TRANSCRIPTOME ANALYSIS IN HPV+/HIV+ TISSUE REVEALS MARKERS OF HPV-DEPENDENT DYSPLASIA

#0277

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ABSTRACT

Background

HPV is accepted today as the necessary but not sufficient etiological agent for anal and cervical neoplasia and HIV appears to be a cofactor in the association between HPV and cervical neoplasia. The objective of our study is the identification of a distinct transcriptomic signature that may serve as predictive markers of dysplasia and/or malignancy in HIV+/+HPV patients.

Method

The study includes a unique cohort of 25 HIV-1 infected individuals co-infected with HPV with a clinical follow-up for more than 20 years. Tissue samples from individuals with signs of a high degree of anal dysplasia were chirurgical collected, together with samples from normal tissue from the same individual as control. After RNA extraction and quality control, RNA library was constructed (Illumina TruSeq RNA stranded) and sequencing was performed (Novaseq, 30M reads/sample). Data analysis was performed as implemented in the computational workflow for the detection of differentially expressed genes and pathways from RNA-seq data. Read alignment and count quantification was conducted using the Rsubread package and the statistical analysis was performed using the edgeR package. The differential expression analysis uses the quasi-likelihood functionality of edgeR.

Results

Whole transcriptome sequencing was performed to examine differential gene expression profiles, and to perform gene annotation based on gene ontology pathway information. Analyses were successfully performed on all 25 paired-ends samples with overall read mapping ratio above 95%. Thirty genes showed significant changes between biopsies showing a high degree of dysplasia and apparently healthy control biopsies. Hierarchic clustering of data demonstrated a clear discrimination between healthy and dysplasic tissues, indicating a common pattern of gene expression changes between individuals. The identified differentially expressed genes include chemokines, potential restriction factors, a miRNA and genes associated to cell proliferation and cell transformation. After filtering the results based on functionality, we selected for further validation a group of 15 genes that fulfill the criteria for becoming a biomarker.

Conclusion

Our analysis allowed the identification of at least 15 potential predictive markers of anal dysplasia in co-infected HIV/HPV individuals. Characterization of the selected genes may result in the development of new therapeutic approaches to treat HIV/HPV induced malignancies.

EXPERIMENTAL METHODS AND WORKFLOW

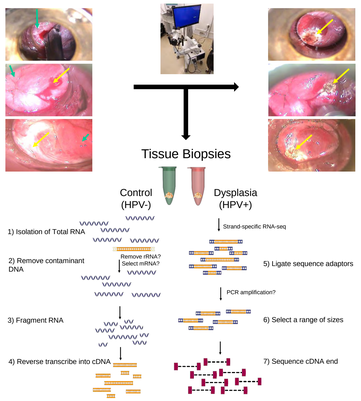


Fig 1. Sample collection and processing for RNAseq analysis. (A) HIV+ Individuals with anal intraepithelial dysplasia were identified and enrolled in the study. Prior to infrared surgery treatment (IFRS), directed biopsy was performed to collect affected tissue (HPV+, yellow arrows) and non-affected tissue (green arrows) from the contralateral side of the each. Biopsies were collected into RNAlater and stored until analysis. (B) Schematic representation of sample processing, sequencing protocol and analysis.

HIERARCHICAL CLUSTERING ANALYSIS OF GENE EXPRESSION IN DYSPLASIA vs NORMAL BIOPSIES FROM HIV+/HPV+ INDIVIDUALS

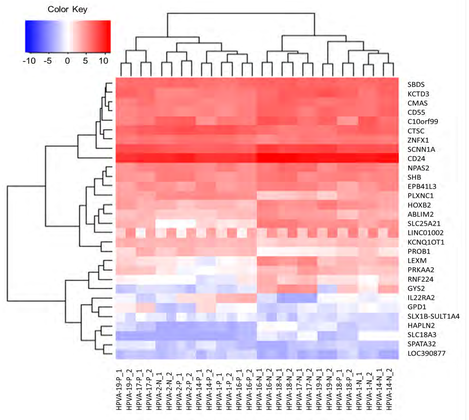


Fig 2. Heat map of differentially expressed genes in tissue biopsies from dysplasia (HPV+) *vs* control (HPV-) biopsies from co-infected HIV+/HPV+ individuals. Heatmap shows result of hierarchical clustering analysis (Euclidean Method, Complete Linkage) which clusters the similarity of genes and samples by expression level (normalized value) from significant list. *HPVA-X-P dysplasia; HPVA-X-N normal control.

