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iTRAQ based investigation of plasma proteins in HIV infected and HIV/HBV coinfected patients — C9 and KLK are related to HIV/HBV coinfection

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ABSTRACT

Objectives: Human immunodeficiency virus (HIV) and hepatitis B virus (HBV) share similar routes of transmission, and rapid progression of hepatic and immunodeficiency diseases has been observed in coinfected individuals. Our main objective was to investigate the molecular mechanism of HIV/HBV coinfections.

Methods: We selected HIV infected and HIV/HBV coinfected patients with and without Highly Active Antiretroviral Therapy (HAART). Low abundance proteins enriched using a multiple affinity removal system (MARS) were labeled with isobaric tags for relative and absolute quantitation (iTRAQ) kits and analyzed using liquid chromatography–mass spectrometry (LC–MS). The differential proteins were analyzed by Gene Ontology (GO) database.

Results: A total of 41 differential proteins were found in HIV/HBV coinfected patients as compared to HIV mono-infected patients with or without HAART treatment, including 7 common HBV-regulated proteins. The proteins involved in complement and coagulation pathways were significantly enriched, including plasma kallikrein (KLK) and complement component C9 (C9). C9 and KLK were verified to be down-regulated in HIV/HBV coinfected patients through ELISA analysis.

Conclusion: The present iTRAQ based proteomic analyses identified 7 proteins that are related to HIV/HBV coinfection. HBV might influence hepatic and immune functions by deregulating complement and coagulation pathways. C9 and KLK could potentially be used as targets for the treatment of HIV/HBV coinfections.

Introduction

Acquired immunodeficiency syndrome (AIDs) due to HIV infection has become a chronic disease, with a lower morbidity due to Highly Active Antiretroviral Therapy (HAART). However, HIV patients who are co-infected with HBV are at an increased risk (Lewden et al., 2005; Wu et al., 2015). Due to the fact that HIV and HBV share similar transmission routes, including sexual and blood–blood contacts, co-infections with human immunodeficiency virus type 1 (HIV-1) and hepatitis B virus (HBV) are common and have become a serious public health problem that leads to accelerated progression of the incidence of severe liver disease (Phung et al., 2014). In previous studies of HIV-infected patients, 5.5–13.2% were coinfected with both HIV and HBV (Thio et al., 2013; Zhang et al., 2014). In HIV/HBV coinfected patients, alanine aminotransferase and aspartate aminotransferase levels were higher and $CD4^+$ T-lymphocyte counts (CD4) cell counts were much lower than in HIV mono-infected patients (Thio et al., 2013; Zhang et al., 2014; Huang et al., 2016). Moreover, the prevalence of liver-related mortality was 17 times greater in HIV/HBV coinfected individuals than in those infected with HBV alone (Sun et al., 2014; Parvez, 2015). The progression of hepatic complications from HBV infection is accelerated in patients that are coinfected with HIV (Chun et al., 2014). Furthermore, patients who had HBV infection in addition to an HIV infection had about 4 times more risk of developing severe hepatic toxicity after administration of HAART (Hoffmann and Thio, 2007; Drake et al., 2004). However, our understanding of HIV/HBV coinfection is still very limited. The proteins that are related to HIV/HBV co-infection could be used as potential treatment targets in the clinical settings and might offer

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new clues for understanding the mechanism of HIV/HBV coinfection. However, few proteins are specifically related to HIV/HBV co-infections.

So as to find proteins that are related to HBV coinfection in HIV infected patients, we selected the patients that had not received HAART, and the patients that were treated with the same drugs to exclude drug effects. Patients with HIV and HBV coinfections have impaired immunity and impaired liver function. In addition to this, drug–drug interactions require a careful balance of HIV and HBV treatments to avoid adverse drug reactions and liver damage. To this end, drugs that are effective against both HIV and HBV and at the same time exhibit limited hepatoxicity are recommended, and these include tenofovir disoproxil fumarate (TDF) and lamivudine (3TC). Moreover, combination antiretroviral therapy (ART) with TDF, 3TC, and efavirenz (EFV) has been shown to be safe and effective (Wu et al., 2016). However, despite these patient selection criteria, it remained difficult to identify proteins that are related to HIV/HBV coinfection, thus warranting the use of novel technologies with high throughout and sensitivity.

