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Research Article

Conservation Analysis of HIV-1 Protein Sequences Reveal Potential Drug Binding Sites:ACase of Viral Protein U and Protease

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Abstract

The HIV-1 viral protein U (Vpu) and protease play a pivotal role in the infectious lifecycle of human immunodeficiency virus-1. The objective of this study is to find the degree of conservation of the viral protein U (Vpu) and protease protein and to detect conserved binding sites, which might be used as target sites for potential anti-Vpu and anti-protease drugs. The conservation analysis was based on 4231 amino acid sequences for Vpu and 13,457 amino acid sequences for protease. The conservation analysis revealed a number of conserved and variable residues. The universally conserved residues identified in this study might be involved in either structure stabilizing or protein-protein interactions. The novel conserved potential binding sites which have been identified are: Vpu (Ile39, Arg45, Ile46 and Gly71) and protease (Pro9, Thr80 and Asn83). Along with conservational analysis, structural analysis revealed novel binding sites, namely four conserved sites on Vpu (Arg49, Ala50, Ser53, Gly54, Gly59; Glu56, Ser57, Asp60; Glu56, Gly71; Glu48, Glu51, Asp52, Gly54-Glu56) and single novel conserved site on protease (Thr4, Trp6 and Arg87, Asn88, Thr91, Gln92). The outcome of this study provides the basis for developing anti-Vpu and anti-protease drugs which have abridged potential to induce drug resistance through mutations.

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Introduction

Acquired immunodeficiency syndrome (AIDS) is caused by the infectious agent human immunodeficiency virus (HIV), affecting estimated 2.5 million people and results in 1.5 million deaths in the year 2014 [1]. In the past few years, owing to substantial rise in the usage of anti-retroviral therapy, there has been a stable decline in the AIDS associated deaths [1].There are two types of HIV namely, human immunodeficiency virus type 1 (HIV-1) and human immunodeficiency virus type 2 (HIV-2) [2, 3]. Since the discovery of HIV-1 (which was way back in 1983) up till now, HIV-1 remains to be the foremost concern regarding public health globally [4]. By realizing the devastating nature of HIV, the researchers globally are unified to uncover solutions which will facilitate in fighting this elusive virus. As a result of strong and determined efforts, the highly active antiretroviral therapy (HAART) was developed [5-7]. HAART has drastically improved and raised the lifespan of patients affected with HIV; yet it is unable to eradicate the virus totally from the HIV patients. This is down to the fact that infected cells are resistant to the apoptosis which is bestowed by the HIV.Without eliminating these cells it is impractical to eradicate the virus absolutely from an infected individual. Therefore is of urgent importance to develop antiretroviral drugs against those proteins which are accountable for conferring apoptosis resistance to the infected cell.

HIV-1 is a RNA virus which belong to lentivirus class of retrovirus, whose genome encodes three structural proteins (gag, pol and env), two regulatory proteins (tat and rev) and four accessory proteins (vpu, vif, nef and vpr) [8, 9]. The viral protein U (Vpu) is a 16 KDa trans-membrane protein and is about 81 amino acids long [10]. Vpu is known to exist as tetramer, pentamer and hexamer [11]. The Vpu monomer is predicted to have three domains namely, N-terminal domain (NTD; residues 1-3; extra-cellular), transmembrane domain (TD; residues 4-27) and C-terminal domain (CTD; residues 28-81; intracellular) [12, 13]. The multiple roles of Vpu identified are: (i) CD4 degradation via proteasome pathway [14, 15] (ii) elevate the release of virion progeny from the infected cells via inhibition of tetherin[16, 17] (iii) down regulation of major histocompatibility complex I and major histocompatibility complex II [18, 19] (iv) hindrance in degranulation of natural killer cells by down regulating the natural killer-T and B cell antigen [20] (v) down regulation of poliovirus receptor activating ligand of natural killer cells [21] (vii) modulation of CD1d [22, 23] (vii) promotes apoptosis, mediated by p53 [24].

The HIV-1 protease (M_W= 10.7 KDa) is a constituent of aspartic protease family of about 99 amino acid residues long [25]. The essential function of HIV-1 protease is to cleave the peptide bonds in the polyproteins of gag and pol genes during the maturation process of virus [25-28]. HIV-1 protease exists as a homodimer with identical contribution of catalytic residues from both the subunits to the active site [29, 30]. The active site of the protease consists of two triads composing of Asp25-Thr26-Gly27 contributed by the two subunits [29, 30]. The active site is gated by an extended beta hairpin loop known as flaps; the region corresponding to the flaps is residues 46-56 [29, 30]. The two subunits of the protein are joined by the non-covalent interactions between N-terminal (residues 1-5) and C-terminal (residues 95-99) residues and these interactions are important in dimer stability [31].

