TGF-β Suppression of HBV RNA through AID-Dependent Recruitment of an RNA Exosome Complex

【総説】

第14回　高安賞最優秀論文賞受賞

論文　「TGF-β Suppression of HBV RNA through AID-Dependent Recruitment of an RNA Exosome Complex」
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TGF-βはAID依存性にRNAデグラトゾームをリクルートする事でB型肝炎ウイルスのRNAを低下させる

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Background

HBV is one of the causative factors of hepatocellular carcinoma. Recent studies have shown that the members of the APOBEC deaminase family are antiviral factors that suppress the replication of viruses, such as HIV-1 and HBV. APOBEC3G suppresses viral replication by either hypermutation of nascent DNA or inhibition of reverse transcription. Recent studies have been suggested that AID, another APOBEC family member, restricts viruses and retrotransposons that use reverse transcription for their replication. However, little is known about the antiviral mechanisms of AID. TGF-β is a pleiotropic cytokine involved in the suppression of HBV replication[1], but the mechanism underlying its anti-HBV activity is unclear. In this study, we found that AID plays a role in the anti-HBV activity of TGF-β. Further study revealed that AID physically associates with a viral RNP complex containing reverse transcriptase and recruits the RNA degradosome (RNA exosome) to the RNP complex to degrade the viral RNA. To the best of our knowledge, this study is the first to reveal a novel antiviral pathway in which AID triggers viral RNA degradation by tethering the RNA exosome to the viral reverse transcriptase/RNA complex. Viral RNA may be another target for APOBEC antiviral activity.

Results

To investigate the involvement of APOBEC deaminases in TGF-β1-mediated antiviral activity against HBV, HBV replication was evaluated by measuring HBV transcript levels by using quantitative reverse transcription -polymerase chain reaction (qRT-PCR) (Fig 1a) and Northern blotting (Fig 1d). In further experiments, qRT-PCR was used to determine the expression of APOBEC deaminases in the presence and absence of TGF-β1. A3G and A3C were highly expressed among A3 deaminases (Fig 1b). In TGF-β1-treated Huh7 cells, expression of

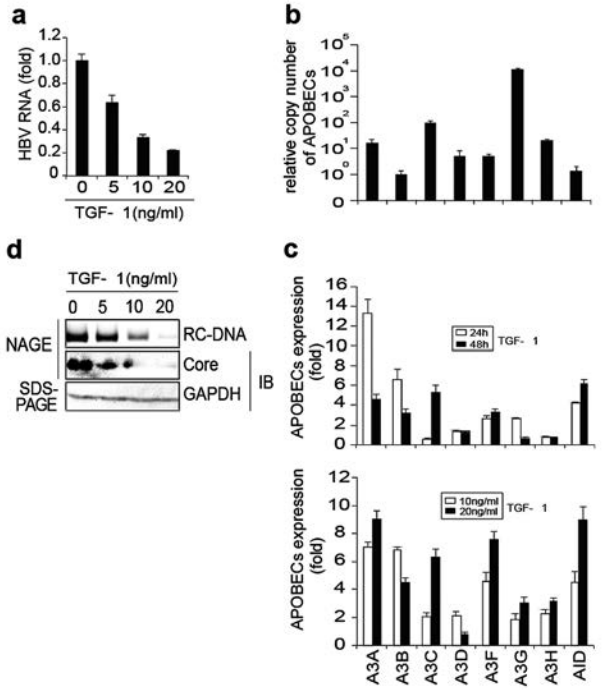

Fig 1. TGF-β1 upregulates APOBEC3 expression and suppresses HBV replication in Huh7 cells.
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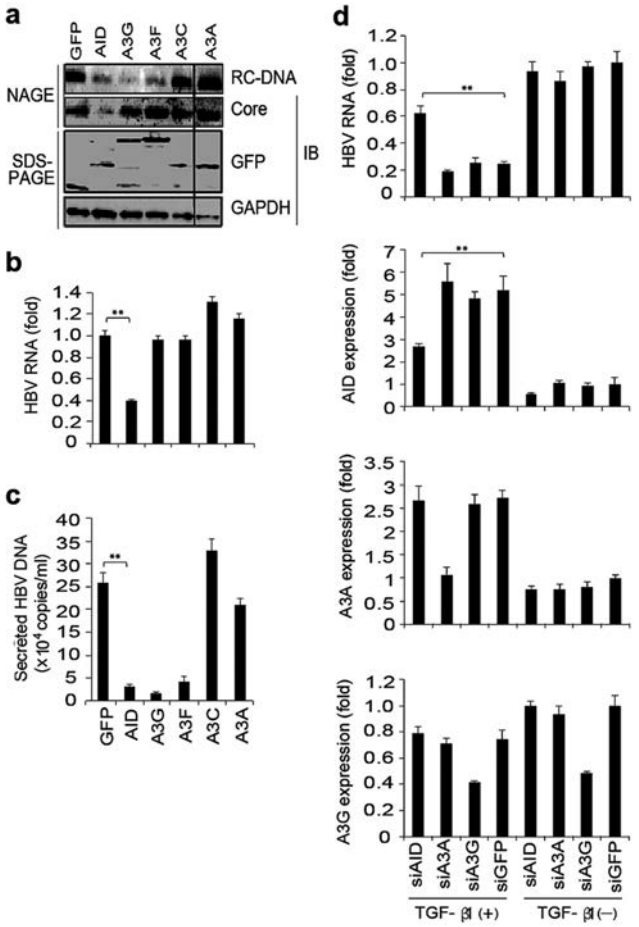

