Mutations in the HIV-1 Envelope Glycoprotein Confer Broad Resistance to Antiretrovirals

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Abstract

Background

We recently reported that, in vitro, HIV-1 can acquire resistance to the potent IN inhibitor, dolutegravir (DTG), by acquiring mutations in the envelope (Env) glycoprotein that enhance viral spread via cell-to-cell transmission. The aim of this study is to clarify the mechanism of Env-mediated HIV-1 drug resistance.

Methods

Virus replication in the presence of ARVs was measured by propagating viruses in a spreading infection in SupT1 cells. Sensitivity to neutralizing antibodies (NAbs) was measured using TZM-bl indicator cells. gp120 shedding from virus particles was measured by western blotting. To examine possible in vivo relevance of Env-mediated drug resistance, we performed single-genome sequencing using plasma from 5 patients failing a raltegravir (RTG)-containing regimen (La Rosa et al., 2016).

Results

We demonstrated that the gp41 mutations Env-A539V and A556T, which enhance viral cell-to-cell transmission, provide a replication advantage over WT in the presence of not only DTG but also other classes of ARVs targeting RT and PR. Moreover, Env-A539V compensated for viral fitness defects induced by drug resistance mutations in the viral enzymes and increased resistance to ARVs when coupled with these mutations, suggesting that the Env mutations may be a "stepping-stone" on the path to high-level drug resistance. Interestingly, Env mutations that show enhanced cell-to-cell transmission cluster in the gp120 C1 domain and gp41 heptad repeat 1, which are located at the gp120/gp41 interface and are crucial for stabilizing the gp120-gp41 interaction. Indeed, we observed that the Env mutations reduced gp120 shedding from virus particles. In addition, Env-A539V decreased the sensitivity of HIV-1 to NAbs that recognize the CD4-induced Env conformation, suggesting that this mutation decreases the efficiency with which Env transitions from the unliganded state. Sequencing of viruses from RTG-failure patients revealed the presence of several mutations in the highly conserved gp120/gp41 interface in the absence of resistance mutations in IN.

Conclusion

These results demonstrate that mutations in Env can contribute to broad HIV drug resistance *in vitro*. Moreover, we established a relationship between Env stability, drug resistance, and the efficiency of viral cell-to-cell transmission. These findings offer mechanistic insights into Env-mediated drug resistance in culture, and suggest, preliminarily, that Env-mediated drug resistance may also occur *in vivo*.

Env trimer (closed conformation) **gp120-gp41 interface**

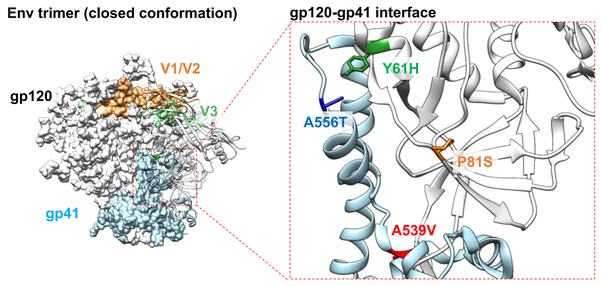
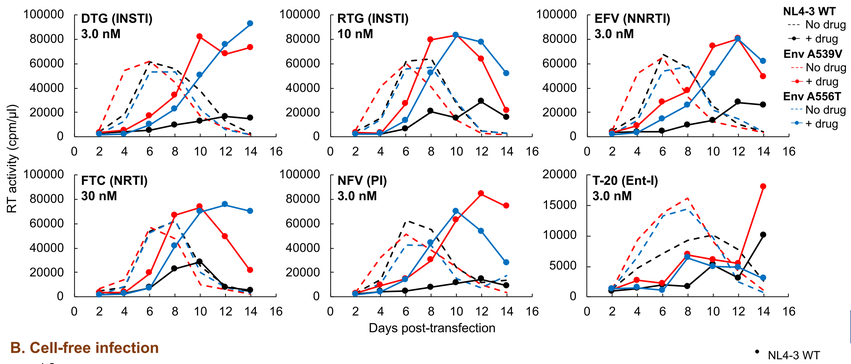


Fig.1. Location in gp120 and gp41 of the mutations that enhance cell-to-cell transfer and confer resistance to DTG. The residues we identified in Van Duyne et al., PNAS, 2019 were mapped onto a structure of BG505 SOSIP.664 trimer (PDB: 5I8H).

