Published in final edited form as:

J Proteome Res. 2013 May 3; 12(5): 2045–2054. doi:10.1021/pr300918r.

The Conserved Set of Host Proteins Incorporated into HIV-1 Virions Suggests a Common Egress Pathway in Multiple Cell Types

Michael E. Linde[†], David R. Colquhoun[‡], Ceereena Ubaida Mohien[‡], Thomas Kole[§], Veronica Aquino[‡], Robert Cotter[§], Nathan Edwards^{||}, James E.K. Hildreth[⊥], and David R. Graham^{*‡}

[†]Graduate Program in Immunology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, United States

[‡]Department of Molecular and Comparative Pathobiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, United States

[§]Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, United States

^{||}Department of Biochemistry and Molecular & Cellular Biology, Georgetown University Medical Center, Washington, D.C. 20057, United States

[⊥]Molecular and Cell Biology, University of California Davis, Davis, California 95616, United States

Abstract

HIV-1 incorporates a large array of host proteins into virions. Determining the host protein composition in HIV virions has technical difficulties, including copurification of microvesicles. We developed an alternative purification technique using cholesterol that differentially modulates the density of virions and microvesicles (density modification, DM) allowing for high-yield virion

© 2013 American Chemical Society

*Corresponding Author: dgraham@jhmi.edu.

Supporting Information Supplemental Figure 1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Notes The authors declare no competing financial interest.

DEDICATION We dedicate this study to the memory of Dr. Robert J. Cotter, who passed away during the preparation of this manuscript. His guidance, mentorship, and friendship will not be forgotten.

purification that is essential for tandem mass spectrometric and quantitative proteomic (iTRAQ) analysis. DM purified virions were analyzed using iTRAQ and validated against Optiprep (60% iodixanol) purified virions. We were able to characterize host protein incorporation in DM-purified HIV particles derived from CD4+ T-cell lines; we compared this data set to a reprocessed data set of monocyte-derived macrophages (MDM) derived HIV-1 using the same bioinformatics pipeline. Seventy-nine clustered proteins were shared between the MDM derived and T-cell derived data set. These clusters included an extensive collection of actin isoforms, HLA proteins, chaperones, and a handful of other proteins, many of which have previously been documented to interact with viral proteins. Other proteins of note were ERM proteins, the dynamin domain containing protein EH4, a phosphodiesterase, and cyclophilin A. As these proteins are incorporated in virions produced in both cell types, we hypothesize that these proteins may have direct interactions with viral proteins or may be important in the viral life cycle. Additionally, identified common set proteins are predicted to interact with >1000 related human proteins. Many of these secondary interacting proteins are reported to be incorporated into virions, including ERM proteins and adhesion molecules. Thus, only a few direct interactions between host and viral proteins may dictate the host protein composition in virions. Ultimately, interaction and expression differences in host proteins between cell types may drive virion phenotypic diversity, despite conserved viral protein–host protein interactions between cell types.

Keywords

HIV; envelope; iTRAQ; mass spectrometry; host protein; macrophage; T-cell; budding

INTRODUCTION

During HIV replication and packaging, HIV relies on the coordinated interactions between viral and host proteins.¹ As HIV buds, it incorporates hundreds of cellular host proteins into the nascent virion, either into its lipid bilayer or inside the HIV virion.² Several studies have indicated that host protein incorporation affects both HIV attachment and infectivity.³ Other proteins, such as cyclophilin A, have been implicated in the HIV lifecycle;^{2b,3a,4} however, due to the large number of host proteins reported to be incorporated into HIV virions, it is difficult to determine the biological relevance, if any, of many of these proteins.

We hypothesized that host proteins that play a significant role in the HIV-1 virion lifecycle or those that significantly affect HIV spread through the host would be conserved in the virus regardless of the progenitor cell type. HIV-1 infects multiple cell types, most prominently macrophages and CD4+ T-cells. As these cell types have different protein expression patterns and surface protein composition, it is expected that HIV-1 virions budding from these different cell types carry different sets of host proteins. Further, it is likely that many of the proteins incorporated by the virus are done so through secondary or higher interactions. Here, we define a minimal set of relevant host proteins that are incorporated into HIV-1 virions from multiple cell types.

Mass spectrometry (MS) analysis of purified viral particles is one tool for determining which host proteins are incorporated in HIV virions on a global scale. While there are

biochemical and proteomic techniques that can be used to identify HIV-associated proteins, the success of these studies are limited by HIV purification techniques that often result in copurification of contaminating microvesicles.⁵ HIV virions are small, dense particles of approximately 100 nm diameter; HIV-infected cells produce microvesicles of similar size and density to that of the HIV virion, which have also been shown to share many of the components of HIV.⁶ A variety of techniques have been employed to reduce microvesicle contamination, including CD45 depletion of microvesicles and affinity purification using viral envelope proteins.⁷ Due to the large quantities of virions needed, affinity purification of virions or depletion of microvesicles is not a practical option for many biochemical studies. Therefore, we developed a novel strategy for purifying large quantities of HIV-1. By using cholesterol to manipulate the density of the particles in HIV-1 preparations (density modification; DM), we were able to separate virions from contaminating vesicles by centrifugation, making them suitable for analysis by tandem MS. Using this strategy, we were able to characterize host protein incorporation in HIV particles derived from CD4+ T-cell lines. We compared our DM purification method with an orthogonal purification approach using inoxidol (OptiPrep) gradients by quantitative proteomics. Lastly, we tested our hypothesis that conserved proteins would reveal critical shared pathways by comparing our data set of T-cell derived HIV-1 virions to a reprocessed data set of monocyte-derived macrophages (MDM) derived HIV-1 using the same bioinformatics pipeline.

We identified clusters of conserved proteins between MDM and T-cell derived HIV-1. These clusters included an extensive collection of actin isoforms and other core interacting proteins, many of which have previously been documented to interact with viral proteins.⁸ These data suggest that a limited number of viral-host protein interactions can explain the phenotypic diversity of HIV-1 virions produced from MDM or T-cells allowing HIV-1 to be incredibly plastic and opportunistic in its final protein composition depending on the cell type it is produced from. The common incorporation of syntenin-1 (a component of tetraspanin enriched membranes, TEMs) and CD44 (hyaluronic acid receptor) is suggestive of a common cellular egress pathway involving TEMs and a common vesicle population that targets hyaluronic acid enriched microenvironments.

EXPERIMENTAL SECTION

Apparatus

MALDI-MS and MS/MS spectrum were obtained using an ABI 5800 MALDI TOF/TOF analyzer (AB Sciex) using a 2 KeV extraction method with CID turned off using dynamic exit.

Reagents

HIV_{MNCL4} from either H9 (T-cell line) or CEMx174 (B-cell/T-cell hybrid line) cells was obtained from the AIDS and Cancer Vaccine Program (SAIC-Frederick).

