ABSTRACT

Human immunodeficiency virus is the causative agent of the Acquired immunodeficiency syndrome (AIDS) which is primarily characterized by the progressive loss of CD4+ T helper cells thereby leading to the weakening of the immune system. This condition results in susceptibility to various opportunistic infections ultimately leading to death of the infected subjects. According to the UNAIDS report 2015, a total of 36.9 million people living with HIV globally. Human immunodeficiency virus type 1 (HIV-1) is a positively stranded RNA virus which belongs to the family Retroviridae and genus Lentivirus. The genetic information of HIV-1 is carried by an RNA genome of approximately 9.8 kb which is packaged into viral particles as a non-covalent dimer. The RNA genome comprises of 9 open reading frames (ORFs) which encode for 15 different proteins including structural proteins (matrix, capsid, nucleocapsid and p6), envelope glycoproteins (gp120 and gp41) and viral enzymes (protease, reverse transcriptase and integrase) as well as regulatory proteins (Tat and Rev) and accessory proteins (Nef, Vif, Vpr and Vpu). The accessory viral proteins have complex and diverse functions and are generally found to interact with a wide range of host cellular factors thereby exploiting these factors to promote the viral replication and production.

A major cellular change brought about by HIV-1 infection in activated CD4+ T cells is cell cycle dysregulation in the host cells leading to arrest at the G2/M phase of the cell cycle. Accumulation of cells in G2/M phase is believed to be essential to enhance the replication of HIV. This is followed by cell death leading to apoptosis of the infected cells. Majorly, the viral accessory protein, Viral protein R (Vpr) has been associated with this dysregulation of the host cell cycle. Another viral protein, Viral infectivity factor (Vif) has also been shown to have similar effects. CD4+ T cell proliferation defects have also been reported in the case of HIV-1 patients. Understanding mechanisms that underlie these proliferative defects leading to apoptosis in HIV-1 infection is hence important. It is possible that several host cellular factor modulations might be mediated by the virus to enable its replication and production. In addition, there could also be many cellular factors that negatively influence the viral replication which the virus has to encounter with. Understanding the molecular alterations of cell cycle/apoptosis associated genes involved in HIV-1 infection is therefore important.

In recent years, increasing evidences of the role of different Cyclins, CDKs and CDK inhibitors in HIV-1 infection have been elucidated. Cyclin T1-CDK9 functions as a cofactor of HIV-1 Tat induced transcription elongation of viral genome. A related cyclin, Cyclin K has been shown to function as a competitor of this complex as it competes to bind with CDK9 and hence negative regulates HIV-1 replication. This is
an example of differential roles are exhibited by even related cell cycle associated proteins on HIV-1 infection. Another Cyclin-CDK complex, Cyclin A2/CDK1 have shown to be involved in the inactivation of a host restriction factor SAMHD1, by phosphorylating it, thereby involving in the positive regulation of HIV-1 infection. Cyclin L2 has been shown to be an HIV-1 dependency factor in macrophages as it causes SAMHD1 degradation. A CDK inhibitor, p21/CDKN1A have been shown to be an intrinsic inhibitor of HIV-1 reverse transcription in CD4+ T cells of elite controllers.

On the basis of the above literature, to understand the underlying expression changes exhibited by cell cycle/apoptosis associated genes, in the first part of the study we have carried out a differential gene expression analysis for cell cycle/apoptosis associated genes during HIV-1 infection. The analysis was performed using a Cell Cycle PCR array coated with primers for 84 cell cycle pathway associated genes. Some of the gene primers in the array were common to both cell cycle and apoptosis pathways. Real-time based gene expression analysis revealed that several genes associated with cell cycle/apoptosis were being differentially modulated during HIV-1 infection. Among the genes which were found to be show highest modulations, Cyclin F (CCNF/FBXO1) was identified to be highly down-modulated. Further analysis of the modulated genes in the PCR array data showed significant down-modulation of Cyclin F during HIV-1 infection in primary CD4+ T cells and T cell lines.

Cyclin F is the largest and in many respects one of the most interesting members of the cyclin family of proteins. Cyclin F is characterized as a cyclin belonging to the Cyclin A/B family owing to the presence of cyclin box in it and due to the sequence similarity to cyclins A and B. In addition to this, Cyclin F also possesses an F-box domain and is the founding member of the F-box family of proteins. F-box proteins are the substrate binding subunits of the SCF E3 ubiquitin ligases. In the SCF E3 ubiquitin ligases, F-box protein varies depending on the substrate specificity.

As Cyclin F was found to be down-modulated during infection, the second part of the study focused on the identification of the role of Cyclin F in HIV-1 infection. Overexpression and knockdown studies of Cyclin F were performed to analyse the effect of Cyclin F on viral infection. Results from these experiments consistently showed that Cyclin F expression negatively influences the infectivity of viral particles produced with seemingly no effect on the total viral production. As infectivity of viral particles directly correlates with the expression of HIV-1 Vif (Viral infectivity factor) accessory protein, expression analyses of Vif with Cyclin F overexpression/silencing was analysed which revealed a negative regulatory role of Cyclin F on Vif protein expression.

On further analysis of the Cyclin F-Vif association using co-immunoprecipitation assays, we identified a novel host-pathogen interaction between the cellular factor
Cyclin F and viral protein Vif. We confirmed the interaction between these two proteins in the presence and absence of virus using expression constructs of the two proteins as well as in endogenous conditions using infected cell lysates for co-immunoprecipitation analysis. We further went ahead to characterize the amino acid residues on Vif which are involved in Cyclin F-Vif interaction. Bioinformatic-based molecular docking analysis predicted that a putative amino acid motif in the C-terminal region of Vif is involved in the interaction with residues in the hydrophobic patch of cyclin domain of Cyclin F which was further confirmed by experimental mutational analysis of Vif followed by co-immunoprecipitation analysis with Cyclin F.

We further looked into the functional relevance of this newly identified Cyclin-Vif interaction. As Cyclin F is an F-box protein, which physically interacts with its substrates leading to their proteasomal degradation, we presumed the role of SCF$^{\text{Cyclin F}}$ E3 ligase in regulating the expression of Vif. Analysis using ubiquitination and proteasomal assays revealed that HIV-1 Vif is a substrate of SCF$^{\text{Cyclin F}}$ E3 ligase, thereby leading to the proteasomal degradation of Vif with Cyclin F expression. As Vif maintains or enhances viral infectivity by mediating proteasomal degradation of host restriction factor APOBEC3G, we analysed the effect of Cyclin F silencing on APOBEC3G expression. Results showed that Cyclin F silencing decreases APOBEC3G expression. This could possibly be because of Vif stabilisation with Cyclin F silencing. Silencing or down-regulation of Cyclin F expression is therefore beneficial to the virus to maintain its infectivity which could be the possible explanation for Cyclin F down-regulation observed during HIV-1 infection in CD4$^+$ T cells.

Thus, our results identify a novel host-pathogen interaction between Cyclin F and Vif and have been able to elucidate the functional relevance of this interaction. This could contribute to the therapeutic approaches that focus to regulate Vif-APOBEC3G interaction as we have been able to show Cyclin F as an intrinsic cellular regulator of HIV-1 Vif protein.