



Review article

HIV-1 Nef and host proteome analysis: Current perspective

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ABSTRACT

Proteome represents the set of proteins being produced by an organism at a given time. Comparative proteomic profiling of a healthy and diseased state is likely to reflect the dynamics of a disease process. Proteomic techniques are widely used to discover novel biomarkers and decipher mechanisms of HIV-1 pathogenesis. Proteomics is thus emerging as an indispensable tool of monitoring a disease process and intense interactions between HIV-1 and host. Nef is known to regulate various functions in the host to establish the state of infection. This review gives an overview of all proteomic studies done on HIV infection and HIV associated disorders including recent developments in Nef-host proteomic profiling. Here, we propose an emphasis on Nef based proteomic studies. We also discuss the future prospects and the technical and biological challenges involved in proteomic studies. Future studies with Nef related proteomic investigation are likely to identify more targets for diagnosis and therapy.

1. Introduction

Human immunodeficiency virus (HIV) is the causative agent of Acquired Immuno Deficiency Syndrome (AIDS) and has evolved as one of the most successful pathogens infecting humans. Despite several research efforts, it remains unabated. Application of proteomics studies in the analysis of host-virus interaction leading to differential expression of host cellular proteins has emerged as a promising domain of extensive research and opens new avenues in the field of drug discovery. The search for disease biomarkers as potential therapeutic targets has become a favorable approach towards drug development throughout the past few years [1–3].

The advent of proteomics has contributed significantly to drug development by deciphering the disease mechanisms and the identification of effective diagnostic and therapeutic biomarkers [4]. Various cellular responses emerging during HIV infection can be characterized by understanding the proteomic changes including the forms and functions of proteins using different techniques [4–6]. A recent review on proteomics studies discusses the different proteomics techniques used in HIV -host interaction studies [7]. We focus on proteomic investigations of HIV related disorders, primary target cells, and its own viral proteins in particular HIV-1 Nef.

The utilization of proteomic techniques to globally identify differentially expressed proteins in immune and nerve cells in response to

HIV-1 infection represents a significant undertaking towards the investigation of disease. Intense interest in applying proteomics technology creates numerous opportunities as well as challenges to meet the needs for high sensitivity and high throughput required for disease-related investigations. Particularly promising areas of research include: delineation of altered protein expression, from different samples like whole-cell or tissue, also in subcellular structures, in protein complexes and in biological fluids and the identification of new targets for therapeutics and the potential to evaluate therapeutic effect and toxicity for accelerating drug development [1,4]. An overview of host proteome research is given in Fig. 1. Displaying the source, methods, outcome, and relevance of Proteomic studies in the field of drug development.

Due to the fact that HIV-1 infection of cell results in the production of foreign viral RNAs and proteins, changes in cell cycle, morphology, cellular signaling, and protein synthesis dysregulation and degradation, it is reasonable to expect alterations in protein expression between a normal and infected cell. Proteomic techniques are able to characterize these overall changes, which lead to the understanding of the role of viral proteins in manipulating the cell. Host proteins modulation by viral proteins is of vital importance to understand crucial Host-virus interplay. Of HIV-1 accessory proteins including tat, rev, Nef, vpr and vpu; Nef is primarily involved in regulation of cellular signaling machinery. The HIV-1 protein Nef is a multifunctional protein that contributes to HIV pathogenesis through alteration of endocytosis, signal

