Associations between activation-induced cytidine deaminase/apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like cytidine deaminase expression, hepatitis B virus (HBV) replication and HBV-associated liver disease (Review)

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Abstract. The hepatitis B virus (HBV) infection is a major risk factor in the development of chronic hepatitis (CH) and hepatocellular carcinoma (HCC). The activation-induced cytidine deaminase (AID)/apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) family of cytidine deaminases is significant in innate immunity, as it restricts numerous viruses, including HBV, through hypermutation-dependent and -independent mechanisms. It is important to induce covalently closed circular (ccc)DNA degradation by interferon-α without causing side effects in the infected host cell. Furthermore, organisms possess multiple mechanisms to regulate the expression of AID/APOBECs, control their enzymatic activity and restrict their access to DNA or RNA substrates. Therefore, the AID/APOBECs present promising targets for preventing and treating viral infections. In addition, gene polymorphisms of the AID/APOBEC family may alter host susceptibility to HBV acquisition and CH disease progression. Through G-to-A hypermutation, AID/APOBECs also edit HBV DNA and facilitate the mutation of HBV DNA, which may assist the virus to evolve and potentially escape from the immune responses. The AID/APOBEC family and their associated editing patterns may also exert oncogenic activity. Understanding the effects of cytidine deaminases in CH virus-induced hepatocarcinogenesis may aid with developing efficient prophylactic and therapeutic strategies against HCC.

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Abbreviations: AID, activation-induced cytidine deaminase; APOBEC, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; HCC, hepatocellular carcinoma; cccDNA, covalently closed circular DNA; ApoB48, apolipoprotein B; IFN-α, interferon-α; PHH, primary human hepatocytes; ssDNA, single stranded DNA; SHM, somatic hypermutation; HMM, high molecular mass; rcDNA, relaxed circular DNA; pgRNA, pregenomic RNA; UNG, uracil DNA glycosylases; RT, reverse transcriptase; FRET, fluorescence resonance energy transfer; CH, chronic hepatitis

Key words: hepatitis B virus, activation-induced cytidine deaminase, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like, polymorphism, hepatocellular carcinoma

Contents

1. Introduction

The hepatitis B virus (HBV) infection is a prevalent type of infectious disease that is causing a global concern for public health (1). Although there has been considerable improvement in understanding the molecular virology and pathogenesis of the HBV infection over the past decade and effective therapeutic measures have been developed for its treatment, there are currently 240 million individuals globally who are chronic HBV carriers, and ~620,000 succumb per year due to late sequelae of liver cirrhosis or hepatocellular carcinoma (HCC) (1). The treatment of chronic hepatitis (CH) B is currently limited; predominantly consisting of interferons (IFNs) and nucleoside analogs (NUCs) (2). NUCs are efficient antiviral agents, however, NUCs only control rather

than cure HBV infections due to persistent viral covalently closed circular (ccc)DNA. Therefore, a long-term treatment is required, which is expensive and may lead to concomitant resistance (2). IFN therapy is associated with side effects, and treatment with this cytokine can result in viral clearance in a small proportion of patients (3). Thus, it is important to continue conducting research in order to identify therapeutic targets to stimulate the development of novel antiviral agents and immunotherapies.

The activation-induced cytidine deaminase (AID)/apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) family, which was first described for inhibition of human immunodeficiency virus type 1 (HIV-1), is important in the innate immune system, as it defends against viruses, including HBV through hypermutation-dependent and -independent mechanisms. Nine of 11 APOBEC3 family members have been identified to exert varying levels of activity against HBV under experimental conditions (Table I). APOBEC-3A and APOBEC-3B are essential for cccDNA degradation by IFN-α or the lymphotoxin-β receptor-agonist without damaging the infected host cells (4). Thus, targeting the formation and subsequent processing of viral cccDNA may be more rational approaches.

The aim of the present study was to review the rapidly accumulating evidence for the involvement of AID/APOBEC cytidine deaminase expression and HBV replication, and to summarize the current knowledge surrounding the role of editing in HBV-associated liver disease as a consequence of its actions in host cells.