The emergence of HIV-1 mutants which are drug resistant and the spreading of these mutants globally are serious cause of concern. To overcome this problem one has to exploit HIV-1 proteins as a potential drug targets. In order to achieve this target one has to understand the conservation

pattern of these proteins. At present, there are no licensed drugs available targeting the Vpu protein of HIV-1. There are many licensed drugs available which target the protease; they are saquinavir, Ritonavir, Indinavir, nelfinavir, Amprenavir, fosamprenavir, Lopinavir, atazanavir, Tipranavir and darunavir[32]. There are some promising compounds at clinical trials stage, which are PL-100, Brecanavir, SPI-256 and GS 8374 [32]. But the main problem with the above said drugs are that these drugs get ineffective as a result of mutations in the HIV-1 protease. To overcome this problem one has to exploit the conserved residues in the HIV-1 protease. The aims of this study are to identify the degree of conservation of Vpu and protease proteins to facilitate the detection of regions with universal conservation. By mapping the obtained conservational scores on to the protein structure in conjunction with binding site analysis suggests binding sites for antiviral compounds which are resistant to mutations that may arise in the future.

Methods

Sequence analysis and protein structure

The full length sequences of the Vpu protein were obtained from UniProt[33] belonging to all the HIV-1 subtypes were selected. For alignment of the collected proteins sequences, MUSCLE version 3.8 [34] was used with default parameters. Multiple refinements of the obtained alignment were carried out resulting in 26-30 iterations, until no further improvement was attained. The structures of Vpu protein (residues 7-25 and residues 37-81) and protease protein (residues 1-99) were obtained from Protein Data Bank (PDB) [35] with the entries 1PI8 [36]; 1VPU [37] and 3GGX [38] respectively.

Conservation analysis

By providing multiple sequence alignment and protein structure file as an input, conserved regions were identified and mapped onto the experimental protein structures using ConSurf server (http://consurf.tau.ac.il/) [39-42]. By taking evolutionary relationships among protein sequences into account, ConSurf algorithm produces consequential conservation scores. ConSurf algorithm gives more emphasis to those protein sequences which are evolutionarily distant, thus producing conservation scores which are significant [39-42]. The ensuing conservation scores are criterion scores with an average of 0 and a standard deviation of 1. The residues with score < 0 denote higher conservation and those with score >0 are variable residues [39-42].The Bayesian algorithm is used to evaluate the confidence intervals of calculated conservation scores [39-42]. The conservation score given by ConSurf server is divided into scale of nine grades which are given for the purpose of visualization [39-42]. Most variable positions are placed in grade one (turquoise), intermediately conserved positions are placed in grade five (white), and most conserved positions are placed in grade nine (maroon) [39-42].

Binding site analysis

Ligand binding sites (LBS) on Vpu protein structure were identified using COFACTOR (http://zhanglab.ccmb.med.umichedu/COFACTOR/), identifies the LBS using both global and local with templates from PDB and match local motifs of the identified template with that of query

structure [43-45]; TM-site (http://zhanglab.ccmb.med.umich.edu/COACH/), identifies the LBS using intermediary approach if both local and global alignments [45, 46]; S-site(http://zhanglab.ccmb.med.umich.edu/COACH/), identifies the LBS by explicitly comparing binding site specific sequence profiles [45, 46]and SiteHound (http://scbx.mssm.edu/sitehound/sitehound-web/Input.html) which uses energy based method to find regions with high potential for ligand interactions [47].

Results

Multiple alignments of protein sequences and Vpu protein structure

For the HIV-1 Vpu protein, 4231 sequences and for the protease protein 13,457 sequences were obtained from UniProt [33]. None of the templates identified in the Protein Data Bank (PDB) [35] has covered the entire Vpu protein. The region between the residues Leu7-Ile25 and Arg37-Leu81 of the Vpu are the only ones whose structures are experimentally solved. The experimental structure for the fragment Leu7-Ile25 consists of a single • -helix which is part of transmembrane domain of Vpu. The experimental structure for the fragment Arg37-Leu81 consists of four • -helices: helix 1 (39-49), helix 2 (53-55), helix 3 (60-63) and helix 4 (75-78); which are connected by loops. The obtained experimental structure (residues 1-99 amino acids) consists of eleven • -sheets (residues 1-3; 9-15; 18-25; 30-35; 43-47; 54-59; 62-67; 69-73; 75-78; 83-86 and 96-99) which are numbered sequentially and a lone • -helix (residues 87-92).

