Computational Repurposing of Chemotherapeutics for Pulmonary Hypertension

Vinny Negi[1]; Adam Handen[1]; Gil Speyer[3]; Asma Naqvi[2]; Seungchan Kim[3]; Imad Al Ghouleh[2]; Stephen Y Chan[1].

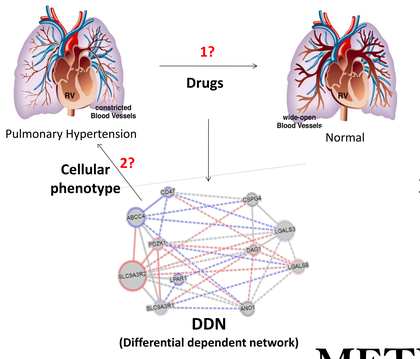
[1,2]Division of Cardiology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA; and Center for Pulmonary Vascular Biology and Medicine, Pittsburgh Heart, Lung, and Blood Vascular Medicine Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

[3]Integrated Cancer Genomics Division, Biocomputing Unit, Translational Genomics Research Institute, Phoenix, AZ 85004, USA

BACKGROUND

Repurposing is an accelerated way of directing new and efficient drugs for lesser studied diseases such as pulmonary hypertension (PH). There have been various similarities reported between cancer and PH[1]. The availability of high throughput data in cancer research combined with cutting edge computational technology allows for discovery of novel biology and new drugs affecting PH. In this study we are utilizing computational method to investigate applicability of chemotherapeutics in PH. RNA sequencing data from >800 cancer cell lines exposed to 368 chemotherapies (via the CTRP (Cancer Therapeutics Response Portal) and CCLE (Cancer Cell Line Encyclopedia) databases) were combined with a compilation of gene clusters important in PH and a novel computational algorithm, EDDY (Evaluating Differential DependencY), to define the re-wiring of gene dependency networks of these PH gene clusters in response to specific chemotherapies.

HYPOTHESIS



1) To identify drugs, alone or in combination that affect pulmonary hypertension.
2) To determine novel gene networks regulating pulmonary hypertension.

METHODS

1) PH relevant genes were clustered into smaller networks

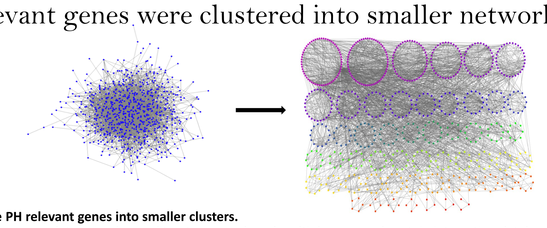


Figure 1. Demarcate PH relevant genes into smaller clusters.
All the PH related genes was determined using literature search and multiple gene and protein interaction databases. The 747 genes obtained were then dissected into 55 clusters using Map Equation. These clusters were then fed into EDDY to determine the drugs able to target PH.

2) PH clusters were fed into EDDY to determine differential dependent networks (DDN)

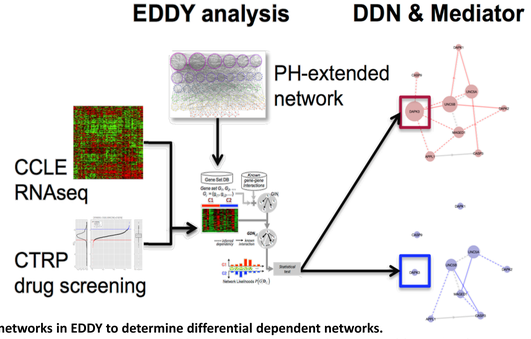


Figure 2. Overlay PH networks in EDDY to determine differential dependent networks.
EDDY determine differential dependent networks (DDN) using CCLE and CTRP in drug sensitive and resistant conditions. PH relevant networks were overlaid on it to determine DDN and critical mediators with respect to PH.

