Species-specific splicing of DNA editing enzymes: Implications in HIV and SIV mutagenesis

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BACKGROUND

Humans and nonhuman primates (NHPs) encode up to seven APOBEC3 proteins (A3A/B/C/D/F/G/H) that can mutate viral and host genomes. A3G is the most potent anti-HIV enzyme, primarily inducing GG>AG mutations that can create stop codons in the HIV/SIV genomes. A recent study (PMID: 31776266) found that macaque A3Gs have a splicing defect, leading to significantly fewer GG>AG mutations in SIV than in HIV. Here, we conduct a large-scale analysis of HIV and SIV mutation profiles alongside A3 splicing patterns across humans and diverse NHPs to investigate differences in A3-mediated antiviral immunity across human and NHPs.

QUESTION & HYPOTHESIS

Q: Do A3 enzymes differentially splice across human and NHP models?

H: A3G and possibly also other A3 enzymes undergo differential splicing across human and NHP models.

APPROACH

1. LANL HIV/SIV sequences download and analysis
2. Human and NHP bulk RNAseq data analysis
3. Oxford Nanopore Technologies (ONT) long-read adaptive sampling (AdS)
4. Deaminase assay
5. Western Blot
6. Infectivity assay and HIV-1/SIV packaging assay

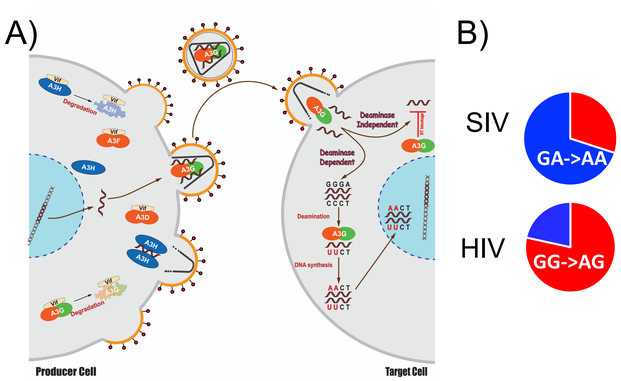


Figure 1. Mutation of HIV by A3 enzymes. A) A3 enzymes package into HIV-1 particles and induce G>A mutations during reverse transcription; B) Rate of GG>AG mutations in lower in SIV compared to HIV.