Procedures

Virus Purifications—DM purification was accomplished by incubating virus in 420 μ g/mL of cholesterol and 20 mM 2-hydroxy-beta cyclodextrin (β CD) in TNE as indicated,

filtration through a 5 μ m filter on ice and pelleted through 20% sucrose for 1 h at 100 000 $\times$ g. OptiPrep purification was performed as previously described.⁹ SDS page, Western blotting and EM were performed as described.¹⁰

Quantitative MS—Virus (normalized by p24) was ultracentrifuged and resuspended in 0.5 M triethylammonium bicarbonate with 1% rapigest, reduced (TCEP) and alkylated (MMTS) and subjected to tryptic digestion as previously described.¹¹ Peptides were labeled with iTRAQ reagents as follows for HIV-1 derived from CEMx174 cells: m/z 113: Control, m/z 114: DM, m/z 115: OptiPrep, and for HIV-1 derived from H9 cells: m/z 117: Control, m/z 118: DM, m/z 115: OptiPrep. Peptides were then subjected to nano rHPLC on a TEMPO-LC MALDI spotting system using a 90 min gradient from 5% to 80% B (98% ACN, 0.1% TFA) at 500 nL min⁻¹, Matrix (CHCA, 5 mg/mL in 75% ACN) was then supplemented to the flow postcolumn at 500 nL min⁻¹, and samples were deposited onto a stainless steel plate at 10 s intervals. One-thousand MALDI-MS and up to 1500 MS/MS spectrum were obtained using an ABI 5800 MALDI TOF/TOF analyzer (AB Sciex) using a 2 KeV extraction method with CID turned off using dynamic exit. Spectral quality settings were set to high for both the spectrum and the iTRAQ reporter regions, according to manufacturer's suggestions (AB Sciex). ProteinPilot 3.0 was used to search UniProt-Swiss-Prot with contaminants appended (2007.01.23; 254,765 sequences) with peptide threshold of 99.9%, and fixed modifications of iTRAQ (K, N-term), MMTS (C). Due to variable processing of gag, we subsequently normalized virus from different treatments and lines using cyclophilin A, a known gag interacting host protein,^{4a} using the iTRAQ reporter bias correction feature built into ProteinPilot.

Tandem Mass Spectrometry (LCQ)—500 μ g of capsid equivalents of DM purified HIV-1_{MNCL4}/H9 virus was desalted and subjected to reverse phase HPLC analysis on a Beckman PF2D system as previously described into 37 fractions, digested and tandem MS performed as described.¹² Briefly, ESI-MS/MS of tryptic peptides was performed on an LCQ-ion trap-MS/MS instrument using a 60 min gradient as previously described.¹³ Data were acquired using Xcalibur 2.07 (Thermo, San Jose, CA). The three most intense ions (minimum signal of 100 000 ions) were selected for MS/MS fragmentation using a normalized collision energy of 35. Dynamic exclusion was applied for 30 s after 1 MS/MS acquisition, with a mass window of 2 Da.

Comparison between MDM Derived Virus and T-cell HIV-1—Seventeen raw files for the analysis of MDM derived virus from a study published by Chertova et al.^{2a} were obtained from the authors and analyzed in parallel with 37 DM purified fractions of HIV-1/H9. Briefly, peaks were selected and deisotoped using DeconMSn for MDM derived LTQ data and using ReAdW (2009v) for LCQ derived HIV-1/H9. The data were then searched using PepArML,¹⁴ which uses multiple different search algorithms (OMSSA, X! Tandem with native, k-score and s-score scoring, MASCOT, MyriMatch, and InSpecT) as previously described.¹⁵ Carbamidomethylation was set as a fixed modification and oxidized methionine was set as variable modification. Mass tolerances on precursor and fragment ions were set at 1.5 and 0.8 Da, respectively, and missed cleavage as 1 using a specific search. The database used for search was the UniProt-Swiss-Prot database (version

2010.11.02; 522,019 sequences). Peptides were combined on PepARML using a random forest approach (Weka)¹⁶ and the results were then parsed into MASPECTRAS 2.0¹⁷ with minimum 2 peptide for a protein and a spectrum false discovery rate of 5%. Peptides assigned to keratin were excluded, and since the analysis was focused on host proteins, viral peptide assignments were excluded. Protein redundancy was removed by MS based evidence clustering.¹⁷ The data analysis pipeline meets all MIAPE standards¹⁸ and the proteomics data have been deposited in the ProteomeExchange via the Protein Identifications database (PRIDE) partner repository with the data set identifier PXD000064.¹⁹

HIV Protein Interactions and Network/Pathway Analysis—Network and pathways analysis was performed using the GeneMANIA gene network tool, which contains 353 human interaction networks based on data from BIND, IntAct and other interaction databases, using association data from protein and genetic interactions, known and predicted pathways, coexpression, colocalization and protein domain similarity.²⁰ Our analysis was performed using only protein physical interaction data from GeneMANIA with GeneMANIA Cytoscape plugin. Identified host proteins were searched in the HIV-1, Human Protein Interaction Database for reported interactions in the literature.²¹

Safety Considerations—All work with infectious HIV was performed in a biosafety level 3 environment using standard safety practices.

RESULTS

DM Virion Preparations Allows for the Separation of Virus from Microvesicles

The DM purification method is based upon our previous studies using beta-cyclodextrin to manipulate cholesterol in HIV-1 particles.^{10,22} As viral purification through a 20% sucrose gradient results in copurification of HIV and microvesicles of similar density, we differentially modified microvesicle and virion density by adding excess cholesterol to purified viral stocks. Cholesterol is differentially incorporated into microvesicles and virions, resulting in density changes that allow for the separation of highly purified virions from microvesicle contaminants. DM HIV-1 purity was assessed using CD45, a well-defined marker of vesicle contamination, resulting in a >90% reduction of material and elimination of microvesicles (Figure 1A–C). DM HIV-1 had a <1 log infectivity decrease (not shown) and virion morphology was substantially altered (Figure 1D).

To rule out artifact, we validated DM purified samples with an alternative purification method (OptiPrep; 60% iodixanol).⁹ The relative abundances of proteins by quantitative proteomics (iTRAQ) from both samples were compared against virus pelleted through a 20% sucrose gradient. Virus preparations were carefully normalized by capsid protein (p24) by ELISA, and subsequently validated by SDS-PAGE (data not shown) prior to digestion with trypsin and labeling with iTRAQ reagents.

In an iTRAQ experiment, posthoc corrections for the relative abundance of reporter ions are made to ensure that no single reporter is over-represented in the data analysis.²³ This can occur for a multitude of reasons, including variations in manufacturing of reagents or sample

preparation conditions during labeling. Recently Breitwieser and colleagues showed that iTRAQ reporter intensities are valid over one log of dynamic range.²³ In this study, we manually adjusted iTRAQ reporter bias to ensure that no purification method resulted in a reporter ratio >1 when compared to the control preparation, as protein can only be depleted in the purification process.

For comparisons between viruses (CEMx174 vs H9 derived HIV-1), we initially attempted to adjust iTRAQ reporter bias based upon spectra assigned to HIV-1 capsid protein (p24). However, p24 resulted in unreliable bias estimation secondary to the extreme sequence divergence of HIV capsid protein and improperly assigned viral peptides by ProteinPilot. Instead, we investigated the use of spectra assigned in control preparations to the host protein cyclophilin A (CypA). The host protein CypA has been reported to be included in virions,^{4a} but has not been reported to be present in microvesicles except under conditions of extreme cellular stress, such as cellular irradiation.²⁴ CypA incorporation has also been reported to be important for maximal HIV infectivity and it has been suggested that the absence of CypA incorporation leads to HIV restriction.^{4b} HIV recruits CypA to ~10% of its capsid monomers in newly assembled cores and the CypA binding site on capsid is highly conserved in all primate lentiviruses.^{4a,b} We therefore adjusted iTRAQ reporter bias using CypA peptides, while ensuring that the most abundant protein was normalized to a 1:1:1 ratio between different preparations. Indeed, this method did not violate our rule of host proteins in the purification groups being less than control. Final adjustments were minor and accounted for a 25% decrease in CypA for CEMx174 derived HIV-1 and a 35% decrease in CypA for H9 derived HIV-1 using DM purification. Comparatively, a 10% and 40% decrease in CypA were observed using OptiPrep purification for CEMx174 and H9 derived HIV-1, respectively. These results suggest that CypA may indeed be present in microvesicles induced by HIV-1 infection, like other forms of cellular stress.