2. AID/APOBEC family of deaminases

AID/APOBEC family members. All members of the AID/APOBEC family possess one or two catalytic domains that deaminate cytidine in RNA and DNA. The deaminases mediate the hydrolytic removal of an amino group at the C4 position of a cytidine (C) or deoxycytidine (dC) generating a uridine (U) or deoxyuridine (dU), respectively (5). The presence of the enzyme in cells producing RNA virus results in C-to-U conversion of minus strand reverse transcripts and G-to-A in plus strand DNA. The binding to the target DNA creates a U-G mismatch, which generates a C-to-T transition in minus strand DNA and a G-to-A transition in plus strand DNA during general DNA replication without repair pathways (Fig. 1) (6). In humans, the family comprises 11 members with distinct functions, including AID, APOBEC1 (A1), APOBEC2 (A2), APOBEC4 (A4) and APOBEC3 (A3) subgroups. The A3 group consists of seven proteins: A3A, A3B, A3C, A3DE, A3F, A3G and A3H (7-10). The seven A3 genes are arranged in a tandem gene cluster on chromosome 22 in humans (11). The presence of the AID/APOBEC family is restricted to vertebrates. AID and A2 are likely to be the ancestral members, while A1 and A3 are later evolutionary arrivals (12), A3s are restricted to placental mammals, and their gene copy number is species-specific. For example, mice only possess a single A3 gene, pigs have two, sheep and cattle have three, cats have four, horses have six and primates have at least seven A3 genes (13). The rapid expansion of the A3 locus in humans indicates an important role in the host genome defense against exogenous viruses and endogenous retroelements (12-14). The role of the AID/APOBEC family in the inhibition of viral infection was initially described for HIV-1. Various studies have shown that the genome of hepadnaviruses is hyperedited by cytidine deaminases (15-21). Recent reports demonstrated that HBV DNA replication is restricted by A1, AID, A3A, A3B, A3C, A3G, A3F, but not A3DE (4,15,17,22-26), specifically via the degradation of HBV cccDNA (4), which was investigated in an experimental setting through deaminase-independent and -dependent mechanisms (27,28).

AID/APOBEC family gene expression. A1 is primarily expressed in the gastrointestinal compartment and catalyzes the expression of a truncated form of apolipoprotein (Apo) B (ApoB48) with the distinct biological function of ApoB100 (10). AID is expressed in germinal center B cells and randomly edits dC residues to dU in the variable region of immunoglobulin gene loci, which is essential for the process of antibody diversification (29,30). As expected, AID and A1 were not detected in the HCC cell lines and liver tissue (20,25). A2 is widely expressed in muscle; predominantly in cardiac and skeletal muscle, thus, is exclusively associated with the development of cardiac and skeletal muscle, as well as early embryogenesis (9,31-33). A4 was discovered by a computational homology search and was found to be expressed in the testicles, although its function remains undetermined (8). A3 (particularly A3G and A3F) is widely expressed in human tissues, where the mRNA levels broadly correlate with the lymphoid cell content (with the exception of gonadal tissues) (34). In the healthy human liver, low to moderate APOBEC expression levels have been observed (11,20,26,35,36), with A3G expression levels identified to be the highest, followed by A3C and A3H expression (18). Positive transcription and expression has been identified for A3G, A3C and A3H in HepG2 cells, although not for A3B and A3F. Amplification experiments with A2- and A3A-specific primers resulted in particularly faint signals, and A1- and A3DE-specific sequences were not detected (18).

Onset of liver disease results in changes to the levels of these deaminases. For example, in hepatitis/cirrhotic samples, five of seven A3 genes were significantly upregulated in the following order: HCV ± HBV > HBV > alcoholic cirrhosis. A3C and A3D were upregulated for all groups, while IFN inducible A3G was overexpressed in virus-associated cirrhotic patients, and AID was present in 50% of the HBV/HCV samples (21). The expression levels of A1, A2, and A4 were not identified to be significantly different between cirrhotic liver and normal tissue samples (21). Furthermore, the A3B cytidine deaminase was widely upregulated in HCC tumor tissue samples (37).

Upregulation by IFNs and other cytokines. The expression of APOBEC genes is induced by IFNs, as well as other cytokines. IFN-α has been observed to trigger the expression of A3 in numerous reports. For example, treatment with IFN-α in primary human hepatocytes (PHHs) and HCC cell lines resulted in induction of A3-associated genes, particularly A3G; however, no changes were triggered in the expression of A1, A2 or AID (25,26,36). In addition, A3A was demonstrated to be significantly expressed in peripheral blood mononuclear cells, HBV-infected differentiated HepaRG cells and PHHs that were treated with IFN-α (4,36,38). IFN-α enhances A3 expression levels in human macrophages (39), phagocytes (38) and peripheral plasmacytoid dendritic cells (40). This indicates that A3

Table I. Reported hyperediting and restriction activity of AID/APOBECs against HBV DNA.