RESULTS

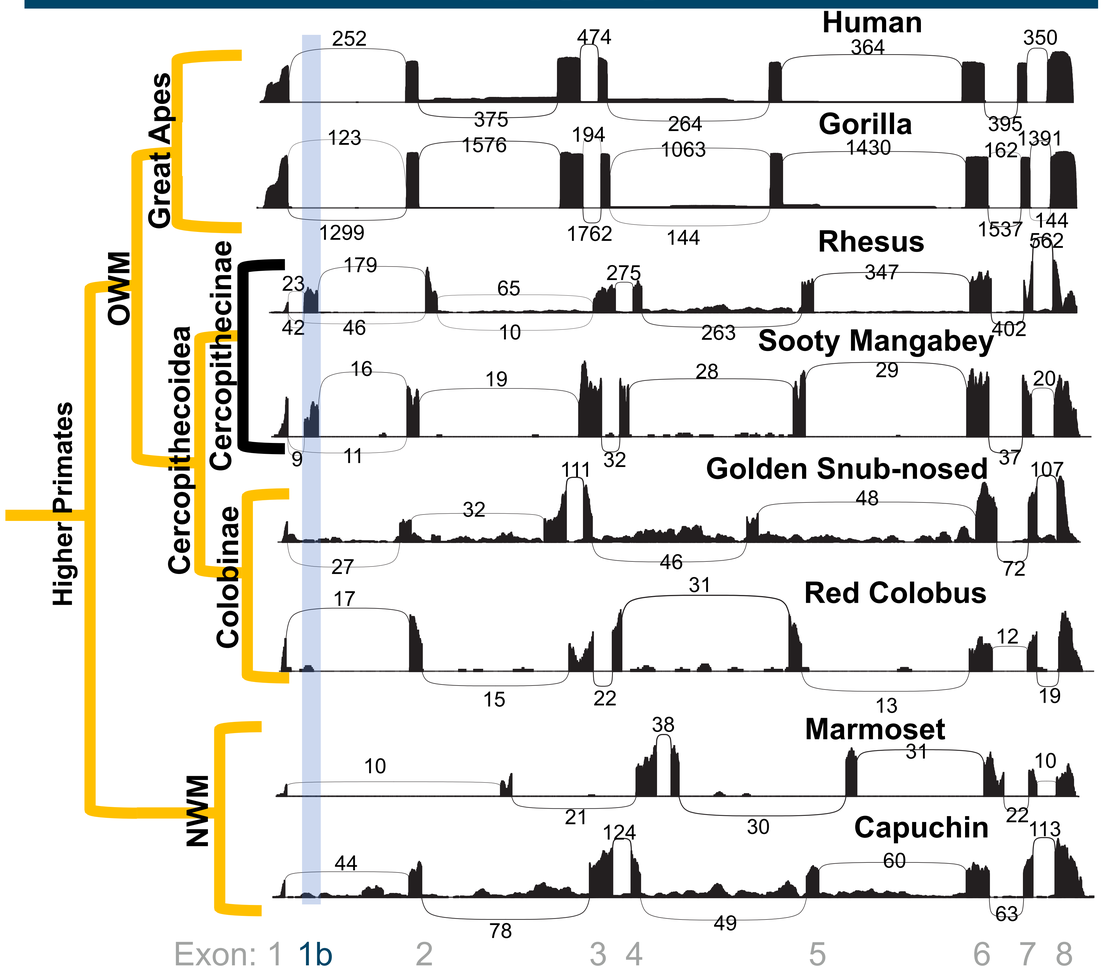


Figure 2. Aberrant A3G splicing is present in the Cercopithecinae subfamily. Sashimi plots depicting A3G splicing across human and NHPs in both Old World (OWM) and New World (NWM) monkeys. *A3G* mRNA in the Cercopithecinae subfamily contains an intronic region that has two stop codons (exon 1b).

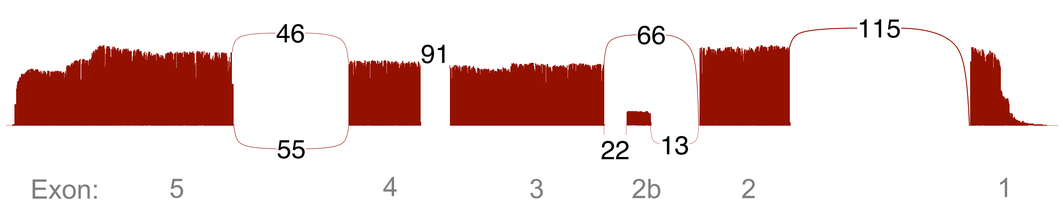


Figure 3. Novel A3H isoform in rhesus macaques. Sashimi plot depicting A3H splicing pattern from ONT long-read sequencing using AdS on PBMCs from three Indian-origin rhesus macaques.

RESULTS

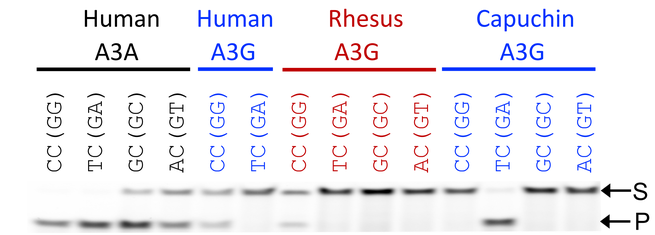


Figure 4. New World Monkey A3Gs have a GA-to-AA preference. Deaminase assay using HEK293T transfected cell lysates to determine sequence motif preference.

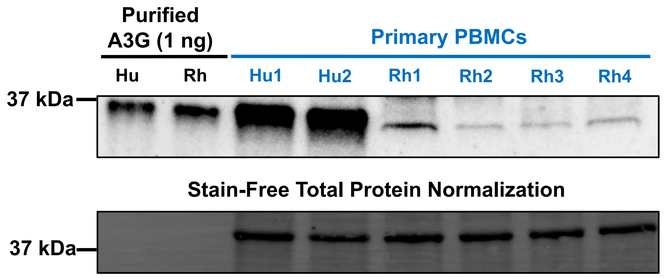


Figure 5. Rhesus has less A3G protein than human. Western blot analysis using purified human and rhesus A3Gs, along with primary PBMCs from two human and four rhesus donors. Hu = Human, Rh = Rhesus macaque.

SUMMARY

Alternative splicing of A3 enzymes can influence viral mutagenesis and antiviral immunity across humans and nonhuman primates. Rhesus macaques and other Cercopithecinae subfamily members, commonly used as animal models for infectious disease research, exhibit aberrant A3G splicing, whereas other Old World and New World monkeys retain normal A3G splicing. Understanding these molecular differences between human and NHP models is crucial, as they may have functional implications for immune responses, particularly in translational research.

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