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Differential gene expression profile of CD4+/CD8+ T cells in patients with hepatitis C virus and/or human immunodeficiency virus infection

L Yi, J Zhao, J Lu, Y Chen, D Huang, YF Li, L Chen, ZR Yang, HF Kung, ML He *

KEY MESSAGES

1. Coinfection of a host organism with hepatitis C virus (HCV) and human immunodeficiency virus (HIV) alters the course of infection of each virus.
2. Treatment-naive HIV/HCV mono-/co-infected patients with CD4+ T cell count >300/μL were recruited, and the gene expression profile of their CD8+ and CD4+ T cells was investigated by microarray assay, bioinformatics analysis, and quantitative real time PCR validation.
3. Genes involved in cell proliferation, activation, differentiation, and regulation and cytokine responses were significantly altered in CD8+ T cells. In CD4+ T cells, innate immune response, cell cycle regulation, GPCR signalling pathway, transcriptional regulation, and metabolic pathways were significantly affected by HIV/HCV co-infection, compared with HCV mono-infection.
4. These findings offer new insight into disease progression in HIV/HCV co-infection, and may help to identify new markers for its management.

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Introduction

Co-infection with hepatitis C virus (HCV) and human immunodeficiency virus (HIV) is common because of their similar routes of transmission (eg drug injection).[1] HIV co-infection significantly affects the natural history of hepatic fibrosis in HCV-infected persons.[2] Concomitant HIV infection increases the evolution of HCV quasispecies, the level of viraemia, and the extrahepatic viral reservoirs.[3] HIV viral load displays additive effects on HCV-triggered mitochondrial DNA depletion that may be associated with nucleoside analogue toxicity.[4] Although highly active antiretroviral treatment is effective for treating HIV mono-infection, its impact on HCV is controversial.[5] Co-infected patients have an increased risk of hepatotoxicity after such treatment.[6] Similarly, the toxicity of interferon-based HCV therapies is also exacerbated in HIV/HCV co-infected patients with a higher rate of relapse.[7] The underlying mechanisms of highly increased mortality remain elusive.

In this study, the gene expression profile of CD8+ and CD4+ T cells from treatment-naïve HIV/HCV mono- and co-infected individuals was analysed. Using gene set enrichment analysis, a network of enriched pathways related to the pathogenesis of disease progression in the co-infected patients was identified and confirmed by quantitative real-time PCR (qRT-PCR) assays. These findings offer new insight into the impact of co-infection at the gene transcription level.

Methods

This study was conducted from November 2009 to October 2011. A Chinese population with CD4+ count >300/μL was recruited from an ongoing voluntary-based HIV/AIDS surveillance study in Shenzhen, China from September 2009 to December 2010. The status of HCV, HIV, and HBV was strictly monitored according to the stringent guidelines of the Chinese Center for Disease Control and Prevention.

Fresh CD4+ and CD8+ T cells were isolated from 30 mL whole blood by microbead immunoselection according to the manufacturer's instructions (Miltenyi Biotec, Oslo, Norway). In each patient, equal amounts of CD4+ or CD8+ T cells were pooled together to form three biological replicas in each group. The remaining CD4+ or CD8+ T cells were separately stored at -80°C for validation. RNA was isolated using RNAeasy Total RNA Isolation Kit (Qiagen, Germany) and applied for microarray assays.

Transcriptomic analysis by microarray assays was performed using Affymetrix GeneChip Human Gene 1.0 ST Array (Affymetrix, Santa Clara [CA], USA). The images were acquired by the Affymetrix Scanner 3000 7G Plus and the CEL files were imported into the program Partek Genomic Suite

(version 6.4, Partek, St Louis [MO], USA) and the robust multi-chip average was normalised. Two-way ANOVA test was applied to identify differentially expressed genes (fold change >2 and adjusted P<0.05).

Gene set enrichment analysis (version 2.0, Broad Institute, Cambridge [MA], USA) was conducted. A nominal P value was calculated by permuting the genes 1000 times. Gene sets were collected from online databases such as Bio-Carta, React, and Kyoto Encyclopedia of Genes and Genomes (KEGG).

The qRT-PCR assay was carried out using ABI 7500 Real-Time PCR system with Power SYBR Green Master Mix (Applied Biosystems, Foster City [CA], USA).

