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Identification of glycoproteins associated with HIV latently infected cells using quantitative glycoproteomics

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Abstract

HIV infection is not curable due to viral latency. Compelling reports suggest that there is a distinct profile of surface proteins that can be used for targeting latently infected cells. We have recently reported that glycoproteins were differentially secreted from HIV latently infected ACH-2 cells compared to the parental A3.01 cells. This finding suggests that glycophenotype might be different in these two cell lines. To determine the difference, the ACH-2 and A3.01 cell lines were subjected to a glycoproteomic analysis. A total number of 940 unique N-linked glycosite-containing peptides from 515 glycoproteins were identified. Among the glycoproteins, 365 and 104 were annotated as cell surface and membrane-associated proteins, respectively. Quantitative LC-MS/MS analysis revealed a change of 236 glycosite-containing peptides from 172 glycoproteins between the two cell lines without reactivation. Bioinformatic analysis suggests that cell adhesion, immune response, glycoprotein metabolic process, cell motion, and cell activation were associated with the changed proteins. After reactivation of latency, changes in glycosite-containing peptides were observed in both cell lines. The changed proteins suggest that cell migration, response to wounding and immune response might be impaired in reactivated latently infected cells. Glycoproteomics merits future application using primary cells to discover reveal mechanisms in HIV pathogenesis.

Keywords

Glycoproteins; Glycoproteomics; HIV; Label-free quantitation; Latency

1 Introduction

In 2013, it was estimated that 35 million people were living with HIV and there are approximately 2.1 million new infections annually worldwide (<http://www.cdc.gov/hiv/statistics/basics/>). HIV infection has now become a chronic disease due to highly active antiretroviral therapy (HAART) [1]. Lifelong therapy will require a significant amount of financial support for drugs and public health management and providing lifelong therapy to

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the large infected population may emerge as a challenge [2]. Thus it will be essential to develop a cure that will ultimately end the epidemics of HIV. HIV latency is considered as a major impediment to curing HIV [3]. The virus inserts its DNA into the host genome to become a provirus [4]. During latency, the provirus is transcriptionally silent with little or no viral protein expressed [5]. Consequently, the immune system fails to recognize and eliminate the latently infected cells in the human body [5, 6]. The latently infected cells, occasionally, can be reactivated, likely due to antigen stimulation, to produce infectious virus, which constantly fuel the chronic infection [5]. It is estimated that latently infected cells persist in the host with half-lives of 44 months resulting in an estimation of 60–70 years for HAART to cure HIV infection [7,8]. However, the size of latent reservoir may be up to 60-fold greater than previously estimated, suggesting that curing HIV becomes even harder [7].

Latently infected CD4⁺T cells in vivo are very rare (one in a million cells) [5,8]. This rare cell population impedes in-depth study of HIV latent infection [4]. To identify, isolate and target the rare latently infected cells in vivo, identifying glycosylated biomarkers of the cells is meritorious. Early study profiling plasma membrane proteome of the latently infected cells has identified latent infection associated proteins that could be targeted by drugs [9]. Recently, few cell surface proteins including CD2, PD-1, LAG-3, TIGIT, CTLA-4, BTLA, CD160, 2B4, TIM-3 have been studied to reveal their correlation with latently infected cells ex vivo [10, 11]. Association of CD2, PD-1, LAG-3, and TIGIT with latently infected cells suggests that the latent cells do have special characterization of glycoproteome being display on cell surface [10, 11]. Interestingly, all nine cell surface proteins investigated above are N-linked glycosylated proteins (glycoproteins). The specific change of these proteins during the establishment of latent infection remains to be elucidated. It is estimated that there are over 1000 cell surface glycoproteins that form a complex and dynamic cell phenotype [12]. It may not be practical to study the glycoproteome using antibody-based approaches such as flow cytometry and ELISA. It thus appears that interrogating the cell glycoproteome using high-throughput glycoproteomics would uncover specific glycoproteins for targeting latently infected cells and improve our understanding of HIV pathogenesis.

