ACCELERATED AGING AND OXIDATIVE STRESS DISTINGUISH HIV FROM NON-HIV SMALL AIRWAY EPITHELIAL CELLS

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Introduction

- There is an increased prevalence of chronic obstructive pulmonary disease (COPD) in patients with human immunodeficiency virus (HIV).[1]
- This increased prevalence does not appear to be due solely to cigarette smoke exposure and suggests an accelerated aging phenomenon. The molecular underpinnings of this accelerated aging hypothesis have yet to be determined.
- Investigation of DNA methylation and gene expression profiles may reveal how the small airways of HIV-infected individuals differ from those of HIV-uninfected individuals.

Hypothesis

The small airway epithelial cells of HIV-infected individuals demonstrate methylomic and transcriptomic signatures of accelerated aging when compared to those of HIV-uninfected individuals.

Methods & Materials

- *Patient populations:* HIV-infected individuals undergoing bronchoscopy for clinical indications (i.e. lung nodules, pneumonia, or bronchiectasis) were matched to HIV-uninfected individuals undergoing bronchoscopy for lung cancer screening by age and smoking status.
- *Sample acquisition:* Airway epithelial cells were collected from both HIV and non-HIV patients via bronchoscopic brushings. DNA was extracted from epithelial cells and then bisulfite converted using the EZ DNA Methylation Kit (Zymo). RNA was extracted from epithelial cells using the miRNAeasy MiniKit (Qiagen).
- *DNA Methylation and Gene Expression Profiling:* DNA methylation profiles were obtained using the Illumina Infinium 450K Human Methylation array. Gene expression profiles were obtained using the Affymetrix GeneChip® Human Gene 2.0 ST array.
- *DNA Methylation Analysis:* Methylation status at a CpG site is expressed as a β-value:

$$\beta\text{-value} = \frac{\text{Average signal of the methylated probe}}{\text{Total average signal of both methylated and unmethylated probes}}$$

A probe was considered hypermethylated or hypomethylated if 1) the average difference in β-values between HIV and non-HIV was ≥0.15 or ≤-0.15 and 2) a non-parametric Mann-Whitney U test p-value ≤0.05 after correcting for multiple comparisons using the Benjamini-Hochberg method. Genes associated with senescence-associated secretory phenotype (SASP) and senescence-associated inflammatory response (SIR) were preferentially assessed.[2]

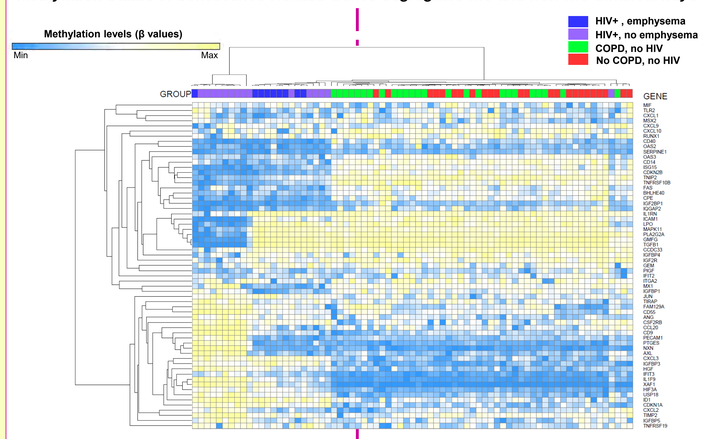
- *DNA Methylation Age:* A methylation age was calculated using methods from Horvath.[3] The methylation age is a weighted average of the regression coefficients of the 353 significant CpG sites generated by regressing methylation status on chronologic age. The age acceleration residual, the difference between the observed methylation age and the predicted age derived from the regression line of the methylation age on chronologic age is the measure of age acceleration, with greater positivity in the residual indicative of faster age acceleration.
- *DNA Methylation and Gene Expression Integration*: Genes considered differentially methylated were aligned to expression levels that had a permutation p-value <0.05 and an average fold change of >1.2 or <0.8. Genes considered hypomethylated and overexpressed or conversely hypermethylated and underexpressed were retained for enrichment analysis using Ingenuity Pathway Analysis®.
- *Statistical Software:* Analyses were performed using R.