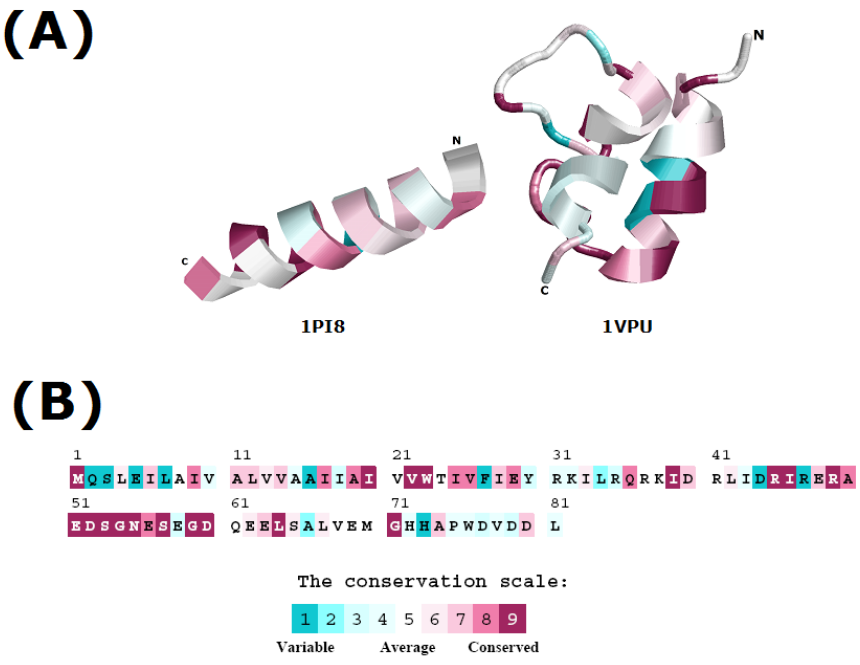

Figure 1 The conservation obtained by projecting conservation scores on to the ribbon structure of the Vpu protein (A) and the residue conservation of the Vpu protein obtained from ConSurf server [39-42].

Conserved and variable residues

The variable and conserved residues in the Vpu and protease proteins were identified using ConSurf server [39-42] and are illustrated in Figure 1 and Figure 2. The variable residues of grades 1-3 and conserved residues of grades 7-9 are clustered together in Table 1. The conservation scores were projected onto the Backbone ribbon structures of the Vpu protein and protease protein and are shown in Figure 1 and Figure 2.

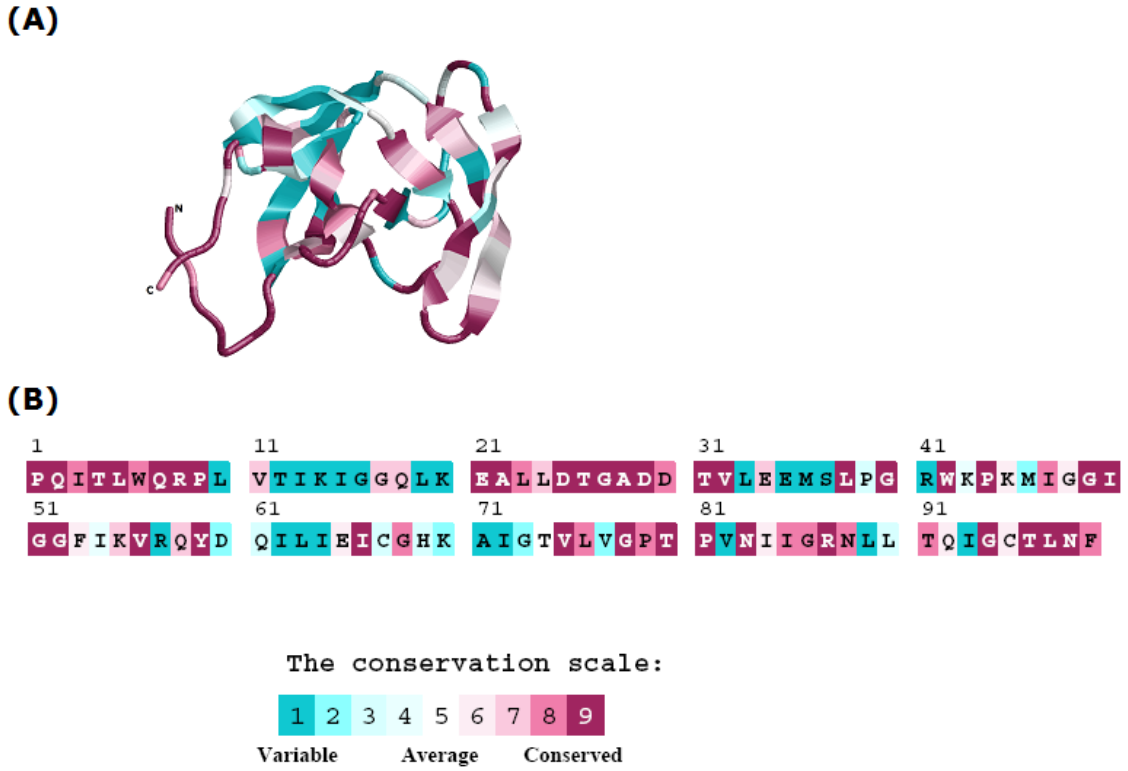

Figure 2 The conservation obtained by projecting conservation scores on to the ribbon structure of the protease protein (A) and the residue conservation of the protease protein obtained from ConSurf server [39-42].

