APOBEC-mediated mutagenesis is a major driver of human papillomavirus variant diversity and evolution

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INTRODUCTION

APOBEC3 proteins are an important component of antiviral innate immune response. These enzymes are able to introduce an excessive number of mutations in viral genomes, leading to loss of genetic integrity and impairment of viral replication. On the other hand, there is mounting evidence that APOBEC activity and its selective pressure has shaped/impacted the evolution of different viral species. Here we investigated the impact of hypermutation on the course of HPV infection and viral evolution.

RESULTS

Initially the presence of hypermutation in HPV sequences was investigated in DNA from cervical samples from women belonging to a cohort followed longitudinally between 2009 and 2011. Using 3D-PCR hypermutation was detected only from one out of 39 patients (2.5%, Figure 1).

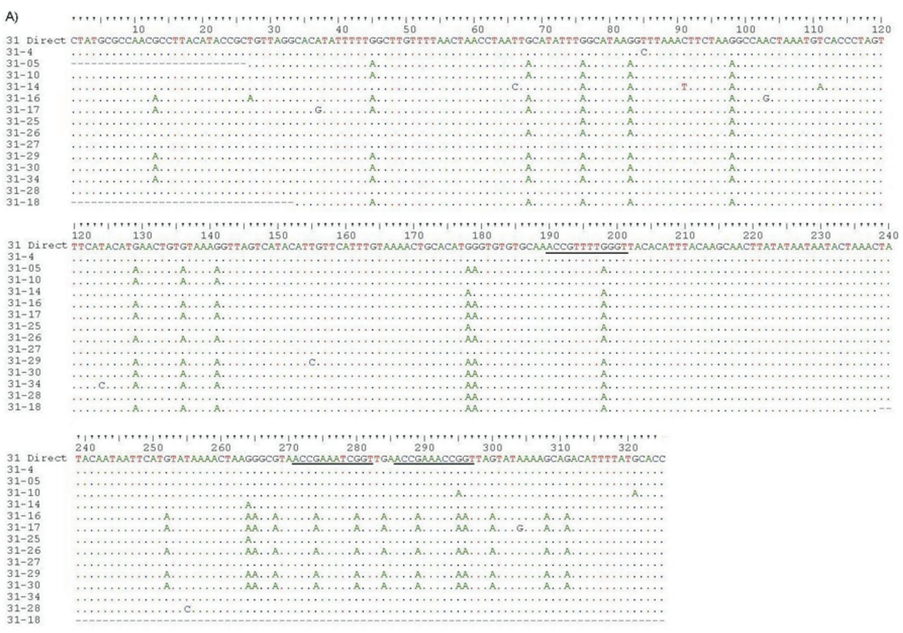

Figure 1: APOBEC editing of HPV16 genome *in vivo*. A selection of edited HPV16 LCR sequences derived from a cervical sample (sample 31).

Next the impact of mutations introduced by APOBEC3 in the HPV16 LCR regions *in vivo* was tested. A significant reduction in the LCR promoter activity in the presence of the mutations analyzed was observed (Fig. 2). Considering that the early promoter, present in the LCR region, controls the expression of the viral oncogenes, such changes should impact the oncogenic potential of these viruses and impact the course of the infection.

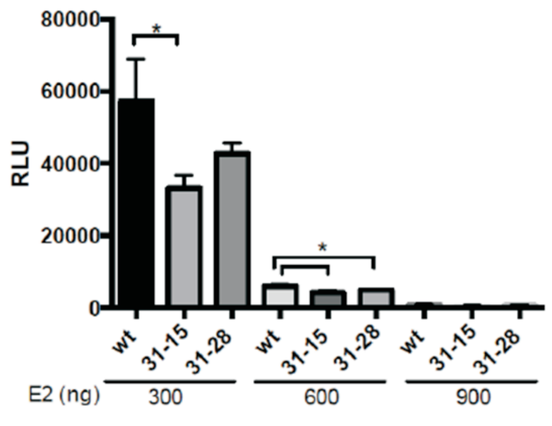

Figure 2. Effects of hypermutation in HPV transcriptional activity. The transcriptional activity of HPV16 LCR in the presence of APOBEC3 mediated mutations (31-15 and 31-28) compared to the wild-type LCR (WT) after co-transfection with HPV E2 in different concentrations (300, 600 or 900ng).

