Human immunodeficiency virus (HIV) is recognized to be one of the most destructive pandemics in recorded history. By 2011, approximately 34 million people had been infected globally. There were 2.5 million new HIV infections just in 2011 alone according to the Joint United Nations Programme on HIV/AIDS (UNAIDS) Report (2012). With the use of highly active antiretroviral therapy (HAART), the replication of HIV virus and the progression of HIV disease can be suppressed. However, during the life-long treatment of AIDS, HIV resistance and adverse drug reactions have become serious problems. It is necessary to monitor the clinical effect consistently. In most HIV-infected patients, there is a progressive depletion of CD4\(^+\) lymphocytes and eventually a rise in plasma viral load, and when HAART is used the CD4\(^+\) lymphocyte count rises and the viral load becomes suppressed. The CD4\(^+\) lymphocyte count and the HIV viral load measurement have thus become the main indicators for evaluating the clinical effect. It may be useful, however, to develop new biomarkers related to virus load for monitoring the efficacy of treatment as viral load testing is very expensive and the CD4\(^+\) lymphocyte count is a delayed measure of the response to HAART treatment. At present, comparative proteomics provides a powerful approach in screening proteins related to HIV infection that might be potential new therapeutic targets or clinical evaluation biomarkers [1,2]. As reviewed by Zhang, proteomics has been used to search for biomarkers related to HIV-associated neurocognitive impairment, and to virus load in HIV-infected children with renal disease [3]. Potential biomarkers such as Chitinase-3-like protein 1 (YKL-40) [4], talin 1 [2], and antitrypsin [1] have been found. However, global proteomic profiling of HIV-positive human peripheral blood mononuclear cells (PBMCs) for HAART treatment efficacy evaluation has not been studied before. In this work, PBMCs of AIDS patients after \(>6\) months HAART treatment (successfully and unsuccessfully treated groups) were collected and the extracted proteins were analyzed by iTRAQ-based quantitative differential LC/MS/MS analysis. Several differentially expressed proteins apparently related to HAART clinical effect were found and their functions were evaluated by bioinformatic methods.

HIV-positive patients under HAART therapy for \(>6\) months and with no tuberculosis, HBV or HCV infection were hospitalized during June 2010 to February 2011 in Shanghai Public Health Clinical Center affiliated to Fudan University (Shanghai, China) and their written consents were obtained and approved by the Ethics Committee of Shanghai Public Health Clinical Center. The enrolled HIV-positive patients were tested for viral load and divided into HIV-uncontrolled group (also named as unsuccessfully HAART-treated group) (HIV load \(>50\) copies/ml, \(n = 11\) ) and HIV-controlled group (successfully HAART-treated group) (HIV load \(<50\) copies/ml, \(n = 10\) ). The gender, age, and treatment had no difference between these two groups. The characteristics of the enrolled patients are shown in Table 1.

Peripheral whole blood samples (5 ml) were collected into EDTA-anticoagulated tubes. PBMCs were isolated by density gradient centrifugation using Ficoll-Paque™ Plus (GE Healthcare, Piscataway, USA) as previously described [2]. After Ficoll density gradient centrifugation, the upper suspension was removed carefully, and the PBMCs were collected and washed twice with PBS by low-speed centrifugation (200 \(g\)) at 20°C for 10 min. The PBMCs were lysed by 2-DE lysis buffer (8 M urea, 2 M thiourea, 4% CHAPS, 1% Nonidet P-40, 65 mM DTT, and 0.5% Pharmalyte, pH 3–10). Protein concentrations were determined by the Bradford assay. Proteins from each sample of the same group (controlled group or uncontrolled group) were split into equal amounts (100 or 50 \(\mu g\)) and stored at \(-80^\circ C\) for further protein digestion.

