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Quantitative proteomic analysis of exosomes from HIV-1 infected lymphocytic cells

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Abstract

HIV-1 infection causes profound effects both inside and outside of cells through multiple mechanisms, including those mediated by exosomes. Using the technique of Stable Isotope Labeling by Amino acids in Cell culture (SILAC), we compared protein expression patterns in the exosomal compartment of HIV-1 infected and uninfected lymphocytic H9 cells. Of 770 proteins identified in two independent sets of exosomal samples, 14 proteins were found to be differentially expressed in the exosomal fraction of HIV-1 infected cells vs. uninfected controls. Gene Ontology survey and DAVID analysis revealed that identified proteins were enriched for functional categories such as binding. Of these 14 proteins, three immunomodulatory molecules were reproducibly identified in both replicates and included ADP-ribosyl cyclase 1 (CD38), L-lactate dehydrogenase B chain (LDHB) and Annexin A5 (ANXA5). In addition to previously reported HIV-1 associations with CD38 and LDHB, new interactions were identified and validated for ANXA5, CD38 and LDHB, which were found to bind to HIV-1 p24 and Tat. In summary, our studies reveal that exosomes released from HIV-1 infected cells are composed of a unique and quantitatively different protein signature and harbor regulatory molecules that impact the processes of cellular apoptosis (ANXA5 and LDHB) and proliferation (CD38).

Abbreviations

HIV-1; SILAC; MS

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CONFLICT OF INTEREST

All authors declare no financial/commercial conflicts of interest.

Keywords

HIV-1; exosome; SILAC; mass spectrometry

1. INTRODUCTION

Advances in cell biology have led to new tools that allow the global analysis of host factors that impact HIV-1 infection [1]. Such investigations have been undertaken in an attempt to better understand disease pathogenesis, prevention and treatment. For example, a recent genome wide small interfering RNA screen identified more than 250 host factors required by HIV-1 to complete its life-cycle [1]. While proteomics based studies have been applied to HIV-1 biology and improved our understanding of the intracellular sequelae of infection [2, 3], few systematic approaches have been described that detail how an infected cell may potentially impact its neighbors. The relevance of this investigation derives from the bystander effect playing a prominent role in HIV-1 pathogenesis whereby an infected cell influences its immediate and more distant neighbors by releasing a wide range of biologically active molecules [4].

In multicellular organisms, communication between cells mainly involves the cellular release of proteins that can then potentially bind to receptors on neighboring cells and initiate signaling cascades. Another mode of intercellular communication is mediated by exosomes [5], which are 30–90 nm vesicles secreted by a wide range of mammalian cell types [6]. Exosomes contain both protein and RNA molecules, and are believed to modulate cellular functions including presenting tumor antigens to T cells, promoting cell-to-cell spread of infectious agents and pathogen immune surveillance [5, 7]. Proteomic analysis of exosomes isolated from neoplastic cells [8], hepatocytes [9], and urine [10] reveals unique protein signatures suggesting that disease state and tissue of origin impact exosome cargo. With respect to HIV-1, recent studies have revealed that exosomes from infected cells harbor the Nef viral protein [11, 12]. Ectopic expression of Nef in cell lines and primary cells was associated with a 4-fold increase in exosome production. Treatment of primary cells and cell lines with exosomes led to Nef transfer and recipient cell apoptosis [12, 13]. These results prompted our interest in asking whether HIV-1 infection was associated with perturbations of any host proteins in the exosomal fraction.

As exosomes continuously bud out from cells, in order to discriminate between protein secretion patterns that were reflective of the normal state of a cell and those induced by infection, we chose Stable Isotope Labeling by Amino acids in Cell Culture (SILAC) to specifically assign a quantitative proteomic profile to HIV-1 infection [14]. As detailed below, we have used SILAC to compare the proteomes of the exosomal compartment of HIV-1 infected and uninfected lymphocytic cells (Supp. Figure 1). With ~ 770 proteins identified from two separate experiments, 14 proteins were found to be differentially expressed in the exosomal fraction of HIV-1 infected cells when compared to that of uninfected cells. Of these 14 proteins, three immunoregulatory molecules (CD38, LDHB and ANXA5) had similar expression profiles in experimental replicates and were studied in detail. Exosomal levels of CD38 were up-regulated by HIV-1 infection, while the expression of LDHB and ANXA5 was dramatically down-regulated. Notably, CD38 [15], LDHB [16] and ANXA5 [17] have had previously reported associations with proliferation and apoptosis suggesting that their exosomal release may play a role in the viral bystander effect. Further experimentation revealed that CD38, ANXA5 and LDHB were also physically associated with HIV-1 p24 and Tat proteins. Our results suggest that cells infected by HIV-1 may impact the apoptotic and proliferative capacity of neighboring cells by exosomal delivery of HIV-1 binding modulatory molecules.

