APOBEC3 Deaminases Induce Hyptermutation in Human Papillomavirus 16 DNA upon Beta Interferon Stimulation

【総説】

第12回　高安賞優秀論文賞受賞

論文　「APOBEC3 Deaminases Induce Hyptermutation in Human Papillomavirus 16 DNA upon Beta Interferon Stimulation」
Journal of Virology
88(2) : 1308-1317　2014年1月掲載
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APOBEC3デアミナーゼはインターフェロンベータ刺激にてパピローマウイルス16型のウイルスDNAに高頻度変異を導入する

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Background

Recent studies have shown that human papilloma virus (HPV) and subsequent persistent infection are strongly associated with cancer. An increasing number of studies imply that integration of viral DNA into the host genome is a key step in HPV-induced cancer. APOBEC3 proteins (A3s) are antiviral factors that counteract various viruses such as Hepatitis B virus (HBV) and HIV-1 by inducing cytidine (C)-to-uracil (U) mutations in viral DNA and inhibiting reverse transcription. However, it remains unknown whether A3s hypermutate HPV viral DNA and exhibit antiviral activity in human keratinocyte. Thus, in this study, we focus on A3A- and A3G-mediated hypermutation of HPV viral DNA. Our results demonstrate that endogenous A3s upregulated by IFN-β induce viral DNA hypermutation of HPV16 in cervical keratinocytes, and a pathogenic consequence of viral DNA hypermutation is discussed.

Results

To detect hypermutation of HPV viral DNA, we first established Differential-DNA denaturation PCR (3DPCR) to detect hypermutation in E2 / Long control region (LCR). W12 cells are immortalized human keratinocytes, which were established from a HPV16+CIN 1 lesion. The results showed that E2 ORF was hypermutated in HPV DNA following the overexpression A3A and A3G, but not other A3s (Fig. 1A). In agreement with the findings of a previous study[1], DNA sequence analysis revealed that the dinucleotide preference of A3A and A3G was 5'-CpC and -TpC, respectively (Fig. 1B and C). In addition, C to T mutation was observed in LCR DNA after A3A overexpression (Fig. 2). These results suggest that A3A and A3G may hypermutate HPV viral DNA in W12 cells. Moreover, UNG enhances the frequency of hypermutation.

To explore endogenous A3 function, human recombinant IFN-β was used as a stimulator. Type I IFN is an inducer cytokine which increases A3 expression levels in virus-infected cells[2]. In W12 cells, RT-qPCR reavealed that the expression levels of A3A and A3G were significantly enhanced by IFN-β (Fig. 3A). These results were further confirmed by Western blotting (Fig. 3B). To determine their hypermutation ability against HPV viral DNA, total DNA was harvested from IFN-β -treated W12 cells and then subjected into E2 3DPCR assay. Results showed that IFN-β treatment showed

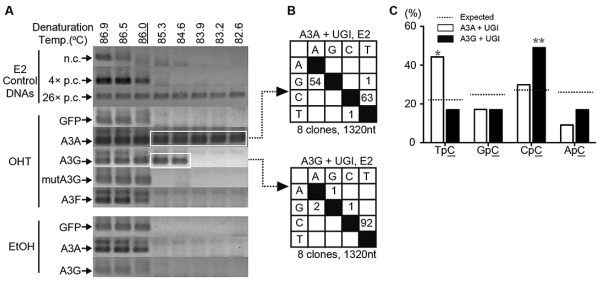


Fig. 1. A3A and A3G heavily hypermutate the E2 gene in the UNG-inhibited W12 cells.

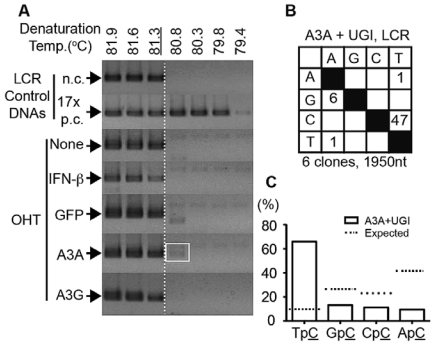


Fig 2. A3A expression causes LCR hypermutation in UGI-ER transfectants.

more hypermutated viral DNA than control (Fig. 4A). Moreover, DNA sequence showed C-to-T hypermutation with a strong CpC bias of the 5'-dinucleotide preference, suggesting that A3G mediates IFN-β-induced viral DNA hypermutation (Fig. 4B and C). Transfection of siRNA was used as another approach to evaluate the A3 function. Thus, we used individual siRNA against A3A or A3G in IFN-β-treated W12 cells. Interestingly, viral DNA hypermutation was almost abolished by siA3G transfection, whereas siA3A had a marginal effect (Fig. 4F). Importantly, siA3G altered the 5'-dinucleotide preference with a drastic decrease in CpC preference (Fig. 4G and H). Transfection by siA3A did not alter these patterns significantly. A Knocking down effect on target gene was shown by both qPCR and Western blotting (Fig. 4D and E). These results suggest that A3G, but not A3A, mediated IFN-β-induced HPV viral DNA hypermutation.

