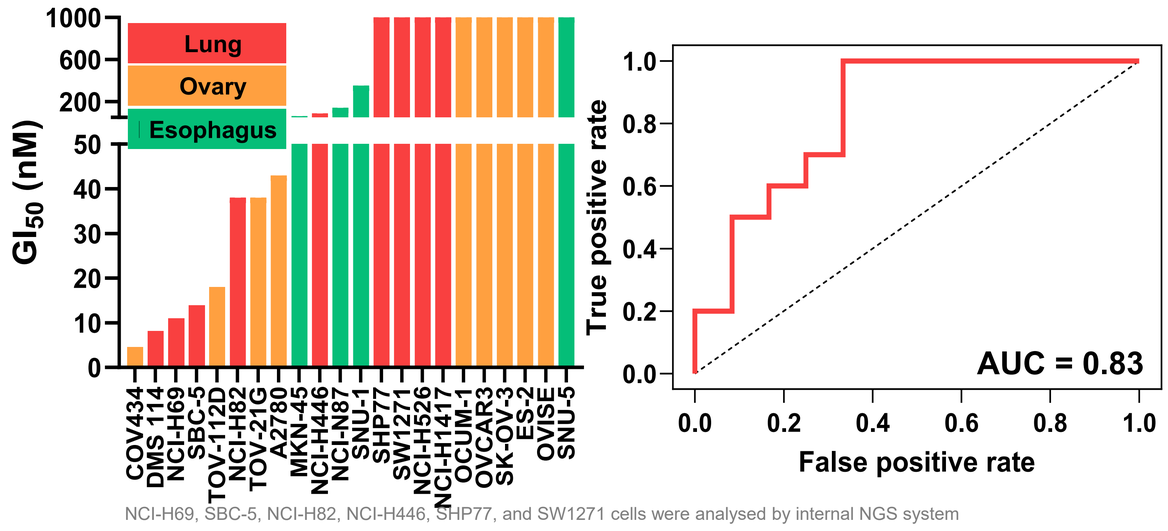


(C) ROC curves for the EMT gene set predicting EZH2 CRISPR effect

Lineage	nEMT	# of cell lines
Lung	16	37
Breast	18	14
Ovary	15	16
Esophagus	17	20



(D) ROC curves for the EMT gene set predicting HM97662 response



NCI-H69, SBC-5, NCI-H82, NCI-H446, SHP77, and SW1271 cells were analysed by internal NGS system

Summary

- The EMT gene set was the most potent distinguisher of EZH2 knock-out sensitivity across 18 cancer cell lineages.
- The EMT gene set was significantly downregulated in the EZH2 CRISPR sensitive group in various solid cancers including lung, breast, ovary, and esophagus cancer.
- The EMT gene set exhibited great performance in predicting sensitivity to EZH2 CRISPR and HM97662, an EZH1/2 dual inhibitor.
- Taken together, these bioinformatics analysis demonstrated that the EMT gene set holds promising potential as a novel biomarker for maximizing the benefits of EZH2-targeted therapies.
- Currently, a first-in-human phase 1 dose escalation study of HM97662 in advanced or metastatic solid tumors in underway in KR/AU (NCT05598151).

References

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2. Gan L. et al., *Biomark Res*. **2018**, 6, 10;
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4. Bull C. et al., *Sci Rep*. **2024**, 14(1):30202.

Acknowledgements

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Abbreviations

*EMT Score = NES x nEMT
NES = Normalized enrichment scores of EMT pathway via GSEA (S vs. R)
nEMT = Number of significantly downregulated genes in EMT pathway gene set