2. MATERIALS AND METHODS

2.1. Cell culture and HIV-1 challenge

We used H9 cells (ATCC, Manassas, VA; HTB-176), a cell line derived from a human leukemia that can be readily infected by HIV-1. Cells were cultured either in SILAC®RPMI-1640 labeling medium, designated as “Heavy” medium (containing 10% dialyzed FBS and supplemented with 100 mg/L [U-¹³C₆]-L-Lysine (K+6), 100 mg/L [U-¹³C₆, ¹⁵N₄]-L-Arginine (R+10) and 1X L-Glutamine (Invitrogen, Carlsbad, CA)) or in RPMI-1640 medium (Invitrogen), designated as “Light” medium (containing 10% dialyzed FBS and supplemented with 100 mg/L L-Lysine, 100 mg/L L-Arginine, 1X L-Glutamine). Cells in the “Heavy” medium were expanded for six doublings to achieve complete labeling of cellular proteins with heavy labeled amino acids, while cells in “Light” medium were independently maintained. SILAC labeled cells were subsequently infected by HIV-1_{NL4.3} (starting with ~10×10³ TCID₅₀/million cells) for 7 days (AIDS Repository Reagent Program, Germantown, MD). Infection was monitored by p24 quantification in culture supernatants with Alliance HIV-1 p24 Antigen ELISA kit from PerkinElmer (Shelton, CT).

2.2. Exosome isolation

Cellular supernatants from both infected and uninfected H9 cells were independently enriched for the exosomal compartment using techniques as noted below. All of the followings steps were performed at 4°C. Briefly, cells were removed by centrifuging at 300g for 10 minutes followed by another 20 minutes at 2,000g. The cleared supernatant was transferred to ultracentrifugation tubes and underwent multi-step centrifugation (30 minutes at 10,000g followed by 70 minutes at 100,000g) (Sorvall Discovery 100, rotor TH-641). The cellular pellet was transferred into fresh PBS, washed once with PBS then centrifuged at 100,000g for 70 minutes before resuspension in 50 µl of fresh PBS [18]. Protein was extracted from pellets as described below.

2.3. Protein extraction and preparation

Exosomal pellets were mixed with CellLytic® M reagent (Sigma, St. Louis, MO) for 15 minutes on a shaker before 10 minutes of centrifugation at 12,800g to separate lysed exosomal protein from supernatant. Micro BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, IL) was used to determine protein concentration. Heavy (2µg) and light (2µg) exosomal protein samples were mixed 1:1 before running on NuPAGE® 4–12% Bis-Tris precast Gel (Invitrogen) by using XCell SureLock® Mini-Cell (Invitrogen). The gel was stained by SimplyBlue® SafeStain (Invitrogen). The entire sample lane from the destained gel was cut into ten equal gel pieces for fractionation. Each piece was washed three times with 50% acetonitrile/50% HPLC grade water prior to mass spectrometric analysis.

2.4. Mass spectrometry and quantitative analysis

Gel pieces were washed, reduced with DTT and alkylated with iodoacetamide. Gels were then digested with modified trypsin (Promega, Madison, WI) at pH 8.3 overnight at 37°C with shaking. The resulting peptide mixtures from each gel piece were analyzed separately by data dependent microcapillary reversed phase liquid chromatography tandem mass spectrometry (LC/MS/MS). LC/MS/MS was performed using an EASY-nLC nanoflow HPLC (Thermo Fisher Scientific, Waltham, MA) with a self-packed 75 µm id×15 cm C₁₈ column in buffer A (0.1% formic acid in water) coupled to a LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific) using a MS-FT target value of 5.0E5 and a MS/MS-IT target value of 3.0E4 in data dependent acquisition (DDA) / positive ion mode. The LC gradient was produced over 60 min from 2% B to 38% B. Buffer B contained acetonitrile.