One of the current eradication strategies is to kill the latently infected cells following expression of viral proteins reactivated by different latency-reversing reagents [13]. The strategy is known as a “shock-and-kill” approach. However, it has been shown that the reactivated cells are not killed by viral cytopathic effect or CTL [14]. This is because, in part, that CTL is compromised in most HIV⁺ individuals and thus restimulation of broad CTL response is required to clear latent HIV-1 [6]. It is not clear whether the stimulated CTL response is long-lasting and broad-reacting enough to clear all latent reservoirs. The challenge to use shock-and-kill for a cure remains significantly great [15]. Thus, it might be more feasible to target latently infected cells without reactivation.

Cell surface proteins are commonly glycosylated and play various roles such as cell receptor, cell–cell communication, cell migration, and immune regulation [16]. In HIV neuropathogenesis, cell surface glycoproteins JAM-A and ALCAM are important for CD14⁺ CD16⁺ monocytes to cross the blood brain barrier that is an important step in the development of HIV associated neurocognitive disorders [17]. We have previously shown

that HIV infection caused changed secretion of glycoproteins from latently infected cells that might interplay with the immune system and contributed to significant change in the glycoprotein profile in sera from HIV+ people with acquired immune deficiency syndrome (AIDS), HAART-treatment or elite suppression [18, 19]. The changed secretion of glycoproteins from latently infected cells indicates that cellular glycoproteome may also change. To determine whether this is the case, we applied the same solid phase extraction of glycopeptides (SPEG) based glycoproteome to study the established model of latency ACH-2 and A3.01 cell lines [20]. The A3.01 cell is the parent cell line of ACH-2 and not a HIV latently infected T-cell line while ACH-2 harbors latent infection [21, 22]. In this study, N-linked glycosite-containing peptides were isolated by hydrazide chemistry and released using PNGase F [20]. The glycosite-containing peptides were subjected to label-free quantitative nano-LC-MS/MS analysis [18, 19]. Bioinformatic analysis was used to reveal roles of glycoproteins with changed glycosite-containing peptides.

2 Materials and methods

2.1 Chemicals

Sodium periodate and hydrazide beads were purchased from Bio-Rad (Hercules, CA); bicinchoninic acid protein assay (BCA) assay kit was purchased from Pierce (Rockford, IL); sequencing-grade trypsin was from Promega (Madison, WI); Sep-Pak C18 1cc Vac Cartridge was from Waters (Milford, MA); PNGase F was from New England Biolabs (Ipswich, MA); Other chemicals such as urea, ammonia bicarbonate (AB), ACN, TFA, tris (2-carboxyethyl) phosphine (TCEP), iodoacetamide, 5 M NaCl, sodium sulphite and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma-Aldrich (St. Louis, MO).

2.2 Cell culture and reactivation of latency

The ACH-2 and A3.01 cells were obtained from Dr. Thomas Folks through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH [23–25]. The ACH-2 cells were a subclone of A3.01 cells with HIV-1 latent infection. The HIV DNA is integrated into the genome of the T-cell line and low levels of supernatant RT and p24 antigen are constitutively produced in ACH-2 cells. PMA can be used to induce HIV-1 replication to produce high levels of infectious HIV-1 virions. Therefore, the provirus in ACH-2 cells is replication competent. Both cell lines were cultured in RPMI 1640 supplemented with 2 mM L-glutamine (Gibco Laboratories, Grand Island, NY), 100 U/mL penicillin, 100 g/mL streptomycin (Invitrogen, Carlsbad, CA) and 10% v/v heat-inactivated FCS (Hyclone Laboratories, Logan, UT). To reactivate cells, PMA (20 ng/mL) was added to medium. After 48 h of incubation at 37°C in 5% CO₂, the cells were washed three times using phosphate buffered saline. After a final wash, the cell pellets were lysed in 8 M urea in 1 M AB buffer. Protein concentration was determined by BCA assay.

2.3 Western blot confirmation of gp120 and p24 expression

Cell lysate were resolved on SDS-PAGE and were transferred electrophoretically onto a nitrocellulose membrane. The membrane was blocked by 5% nonfat milk, 0.1% TBS-Tween 20 at room temperature for 2 h. Then membrane was probed with anti-HIV-1 gp120 antibody (1:2000, abcam) or anti-HIV-1 p24 antibody (1:2000, biotin, abcam) at 4°C

overnight, followed by three 15-min washes of 0.1% TBS-Tween 20. Horseradish peroxidase-conjugated secondary antibody anti-goat or biotin (Thermo Scientific) were added at 1:2000 dilution, followed by a 1-h incubation at room temperature. After three washes with 0.1% TBS-Tween 20, the signals were visualized by SuperSignal West Femto maximum sensitivity substrate (Thermo Scientific).

