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Comparative transcriptome analysis of PBMC from HIV patients pre- and post-antiretroviral therapy

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ABSTRACT

Infections of the human immunodeficiency virus (HIV) trigger host immune responses, but the virus can destroy the immune system and cause acquired immune deficiency syndrome (AIDS). Highly active antiretroviral therapy (HAART) can suppress viral replication and restore the impaired immune function. To understand HIV interactions with host immune cells during HAART, the transcriptomes of peripheral blood mononuclear cells (PBMC) from HIV patients and HIV negative volunteers before and two weeks after HAART initiation were analyzed using RNA sequencing (RNA-Seq) technology. Differentially expressed genes (DEGs) in response to HAART were firstly identified for each individual, then common features were extracted by comparing DEGs among individuals and finally HIV-related DEGs were obtained by comparing DEGs between the HIV patients and HIV negative volunteers. To demonstrate the power of this approach, minimum numbers of patients (one HIV alone; one HIV+ tuberculosis, TB; one HIV+ TB with immune reconstitution inflammatory syndrome during HAART) and two HIV negative volunteers were used. More than 15,000 gene transcripts were detected in each individual sample. Fourteen HAART up-regulated and eleven down-regulated DEGs were specifically identified in the HIV patients. Among them, nine up-regulated (*CXCL1*, *S100P*, *AQP9*, *BASP1*, *MMP9*, *SOD2*, *LIMK2*, *IL1R2* and *BCL2A1*) and nine down-regulated DEGs (*CD160*, *CD244*, *CX3CR1*, *IFIT1*, *IFI27*, *IFI44*, *IFI44L*, *MX1* and *SIGLEC1*) have already been reported as relevant to HIV infections in the literature, which demonstrates the credibility of the method. The newly identified HIV-related genes (up-regulated: *ACSL1*, *GPR84*, *GPR97*, *ADM*, *LRG1*; down-regulated: *RASSF1*, *PATL2*) were empirically validated using qRT-PCR. The Gene Set Enrichment Analysis (GSEA) was also used to determine pathways significantly affected by HAART. GSEA further confirmed the HAART relevance of five genes (*ADM*, *AQP9*, *BASP1*, *IL1R2* and *MMP9*). The newly identified HIV-related genes, *ADM* (which encodes Adrenomedullin), a peptide hormone in circulation control, may contribute to HIV-associated hypertensions, providing new insights into HIV pathology and novel strategies for developing anti-HIV target. More importantly, we demonstrated that comparative transcriptome analysis is a very powerful tool to identify infection related DEGs using a very small number of samples. This approach could be easily applied to improve the understanding of pathogen-host interactions in many infections and anti-infection treatments.

1. Introduction

Human immunodeficiency virus-1 (HIV-1) is a retrovirus that primarily infects components of the human immune system, such as CD4+ T cells, macrophages and dendritic cells (Clapham and McKnight, 2001). HIV directly and indirectly destroys CD4+ T cells, which leads to severe immunodeficiency and increased susceptibility to opportunistic infections in most infected patients (Veazey et al., 1998). In addition, HIV also induces chronic immune activation,

Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; HAART, highly active antiretroviral therapy; PBMC, peripheral blood mononuclear cells; DEGs, differentially expressed genes; GSEA, Gene Set Enrichment Analysis; RIN, RNA integrity number; GO, Gene Ontology.

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including cells involved in the innate immunity and acquired immunity, not only during the early phases of the infection but also throughout the chronic phase (Guha and Ayyavoo, 2013). The state of chronic immune activation contributes to the loss of CD4 + T cells and changes in the immune responses, ultimately leading to disease progression (Sivro et al., 2014). Highly active antiretroviral therapy (HAART) can suppress viral replication, reduce the virus load in a patient's body and partially restore circulating CD4 + T cells to allow the immune system to combat HIV infections (Zhang et al., 1999). However, the side effects of this treatment may accumulate and problems including HIV-associated hypertension disorders (Mateen et al., 2013) and cardiovascular disease (Palella and Phair, 2011) may emerge in certain patients during antiretroviral therapy (ART). Because an increasing number of patients suffer from drug toxicity, the emergence of drug resistant viruses and immune reconstitution inflammatory syndrome (IRIS) following the initiation of HAART represent new challenges in the battle against AIDS (Muller et al., 2010; Chan et al., 2003).

Genome-wide gene expression profiling is an informative method used to reveal global changes of the immune system in health and/or disease conditions. It has been particularly useful in identifying biomarkers, examining disease states and investigating immune responses (Chaussabel et al., 2010). Although a number of transcriptomic studies of HIV infection have been conducted, most were based on microarray technologies that focused on a limited number of genes (Ryndak et al., 2014; Wu et al., 2013a; Giri et al., 2006; da Conceicao et al., 2014; Rotger et al., 2010). Thus, these methods are limited in their capacity to detect novel gene products that interact with the virus infection. Recently, next-generation sequencing (NGS) technology has provided a new methodology to both identify and quantify the gene transcripts detected in transcriptome studies (Metzker, 2010). This method, termed RNA-Seq (RNA sequencing), provides highly accurate measurements of genome-wide gene expression via high-throughput NGS sequencing and generates high quality transcriptomic data. This approach yields a plethora of information, including transcript abundance, gene structure, alternative splicing, profiles of non-coding RNA species and genetic polymorphisms (Nagalakshmi et al., 2010; Wang et al., 2009; Yeung et al., 2009). RNA-Seq has been applied in HIV-1 studies. For example, Stewart T. et al. used this technology to examine mRNA and MicroRNA changes in the transcriptome of CD4 + T cells infected with HIV in culture (Pacheco et al., 2013; Ostrowski et al., 2005). Ming D. et al. sequenced RNA transcripts in the brain of HIV-1 transgenic rats to identify differentially expressed genes (DEGs) and enriched pathways affected by the HIV transgene in different areas of the brain (Yamamoto et al., 2011). However, few studies have examined the utility of comparative transcriptomic analysis based on RNA-Seq to investigate HIV-host interactions in samples from HIV patients, especially the transcriptional changes of host genes after HAART.