RESULTS

1) Shortlisted drugs and clusters

Drug	# of Clusters	# of Mediators	–log (p-values)	Score
I-BET151/762	7	18	1.86	2.34
MK-1775	8	14	2.05	2.29
NVP-231	5	18	1.58	2.02
BRD-K11533227	7	12	1.85	2.00
Crizotinib	7	12	1.76	1.98
BRD-K34222889	6	11	2.36	1.95
WAY-362450	6	13	1.68	1.89
Momelotinib	6	11	2.04	1.87
selumetinib: decitabine	6	11	1.68	1.78
PLX-4720	6	11	1.67	1.78

Table 1. Shortlisted PH relevant drugs.
The drugs were shortlisted based on the number of clusters they target and number of critical mediators involved. Further those drugs were selected that were already in clinical trials.

Cluster	Common Drugs
C41	UNC0321
C28	CAY10594
C6	I-BET151, NVP-231
C31	RO4929097
C30	hyperforin
C17	WAY-362450
C15	PL-DI, momelotinib, NVP-231, I-BET151, BRD-K34222889, GSK-3 inhibitor IX, mitomycin, crizotinib, MK-1775, BRD-K11533227

Table 2. Shortlisted PH clusters.
Cluster 15 was selected as it was found to be targeted by various drugs.

2) Effect of IBET762 and MK-1775 on cluster15

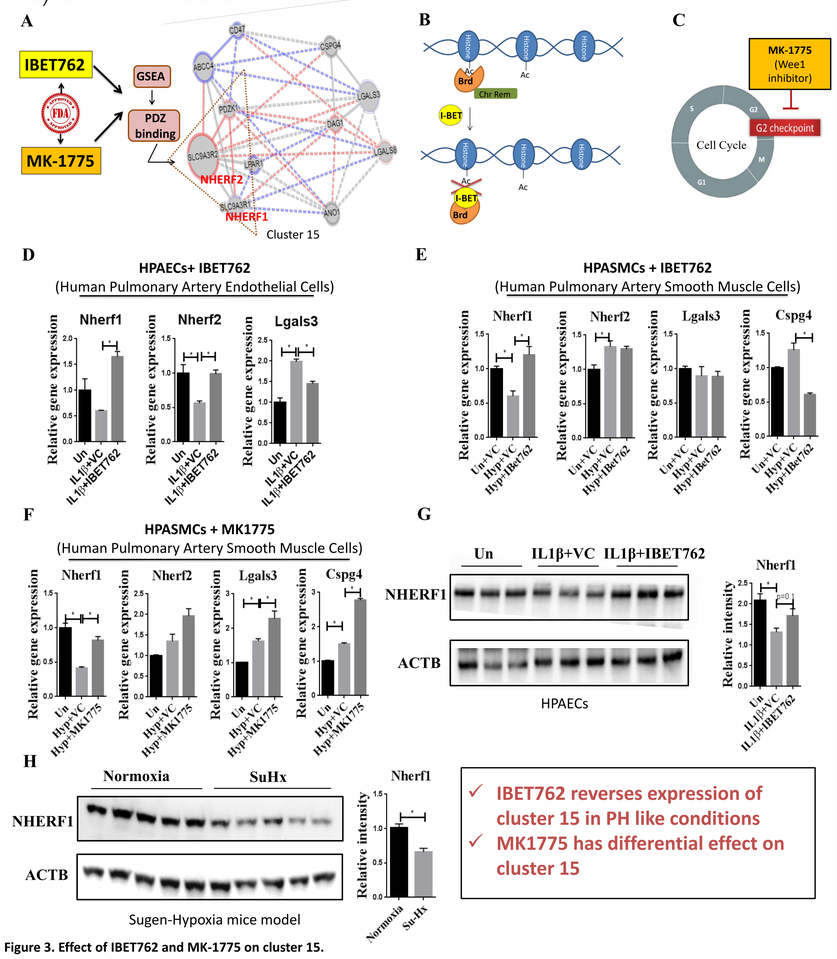


Figure 3. Effect of IBET762 and MK-1775 on cluster 15.
A. Cluster 15 was found to be the target of most of the drugs. FDA approved drugs, IBET762 and MK-1775 were selected for further studies. Gene enrichment analysis (GSEA) resulted in PDZ binding domain to be the major functional class in cluster 15 including NHERF1, NHERF2 and PDZK1. **B.** Mode of action of IBET762; it binds to the acetylated lysine residue and prevents the binding of bromodomain containing epigenetic regulator. **C.** Mode of action of MK-1775.; it inhibits Wee-1 kinase, which is a cell cycle checkpoint kinase. **D**. HPAECs and **E.** HPASMCs were treated with IL-1β/hypoxia along with vehicle control (VC) i.e DMSO or IBET762 for 24h. The relative gene expression of cluster 15 was determined using RT-PCR. **F**. HPASMCs were treated with hypoxia along with vehicle control (VC) i.e DMSO or MK-1775 for 24h and relative gene expression of cluster 15 was determined. **G**. Western blot of NHERF1 in HPAECs and its respective densitometry. **H.** Western blot of NHERF1 In in Sugen-hypoxia model of PH and its respective densitometry.

