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Characterization of Gp41 Polymorphisms in the Fusion Peptide Domain and T-20 (Enfuvirtide) Resistance-Associated Regions in Korean HIV-1 Isolates

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HIV-1 gp41 is an envelope protein that plays an essential role in virus entry. The mutation of gp41 affects HIV-1 entry and susceptibility to the fusion inhibitor T-20. Therefore, we analyzed the natural polymorphism of gp41 of 163 HIV-1 isolates from T-20-naïve Koreans infected with HIV-1. This study of gp41 polymorphisms showed that insertions in the fourth threonine (74.8%) and L7M substitutions (85.3%) were more frequent in the fusion peptide motif in Korean HIV-1 isolates compared with those from other countries. Minor T-20 resistance mutations such as L45M (1.2%), N126K (1.2%), and E137K (6.7%) were detected, but the critical T-20 resistance mutations were not detected in the gp41 HR1 and HR2 regions. In addition, the N42S mutation (12.9%) associated with T-20 hypersusceptibility was detected at a high frequency. These results may serve as useful data for studies considering T-20 for use in the development of a more effective anti-retroviral treatment in Korea.

Keywords: Fusion Inhibitor; Fusion Peptide; Gp41 Polymorphism; HIV-1 Envelope Glycoprotein; T-20 Resistance Mutation

AIDS is a disease caused by HIV infection, which destroys the CD4 T cells responsible for cell-mediated immunity in the human body, thereby undermining the immune system. HIV infections have spread gradually since the first case was reported in the USA in 1981 (1). In Korea, the first patient was identified in 1985, with the number of HIV-infected patients rising to 8544 by 2011 (2). The increasing number of HIV-1-infected patients in Korea is causing novel clinical problems such as increased drug resistance in patients treated with highly active antiretroviral therapy (HAART), thus raising the need to develop more effective therapies for patients for whom antiretroviral treatment is or has been unsuccessful (3).

Recently, the HIV-1 envelope glycoprotein was highlighted as an important alternative target for the development of new antiretroviral drugs and vaccines. The glycoprotein is expressed as a polyprotein, which is subsequently cleaved into two subunits, gp120 and gp41, by the host furin (subtilysin proteinase) (4). Despite the more stable genetic characteristics of gp41 compared with gp120, mutations in gp41 may affect HIV-1 entry, pathogenesis, escape from host immune pressure, and resistance to the fusion inhibitor T-20 (enfuvirtide, Fuzeon®). HIV-1 gp41 protein plays an essential role in virus-cell fusion and has a complex structure with three functionally critical domains. The fusion peptide (FP) at the amino (N) terminal of gp41 normally contains 20 amino acids, which attach directly to membrane of the host cell during the first step of fusion. The 16 residues of FP (AVGIGALFLGFLGAAG) are mainly hydrophobic, and facilitate attachment to the cell's membrane. In particular, the conserved FLGFL motif at positions 8-12 has been reported to be essential for fusogenic activities (5). Recent studies have shown that mutations with a polar residue inserted into this motif, such as V2E and L9R substitutions, reduced its fusogenic activity significantly (6).

Other structures in gp41 that are important for viral fusion are two helical repeats, heptad repeat 1 (HR1) and HR2, which include hydrophobic regions that interact with each other during virus-cell fusion via the formation of a six-helix bundled structure (7). The integrity of both repeats is important for the formation of a functional fusogenic complex. Therefore, the HR1 and HR2 domains of gp41 have been considered as potential targets for anti-HIV-1 therapy.

The HIV fusion inhibitor T-20, a synthetic 36-amino acid pep-

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tide, which is a homolog of amino acids 127-162 in the HR2 region, was approved as a new class of antiretroviral drug that targets the HR1 domain of gp41 (7). T-20 can restrict HIV-1 replication in an efficient manner (7), but several T-20 resistance mutations have been reported in HR1 as follows: G36D/S, I37V, V38A/M/E, Q39R, Q40H, N42S/T/D/E, N43D/K/S, L44M, L45M, G36S/L44M, G36S/V38M, V38A/N42D, V38A/N42T, V38E/N42S, N42T/N43S and N42T/N43K (8). Recently, it has also been reported that T-20 resistance is greatly increased by a combined mutation of HR1 with secondary and/or compensatory mutations in the HR2 region (N126K, E137K, S138A) (9). Although T-20 therapy is known to efficiently control viral load in HIV-1 infected patients, mutation associated with T-20 resistance was observed to allow persistent replication under T-20 treatment in a clinical study (10).