Fig 2. AID is responsible for TGF-β1-mediated reduction of HBV transcripts.
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most APOBEC deaminases, including A3A, A3B, A3C, A3F, and AID (Fig 1c, upper and lower) were upregulated.

It has been demonstrated that APOBEC3 proteins suppress HBV replication *in vitro*. The overexpression of AID but not A3G reduced HBV transcript levels, nucleocapsid formation, and virion secretion (Fig 2a-2c). In accordance with the HBV life cycle, these data suggest that AID-mediated reduction of HBV transcripts leads to the downregulation of nucleocapsid core protein and NC-DNA. Small interfering (si) RNAs targeting specific deaminases were transfected to investigate the contributions of APOBEC deaminases to TGF-β1-mediated anti-HBV activity. These data suggest that TGF-β1-mediated downregulation of HBV transcripts is dependent on endogenous AID expression not A3A or A3G (Fig 2d). Partial rescue of HBV transcript levels in siAID-transfected cells also suggests the involvement of either residual AID or other unidentified effectors in TGF-β1-mediated reduction of HBV transcripts.

To investigate the mechanism of AID-mediated downregulation of HBV transcripts, we initially focus on the viral P protein, because AID, P protein and HBV transcripts form RNP complex. As shown in Fig 3a, AID-mediated downregulation of HBV transcripts was not observed in pPB-ΔP-transfected Huh7 cells, indicating that AID-mediated downregulation of HBV transcripts requires intact viral P protein.