Env A539V and A556T confer resistance to multiple classes of ARV

A. Replication kinetics in SupT1cells



B. Cell-free infection

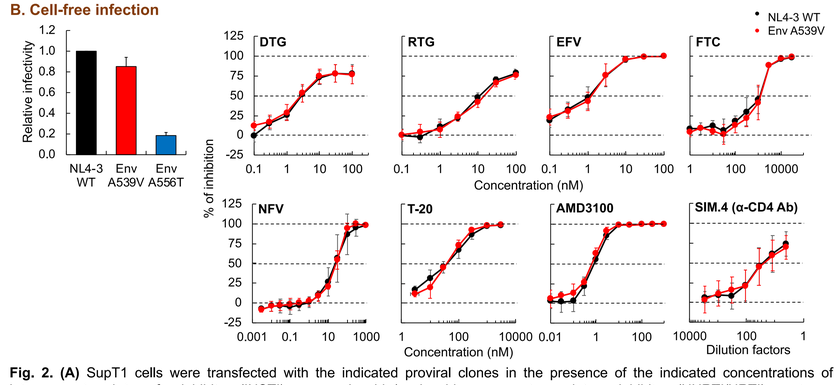
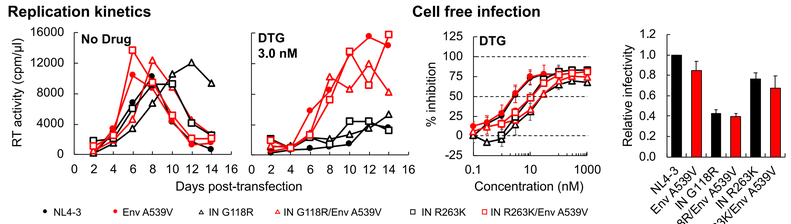


Fig. 2. (A) SupT1 cells were transfected with the indicated proviral clones in the presence of the indicated concentrations of integrase strand transfer inhibitor (INSTI), non-nucleoside/nucleoside reverse transcriptase inhibitor (NNRTI/NRTI), protease inhibitor (PI) and entry inhibitor (Ent-Is) **(B)** RT normalized viruses were infected with TZM-bl cells to examine cell-free infectivity. For drug sensitivity assay, TZM-bl cells were exposed 100 $TCID_{50}$ of the indicated virus. Data are shown as mean ± SD.

Env-A539V compensates for fitness defects of IN/PR mutants

A. IN mutants (IN G118R and IN R263K)



B. PR mutant (PIR: L10R/M46R/L63P/V82T/I84V)

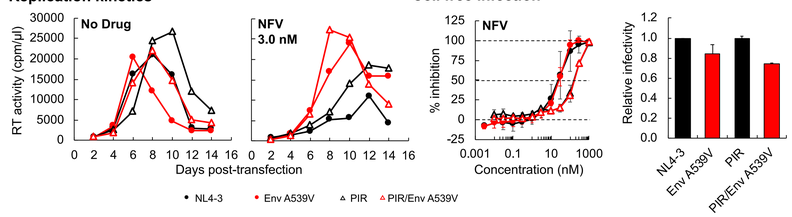
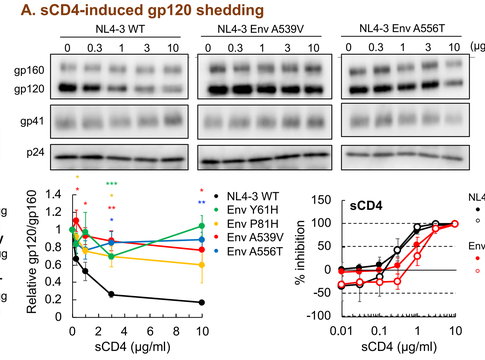


Fig. 3. (A) IN G118R and IN R263K are known major INSTI-resistance mutations. **(B)** PIR, which was isolated from HIV-1 patients failing treatment with Indinavir, contains L10R, M46I, L63P, V82T and I84V in HIV-1 PR (Condra et al., Nature, 1995). Replication kinetics assay and cell-free infection assay were performed as indicated in Fig. 2. Data are shown as mean ± SD.

Env mutations stabilize gp120-gp41 interaction

A. sCD4-induced gp120 shedding



B. Time-dependent gp120 shedding

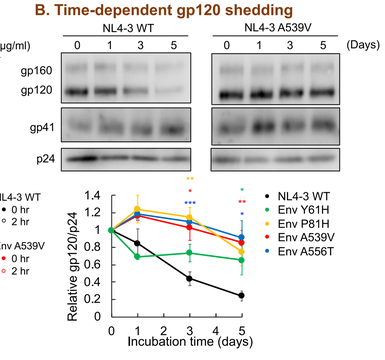


Fig. 4. (A) Virions were incubated with increasing concentrations of sCD4 at 37°C for 2 hrs. Virus particles were subsequently purified by centrifugation through 20% sucrose and were subjected to SDS-PAGE, and analyzed by western blot with anti-gp120, anti-gp41 and anti-Gag antibodies. Data from three independent experiments are shown as mean ± SE. *p*-values < 0.001 (***), < 0.01 (**), < 0.05 (*). **(B)** Virions were incubated at 37°C for 0, 1, 3 and 5 days. Virus particles were subsequently purified by centrifugation through 20% sucrose and were subjected to SDS-PAGE and analyzed by western blot with anti-gp120, anti-gp41, and anti-Gag antibodies. Data from at least 3 experiments are shown as mean ± SE. *p*-values < 0.001 (***), < 0.01 (**), < 0.05 (*).