For virions produced in either H9 or CEMx174 cells, we observed a decrease in protein abundance for both DM and OptiPrep purified virions compared to control methods. DM purification significantly reduced the abundance of 34 proteins for CEMx174-derived HIV-1 virions, whereas OptiPrep purification resulted in significant reductions of 8 proteins (Table 1). Similar results were observed for H9 cells (data not shown). Many proteins that were reduced in quantity for either DM or OptiPrep purification have been shown to be in microvesicles;²⁵ the greater reduction in proteins using DM purification suggests that this method is a more stringent purification measure than OptiPrep purification. However, many of the reduced proteins have also been shown to be incorporated into HIV virions and we cannot rule out the loss of a subset of viral particles in either purification method.⁶

iTRAQ Analyses Can Differentiate HIV-1 Virions Derived from a T-cell Line and a B-cell/T-cell Hybrid Cell Line

Viral stocks produced from different cell lines displayed unique phenotypes, and virion composition reflected the progenitor cell type. DM purified viral stocks derived from CEMx174 and H9 cells were compared. Table 2 shows that 15 proteins can be used to differentiate between the cell lines. Notably, virions produced from CEMx174, which is a T-

cell/B-cell hybrid,²⁶ contained higher levels of CD48 antigen precursor, a marker of B-cell activation.²⁷

Shotgun Analysis of DM Modified HIV-1 Identifies 283 Host Proteins

While our iTRAQ experiments provided us with a powerful method of determining our relative purification efficiency, no multidimensional protein separation strategies were used for this experiment. Therefore, to extend our coverage of the DM HIV proteome, we performed HPLC separation of DM-HIV-1, and collected 37 fractions that were then subject to analysis by MS/MS on LCQ-duo equipped with an Agilent nano-HPLC system. The resulting spectra were pooled and searched on our MS-analysis pipeline. We found that the peptides were assigned to >1800 proteins, including redundant assignments; these proteins clustered to 283 individual host proteins using a spectrum false discovery rate of 5%.

MDM and T-cell-derived HIV-1 Virions Incorporate a Limited Number of Shared Host Proteins

To determine which host proteins were incorporated to the virion both in T- and in macrophage-cell types, we compared our T-cell data set to the MDM-derived HIV-1 data set generated by Chertova and colleagues.^{2a} To ensure that the data sets were comparable, the Chertova data set was reanalyzed using our data analysis pipeline.²⁸ 136 (38 clustered) proteins were unique to the macrophage derived virus, and 1339 (241 clustered) proteins were unique to the T-cell derived virus. 544 (79 clustered) proteins were shared between the MDM derived and T-cell derived data set (Supplemental Figure 1, Supporting Information). Of the 79 common proteins, many of these proteins were isoforms of the same protein with different peptides identified (Table 3). The majority of these proteins were distinct actin isoforms. Other proteins of note were ERM proteins, the dynamin domain containing protein EH4, a phosphodiesterase, CypA and heat shock proteins. The only conserved membrane proteins identified were syntenin-1 (a TEM protein) and CD44 (hyaluronic acid receptor), a marker presently used in commercial kits to enrich HIV.

As the conserved set of proteins may represent important cellular partners for the HIV virion, we conducted a literature search to determine whether the identified host proteins have been reported to be relevant in the HIV lifecycle and interact with viral proteins (Table 4). Out of the 26 protein clusters reported, 16 have previously been described in association with HIV-1, and 10 represent previously undefined associations.

To determine if the conserved set of proteins between MDM and T-cell derived HIV-1 could be used to reconstruct the protein composition of each virion, we seeded the GeneMANIA human network database with the core set of proteins and allowed for the 1000 most-related interacting partners. We found that 29 and 53% of host proteins from T-cell derived or MDM derived HIV-1, respectively, could be explained by primary interactions with the conserved set.

DISCUSSION

Using a novel HIV purification assay we have found a common set of host proteins that are incorporated into virions produced from monocyte-derived macrophages (MDMs) and T-

cells. DM purification modifies the density of microvesicles, allowing for the purification of large quantities of microvesicle-free viral stocks. This method may not be necessary for virion purification from infected MDMs, since these cells have longer half-lives than T-cells, allowing for higher virion yields, and MDMs produce a lower level of contaminating microvesicles compared to lymphocytes.^{2a,5} We found that DM clears >90% of microvesicles using CD45 as a marker protein (by densitometry, not shown). We compared this method to the OptiPrep (60% iodixanol) method for microvesicle-free HIV-1 purification.⁹ Results were normalized by CypA, as CypA has been reported to be incorporated into viruses in an approximately 1:10 ratio to gag particles.^{4a,b} While using a viral protein for normalization might seem like an adequate normalization tool, viral protein sequence divergence and differential processing by progenitor cell type make it difficult to normalize by these proteins. Both OptiPrep and DM purification methods produce consistent results, reducing levels of proteins known to be incorporated in microvesicles. DM purification proved to be a more stringent approach, as there was a greater reduction in CypA levels coupled with a higher number of significantly reduced proteins compared to OptiPrep methods. Ott and colleagues developed a purification technique based on similar principles, in which proteins in microvesicles are digested with the nonspecific serine protease subtilisin.^{8a} The subtilisin digestion decreases microvesicle density, allowing for purification of HIV particles by density gradients, allowing for >95% purification of virions.⁷ Subtilisin treatment digests membrane proteins, though, and is only suitable for determining the composition of proteins inside the virion.^{8a}

We cannot rule out that DM purification also modifies viral composition in some manner, as cholesterol has been reported to be an integral component of the viral membrane, and that this may account for some of the protein reduction.^{10,22} Notably, electron micrographs of DM-treated virions show some membrane irregularities, which could impact protein composition of the purified viral stocks. This may explain the absence of some host membrane proteins which have been reported to be incorporated into HIV virions. Of note, tetraspanin proteins were not detected in our analysis. This may not be surprising, though, given that tetraspanin interactions are affected by cholesterol and the DM assay may have disrupted TEMs.²⁹ It is of note that very few membrane-bound proteins were observed in the common set of proteins, particularly given that TEMs have been shown to be of importance in HIV-1 biology.³⁰ We did detect the PDZ-containing protein syntenin-1 in both MDM- and T-cell-derived virions. Syntenin-1 has been shown to have a large variety of interaction partners, including syndecan, Rab 5, Rab 7, CD63, and phosphoinositol lipids. Many of the partners for syntenin-1 are involved in membrane trafficking, including tetraspanin and TEM-associated proteins.³¹ Since TEM components are frequently reported in the viral envelope, but were not detected in this analysis, it is possible that syntenin-1 is involved in the HIV-1-TEM interaction, and that the syntenin-1-TEM interactions were disrupted by our purification process. This may implicate syntenin-1 as an important mediator of viral envelope composition. However, preliminary data with siRNA knockdown of syntenin-1 in HIV-infected Jurkat cells has not demonstrated any effect on virion production (data not shown), so the importance of syntenin-1 in the HIV lifecycle remains speculative.