Nonparametric test was used for pairwise comparisons. A P value of <0.05 was considered statistically significant.

Results

Differentially expressed genes in CD8+ T cells

A total of 28 869 genes were scanned. Of 110 transcript identifiers (IDs) differentially expressed in the HCV and HIV mono-infected groups, 81 were known genes (40 up-regulated, 41 down-regulated) and 29 were transcript IDs without known functions. Of these, 72 transcript IDs were differentially expressed in the HCV/HIV co-infected and the HCV mono-infected groups. The expression level of 47 of 55 genes in HCV/HIV co-infected group was lower than in the HCV mono-infected group. The Gene Ontology annotation assignment of these 55 differentially expressed genes was similar to those identified in HIV and HCV mono-infected groups. In the biological process category, biological regulation (64.15%), response to stimulus (49.06%), and metabolic process (47.17%) were the most dominant subcategories. In the molecular function and cellular component categories, cytokine binding, molecular transducer, and plasma membrane were significantly enriched. The KEGG pathways showed altered expression between the HCV/HIV co-infected and the HCV mono-infected groups including cytokine-cytokine receptor interaction, cell adhesion molecules, lysosome, chemokine signalling pathway, and antigen processing/presentation. In a Venn diagram for the gene expression relationship among the HIV/HCV mono-/co-infected groups (Fig), 18 IDs (17 genes, 1 transcript IDs) were shared between circles H and J and referred to genes that had ≥2-fold change in expression in both the HIV mono-infected and the HCV/HIV co-infected groups, compared with the HCV mono-infected group. In HIV mono-infection, 8 IDs (1 gene, 7 transcript IDs) were differentially expressed, compared with HCV mono-infection or HCV/HIV co-infection. Comparing the gene expression profile of the HIV/HCV co-infected group with the HIV or HCV mono-infected groups, 3 IDs (1 gene, 2 transcript IDs) displayed similar expression changes.

To confirm the observations from the microarray analysis, the mRNA level of the selected differentially expressed genes was measured using qRT-PCR. Blood samples from uninfected males with matched age were used as controls. Differentially expressed genes in the KEGG pathway of cytokine-cytokine receptor interaction were selected for qRT-PCR confirmation, as this pathway was enriched when comparing HIV with HCV mono-infection (R=9.06, 4 genes: CXCL16, TNFRSF9, CCR4 and CX3CR1) and HCV/HIV co-infection with HCV mono-infection (R=25, 8 genes: CXCL16, TNFRSF9, CCR4, CX3CR1, IL13RA1, IFNGR2, CD40LG, and PPBP). The mRNA isolated from CD8+ T cells was used for qRT-PCR experiments. The expression profile of 10 genes (CCR4, CD40LG, CX3CR1, CXCL16, IFNGR2, IL13RA1, EGR1, KLF4, GPR56, and TNFRSF9) exactly matched the observation from the microarray assays; another three genes (PPBP, OAS1, and P2RY13) matched at least one expression pattern out of three pairwise comparisons.

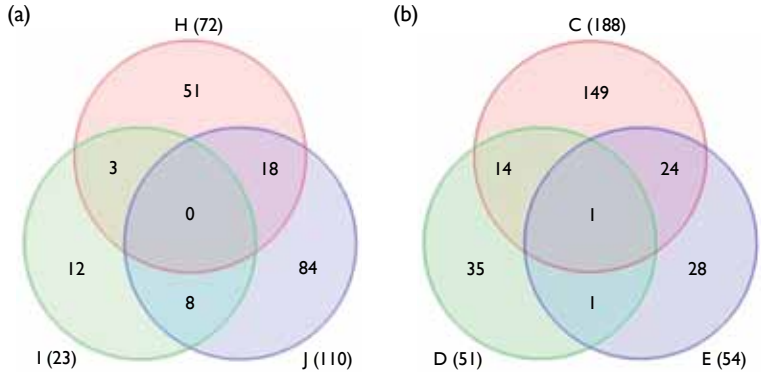

FIG. Venn diagram for differentially expressed transcript identifiers in (a) CD8+ T cells and (b) CD4+ T cells in paired comparisons of HCV-mono- versus HIV-mono-infection (circles J and E), HCV/HIV-co-infection versus HCV-mono-infection (circles H and C), and HCV/HIV-co-infection versus HIV-mono-infection (circles I and D)