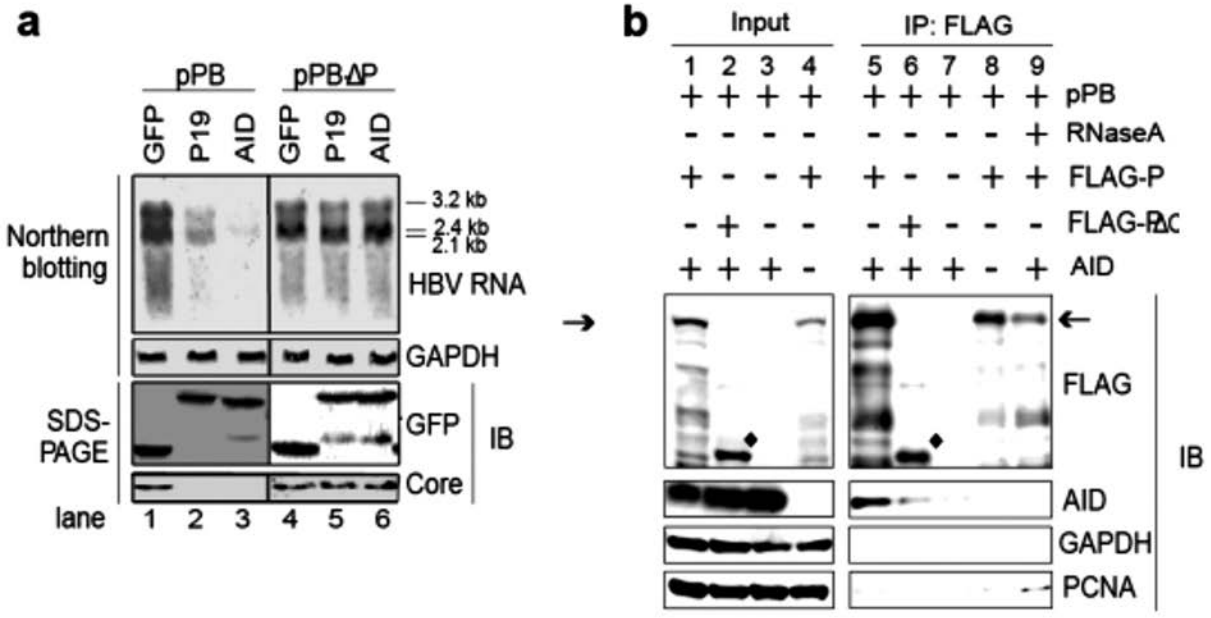

Fig 3. Intact P protein is required for AID-mediated downregulation of HBV transcripts and AID associates with HBV P protein.
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In subsequent experiments, immunoprecipitation analyses showed that wild type P protein co-precipitated AID in an RNase A-sensitive manner (Fig 3b), whereas the mutant P protein precipitated only trace levels of AID protein, suggesting that AID may not efficiently form RNP complex with the mutant P protein in pPB-ΔP-transfected cells.

AID was recently shown to physically interact with RNA exosome proteins and promote CSR in transcribed immunoglobulin genes[3]. Exosome component 3 (Exosc3, also known as Rrp40) is non-catalytic but is essential for the degradation and processing of target RNA. Thus, we investigated whether Exosc3 is involved in TGF-β1-mediated downregulation of HBV transcripts in Huh7 cells. As shown in Fig 4a, immunoprecipitation of AID co-purified Exosc3, but did not precipitate GAPDH or GFP. Exosc3 immunoprecipitation also co-purified AID but not GAPDH or GFP (Fig 4b), indicating a physical association between AID and Exosc3 proteins. This study found a physical association between AID and the RNA exosome proteins (Exosc 2, 3, 7) in Huh7 cells in the absence of HBV replication (Fig 4d). In current study, we examined whether Exosc3 associates