2.4 Isolation of N-linked glycosite-containing peptides

Cell lysates (1 mg) of each sample were reduced in 10 mM TCEP at room temperature (RT) for 1 h. Proteins were alkylated in 10 mM iodoacetamide at RT for 30 min in the dark. Samples were diluted into 6 mL using 0.2 M AB buffer to decrease the concentration of urea and proteins. Trypsin (0.5 µg/µL, enzyme/protein ratio of 1/40 w/w) was added to digest proteins at 37°C over night. After digestion, 5 µL of sample was resolved on a tricine gel and silver staining was used to determine the completion of tryptic digested peptides. The peptides were desalted by a C18 cartridge according to the manufacturer's instructions and oxidized by incubating with 10 mM sodium periodate in 60% ACN v/v 0.1% TFA v/v at RT for 1 h in the dark. Sodium sulphite was added at a final concentration of 60 mM to quench the oxidation reaction. The samples were then mixed with 50 µL of (50% slurry) hydrazide support prewashed with 1 mL deionized water. The mixture was incubated with gentle shaking at RT overnight for the coupling reaction. The glycopeptide conjugated hydrazide beads were sequentially washed with 800 µL of 50% ACN v/v 0.1% TFA v/v, then 1.5 M NaCl and water, three times/solution to remove noncoupled peptides. The beads were finally washed with 200 µL of 1 × G7 buffer twice and incubated with 3 µL of PNGase F in 50 µL of 1 × G7 buffer at 37°C overnight to specifically release N-linked glycosite-containing peptides, which were desalted by a C18 cartridge, dried in a speed-vac and resuspended in 40 µL of 0.1% formic acid prior to MS analysis.

2.5 LC-MS/MS analysis

The N-linked glycosite-containing peptides (~1 µg) were separated through a Dionex Ultimate 3000 RSLC nano system (Thermo Scientific) with a 75 µm × 15 cm Acclaim PepMap100 separating column (Thermo Scientific) protected by a 2 cm guarding column (Thermo Scientific). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid 95% acetonitrile (B). Flow rate was 300 nL/min. The gradient profile was set as following: 4–35% B for 70 min, 35–95% B for 5 min, 95% B for 10 min and equilibrated in 4% B for 15 min. MS analysis was performed using an Orbitrap Velos Pro mass spectrometer (Thermo Scientific). The spray voltage was set at 2.2 kV. Orbitrap spectra (AGC 1 × 10⁶) were collected from 400–1800 *m/z* at a resolution of 60K followed by data-dependent HCD MS/MS (at a resolution of 7500, collision energy 35%, activation time 0.1 ms) of the ten most abundant ions using an isolation width of 2.0 Da. Charge state screening was enabled to reject unassigned and singly charged ions. A dynamic exclusion time of 35 sec was used to discriminate against previously selected ions.

2.6 Protein identification and quantification

Proteins were identified using SEQUEST in Proteome Discoverer software (Thermo Fisher Scientific, version 1.4). A NCBI human database containing 53,918 protein entries was used [26]. The precursor mass tolerance was set at 20 ppm and the MS/MS tolerance at 0.06 Da.

Parameters of the search were modified as follows: oxidized methionines (add Met with 15.995 Da), a (PNGase F-catalyzed) conversion of Asn to Asp (add Asn with 0.984 Da) and Cys modification (add cysteine with 57 Da). A maximum of two missed tryptic cleavage sites were allowed. Percolator node in SEQUEST in Proteome Discoverer was used to filter protein identification at 1% FDR. Annotation node in Proteome Discoverer and Cell Surface Protein Atlas [27] were used to determine cellular component. Only peptides unique to one protein group and with at least two spectral counts were reported and used for quantification. The label-free, semiquantitative differential expression analysis of the identified protein was conducted using SIEVE software (Thermo Scientific, version 2.0). The raw files were imported into SIEVE and the chromatograms were aligned. Frame parameters were set as follows: frame for all MS2 peak intensity threshold 1-E4, retention time from 0 to 60 min, m/z from 350 to 1800, frame time retention time width 2.5 min and frame m/z width 10 ppm. After framing, the Proteome Discoverer result files were imported with a 1% FDR. The integrated intensity (peak area) of glycosite-containing peptides was calculated and normalized by the median of each sample and used to calculate a p -value. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [28] partner repository with the dataset identifier PXD003561.