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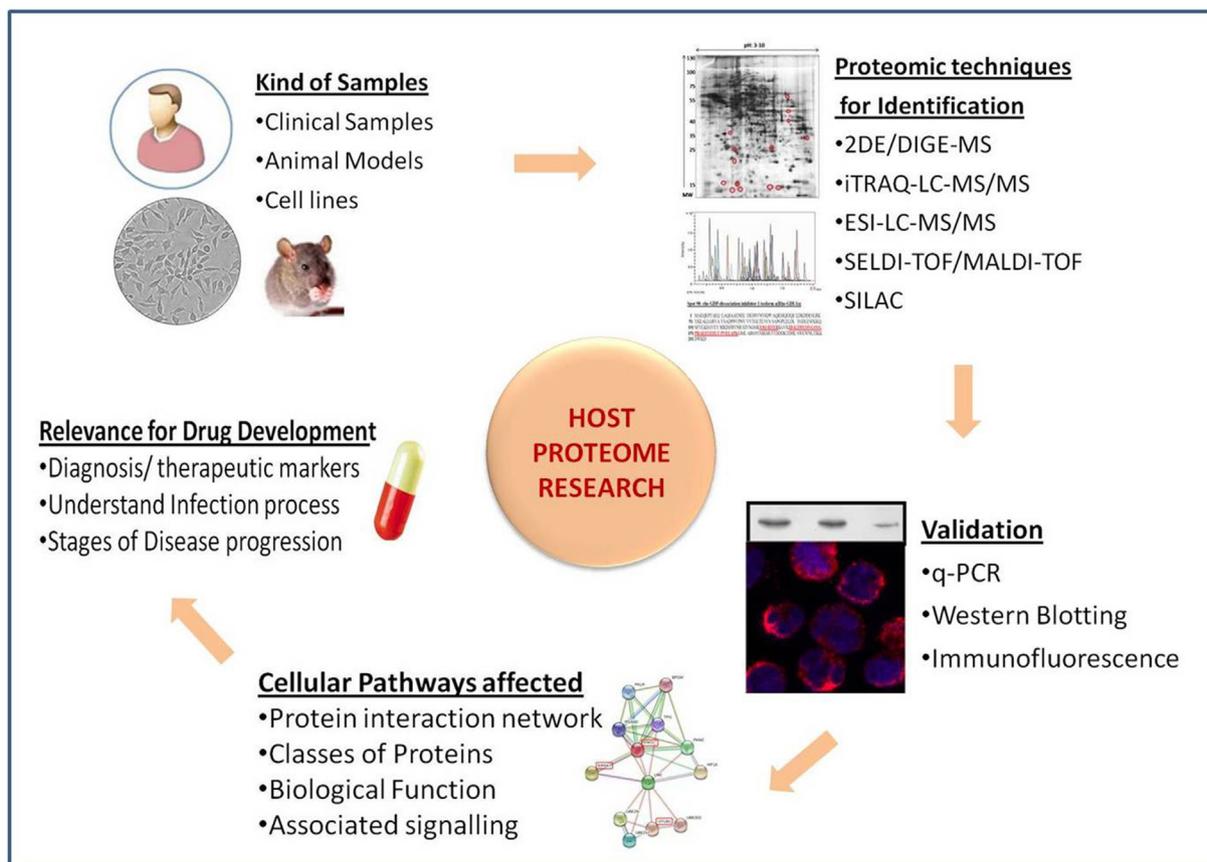


Fig. 1. Schematic representation of host proteome research. The figure gives an overview of the sources, techniques, outcome, and relevance of proteomic studies in the field of drug development.

transduction, cell survival, vesicular trafficking, and immune evasion. It has been reported that Nef deletion indicates poor viral replication and lower infectivity leading to delayed or no immunodeficiency syndromes. It is well established that Nef promotes HIV-1 replication and facilitates viral immune evasion by interacting with various host factors [8–10]. But until now Nef has not been evaluated as an antiretroviral drug target. Thus analysis of differential protein expression upon HIV-1 Nef infection through proteomics studies, and convert it into clinically useful information relating to the pathogenesis, transmission and therapeutic response of Nef will facilitate the research for drug development in HIV-1. This review covers all the proteomic studies in HIV-1 research reported so far and emphasizes the need for more Nef related proteomic studies.

2. Monitoring of HIV-1 infection and HIV-1 associated disorders through proteomics: reports so far

A number of studies have been initiated to seek biomarkers relevant for HIV-1 diagnosis as well as use in therapeutic monitoring for anti-retroviral responses. The host-virus proteome studies of HIV-1 have been divided into few sections based on the subjects and the purpose of the study.