The incorporation of CD44, a receptor for hyaluronic acid, also provides potential fitness benefits for a lentivirus. HIV replicates in activated T-cells, so an attachment to hyaluronic acid may allow the virus to target areas of inflammation, as CD44 induction is a first step of immune activation and also involved in T-cell trafficking.³² Notably, it has been reported that CD44 cell-surface expression is lost in HIV-infected monocytic cell lines, resulting in cell aggregation.³³

Using quantitative proteomic analyses on DM-purified input, we were able to differentiate between viral stocks prepared from the H9 T-cell line and the CEMx174 B-cell/T-cell hybrid line. There were several proteins which could differentiate between the two stocks, including the B-cell activation marker CD48 precursor, which is not unexpected considering the cell line origins.²⁷ These results indicate that proteomic analysis in combination with several purification techniques can be used to differentiate viral stocks from multiple sources. These methods may be applicable to identifying the cell source of virus produced from latently infected cells. These methods may also be used to differentiate between viruses from various cellular reservoirs within the host.

We further compared DM purified viral stocks from H9 cells to a published database of MDM-derived viral stocks. To increase the validity of this comparison, raw data from the Chertova study collected using similar MS instrumentation employed in the current study was reanalyzed using the bioinformatics pipeline developed by our group. This method ensured that both data sets were analyzed using the same stringent search criteria. Using this comparison, we identified a common set of 26 proteins that are incorporated into HIV virions produced in both MDM and T-cell lines. As these proteins are incorporated in virions produced in both cell types, we hypothesize that these proteins may have direct interactions with viral proteins or may be important in the viral lifecycle.

The base host protein composition in virions is mainly actin, chaperones, CypA and a handful of other proteins. Many of these have been shown to have functional impact on the HIV lifecycle. The importance of CypA on the viral lifecycle has been well documented.³⁴ CypA is believed to regulate the capsid interaction with host factors, either during uncoating or during other lifecycle processes.³⁵ The protease $\alpha 1$ -antitrypsin (SERPINA1; AAT) blocks protease cleavage of gp160 and gag polyprotein. Thus, it is not surprising that HIV protease binds and cleaves AAT, and this binding may account for the incorporation of AAT in HIV particles.³⁶ Our results suggest that HIV-1 virions have evolved to target a pathway enriched in ERM family proteins and vesicle trafficking, based on the conserved incorporation of EHD4 in T-cell and MDM derived virions. EHD4 has been shown to regulate transport from the early endosome to the recycling endosome and the late endocytic pathway.³⁷ EH domains bind Rab proteins, which are known to interact with the HIV protein rev.³⁸ A recent study has suggested a role of HSP90AB in HIV replication. Inhibition of HSP90AB resulted in anti-HIV activity *in vitro*, with ritonavir-resistant viruses showing hypersensitivity to the inhibitor.³⁹ Another agent with anti-HIV activity was found to bind HSP90AB and prevent dimerization.⁴⁰ Annexin A2, a protein involved in membrane trafficking, likely bridges the gap between the cytoskeletal proteins and the viral membrane. Annexin A2 binds HIV gag and siRNA knockdown of the protein can reduce infectivity of virions generated in MDMs, but this may be cell type dependent.⁴¹

Other identified proteins, including cytoskeletal and HLA proteins have repeatedly been reported in the literature as interacting with HIV proteins.^{2a,8a,42} Further, many of the proteins identified in this study, including actin, 2',3'-cyclic-nucleotide 3'-phosphodiesterase, CypA, EEF1A-1, ezrin, annexin 2, HSP70, and HSC71, have also been identified in a quantitative proteomic analysis of an HIV-1 lentivirus vector produced in 293T-cells.⁴³ Thus, this common set of proteins identified in this study is recapitulated by findings by other groups.

Given the large number of host proteins incorporated into the virus and the limited number of viral proteins, it is plausible that only a few specific interactions between virus and host proteins allows it to package a large array of host proteins. Host proteins that have a direct interaction with HIV proteins would serve as protein hubs. The 26 proteins identified to be common to the two different cell types are predicted to interact with >1000 related human proteins. Many of these secondary interacting proteins are commonly reported to be incorporated into virions, including ERM proteins and adhesion molecules. By assigning query based network weights (GeneMANIA, see methods), these associational proteins are predicted to interact with 62% and 38% of the proteins common to T-cell and MDM derived HIV-1, respectively. However, it is important to note that many more T-cell derived proteins were identified than MDM HIV-1 derived proteins, so this may skew this analysis. Additionally, protein prediction network algorithms are based on protein-protein interactions; other interactions (e.g., lipid or nucleic acid mediated) are not modeled. Thus, only a few direct interactions within the virus may dictate the host protein composition in nearly limitless dimensions. Ultimately, the host protein composition, as well as interaction differences between cell types, may drive virion phenotypic diversity, despite conserved viral protein-host protein interactions between cell types (Figure 2). While we do not intend to minimize the functional importance of other host proteins incorporated into HIV-1 outside of this minimal set of proteins, it is likely that therapeutic strategies targeting proteins other than these core proteins would result in limited efficacy due to the high degree of plasticity apparent with HIV. Therefore, we would propose that therapeutic or drug development efforts targeting host-virus interactions be focused on interacting proteins showing direct interaction with HIV proteins that are conserved between T-cells and macrophages.

Finally, this study demonstrates the critical nature of harmonized data analysis when making interstudy protein comparisons. Existing studies have demonstrated the lab-to-lab and instrument-to-instrument variability in proteomics studies of identical samples, as well as search results from different search algorithms.⁴⁴ Our reanalysis of the historical data from Chertova et al. using current FDR-driven statistical analysis resulted in a truncated list of virally incorporated host proteins, comparable to what we observed experimentally in our work. This demonstrates the need for archiving of instrument raw data files so they may be subject to reinterpretation as bioinformatics improvements are developed, and highlights the danger of making protein comparisons from tables in the published literature, particularly with regard older data that was not filtered by FDR or another stringent statistical measure.

CONCLUSIONS

Using a proteomic analysis approach, this study identifies proteins that are incorporated into the virus in multiple cell types, and many of these proteins have been shown to be relevant to the HIV lifecycle. These proteins may represent important conserved interactions and, therefore, could be targets for interventional strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge funding from the NHLBI under contract number NHLBI-HV-10-05_(2) that helped to support the development of the bioinformatics infrastructure used in these analysis. We would also like to acknowledge the NIH for funding under grant number P01 MH070306, and to the Johns Hopkins University Dean's office (startup funds to DG). We acknowledge Simon Sheng and the Van Eyk Laboratory for assistance with the HPLC separation of HIV for these studies. Electron micrographs were performed in the laboratory of Kunio Nagashima at NCI-Frederick. The author's also gratefully acknowledge Drs. Ott and Veenstra for providing the original RAW files for reanalysis. The authors wish to thank Dr. Rhoel Dinglasan for his support of shared mass spectrometry resources.