Results

Demographic Characteristics of Study Cohort

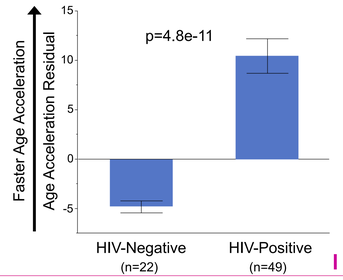
Parameter	HIV+ (n=22)	HIV- (n=49)	p-value
Age (years)	58.36 ± 9.34	61.93 ± 6.60	0.115
Male Sex (%)	81%	53%	0.021
Smoking Status Current (%) Former (%) Never (%)	 50.0% 40.9% 9.1%	 44.9% 55.1% 0%	0.060
Smoking Pack-Years	30.69 ± 32.36	46.54 ± 13.60	0.040
Caucasian Ethnicity (%)	95.5%	98.0%	0.555
FEV1/FVC (%)	68.94 ± 13.27	68 ± 15.03	0.810
Viral Load Detectable	31.82%	N/A	------
CD4 count (cells/mm^3)	427.27 ± 294.75	N/A	------

Methylation Status of Senescence-Related Genes Segregates HIV and Non-HIV Small Airways

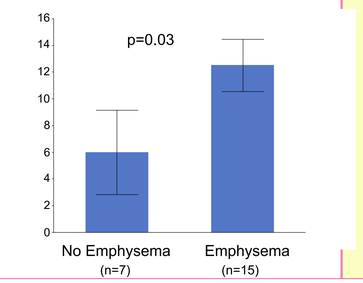


Advanced Methylomic Age in HIV and HIV-Associated Emphysema

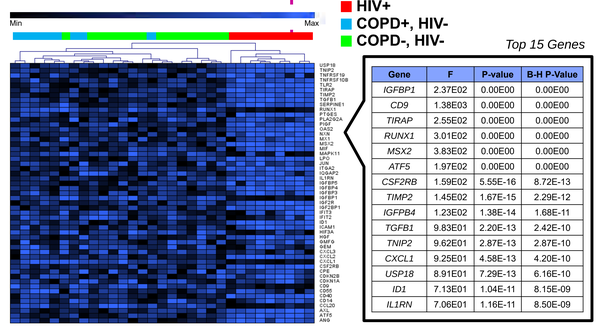
HIV vs. Non-HIV



Emphysema vs. Non-Emphysema, HIV-Only



Senescence-Related Gene Expression Segregates HIV and Non-HIV Small Airways



Top 15 Genes

Gene	F	P-value	B-H P-Value
IGFBP1	2.37E02	0.00E00	0.00E00
CD9	1.38E03	0.00E00	0.00E00
TIRAP	2.55E02	0.00E00	0.00E00
RUNX1	3.01E02	0.00E00	0.00E00
MSX2	3.83E02	0.00E00	0.00E00
ATF5	1.97E02	0.00E00	0.00E00
CSF2RB	1.59E02	5.55E-16	8.72E-13
TIMP2	1.45E02	1.67E-15	2.29E-12
IGFPB4	1.23E02	1.38E-14	1.68E-11
TGFB1	9.83E01	2.20E-13	2.42E-10
TNIP2	9.62E01	2.87E-13	2.87E-10
CXCL1	9.25E01	4.58E-13	4.20E-10
USP18	8.91E01	7.29E-13	6.16E-10
ID1	7.13E01	1.04E-11	8.15E-09
IL1RN	7.06E01	1.16E-11	8.50E-09

Oxidative Stress Response Pathways Highly Enriched In HIV Small Airways After Integration of DNA Methylation and Expression Profiles

Conclusions

- Small airway epithelial cells from HIV-infected patients have distinct DNA methylation and gene expression patterns from HIV-uninfected patients.
- These patterns are particularly enriched for impaired oxidative stress response and age acceleration, two pathways which may contribute to the increased prevalence of COPD in HIV.
- Extension of these findings by evaluating disease-specific DNA methylation and gene expression airway epithelial cell patterns is warranted for future study.

References

1. Crothers K, Huang L, Goulet JL, *et al.* HIV infection and risk for incident pulmonary diseases in the combination antiretroviral therapy era. Am J Respir Crit Care Med 2011;183(3):388-95.
2. Pribluda A, Elyada E, Wiener Z, *et al.* A senescence-inflammatory switch from cancer-inhibitory to cancer-promoting mechanism. Cancer Cell 2013;24(2):242-56.
3. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol 2013;14(1):R115.

ACKNOWLEDGEMENTS: Funding for this study was provided by the Canadian Institutes of Health Research, the National Institutes of Health, the Terry Fox Research Institute, and the Canadian Partnership Against Cancer.