LC/MS grade solvents were used. The full scan range was acquired between 390 m/z to 1500 m/z at 30,000 resolution using a Top5 DDA method via CID. Precursor ions were excluded for a duration of 2 min. Nano-ESI spray voltage of +3kV was used without sheath or auxiliary flow gas. The ion count threshold for MS/MS selection was 700 counts. Activation time and Q values were set as default. Generated MS/MS spectra were searched against the reversed and concatenated non-redundant Human IPI database (version 3.68) by using Mascot (Matrix Science, London, U.K.; version 2.2). Mascot searches used the fixed modification of carbamidomethyl Cys and variable modifications of oxidation of Met and acetylation of protein N-terminus. Two missed cleavages and Trypsin with Pro restriction were used during the searches and a minimum of 6 amino acid residues were allowed. An MS tolerance of 30 ppm and MS/MS tolerance of 0.8 Da were used during Mascot searches. The peptide and protein false discovery rate (FDR) was set at 1%. Protein quantitation was achieved by employing MaxQuant software (version 1.0.13.13) (<http://www.maxquant.org>) requiring at least 2 peptides per protein (1 razor and 1 unique) for quantitation. Normalized SILAC ratios were used for all subsequent interpretation.

2.5. Immunoprecipitation

Crosslink Magnetic IP and Co-IP Kit from Thermo Fisher Scientific (Rockford, IL) was used to immune-isolate potential binding partners of identified proteins by cross-linking IgG (Cell signaling, Danvers, MA), CD38, ANXA5 (Santa Cruz Biotechnology, Santa Cruz, CA) and LDH8 (Epitomics, Burlingame, CA) to magnetic Protein A/G beads. Standard protocols were followed according to manufacturer's instructions.

2.6. Western blotting

Cellular protein was harvested, quantified by micro BCA assay and run on NuPAGE® 4–12% Bis-Tris precast Gel (Invitrogen) by using XCell SureLock® Mini-Cell (Invitrogen). After electrophoresis, the resolved proteins were transferred onto PVDF membrane by using XCell II® Blot Module. Upon the completion of transfer, membranes were blocked for 1 hour before adding anti-ACTB (1/10000 dilution, BD Biosciences, San Diego, CA), anti-CD38 (1/2000 dilution), anti-ANXA5 (1/1000 dilution) and anti-LDH8 (1/5000 dilution, Epitomics) and incubating overnight at 4°C. Finally, proteins were detected and analyzed by Odyssey® CLx Infrared Imaging System (LI-COR, Lincoln, NE) after incubation with IRDye® 680LT or 800CW conjugated secondary antibodies for 30 minutes followed by 4 PBST washes.

2.7. Data analysis

1. Protein quantitation—Two prerequisites were applied to ensure greater confidence in the accuracy of the MS derived peptide ratios: 1.) Two or more quantifiable peptides were required to be associated with a given protein; 2.) In order to determine significantly up- and down-regulated protein candidates, conservative cut-off values were calculated as follows: median and standard deviation () of normalized H/L ratios were calculated using log transformed data of all quantified proteins. The cut-off of value (median $\pm$ 2) was calculated in log space which was then transformed back into linear space. (<http://proteome.moffitt.org/proteomics/silacratioanalyser/silacratioanalyser.jnlp>). All identification and quantitation was also manually inspected using previously published methodologies [19].

2. Histogram of SILAC ratio distribution—Normalized H/L Ratios of all quantified proteins were log₂ transformed, and plotted by using SigmaPlot (version 10.0).

3. Gene Ontology (GO) analysis—Protein candidates listed in Table 1 were searched against UniprotKB, EBI and GO databases via Software Tool for Rapid Annotation of Proteins (STRAP, version 1.1.0.0) [20]. Visualization of GO analysis results was finalized in Excel by importing analyzed data from STRAP.

4. DAVID analysis—To find statistically significant overrepresented gene ontology (GO) terms, the Database for Annotation, Visualization and Integrated Discovery (DAVID, <http://david.abcc.ncifcrf.gov>, version 6.7) was employed [21, 22] and Functional Annotation Charts were created with default settings.

5. ExoCarta retrieval—The most recent (Jan., 2012) exosome database (<http://www.exocarta.org/>) was searched against protein candidates from SILAC and network analysis [23, 24]. Notably, the top 25 exosomal marker proteins listed at ExoCarta were also searched against SILAC generated lists.