2.7 Statistics and bioinformatics

Student's t -test was used to calculate the p -value of differentially expressed glycoproteins. Fold change threefold and p -value 0.05 was considered significant. Hierarchical clustering was used to cluster the samples and analysis based on normalized intensity of glycosite-containing peptides using QCanvas version 1.2 [29]. Finally, the database for annotation, visualization, and integrated discovery (DAVID) and UniProt (<http://www.uniprot.org>) were used for GO, pathway analysis and annotation [30].

3 Results

To determine change of glycoproteins in latently infected cells with and without reactivation of latency, we analyzed HIV-1 latently infected human T lymphocyte ACH-2 cells and the parental cell line A3.01 cells in the presence/absence of PMA using label-free quantitative glycoproteomics. The HIV provirus in ACH-2 cells is replication-competent while A3.01 does not contain provirus. Expression of gp120 and p24 was confirmed in PMA-treated ACH-2 cells but not in ACH-2 cells without PMA and A3.01 cells (Fig. 1). To extract N-glycosite-containing peptides, equal amounts of cell lysates were processed using SPEG method as described in Method section. Next, the peptides from each sample were analyzed by LC-MS/MS in technical triplicates. Data are available via ProteomeXchange with identifier PXD003561. Trans Proteomic Pipeline generated Pep3D images showed the reproducibility and calculated CV among LC-MS/MS runs (Supporting Information Fig. 1). SEQUEST in Proteome Discoverer identified 940 unique glycosite-containing peptides from 515 unique glycoproteins with 1% FDR, at least two spectral counts per protein and uniqueness to one protein group (Supporting Information Table 1). Protein annotation of these glycosite-containing peptides from both cell lines showed that 365 and 104 proteins, accounting for approximately 70.9 and 20.2% of the identified proteins, were cell surface and membrane proteins, respectively (Supporting Information Fig. S2). This analysis

demonstrated that SPEG method favored enrichment of cell surface and membrane glycoproteins that is a desired feature to find cell membrane associated biomarkers for latently infected cells. To quantify the data, SIEVE was used and alignment of runs was good indicated by the scores being above 0.73, which is the minimal acceptable score (Supporting Information Fig. 3). In total, SIEVE quantified 900 unique glycosite-containing peptides from 497 unique glycoproteins with 1% FDR (Supporting Information Table 2). Using relative intensities of the 900 unique peptides, unsupervised hierarchical clustering showed that ACH-2 cells had a distinct profile of glycoproteins compared to A3.01 cells, even after PMA treatment (Fig. 2A). It should be noted that changes of glycosite-containing peptides detected by SPEG method could be caused, separately or in combination, by different changes in glycoproteins including changes in glycoprotein expression, glycosylation occupancy, or glycoprotein isoforms. Using a cut off ratio of threefold and a p -value 0.05, 236 glycosite-containing peptides from 172 proteins were changed between ACH-2 and A3.01 cells without PMA treatment (Fig. 2B and Supporting Information Table 3). Of the changed proteins, 14 proteins had more than three changed peptide including ten upregulated proteins, i.e. galectin-3-binding protein (LG3BP), CD109 antigen (CD109), dipeptidase 1 (DPEP1), angiotensin-converting enzyme (ACE), lymphocyte antigen 75 (LY75), N-acetylglucosamine-1-phosphotransferase subunits alpha/beta (GNPTAB), sortilin-related receptor (SORL1), trophoblast glycoprotein (TPBG), KDEL motif-containing protein 2 (KDEL2), 5'-nucleotidase (NT5E), and four downregulated proteins, i.e. prolowl-density lipoprotein receptor-related protein 1 (LRP1), cholinesterase (BCHE), neuroserpin (SERPINI1), and peroxidasin homolog (PXDN). The other changed peptides and their corresponding proteins are listed in Supporting Information Table 3. Noticeably, 29 cell surface proteins appeared to have changed (Table 1). It is likely that change of protein glycosylation might affect activity of the proteins. To gain insight of impacted pathways, the proteins with changed glycosite-containing peptides were analyzed using DAVID bioinformatics, and GO term analysis revealed affected pathways including cell adhesion (31 proteins), immune response (26 proteins), glycoprotein metabolic process (12 proteins), cell motion (15 proteins), cell activation (11 proteins), and others were significantly changed with a p -value < 0.01 (Supporting Information Table 4). These data suggest that ACH-2 cells have a different profile of glycoproteins from A3.01 cells that might result in different cell behavior and response to stimuli. It was noted that among the changed proteins between ACH-2 and A3.01 cells without PMA treatment, trend changes of 24 proteins including LGALS3BP, ACE, DPEP1, deoxyribonuclease-2-alpha (DNASE2), Golgi membrane protein 1 (GOLM1), CD109, inositol monophosphatase 3 (IMPAD1), SORL1, group XV phospholipase A2 (PLA2G15), hereditary hemochromatosis protein (HFE), LY75, V-type proton ATPase subunit S1 (ATP6AP1), junctional adhesion molecule B (JAM2), integrin alpha-2 (ITGA2), cadherin-2 (CDH2), cyclic AMP-dependent transcription factor ATF-6 beta (ATF6B), CMP-N-acetylneuraminate-poly-alpha-2,8-sialyltransferase (ST8SIA4), lysosomal protective protein (CTSA), CMP-Nacetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 1 (ST3GAL1), neogenin (NEO1), neuropilin-1 (NRP1), tetraspanin-15 (TSPAN15), T-lymphocyte surface antigen Ly-9 (LY9), and cystatin F (CSF7) were consistent with our previous study analyzing secreted glycoproteins in the culture medium of the same cell lines suggesting these proteins were likely to be differentially