The protein mixtures (100 \(\mu g\)) were then acetone precipitated overnight at \(-20^\circ C\) and resuspended in a 30-\(\mu l\) iTRAQ\(^{TM}\) Dissolution Buffer (ABI, Foster City, USA). After reduction and alkylation, protein solutions were digested overnight at 37°C with sequence grade modified trypsin.
The peptide and proteins were exported and saved as Excel files.

After LC–MS/MS analysis, a total of 1231 proteins were quantified in a double analysis with $P > 0.05$ confidence level (ProteoScore > 1.3) (Supplementary Table S1). For the identified proteins, 35.4% (436 out of 1231) proteins were identified by more than five peptides, 8.5% (105 out of 1231) by four peptides, 11% (136 out of 1231) by three peptides, 16.5% (203 out of 1231) by two peptides, and only 28% (350 out of 1231) by one peptide (Supplementary Table S1). For quantification purposes, we selected the proteins that were identified with $P < 0.05$, EF < 2, and fold changes $>1.5$ or $<0.66$. A total of 65 non-redundant differentially expressed proteins were screened in two experiments. In the first experiment, 41 differently expressed proteins were identified, including 16 up-regulated and 25 down-regulated (controlled/uncontrolled, 117/115) with $P < 0.05$ (Supplementary Table S2). In the second, 39 differently expressed proteins ($P < 0.05$) were detected, including 20 up-regulated and 19 down-regulated (controlled/ uncontrollable, 116/114) (Supplementary Table S3). And 16 of the differentially expressed proteins were detected to be consistent in the two independent experiments including eight up-regulated and eight down-regulated ones (Supplementary Table S4). Representative MS/MS spectra for three peptides identified from vimentin (VIM) are shown in Fig. 1. Consistent changes were found in these three peptides (EEAENTLQSFR, ILLAELEQLK, and NLQEAEE) in Fig. 1A–C). Almost all of the $y$ or $b$ ions were detected in the MS/MS spectrum of peptide VLGAFFSDG ALHLDNLK (Fig. 1D).

The subcellular location and function of the identified proteins were elucidated by UniProt knowledgebase (Swiss-Prot/TrEMBL) and Gene Ontology (GO) database. As annotated by GO database, the expression of proteins involved in the following molecular functions changed more frequently: binding activity (100%), enzyme activity (50%), transporter activity (12.5%), and receptor activity (12.5%) (Supplementary Table S4).
As is now understood, HIV is a very efficient virus, which only encodes nearly 10 proteins. In its life cycle such as invasion and replication, HIV mainly depends on host cell proteins. In order to systematically understand the proteins involved in HIV infection and HAART treatment, a protein–protein interaction network was drawn using differentially expressed proteins identified in this work by STRING 9.05 (http://string.embl.de/) which is a powerful online tool to check the known and predicted protein interaction. The interaction between the differentially expressed proteins and HIV proteins was checked by HIV interaction database (http://www.ncbi.nlm.nih.gov/projects/RefSeq/HIVInteractions/). As shown in Fig. 2, a wide protein–protein interaction network was affected in HIV-positive patients under HAART therapy according to the information in the String interaction database (http://string.embl.de). Seen from the KEGG network (Fig. 2), the more affected proteins appear to be the cell adhesion molecules including integrin, calpain, CD45 (PTPRC), and VIM. Cell adhesion molecules are expressed on the cell surface and play a critical role in a wide array of biologic processes including the immune response, inflammation, embryogenesis, and development of neuronal tissue. One of the node proteins, VIM, has a wide connection with other differentially expressed proteins identified in this work such as PTPRC, carbonic anhydrase 2 (CA2), serotransferrin (IF), calpain-1 catalytic subunit (CAPN1), and serum albumin (ALB). VIM, up-regulated in the controlled group, is a class-III intermediate filament found in various non-epithelial cells, especially in mesenchymal cells. CA2, down-regulated in the controlled group, is essential for bone resorption and osteoclast differentiation. CAPN1, down-regulated in the controlled group, is a calcium-regulated non-lysosomal thiolprotease which catalyzes limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction. Furthermore, VIM, up-regulated in the HIV-controlled group, has interactions with several HIV proteins such as gp120, Vif, Vpr, and Pol (http://www.ncbi.nlm.nih.gov/gene/7431) according to the HIV interaction database we used. VIM is a...
known substrate for HIV-1 PR. Envelope surface glycoprotein gp120 decreases phosphorylation of VIM [6,7]. Vif has been reported to colocalize with VIM filaments [8]. Due to its key location in the network, VIM was selected for western blot analysis in order to further verify our proteomic results and offer useful information for HAART treatment efficiency evaluation.

