SIVmac239 Integrase as a Model of HIV Drug Resistance Against Integrase Inhibitors

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Background

Integrase strand transfer inhibitors (INSTIs) are the most recent class of antiretroviral used to treat HIV-positive individuals. Given the recent approval of dolutegravir (DTG, an INSTI) by the U.S. Food and Drug Association, it is important to better understand how viruses resistant to INSTIs in general and to DTG in particular affect HIV pathogenesis *in vivo*. The development of a predictive animal model may help to identify impending resistance mutations and possibly to inform treatment decisions. Previous studies have shown that HIV-infected humans and SIV-infected macaques share similar patterns of drug resistance substitutions (N155H mutation) (Hazuda *et al.* Science 2004). A long-acting form of a new INSTI termed S/GSK-1265744, a DTG analogue, was shown to protect macaques against repeated vaginal and rectal exposures of SHIV (Andrews *et al*. Science 2014; Dobard *et al*. Sci. Transl. Med. 2014). Furthermore, we have shown in tissue culture studies that the same substitutions that confer resistance against INSTIs in HIV also do so in SIV, and SIV is susceptible to INSTIs with IC_{50}s in the nanomolar range.

Objectives

- Validate simian immunodeficiency virus macaque isolate"239" (SIVmac239) as a model to study the effects of resistance to INIs on viral pathogenesis
- Observe whether HIV and SIV share similar resistance pathways under INSTI pressure in selections and cell free assays
- Test the effect of HIV-1 IN resistance mutations on SIV IN activity

Methods

To elucidate the molecular mechanism of resistance, SIVmac239 IN protein was cloned into a bacterial expression vector, pET15b. Site-directed mutagenesis was used to introduce into SIVmac239 HIV-equivalent mutations that were previously observed in HIV to be responsible for drug resistance against INSTIs. Purified recombinant SIVmac239 WT, E92Q, T97A, G118R, Y143R, Q148R, N155H, R263K, E92Q/T97A, E92Q/Y143R, R263K/H51Y or G140S/Q148R IN enzymes were generated and strand transfer activities and INSTI inhibitory constants were assessed using cell-free based assays.

Multiple Sequence Alignments of Integrase Gene From HIV-1 subtype B, HIV-2 and SIVmac239

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HIV-1_Subtype_B   FLDGIDKAQEEHEKYHSNWRAMASDFNLPPVVAKEIVASCDKCQLKGEAMHGQVDCSPGI 60
HIV-2             FLEKIEPAQEEHEKYHSNVKELSHKFRLPKLVARQIVNTCAQCQQKGEAIHGQVNAELGT 60
SIVmac239         FLEKIEPAQEEHDKYHSNVKELVFKFGLPRIVARQIVDTCDKCHQKGEAIHGQANSDLGT 60

HIV-1_Subtype_B   WQLDCTHLEGKVILVAVHVASGYIEAEVIPAETGQETAYFLLKLAGRWPVKTVHTDNGSN 120
HIV-2             WQMDCTHLEGKVIIVAVHVASGFIEAEVIPQETGRQTALFLLKLASRWPITHLHTDNGAN 120
SIVmac239         WQMDCTHLEGKIIIVAVHVASGFIEAEVIPQETGRQTALFLLKLAGRWPITHLHTDNGAN 120

HIV-1_Subtype_B   FTSTTVKAACWWAGIKQEFGIPYNPQSQGVIESMNKELKKIIGQVRDQAEHLKTAVQMAV 180
HIV-2             FTSQEVKMVAWWIGIEQSFGVPYNPQSQGVVEAMNHHLKNQISRIREQANTVETIVLMAV 180
SIVmac239         FASQEVKMVAWWAGIEHTFGVPYNPQSQGVVEAMNHHLKNQIDRIREQANSVETIVLMAV 180

HIV-1_Subtype_B   FIHNFKRKGGIGGYSAGERIVDIIATDIQTKELQKQITKIQNFRVYYRDSRDPVWKGPAK 240
HIV-2             HCMNFKRRGGIGDMTPSERLINMITTEQEIQFLQAKNSKLKNFRVYYREGRDQLWKGPGE 240
SIVmac239         HCMNFKRRGGIGDMTPAERLINMITTEQEIQFQQSKNSKFKNFRVYYREGRDQLWKGPGE 240

HIV-1_Subtype_B   LLWKGEGAVVIQDNSDIKVVPRRKAKIIRDYG--KQMAGDDSVASRQDED---------- 288
HIV-2             LLWKGDGAVIVKVGTDIKIIPRRKAKIIRDYGGRQEMDSGPHLEGTREDGEVA------- 293
SIVmac239         LLWKGEGAVILKVGTDIKVVPRRKAKIIKDYGGGKEVDSSSHMEDTGEAREVA------- 293
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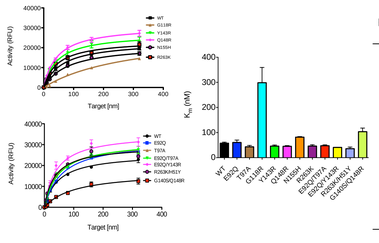
Results

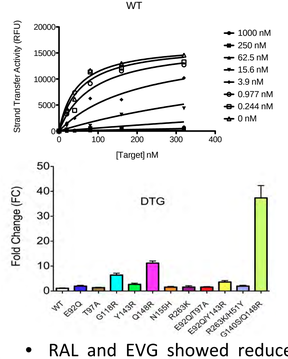
Relative Strand Transfer Activity When Varying Target [DNA]