6. Protein interaction analysis

a. Interaction analysis of exosomal proteins: STRING (Search Tool for the Retrieval of Interacting Genes/Proteins, version 9.0) was used to check the known and predicted associations, including direct (physical) and indirect (functional) ones, among SILAC generated exosomal candidates (<http://string.embl.de>) [25]. The top ten known partners associated with each exosomal candidate were also mined.

b. HIV-1/host exosomal proteins network analysis: The most recent (Jan., 2012) HIV-1, human protein interaction database (<http://www.ncbi.nlm.nih.gov/RefSeq/HIVInteractions/>) [26] was manually curated to identify species shared by the database and our SILAC generated results. The database contains 1443 human proteins, which have been reported to interact with HIV-1 encoding proteins (*env*, *gag*, *nef*, *pol*, *rev*, *tat*, *vif*, *vpr*, *vpu*).

3. RESULTS

3.1. HIV-1 infection alters the exosomal proteome

Before submitting SILAC labeled HIV-1 samples for mass spectrometry analysis, highly productive infection was confirmed by quantifying HIV-1 p24 levels in culture supernatant which revealed a 3 log increase by day 7 post-infection.

To minimize random effects and increase fidelity, two independent replicates of exosomal fractions were isolated and prepared for LS-MS/MS identification and MaxQuant quantitative analysis. For the first set of exosomal fractions, 766 unique proteins were identified and 293 proteins had quantified ratios (Supp. Table 1). For the second set of exosomal fractions, 772 unique proteins were identified and 358 proteins were quantified (Supp. Table 2). In total, 485 unique proteins were identified from either the first or the second set. From these 485 proteins, a total of 342 proteins had quantified ratios in either the first or second set. Out of the 342 proteins, 222 appeared in both the first and second sets (Supp. Table 3).

Prior to in-depth analysis, we plotted log₂ transformed H/L ratios of all proteins from the two sets. The normal distribution of protein ratios indicated that our experimental techniques did not produce bias (Fig. 1a).

Although an established standard exosome isolation protocol was followed [18], we wanted to further validate our isolation techniques by profiling MS identification of the top 25 proteins that are often identified in exosomes (http://exocarta.org/exosome_markers). We

identified 23 exosome markers from our MS list (Supp. Table 4). And notably, except for ANXA5, the remaining 22 exosome makers in our list had SILAC ratios close to 1 indicating that the expression of most exosome makers in this study was not impacted by HIV-1 infection.

Next, to find significant exosome protein candidates impacted by HIV-1, we calculated the cut-off of values for the first (>1.66 and <0.56) and second set of data (>1.71 and <0.54) by using silacratioanalyser (Supp. Table 1 & 2). Not surprisingly, this cutoff value is in line with the previously published cutoff value of 1 ± 0.5 – 0.6 [27, 28]. In addition, we excluded proteins found to be significantly differentially expressed in one set of data but not in the other. Based on the above criteria, 14 protein candidates were selected for further analysis (Table 1). Finally, exosomal protein fractions from uninfected and infected H9 cells were blotted with antibodies against the three highly (CD38, ANXA5 and LDHB) differentially expressed candidates. They were found to have similar expression profiles by western blot indicating that the MS data were indeed reliable in terms of assigning relative protein abundance (Fig. 1b).

3.2. HIV-1 related exosomal protein perturbations impact multiple cellular processes

To gain broader and more accurate understanding of the 14 candidates and their putative role in HIV-1 biology, we applied Gene Ontology (GO) analysis. When considering Biological Process (BP), the largest percentage of proteins was associated with cellular processes, followed by regulation and other processes (Fig. 2a). In the category of Cellular Component (CC), proteins were predicted to be mainly localized to the cytoplasm, nucleus and plasma membrane (Fig. 2b). As for the Molecular Function (MF), the majority of proteins (60%) appeared to participate in binding (Fig. 2c). We next used DAVID to interrogate our SILAC generated data set. As shown in Supp. Table 5, regulation of cell activation and/or apoptosis was noticeably enriched in the BP category. Cytoplasmic and intracellular fractions were significantly enriched in the CC category. For MF, protein binding was dramatically enriched ($p < 0.03$).

3.3. HIV-1 related exosomal proteins have associations with HIV-1 gag and tat encoding proteins

We next explored the possible associations between identified exosomal proteins and HIV-1. For the 14 candidates, 11 of them were found significantly differentially expressed in one set of SILAC replicate experiments. The three remaining candidates (ANXA5, LDHB and CD38) were decreased or increased at least 2.5 fold in both sets of exosomal proteins isolated from cells infected with HIV-1 vs. controls and their reproducible identification prompted further experimentation.