regulated and prone to secretion (Supporting Information Table 5). These differentially secreted proteins might play a role in immune regulation and chronic inflammation [18].

To study whether viral replication during latency reactivation affected glycoproteins, cells treated with PMA for 48 h were analyzed. At this time point, viral replication occurs and reaches peak in essentially all cells [31]. PMA not only reactivates HIV latency through protein kinase C and NF κ B, but also causes many changes due to T-cell activation [32]. We observed change of 155 glycosite-containing peptides from 125 glycoproteins in A3.01 cells after PMA treatment (Fig. 3 and Supporting Information Table 6). Similarly, 161 glycosite-containing peptides from 127 glycoproteins were changed in ACH-2 cells (Fig 3 and Table S7). Changes of glycosite-containing peptides in ACH-2 cells than in A3.01 cells suggest that the response of ACH-2 and A3.01 cells to PMA shares similarity but also contains some difference in respect to N-glycoproteome. The difference could be due to HIV latency and/or years of passages of the cell lines. For the 29 changed cell surface proteins between ACH-2 and A3.01 cells without PMA treatment, 17 and 16 proteins were seen to have change after PMA treatment in each cell line. Finally, we observed changes of 49 glycosite-containing peptides from 45 glycoproteins that might be caused by viral replication (Fig. 3 and Supporting Information Table 8). GO term analysis of the 45 glycoproteins revealed positive regulation of cell migration (five proteins, 8.8×10^{-5}), response to wounding (nine proteins, 6×10^{-5}) and immune response (eight proteins, 2×10^{-3}) (Supporting Information Table 9).

4 Discussion

HIV has evolved a surprisingly sophisticated mechanism for dormancy in the host cells. Maintenance of HIV latency involves complex interplay at transcriptional, translational, and microRNA levels [33–35]. To study the latent infection, several cell model systems have been established, which include transformed cell lines and primary cells [21]. However, it is problematic in the field that none of the latent cell models fully recapitulates latency in vivo [36]. ACH-2 and A3.01 cell lines are established transformed cell models used to dissect mechanisms governing latency [24, 25]. However, we could not rule out the possibility that the changed glycoproteins were a result of differences acquired over time in the two cell lines that have diverged through long-term passage and culture. To overcome the drawback of using ACH-2 and A3.01 cell lines, a latently infected primary cell model would be more physiologically relevant [36]. By using cell lines, we established the applicability of glycoproteomics that has advantages to study the glycoproteins that are important to understand and target latently infected cells.