Although the frequency of detection of hypermutation was low using 3D-PCR, this frequency was much higher using ultradeep sequence techniques (22%; Fig. 3). Moreover, when the viral sequences from patients with hypermutation were analyzed longitudinally, both extinction and fixation of viruses carrying APOBEC-mediated mutations were observed. Interestingly, all women with hypermutated sequences had or developed cervical lesions during follow up.

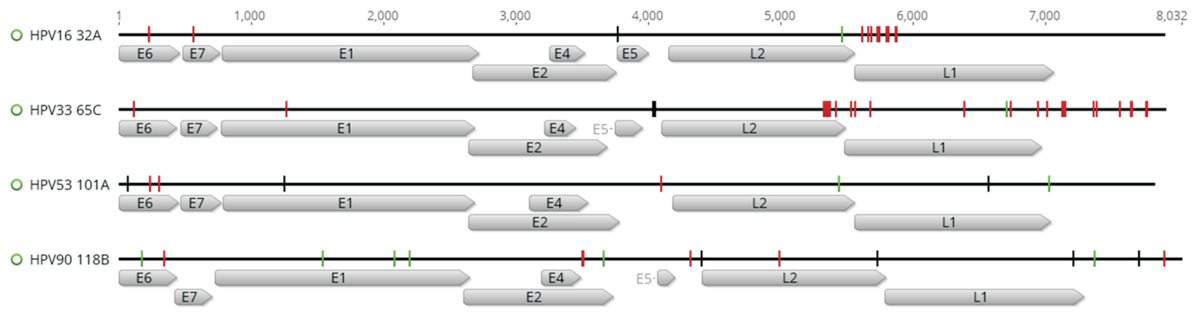

Figure 3: Distribution of mutations in hypermutated HPV genomes obtained and assembled from next-generation sequencing data. G-to-A mutations are shown in green, C-to-T are shown in red and others mutations in black.

Next, considering that APOBEC mutation seemed to be the main source of intrahost viral diversity we sought to understand its possible influence in the generation of HPV established and novel variants. For this, we first compared the different whole genome HPV sequences obtained from our cohort with the prototype sequences for the respective HPV types. Surprisingly, a great part of the diversity observed could be attributed to mutations harboring APOBEC signature. To confirm this, we next analyzed HPV variants deposited in publicly available databases, and it was observed that at least 57% of the HPV types analyzed presented lineages or sublineages that carried APOBEC3 signature. Among those, approximately 68% of the sequences analyzed were considered significantly enriched for mutations in the APOBEC3 preferred dinucleotide context (Table 1).

Table 1: APOBEC3 impact on HPV lineages and sublineages diversification.

HPV Type	HPV species	Oncogenic potential	Lineages/ sublineages (n)	Percentage hypermutated*
6	Alpha 10	Low	3	100
11	Alpha 10	Low	1	100
16	Alpha 9	High	9	66.67
18	Alpha 7	High	6	66.67
30	Alpha 6	Undetermined	3	33.33
31	Alpha 9	High	6	16.67
33	Alpha 9	High	2	0
34	Alpha 11	Undetermined	4	25
35	Alpha 9	High	1	100
39	Alpha 7	High	2	0
45	Alpha 7	High	4	0
51	Alpha 5	High	5	60
52	Alpha 9	High	6	66.67
53	Alpha 6	Probably high	6	50
54	Alpha 13	Low	2	0
56	Alpha 6	High	2	50
58	Alpha 9	High	7	100
59	Alpha 7	High	3	0
61	Alpha 3	Low	3	100
66	Alpha 6	High	2	0
67	Alpha 9	Undetermined	2	0
68	Alpha 7	Probably high	8	0
69	Alpha 5	Undetermined	3	0
70	Alpha 7	Low	1	0
73	Alpha 11	Probably high	2	0
82	Alpha 5	Probably high	9	22.22

* Compared to the prototype sequence for each HPV type.

CONCLUSION

Altogether, these results unveil a pivotal role of the APOBEC enzymes on HPV sequence diversity, highlighting the central function of these enzymes on HPV diversification and evolution at both intrahost and populational levels, and may also impact the course of HPV infection and cancer development.

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