REFERENCES

- (1). von Schwedler UK, Stuchell M, Muller B, Ward DM, Chung HY, Morita E, Wang HE, Davis T, He GP, Cimbara DM, Scott A, Krausslich HG, Kaplan J, Morham SG, Sundquist WI. The protein network of HIV budding. *Cell*. 2003; 114(6):701–13. [PubMed: 14505570]
- (2) (a). Chertova E, Chertov O, Coren LV, Roser JD, Trubey CM, Bess JW Jr. Sowder RC 2nd, Barsov E, Hood BL, Fisher RJ, Nagashima K, Conrads TP, Veenstra TD, Lifson JD, Ott DE. Proteomic and biochemical analysis of purified human immunodeficiency virus type 1 produced from infected monocyte-derived macrophages. *J. Virol*. 2006; 80(18):9039–52. [PubMed: 16940516] (b) Guo MM, Hildreth JE. HIV acquires functional adhesion receptors from host cells. *AIDS Res. Hum. Retroviruses*. 1995; 11(9):1007–13. [PubMed: 8554897] (c) Graham DR, Mitsak MJ, Elliott ST, Chen D, Whelan SA, Hart GW, Van Eyk JE. Two-dimensional gel-based approaches for the assessment of N-Linked and O-GlcNAc glycosylation in human and simian immunodeficiency viruses. *Proteomics*. 2008; 8(23–24):4919–30. [PubMed: 19072736]
- (3) (a). Liao Z, Roos JW, Hildreth JE. Increased infectivity of HIV type 1 particles bound to cell surface and solid-phase ICAM-1 and VCAM-1 through acquired adhesion molecules LFA-1 and VLA-4. *AIDS Res. Hum. Retroviruses*. 2000; 16(4):355–66. [PubMed: 10716373] (b) Hioe CE, Bastiani L, Hildreth JE, Zolla-Pazner S. Role of cellular adhesion molecules in HIV type 1 infection and their impact on virus neutralization. *AIDS Res. Hum. Retroviruses*. 1998; 14(Suppl 3):S247–54. [PubMed: 9814951]
- (4) (a). Franke EK, Yuan HE, Luban J. Specific incorporation of cyclophilin A into HIV-1 virions. *Nature*. 1994; 372(6504):359–62. [PubMed: 7969494] (b) Thali M, Bukovsky A, Kondo E, Rosenwirth B, Walsh CT, Sodroski J, Gottlinger HG. Functional association of cyclophilin A with HIV-1 virions. *Nature*. 1994; 372(6504):363–5. [PubMed: 7969495] (c) Luban J, Bossolt KL, Franke EK, Kalpana GV, Goff SP. Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell*. 1993; 73(6):1067–78. [PubMed: 8513493]
- (5) (a). Bess JW Jr. Gorelick RJ, Bosche WJ, Henderson LE, Arthur LO. Microvesicles are a source of contaminating cellular proteins found in purified HIV-1 preparations. *Virology*. 1997; 230(1): 134–44. [PubMed: 9126269] (b) Gluschkof P, Mondor I, Gelderblom HR, Sattentau QJ. Cell membrane vesicles are a major contaminant of gradient-enriched human immunodeficiency virus type-1 preparations. *Virology*. 1997; 230(1):125–33. [PubMed: 9126268]
- (6). Gould SJ, Booth AM, Hildreth JE. The Trojan exosome hypothesis. *Proc. Natl. Acad. Sci. U.S.A.*. 2003; 100(19):10592–7. [PubMed: 12947040]

- (7). Ott DE. Purification of HIV-1 virions by subtilisin digestion or CD45 immunoaffinity depletion for biochemical studies. *Methods Mol. Biol.* 2009; 485:15–25. [PubMed: 19020815]
- (8) (a). Ott DE, Coren LV, Kane BP, Busch LK, Johnson DG, Sowder RC 2nd, Chertova EN, Arthur LO, Henderson LE. Cytoskeletal proteins inside human immunodeficiency virus type 1 virions. *J. Virol.* 1996; 70(11):7734–43. [PubMed: 8892894] (b) Ott DE, Coren LV, Johnson DG, Kane BP, Sowder RC 2nd, Kim YD, Fisher RJ, Zhou XZ, Lu KP, Henderson LE. Actin-binding cellular proteins inside human immunodeficiency virus type 1. *Virology.* 2000; 266(1):42–51. [PubMed: 10612659]
- (9). Cantin R, Diou J, Belanger D, Tremblay AM, Gilbert C. Discrimination between exosomes and HIV-1: purification of both vesicles from cell-free supernatants. *J. Immunol. Methods.* 2008; 338(1–2):21–30. [PubMed: 18675270]
- (10). Graham DR, Chertova E, Hilburn JM, Arthur LO, Hildreth JE. Cholesterol depletion of human immunodeficiency virus type 1 and simian immunodeficiency virus with beta-cyclodextrin inactivates and permeabilizes the virions: evidence for virion-associated lipid rafts. *J. Virol.* 2003; 77(15):8237–48. [PubMed: 12857892]
- (11). Guo Y, Singleton PA, Rowshan A, Gucek M, Cole RN, Graham DR, Van Eyk JE, Garcia JG. Quantitative proteomics analysis of human endothelial cell membrane rafts: evidence of MARCKS and MRP regulation in the sphingosine 1-phosphate-induced barrier enhancement. *Mol. Cell. Proteomics.* 2007; 6(4):689–96. [PubMed: 17210631]
- (12). Sheng S, Chen D, Van Eyk JE. Multidimensional liquid chromatography separation of intact proteins by chromatographic focusing and reversed phase of the human serum proteome: optimization and protein database. *Mol. Cell. Proteomics.* 2006; 5(1):26–34. [PubMed: 16188874]
- (13). McDonald T, Sheng S, Stanley B, Chen D, Ko Y, Cole RN, Pedersen P, Van Eyk JE. Expanding the subproteome of the inner mitochondria using protein separation technologies: one- and two-dimensional liquid chromatography and two-dimensional gel electrophoresis. *Mol. Cell. Proteomics.* 2006; 5(12):2392–411. [PubMed: 17000643]
- (14). Edwards N, Wu X, Tseng C-W. An Unsupervised, Model-Free, Machine-Learning Combiner for Peptide Identifications from Tandem Mass Spectra. *Clin. Proteomics.* 2009; 5(1):23–36.
- (15). Parish LA, Colquhoun DR, Ubaida Mohien C, Lyashkov AE, Graham DR, Dinglasan RR. Ookinete-interacting proteins on the microvillar surface are partitioned into detergent resistant membranes of *Anopheles gambiae* midguts. *J. Proteome Res.* 2011; 10(11):5150–62. [PubMed: 21905706]
- (16). Hall M, Frank E, Holmes G, Pfahringer B, Reutemann P, Witten IH. The WEKA Data Mining Software: An Update. *SIGKDD Explorations Newslett.* 2009; 11(1):10–8.
- (17). Ubaida Mohien C, Hartler J, Breitwieser F, Rix U, Remsing Rix L, Winter GE, Thallinger GG, Bennett KL, Superti-Furga G, Trajanoski Z, Colinge J. MASPECTRAS 2: An integration and analysis platform for proteomic data. *Proteomics.* 2010; 10(14):2719–22. [PubMed: 20455215]
- (18). Taylor CF, Paton NW, Lilley KS, Binz PA, Julian RK Jr, Jones AR, Zhu W, Apweiler R, Aebersold R, Deutsch EW, Dunn MJ, Heck AJ, Leitner A, Macht M, Mann M, Martens L, Neubert TA, Patterson SD, Ping P, Seymour SL, Souda P, Tsugita A, Vandekerckhove J, Vondrisk TM, Whitelegge JP, Wilkins MR, Xenarios I, Yates JR 3rd, Hermjakob H. The minimum information about a proteomics experiment (MIAPE). *Nat. Biotechnol.* 2007; 25(8): 887–93. [PubMed: 17687369]
- (19). Vizcaino JA, Cote R, Reisinger F, Barsnes H, Foster JM, Rameseder J, Hermjakob H, Martens L. The Proteomics Identifications database: 2010 update. *Nucleic Acids Res.* 2010; 38(Database issue):D736–42. [PubMed: 19906717]
- (20). Montojo J, Zuberi K, Rodriguez H, Kazi F, Wright G, Donaldson SL, Morris Q, Bader GD. GeneMANIA Cytoscape plugin: fast gene function predictions on the desktop. *Bioinformatics.* 2010; 26(22):2927–8. [PubMed: 20926419]
- (21) (a). Ptak RG, Fu W, Sanders-Beer BE, Dickerson JE, Pinney JW, Robertson DL, Rozanov MN, Katz KS, Maglott DR, Pruitt KD, Dieffenbach CW. Cataloguing the HIV type 1 human protein interaction network. *AIDS Res. Hum. Retroviruses.* 2008; 24(12):1497–502. [PubMed: 19025396] (b) Fu W, Sanders-Beer BE, Katz KS, Maglott DR, Pruitt KD, Ptak RG. Human immunodeficiency virus type 1, human protein interaction database at NCBI. *Nucleic Acids Res.*

