Poster 194

High-frequency Illegitimate Strand-transfers of Nascent DNA Fragments during Reverse Transcription Result in Defective Retrovirus Genomes

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Introduction

Human immunodeficiency viruses are retroviruses that contain diploid viral RNA genomes in each virion particle. Two precise strand-transfers (disassociation and realignment) of nascent DNA fragments at the ends of the templates are required to complete the synthesis of the double-stranded proviral DNA genome with both long terminal repeats (LTR). However, whether strand-transfers occur at illegitimate sites and how this may affect retrovirus replication are not well understood.

Methods

Reverse transcription was carried out with reverse transcriptases from HIV-1, HIV-2 and murine leukemia virus (MLV). The nascent cDNA fragments were directly cloned and sequenced without PCR amplification. Sequences were compared to the RNA template sequence to determine if new sequences contained fragments caused by illegitimate strand-transfers.

Results

Among 1067 nascent reverse transcript sequences, most of them (72%) matched to the template sequences, although they randomly stopped across the RNA templates. The other 28% of them contained mismatched 3' sequences due to illegitimate strand-transfers. The majority of the illegitimate strand-transfers (81%) were disassociated from RNA templates and realigned onto opposite complementary DNA strands. Up to three strand-transfers were detected in a single sequence while the majority of them (93%) contained one strand-transfer. Since the majority of illegitimate strand-transfer fragments were generated from templates at two opposite orientations, they resulted in defective viral genomes and could not been detected by previous methods. Further analysis showed that mutations at pause/disassociation sites resulted significantly higher strand-transfer rates. Moreover, illegitimate strand-transfer rates were significantly higher for HIV-2 RT (38.2%) and MLV RT (44.6%) than for HIV-1 RT (5.1%).

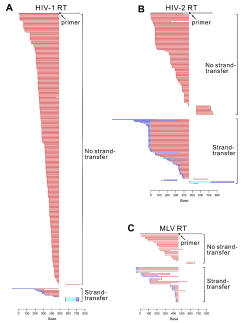


Figure 1. Pausing and strand-transfers of nascent DNA fragments during reverse transcription. Nascent DNA sequences generated by different RTs: HIV-1 (A), HIV-2 (B) and MLV (C).

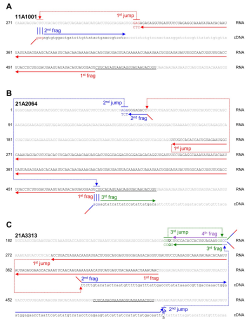


Figure 2. Illegitimate strand-transfers among RNA and cDNA templates with or without homologous sequences at misalignment sites. Single strand-transfer: RNA to cDNA (A). Two strand-transfers: RNA to RNA to cDNA (B). Three strand-transfers: RNA to cDNA to RNA to RNA (C).

Table 1. Frequency of illegitimate strand-transfers among different reverse transcriptases

RNA Template	RT	Source	Primer	No. of sequences	No. of sequences with strand-transfer (%)
HIV-2	HIV-1	Abcam	env3	342	16 (4.7)
			env4	67	5 (7.5)
			Subtotal	409	21 (5.1)
	HIV-2	Abcam	env3	199	78 (39.2)
			env4	50	18 (36.0)
		HIV-2_{ROD}F11	Plasma	env3	10	4 (40.0)
		HIV-2_{ROD}F17	Plasma	env3	38	13 (34.2)
		HIV-2_{ROD}F22	Plasma	env3	10	5 (50.0)
		HIV-2_{ROD}	Cell culture	env3	7	2 (28.6)
				Subtotal	314	120 (38.2)
	MLV	Abcam	env3	55	30 (54.5)
			env4	120	48 (40.0)
			Subtotal	175	78 (44.6)
HAV	HIV-1	Abcam	HA5	57	6 (10.5)
	HIV-2	Abcam	HA5	61	43 (70.5)
	MLV	Abcam	HA5	51	32 (62.7)
Total				1067	300 (28.1)

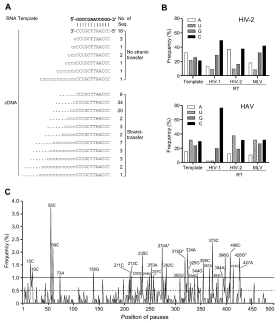


Figure 3. Base preference for strand-transfers. High frequencies of addition mutations and strand-transfers after cDNA reached the start of the RNA template. (A). Preference of bases for nascent cDNA pausing (B). Hot-spots for RT pausing (C).

Table 2. Template switches between RNA and newly synthesized cDNA

Template	RT	No. of sequences with jumps	Number of jumps between templates																	
			1				2						3							
			RNA ↓ cDNA	RNA ↓ RNA	RNA ↓ cDNA	RNA ↓ cDNA	RNA ↓ cDNA	RNA ↓ RNA	RNA ↓ cDNA	RNA ↓ cDNA	RNA ↓ cDNA	RNA ↓ RNA	RNA ↓ cDNA	RNA ↓ RNA	RNA ↓ RNA	RNA ↓ cDNA	RNA ↓ RNA	RNA ↓ cDNA	RNA ↓ cDNA	RNA ↓ cDNA
HIV-2	HIV-1	21	11	4	5			1												
	HIV-2	120	90	9	5	4	3	1	2	2	2	1	1							
	MLV	78	75	3																
HAV	HIV-1	6	2	4																
	HIV-2	43	39	1		1	2													
	MLV	32	30					1									1			
Total		300	247	21	10	5	5	3	2	2	2	1	1	1						

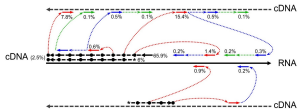


Figure 4. Frequency of pausing and illegitimate strand-transfers of nascent DNA fragments. The first, second and third strand-transfers are indicated by red, blue and green dotted lines, respectively

Conclusions

- Illegitimate strand-transfers frequently occur during reverse transcription and can result in a large portion of defective retrovirus genomes.
- Illegitimate strand-transfer rates are higher for HIV-2 and MLV RTs than HIV-1 RT.
- Illegitimate strand-transfers occur more frequently from RNA to DNA templates than from DNA to RNA templates.
- The majority of Illegitimate strand-transfers happen once during reverse transcription on short RNA templates.
- The majority of reverse transcripts are modified by adding extra bases and jump to illegitimate sites after reaching to the 5'-end to the RNA templates.
- Increasing illegitimate strand-transfer rate may serve as a new therapeutic target.