Table 1 Variable and conserved residues of Vpu and protease proteins of HIV-1 identified using the ConSurf server [39-42].

Residues	Viral protein U	Protease
Conserved (grades 7-9)	Met1, Ile6, Ile9, Ala11, Leu12, Val14, Ile17, Ala19, Ile20, Val22, Trp23, Ile25, Val26, Ile28, Glu29, Gln36, Ile39, Asp40, Arg45, Ile46, Glu48-Ser57, Gly59, Asp60, Glu63, Leu64, Gly71, Ala74, Asp80	Pro1-Pro9, Val11, Gly17, Glu21-Val32, Glu34, Leu38, Gly40, Trp42, Pro44, Lys45, Ile47, Gly49-Gly52, Lys55, Val56, Gln58, Tyr59, Glu65, Ile66, Gly68, Val75, Leu76, Gly78-Pro81, Asn83, Ile85-Asn88, Thr91, Gln92, Gly94, Thr96- Phe99
Variable (grades 1-3)	Gln2, Ser3, Glu5, Leu7, Ala16, Ile18, Phe27, Tyr30, Leu34, Arg35, Asp44, Arg47, Glu58, Ala66, His73, Asp77, Asp79	Leu10, Thr12-Gly16, Leu19,Lys20, Leu33, Glu35-Ser37, Arg41, Met46, Arg57, Asp60, Ile62- Ile64, Cys67, His69, Lys70-Gly73, Val77, Val82, Leu89, Leu90, Ile93

Conservation scores for the Vpu protein were obtained between -0.929 (maximum conservation) and 3.782 (maximum variability) by the ConSurf server [39-42]. In general the Vpu protein is conserved with approximately 45.7% of the residues belong to grades 7-9 (conserved), while 21% of the residues belong to grades 1-3 (variable). Altogether, eighteen residue positions (approximately 22.3% of total residues) were found to be highly conserved (grade 9) and are shown in the Table 1. The residue positions 1, 53, 55 and 57 showed highest conservation among all the sequences analysed. In total, nine residues (11.2% of total residues) were found to be highly variable (grade 1). The residue positions 5, 7, and 27 showed highest variation among all the sequences analysed.

The transmembrane • -helix (Leu7-Ile25) was found to be intermediately conserved, with residues Ile20, Val22 and Trp23 were found to be highly conserved (grade 9). The • -helix 1 of the CTD fragment was found to be intermediately conserved with about 55% conservation. Residues Ile39, Arg45, Ile46 and Arg49 were found to be highly conserved (grade 9), while residues Asp40 and Glu48 were found to be conserved (grade 7). The second • -helix was found to be highly conserved with residues Ser53, Gly54 and Asn55 belong to grade 9. The third • -helix was found to be intermediately conserved with Asp60 (grade 9) andGlu63 (grade 7) were only residues to be found conserved. The fourth • -helix was also found to be variable with its residue either in grade 3 or grade 4. The loop joining the • -helix 1 and • -helix 2 was found to be highly conserved, while the loop joining the • -helix 2 and • -helix 3 was found to be conserved. The loop joining the • -helix 3 and • -helix 4 was found to be variable. Apart from the conserved residues in the known functional regions of Vpu, novel highly conserved residues were identified, namely Ile39, Arg45, Ile46 and Gly71.

Conservation scores for the protease protein were obtained between -0.770 (maximum conservation) and 5.304 (maximum variability) by the ConSurf server [39-42]. In general the protease protein is conserved with approximately 61% of the residues belong to grades 7-9 (conserved), while 31.1% of the residues belong to grades 1-3 (variable).Altogether, forty one residue positions (approximately 41% of total residues) were found to be highly conserved (grade 9) and are shown in the Table 1. The residue positions 1, 9, 26-29, 31, 44, 56, 80 and 83 showed highest conservation among all the sequences analysed. In total, twenty two residues (20.5% of total residues) were found to be highly variable (grade 1). The residue positions 10, 19, 37 and 63 showed highest variation among all the sequences analysed.

The conservation analysis of protease protein revealed a pattern in residue conservation across its secondary structures. The • -sheet 1 and • -sheet 11 were found to be highly conserved, whereas • -sheet 8 was found to be highly variable. The • -sheet 3, • -sheet9 and • -sheet 10 showed similar level of amino acid conservation where as residue conservation of • -sheet 4, • -sheet 5 and • -sheet 6 were alike. A very low amount of residue conservation was observed in the • -sheet 2 and • -sheet 7. The loop 1, loop 3 and loop 8 were found to be completely conserved, while loop 7 and loop 9 were found to be variable. Intermediate conservation was observed in the loop 2 and loop 11, whereas loop 5 and loop 10 were found to be mostly conserved. The lone • -helix showed moderate level of conservation among its amino acid residues.