Many genome-wide expression studies of HIV infection are based on an analysis of total peripheral blood mononuclear cells (PBMCs) (da Conceicao et al., 2014; Twine et al., 2003; Showe et al., 2009; Pecankova et al., 2015), which consist of over a dozen cell subsets, including T cells, B cells, NK cells and monocytes. Although the specific gene expression signals of particular cell subsets will be diluted by those from the other cells and thus reduce the specificity of this approach (Wu et al., 2013a; Rotger et al., 2010; Ostrowski et al., 2005), PBMC is a good starting material to obtain generic information against HIV infection.

In this study, we investigated changes in the transcriptomes of PBMCs from HIV positive patients and HIV negative volunteers before and two weeks after HAART using RNA-Seq technology. To demonstrate the power of this approach, small cohorts (three HIV patients and two HIV negative volunteers) were used. We firstly identified the differentially expressed genes (DEGs) for the time course of each individual. The shared DEGs among individuals were then used to enable comparisons between the HIV patients and HIV negative volunteers. All DEGs were validated empirically by qRT-PCR. The Gene Set Enrichment Analysis (GSEA) was also used to identify pathways that were significantly affected by HAART. These analyses revealed new gene expression patterns of PBMCs and provided new insights into the pathogenesis of HIV-induced immune suppression and HIV-TB associated gene expression changes during HAART. Such an individual comparative transcriptome approach did not require large sample cohort thus could be valuable for future practice of precision medicine.

2. Materials and methods

2.1. Ethics statement

The study protocol was approved by the Institutional Review Board of the Shenzhen Third People's Hospital. Written informed consent was obtained from all participants.

2.2. Treatment-naïve HIV infected and HIV negative individuals

Three HIV-infected individuals and two HIV-negative volunteers were recruited at the Shenzhen Third People's Hospital from April 2013 to September 2013 (Table 1). P1 is 31-year-old Chinese man who is an office worker. He had 10 years' man-man's sex. Two years before admission, he first was found positive in HIV testing. The HIV testing result was confirmed by Shenzhen CDC. P2 is 45-year-old Chinese man presented to our hospital with fever and cough that had persisted for more than one month. Positive history of dozens of homosexual partners for >10 years was self-reported. P3 is 32-year-old Chinese man. He was found to be infected with HIV in medical examination before exodontia. He couldn't recall when he may be infected, but unprotected sex in the last 5 years were self-reported (more details were added in the Supplementary material P1–3info.docx). Two healthy persons who putatively exposed to HIV and proactively requested HAART were proved to be free of HIV infection by ELISA and HIV RNA testing one month later. All participants were screened for HIV antigens and antibodies via standard ELISA analyses, and these findings were further confirmed by Western Blotting. CD4 counts were obtained by flow cytometry, and the viral loads were measured by qRT-PCR. Two of the three HIV serum-positive patients were also diagnosed as being positive for *B. tuberculosis*, as assessed by microbiology tests (sputum acid fast bacilli stain or cultures on Lowenstein–Jensen media). All clinical tests mentioned above were performed by the hospital clinical laboratory, which has a certified license issued by the National AIDS Reference Laboratory at China CDC.

Treatment for the diagnosed HIV-TB patients consisted of standard fixed-dose chemotherapy for two weeks with isoniazid (H), ethambutol (E), rifampicin (R) and pyrazinamide (Z), followed by a combination of highly active antiretroviral therapy (HAART) and anti-TB treatment (HERZ) (WHO, 2014). The HAART regimen consisted of zidovudine plus lamivudine with efavirenz, which is the recommended anti-HIV treatment regimen in China (Fujie, 2012).

2.3. Sample collection, PBMC isolation, RNA extraction and RNA-Seq sequencing

In total, 10 blood samples (5 mL per sample) from five participants were collected at two different time points: immediately before and two-weeks after the start of HAART. The total RNA was extracted from each PBMC sample using the Qiagen RNeasy kit (QIAGEN). The RNA concentration and quality were measured using an Agilent 2100 Bioanalyzer (Agilent), and these analyses showed that all RNA samples had an RNA integrity number (RIN) of >7.5 and a 28S:18S rRNA ratio of >1.8. Beads containing oligo (dT) were used to isolate poly(A)-tailed mRNA from the total RNAs. The Purified mRNAs were used to construct RNA-Seq libraries, which were sequenced on an Illumina HiSeq 2000 sequencing platform (using TruSeqV3 sequencing reagents) at the BGI-shenzhen (Expected library size: 200 bp; Read length: 90 nt; and