2.1. Proteomic investigation of HIV associated disorders

There have been several reports comparing the proteomic profiling of healthy donors and HIV-1 infected donors with HIV-1 associated disorders. In a recent study, 2-dimensional electrophoresis (2DE) with Differential Gel Electrophoresis (DIGE) profiling method of immunodepleted CSF or sera from HIV-1 infected individuals with or without HAD was done and demonstrated several differentially

expressed proteins which can be potential biomarkers. Differential expression of 6 proteins was observed on the cerebrospinal fluid proteomic study as affected by HIV-1 associated Dementia and can be positioned as potential HAD biomarkers. These included vitamin D binding protein, clusterin, gelsolin, complement C3, procollagen C-endopeptidase enhancer 1, and cystatin C [11,12]. Results of proteomic analysis of sera from HIV-infected individuals with or without cognitive impairment reported gelsolin and pre-albumin as differentially expressed proteins [2]. Proteins associated with cell signaling, structural function, and antioxidant activities were found specific to patients with cognitive impairment. Unique serum proteins were identified in long-term nonprogressors and are indicative of natural immunity with disease progression. Specifically, p16INK4A, a cdk4/6 inhibitor was identified, as preferentially present in the pooled serum of HIV-1 LTNP patients, as opposed to HIV-1 infected individuals responding to HAART treatment [5]. 2D-gel electrophoresis and Tandem mass spectrometry peptide sequencing were employed to study the protein profile of the HIV-1 infected macrophages on human brain microvascular endothelial cell (HBMEC). An upregulation of proteins involved in metabolism, transport, heat shock, cytoskeleton formation, and signaling was found [13]. A study of proteomic changes in oral HPV lesions in HIV patients utilized iTRAQ and identified nine differentially expressed proteins [14]. An elevated level of iron and iron-related proteins was found in the urine of children with HIV-associated renal diseases and thus emerge as a new therapeutic target [15].

Proteins associated with protein folding and anti-inflammatory responses heat-shock proteins, Cryab, Calr, IL-1RA, and Galectin-3-binding protein and also the proteins linked to redox homeostasis and detoxification (e.g., Gstp1, Prdx1, and Ero1) were underexpressed in the proteomic investigation of Human oral epithelial cells in HIV-infected subjects. However, an increase of protein disulfide isomerases

and vimentin was seen in the same study [3]. To determine the effects of methamphetamine on plasma proteins in HIV-infected persons, plasma proteomic profiling was carried and 28 proteins were found differentially expressed uniquely to Methamphetamine HIV abusers [16].

In a study of saliva of Heroin addicted HIV patients revealed cognition related proteins and also their association with exosomes was observed. Thus salivary glands cause possible modulation of HIV-associated neurocognitive disorders (HAND) through the release of proteins in form of exosomes [17]. Assessment of cerebrospinal fluid proteins from HIV-1 Hispanic women with and without cognitive impairment was done through integrated proteomic techniques involving SELDI-TOF and RP-HPLC and identified modulation of proteins involved in apoptosis, chemotaxis, inflammation and redox metabolism [18]. An interesting study identifying proteomic signatures in cervical mucosa of HIV-1 resistant sex-workers and control groups through 2D-DIGE: two-dimensional difference in-gel electrophoresis was carried. More than 15 proteins as potential biomarkers of HIV resistance were revealed with overexpression of antiproteases and cystatin A [19]. A recent report of CSF proteomic analyses characterized novel targets linked to HIV associated CNS disorders with a role in neuronal plasticity and immune response modification [20]. Macrophages of HIV-1 patients with HAD were examined through proteomics (SELDI-TOF) and showed loss of lysozyme [21]. Specific proteins belonging to different classes have been found to be influenced at different stages of HIV-1 infection. A change in their relative abundance with progression to disease defines their significance as biomarkers regarding the impact of that stage on the host cellular factors. Several studies initiated in this direction provide unique proteins showing upregulation and/or downregulation upon HIV-1 infection and have been found to be associated with alteration in cellular pathways causing metabolic re-routing.

Through such studies more specific drug targets could be achieved with relevance in controlling HIV-1 and in particular, the HIV-1 associated condition.