Small molecule binding potential

One of the important objectives of the present study was to detect potential drug binding sites which are spatially in close proximity to the conserved regions. Putative ligand binding sites were

identified using COFACTOR (detects LBS by global and local alignments with template structures in PDB), TM-site (detects LBS by transitional approach balancing global and local alignments), S-site (detects LBS by comparing sequence profiles which are binding site specific) and SiteHound (detects LBS by positive interaction between a chemical probe and protein structure) algorithms [43-47].By amalgamating the binding site results with that of evolutionary analysis, binding sites which are in the regions of conservation were identified on both Vpu and protease proteins which are shown in Table 2.

Table 2 The putative conserved ligand binding sites identified on Vpu and protease protein using COFACTOR, TM-site, S-site and SiteHound algorithms [43-47].

Site	Binding sites located in conserved regions of Vpu protein	Binding sites located in conserved regions of protease protein
1	**Arg49, Ala50, Ser53, Gly54, Gly59**	**Asp25, Gly27, Asp29, Asp30, Val32, Gly49, Ile50, Pro81**
2	**Glu56, Ser57, Asp60**	**Asp25, Gly27, Gly49, Ile50**
3	**Glu56, Gly71**	**Thr4, Trp6**
4	**Glu51, Asp52**	**Leu5, Trp6, Gln7, Arg8, Pro9, Leu23, Leu24, Asp25, Thr26, Gly27**
5	**Glu48, Glu51, Asp52, Gly54, Asn55,Glu56**	**Ala28, Asp29, Asp30, Ile47**
6	**Ile46, Arg49**	**Gln2,Ile3, Thr4, Leu5, Trp6, Gln7**
7	**Asp60, Glu63**	**Pro1, Gln2, Ile3, Thr96, Leu97, Asn98, Phe99**
8	**Glu48, Glu51**	**Arg87, Asn88, Thr91, Gln92**
9	**Arg45, Glu48**	

Note: Conserved residues (grade 7-8) are shown in bold face while highly conserved residues (grade 9) are shown in bold face and underlined.

In total nine potential binding sites with high conservation were found on the HIV-1 Vpu protein. Binding sites 1 to 9 are located in the conserved regions. Site 4 and site 6 are found to be highly conserved, while site 1, site 2, site 5 and site 7 were found to contain highly conserved residues. In total eight potential binding sites with high conservation were found on the HIV-1 protease. Highly conserved sites were found in close proximity to the active site, namely site 1, site 2, site 4 and site 5. Conserved sites namely, site 3, site 6 and site 7 are found to be located in the region which is involved in non-covalent interactions. Binding site 8 is located in close proximity to the region which is important in the formation of the dimer.

Discussion

Sequence conservation

The objective of the present study was to determine the degree of conservation of the Vpu protein among all the subtypes of human immunodeficiency viruses. The Vpu and protease proteins from all subtypes were analyzed together to facilitate the identification of universally conserved residues of potential pandemic HIV viruses that might arise in future due to either a event of mutation. The

conserved residues detected on the Vpu and protease proteins may have either functional importance or structural importance [48]. On the contrary, variable sites arise as a result of either adaptation or evolutionary pressure to evade the host immune system [48].

In the regions which are responsible for the differential ability of Vpu belonging to HIV-1 and simian immunodeficiency virus-chimpanzee (residues 1-8 and 14-22) [49], residues Met1, Ile20 and Val22 were found to be highly conserved (grade 9), while residues Ile6, Val14, Ile17 and Ala19 were found to be conserved (grades 7-8). Likewise in the region which is recognised by beta transducing repeat-containing protein (residues 52-57) [50], except Glu56 which was found to be conserved (grade 8), all the other residues were found to be highly conserved (grade 9). The regions which are assigned for trafficking signals (residues 29-34 and residues 63-68) [51, 52], residues Glu29 and Glu63 were found to be conserved (grades 7-8), while residue Leu64 was found to be highly conserved (grade 9). Amino acid residues which were found to be critical in the interaction between Vpu and tetherin proteins (residues 11, 14-16, 18, 22, 26-28, 34, 37 and 41) [53-55], residues Ala11, Val14, Val26 and Ile28 were found to be conserved residues (grades 7-8), while residue Val22 was found to be highly conserved.

In the functionally important regions of HIV-1 protease, high residual conservation was found. In the regions (1-5 and 95-99) which are important in the formation and maintaining the stability of the dimer, residues Pro1, Gln2, Thr4, Leu5, Thr96, Leu97 and Asn98 were found to be highly conserved (grade 9), while residues Ile3 and Phe99 were found to be conserved (grade 8). The active site triad residues (Asp25, Thr26 and Gly27) were found to be highly conserved (grade 9). In the flap region which gates the active site of the protease, residues Ile47, Gly49, Ile50, Gly51, Gly52 and Val56 were found to be highly conserved (grade 9), while residue Lys55 was found to be assigned to grade 7 (conserved). Apart from the residues with highest conservation in the known functional region, novel highly conserved residues elsewhere in the protein were detected, namely Pro9, Thr80 and Asn83. These residues with highest conservation might have functional or structural importance which is currently not known.