KEGG ENRICHMENT ANALYSIS

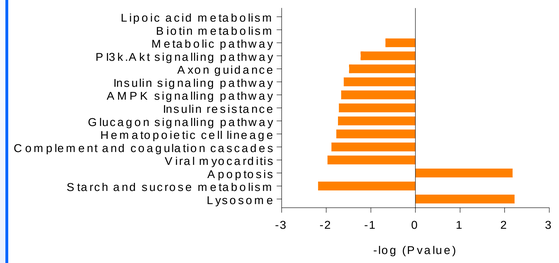


Fig 4. Identification of differential expression of molecular pathways in dysplasia vs normal biopsies from HIV+/HPV+ individuals. Results of the KEGG pathway analysis for the differentially expressed mRNAs showing the most significant KEGG pathways upregulated or downregulated. The length of the column in the figure represents the enrichment score of the each pathway.

GENE EXPRESSION OF IMMUNE RELATED GENES IN DYSPLASIA vs NORMAL BIOPSIES

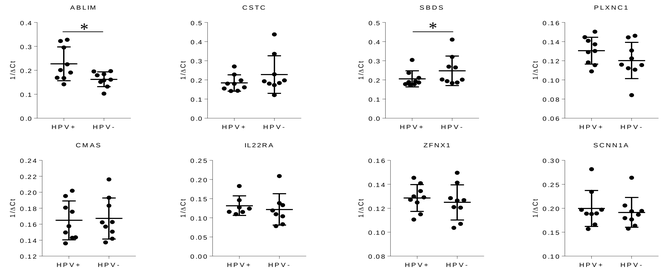


Fig 5. Determination of gene expression (mRNA) of candidate genes by qPCR. A subgroup genes identified by the RNAseq array were selected according to their role in immune function, complement system regulation, cell proliferation/transformation and cell-to-cell signaling/interactions. N=9, Mean±SD; Paried t-Test * p<0.05.
ABLIM LIM zinc-binding domain-containing protein; **CD55** Decay-accelerating factor; **CTSC** cathepsin C; **CMAS** cytidine monophosphate N-acetylneuraminic acid synthetase; **PLXNC1** plexin C1; **SBDS** ribosome maturation factor; **SCNN1A** sodium channel epithelial 1 alpha subunit; **IL22RA** interleukin 22 receptor subunit alpha; **ZFNX1** zinc finger NFX1-type containing 1.

CONCLUSIONS

- We have identified a number of factors that have the potential to be used as biomarkers of HPV-associated dysplasia and early detection of disease.
- Characterization of the role of selected genes may result in the development of new diagnostics and/or therapeutic approaches to treat HPV-induced malignancies.

COHORT OF HIV+/HPV+ CO-INFECTED INDIVIDUALS

Identifier	CD4 count (cells/µL)	Viral Load (copies/mL)	ART	Proctology Follow-up (years)	Treatment Dysplasia
HPVA-01	677	<40	ELV/C/E/TAF	5	IFRS
HPVA-02	391	<40	DRV/C/TRU	3	IFRS
HPVA-03	422	<40	DRV800/DTG/RTV	14	IFRS
HPVA-04	1244	69	DRV/C	12	IFRS
HPVA-05	93	<40	DTG/ABC/3TC	11	IFRS
HPVA-06	669	49	DTG/RPV	4	IFRS
HPVA-07	975	<40	DRV/C	6	IFRS
HPVA-08	538	<40	DTG/ABC/3TC	3	IFRS
HPVA-09	396	<40	ELV/C/F/TAF	3	IFRS
HPVA-10	782	4332	-	5	IFRS
HPVA-11	372	<40	ABC/3TC/LPV/RTVmeltrex	9	IFRS
HPVA-12	849	<40	DRV/C	9	IFRS
HPVA-13	756	<40	DTG/ABC/3TC	2	IFRS
HPVA-14	510	<40	ELV/C/E/TAF	2	IFRS
HPVA-15	466	<40	ABC/3TC/NVP	3	IFRS
HPVA-16	758	<40	ELV/C/E/TAF	2	IFRS
HPVA-17	1080	<40	ABC/3TC/RAL	4	IFRS
HPVA-18	1200	<40	DTG/ABC/3TC	11	IFRS
HPVA-19	439	<40	ELV/C/E/TDF	4	IFRS
HPVA-20	503	<40	ATV30/RTV /TRU	9	IFRS