In this study, we analyzed the distributions of HIV-1 gp41 polymorphisms and T-20 resistance mutations in order to determine whether T-20 is suitable for treating Korean patients infected with HIV-1. We studied HIV-1 isolates from 163 T-20-naive HIV-1 infected patients who were diagnosed in Korea National Institute of Health (KNIH) in the period 2009-2011. The median CD4+ count was 291 cells/μL, and fifteen patients received effective ART (9.2%). Peripheral blood mononuclear cells (PBMCs) were obtained from these patients. The HIV-1 gp41 genes were amplified by nested DNA PCR. The HIV-1 gp41 polymorphisms were analyzed using bioinformatics software DNASTAR® (DNASTAR Inc, Madison, Wisconsin, USA) (11) and compared with the gp41 regions of then reference strain HXB2.

Previously, we reported that the dominant Korean HIV-1 subtype was Korean B subtype, which was classified into a separate branch compared with isolates obtained from North Americans and Europeans (12). In the present study, the distribution of the HIV-1 subtypes in our 163 HIV-1-infected patients comprised 148 patients with subtype B and 15 with non-B subtypes, which were identified as subtypes G (n = 6), A (n = 1), AB (n = 1), AE (n = 3), C (n = 1), and E (n = 3).

In our FP analysis, we found that there were no variations in the FLGFL motif, but that there were many specific insertional mutations of polar uncharged (threonine: T) and non-polar hydrophobic amino acids (valine, V; alanine, A; isoluecine, I; and methionine, M) in position 4. Table 1 shows that the dominant mutation was a T-insertion mutation (n = 122, 74.8%), while an L7M substitution near the FLGFL motif was also frequent in HIV-1-infected patients (n = 139, 85.3%). Glycine insertions were observed in the reference J, K, and N subtypes obtained from the database of the Los Alamos National Laboratory (http://www.hiv.lanl.gov/content/index), but insertions of polar and other non-polar residues were detected for the first time in our study. In addition, many of the FPs in Korean HIV-1 isolates had L7M substitutions near the FLGFL motif. These mutated residues generally have hydrophobic side chains, with the exception of the fourth threonine insertion, but further investigations should be performed to determine the mutation-related fusion activities of Korean HIV-1 gp41 (Table 1).

Table 1. Polymorphisms in the gp41 fusion peptide domain*

FP	HXB2	163 isolates from HIV-1 infected Koreans		Reference sequences
		Amino acid polymorphism in clade B (n = 148)†	Amino acid polymorphism in non-clade B (n = 15)	Amino acid polymorphism in subtype reference (n = 39)
1	A	D (1)		
2	V	I (3), L (2)		A (1), I (6), F (2)
3	G	T (2) **A (6), I (1), M (2), T (118), V (4)‡**	**T (4)**	T (1), A (2) **G (2)**
4	I	F (5), L (55), M (3), V (2)	L (5)	L (21), M (7), F (1), V (2)
5	G			
6	A			
7	L	M (130), V (9)	F (2), M (9), V (4)	M (5), V (18), F (2), I (1)
8	F	L (1)	I (6), L (1)	L (3)
9	L		F (6)	I (2), F (2)
10	G			R (1)
11	F	I (1), L (3)		V (3)
12	L			
13	G			S (4)
14	A	T (7)	T (3)	T (6)
15	A			
16	G			
17	S			
18	T			
19	M			N (1)
20	G			I (1)

*This table shows the single-letter codes for the amino acids at each position in the HXB2 reference strain. †Number of isolates. ‡Amino acids identified as insertional mutations. FP, Fusion Peptide; HXB2, HIV-1 reference strain.