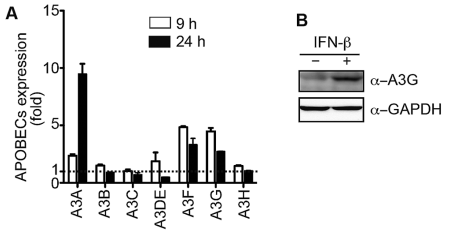


Fig 3. IFN-β induces APOBEC3 expression in W12 cells.

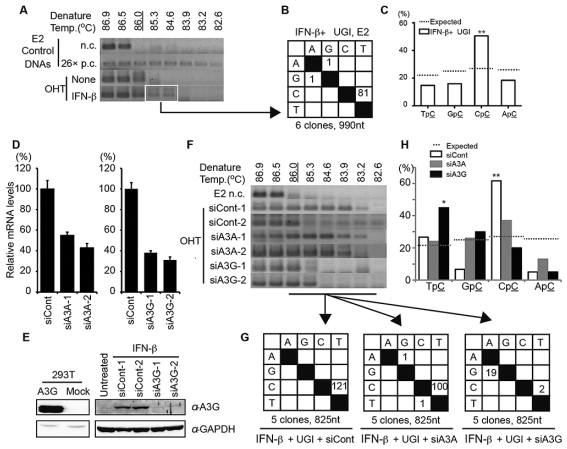


Fig 4. EndogenousAPOBEC3 proteins hypermutate E2 in UNG-inhibited W12 cells.

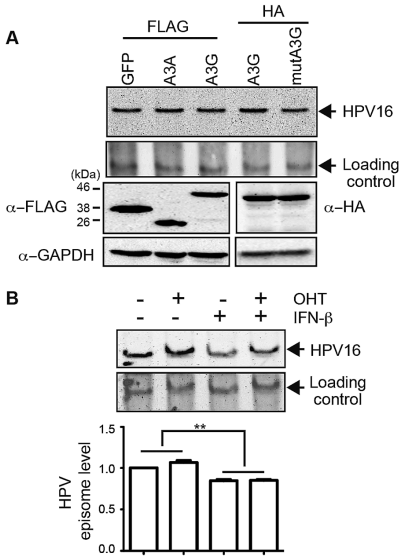


Fig 5. A3G and A3Aexpression did not change episomal DNA levels in W12 cells.

Interestingly, viral DNA hypermutation was almost abolished by siA3G transfection, whereas siA3A had a marginal effect (Fig. 4F). Importantly, siA3G altered the 5'-dinucleotide preference with a drastic decrease in CpC preference (Fig. 4G and H). Transfection by siA3A did not alter these patterns significantly. A Knocking down effect on target gene was shown by both qPCR and Western blotting (Fig. 4D and E). These results suggest that A3G, but not A3A, mediated IFN-β-induced HPV viral DNA hypermutation.

The antiviral functions of A3s have been extensively studied for HIV-1 and HBV[2]. Therefore, we determined whether A3s inhibit viral replication in W12 cells. Transfection with A3A and A3G expression vectors did not cause a significant decrease in HPV viral DNA levels in W12 cells (Fig. 5A). The A3A and A3G expression levels were measured by western blotting (Fig. 5A). The UNG function also did not alter viral DNA level although IFN-β moderately reduced viral DNA level (Fig. 5B).

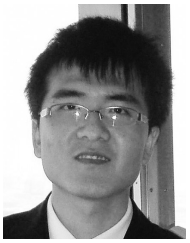
Conclusion

HPV viral DNA is hypermutated due to A3G expressed by either transfection or IFN-β stimulation in W12 cells, although the hypermutation load is too low to be detected by a conventional sequencing. Therefore, we speculate that viral replication was not significantly affected by A3s. HPV integration may require DNA breakage because broken DNA is more suitable for integration than circular DNA. In this study, we showed that A3 can introduce uracils on viral DNA. Uracil containing DNA is converted to a DNA strand break during base excision repair. Therefore, it is possible that A3s play a role during HPV integration through the hypermutation of viral DNA. Our study may give rise to a new possibility for explaining viral DNA integration.

Reference

1) Stenglein, M.D. *et al*. APOBEC3 proteins mediate the clearance of foreign DNA from human cells. *Nat Struct Mol Biol* 17, 222-9 (2010).

2) Chiu, Y.L. *et al*. The APOBEC3 cytidine deaminases: an innate defensive network opposing exogenous retroviruses and endogenous retroelements. *Annu Rev Immunol* 26, 317-53 (2008).



Profile

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