Invited Review [210414-007276]

Accumulation of Host Cell Genetic Errors following High-Risk HPV Infection

Tim R. Fenton[1,2]

[1]School of Biosciences, Division of Natural Sciences, University of Kent, Canterbury, UK
[2]School of Cancer Sciences, Faculty of Medicine, University of Southampton, Southampton, UK

Email: t.fenton@soton.ac.uk

Abstract

APOBEC3 cytidine deaminases convert deoxycytidine to deoxyuridine in single-stranded DNA, forming part of the innate immune response to HPV infection but also contributing to mutagenesis of the host genome of infected cells during HPV-associated carcinogenesis. Of the seven human APOBEC3 genes, two (*APOBEC3A* and *APOBEC3B*) have been implicated in both processes, with evidence increasingly pointing to APOBEC3A as the main culprit in somatic mutagenesis. This review discusses recent developments in host and viral genome sequencing that suggests viral editing by one or more APOBEC3 enzymes plays an important role in viral clearance, while bursts of APOBEC3A activity may drive carcinogenesis in persistently infected cells. Progress in our understanding of HPV replication is also discussed and a model is presented in which chronic activation of the DNA damage response by HPV, together with suppression of p53 function acts to create a perfect storm for APOBEC3 activity against the host cell genome.

Highlights

- **APOBEC3 editing of HPV genomes plays a role in viral clearance but deamination of the host genome can drive carcinogenesis.**
- **Induction of DNA damage responses by HPV establishes a feed-forward loop, or 'perfect storm' for APOBEC3 activity.**
- **APOBEC3 induction may allow cells to survive the DNA damage associated with viral genome replication.**

Short title

Somatic mutagenesis in human papillomavirus-driven cancer

Background

Apolipoprotein-B mRNA editing enzyme catalytic subunit-like (APOBEC) enzymes are polynucleotide (deoxy)cytidine deaminases, which catalyse the deamination of cytosine bases in single-stranded DNA (ssDNA), and in some cases RNA, converting it to uracil. In humans, a family of seven closely related APOBEC genes (*APOBEC3A*, *B*, *C*, *D*, *F*, *G* and *H*) reside in tandem array on the long arm of chromosome 22[1]. Several APOBEC3 genes restrict the replication of viruses and endogenous retroelements through both deaminase-dependent and independent mechanisms[2] and in 2008, Vartanian and colleagues implicated APOBEC3 enzymes in editing human papillomavirus (HPV) genomes in plantar warts and precancerous cervical lesions[3]. Several years later, evidence from cancer exome-sequencing studies, functional experiments in cell lines and heterologous APOBEC3 expression in yeast combined to reveal a role for off-target APOBEC3 activity in the generation of C>T transitions and C>G transversions at TpC dinucleotides in human cancer[4,5]. Such APOBEC3 signature mutations, including oncogenic mutations in *PIK3CA* and other cancer driver genes are strongly enriched in HPV-associated cancers, suggesting a link between the host APOBEC3 response elicited by the virus and the somatic mutagenesis that eventually leads to host cell transformation[6–10].

Here I shall discuss recent developments in our understanding of APOBEC3-mediated mutagenesis in the context of HPV infection and HPV-associated malignancies. I'll focus on the possible link between mutagenesis of host and viral genomes by specific APOBEC3 enzymes and will explore how activation of the DNA damage response (DDR) by HPV may establish a feed-forward cycle of APOBEC3 activity in host cells. I will also discuss why papillomaviruses appear to induce rather than suppress APOBEC3 activity and will propose that APOBEC3 activation may be necessary to enable survival of host cells following DDR activation by HPV.

