Chapter 6

SUMMARY AND CONCLUSIONS
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Massive apoptosis of infected as well as non-infected CD4 T lymphocytes subsets of immune cells is one of the important outcomes of HIV infection ultimately leading to immunosuppression and AIDS. The exact molecular mechanism of how HIV-1 is able to hijack the apoptotic machinery of the infected cells possibly to facilitate virus spread or to avoid innate or adaptive immune responses of the host is not known. Vpu is an accessory gene exclusive to HIV-1 not present in HIV-2 and in most Simian immunodeficiency viruses. Previous studies in macaque model using chimeric simian human immunodeficiency virus (SHIV) revealed high viral load and rapid loss of CD4+ T lymphocytes with severe depletion within lymphoid organs because of functional vpu gene. Hence vpu make HIV-1 more pathogenic and virulent.

During viral life cycle, CD4 as well as BST-2 degradation is very critical for optimum production of infectious virus particles and is achieved by binding of Vpu to CD4 receptor and an F-box protein, β-TrcP the substrate recognition subunit of SCF β-TrcP ubiquitin ligase. This interaction involves highly conserved and constitutively phosphorylated DS52GNES56 motif (β-TrcP binding motif) of HIV-1 Vpu and WD40 repeat domain of β-TrcP, leading to ubiquitination and subsequent degradation of CD4 and BST-2. However as this process involves molecular hijacking of SCF β-TrcP ubiquitin ligase complex, there is strong competitive inhibition of degradation of natural β-TrcP substrates. While looking into literature for some other novel cellular proteins as substrates of SCF β-TrcP ubiquitin ligase complex as well as key players of HIV-1 pathogenesis, we found tumor suppressor protein p53 as important substrate of β-TrcP. Since HIV-1 Vpu was also known to bind and sequester β-TrcP, we speculated it to result in alteration of β-TrcP mediated p53 ubiquitination.

We firstly analyzed the novel impact of HIV-1 vpu expression on β-TrcP dependent ubiquitination and subsequent proteosomal degradation of p53 with following physiological consequences.
Cellular expression of wild type Vpu leads to upregulation of total as well as β-TrcP substrate form of p53 (p-ser-362/366 p53).

Cellular expression of wild type but not mutant Vpu leads to higher intracellular stability of p53.


Vpu (wt) competitively inhibits cellular ubiquitination and subsequent degradation of p53.

Vpu potentiates apoptotic activity of p53 and vice versa.

Vpu contributes to p53 dependent apoptosis in HIV-1 infected MOLT cells.

Also, for a better understanding of subtype specific differences, it is essential to determine what functional differences might arise due to sequence variation among different subtypes. We reasoned that genetic differences that exist between subtypes B and C Vpu may explain known differences in its biological activities. We found that subtype C Vpu gene showed significant functional differences with respect to the two known functions (Virus release and Apoptosis) as:

Vpu from subtype B is more potent in degrading BST-2 and hence viral release process than Vpu subtype C.

The higher viral release activity was found to be contributed by N-terminal transmembrane domain of Vpu B which is known to assist viral release process.

In contrast to viral release, higher cell death potential is associated with Vpu C as contributed by determinants other than β-TrcP binding motif in Vpu C cytoplasmic helix-2 of high variations.

Also, we further analyzed the possible implication of our results with respect to naturally occurring mutations at Vpu locus among infected individuals from North
India. Our purpose was to identify the functional differences arising due to sequence variations among subtypes and subtype variants to elucidate their role in HIV pathogenesis. We were able to shed some light on the functional differences with respect to their ability to promote virus release and cause apoptosis as:

- We observed extreme heterogeneous nature of Vpu locus in HIV-1 genome displaying notable variations in sequence as well as length.
- Differences in rate and pattern of variation among subtype B and C HIV-1 Vpu gene variants.
- We noted a substitution of a phosphorylable serine residue in cytoplasmic tail of Vpu from all of the subtype B variants.
- Results of Cycloheximide chase assay confirmed higher kinetic stability associated with S61 mutants. Ubiquitination profile also suggested lesser intracellular poly-ubiquitinated species corresponding to Vpu S61 mutants then S61 wild type variants.
- Despite achieving superior viral release activity as well as expression levels, subtype B variants retained the moderate cell death potential associated with wild type Vpu B.
- A DNAzyme was designed targeting common conserved region from Vpu isolates and it showed very impressive knockdown efficiency against both Vpu B and Vpu C. Hence, despite numerous genetic variations, it is possible to achieve optimum knockdown efficiency by specifically targeting the genetically stable regions of viral proteins.

This study identified Vpu as a novel contributor to apoptosis, the pathological hallmark of AIDS, via exploitation of p53 pathway in infected cells. Also, unlike known biological activities, the previously unknown determinants for subtype specific differences were demonstrated to be β-TrcP independent. Therefore,
cytoplasmic and transmembrane region of high variations has a role in modulating known biological activities of Vpu. In support to this notion we also observed positive selection of natural isolates of Vpu with respect to currently ongoing epidemic of HIV-1 in North India. We identified another β-TrCP independent and S61 dependent mechanism assisting enhanced viral replication and moderate apoptosis in selected isolates. In summary, this thesis work adds into literature another example of ongoing struggle between host and viral determinants of pathogenesis for selection of alleles best suitable for viral persistence.