Cytidine deaminases	Hyperediting activity	Dinucleotide preference	Restriction activity against HBV DNA	Reference
AID	Deaminates each viral RNA and HBV minus-strand DNA	GpC, ApC	Active	25,27
A1	Deaminates HBV minus-strand DNA	TpC	Active	19,21,25
A2	NI	NI	Not active	25
A3A	Deaminates each strand of HBV DNA	Weak bias	Active/not active[a]	4,17,25
A3B	Deaminates each strand of HBV DNA	GpC, ApC	Active	4,16,24-26,28,37
A3C	Deaminates HBV minus-strand DNA	No bias	Active but weak/not active[a]	16-18,20,26,37
A3DE	Not active	N/A	NI	17
A3F	Deaminates each strand of HBV DNA	TpC, GpC	Active	16-18,20,25,26,37
A3G	Deaminates each strand of HBV DNA	CpC	Active	16-18,20,26,37,75
A3H	Deaminates each strand of HBV DNA	TpC	NI	17,20

[a]Controversial experimental results exist in the article. NI, no information found; N/A, not applicable; AID, activation-induced cytidine deaminase; APOBEC, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; HBV, hepatitis B virus.

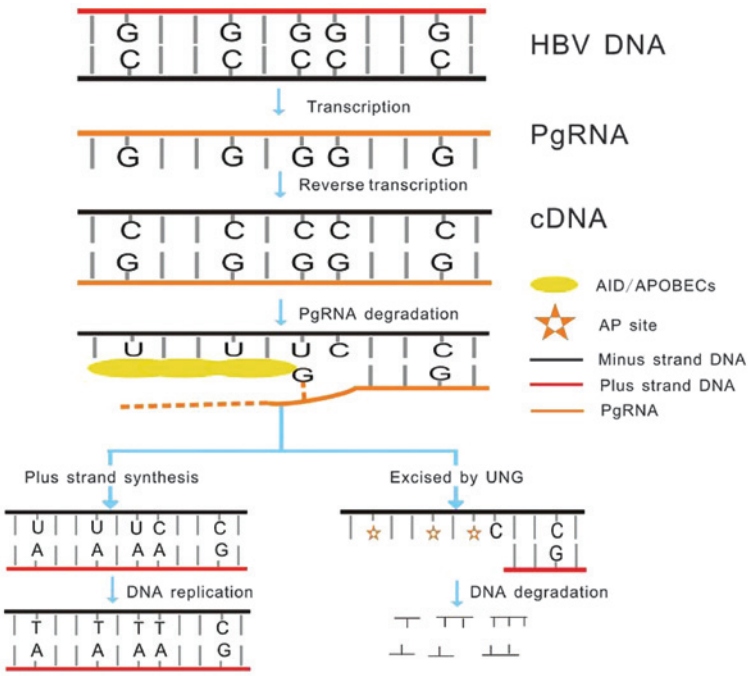

Figure 1. HBV DNA is transcribed to pgRNA as a replicative RNA intermediate, according to which the minus DNA strand (cDNA) forms. ssDNA is formed as the pgRNA is degraded. AID/APOBECs catalyze cytosine deamination of HBV DNA on the cDNA, producing uracil during reverse transcription. Uracils in DNA (including cccDNA) are recognized and excised by UNG leading to formation of AP sites. These AP sites are recognized by cellular AP endonucleases leading to DNA digestion. The HBV DNAs that do not undergo degradation, generate C-to-T transitions in minus strand DNA and G-to-A transitions in plus strand DNA during general DNA replication. HBV, hepatitis B virus; pgRNA, pregenomic RNA; ssDNA, single stranded DNA; AID, activation-induced cytidine deaminase; APOBEC, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; cccDNA, covalently closed circular DNA; UNG, uracil DNA glycosylases; AP, apurinic/apyrimidinic.

expression may contribute to the antiviral effects of IFN-α against HBV DNA. In addition to IFN-α, other IFN family members, such as IFN-γ and IFN-β, directly upregulate A3G expression in macrophages (39,41). IFN-γ is known to control HBV in a dose-dependent reduction manner, which was consistent with the observation of a dose-dependent increase in A3G and A3F protein expression following IFN-γ therapy (42). In addition, the expression of AID is induced in response to tumor necrosis factor (TNF)-α. Interleukin (IL)-1β stimulation in cultured human hepatocytes and pro-inflammatory cytokine-mediated expression was meditated by nuclear factor-κB signaling pathways (43,44) and by IL-4 and -13 through *STAT6* (45). Transforming growth factor-β is a cytokine that induces AID in B and HepG2 cells (46), whereas TNF-α and IL-1β stimulate the expression of A2 in human hepatocytes (44).