3) Global expression analysis suggested cluster 15 to be associated with respiratory electron transport and EndMT pathways

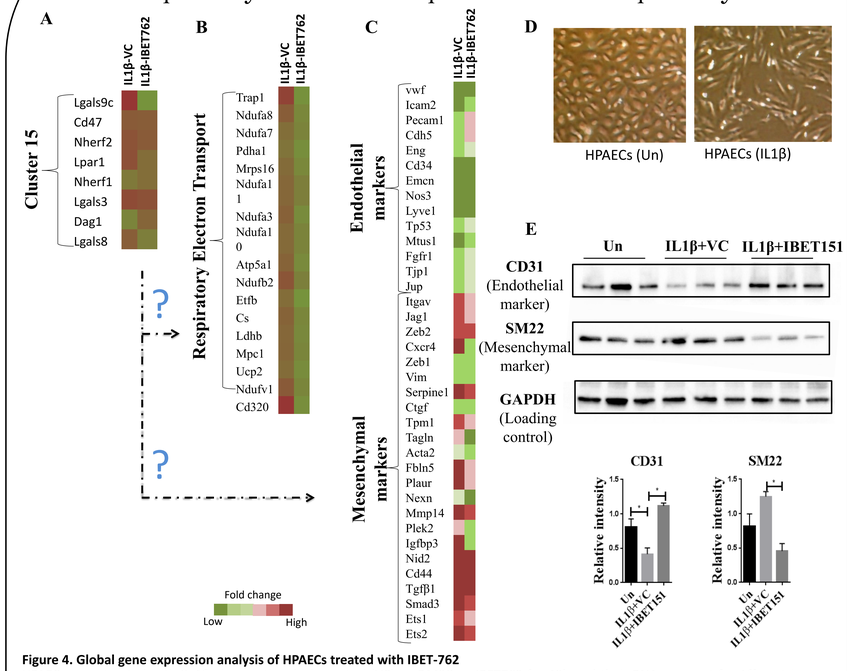
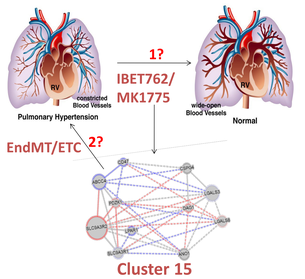


Figure 4. Global gene expression analysis of HPAECs treated with IBET-762
HPAECs were treated with IL-1β along with vehicle control (VC) i.e DMSO or IBET762 for 48h and their RNA was sent for Affymetrix gene expression array. **A**. Heat map of the cluster 15 gene expression. Gene enrichment analysis revealed respiratory electron transport and endothelial to mesenchymal transition to be among the targeted pathways **B.** Heat map of respiratory electron transport genes that were differentially expressed **C.** Representative expression of endothelial and mesenchymal genes that were differentially expressed. **D.** Pictorial representation of the morphological changes in HPAECs upon induction with IL1β. **E.** Western blot of endothelial marker CD31 and mesenchymal marker SM22 in HPAECs treated with IL1β along with VC or IBET762.

CONCLUSIONS



1. A computational approach can be used to repurpose drugs for PH.
2. IBET762, MK1775 and Momelotinib are potential drugs to treat PH.
3. Cluster 15 was identified as a novel network potentially involved in pathophysiology of PH.

FUTURE DIRECTIONS

1. Determine the effect of IBET-762 in mouse model of pulmonary hypertension.
2. Determine whether cluster 15 affects processes such as the respiratory electron transport chain and endothelial to mesenchymal transition.
3. Validate the connections in cluster 15 as a pathway.
4. Determine the effect of other drugs such as MK-1775 and Momelotinib both in *in vitro* and mouse model of PH.

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