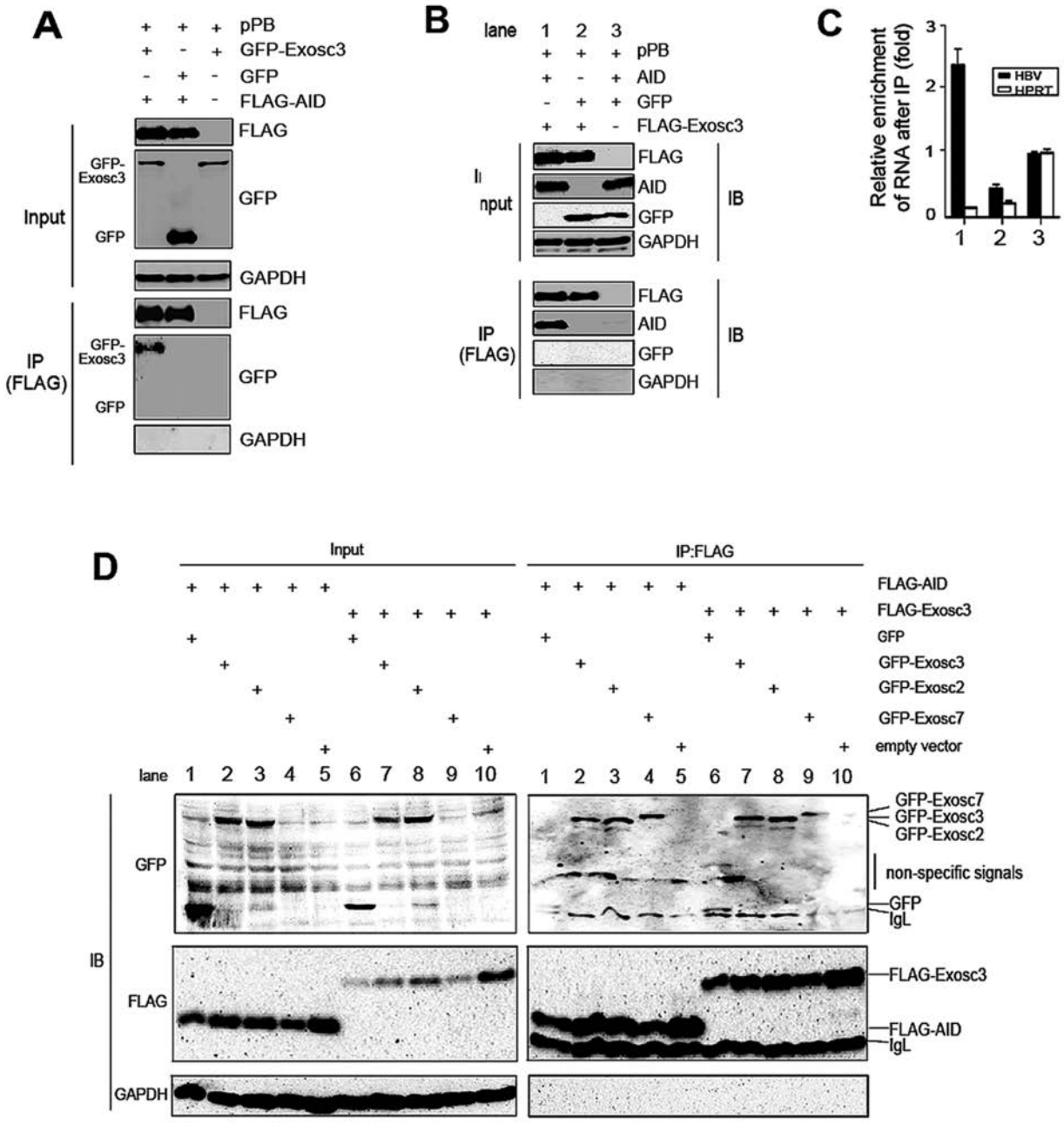

Fig 4. AID inducing HBV RNA reduction depends on Exosc3. Converted from PLoS Pathong,11(4), e1004780,2015

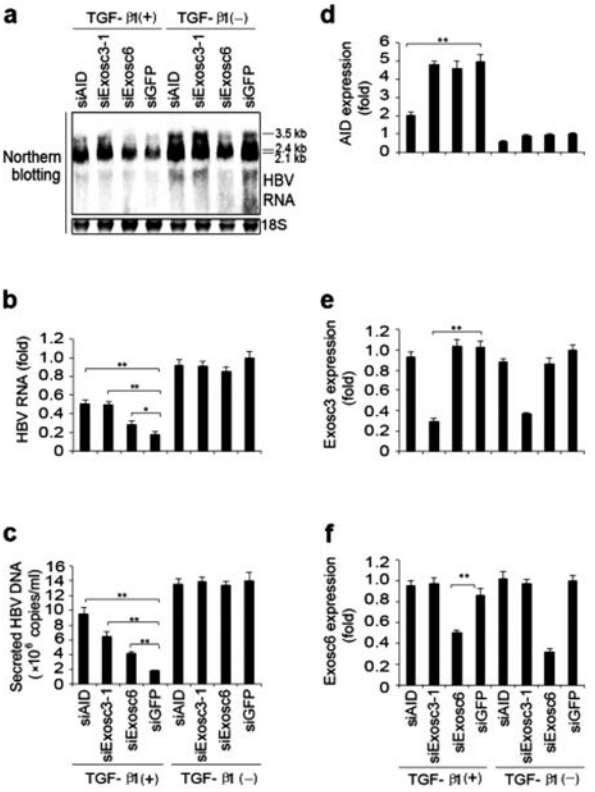

Fig 5. TGF-β1-mediated downregulation of HBV transcripts requires RNA exosome proteins.
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with HBV transcripts. As shown in Fig 4c, qRT-PCR analysis demonstrated enrichment of HBV but not HPRT transcripts in Exosc3 immunoprecipitates, which was observed only when AID was present (Fig 4c, lane 1). These results suggest that AID recruits the RNA exosome proteins to HBV transcripts.

To further confirm that the RNA exosome is involved in TGF-β1-mediated downregulation of HBV transcripts, we used the siRNA knockdown of Exosc3, which is essential for the RNA exosome function. Importantly, knockdown of another RNA exosome component Exosc3 or Exosc6 attenuated TGF-β1-mediated downregulation of HBV transcripts and nucleocapsid formation (Fig 5a-5f).

Conclusion

Reduction of HBV transcripts by TGF-β is dependent on AID expression, which significantly decreases both HBV transcripts and viral DNA, resulting in inhibition of viral replication. Immunoprecipitation reveals that AID physically associates with viral P protein that binds to specific virus RNA sequence called epsilon. AID also binds to an RNA degradation complex (RNA exosome proteins), indicating that AID, RNA exosome, and P protein form an RNP complex. Suppression of HBV transcripts by TGF-β was abrogated by depletion of either AID or RNA exosome components, suggesting that AID and the RNA exosome involve in TGF-β mediated suppression of HBV RNA. Moreover, AID-mediated HBV reduction does not occur when P protein is disrupted or when viral transcription is inhibited. Induced expression of AID by TGF-β causes recruitment of the RNA exosome to viral RNP complex and the RNA exosome degrades HBV RNA in a transcription-coupled manner.

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Reference

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Profile

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