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Transcriptional profiles in CD8+ T cells from HIV+ progressors on HAART are characterized by coordinated up-regulation of oxidative phosphorylation enzymes and interferon responses

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ABSTRACT

The functional impairment and numerical decline of CD8+ T cells during HIV infection has a profound effect on disease progression, but only limited microarray studies have used CD8+ T cells. To understand the interactions of HIV and host CD8+ T cells at different disease status, we used the Illumina Human-6 BeadChips to evaluate the transcriptional profile (>48,000 transcripts) in primary CD8+ T cells from HIV+ therapy-naive non-progressors and therapy-experienced progressors. 68 differentially expressed genes were identified, of which 6 have been reported in HIV context, while others are associated with biological functions relevant to HIV pathogenesis. By GSEA, the coordinated up-regulation of oxidative phosphorylation enzymes and interferon responses were detected as fingerprints in HIV progressors on HAART, whereas LTNP displayed a transcriptional signature of coordinated up-regulation of components of MAPK and cytotoxicty pathways. These results will provide biological insights into natural control of HIV versus HIV control under HAART.

Introduction

HIV infects all major blood cell types and distinct HIV-1 strains evolve independently in different blood leukocytes *in vivo* (Potter et al., 2004, 2006). The interactions between HIV and different blood cell types remain unclear and studying the subversion of human gene machinery by HIV in different cell types may help to answer the above question. Among different blood cell types, the paramount role of CD8+ T cells in HIV pathogenesis and their importance in secreting CD8 antiviral factor are well known (Mackewicz et al., 1995; Tsubota et al., 1989; Walker et al., 1989). During acute infection, the expansion of HIV-specific CD8+ T cells is associated with a decline in viremia (Koup et al., 1994; Pantaleo et al., 1994). Despite their antiviral activity, CD8+ T cells can also be productively infected by HIV (Imlach et al., 2001; McBreen et al., 2001; Saha et al., 2001). HIV-infected, therapy-naive, long-term non-progressors (LTNP) usually maintain highly functional CD8+ T cells, whereas HIV progressors display impaired CD8+ T cell functions (Betts et al., 2006; Migueles et al., 2002). Although the multiple roles of CD8+ T cells during HIV infection are well characterized, the genetic basis of interactions between host CD8+ T cells and HIV in relation to disease stages remain poorly understood.

Previous gene microarray studies in relation to HIV disease have used whole PBMC or cell lines, monocytes, macrophages, CD4+ T cells, lymphoid and gut tissue, etc (Giri et al., 2006; Ryo et al., 1999), but only a few studies have used CD8+ T cells to understand gene regulation during HIV infection. These include studies using CD8+ T cell gene expression profiling to detect genes responsible for non-cytotoxic activity of CD8+ T cells (Diaz et al., 2003; Martinez-Marino et al., 2007); the study by Martinez-Marino et al. identified 52

Abbreviations: HAART, highly active antiretroviral therapy; NEG, HIV sero-negative individuals; VIR, viremic patients; LTNP, long-term non-progressor; GO, gene ontology; GSEA, gene set enrichment analysis; FDR, false discovery rate; OXPHOS, oxidative phosphorylation; NRTI, nucleoside reverse transcriptase inhibitors; AZT, zidovudine; ES, enrichment score; NES, normalized enrichment score; ISG, interferon stimulated genes; CTL, cytotoxic T lymphocyte.

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[4] Contributed to the writing.
[5] Designed the research project, supervised this work and contributed to the writing.

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differentially expressed genes between infected subjects with high CD8+ cell non-cytotoxic anti-HIV responses and uninfected controls that lack this activity. Recently, the transcriptional profiling of CD4+ and CD8+ T cells from early infection, chronic infection and LTNP patients have been studied and interferon responses were characterized as a transcriptional signature of T cells from early and chronic HIV infected individuals (Hyrcza et al., 2007). It has also been shown that CD8+ T cells contain more differential genes than CD4+ T cells, which is consistent with our recent antibody microarray study illustrating that CD8+ T cells have more cell surface molecules differentiating disease status then CD4+ T cells (Wu et al., 2007). Despite the aforementioned microarray studies on CD8+ T cells, a clear and comprehensive comparison at the transcriptional level on human genome scale between primary CD8+ T cells from therapy-naive LTNPs and HIV progressors on HAART is lacking.

Using Illumina Human-6 v2 Expression BeadChips, we have compared expression profiles of the whole human genome (>48,000 gene transcripts) in *ex vivo*-derived CD8+ T cells from HIV+ therapy-naive LTNP and viremic (VIR) patients under HAART to identify transcriptional features related to HIV disease stages and HAART effect. The comprehensive approach of profiling whole human genome in these two HIV+ patient groups has provided new insights into virus-host interactions at the CD8+ T cell transcriptome level in relation to disease stages and antiretroviral therapy. This will facilitate the understanding of HIV pathogenesis and HAART effects.