Glycoproteomics is a subfield of proteomics that preferentially analyses the glycoproteome in a complex sample [20]. N-linked glycosylation commonly modifies cell surface receptors, membrane proteins, and signal of cell migration [37]. Our SPEG method substantially reduces the complexity of sample that allows identification of low abundant but biologically relevant glycosite-containing peptides [38]. To quantify the samples, we apply label free LC-MS/MS analysis that avoids the use of expensive labeling reagents and enables an unlimited number of experiments for comparison [19]. Reproducibility of the LC-MS platform is crucial in the label free quantification [19]. To ensure the reproducibility, we inspected the

Pep3D image and used the alignment-scoring algorithm in the SIEVE software. These steps were essential and showed the reproducible data generated in this study.

In a total 12 LC-MS/MS experiments, 940 unique glycosite-containing peptides from 515 unique glycoproteins were identified using SEQUEST in Proteome Discoverer software. Three hundred sixty-five and 104 glycoproteins were annotated as cell surface and membrane proteins, respectively. It was found that many of the glycoproteins had only one glycosite-containing peptide identified indicating single N-linked glycosylation site for the protein or even lower abundance for other glycosite-containing peptides from the protein. As a result, SIEVE was only able to quantify 900 glycosite-containing peptides from 497 glycoproteins with 1% FDR. Two hundred thirty-six peptides from 172 proteins were changed between ACH-2 and A3.01 cells without latency reactivation. A change of 29 cell surface proteins was observed. However, the role and clinical value of the changed proteins will need careful investigation in a latently infected primary cell model and primary cells from HIV+ individuals.

Change of glycosite-containing peptides due to PMA mediated reactivation of latency has not been described and may provide potential new insight into reactivated latently infected cells. We used A3.01 cells as the uninfected and matched HIV-control. PMA treatment affected glycosite-containing peptides in both cells. Comparison between the cell lines with PMA treatment showed changes of 49 glycosite-containing peptides from 45 glycoproteins. Because reactivation of latency is inextricably associated with T-cell activation, these changed glycosite-containing peptides might not be directly associated with viral replication. Pathway analysis suggests that the reactivated cells might have an altered behaviour in cell migration, response to wounding and immune response. Changed behaviour of immune cells due to changes of glycoproteins could also be an important factor in HIV pathogenesis [17].

4.1 Conclusion

Our data show changes of glycoproteins in an established HIV latently infected cell line model (ACH-2 and A3.01 cell lines). We found that the SPEG method preferentially enriched cell surface and membrane proteins. This favored the discovery of biomarkers of HIV latently infected cells. Analysis revealed that the profile of glycoproteins was significantly changed between ACH-2 and A3.01 cells. Two hundred thirty-six glycosite-containing peptides from 172 glycoproteins were changed between the cell lines without latency reactivation. These proteins were mainly associated with cell adhesion, immune response, glycoprotein metabolic process, cell motion, and cell activation. PMA treatment, which reactivated the latent infection, also changed many glycoproteins in both cell lines. Cell migration, response to wounding and immune response might be associated with changed glycoproteins suggesting the cells with reactivated latency might have changed cell biology. This observation will need investigation in primary latent cells. This first glycoproteomic analysis of HIV latently infected cells provided valuable data in respect to glycoproteome in HIV latency. Considering the importance of glycoproteins in cell biology, the study of glycoproteome in latently infected primary CD4+ T cells certainly merits detailed investigation, which is currently ongoing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ACE	angiotensin-converting enzyme
AIDS	acquired immune deficiency syndrome
BCA	bicinchoninic acid protein assay
DAVID	database for annotation, visualization, and integrated discovery
HIV	human immunodeficiency virus
PMA	phorbol 12-myristate 13-acetate
RT	room temperature
SPEG	solid phase extraction of glycopeptides
TCEP	tris (2-carboxyethyl) phosphine

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Significance of the study

Human immunodeficiency virus (HIV) is the etiological agent responsible for human acquired immune deficiency syndrome (AIDS). Cure of the disease is not feasible due to latent infection, which has become a key focus of biomedical research. Latently infected cells harbor replication-competent viral DNA but express little or no viral proteins. This feature enables these cells to evade immune clearance. Lack of a method to isolate these cells causes a significant gap in understanding and targeting of these cells. It is assumed that the latently infected cells have the same cell surface feature as uninfected counterparts. However, recent ex vivo studies suggest that there is a unique profile of glycoproteins for latently infected cells. It thus becomes meritorious to assess glycoproteome of the latently infected cells. Our study here used quantitative glycoproteomics to study an established HIV-1 latently infected human T-cell model. We showed that latently infected cells without latency reactivation had changed glycoproteome. This important observation highlights the necessity to assess glycoproteome of latently infected primary cells using glycoproteomics. Targeting the changed glycoproteins would become an effective approach toward isolation and eradication of the latently infected cells.