In order to explore whether these three candidates directly (physical) and/or indirectly (functional) associated with HIV-1 viral proteins, we mined the NIH HIV-1 human protein interaction database. We found that CD38 had previously reported associations with *env* encoding protein gp120 [29–31] and that LDHB also has known associations with *env* encoding protein gp120 and gp41 [32, 33] as well as Tat protein [34]. However, no such findings had been reported for ANXA5 (Table 2).

We cross linked antibodies against ANXA5, CD38 and LDHB onto protein A/G magnetic beads, followed by immunoprecipitation and western blotting with a panel of antibodies against gp160 (*env*), p24 (*gag*), Nef (*nef*), integrase, protease and reverse transcriptase (*pol*), Rev (*rev*), Tat (*tat*), Vif (*vif*), Vpr (*vpr*) and Vpu (*vpu*). We found that p24 and Tat could be recovered from immunoprecipitated samples of ANXA5, CD38 and LDHB (Fig. 3a). The

associations were relatively strong for LDHB and ANXA5 and weaker for CD38. No physical associations were found for the remaining HIV-1 encoding proteins.

4. DISCUSSION

HIV-1 infection leads to profound changes in the cellular transcriptome and proteome. Previously reported proteomic surveys have used a variety of substrates from cell lines to primary patient derived biologic samples and, while results vary, a consistent experimental observation has been the broad range of protein deregulation that follows HIV-1 infection [35–41]. Here, we focused on quantifying perturbations at the extracellular level by studying exosomes isolated from infected lymphocytic cells.

By applying a series of filters, including sample replicates and selection of a cutoff for protein ratios, we initially identified 14 proteins as differentially expressed in exosomes derived from infected cells. Interestingly, when we checked the most recent exosome database (ExoCarta), all identified proteins except CD38 had been previously identified in exosomes by other groups (Table 2). By searching CD38 in STRING, a database of known and predicted protein interactions, three out of the first ten partners for CD38 were found to be exosomal proteins (Supp. Fig 2). These results support CD38 as an exosome associated protein especially in the setting of HIV-1 infection.

To find possible internal links among ANXA5, CD38 and LDHB, we mined the first 10 associated partners from STRING for each protein. From a total of 30 partner candidates, proteins found to be both exosome and HIV-1 associated were selected for creating a network map. Surprisingly, we found that ANXA5 and CD38 had direct associations and indirect links through CD9 and CD63, while LDHB connected with ANXA5 through G6PD-EGF bridge (Fig. 3b).

To explain the potential biologic implications of the decreased level of ANXA5 and LDHB and elevated level of CD38 in exosomes from HIV-1 infected cells, we propose a model combining our findings in this study with the fact that both ANXA5 [17, 42] and LDHB [16] are known apoptosis makers, while CD38 promotes cell proliferation [43–45] (Fig. 3c). Compared to uninfected cells, by secreting exosomes containing less ANXA5 and LDHB and more CD38, HIV-1 infected cells may end up delivering exosomes with less “negative” effectors to neighboring cells, giving recently produced virions a greater chance of survival and intercellular transmission. However, for neighboring HIV-1 infected cells, the “positive” effects could potentially be regulated by binding to HIV-1 viral proteins through differentially expressed exosomal proteins. In conclusion, by regulating the protein content in exosomes, our results suggest that HIV-1 may be able to maintain a more “favorable” extracellular environment that favors cell to cell transmission and infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Antibody (5B2B2) from Dr. D. Helland and Dr. A. M. Szilvay [53], HIV-1 Protease Antiserum, HIV-1 Rev Monoclonal Antibody (1G7) from Dr. A. M. Szilvay [54, 55], HIV-1 Tat Monoclonal Antibody (1D9) from Dr. Dag E. Helland [56], HIV-1 Vif Monoclonal Antibody (#319) from Dr. Michael H. Malim [49, 57], HIV-1 Vpr (1–50) Antiserum from Dr. Jeffrey Kopp, HIV-1_{NL4-3} Vpu Antiserum from Dr. Klaus Strebel [58].