Results

Cluster and differential gene analysis

CD8+ T cell-derived total cellular RNA from 9 HIV+ patients (4 LTNP and 5 VIR, Table 1) and 5 HIV sero-negative (NEG) healthy individuals were hybridized to the Sentrix Human-6 v2 Expression BeadChip. The data were subjected to gene expression analysis using software BeadStudio v3. To determine the similarity of global expression between samples, hierarchical clustering was performed based on the Pearson correlation metric and Correlation agglomeration method implemented in BeadStudio. Cluster analysis revealed that the VIR group formed an independent cluster away from LTNP and NEG groups (Fig. 1). Differential expression analysis between LTNP and NEG showed limited differences (7 differentially expressed genes, data not shown), which is consistent with a previous study showing lack of differences of gene expression profiles between CD8+ T cells from HIV non-progressors and negative controls (Hyrcza et al., 2007). Therefore, our subsequent rationale was to analyze differential gene expression in CD8+ T cells derived from VIR and LTNP groups.

Differential expression analysis between LTNP and VIR groups revealed a candidate list of 68 genes. Each differential gene had a detection p value <0.01 (indicating that the transcript was detected) in at least one group, with a differential score of <−20 or >20

Table 1
Patient clinical details of viral load, CD4+ and CD8+ T cell counts[a]

Patient	Disease group	Age	Viral load (copies/ml)	CD4 counts (cells/μl)	CD8 counts (cells/μl)	RNA integrity number
V1	VIR	55	334	282	2281	7.0
V2	VIR	39	2130	296	905	8.8
V3	VIR	40	4940	387	1239	8.9
V4	VIR	44	668,000	86	335	9.0
V5	VIR	61	713,000	40	70	9.3
L1	LTNP	59	<50	630	579	9.1
L2	LTNP	51	<50	714	476	8.5
L3	LTNP	79	<50	920	900	9.3
L4	LTNP	33	57	780	900	9.7

[a] Plasma viral load was measured using the Quantiplex HIV RNA3.0 (Chiron bDNA) assay with a lower limit of detection of 50 HIV-1 copies/ml (Chiron Diagnostics, Halstead, United Kingdom).

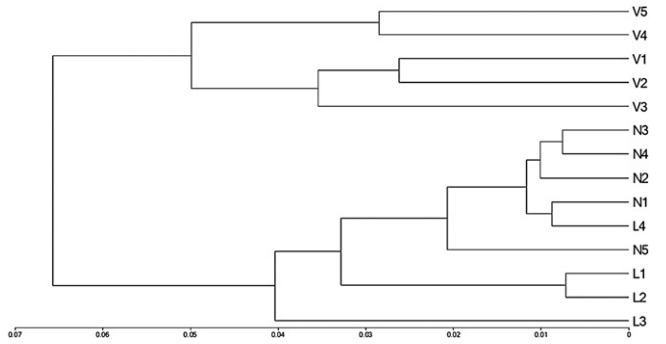

Fig. 1. Clustering analysis of global gene expression profiles from CD8+ T cells. Similarities in the gene expression patterns among individuals were evaluated and visualized with BeadStudio v3 Cluster Analysis tool. The algorithm used is named Correlation, which computes the Pearson correlation using a 1-r distance measure. The distance on the X axis represents the similarity relationships among samples.

corresponding to significance level of $p<0.01$ (down-regulation or up-regulation, respectively) and fold change >2.7. When compared to the LTNP group, 57 genes were up-regulated and 11 down-regulated in the VIR group.

For annotation of these 68 differentially expressed genes, the Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/) (Huangda et al., 2007) was used as well as exhaustive searches in the PubMed literature (http://www.ncbi.nlm.nih.gov/Literature/). These 68 genes were assigned to eight categories according to biological functions relevant to HIV pathogenesis as follows: (1) genes previously reported to be involved in HIV infection and disease (6/68); (2) Interferon induced, apoptosis and actin-related genes (11/68); (3) cell cycle, proliferation and activation genes (5/68); (4) adhesion and cell surface molecules (6/68); (5) endosome, lysosome and cytotoxicity (6/68); (6) mitochondria, lipid and carbohydrate metabolism associated genes (8/68); (7) genes lacking relevance to HIV pathogenesis (13/68) and (8) less characterized genes (13/68) (Table 2).

To confirm the differential expression of genes from Illumina microarray, mRNA expression levels of 18% of the randomly selected above genes were measured by quantitative PCR (Table 3). The mRNA from the CD8+T cells of the same patient at the same time point was used for qPCR analysis. The fold changes evaluated by qPCR were generally consistent with the results from microarray except for genes SLC25A20 and PYCARD, which repeatedly failed to be quantified by qPCR.

L2L analysis

To facilitate our understanding of the biological implications of these 68 differential genes, we compared our gene list containing these 68 differential genes to 953 differential gene lists derived from 197 published microarray data as well as 3 additional gene ontology (GO) lists (biological process, cell component and molecule function) using L2L microarray analysis tool (Newman and Weiner, 2005). Detection of significant overlap between lists may indicate the inclusion of genes with one or more similar biological functions defined in previously published studies.

For genes up-regulated in the VIR group, several relevant themes emerged from the L2L analysis. These include (1) interferon and TGFβ responses and apoptotic pathways involving JAK-STAT and caspase activation, (2) genes encoding mitochondrion components and (3) endosome and lysomsome transport (Table 4). Although the biological meanings derived from these lists are relevant to HIV pathogenesis and the p values are highly significant, the number of the actual overlapping genes is only around 2–3 in the majority of list comparisons, which may limit the derivation of the biological pathway or mechanism involved. Thus, we attempted a complementary approach using Gene Set Enrichment Analysis (GSEA) (Subramanian