Small molecule binding potential

Most of the predicted LBS identified on the Vpu and protease proteins are surrounded by conserved residues. Targeting these LBS using small drug molecule will most probably result in disruption of Vpu and protease function. Among the predicted binding sites identified by different methods, few novel binding sites were detected on both the proteins. These novel sites may be either involved in new protein-protein interactions or sites of known protein-protein interactions.

The novel binding sites identified on Vpu protein are binding site 1 to binding site 9. These novel sites are completely conserved. Targeting these sites using small ligand molecule would inhibit the functions of the Vpu protein. These sites might be functionally important which can be explained by the degree of conservation found in these sites and the functions of these sites are not elucidated yet.

In order to significantly inhibit the main activity of the HIV-1 protease that is cleavage of the peptide bonds in the polyproteins of gag and pol genes, binding sites- site 1, site 2, site 5 and site 6 should be targeted with small drug like molecules. Upon binding to these sites, these drug molecules either inhibit the ligand binding to the active site or induce a conformational change in the structure of the protein such that the protein is rendered useless. The HIV-1 protease is known to be functional only in the dimer state that is homodimer, to inhibit the protein from forming dimer

binding sites: site3, site 7, site 8 and site 9 should be targeted with small drug like organic molecules. After binding to these sites, the drug molecules will inhibit the formation of non-covalent interactions between the residues which leads to dissociation of the two homomer. More over by targeting the binding sites identified in this study, it is highly unlikely that the drugs will become ineffective by an event of mutation in the HIV-1 protease that may arise in the future.

Conclusion

In conclusion the study of 4231 Vpu protein sequences and 13,457 protease protein sequences divulge an elevated level of sequence conservation pattern that intersects with prospective ligand binding site analysis rendering the Vpu and protease proteins as an exceptional drug target. Targeting the conserved binding sites identified in this study using small drug molecule will effectively diminish the activity of the both the proteins. Moreover, anti-Vpu and anti-protease drugs targeting these conserved sites are less likely to become ineffective due to drug resistance in the future.

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References

1. Singh J, Chhikara BS. Comparative global epidemiology of HIV infections and status of current progress in treatment. ***ChemBiol Lett.*** 2014, 1(1): 14-32
2. Barré-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T–lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome. ***Science.***1983, 220:868-870
3. Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. ***Science.***1984, 224: 500-504
4. United nations joint programme on HIV/AIDS, by the number, Geneva, Switzerland: ***UNAIDS***, 2013
5. Cohen MS, Chen YQ,McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, Hakim JG, Kumwenda J, Grinsztejn B, Pilotto JH, *et al.* Prevention of HIV-1 infectionwith early antiretroviral therapy. ***N Eng J Med***. 2011, 365: 493-505
6. Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS, Eron JJ Jr, Feinberg JE, Balfour HH Jr, Deyton LR, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection andCD4 cell counts of 200 per cubic millimeter or less. AIDS clinical trials group 320 study team. ***N Engl J Med.*** 1997, 337: 725-733
7. Davey RT Jr, Bhat N, Yoder C, Chun TW, Metcalf JA, Dewar R, Natarajan V, Lempicki RA, Adelsberger JW, Miller KD, et al.HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. ***Proc. Natl. Acad. Sci.***1999, 96: 15109-15114
8. Barré-Sinoussi F. HIV as the cause of AIDS. ***Lancet.*** 1996, 348: 31-35