SIV$_{mac239}$ Recombinant IN	Fixed [LTR] - strand transfer activity			
	Activity ± SEM (RFU/hr)	Target DNA affinity ± SEM (nM)	Activity/Target DNA Affinity (RFU/h/nM)	FC V_{max}/K_m
WT	24432 ± 587.6	54.62 ± 3.744	447	1
E92Q	33247 ± 1808	60.66 ± 9.523	548	1.2
T97A	31062 ± 1331	43.18 ± 5.693	719	1.6
G118R	28266 ± 3393	298.7 ± 60.85	94.6	0.2
Y143R	27142 ± 858.5	45.96 ± 4.398	591	1.3
Q148R	31008 ± 625.1	45.40 ± 2.778	683	1.5
N155H	21494 ± 236,3	81.85 ± 2.195	263	0.6
R263K	23851 ± 46.18	46.18 ± 5.280	517	1.2
E92Q/T97A	31916 ± 1175	49.48 ± 5.401	645	1.4
E92Q/Y143R	35339 ± 228.4	40.84 ± 0.8202	865	1.9
R263K/H51Y	30003 ± 374.1	27.80 ± 1.062	1097	2.5
G140S/Q148R	16976 ± 973.5	103.7 ± 14.17	163.7	0.4

- G118R, N155H, and G140S/Q148R substitutions decreased target DNA affinity and enzyme efficiency
- G140S/Q148R negatively impacted strand transfer activity

Susceptibilities of SIV Recombinant Integrase Proteins to INSTIs



INTEGRASE PROTEINS	Dolutegravir		Elvitegravir		Raltegravir	
	K_i ± SEM (nM)	~ FC	K_i ± SEM (nM)	~ FC	K_i ± SEM (nM)	~ FC
WT	1.073 ± 0.1502	1	3.016 ± 0.4996	1	1.903 ± 0.2619	1.0
E92Q	1.960 ± 0.3212	1.83	16.70 ± 2.3880	5.54	6.310 ± 1.228	3.3
T97A	1.351 ± 0.1480	1.26	5.316 ± 1.1670	1.76	5.977 ± 1.067	3.1
G118R	6.394 ± 0.7521	5.96	18.13 ± 2.9310	6	7.612 ± 1.097	4.0
Y143R	2.665 ± 0.4933	2.48	2.423 ± 0.6423	0.8	21.68 ± 3.202	11.4
Q148R	11.20 ± 0.8548	10.4	602.3 ± 150.50	199.7	456.0 ± 110.0	240.0
N155H	1.539 ± 0.3070	1.43	13.19 ± 2.5680	4.37	13.89 ± 2.231	7.3
R263K	1.578 ± 0.5452	1.47	3.525 ± 1.1230	1.17	2.846 ± 0.4498	1.5
E92Q/T97A	1.569 ± 0.2188	1.46	7.132 ± 1.6160	2.36	10.66 ± 2.681	5.6
E92Q/Y143R	3.602 ± 0.5841	3.36	31.33 ± 6.6210	10.4	248.5 ± 30.25	131.0
R263K/H51Y	1.945 ± 0.3518	1.81	10.15 ± 1.9880	3.37	4.792 ± 0.5721	2.5
G140S/Q148R	37.36 ± 4.9620	34.8	> 1000	> 300	684.5 ± 186.6	360.0

- RAL and EVG showed reduced activity against the Q148R, E92Q/Y143R and G140S/Q148R variant enzymes
- The Q148R and G140S/Q148R enzymes showed moderate resistance to DTG

Tissue Culture Selections in rhesus PBMCs Infected with SIVmac239 in the Presence of INSTIs

Tissue culture selections were performed in rhesus macaques' peripheral blood mononuclear cells (PBMCs) infected with SIVmac239 viruses in the presence of RAL, EVG and DTG. Viral RNA was extracted from cell culture fluids and sequenced for any changes in the integrase coding region.

Drug	Week 22		Week 29	
	Concentration µM	Acquired mutations	Concentration µM	Acquired mutations
RAL	0.1	A54A/V, V277M/V	0.5	A54A/V, V277M/V
EVG	0.01	A54A/V, V277M/V, A265A/G	0.05	E92Q
DTG	0.05	R263R/K, A54V, V277M	ND	ND

- After 22 weeks of DTG pressure, a mixture including 263R/K mutation was noted in rhesus macaque PBMCs infected with SIVmac239
- SIV in tissue culture selections under EVG pressure detected the E92Q mutation

Conclusions

- SIVmac239 DTG-treated viruses led to the emergence of R263K which is similar to a major pattern of DTG resistance in participants in the SAILING study
- Detection of E92Q mutation in SIV treated with EVG is important since this primary mutation is associated with virological failure on EVG-containing regimen in humans
- This study further confirms that the same mutations associated with drug resistance in HIV exhibits similar profiles in SIV
- SIVmac239 represents a good model to study resistance against INSTIs and the effects of substitutions in IN on infectivity and pathogenesis

Suggested Readings

- Hassounah SA, Mesplède T, Quashie PK, Sandstrom PA, Wainberg MA. Effect of HIV-1 integrase resistance mutations when introduced into SIVmac239 on susceptibility to integrase strand transfer inhibitors. J Virol. 2014 Sep 1;88(17):9683-92
- Wares M, Hassounah S, Mesplède T, Sandstrom PA, Wainberg MA. Simian-tropic HIV as a model to study drug resistance against integrase inhibitors. Antimicrob Agents Chemother. 2015 Jan 12

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