Immune Gene Expression in Anal Dysplastic Lesions by HIV Status and Ablation Outcomes

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INTRODUCTION

- Human papilloma virus (HPV) associated high-grade squamous intraepithelial lesions (HSIL, AIN 2/3) are prevalent in HIV infected (HIV+) patients and are precursors to invasive carcinoma.
- There has been limited study of the interactions between HIV infection, the immune microenvironment of HSIL, and their natural history

OBJECTIVE

- To compare gene expression profiles in HSIL lesions by HIV status and to identify genes associated with post-ablation recurrence

METHODS

Population

- 44 patients from the anal cancer screening program at the Mount Sinai Health System, diverse in terms of gender, race/ethnicity, and sexual behavior
- 24 HIV+ and 20 HIV- samples were included in our analysis

Gene Expression Analysis

- The Nanostring nCounter assay was used to multiplex ~800 genes with mRNA transcript levels measured for each of 44 samples.
- The Nanostring immunooncology gene expression panel was used to quantify the RNA expression.
- Data was normalized with the NanoStringDiff R package.
- Normalized data was analyzed with likelihood ratio testing with corrections for multiple comparisons using the procedure of Benjamini and Hochberg
- Two comparisons were made: 1) by HIV status; 2) by HSIL outcome (persist vs. cure)

Confirmatory and Multiplex Immunohistochemistry (IHC)

- IHC of differentially expressed genes by HIV status was performed
- Additional samples were stained for CD4, CD8, PD-1, PDL-1 and quantitative density of target fluorophores were calculated.
- These data were analyzed for statistically significant differences, comparing HSIL images to benign anal tissue.

RESULTS

Table 1. Cohort Characteristics

	HIV (-)	HIV (+)
Samples	20	24
Median Age*	46	46.5
% Men Who Have Sex With Men	90%	96%
% Virally Suppressed	N/A	71%
CD4 per mm^3	N/A	749

Table 2. Gene Expression Fold Change by HIV Status

Gene	Fold-Change (HIV-/HIV+)	Q-value
CCL27	38.6	1.1E-4

Table 3. Gene Expression Fold Change by Post-Ablation Lesion Status

Gene	Fold-Change (Persist/Cure)	Q-value
HAMP	0.13	0.034
IFI35	0.37	0.019
IFNL1	5.10	0.0003
IL4	0.66	0.034
IRF5	1.76	0.037
MAP3K5	10.03	0.034
MAPK11	19.05	0.025
MR1	1.27	0.034
PASD1	0.59	0.035
PLA2G1B	0.47	0.0002
PRAME	36.91	0.012
PRKCE	0.58	0.019
TRAF3	3.78	0.001
ZNF205	1.22	0.011

Gene	Fold-Change (Persist/Cure)	Q-value
ADA	3.66	0.030
ARG1	249.18	<0.001
CAMP	3.38	0.019
CCL2	2.00	0.042
CD1A	10.07	0.034
CD1B	50.47	0.011
CD1E	5.56	0.016
CD207	252.25	0.043
CD3EAP	1.97	0.019
CREBBP	2.54	0.011
FADD	14.57	0.022
FUT7	21.83	0.029
GATA3	12.90	0.001

- Genes associated with recurrence were involved with T cell proliferation, localization, and antigen presentation.
- IL4, a Th2 specific gene, was significantly more expressed in lesions that did not recur or persist after electrocautery ablation

Figure 1. CCL27 IHC on HIV- and HIV+ AIN 3

CCL27 is a cutaneous cytokine associated with attracting T-cells to squamous epithelium during inflammatory responses

Positive nuclear staining, Human CCL27 Polyclonal Antibody (R&D Catalog # AF376)

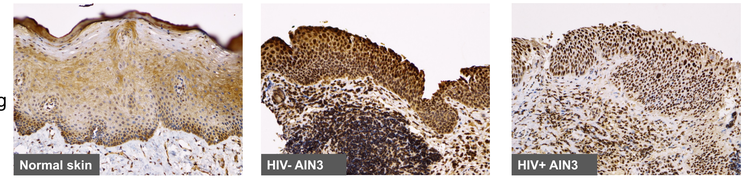
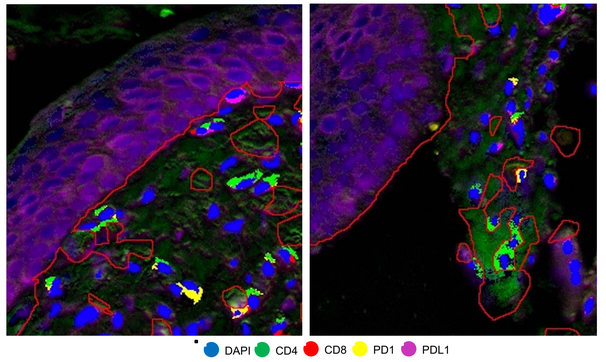


Figure 2. Preliminary Multiplex Immunohistochemistry Results for HIV- Uninfected Samples

- Preliminary data includes 9 HIV- samples (3 benign, 6 dysplastic
- PD-1 density in higher in stroma for HSIL versus benign tissue (p=0.01)
- PD-1 more prevalent within stromal CD4+ T cells of HSIL compared to benign tissue (p=0.009)

Immunohistochemistry of HIV- HSIL



CONCLUSIONS

- We found significant overlap in the host immune response, as measured by gene transcription, in anal HSIL by HIV status (only 1 gene was differentially expressed)
- HSILs that persisted after ablation showed different expression of 27 genes associated with host immune response at baseline when compared to HSILs that did not persist/recur

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