SAHA Dampens TCR-mediated Reactivation of Latently Infected Cells

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Grants:

Swiss National Science Foundation
Bill & Melinda Gates foundation
Seventh Framework Programme

ABSTRACT

Background: Cell activation via T-cell receptor (TCR) stimulation represents a standard step in the analysis of primary cell models of HIV latency. Reactivation from latency using other pharmacological or immunological stimuli is generally benchmarked against the results of TCR signaling. We analyzed the molecular interaction between TCR stimulation and SAHA (a histone deacetylase inhibitor that increases viral transcription) to assess whether these two approaches were synergistic or antagonistic at the molecular level - with the goal of understanding post-transcriptional processing of viral transcripts.

Methodology: We use a CD4+ T_{CM} latency model (Sahu *et al.* 2006, Tyagi *et al.* 2010) that requires sorting of activated and infected cells, followed by reversion to a resting memory phenotype and maintenance of latent cells by co-culture with H80 feeder cells. After 4 weeks of latency, cells were reactivated with anti-CD3/anti-CD28/IL-2 (TCR stimulation), SAHA, or both. Materials were collected at 8h and 24h post-stimulation for viral reporter analysis by FACS and for RNA-Seq analysis. RNA data were normalized by added spike-ins, and results are reported under 5% false discovery rate.

Results: In this primary cell model, SAHA was characterized by an increase in viral transcripts at both 8h and 24h post-treatment as compared to DMSO, while increase in viral transcripts upon TCR stimulation was observed mostly at 24h. Enhancement of virally-encoded GFP translation was observed mainly upon TCR stimulation, regardless of SAHA adjunction. Analysis of variance on transcriptome profiles performed over 14,036 genes revealed that TCR stimulation altered the expression of 8384 genes, enriched for cell metabolism and cell cycle among other pathways, and consistent with the induction of a cellular environment supportive of viral replication. Addition of SAHA dampened the transcriptional response to TCR stimulation: the two responses were anti-correlated (r=-0.19, $p<10^{-109}$). In particular, a fraction of TCR regulated genes (n=1064, 13%) showed a significant non-additive response upon SAHA co-administration, which was highly dominated by antagonistic effects (n=953, 90%), suggesting a negative interaction between the two treatments.

Conclusions: The current data suggest that activation of cells treated with SAHA does not improve translation of viral transcripts beyond effects from cellular activation alone. On the contrary, transcriptome analysis indicates that SAHA antagonizes the expression of numerous genes that depend on TCR signaling.

AIM

- Understand the failure of SAHA to stimulate viral particle production *ex vivo*.
- Investigate the features of TCR stimulation in a primary model of HIV latency.
- Investigate the features of SAHA exposure in a primary model of HIV latency.
- Investigate the features of combining TCR + SAHA in a primary model of HIV latency.

METHODS

Experimental Set-up:

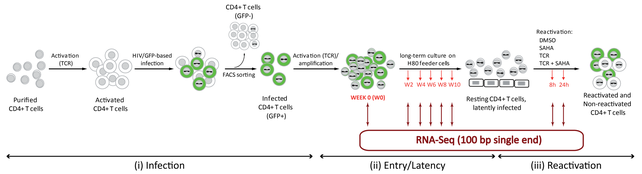


Figure 1. Experimental model of latency.
The experimental setting consists of 3 different phases: (i) The infection process. (ii) Co-culture of activated CD4+ T cells with a feeder layer to promote entry and maintenance of latency, and (iii) Reactivation of viral transcription using a histone deacetylate inhibitor (vorinostat/SAHA, 0.5 mM) or/and a full TCR co-stimulation using anti-CD3/anti-CD28/IL-2.

Bioinformatic Analysis:

1. Data Cleaning
- Adapter trimming: Illumina (cutadapt)
- Trim low quality end of reads (prinseq)
- Trim low overall quality reads (prinseq)
- Remove polyA tail (prinseq)

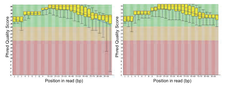


Figure 2. Read Quality Distribution.
Boxwhisker plot of base quality before (left panel) and after (right panel) data cleaning. X axis represents the base position in the read and the Y axis the quality score. The background of the graph divides the Y axis into very good quality calls (green), call of reasonable quality (orange) and calls of poor quality (red).

2. Alignment
- Splice junction aligner (tophat)
- Reference genome = hg19 + HIV vector

3. Analysis
- Data normalization and differential expression analysis (DESeq R package)
- Principal component analysis and other statistical analyses were performed using R and python languages

Sources:
http://www.bioinformatics.bbsrc.ac.uk/project/fastqc/
http://code.google.com/p/cutadapt/
http://prinseq.sourceforge.net/
http://cran.r-project.org7
http://www-huber.embl.de/users/anders/DESeq/
http://www.python.org/
http://tophat.cbcb.umd.edu/

RESULTS

SAHA vs TCR in reactivation of latently infected cells:

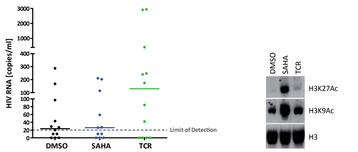


Figure 3. SAHA fails to stimulate viral particle production from latently infected cells *ex vivo*.
Left panel: HIV RNA copies/ml measured in the supernatant of resting CD4+ T cells isolated from treated HIV+ individuals (S: cells from single individuals, P: pooled cell from 2-3 individuals) and stimulated *ex vivo* with DMSO (control, black), SAHA (blue), or TCR (green) for 1, 2 or 4 days.
Right panel: Western blot assessing SAHA efficiency (post-24h) at the level of histone acetylation.

