408 - Donor-to-Donor Variation in the Host Gene Expression Response to SAHA

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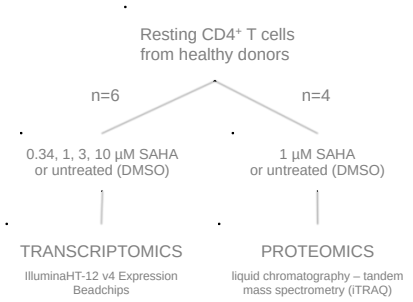
Introduction

Reactivation of latent provirus is central to the "shock and kill" strategy for eradication of the latent HIV reservoir. One of the therapeutics currently under investigation is the histone deacetylase (HDAC) inhibitor suberanilohydroxamic acid (SAHA). SAHA treatment was shown to increase HIV transcription in patients on anti-retroviral therapy, but the increase in HIV transcription levels varied widely between patients (Archin 2012, Nature; Elliot 2014, PLoS Pathogens). It is not currently understood whether viral factors, host factors or a combination of both are responsible for the observed patient-to-patient variability in response to SAHA.

Aim

Here, we hypothesize that differences in host factors may explain why some patients show a more robust response to SAHA treatment. By investigating donor-to-donor differences in gene expression upon SAHA treatment of primary resting CD4$^+$ T cells we have sought to identify mechanisms that impede or facilitate the activation of HIV transcription.

Methods



DATA ANALYSIS

1. Filtering out genes and proteins with high variance (SD > 25th percentile) in untreated controls, resulted in 4083 genes and 387 proteins.
2. Co-expression analysis performed using Short Time-series Expression Miner (STEM) with a permutation test to calculate significance for each profile.
3. Protein interaction networks were generated using Metacore. ToppGene was used for gene ontology.

Acknowledgements

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Results

1. Enrichment strategy reveals genes and proteins that varied the most between donors after exposure to SAHA

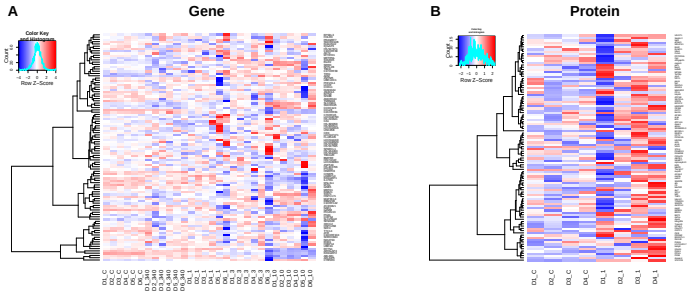


Figure 1. The top 100 most variable genes (A) and proteins (B) demonstrate varying expression levels between donors. Amongst the genes and proteins with highest variability were the interferon-responsive transcriptional activators *STAT1* (gene) and STAT6 (protein), *SIN3B* and *L3MBTL3* which are involved in nucleosome remodeling, and the protein HMG20 which is part of a chromatin remodeling complex.

2. Co-expression analysis identifies groups of genes and proteins that behave differently between donors



Figure 2. A). Within the microarray data, profiles 25 (0,0,0,0,-1,4) and 26 (0,0,0,0,1,-3) contained significantly higher numbers of genes than predicted by permutation testing. B). Within the proteomics data at 1 µM, profiles 9 (0,-3,1,2) and 22 (0,-1,2,1) displayed high variability across donors.

Table 1. Details of selected genes and proteins within profiles

Profile	Dose	Gene symbol	Group	Associated GO ontologies	Largest difference in response between donors
26	1 µM, 10 µM	SMARCA4 (BRG1)	SWI/SNF superfamily-type complex	Nucleosome binding, ATP-dependent chromatin remodeling, SWI/SNF superfamily-type complex	2.22
26	3 µM	SMARCA5 (SNF2H)			2.75
22	1 µM	ARID1A (BAF250)			4.35
22	1 µM	CHD4			7.19
9	1 µM	HMG20B	HDAC complex	Chromatin remodeling, BRCA2-BRAF35 complex	19.75
25	3 µM	SIN3A		Chromatin binding, histone deacetylation, histone deacetylase complex	2.30
26	10 µM	BRMS1		NF-kappaB binding, protein deacetylation, mSIN3A complex	2.77
26	3 µM	TRRAP	HAT complex	Histone acetylation, HAT complex, PCAF complex	2.17
25	3 µM	MORF4L2		Chromatin modification, HAT complex, mSIN3A complex	2.31
26	1 µM, 3 µM	CUX1	Transcription factor	Chromatin binding, transcription regulatory region DNA binding	2.57
26	1 µM	ZNF143		sequence-specific DNA-binding, transcription from RNA polymerase II promoter	2.43
26	1 µM	STAT1		regulatory region DNA binding, negative regulation of I-kappaB kinase/NF-kappaB signaling	2.07

3. Transcription factors ZNF143 and CUX1 are major hubs within protein interaction network of variable genes and proteins

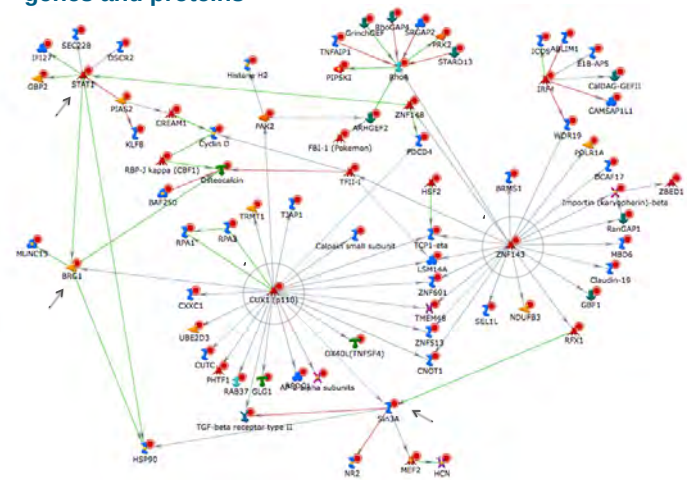
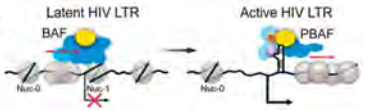


Figure 3. A combined protein interaction network for profiles 9, 22, 25 and 26. Cut-like homeobox 1 (CUX1, 40 edges) and Zinc Finger Protein 143 (ZNF143, 26 edges) are major hubs. STAT1 (9 edges), BRG1 (SMARCA4, 6 edges) and SIN3A (6 edges) are among the smaller hubs.

Conclusions

Expression levels of genes and proteins driving repressive chromatin structure at HIV LTR are variable between donors

- Members of nucleosome remodeling complexes were among the top 100 genes and proteins that varied the most across donors upon SAHA treatment and were consistently found within the co-expression profiles.
- 3 core ATPases (BRG1, SNF2H and CHD4), representing the 3 major classes of the SWI/SNF superfamily of ATP-dependent chromatin remodeling complexes were identified as being variable at either the gene (*BRG1, SNF2H*) or protein (CHD4) level in response to SAHA.
- Variability in these factors may reflect temporal differences between donors in returning to repressive chromatin structure after activation by SAHA.
- Future studies will be required to correlate variability in these factors to differences in increase of HIV transcription upon reactivation with SAHA.



Modified from: Mahmoudi et al. Transcription, 3:4, 171-176

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