- 2009; 37(Database issue):D417–22. [PubMed: 18927109] (c) Pinney JW, Dickerson JE, Fu W, Sanders-Beer BE, Ptak RG, Robertson DL. HIV-host interactions: a map of viral perturbation of the host system. *AIDS*. 2009; 23(5):549–54. [PubMed: 19262354]
- (22). Liao Z, Graham DR, Hildreth JE. Lipid rafts and HIV pathogenesis: virion-associated cholesterol is required for fusion and infection of susceptible cells. *AIDS Res. Hum. Retroviruses*. 2003; 19(8):675–87. [PubMed: 13678470]
- (23). Breitwieser FP, Muller A, Dayon L, Kocher T, Hainard A, Pichler P, Schmidt-Erfurth U, Superti-Furga G, Sanchez JC, Mechtler K, Bennett KL, Colinge J. General statistical modeling of data from protein relative expression isobaric tags. *J. Proteome Res*. 2011; 10(6):2758–66. [PubMed: 21526793]
- (24) (a). Yu X, Harris SL, Levine AJ. The regulation of exosome secretion: a novel function of the p53 protein. *Cancer Res*. 2006; 66(9):4795–801. [PubMed: 16651434] (b) Welton JL, Khanna S, Giles PJ, Brennan P, Brewis IA, Staffurth J, Mason MD, Clayton A. Proteomics analysis of bladder cancer exosomes. *Mol. Cell. Proteomics*. 2010; 9(6):1324–38. [PubMed: 20224111]
- (25) (a). Buschow SI, van Balkom BW, Aalberts M, Heck AJ, Wauben M, Stoorvogel W. MHC class II-associated proteins in B-cell exosomes and potential functional implications for exosome biogenesis. *Immunol. Cell Biol*. 2010; 88(8):851–6. [PubMed: 20458337] (b) Escola JM, Kleijmeer MJ, Stoorvogel W, Griffith JM, Yoshie O, Geuze HJ. Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes. *J. Biol. Chem*. 1998; 273(32):20121–7. [PubMed: 9685355] (c) Staubach S, Razawi H, Hanisch FG. Proteomics of MUC1-containing lipid rafts from plasma membranes and exosomes of human breast carcinoma cells MCF-7. *Proteomics*. 2009; 9(10):2820–35. [PubMed: 19415654]
- (26). Salter RD, Howell DN, Cresswell P. Genes regulating HLA class I antigen expression in T-B lymphoblast hybrids. *Immunogenetics*. 1985; 21(3):235–46. [PubMed: 3872841]
- (27). Klyushnenkova EN, Li L, Armitage RJ, Choi YS. CD48 delivers an accessory signal for CD40-mediated activation of human B cells. *Cell Immunol*. 1996; 174(1):90–8. [PubMed: 8929458]
- (28). Ubaida Mohien C, Colquhoun DR, Mathias DK, Gibbons JG, Armistead JS, Rodriguez MC, Rodriguez MH, Edwards NJ, Hartler J, Thallinger GG, Graham DR, Martinez-Barnette J, Rokas A, Dinglasan RR, Bioinformatics A. Approach for Integrated Transcriptomic and Proteomic Comparative Analyses of Model and Non-sequenced Anopheline Vectors of Human Malaria Parasites. *Mol. Cell. Proteomics*. 2013; 12(1):120–31. [PubMed: 23082028]
- (29). Charrin S, Manie S, Thiele C, Billard M, Gerlier D, Boucheix C, Rubinstein E. A physical and functional link between cholesterol and tetraspanins. *Eur. J. Immunol*. 2003; 33(9):2479–89. [PubMed: 12938224]
- (30) (a). Kremontsov DN, Weng J, Lambele M, Roy NH, Thali M. Tetraspanins regulate cell-to-cell transmission of HIV-1. *Retrovirology*. 2009; 6:64. [PubMed: 19602278] (b) Weng J, Kremontsov DN, Khurana S, Roy NH, Thali M. Formation of syncytia is repressed by tetraspanins in human immunodeficiency virus type 1-producing cells. *J. Virol*. 2009; 83(15):7467–74. [PubMed: 19458002] (c) Grigorov B, Attuill-Audenis V, Perugi F, Nedelec M, Watson S, Pique C, Darlix JL, Conjeaud H, Muriaux D. A role for CD81 on the late steps of HIV-1 replication in a chronically infected T cell line. *Retrovirology*. 2009; 6:28. [PubMed: 19284574]
- (31) (a). Yanez-Mo M, Barreiro O, Gordon-Alonso M, Sala-Valdes M, Sanchez-Madrid F. Tetraspanin-enriched microdomains: a functional unit in cell plasma membranes. *Trends Cell Biol*. 2009; 19(9):434–46. [PubMed: 19709882] (b) Beekman JM, Coffey PJ. The ins and outs of syntenin, a multifunctional intracellular adaptor protein. *J. Cell Sci*. 2008; 121(Pt 9):1349–55. [PubMed: 18434645]
- (32). Johnson P, Ruffell B. CD44 and its role in inflammation and inflammatory diseases. *Inflamm. Allergy Drug Targets*. 2009; 8(3):208–20. [PubMed: 19601881]
- (33). Guo MM, Hildreth JE. HIV-induced loss of CD44 expression in monocytic cell lines. *J. Immunol*. 1993; 151(4):2225–36. [PubMed: 8345206]
- (34). Luban J. Cyclophilin A, TRIM5, and resistance to human immunodeficiency virus type 1 infection. *J. Virol*. 2007; 81(3):1054–61. [PubMed: 16956947]
- (35). Strebel K, Luban J, Jeang KT. Human cellular restriction factors that target HIV-1 replication. *BMC Med*. 2009; 7:48. [PubMed: 19758442]