2.2. Proteomics of HIV infected cells viz. T cells, macrophages, and dendritic cells

A recent study demonstrated that infected astrocytes show modest changes in protein elements compared to uninfected cells. In contrast, infected astrocytes induce robust changes in the proteome of HIV-1-infected microglia [22]. To understand the influence of virus on the functions of macrophages, another study of HIV-1 infected macrophages was carried which discovered differentially expressed proteins involved in cytoskeleton formation, enzymatic reactions and immunoregulation like cystatin C, cystatin B, chitinase 3-like 1 protein, cofilin-1, α -plactin, superoxide dismutase, leukotriene A4 hydrolase, and α -enolase [23,24]. Another study on highly purified HIV-1 virions produced from human monocyte-derived macrophages (MDM), host cell proteins incorporated into virions was characterized. Proteins involved in different functions linked to the cytoskeleton, adhesion, signaling, intracellular trafficking, immunoregulation, modulation of cell growth and differentiation, chaperone, metabolic, ubiquitin/proteasomal, and immune response systems were identified [25]. In a study with emphasis on the effects of drug abuse, the response of the immune system towards pathogens associated with drug abuse was studied through proteomic techniques. Changes in the proteome of peripheral blood mononuclear cells (PBMC) derived from HIV-1 infected patients by drugs of abuse were studied and found modulation of several functional classes of proteins like heat shock proteins, structural proteins [26]. Modulation of protein profile of HIV-1 infected brain macrophages induced by T regulatory cells was assessed and changes in protein profile were found. Proteins involved in interferon-mediated antiviral immunity were induced and thus the effect of T reg cells on virus-infected MP functions was seen [27].

Proteomic analysis of human synaptosomes from frontal neocortex in HIV-1 infected was done to determine the influence of HIV-1 infection over the protein profile of human synapses. Synapsin 1b and stathmin were inversely related to brain HIV-1 load; 14-3-3 ζ and 14-4-4 ϵ proteins were induced in subjects with HIV-1 loads [28]. While studying the proteome of monocytes upon virus exposure, facilitation of protein transformation from cytosol to plasma membrane was seen. Fifty-three percent of proteins were found to be PM associated and were largely involved in cellular activation and oxidative stress [29].

Monocyte-derived dendritic cells treated with cocaine or methamphetamine was subjected two 2DGE and proteins that alter apoptosis, protein folding, protein kinase activity, signal transduction and metabolism were differentially expressed [30].

Proteomic studies in T cells revealed changes in protein expression and compared the differences between uninfected and HIV-1-infected T cells. Classes of proteins and general pathways that are altered upon HIV infection were identified [31]. Identification of unique proteins serves as an indication of altered viral states in response to infection as well as natural phenotypic variability in response to HIV-1 infection in a given population.

HIV-1 infected T cells proteomic investigation revealed differential expression of proteins involved in Protein Synthesis, Cell Proliferation, and T-cell Activation [31]. A study of the proteome of the viral cores purified from HIV-1 virions assembled in T cells and monocytes identified groups of proteins belonging to class of RNA binding, DNA binding, cytoskeleton, cytoskeleton regulation, chaperone, vesicular trafficking-associated and ubiquitin-proteasome pathways. More RNA binding proteins were derived from T cells as compared monocytes. The cell-specific difference in host protein requirement is thus reflected depending on changes in viral function [32]. Glycoproteomic analysis of plasma of HIV infected T cells detected the presence of glycoproteins. Of 59 total glycoproteins detected 6 proteins (galectin-3-binding protein, α -selectin, neogenin, adenosine deaminase CECR1, ICOS ligand, and phospholipid transfer protein) were significantly higher in HIV+ T cells. Such studies give an insight into understanding the host response to HIV infection [33]. Comparison of proteomic profiling of exosomal compartment of HIV-1 infected and uninfected lymphocytic H9 cells demonstrated 14 differentially expressed proteins majorly affecting cellular apoptosis, proliferation, and immunomodulation [34]. Major pathways affected by HIV-1 in different cell types have been displayed in Fig. 2.

2.3. Proteomics of HIV proteome for determining modulation of viral proteins during pathogenesis

In-depth understanding of how the virus infects a host, proteomic changes of the virus must be studied well. Few proteomic studies of viral proteome modulation are discussed here. Association of three forms of Cyclophilin A with HIV-1 was found through 2DGE and MALDI-TOF [35]. Novel viral proteins were identified through LC-MS/MS and comparative proteomics of wildtype and mutant viruses demonstrated host specificity [36]. Proteomic investigation of HIV proteome with emphasis on HIV modulated molecules involved in angiogenic pathways was done. Proteomic Analysis of Early HIV-1 Nucleoprotein Complexes demonstrated alterations in protein profile at all stages of viral replication [37].