The expression change of VIM was validated using western blot analysis with PBMC proteins from the HIV controlled group and uncontrolled group. Fifty micrograms of PBMC proteins from the controlled or uncontrolled group were separated by 1-DE and transferred to PVDF membrane (GE Healthcare). Then the membrane was incubated overnight at 4°C with the mouse monoclonal primary antibody against VIM (1:200; Santa Cruz, Dallas, USA). After three times wash with TBS-Tween, the membrane was incubated for 1 h at room temperature with goat anti-mouse IgG-HRP secondary antibody (1:2000; Santa Cruz). After further washes, the immune complexes were revealed by enhanced chemiluminescence and detected by X-ray films. Finally, the X-ray films were scanned. Each reaction was performed in triplicate. As shown in Fig. 3, VIM was verified to be significantly increased in the PBMCs from the controlled group compared with the uncontrolled group, which was consistent with the results from the iTRAQ label quantification result.

By systemically checking the HIV interaction database [9], we found that some other proteins, besides VIM in network nodes, also have interactions with HIV proteins (Fig. 2). Integrin beta 2, up-regulated in the HIV controlled group, is identified to have a physical interaction with HIV-1 gp160 in human HEK293 and/or Jurkat cell lines [10]. The interaction of integrin beta 2 with gp120 affects the binding of virus and the invasion of cells [11]. HIV-1 Tat-mediated inhibition of autophagy in bystander macrophages/monocytic cells requires CXCR4, VEGFR1, and beta-integrins [12]. HIV-1 MA (matrix) colocalizes with two integrin CD18, aM and aX integrins (CD11b and CD11c) [13]. Myeloperoxidase (MPO), up-regulated in the HIV-controlled group, is a part of the host defense system of polymorphonuclear leukocytes. HIV-1 Tat up-regulates MPO in HEK293T cells [14]. Apolipoprotein A-I (APOA1) is down-regulated in the HIV-controlled group. HIV-1 Nef impairs cholesterol efflux from macrophages and inhibits internalization of apolipoprotein A1 from the plasma membrane [15]. CAPN1, down-regulated in the HIV-controlled group, is a calcium-regulated non-lysosomal thiol-protease. Envelope surface glycoprotein gp120 can activate calpain-1 catalytic subunit and result in cytotoxic effects in neuroblastoma cultures [10]. HIV-1 Tat increases calpain protease activity at the PM [4]. The cleavage of Tat by CAPN1 increases neurotoxic effect of this viral protein.
In conclusion, by using the iTRAQ labeling method and proteomics analysis of HIV-positive PBMCs from AIDS patients, many proteins were detected to be differentially expressed, and several of them have known functions during HIV infection. VIM was further verified to be related to HAART treatment and virus loading. Our research might provide some clues to further understanding the mechanism of HIV infection and offer novel biomarkers for HAART treatment efficiency evaluation. However, there are certain limitations of this study. For example, the protein–protein interaction was database dependent; limited clinical samples were obtained to verify the expression level of VIM. Further study is needed to verify the protein–protein interaction of VIM predicted by STRING and by antibody-based technologies, such as co-immunoprecipitation, and a larger size of clinical samples should be collected to verify the potential of VIM as a biomarker for HAART treatment efficiency.

Supplementary Data

Supplementary Data are available at ABBS online.

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