Differentially expressed genes in CD4+ T cells

The number of differentially expressed transcript IDs in each comparison was identified. Of the 54 differentially expressed transcript IDs identified following comparison between HCV and HIV mono-infected groups, 24 showed significant matches with known genes involved in both immune system development and immune response (CD38, CXCL10, OAS3, FCGR3A, and IFI44L) and G-protein coupled receptor protein signalling pathway (P2RY13, GPR56, CX3CR1, and FFAR2). In HCV/HIV co-infection versus HCV mono-infection, 72 genes with known function were identified; 16 of them played important roles in stimulus response and the immune system process, including IL6,

FCGR1C, RSAD2, LILRA3, C1QC, IGSF6, IL1RN, and OAS3. In addition, genes regulating locomotor behaviour (such as CCRL2, FPR2, and IL8RA) also had differential expression profiles. Comparing HCV/HIV co-infection and HIV mono-infection, only seven genes were identified, including three encoding small nucleolar RNAs and four encoding AQP9, TNFAIP6, IL6, and PTX3. In a Venn diagram for the gene expression relationship among the HIV/HCV mono-/co-infected groups (Fig), only one cDNA clone was significantly differentially expressed among all three groups. The 24 IDs (8 cDNA clones and 16 genes, including IFIT1, FAM72D, SERPING1, FCGR3A, and SIAE) were found to have >2-fold change in expression in both the HIV mono-infected and the HCV/HIV co-infected groups, compared with the HCV mono-infected group. Comparing the gene expression profile of HIV/HCV co-infected patients with HIV or HCV mono-infected patients, 14 IDs (10 cDNA clones and 4 genes: SNORD116-6, SNORA38B, IL6, and PTX3) displayed similar expression changes.

Interestingly, the majority of gene sets up-regulated in HIV mono-infection also showed enrichment in HIV/HCV co-infection when compared with HCV mono-infection. Based on the biological functions, they could be mainly divided into cell cycle, immune response, and gene expression (regulation). In the cell cycle category, 32 of 88 and 18 of 67 gene sets were significantly up-regulated in the HIV mono-infected and HIV/HCV co-infected groups, respectively. The leading edge analysis revealed that the majority of them engaged in G1/S and G2/M transitions. Genes involved in the innate immune response, particularly in pathogen-associated molecular pattern recognition, showed increased expression in HIV mono-infection and contributed most to ES. In the HIV/HCV co-infected group, the most generally up-regulated gene sets were innate immune signalling including natural killer cell mediated cytotoxicity, toll-like receptor signalling pathways, NOD-like receptor signalling pathways, and complement activation.

In addition, the gene sets involved in signal transduction were important. Almost the entire gene set could be directly or indirectly associated with GPCR signalling. The mRNA levels from each paired comparison were randomly selected and validated by qRT-PCR.

Discussion

This study evaluated the global gene expression pattern of HCV/HIV co-infection against mono-infection in primary CD8+ and CD4+ T cells. Both HIV and HCV have evolved their own pathogenesis; cellular processes in the HIV/HCV mono-/co-infected patients were altered. This played a crucial role in cell proliferation/activation/differentiation; T cell regulation and cytokine responses were noted in CD8+ T cells. Gene sets involved in cell cycle progression, innate immune response, and some transcription factors in CD4+ T cells were affected mainly by HIV, whereas genes associated with extracellular matrix, beta cell development, and insulin synthesis and secretion were the major targets of HCV. Metabolic pathways appeared to be modulated by both viruses. In addition, the role of GPCR signalling pathway during HIV/HCV infection was revealed.

Future studies should validate the genes involved in T cell proliferation (such as TNFRSF9, CD160, CD38, CDCA7, CCNA2, and NUSAP1) and cytokine production that induces an innate immune response (such as MS4A1, CD40LG, and IL13RA1). As long as one gene is differentially expressed, the correlation between its expression level and disease status or progression should be investigated, as should its biological functions in cell culture or animal models using loss-of-function (such as RNA interference) and gain-of-function (such as lentiviral vector mediated gene transfer) studies. This will enable identification of biomarkers for better management of HCV/HIV co-infected patients and reveal new drug targets.

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