Viral and host genomes display distinct APOBEC3 mutational signatures

APOBEC3B expression is often elevated in tumours compared to normal tissue and it is expressed at higher levels in HPV-positive oropharyngeal cancers compared to their HPV-negative counterparts[8,11]. It is upregulated in HPV-infected cells via multiple mechanisms including activation of TEAD transcription factors, suppression of p53 by E6, and inhibition of the DREAM complex by E7 (reviewed in detail in[12]). Although gene expression and functional data from breast cancer cells initially implicated APOBEC3B in somatic mutagenesis, a compelling body of evidence now favours a predominant mutagenic role for APOBEC3A, acting primarily on the ssDNA of the lagging strand during DNA replication in most cancer types, including in cervical and HPV-associated head and neck squamous carcinoma (HPV+ HNSCC, recently reviewed in[12–14]). The identification of distinct preferences for APOBEC3A and APOBEC3B at the -2 position relative to the target cytosine, in which APOBEC3A prefers pyrimidine (YTCA) while APOBEC3B favours purine (RTCA) was a key finding from heterologous expression of the human enzymes in yeast[15] (Figure 1A). Following the initial demonstration in the same study that many tumour types display a skew towards C>T and C>G mutations at YTCA sites, a recent analysis of whole genome sequence data has confirmed this bias exists in cervical cancer and in a set of HNSCCs, 18 of which were HPV-associated[16], (Figure 1B). This bias towards Y over R at the -2 position is also evident

when visualising the sequence context around all C>T and C>G mutations in whole exome data from TCGA cervical cancer and HPV+ HNSCC cohorts[8] (Figure 1C). Recent studies have reinforced the predominance of APOBEC3A, including the demonstration that of all the APOBEC3 enzymes, only APOBEC3A is oncogenic when expressed as a transgene in mice, and that resulting tumours display the APOBEC3 mutational signature, including the skew towards mutations at YTCW sites (where W = A or T) expected for APOBEC3A-medidated mutagenesis [17]. It is now well-established that *APOBEC3A* gene expression as measured in tumour biopsies is weakly if at all correlated with APOBEC3 signature mutation burden[16,18]. Recent work in cancer cell lines has attributed this disconnect to episodic bursts of APOBEC3A expression and mutagenic activity; similar to the pulsatile pattern of APOBEC3 activity that we speculated may occur during HPV-associated tumour development[12,19] and Petljak et al unpublished]. Several APOBEC3 genes are interferon-inducible and it is possible that changes in the tumour microenvironment, such as T-cell infiltration or immunogenic cell death could trigger such bursts of *APOBEC3A* expression. APOBEC3 signature mutations are associated with several markers of immune cell infiltration in in HPV+ OPSCC, including an IFNγ-associated gene expression signature[20], although functional studies will be required to determine whether APOBEC3 activity is a cause of immune infiltration through the generation of neoantigens, an effect due to IFN signalling, or both.

Both viral and cellular genomes are clearly targeted by APOBEC3 activity but is the same APOBEC3 enzyme responsible for both host and viral genome mutagenesis and if so, are these processes directly linked? In their analysis of HPV16 viral genome sequences from over 5000 cervical lesions, Zhu and colleagues did not observe the bias towards mutations at YTCA sites that implicates APOBEC3A rather than APOBEC3B as the primary mutator of host genomes in cervical and HPV-positive head and neck cancer[21]. It is possible then, that whereas APOBEC3A generates the majority of mutations seen in the genomes of HPV-associated cancers, APOBEC3B may play an equally important role in viral genome editing. Alternatively, one or more additional APOBEC3s that lack a preference for either Y or R at the -2 position could be responsible for viral genome editing. Both *APOBEC3C* and *APOBEC3H* are expressed in mucosal epithelia and were initially proposed, along with *APOBEC3A*, as potential HPV editors by Vartanian and colleagues[3] but as far as I am aware it is unknown whether these or other TpC-specific APOBEC3s are agnostic to the presence of R or Y at the -2 position. Resolution of this question could come from the analysis of viral genomes from patients that are homozygous for the *APOBEC3A_B* deletion polymorphism common among South-East Asian and South American populations[22]. Since the *APOBEC3B* open-reading frame is missing from the *APOBEC3A_B* allele, a lack of YTCA skew in viral genomes from *APOBEC3A_B* carriers would implicate a further APOBEC3 enzyme in HPV editing as opposed to an equal contribution from APOBEC3A and APOBEC3B. Finally, the recent finding that APOBEC3A can target cytosines outside of the TpC motif, provided they are located in its preferred stem-loop context also suggests that assignment of APOBEC3 mutations based solely on TpC could result in the underestimation of APOBEC3A-mediated editing[23,24].