Identifier	CD4 count (cells/µL)	Viral Load (copies/mL)	ART	Proctology Follow-up (years)	Treatment Dysplasia
HPVA-20	687	<40	RIL/FTC/TDF	8	IFRS
HPVA-21	1085	<40	DTG/ABC/3TC	2	IFRS
HPVA-22	540	<40	DTG/ABC/3TC	5	IFRS
HPVA-23	1061	<40	DRV/C	8	IFRS
HPVA-24	508	123	DRV/C	7	IFRS
HPVA-25	918	<40	DTG/ABC/3TC	11	IFRS
HPVA-26	815	<40	RAL/TRU	5	IFRS
HPVA-27	718	<40	ELV/C/E/TAF	7	IFRS

Table 1. Immunological and virological characteristics of patients enrolled in the study at the time of cell sample collection. Patients enrolled for RNAseq array study (Left panel) and patients enrolled for the gene validation study (right panel).
*ELV elvitagravir; TDF tenofovir; TAF tenofovir alafenamide; C cobicistat; DRV darunavir; DRV/C cobicistat-boosted darunavir; ABV abacavir; 3TC lamivudine; RAL raltegravir; NVP nevirapine; DTG dolutegravir; FTC emtricitabine; RIL rilpivirine; TRU truvada; LPV lopinavir; ATV30 atazanavir; E emtricitabine.

GENE ONTOLOGY OF DIFFERENTIALLY EXPRESSED GENES IN DYSPLASIA *vs* NORMAL BIOPSIES FROM HIV+/HPV+ PATIENTS

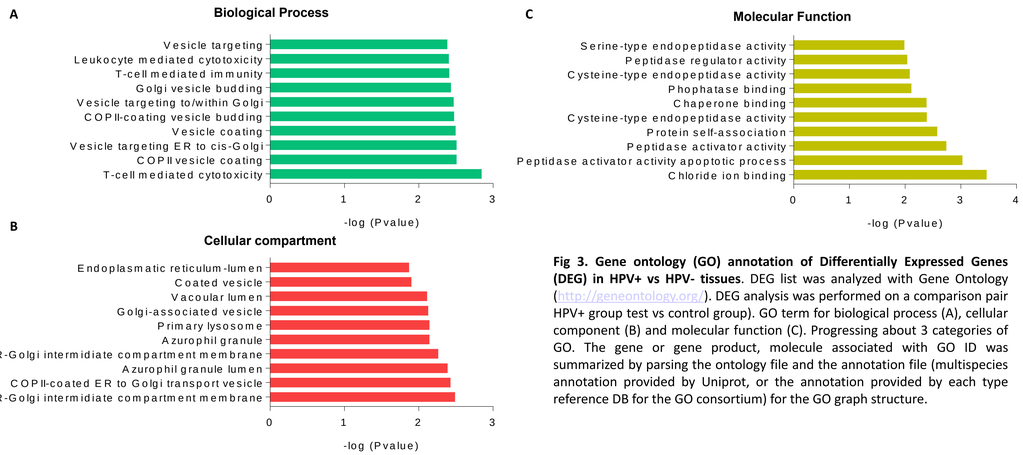


Fig 3. Gene ontology (GO) annotation of Differentially Expressed Genes (DEG) in HPV+ vs HPV- tissues. DEG list was analyzed with Gene Ontology (http://geneontology.org/). DEG analysis was performed on a comparison pair HPV+ group test vs control group). GO term for biological process (A), cellular component (B) and molecular function (C). Progressing about 3 categories of GO. The gene or gene product, molecule associated with GO ID was summarized by parsing the ontology file and the annotation file (multispecies annotation provided by Uniprot, or the annotation provided by each type reference DB for the GO consortium) for the GO graph structure.

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