Proteomics has been used to identify biomarkers for HIV (Zhang et al., 2010; Luo and Muesing, 2014) and HBV infections (Branza-Nichita et al., 2014; Xie et al., 2014), while some proteomic studies have focused on HIV/HCV coinfections (Shetty et al., 2011). Among identified proteins, Apolipoprotein A-II (APOA2), Apolipoprotein C-II (APOC2), Apolipoprotein E (APOE), Complement C3 (C3), and Histidine-rich glycoprotein (HRG) were found to be associated with HIV/HCV coinfection (Shetty et al., 2011). Hence, comparative proteomic technologies for differential proteins that are associated with HIV or HBV infection may facilitate identification of diagnostic biomarkers and drug targets (Luo and Muesing, 2014; Sun et al., 2009). However, comparative proteomic technologies have not yet been used to identify proteins with HIV/HBV mediated alterations that can be used as biomarkers. Therefore, in this study we investigated differential proteins in plasma samples from HIV/HBV coinfected patients compared to HIV mono-infected patients using iTRAQ based proteomic technologies. These analyses identified 7 proteins that were down-regulated in HIV/HBV coinfected patients as compared to HIV mono-infected patients, and these were highly enriched in complement and coagulation signaling pathways. Finally, the proteins C9 and KLK were verified using enzyme linked immunosorbent assay (ELISA).

Methods

Reagents

Hu-14 Multiple Affinity Removal Spin Cartridges (MARS Human-14) were purchased from Agilent technologies (Santa Clara, CA, USA). Strong cation exchange chromatography columns (SCX; 5 μm, 2.1 × 100 mm Polysulfethyl) were purchased from The Nest Group Company (Southborough, MA, USA) and Zorbax300SB-C18 columns (0.3 × 5 mm) were purchased from Agilent Technologies (Santa Clara, CA, USA). 4-plex iTRAQ kit was obtained from Applied Biosystems (Foster City, CA, USA). Formic acid (HPLC grade), ammonium bicarbonate, iodoacetamide, dithiothreitol (DTT), sequencing grade modified trypsin, and the protease inhibitor PMSF were obtained from Sigma (St. Louis, MO, USA). Deionized water from a MilliQ RG ultrapure water system (Millipore, MA, USA) was used in all procedures.

Ethics statement

All procedures involving human plasma were approved by the Shanghai Public Health Clinical Center Ethics committee and written informed consent was obtained from all participants prior to blood collection.

Clinical samples

Recruited patients included 10 HIV mono-infected and 10 HIV/HBV coinfected patients who had not been treated with HAART. Another 10 HIV infected and 10 HIV/HBV coinfected patients who had been treated with HAART for more than 6 months were recruited. HIV and HIV/HBV patient groups were age and gender matched, and 5-mL samples of EDTA anti-coagulated blood were drawn and the plasma was separated. The patients' clinical information is presented in Table 1.

Immunoaffinity depletion of high abundance proteins from human plasma

Low abundance proteins were enriched using a MARS Hu-14 column (Agilent, Santa Clara, California, USA), which removed 14 higher abundance proteins with 94–99% of total plasma proteins, including albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha 2-macroglobulin, alpha 1-macroglobulin, IgM, apolipoprotein A1, apolipoprotin A2, complement C3, and transthyretin, as described previously (Pan et al., 2011; Yadav et al., 2011). In these experiments, 10 μL human plasma samples were diluted 20-fold with buffer A and were filtered through a 0.22-μm spin filter (1.0 min, 14,000 × *g*). Non-bound protein fractions were collected and columns were washed twice with buffer A and were then centrifuged (2.5 min, 100 × *g*). Bound fractions were washed from cartridges with 2.5 mL of buffer B, and non-bound protein fractions were pooled and concentrated using a Nanosep 3K Omega Centrifugal Device (Pall Life Sciences, Port Washington, USA). Collected solutions were acetone precipitated and were lysed in lysis buffer containing 7 M urea, 2 M Thiourea, 50 mM Tris, 50 mM DTT, and 1 mM PMSF, and protein contents were determined using

Table 1
Biochemical data and drug treatments of the four patient groups.

	Without HAART		With HAART		p value[a]
	HIV mono	HIV/HBV	HIV mono	HIV/HBV	
Number	10	10	10	10	–
Age	35.2 ± 6.0	35.5 ± 6.3	39.6 ± 6.9	36.2 ± 9.4	0.914/0.371
Sex	Male	Male	Male	Male(9) Female(1)	1.000/0.343
CD4	282.3 ± 101.8	241.8 ± 149.2	232.3 ± 132.2[b] (489.2 ± 100.1)[c]	234.4 ± 104.3[b] (573.9 ± 115.3)[c]	0.487/0.969
Treatment time	NO	NO	19.3 ± 4.8 months	23.0 ± 3.9 months	0.248

HAART, TDF + 3TC + EFV.
[a] *p* values indicate significant differences between HIV and HIV/HBV patient groups without and with HAART treatments.
[b] Baseline data before treatment.
[c] Data are from within one week of sample collection after treatment for 6 months.