2.4. Proteomics of host with HIV proteins

HIV pathogenesis largely involves host-viral protein interaction. Proteomic profiling of host by HIV proteins would provide deep insight in understanding host-virus interaction. Effects of Tat protein was examined through 2d-DIGE and MS in Jurkat cells. Downregulation of cytoskeletal proteins was the major finding [38]. Proteomic analysis of astrocytes expressing Tat revealed underexpression of phosphatase 2A, isocitrate dehydrogenase, nuclear ribonucleoprotein A1, Rho GDP

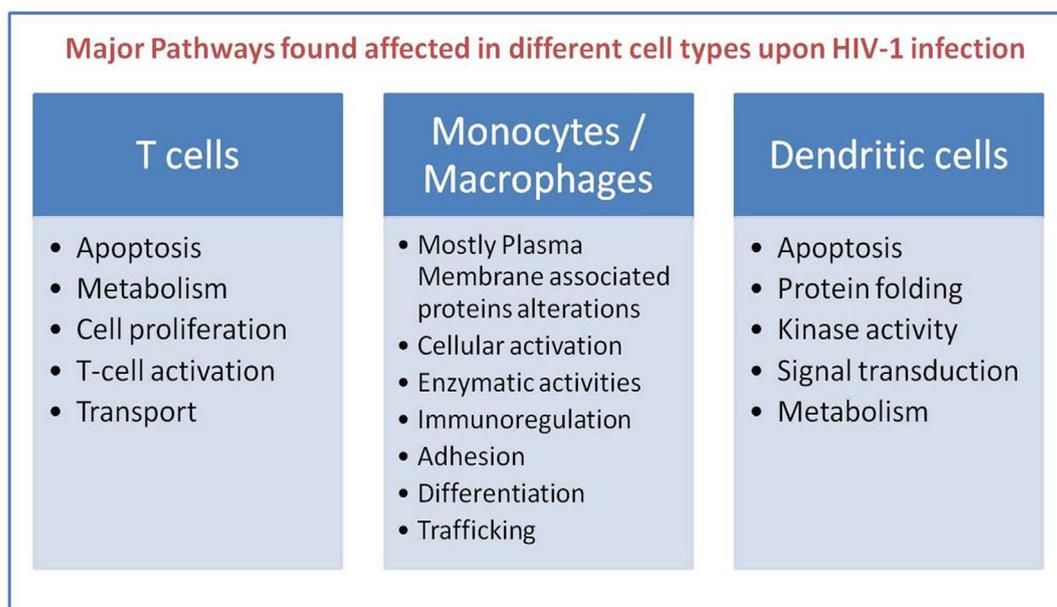


Fig. 2. Proteomic investigation of HIV-1 target cells. The figure shows the major pathways affected by HIV-1 in different HIV-1 target cells viz. T cells, Monocytes/macrophages and Dendritic cells due to modulation of host proteome.

dissociation inhibitor α , h-tubulin, crocalbin like protein/calumenin, and vimentin/ α -tubulin [39]. A very recent study analyzed the effects of Gag and identified candidate proteins interacting with the host [40]. Another protein Nef, which is a multifunctional accessory protein of HIV has also been explored through proteomics techniques for its implications on host protein expression. This review, now discusses Nef proteomics, as more emphasis is required to understand the Nef-mediated modulation of host proteins.

3. HIV-1 Nef: a potential regulator of host cell machinery

It is well known that HIV infection is widely dependent on the host-virus protein interaction. It involves modulation of host cellular machinery by viral proteins. A comprehensive study of cellular factors directly regulated by HIV-1 proteins is required to better understand the disease process and pathogenesis. Proteomic studies with HIV-1 Proteins, such as Nef, must be done to a larger extent to identify potential disease biomarkers. Nef is 27 kD accessory protein of HIV. It does not have a direct role in replication but it increases the efficiency of viral replication by promoting immune evasion and cell survival [10]. It does not have an enzymatic activity of its own but interacts with, and/or regulate different host proteins to create an environment favorable for virus and establish a persistent state of infection [8]. Fig. 3 shows major Nef functions and also displays a network of close interacting cellular proteins to HIV-1 Nef. It shows close interaction of Nef and hosts proteins involved in CD4 downregulation [41,42], MHC-I downregulation [43], vesicular trafficking [44,45], and signal transduction [10]. HIV-1 Nef – Cellular proteins interaction based modulation of functions. Thus, it has been well established that Nef interacts with a number of proteins involved in various cellular activity. Some of the studies based on Interaction of HIV-1 Nef to cellular proteins discussed below.