Env-A539V alters sensitivity to NAbs

NAbs preferring closed conformation **No preference NAb**

NAbs preferring open conformation

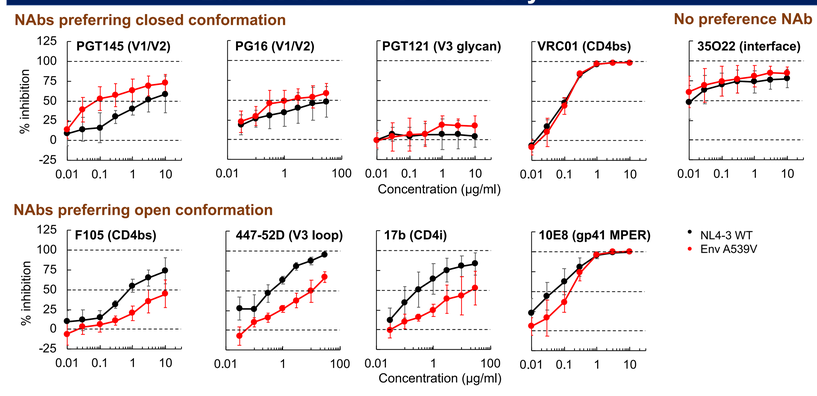


Fig. 5. TZM-bl cells were exposed to 100 $TCID_{50}$ of the indicated viruses in the presence of various concentrations of neutralizing antibodies and incubated for 48 hrs. Data are shown as mean ± SD

IN/Env-coding sequence of Raltegravir (RTG)-failure patients

A. Sample information

Patient	Date	HIV-1 RNA (copes/ml)	RAL conc. (ng/ml)
1	2-Aug-12	805,710	
	30-Jan-13	570	990
	9-Apr-13	11,335	88.6
2	6-Dec-12	17,450	
	19-Jun-13	1,772	92.9
3	13-Sep-12	1,329,415	
	28-Feb-13	433	964.8
	18-Mar-13	1,139	3371.3
4	12-Oct-12	1,617,523	
	5-Apr-13	7,208	1142.3
	29-Apr-13	9,918	480.3
5	26-Apr-12	30,206	
	6-Nov-12	18,806	2728.9

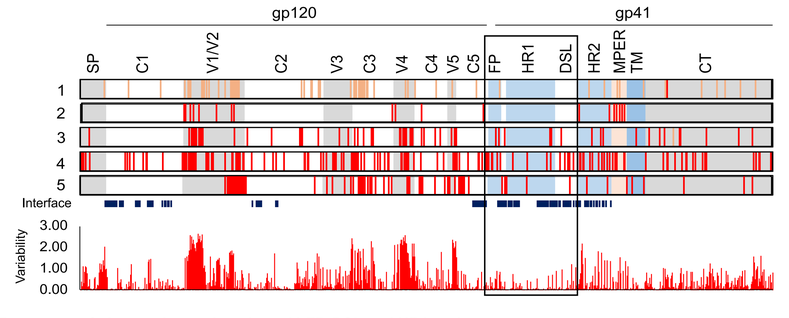
Pre-therapy
Post-therapy

Therapeutic concentrations are above 33 ng/ml.

B. Frequency of major RTG resistance mutations in post-therapy samples

Major mutations	Frequency (%) 1	2	3	4	5
T66**A/I/K**	-	-	-	-	-
E92**Q**	-	-	-	-	-
G118**R**	-	-	-	-	-
E138**K/A/T**	-	-	-	-	-
G140**S/A/C**	-	-	-	-	-
Y143**R/C/H**	-	-	-	-	-
Q148**H/R/K**	-	-	-	-	-
N155**H**	5.6	-	-	-	-
R263**K**	-	-	-	-	-