- (36) (a). Cordelier P, Strayer DS. Mechanisms of alpha1-antitrypsin inhibition of cellular serine proteases and HIV-1 protease that are essential for HIV-1 morphogenesis. *Biochim. Biophys. Acta*. 2003; 1638(3):197–207. [PubMed: 12878320] (b) Shapiro L, Pott GB, Ralston AH. Alpha-1-antitrypsin inhibits human immunodeficiency virus type 1. *FASEB J*. 2001; 15(1):115–22. [PubMed: 11149899] (c) Anderson ED, Thomas L, Hayflick JS, Thomas G. Inhibition of HIV-1 gp160-dependent membrane fusion by a furin-directed alpha 1-antitrypsin variant. *J. Biol. Chem*. 1993; 268(33):24887–91. [PubMed: 8227051]
- (37) (a). George M, Ying G, Rainey MA, Solomon A, Parikh PT, Gao Q, Band V, Band H. Shared as well as distinct roles of EHD proteins revealed by biochemical and functional comparisons in mammalian cells and *C. elegans*. *BMC Cell Biol*. 2007; 8:3. [PubMed: 17233914]
- (b). Sharma M, Naslavsky N, Caplan S. A role for EHD4 in the regulation of early endosomal transport. *Traffic*. 2008; 9(6):995–1018. [PubMed: 18331452]
- (38). Salcini AE, Confalonieri S, Doria M, Santolini E, Tassi E, Minenkova O, Cesareni G, Pelicci PG, Di Fiore PP. Binding specificity and in vivo targets of the EH domain, a novel protein-protein interaction module. *Genes Dev*. 1997; 11(17):2239–49. [PubMed: 9303539]
- (39). Joshi P, Stoddart CA. Impaired infectivity of ritonavir-resistant HIV is rescued by heat shock protein 90AB1. *J. Biol. Chem*. 2011; 286(28):24581–92. [PubMed: 21602280]
- (40). Vozzolo L, Loh B, Gane PJ, Tribak M, Zhou L, Anderson I, Nyakatura E, Jenner RG, Selwood D, Fassati A. Gyrase B inhibitor impairs HIV-1 replication by targeting Hsp90 and the capsid protein. *J. Biol. Chem*. 2010; 285(50):39314–28. [PubMed: 20937817]
- (41) (a). Ryzhova EV, Vos RM, Albright AV, Harrist AV, Harvey T, Gonzalez-Scarano F. Annexin 2: a novel human immunodeficiency virus type 1 Gag binding protein involved in replication in monocyte-derived macrophages. *J. Virol*. 2006; 80(6):2694–704. [PubMed: 16501079] (b) Harrist AV, Ryzhova EV, Harvey T, Gonzalez-Scarano F. Anx2 interacts with HIV-1 Gag at phosphatidylinositol (4,5) bisphosphate-containing lipid rafts and increases viral production in 293T cells. *PLoS One*. 2009; 4(3):e5020. [PubMed: 19325895] Rai T, Mosoian A, Resh MD. Annexin 2 is not required for human immunodeficiency virus type 1 particle production but plays a cell type-dependent role in regulating infectivity. *J. Virol*. 2010; 84(19):9783–92. [PubMed: 20631122]
- (42) (a). Trubey CM, Chertova E, Coren LV, Hilburn JM, Hixson CV, Nagashima K, Lifson JD, Ott DE. Quantitation of HLA class II protein incorporated into human immunodeficiency type 1 virions purified by anti-CD45 immunoaffinity depletion of microvesicles. *J. Virol*. 2003; 77(23):12699–709. [PubMed: 14610192] (b) Esser MT, Graham DR, Coren LV, Trubey CM, Bess JW Jr, Arthur LO, Ott DE, Lifson JD. Differential incorporation of CD45, CD80 (B7–1), CD86 (B7–2), and major histocompatibility complex class I and II molecules into human immunodeficiency virus type 1 virions and microvesicles: implications for viral pathogenesis and immune regulation. *J. Virol*. 2001; 75(13):6173–82. [PubMed: 11390619]
- (43). Denard J, Rundwasser S, Laroudie N, Gonnet F, Naldini L, Radrizzani M, Galy A, Merten OW, Danos O, Svinartchouk F. Quantitative proteomic analysis of lentiviral vectors using 2-DE. *Proteomics*. 2009; 9(14):3666–76. [PubMed: 19639585]
- (44) (a). Falick AM, Lane WS, Lilley KS, MacCoss MJ, Phinney BS, Sherman NE, Weintraub ST, Witkowska HE, Yates NA. ABRF-PRG07: advanced quantitative proteomics study. *J. Biomol. Tech*. 2011; 22(1):21–6. [PubMed: 21455478] (b) Bell AW, Deutsch EW, Au CE, Kearney RE, Beavis R, Sechi S, Nilsson T, Bergeron JJ. A HUPO test sample study reveals common problems in mass spectrometry-based proteomics. *Nat. Methods*. 2009; 6(6):423–30. [PubMed: 19448641]

Figure 1.

Purification of HIV-1 virions using density modification. (A) DM HIV-1 purity was assessed using CD45, a marker of vesicle contamination. Isolated microvesicles (MV) from the (B) H9 or (C) Hut78 cell line were eliminated with this method (>90% of material was lost). (D, E) HIV-1 infectivity was unchanged (not shown), but virion morphology was substantially altered.

Figure 2.

Hypothetical schematic of the impact of primary HIV-host protein interactions on virion phenotype. HIV proteins interact with a common set of host proteins that is found in multiple cell types capable of sustaining HIV infection. These common set proteins have secondary and tertiary interactions with both cell-specific and common protein partners and these interactions determine the phenotype of released virions. Thus, despite a limited number of HIV-host protein interactions, viral diversity is driven by the secondary and higher interactions based on cell-type.

Table 1

Reduced Proteins in HIV-1 Derived from CEMX174 Cells Following Virion Purification using DM or Optiprep

% of sequence coverage	accession number	protein name	peptides (95%)	DM:control	P-value	Optiprep: control	P-value
42.9	P63104	14-3-3 protein zeta/delta	6	0.5151	0.0371	0.7533	0.0613
56.5	P63261	Actin, cytoplasmic 2	29	0.5099	0.0013	0.92	0.1083
41	P06733	Alpha-enolase <i>Homo sapiens</i> (Human)	9	0.5058	0.0001	0.7502	0.0038
33.3	P07355	Annexin A2	9	0.4459	0.0015	0.8047	0.0188
61.2	P80723	Brain acid soluble protein 1	7	0.523	0.0409	0.7312	0.0264
23.5	P09326	CD48 antigen precursor (B-lymphocyte activation marker BLAST-1)	5	0.4887	0.005	0.949	0.5258
41.5	O00299	Chloride intracellular channel protein 1	5	0.5008	0.0012	1.0572	0.5918
28	P31146	Coronin-1A	4	0.5112	0.0003	0.8208	0.011
30.1	P68104	Elongation factor 1-alpha 1	8	0.5466	0.0006	0.9394	0.4253
50	P15311	Ezrin (p81)	12	0.5889	<0.0001	0.8771	0.0974
26.3	P04406	Glyceraldehyde-3-phosphate dehydrogenase	3	0.4113	0.0231	0.8528	0.5458
51.2	P11142	Heat shock cognate 71 kDa protein	18	0.5481	0.0042	0.9216	0.5634
29.1	P08238	Heat shock protein HSP 90-beta	8	0.5316	0.003	1.0241	0.8397
46.6	P30453	HLA class I histocompatibility antigen, A-34 alpha chain precursor	14	0.6886	0.0117	0.9655	0.7305
31.1	P01903	HLA class II histocompatibility antigen, DR alpha chain precursor	7	0.4474	0.007	0.9419	0.4304
52.6	P13760	HLA class II histocompatibility antigen, DRB1-4 beta chain precursor	7	0.5259	0.0458	0.9599	0.7407
41.7	P13761	HLA class II histocompatibility antigen, DRB1-7 beta chain precursor	7	0.5595	0.001	0.9605	0.6705
25.9	P05362	Intercellular adhesion molecule 1 precursor	3	0.5685	0.0489	0.8179	0.1331
57.5	P26038	Moesin	19	0.5428	0.0012	0.9082	0.0732
18.4	Q09666	Neuroblast differentiation-associated protein AHNK	2	0.4589	0.0143	0.7738	0.025
19.2	P43007	Neutral amino acid transporter A	5	0.3368	0.0098	0.8818	0.5752
32.2	Q06830	Peroxisomal protein PEX1	5	0.3815	0.0157	0.7814	0.0282
23	P13796	Plastin-2 (L-plastin)	4	0.5221	0.0042	0.8782	0.1899
16.4	Q8WUM4	Programmed cell death 6-interacting protein	2	0.5772	0.0397	0.8987	0.5737
16.2	P30101	Protein disulfide-isomerase A3 precursor	1	0.4702	0.0423	0.7946	0.3432
23.4	P14618	Pyruvate kinase isozymes M1/M2	5	0.5098	>0.0001	0.926	0.2772
26.6	P15153	Ras-related C3 botulinum toxin substrate 2 precursor	2	0.5057	0.0433	0.963	0.811
24.5	P62834	Ras-related protein Rap-1A precursor	1	0.4817	0.0063	0.7928	0.0465
81.8	P62328	Thymosin beta-4	3	0.4805	0.0215	1.0323	0.7312
31.6	P61586	Transforming protein RhoA precursor	2	0.4166	0.0123	0.9393	0.5786
37.1	P67936	Tropomyosin alpha-4 chain	3	0.506	0.0248	0.8225	0.3176