Is viral genome editing associated with vegetative HPV genome amplification?

Further interesting observations from the HPV sequencing study recently published by Zhu and colleagues were the appearance of C>T but not C>G mutations at TpC sites in the viral genomes and the lack of evidence for mutations having occurred preferentially on the lagging

strand during replication, again in contrast to features of APOBEC3-mediated mutations seen in the cellular genomes of HPV-associated cancers[21]. Several features of HPV genome replication (reviewed in detail elsewhere[25–28]) could potentially explain these observations. Firstly, while viral genome maintenance in the basal layers of the epithelium involves bidirectional theta-type replication and therefore discontinuous replication of the lagging strand as occurs during host genome replication, viral genome amplification in the differentiated intermediate/upper layers of the epithelium appears to involve a switch to recombination-dependent replication (RDR)[29,30]. This mode of replication does not involve a canonical replication fork with simultaneous replication of leading and lagging strand DNA, thus if APOBEC3-mediated deamination occurs during either or both these modes of replication, it could explain the observed lack of bias towards mutations on the lagging strand in HPV genomes. Second, viral genome amplification occurs in cells that are arrested in a G2-like state via the action of viral proteins including E1^E4[31,32]. Both *APOBEC3A* and *APOBEC3B* are preferentially expressed during G2[33–35], both are induced upon differentiation of HPV-infected cells *in vitro*[36] and APOBEC3A is also expressed in the mid-layers of normal oropharyngeal epithelium[37]. Deamination of the viral genome during G2 could potentially explain the lack of C>G transversions seen by Zhu and colleagues, since distinct mutational outcomes have been observed following translesion synthesis (the process primarily responsible for mutagenesis following cytosine deamination) in G2 versus S-phase in mammalian cells [38]. Deamination of the HPV genome during amplification might also explain the increased viral mutation load seen in productive lesions versus cervical cancers[21,39], since the latter are thought to originate from cells in the basal or suprabasal epithelial layers in which amplification is unlikely to have occurred. In this regard, a recent analysis of viral and host mutations in HPV-associated oropharyngeal squamous cell carcinoma (HPV+ OPSCC) found greater evidence of APOBEC-mediated viral genome mutations in those samples with the strongest enrichment for APOBEC-mediated mutations in the host exome, suggesting a link between viral and host mutagenesis at least in tumours, where viral genome editing during amplification is unlikely to have occurred[40]. The authors propose mutagenesis of host and viral genomes co-occurs during infection, although the considerable number of APOBEC signature mutations seen at low variant allele frequency in the host exomes also suggests ongoing mutagenesis, generating subclonal APOBEC signature mutations in these tumours.

A feed-forward model for exacerbation of APOBEC-mediated mutagenesis by HPV

A wealth of evidence underlines the vital role that induction of host cell DNA damage responses (DDR) downstream of the serine/threonine kinases Ataxia Telangiectasia Mutated (ATM) and Ataxia Telangiectasia and Rad3-related (ATR) plays in HPV genome replication (reviewed in[26,27,41]). DDR induction is not restricted to cells in which productive infection is occurring but persists and even increases in HPV-associated cancers, long after exit from the viral life cycle[37,42]. Both E6 and E7 act to induce or enhance the DDR and several recent studies have underlined the importance of this process, including the demonstration that E7 from carcinogenic HPVs interacts with the E3 ubiquitin ligase RNF168, preventing it from facilitating DSB repair via non-homologous end-joining (NHEJ)[43]. As described above, induction of homologous recombination (HR) is critical for HPV genome amplification, however in cells in G1, where HR is not possible, inhibition of NHEJ by E7 serves to increase genomic instability. The recent demonstration that generation of DNA double-strand breaks