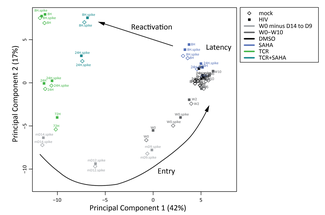
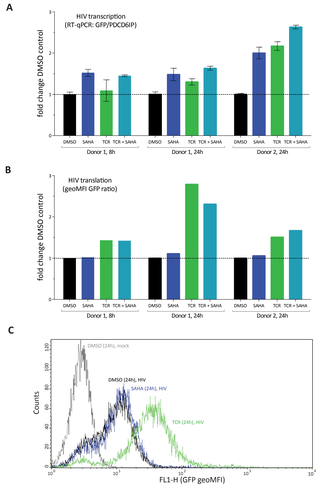


Figure 4. Principal component analysis of the latency and reactivation *in vitro* model.
Cellular samples prior to co-culture with H80 cells (mD14, mD12 and mD9 corresponding to cells collected 14, 12 and 9 days before W0 respectively), as well as cells treated with SAHA, TCR or a combination of TCR + SAHA. This set of CD4+ T cell transcriptomes recapitulates the steps of entry, latency and TCR reactivation. Samples inclued RNA spike-in controls (spike) to control for RNA content differences; normalization data using library size or RNA spike-in were similar.

RESULTS



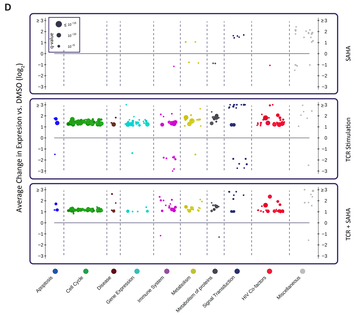


Figure 5. SAHA fails to provide cellular environment necessary for completion of HIV life cycle.
Panel A: Analysis of HIV transcription upon exposure to different stimuli (8h, 24h). Analysis was performed by RT-qPCR (GFP/PDCD6IP) on two different donors and normalized by the DMSO control.
Panel B: Analyis of HIV translation upon exposure to different stimuli (8h, 24h). Analysis was performed by FACS (geoMFI of GFP ratio over mock) on two different donors and normalized by DMSO.
Panel C: Histogram plot of GFP intensity of representative samples: uninfected controls (W10 + 24h DMSO, dotted grey line), latently infected cells upon 24h reactivation with DMSO (black solid line), SAHA (blue solid line) or TCR stimulation (green solid line). Data revealed a shift in intensity of TCR-stimulated cells but not SAHA-treated cells as compared to the corresponding DMSO control.
Panel D: Each panel summarizes over-represented pathways among the differentially expressed genes induced by SAHA (upper panel), TCR stimulation (middle panel), or TCR + SAHA (lower panel). Organized under ten major categories, each individual circle represents one enriched pathway in Reactome. The size is proportional to the adjusted p-value (q-value), and the y-axis corresponds to the average effect of the differentially expressed genes within the reported pathway. Only TCR stimulation using anti-CD3/anti-CD28/IL-2 leads to upregulation of numerous cellular pathways, as well as upregulation of reported HIV cofactors and HIV-related pathways (shown in red).

RESULTS

TCR + SAHA antagonism:

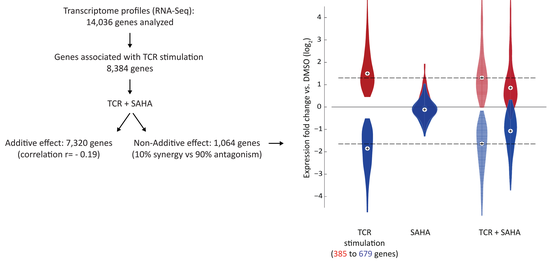


Figure 6. Administration of SAHA combined with TCR stimulation reveals anti-correlated responses.
Distributions of expression fold-change vs. DMSO for all 1064 TCR stimulation-associated genes that exhibit non additive response to SAHA co-administration (as identified by 2-way analysis of variance under 5% FDR). Genes are divided in two categories by their response to TCR stimulation (upregulated: red, downregulated: blue) and their response is followed under three different conditions. The dashed distribution shows the expected response to TCR and SAHA combination derived by adding up individual effects, and on its right side the observed response is plotted. The further shift of observed response toward the expression baseline demonstrates the dominance of the antagonistic interactions in response to SAHA and TCR stimulation combination treatment.

CONCLUSIONS

- SAHA fails to stimulate viral particle production from latently infected cells *ex vivo*.

In one primary cell model of HIV latency:
- SAHA is characterized by an increase in viral transcripts at both 8h and 24h post-treatment.
- TCR stimulation is also characterized by an increase in viral transcripts, although mostly at 24h.
- In contrast to TCR stimulation, SAHA fails to stimulate viral protein expression, highlighting potential post-transcriptional blocks.
- In contrast to TCR stimulation, SAHA does not seem to provide a cellular environment necessary for completion of HIV life cycle.
- The combination of TCR + SAHA results in a negative interaction (antagonistics effects).
- The combination of TCR + SAHA may fail to improve further translation of viral transcripts.