% of sequence coverage	accession number	protein name	peptides (95%)	DM:control	<i>P</i> -value	Optiprep: control	<i>P</i> -value
17.6	Q9BQE3	Tubulin alpha-6 chain	3	0.4275	<i>0.0074</i>	0.8795	0.2247
72.4	P62988	Ubiquitin	3	0.4191	<i>0.0037</i>	0.9396	0.5418

Table 2

Proteins Able to Differentiate between HIV-1 Derived from CEMX174 Cells or H9 Cells

% of sequence coverage	accession number	protein name	peptides (95%)	CEM:H9	<i>P</i> value ^a
61.2	P80723	Brain acid soluble protein 1	7	6.1243	0.042
23.5	P09326	CD48 antigen precursor	5	1.8935	0.0377
45.8	P23528	Cofilin-1	6	2.1352	0.0266
29.4	P04075	Fructose-bisphosphate aldolase A	4	2.2855	0.0409
33	P04899	Guanine nucleotide-binding protein G(i), alpha-2 subunit	5	2.3068	0.0296
46.6	P30453	MHC class I antigen A ^a 34	14	3.3001	0.0184
16.9	P04233	HLA class II histocompatibility antigen gamma chain	2	4.2203	0.0346
47.9	P07737	Profilin-1	4	2.4426	0.0169
23.4	P14618	Pyruvate kinase isozymes M1/M2	5	1.8639	0.016
26.4	Q15286	Ras-related protein Rab-35	3	2.054	0.013
21	P05023	Sodium/potassium-transporting ATPase alpha-1 chain precursor	7	2.0799	0.0344
81.8	P62328	Thymosin beta-4	3	2.4172	0.0273
37.1	P67936	Tropomyosin alpha-4 chain	3	2.7548	0.0016
17.6	Q9BQE3	Tubulin alpha-6 chain	3	1.9302	0.0058
19.1	P07437	Tubulin beta chain	3	1.6285	0.0268

^a*P* < 0.05 cutoff.

Table 3

Identified Proteins Common to MDM- and T-cell Derived HIV-1 Virions

gene name	gene symbol	% of sequence coverage	no. of unique peptides	no. of unique spectra
2',3'-cyclic-nucleotide 3'-phosphodiesterase	CNP	11.17	5	7
6-phosphogluconate dehydrogenase, decarboxylating	Pgd	9.94	3	5
Actin (37 isoforms)	act1	12	827	8432
Alpha-1-antiproteinase	SERPINA1	32.22	17	38
Alpha-2-H	AHSG	25.35	9	16
Annexin A2	ANXA2	52.22	21	62
CD44 antigen	CD44	7.28	4	14
Cell division control protein 42 homologue	CDC42	36.65	6	26
EH domain-containing protein 4	EHD4	24.59	9	12
Elongation factor 1- α (4 isoforms)	eEF1a	10.9	6	78
Ezrin	EZR	18.44	9	23
Heat shock 70 kDa protein (11 isoforms)	HSPA	15.78	132	516
Heat shock cognate 71 kDa protein	HSPA8	39.63	26	126
Heat shock protein H	HSP90AB1	24.45	15	52
Hemoglobin fetal subunit beta		60.69	10	101
Hemoglobin subunit beta (3 isoforms)	HBB	27.9	4	18
HLA A (MHC class I)	HLA-C	19.13	10	39
HLA DR (MHC class II) (3 isoforms)	HLA-DRB1	21.06	5	28
Moesin	MSN	23.4	15	70
P (Ankyrin Repeat Containing)	POTEE	10.33	16	168
Peptidyl-prolyl cis-trans isomerase A (cyclophilin A)	PPIA	56.37	14	103
Phosphoglycerate kinase 1	PGK1	32.86	12	22
Pyruvate kinase isozymes M1/M2	PKM2	40.12	16	40
Ras-related C3 botulinum toxin substrate 2	Rac2	30.73	6	7
Syntenin-1	SDCBP	10.2	7	9
Ubiquitin (Fragment)		39.69	4	27

Table 4**HIV-1-Human Protein Interactions^a Reported in the Literature²¹**

Host protein	HIV protein interaction
2',3'-cyclic-nucleotide 3'-phosphodiesterase	Activated by: tat
Actin	Inhibited by (multiple isoforms): env Binds (multiple isoforms): gag, nef Interacts with (multiple isoforms): gag Associates with (multiple isoforms): nef rearrangement induced by (multiple isoforms): nef, tat Cleaves (multiple isoforms): pol Associates with (beta-actin): rev Downregulated by (multiple isoforms), upregulated by (beta actin): tat Polymerization enhanced by (gamma 1 propeptide): vpr
Alpha-1-antiproteinase	Interacts with: env
Annexin A2	Colocalizes with, interacts with (isoform 1): gag Downregulated by (isoform 2): tat
CD44	Downregulates: vpr
Cell division control protein 42	Inhibited by, interacts with, upregulated by: nef
Eukaryotic translation elongation factor 1 alpha 1	Inhibited by: gag Binds: gag, pol Interacts with: tat
Ezrin	Binds: env Interacts with: env, gag Incorporates: gag Upregulated by: vpr
heat shock protein 70 kDa	Interacts with (protein 5), upregulated by (multiple proteins), inhibits (multiple proteins): env Incorporated by (multiple proteins), stimulates (multiple proteins), inhibits (protein 8): gag Regulates (multiple proteins): tat Inhibits (protein 1a), binds (protein 1a), competes with (multiple proteins): vpr
MHC Class I	Interacts with, complexes with: env Binds: env, gag, nef, pol Upregulated by: env, tat Colocalizes with, inhibited by, modulated by: nef Downregulated by: nef, tat, vpu
MHC Class II	Associates with, incorporated by: env Upregulated by: env, tat Inhibited by, interacts with: env, nef Colocalizes with, relocalizes, relocalized by: gag Downregulated by: gag, nef, tat
Moesin	Binds, relocalized by: env Incorporated by: gag
Peptidyl-prolyl cis-trans isomerase A (cyclophilin A)	Inhibited by, required by: env Incorporated by, modulates, interacts with, stabilized by: gag Binds: gag, nef, vif Isomerizes: gag, vpr
Ras-related C3 botulinum toxin substrate 2	Interacts with: nef Activated by, downregulated by: tat
Syntenin-1	Upregulated by: env
Ubiquitin	Ubiquitinates: gag, rev, tat

^aNo interactions have been reported for 6-phosphogluconate dehydrogenase, decarboxylating; Alpha-2-H; EH domain-containing protein 4; HSP90AB; Hemoglobin fetal subunit; HBB; P (Ankyrin Repeat Containing); Phosphoglycerate kinase 1; Pyruvate kinase isozymes M1/M2.