(DSBs) by Topoisomerase-2β is essential for HPV replication also underlines the importance of DDR activation in the viral life cycle [44]. In addition to these specific mechanisms, the induction of replication stress as a result of unregulated S-phase entry also likely contributes to ATR activation in HPV-infected cells and HPV-associated cancers. The central role of DSB/HR induction to HPV replication and its aberrant induction in cells with deregulated HPV oncogene expression is likely a key driver of APOBEC3 activity in HPV-infected cells, since multiple studies have demonstrated upregulation of APOBEC3B downstream of one or more of the three phosphatidylinositol 3' kinase-related kinases (ATM, ATR and the DNA-dependent protein kinase (DNA-PK)), all of which are activated by DSBs[45–49]. In addition to inducing APOBEC3 expression, generation of DSBs and induction of replication stress also increase the availability of the ssDNA substrate on which APOBEC3s act. In turn, APOBEC3 induction has been shown to activate ATR signalling and cells with high APOBEC3 activity display increased sensitivity to ATR inhibitors[46,50,51]. Taken together, these observations all point to a feed-forward cycle in cells with deregulated E6/E7 expression, in which APOBEC3 activity is potentiated, in turn causing further activation of the DDR (Figure 2). Activation of p53 downstream of the PIKKs would normally be expected to disrupt this cycle by inducing cell cycle arrest or apoptosis and by repressing APOBEC3B expression[52] but in HPV-infected cells, the degradation of p53 by E6 prevents this brake from being applied, creating a 'perfect storm' for APOBEC3-mediated mutagenesis.

Does APOBEC3 induction allow survival of HPV-infected cells?

Multiple lines of evidence indicate that human papillomaviruses have evolved to induce APOBEC3 activity but why is this? Given the recent evidence that APOBEC3 editing of HPV16 genomes is associated with viral clearance[21] and the demonstration that APOBEC3A can restrict HPV replication[53,54], it is maybe surprising that HPV does not appear to have evolved a specific mechanism by which to suppress the APOBEC3 response beyond inhibiting type 1 interferon signalling[12]. Indeed, whereas HIV-1 counters the APOBEC3 response by triggering APOBEC3G degradation[55], HPV16 E7 has been reported to stabilise APOBEC3A[56] in addition to the aforementioned transcriptional upregulation of APOBEC3B via multiple pathways in HPV-infected cells. Low level editing of the viral genome could enable evasion of host immune responses, as has been proposed for APOBEC3 editing of HIV-1 (reviewed in [57]) but it is also possible that APOBEC3 activity plays a protective role, enabling survival of host cells in which the DNA damage response has been triggered to facilitate viral genome replication. Wallace and Munger highlighted the important role that APOBEC3 enzymes could play in countering the de-repression of Long Interspersed Element-1 (LINE1) expression downstream of RB1 inhibition in HR-HPV-infected cells, noting that LINE1 ORF2p can generate DSBs and that LINE1 proteins can be antigenic[58]. Retrotransposon activation also triggers a type 1 interferon response, another reason that papillomaviruses might have evolved to use APOBEC3s to suppress their expression[59,60].

In their study on induction of *APOBEC3B* by chemotherapy drugs, Periyasamy and colleagues recently demonstrated that *APOBEC3B* knockdown or deletion sensitizes breast cancer cell lines to those same DNA damaging agents that activate the DNA-PK pathway[49]. Importantly this sensitization was immediate, implicating *APOBEC3B* directly in sensitivity to these DNA damaging agents, rather than in promoting chemoresistance by driving mutagenesis and therefore evolution of drug-resistant subclones. As the authors speculate, this protective