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A. **B.** **Figure 1.**

a). Histogram of SILAC ratios revealed symmetrical distribution along ratio=1 ($\text{log}_2=0$) trend line. The log_2 transformed ratios were grouped into ratio bins, and the y-axis shows the relative number of detected ratios per bin. The analysis encompasses 293 and 358 proteins, respectively. **b).** Western blotting of representative proteins revealed similar quantitative profiles as suggested by SILAC-MS analysis. CD38, ADP-ribosyl cyclase 1; LDHB, L-lactate dehydrogenase B chain; ANXA5, Annexin A5. Beta-actin (ACTB), which was identified by MS as a component of the exosomes, was not differentially expressed and was used as a loading control.

A. Biological Process

B. Cellular Component

C. Molecular Function

Figure 2.

Gene Ontology (GO) analysis of the fourteen exosomal proteins. **a).** Pie chart of gene ontology analysis on Biological Processes (BF). **b).** Pie chart showing ontology analysis on Cellular Components (CC). **c).** Pie chart of gene ontology analysis on Molecular Functions (MF). Pie charts were created in Excel by importing analyzed data from STRAP (Software Tool for Rapid Annotation of Proteins).

A. **B.** **C.** **Figure 3.**

a.) Western blot of immunoprecipitated lysates by using antibodies against HIV-1 protein p24 and Tat. Immunoprecipitation was performed by cross-linking IgG and antibodies against ANXA5, CD38 and LDHB respectively to magnetic protein A/G beads to isolate potential binding partners in HIV-1 infected lymphocytic cells. **b.)** Predicted network map for ANXA5, CD38, LDHB and its top partners that have both associations with HIV-1 viral proteins and exosomal localization. Network map was created in STRING. **c.)** Proposed model for biological relevance of the differential expression of ANXA5, CD38 and LDHB in exosomes released by HIV-1 infected cells.

Table 1

Up and down-regulated proteins found in exosomal fractions from HIV-1 infected lymphocytic cells. NCBI Reference Sequence (RefSeq) accession number, corresponding gene symbol and SILAC ratios from Set 1 and 2 are given for each protein. ND stands for Not Determined, NV stands for No (quantitative) Values.

Protein Names	Accession No.	Gene Symbol	Ratio H/L (Set 1)	Ratio H/L (Set 2)
THO complex subunit 4	NP_005773	THOC4	ND	4.2656
ADP-ribosyl cyclase 1	NP_001766	CD38	2.9500	3.6223
Heat shock 70 kDa protein 4	NP_002145	HSPA4	0.5418	NV
Adenosylhomocysteinase	NP_000678	AHCY	0.5383	NV
Nuclear transport factor 2	NP_005787	NUTF2	ND	0.5249
Ubiquitin-conjugating enzyme E2 N	NP_003339	UBE2N	NV	0.4883
Signaling threshold-regulating transmembrane adapter 1	NP_055265	SIT1	NV	0.4788
Prostaglandin E synthase 3	NP_006592	PTGES3	ND	0.4733
N(G),N(G)-dimethylarginine dimethylaminohydrolase 2	NP_039268	DDAH2	NV	0.4492
DnaJ homolog subfamily C member 5	NP_079495	DNAJC5	NV	0.4441
Syntaxin-binding protein 2	NP_008880	STXBP2	NV	0.3980
L-lactate dehydrogenase B chain	NP_002291	LDHB	0.3874	0.3938
Obg-like ATPase 1	NP_037473	OLA1	0.3054	NV
Annexin A5	NP_001145	ANXA5	0.1241	0.1710

Note: ND: No identification; NV, identified but no H:L ratios

Table 2

Associations between viral proteins and human HIV-1 exosome related proteins from this study. Exosome localization was checked against ExoCarta and protein associations with HIV-1 were investigated using the HIV-1, Human Protein Interaction Database.

Gene	In Exosome	Previously reported	Newly found in this research	
		Associated with HIV	In Exosome	Associated with HIV
THOC4				
CD38		gp120(<i>env</i>)	MS & western	p24(<i>gag</i>), Tat(<i>tat</i>)
HSPA4		gp120(<i>env</i>), pr55, matrix(<i>gag</i>), Tat(<i>tat</i>), Vpr(<i>vpr</i>)		
AHCY				
NUTF2		matrix(<i>gag</i>)		
UBE2N				
SIT1				
PTGES3		Vpr(<i>vpr</i>)		
DDAH2				
DNAJC5				
STXBP2				
LDHB		gp120, gp41(<i>env</i>), Tat(<i>tat</i>)		p24(<i>gag</i>)
OLA1				
ANXA5				p24(<i>gag</i>), Tat(<i>tat</i>)