3.1. Cellular trafficking

Within the cell, cellular trafficking needs no. of proteins. One of the protein is GCC185 which known to involved in the mannose 6-phosphate receptor (MPR) recycling through Rab9. Recently, it was seen that Nef transfected cell, disruption of GCC185 and Rab9 mediated MPR recycling as a result of Nef-GCC185 interaction [46].

Other Nef interacting protein is COP-I that has been reported to involved in endocytic recycling [47], phagosome formation. An interaction between Nef and β -COP, a component of COP-I coatomers, is important for targeting of Nef and CD4 to acidic late endosomes [10].

3.2. MHC-I downregulation

Antigen presentation by MHC-I is a mechanism by which a cell can communicate with the extracellular environment. Cellular surface with MHC-I-foreign antigen complex detection resulted in initiation of several responses in the CTL, including the release of perforins, granzymes, and proapoptotic factors for the lysis of the infected cell. In viral infected cells, reducing the cell surface expression of MHC-I is crucial for immune evasion. It has been reported that MHC-I-Nef not only blocked from TGN exit but directed from the TGN to lysosomes for degradation [43,45].

3.3. CD4 downregulation

The CD4 is cell surface coreceptor protein required for HIV virus entry into the host cell. However, its continuous expression on HIV-infected cell after viral entry is problematic. It is reported that the presence of CD4 on the cell membrane diminishes the ability of the newly formed particle to properly bud and escape the infected cell resulted in reduced viral infectivity [48]. Therefore, to decrease CD4 expression on the cell surface, it is thought that Nef binds the cytoplasmic tail of CD4. Binding of Nef to the cytoplasmic tail of CD4 and recruits a cellular factor(s) like AP2, ARF and β -COP to transport CD4 from the cell surface to lysosomes for degradation [10,49].

4. HIV-1 Nef mediated modulation of host proteins expression: proteomics approach

Besides protein interaction, we here discuss the proteomic studies with Nef indicating Nef-mediated alteration of host protein expression.