activity could be related to APOBEC3B's role in transcription[61] or through the induction of base-excision repair in response to deamination of genomic DNA, in turn enabling repair of the drug-induced lesions. This hypothesis clearly requires testing in a model of HPV infection but given the importance of the DDR in HPV replication, it could also help to explain why HPV induces rather than suppresses APOBEC3 activation. In the absence of data associating the *APOBEC3A_B* allele with altered susceptibility to HPV infection, persistence or HPV-associated cancer, we must assume that any functions performed by *APOBEC3B* in HPV-infected cells are compensated for in the context of the deletion polymorphism. Both *APOBEC3A*, which is fused to the 3' untranslated region of *APOBEC3B* in the *APOBEC3A_B* allele, and a specific *APOBEC3H* haplotype (*APOBEC3H-I*) have been proposed to substitute for *APOBEC3B*, at least in deamination of cellular genomes in cancer[62–64]. It remains to be seen whether *APOBEC3A* or *APOBEC3H* also confer resistance to DNA damaging agents but the similarity of *APOBEC3A* and *APOBEC3B* in particular suggests they may perform overlapping functions and as noted above, stabilization of APOBEC3A by HPV16 E7 suggests a possible role in the viral life cycle.

Conclusions and future directions

APOBEC3 activity is induced by HPV infection and APOBEC3 mutational signatures are seen in the cellular genomes of HPV-associated cancers. Differences in the APOBEC3 mutational signatures observed in viral and host genomes, together with the fact that viral editing is most commonly associated with viral clearance rather than progression to malignancy suggest host genome mutagenesis is not simply an off-target innate immune response to HPV infection but may instead be driven by multiple factors, including immune cell infiltration into progressing lesions and the chronic activation of DDR required for HPV replication that persists in HPV-associated cancer cells. Furthermore, emerging evidence suggests that different HPV types may be subject to differing levels of APOBEC editing in precancerous cervical lesions [65,66], and that OPSCC driven by different HPV types may display differing enrichment for APOBEC3 signature mutations in the host genome[67]. Both observations are intriguing but will require confirmation in much larger cohorts. Finally, a recent sequencing study of HPV+ OPSCCs arising simultaneously in contralateral tonsils provided a striking demonstration that even in pairs of tumours occurring in the same patient and harbouring the same HPV16 variant, APOBEC3 signature mutations may or may not arise during tumour development[68]. This finding highlights the gap in our understanding of how mutagenic APOBEC activity is triggered and reinforces the need for further investigation. It also highlights that fact that while the APOBEC signature is frequently enriched in HPV-associated cancers, it is not a universal feature of these, or of any other cancer type in which the APOBEC signature has been observed. This mutational process should be seen therefore, as one of several that can generate the somatic mutations necessary for tumour development in HPV infected cells, as opposed to being an absolute requirement for HPV-associated carcinogenesis. Identifying the determinants of APOBEC3 mutagenesis and the alternative mutational processes that can drive transformation of HPV infected cells will be key to understanding the development and progression of HPV-related malignancies.

Conflict of interest statement

Tim Fenton: I am a member of the clinical and scientific advisory board of, and hold stock options in APOBEC Discovery Ltd.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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Figures