9. Frankel A, Young J. HIV-1: fifteen proteins and an RNA. ***Annual Review of Biochemistry.***1998, 67:1-25
10. Strebel K, Klimkait T, Martin MA. A novel gene of HIV-1, vpu, and its 16-kilodalton product. ***Science*** 1988, 241:1221-1223
11. Padhi S, Khan N, Jameel S, Priyakumar UD. Molecular Dynamics Simulations Reveal the HIV-1 Vpu Transmembrane Protein to Form Stable Pentamers. ***PLoS ONE.***2013,8(11): e79779
12. Maldarelli F, Chen MY, Willey RL, Strebel K. Human immunodeficiency virus type 1Vpuprotein is an oligomeric type I integral membrane protein. ***J Virol.*** 1993, 67: 5056-5061
13. Wray V, Federau T, Henklein P, Klabunde S, Kunert O, Schomburg D, Schubert U. Solution structure of the hydrophilic region of HIV-1 encoded virus protein U (Vpu) by CD and 1 H NMR spectroscopy. ***Int J Pept Protein Res.*** 1995, 45: 35-43
14. Chen MY, Maldarelli F, Karczewski MK, Willey RL, Strebel K. Human immunodeficien-cy virus type 1 Vpu protein induces degradation of CD4 invitro: the cytoplasmic domain of CD4 contributes to Vpu sensitivity. ***J Virol.***1993, **67**:3877-3884
15. Bour S, Schubert U, StrebelK.The human immunodeficiency virus type 1 Vpu protein specifically binds to the cytoplasmic domain of CD4: implications for the mechanism of degradation. ***J Virol.***1995, 69:1510-1520
16. Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. ***Nature.***2008, 451: 425-430
17. Nomaguchi M, Fujita M, Adachi A. Role of HIV-1 Vpu protein for virus spread and pathogenesis. ***Microbes Infect.***2008, 10: 960-967
18. Kerkau T, Bacik I, Bennink JR, Yewdell JW, Hunig T, Schimpl A, etal. The human immunodeficiency virus type1 (HIV-1) Vpu protein interferes withan early step in the biosynthesis of major histocompatibility complex (MHC) class I molecules. ***J Exp Med.***1997, 185:1295-1305
19. Hussain A, Wesley C, Khalid M, Chaudhry A, Jameel S. Human immunodeficiency virus type1 Vpu protein interacts with CD74 and modulates major histocompatibility complex class II presentation. ***JVirol.***2008, 82: 893-902
20. Shah AH, Sowrirajan B, Davis ZB, Ward JP, Campbell EM, Planelles V, Barker E, Degranulation of natural killer cells following interaction with HIV-1-infected cells is hindered by downmodulation of NTB-A by Vpu. ***Cell Host Microbe.***2010, 8: 397-409
21. Matusali G, Potestà M, Santoni A, Cerboni C, Doria M. The human immunodeficiency virus type 1 Nef and Vpu proteins downregulate the natural killer cell-activating ligand PVR. ***J Virol.*** 2012, 86:4496-4504
22. Moll M, Andersson SK, Smed-Sörensen A, Sandberg JK. Inhibition of lipid antigen presentation in dendritic cells by HIV-1 Vpu interference with CD1d recycling from endosomal compartments. ***Blood.*** 2010, 116:1876-1884
23. Kelly H, Mandraju R, Coelho-dos-Reis JG, Tsuji M. Effects of HIV-1-induced CD1c and CD1d modulation and endogenous lipid presentation on CD1c-restricted T-cell activation. ***BMC Immunol***. 2013, 14:4
24. Verma S, Ali A, Arora S, Banerjea AC. Inhibition of •-TrcP-dependent ubiquitination of p53 by HIV-1 Vpu promotes p53-mediated apoptosis in human T cells. ***Blood.*** *2*011, 117: 6600-6607
25. Brik A, Wong CH. HIV-1 protease: mechanism and drug discovery. ***Org Biomol Chem.*** 2003, 1(1): 5-14

26. Wartha F, Horn AHC, Meiselbach H, Sticht H. Molecular dynamics simulations of HIV-1 protease suggest different mechanisms contributing to drug resistance. ***J Chem Theory Comput.*** 2005, 1: 315-324
27. Kohl NE, Emmi EA, Schleif WA, Davies LJ, Heimbach JC, Dixon RAF. Active human immunodeciency virus protease is required for viral infectivity. ***Proc Natl Acad Sci.*** 1988, 85: 686-690
28. Katoh I, Yasunaga T, Ikawa Y, Yoshinaka Y. Inhibition of retroviral protease activity by an aspartyl proteinase inhibitor. ***Nature***. 1987, 329(6140): 654-656
29. Navia MA, Fitzgerald PM, McKeever BM, Leu CT, Heimbach JC, Herber WK, Sigal IS, Darke PL, Springer JP. Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1. ***Nature***. 1989, 337: 615-620
30. Wlodawer A, Miller M, Jaskolski M, Sathyanarayana BK, Baldwin E, Weber IT, Selk LM, Clawson L, Schneider J, Kent SB. Conserved folding in retroviral proteases: crystal structure of a synthetic HIV-1 protease. ***Science***. 1989; 245: 616-621
31. Ceccherini-Silberstein F, Erba F, Gago F, Bertoli A, Forbici F, Bellocchi MC, Gori C, D'Arrigo R, Marcon L, Balotta C, Antinori A, Monforte AD, Perno CF. Identification ofthe minimal conserved structure of HIV-1 protease in the presence and absence of drugpressure. ***AIDS***. 2004, 18(12): F11-F19
32. Pokorná J, Machala L, • ezá•ová P, Konvalinka J. Current and Novel Inhibitors of HIV Protease. ***Viruses.*** 2009, 1(3): 1209-1239
33. UniProt Consortium: The Universal Protein Resource (UniProt) in 2010. ***Nucleic Acids Res.***2010, 38(Database issue): D142-D148
34. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. ***Nucleic Acids Res***. 2004, 32: 1792-1797
35. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. ***Nucleic Acids Res.***2000, 28(1):235-242
36. Park SH, Mrse AA, Nevzorov AA, Mesleh MF, Oblatt-Montal M, Montal M, Opella SJ. Three-dimensional structure of the channel-forming trans-membrane domain of virus protein "u" (Vpu) from HIV-1. ***J Mol Biol.***2003, 333: 409-424
37. Willbold D, Hoffmann S, Rosch, P. Secondary structure and tertiary fold of thehuman immunodeficiency virus protein U (Vpu) cytoplasmatic domain in solution. ***EurJ Biochem.*** 1997, 245: 581-588
38. Degoey DA, Grampovnik DJ, Flentge CA, Flosi WJ, Chen HJ, Yeung CM, Randolph JT, Klein LL, et al. 2-pyridyl p1'-substituted symmetry-based human immunodeficiency virus protease inhibitors (a-792611 and a-790742) with potential for convenient dosing and reduced side effects. ***JMedChem***. 2009, 52: 2571
39. Landau M, Mayrose I, Rosenberg Y, Glaser F, Martz E, Pupko T, Ben-Tal N. ConSurf: the projection of evolutionary conservation scores of residues on protein structures. ***Nucl Acids Res***. 2005, 33: W299-W302
40. Glaser F, Pupko T, Paz I, Bell RE, Bechor D, Martz E, Ben-Tal N.ConSurf: Identification of Functional Regions in Proteins by Surface-Mapping of Phylogenetic information. ***Bioinformatics***. 2003, 19:163-164
41. Ashkenazy H, Erez E, Martz E, Pupko T, Ben-Tal, N. ConSurf 2010: calculating Evolution-ary conservation in sequence and structure of proteins and nucleic acids. ***Nucleic Acids Res***. 2010 38: W529-W533