4.1. Comparative proteomics reveal Nef-dependent increase of virus infectivity

In a recent study, Nef ability to increase virus infectivity was

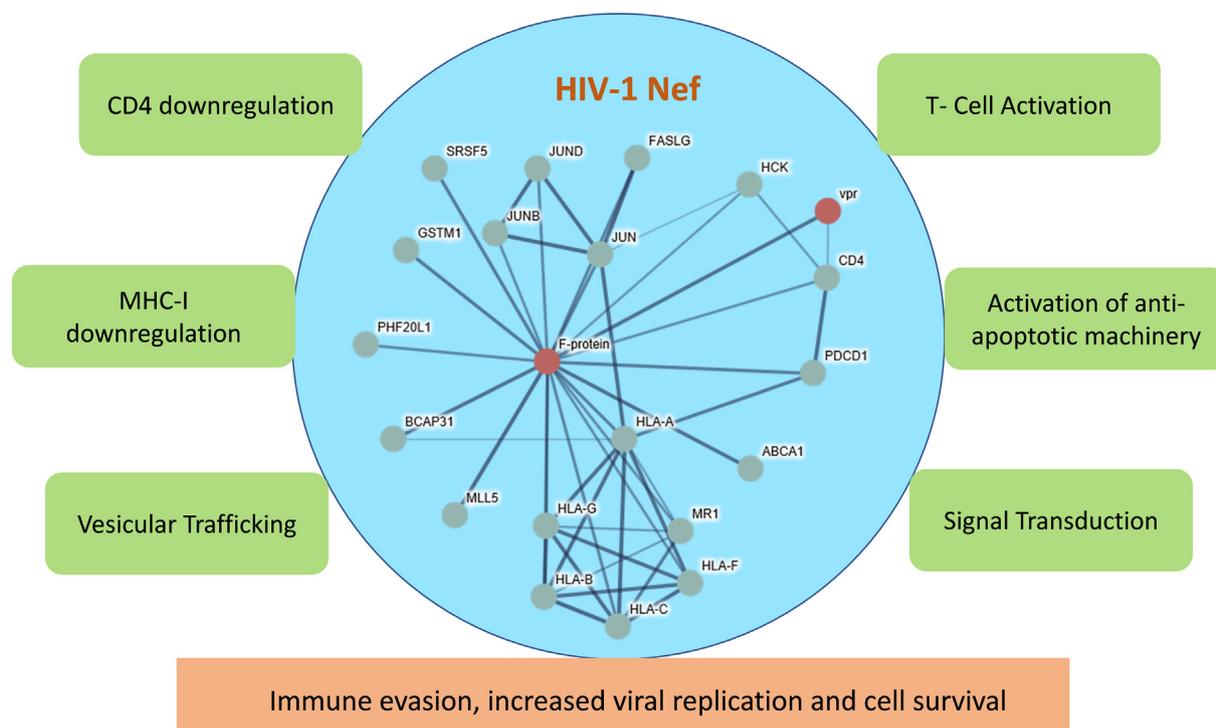


Fig. 3. HIV-1 Nef major functions. The figure shows major Nef functions and interacting network generated by ‘Viruses STRING’ software in support of its functions.

analyzed by the comparative proteomic study of wild-type and nef-deleted viruses. 2D-DIGE and iTRAQ were employed to compare the proteomes and functional analysis showed enrichment of Glucosidase II in WT virions, while Ezrin, ALG-2, CD81, and EHD4 were enhanced in nef-deleted virions [50].

4.2. Proteomic analysis of HIV-1 Nef partners involved in Pak2-association dependent Nef functions

Proteomic profiling of Jurkat cells expressing wild-type Nef and Nef Mutants for Pak-2 association was done. Nef immunocomplexes were analyzed by Tandem mass spectrometry. Exocyst complex proteins (EXOC1, EXOC2, EXOC3, EXOC4, and EXOC6) were identified as key effectors of nanotube formation mediated by Nef [51].

4.3. Nef functionality based on sequence variability

2DGE and LC-MS/MS demonstrated differentially expressed Proteins Cyclophilin A, EIF5A-1 isoform B, Rho GDI 1 isoform a, VDAC1, OTUB1 and α -enolase isoform 1 (ENO1) in control and Nef transfected SupT1 cells. Two Nef forms with variation at protease cleavage site were studied and a contrasting effect of Nef variants was seen upon the expression of these six proteins [52,53].

4.4. Nef-interacting partners identification through proteomics

Nef was used as bait and interacting proteins were subjected to proteomic investigation through LC-MS/MS and Orbitrap. Besides these, other traditional techniques like immunoprecipitation, a mammalian two-hybrid method to study protein-protein interaction and understanding of Nef regulated proteomics [54]. Studies on identification of Nef interacting proteins identified several cellular proteins includes GCC185 [46], ASK1 [55], PACS-1 and PACS-2 [56] etc. whose functions modulated in the presence of Nef.

4.5. Possible Nef association with host proteins in induced exosome formation during HIV-1 infection

Quantitative proteomic analysis of exosomes from HIV-1-infected lymphocytic cells revealed that induced Nef expression increases exosome production. Nef could be involved in the modulation of regulatory molecules in exosomal fractions with impact over cell apoptosis [45].

4.6. Nef networks with differentially expressed proteins during HIV infections

Proteomic analysis of PBMCs from HIV-positive patients and healthy controls characterized HIV associated proteins and determined potential biomarkers like vinculin, filamin-A, and talin-1. Interestingly, bioinformatics studies demonstrated host protein interaction to Nef VCL-TLN1-actin, cytoplasmic 2(ACTG1)-FLNA were found to interact with Nef. Also, Vinculin can be induced to polarize by HIV-1 Nef and induce apoptosis [23].