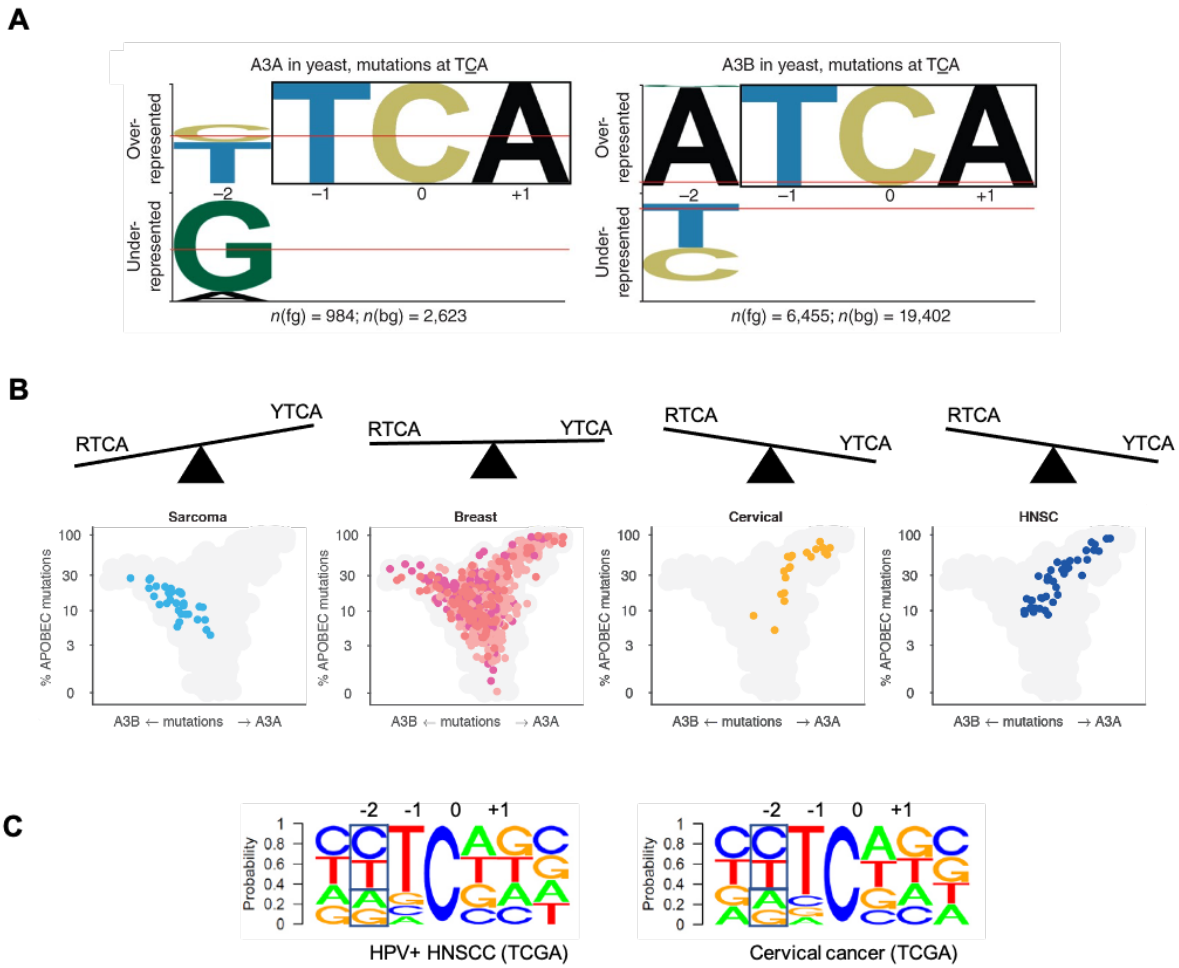

Figure 1: Cervical cancer and HPV-associated head and neck cancer genomes display a mutational signature consistent with APOBEC3A playing the dominant role in mutagenesis. (A) Chan and colleagues used heterologous expression of human *APOBEC3A* or *APOBEC3B* in yeast to demonstrate distinct specificity for pyrimidine (Y) or purine (R) respectively at the -2 position relative to the target cytosine[15]. Figure reproduced from [15] with kind permission from Dr Dimitry Gordenin. **(B)** Jallili and colleagues analysed whole-genome sequencing data from different cancer types, implicating APOBEC3B as the dominant mutator in some tumour types (e.g. sarcoma), both APOBEC3A and APOBEC3B playing roles in breast cancer and APOBEC3A as the dominant mutator in cervical cancer and head and neck cancer (18 of the 43 HNSC samples shown are HPV-positive)[16]. Figure adapted from [16] with kind permission from Dr Rémi Buisson. **(C)** The bias for pyrimidine at the -2 position is clear when considering all C>T and C>G mutations in whole exome data from HPV-associated HNSCC and cervical cancer[8].