Figure 1.
Expression of gp120 and p24 in ACH-2 cell lysate after PMA treatment for 48 h.

Figure 2.

Differential profile of glycosite-containing peptides between ACH-2 and A3.01 cells. (A) Unsupervised hierarchical clustering using medium-normalized relative intensity (log10) of glycosite-containing peptides for each run. Spearman Rank and Euclidean Distance were used for clustering of samples and peptides, respectively. (B) Volcano plot of the glycosite-containing peptides in ACH-2 and A3.01 cells without PMA treatment. Fold change (log2) and p -value ($-\log_{10}$) of glycosite-containing peptides were plotted and colored in red (threefold change and p -value ≤ 0.05) or in blue ($<$ threefold change and p -value > 0.05).

Figure 3. Venn diagrams of changed proteins between two cell lines as well as between the PMA nontreatment and treatment. (A) Proteins changed between ACH-2 and A3.01 cell lines without PMA treatment. (B) Proteins changed in ACH-2 cells before and after PMA treatment. (C) Proteins changed in A3.01 cells before and after PMA treatment. (D) Proteins changed possibly due to viral replication in cells after PMA treatment.

Table 1

Changed cell surface proteins with two glycosite-containing peptides between ACH-2 and A3.01 cells without reactivation

RefSeq ID	Protein name	Peptides	Fold change (log2)	p-value
4503273	Angiotensin-converting enzyme	4	ACH-2	8.2×10^{-3}
29550850	Golgi membrane protein 1	2	ACH-2	6.0×10^{-4}
56237029	Integrin alpha-5	2	ACH-2	5.1×10^{-2}
157419150	Receptor-type tyrosine-protein phosphatase mu	2	ACH-2	4.2×10^{-2}
153218487	KDEL motif-containing protein 2	4	8.27808	4.4×10^{-3}
262205665	Trophoblast glycoprotein	3	8.16615	2.2×10^{-2}
5031863	Galectin-3-binding protein	5	7.468	1.1×10^{-2}
227430301	CD109 antigen	3	4.97164	3.7×10^{-3}
144446030	Lymphocyte antigen 75	5	4.89751	1.7×10^{-3}
7669492	Glyceraldehyde-3-phosphate dehydrogenase	2	4.29127	7.5×10^{-3}
325651886	5'-Nucleotidase	3	4.13465	1.1×10^{-4}
112380628	Lysosome-associated membrane glycoprotein 1	2	3.79967	2.5×10^{-4}
188595677	Integrin beta-2	2	3.70188	2.0×10^{-1}
4507157	Sortilin-related receptor	7	3.42611	3.4×10^{-3}
530424672	Dipeptidase 1	3	2.88665	1.3×10^{-3}
4557251	Disintegrin and metalloproteinase domain-containing protein 10	2	2.77171	5.5×10^{-3}
51317399	Lysosomal acid lipase/cholesterol ester hydrolase	2	2.5324	1.3×10^{-3}
19923669	Protein sel-1 homolog 1	2	2.48395	4.6×10^{-3}
4504061	N-acetylglucosamine-6-sulfatase	2	2.10952	3.6×10^{-2}
42560231	Nectin-1	2	1.91667	7.6×10^{-4}
24638433	Nicastrin	2	1.90526	4.1×10^{-2}
4557351	Cholinesterase	3	-1.5965	2.1×10^{-1}
126012562	Prolow-density lipoprotein receptor-related protein 1	10	-1.6726	3.5×10^{-5}
157694524	Plexin-D1	2	-1.7988	3.6×10^{-3}
4826904	Neuroserpin	3	-1.8537	6.8×10^{-2}
148728162	Receptor-type tyrosine-protein phosphatase eta	2	-2.3996	1.2×10^{-3}
45594240	limbic system-associated membrane protein	2	-2.4586	5.7×10^{-2}
27262661	macrophage colony-stimulating factor 1	2	-2.5891	1.5×10^{-2}
109150416	peroxidase homolog	3	-5.4399	4.4×10^{-2}