4.7. HIV-1 Nef and macrophage activation

It is well established that HIV-1 proteins, including Tat, gp120, and Nef involved in immune activation especially activate macrophages (MΦs). Study on HIV-1 proteins revealed that particularly Nef, preferentially activate M2-MΦs. Human peripheral blood monocyte-derived MΦ treated with extracellular Nef resulted in activation of MAPK and NF- κ B pathways. In the same study Not only signaling activation, but it was also observed that Nef was internalized more rapidly by M2-MΦ than by M1-MΦ and activates anti-inflammatory M2-MΦ [57].

These studies contribute towards understanding the infection process in these HIV-1 target cells and the further spread of HIV-1 through these cells. HIV-1 transmission to various body parts could be well studied through proteomic approaches. Knowledge of host proteins being modulated by viral proteins reflect the pathogenic attributes of these viral proteins, and the mechanism employed for an increase in infectivity could be deciphered.

5. Future prospects and challenges

As we have focused on different proteomic investigations in a variety of HIV-host interaction systems, it is very much clear that proteomic studies have immense potential to contribute for HIV therapeutics. Studies indicate comprehensive information on host-HIV interacting partners which undoubtedly pave a way to understand HIV pathogenesis and also the counter mechanisms adopted by the virus. Broad knowledge of the several host cellular and immune response, viral responses, infection mechanism and cellular targets as prognostic and diagnostic biomarkers, will open new avenues in HIV drug research. We also attempt to emphasize the significance of HIV-1 Nef protein in disease pathogenesis. The implication of Nef in host-virus interplay could be well achieved through proteomic studies with Nef. A concerted network of Nef modulated host proteins would aid in designing inhibitors of Nef actions and exploring Nef and Nef-associated host factors as therapeutic targets.

With assets of proteomic studies, comes the challenges too and need to be tackled in a better way to improve its different aspects. Proteomic studies generate huge sets of information and effective data interpretation is a big challenge. More tools to interpret the findings and understand the precise role of differentially expressed proteins in connection to the virus is required. Most of the studies are limited to identification of biomarkers but lack further investigation on how the set of proteins expressing differentially are involved in the disease process. Reproducibility is another aspect which needs to be improved. A combination of genomics, proteomics, and bioinformatic studies could resolve such issue to a good extent and help in validating and establishing the credibility of the findings. Lack of good models for HIV studies and ethical considerations for the available models is another limitation that slows down the pace of such studies. Also the biological variations among samples and the stage of the disease, early or late, greatly affect the results and needs to be taken account of to correctly understand the disease progression and related biomarkers. A promising approach to overcome the variation due to the infection stage could be extending the studies from cell to organoids. This would maintain the dynamicity of the infection and provide real-time observations. Besides these, there are technical challenges involved in performance and analysis of the study. These techniques are quite expensive and require specialized expertise in these areas due to which current knowledge is very limited and there is need to find ways to lower the expenses involved and find good analyzing software and statistical tools.

6. Conclusion

Advanced proteomic techniques offer promising strategies for investigation of proteome which allows keen insight of host-virus interplay. Studies with proteomics approach will provide a better understanding of the mechanisms of host-virus interactions for drug designing. It enables the identification of proteins involved in host-virus interaction and pathogenicity factors. Studying the difference in regulation of cellular pathways as revealed through the analysis of changes in host cell proteome regulation upon HIV-1 infection and viral proteins is a significant approach towards drug development. Several reports discussed in this review provide an in-depth understanding of host proteins involved in disease progression, HIV-associated disorders and most importantly the infection of immune cells by HIV. These studies reflect the dynamics of the state of cells during infection and provide knowledge of protein networks and alterations in protein abundance during different stages of infection. Proteomic studies allow a broad characterization of cell proteome with a comparative analysis of different strains and physiological states. The identification of altered cellular proteins in HIV-infected patients compared to healthy donors will suggest the probable route of infection and could be used as drug targets. Since Nef represents potent viral protein affecting cellular

machinery, hence Nef proteomics is of prime importance in HIV cure research. Nef proteomics studies have been done to a lesser extent, thus an emphasis on deciphering Nef modulated host proteins is needed. A comprehensive Nef regulated protein network would contribute to the development of relevant protein targets and discover novel diagnostic and therapeutic biomarkers.

Conflict of interest

Authors declare no conflict of Interest.

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