42. Celniker G, Nimrod G, Ashkenazy H, Glaser F, Martz E, Mayrose I, Pupko T, Ben-Tal, N. ConSurf: Using Evolutionary Data to Raise Testable Hypotheses about Protein Function. ***Israel Journal of Chemistry.*** 2013, 53(3-4): 199-206
43. Roy A, Zhang Y. Recognizing protein-ligand binding sites by global structural alignment and local geometry refinement. ***Structure.*** 2012, 20: 987-997
44. Roy A, Yang J, Zhang. COFACTOR: an accurate comparative algorithm for structure-based protein function annotation. ***Nucleic Acids Research.***2012, 40: W471-W477
45. Yang J, Roy A, Zhang Y. BioLiP: a semi-manually curated database for biologically relevant ligand-protein interactions. ***Nucleic Acids Research.*** 2013, 41: D1096-D1103
46. Yang J, Roy A, Zhang Y. Protein–ligand binding site recognition using complementarybinding-specific substructure comparison and sequence profile alignment. ***Bioinformatics.*** 2013, 29 (20): 2588-2595
47. Hernandez M, Ghersi D, Sanchez R. SITEHOUND-web: a server for ligand bindingSite identification in protein structures. ***Nucleic Acids Res.*** 2009, 37: W413-W416
48. Schueler-Furman O, Baker D. Conserved residue clustering and protein structure prediction. ***Proteins.***2003, 52, 225-235
49. Lim ES, Malik HS, Emerman M. Ancient adaptive evolution of tetherin shaped the functions of Vpu and Nef in human immunodeficiency virus and primate lentiviruses. ***J Virol.*** 2010, 84:7124-7134
50. Nakayama KI, Nakayama K. Ubiquitin ligases: cell-cycle control and cancer.***Nat RevCancer.*** 2006, 6:369-381
51. Ruiz A, Hill MS, Schmitt K, Guatelli J, Stephens EB. Requirements of the membrane proximal tyrosine and dileucine-based sorting signals for efficient transport of the subtype C Vpu protein to the plasma membrane and in virus release.***Virology.*** 2008, 378:58-68
52. Bonifacino JS, Traub LM. Signals for sorting of transmembrane proteins to endosomes andlysosomes.***Annu Rev Biochem.*** 2003, **72:**395-447
53. Vigan R, Neil SJ. Determinants of tetherin antagonism in the transmembrane domain of the human immunodeficiency virus type 1 vpu protein.***J Virol.*** 2010, 84**:** 12958-12970
54. Kobayashi T, Ode H, Yoshida T, Sato K, Gee P, Yanamato S, Ebina H, Strebel K, Sato H, Koyanagi Y. Identification of amino acids in the human tetherin transmembrane domain responsible for HIV-1 Vpu interaction and susceptibility.***J Virol.*** 2011, 85:932-945
55. Pang X, Hu S,Li J, Xu F, Mei S, Zhou J, Cen S, Jin Q, Guo F. Identification of novel key amino acids at the interface of the transmembrane domains of human BST-2 and HIV-1 Vpu. ***Retrovirology.*** 2013, 10:84