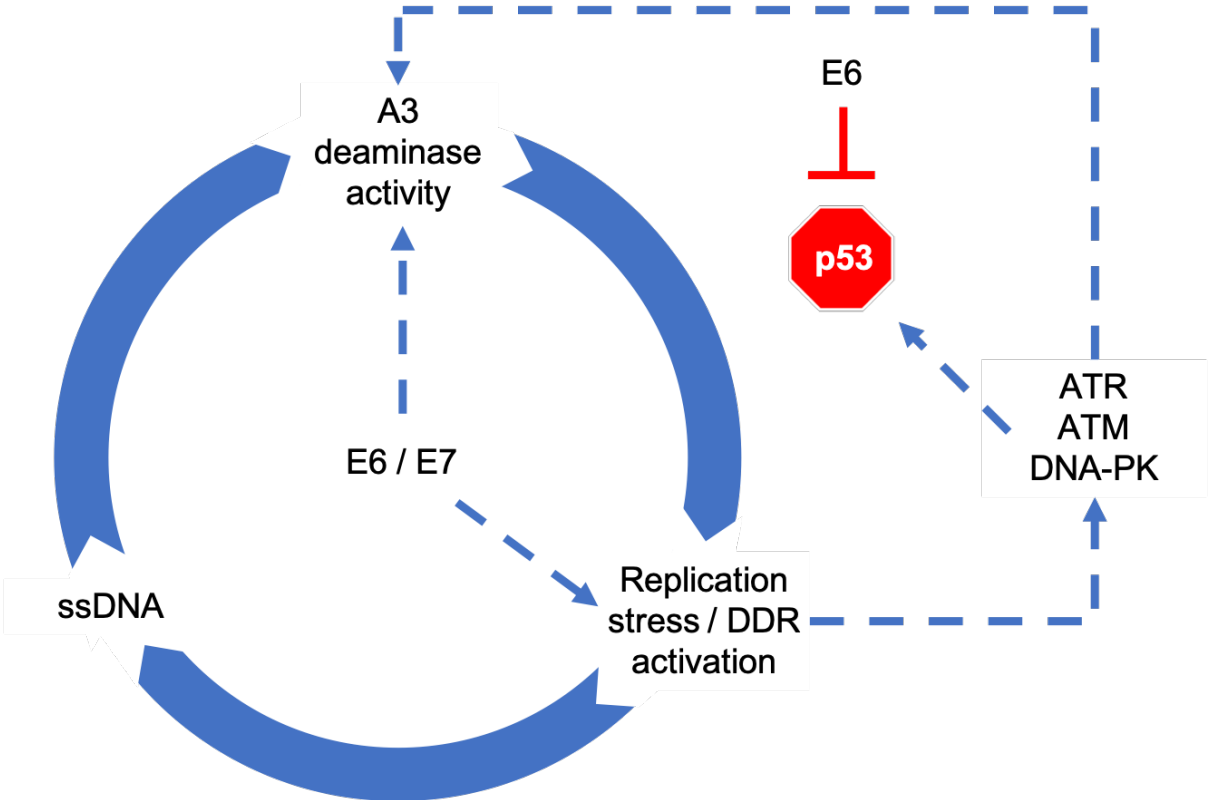

Figure 2: A feed-forward model for exacerbation of APOBEC-mediated mutagenesis by HPV. The E6 and E7 viral oncoproteins induce replication stress / DNA damage response signalling to facilitate viral genome replication. This generates ssDNA at stalled replication forks and possibly also during the repair of DSBs following replication fork collapse. ssDNA is the substrate for mutagenic APOBEC3 activity, which further increase replication stress and ATR-dependent DNA damage responses. APOBEC3B and possibly also APOBEC3A are themselves induced downstream of E6/E7 and by ATR/ATM/DNA-PK signalling. Activation of p53 would break the cycle by inducing cell cycle arrest/apoptosis if it were not for the E6/E6AP-mediated degradation of p53 (see main text for references).