C. Observed mutations in Env during treatment with RTG-containing regimen



D. Frequency of observed mutations in gp41 FP, DSL and HR1 region

Patient	domain	mutation	frequency pre	frequency post	frequency of residues in subtype C Env
1	FP	V525A	6.3%	23%	A: 91.66%, V: 3.31%
	HR2	N636S	3.1%	23%	S: 53.55%, N: 31.36%
		R655K	9.4%	23%	K: 53.89%, R: 10.82%
3	FP	T514G	0%	80%	G: 95.65%, other: 4.35%
		M518V	0%	80%	V: 78.21%, M: 12.27%
		A525V	0%	80%	A: 91.66%, V: 3.31%
	HR1	Q581L	31%	100%	L: 99.62%, other: 0.38%
		V583L	0%	80%	E: 99.57%, other: 0.43%
	DSL	V595M	15%	80%	M: 7.37%, other: 3.58%
5	FP	T514G	17%	95%	G: 95.65%, other: 4.35%
		I518V	25%	95%	V: 78.21%, other: 1.60%
		F520L	25%	95%	L: 90.62%, F: 7.92%
	HR1	A546S	25%	95%	S: 96.90%, other: 3.10%
	DSL	L602R	21%	95%	L: 95.30%, other: 4.70%

Patient	domain	mutation	frequency pre	frequency post	frequency of residues in subtype C Env
4	FP	L515I	44%	94%	I: 67.72%, L: 22.34%
		L519F	22%	100%	F: 70.50%, L: 21.92%
	HR1	A536T	0%	93%	T: 91.71%, A: 6.82%
		S553N	0%	93%	N: 80.23%, S: 12.31%
		A578T	0%	100%	A: 99.57%, other: 0.43%
		I583E	0%	100%	E: 99.57%, other: 0.43%
	DSL	L595I	0%	100%	I: 53.62%, L: 35.42%
		K601R	0%	100%	K: 95.73%, other: 4.27%
		P605T	0%	100%	P: 3.58%, other: 1.59%
		A607N	0%	100%	A: 51.27%, N: 29.84%
		S612D	0%	100%	S: 76.02%, D: 1.79%
		R617K	0%	100%	K: 84.01%, R: 14.68%
		E621K	0%	100%	E: 34.67%, other: 3.79%
		Y622E	0%	100%	other: 0.41%

Fig. 6 (A) Sample information for the present study. Samples are derived from the SELECT study (La Rosa et al., Lanset HIV, 2016). Full-length RNA was extracted from donor plasma samples. Env and IN-coding regions were amplified, and single-genome sequencing was performed. All the viruses are subtype C. **(B)** Information on major RTG resistance mutations from Standard University HIV drug resistance database.

(C) Linear representation of Env gp160 sequences of patient 1 – 5 showing positions of the changes after RAL-treatment. Dominant amino acid changes (frequency >50%) are shown by red bar. Subdominant amino acid changes (frequency 30-50%) are shown by orange bar. Variability of subtype C Env sequences was calculated as entropy by Los Alamos HIV Database. The information of gp120-gp41 interface residues is from PDB 4TVP (Pancera et al., Nature, 2014). **(D)** Frequency of observed mutations in gp41 FP, DSL and HR1 region. Subtype C sequences (n = 5,923) were retrieved from Los Alamos HIV Database. Shared mutations in some patients were shaded in yellow.

Main findings

***In vitro* mutations**

- Env-A539V and A556T in the gp41 ectodomain conferred resistance to INSTIs (DTG and RTG), NNRTI (EFV), NRTI (FTC) and PI (NFV).
- Env-A539V did not alter sensitivity to ARVs in cell-free infections and A556T impairs cell-free infection, indicating that these Env mutations confer broad resistance to ARVs by enhancing cell-to-cell transmission.
- Env-A539V compensated for the virus fitness defects induced by INSTI/PI resistance mutations and increased resistance when coupled with these mutations.
- Env-A539V decreased sensitivity to NAbs that recognize the CD4-induced Env conformation, suggesting that this mutation decreases the efficiency with which Env transitions from the unliganded state.
- Env mutations that show enhanced cell-to-cell transmission are located in the gp120-gp41 interface; these mutations reduce sCD4-induced and time-dependent gp120 shedding, suggesting that they stabilize the gp120-gp41 interaction.

***In vivo* mutations**

- Sequences were analyzed from patients failing a RTG-containing regimen. The IN-coding region did not change following RTG failure; however, many changes accumulated in the Env-coding region.
- Most of the observed gp120 changes were found in the outer domain, whereas several changes in the gp41 ectodomain were observed at the highly conserved gp120-gp41 interface. Some of these gp41 mutations were found in multiple patients.

These findings offer mechanistic insights into Env-mediated drug resistance in culture, and suggest, preliminarily, that Env-mediated drug resistance may also occur *in vivo*.