Several in vitro studies have reported that the loss of T-20 susceptibility is closely related to mutations at positions 36-38 in the GIV motif, which is the T-20-binding domain in the HR1 region (13). Moreover, single-or double-amino acid mutations at positions 36-45 in the HR1 region reduced the susceptibility to T-20 by 5- to 100-fold, while the presence of more than three mutations in the HR1 region reduced the susceptibility by > 400-fold (14). Under selective drug pressure, the low fidelity of HIV-1 reverse transcriptase could lead to the development of T-20 resistance mutations (15). Several mutations (N126K, E137K, and S138A) in the HR2 domain contributed to enhanced viral fusion activity and reduced the susceptibility to T-20 (16). The susceptibility was dramatically reduced by about 1,000-fold, and the structural stability of the HR/HR2 complex was improved when the HR1 resistance mutations were coupled with HR2 resistance mutations (16). In addition, minor mutations associated with reduced susceptibility to T-20 have been reported in several countries as follows: N42S (23%), L44M (2.5%), G36S (0.5%), and S138A (4%) in Spain (17); N43K (1.8%), L44M (2.5%), E137K (10%), and S138A (2.5%) in Brazil (18); N42S (16.4%) and E137K (25%) in Italy (19); and L44M (1.7%), N42S (16%), E137K

Table 2. T-20 resistance-associated mutations in gp41 HR1 and HR2*

Gp41 region	T-20 resistance mutation (HS)†	Amino acid polymorphism in clade B (n = 148)‡	Amino acid polymorphism in non-clade B (n = 15)
HR1	**G36D/E/S/V**§ I37V V38A/E/G/M Q40H **N42T/E**/(S) **N43D/K/S** **L44M** L45M	N42S (10), N42H (1), N42Q (2), L45M (2)	N42S (11)
HR2	N126K E137K S138A	N126K (2) E137A (2), E137D (6), E137K (11), E137L (2), E137Q (8), E137T (3), E137V (1)	E137A (1) , E137G (1), E137T (6)

*This table shows the single-letter codes for the amino acids at each position in the HXB2 reference strain. †The N42S mutation is associated with T-20 hyper-susceptibility. ‡Number of isolates. §Major T-20 resistance-associated mutations (bold).

(15.4%), S138A (8.6%), N42S/S137A (0.5%), and N42S/L44M/E137K (0.5%) in the USA (20). In our study, the naturally acquired T-20 resistance mutation was L45M substitutions (n = 2, 1.2%); and the N42S substitution (n = 21, 12.9%) associated with enhanced T-20 susceptibility was more frequently detected in the HR1 region of HIV-1 gp41 from 163 naïve HIV-1 isolates (14). The N42S mutation has been detected more frequently in subtype non-B isolates than subtype B isolates (73.3% vs 6.8%; $P < 0.001$), whereas the L45M mutation has been detected only rarely in subtype B isolates (1.2%). The minor T-20 resistance mutations detected in the gp41 HR2 region were N126K (1.2%) and E137K (6.7%) in the HIV-1 B subtype only. By contrast, the major T-20 resistance mutations were not detected in the gp41 HR1 region, and the S138A substitution combined with mutations in the HR1 region was not detected in the HR2 regions of the Korean HIV isolates (Table 2). The frequencies of these mutations were also similarly observed in the ART experienced patients (for which the relevant data is not shown). These results demonstrate clearly that the naturally acquired T-20 resistance in the HR1 and HR2 regions of gp41 may be ignored in Korean HIV-1 isolates, while the geographic differences in T-20 resistance mutations are likely to be associated with HIV-1 evolution and HIV-1 subtype distributions in specific countries.

Overall, our results suggest that the genetic variation of gp41 in specific countries may be provided as useful information to consider T-20 for studies considering the use of T-20 in the development of a more effective anti-retroviral treatment in Korea.

DISCLOSURE

The authors declare that they have no competing interests.

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