3. Specific regulation of editing by AID/APOBECs

Mechanism of regulation. The mutagenic activity resulting from AID/APOBEC-mediated deamination is toxic for

retroviruses and beneficial to organisms in host cell defense (13). However, its excessive activity and off-target mutations within the cellular genome are also toxic and oncogenic to the host. Thus, organisms possess multiple mechanisms to regulate the expression of AID/APOBECs; for example, controlling their enzymatic activity and restricting their access to DNA or RNA substrates (47). A1 is the sole family member capable of recognizing and using mRNA as a substrate. Editing only occurs on cytidines 5' of the mooring sequence (48). Although RNA editing occurs in the cytoplasm when A1 is overexpressed (49), cytoplasmic A1 editing activity is suppressed under normal physiological conditions. In addition, RNA editing is restricted to the cell nucleus within a temporal and spatial window that occurs subsequent to pre-mRNA splicing and prior to mRNA nuclear export, only when interacting with cofactor, APOBEC-1 complementation factor (50,51). The sequence requirements for AID/APOBEC deamination are not stringent, however, deamination generally occurs within transcribed or single-stranded regions of DNA. Therefore, single-stranded (ss)DNA is always recognized as a substrate by the AID/APOBEC family. The deaminase activity of AID is responsible for a variety of point mutations and DNA breaks. This mutagenic activity leads to somatic hypermutation (SHM) and class switch recombination (52). Notably, the expression of AID is restricted to activated B cells under physiological conditions, and only targets variable and switch regions of immunoglobulin genes for mutagenesis. A2 is essential for muscle development with its expression being largely restricted to striated muscle. However, *in vitro* (32,53) A2 was identified to lack autonomous deaminase activity. The above-mentioned processes are regulated in a tissue-specific manner during development and in response to metabolic regulation. Furthermore, subcellular localization and RNA binding to AID/APOBECs regulate their deaminase activity. Furthermore, AID is regulated by nuclear-cytoplasm transport and is predominantly located in the cytoplasm. Controlling the abundance of AID exclusion from the nucleus in steady state is one of the major regulatory mechanisms restricting its contact with genomic material (54,55). A3G is strongly retained in the cytoplasm and excluded from chromosomes based on the specialized property of a novel cytoplasmic retention signal (56). A3G is present in two distinct molecular forms within the cell: A low molecular mass form and a high molecular mass (HMM) complex, which contains one or more inhibitory RNAs that inactivate ssDNA deaminase activity (57-59). A3G-RNA complexes in viral particles have been found to be inactive until RNase H activity of reverse transcriptase (RT) degrades the RNA of DNA-RNA hybrids (60). A3C, A3F and A3H all form intracellular HMM complexes (61-63) in addition to A3G. AID exerts no measurable deaminase activity on ssDNA unless the AID is pretreated with RNase to remove the inhibitory RNA, which is bound to the AID (29).

4. Role of AID/APOBECs in HBV-associated disease progression

Promotion of the evolution of HBV virus. The encapsidated HBV genome consists of a 3.2-kb partially double-stranded relaxed circular (rc)DNA molecule. Upon translocation to the nucleus, the rcDNA genome is converted into a cccDNA by cellular repair factors and remains an episomal minichromosome, which transcribes all viral RNAs, including pregenomic (pg)RNA as a replicative RNA intermediate. The pgRNA is translated to form the core protein and internal translation initiation synthesizes the DNA polymerase. The pgRNA, viral core, and polymerase proteins are assembled into the nucleocapsid in the cytoplasm (1). Reverse transcription starts to synthesize the (minus) DNA strand via reverse transcription activity of the viral polymerase. Following degradation of the pgRNA by the viral RNase H, the plus strand is synthesized to form mature core particles, which interact with the preS domain of the membrane-associated viral surface proteins and acquire the viral envelope (64). Approximately 10^{11} viral particles are released per day into the circulation of individuals with the chronic HBV infection and HBV particles are cleared from the plasma with a half-life of ~1.0 day (65,66). In the initial immunotolerant phase of chronic HBV infection, the immune pressure is weak. With the progression of chronic infection, especially during Hepatitis B e antigen (HBeAg) seroconversion, HBV mutations gradually occur (67). The mutation rate of HBV DNA caused by AID/APOBECs in chronic HBV infection patients has been shown to be higher than that of acute HBV infection patients. In the chronic HBV infection group, the frequency of hypermutated genomes was found to be higher in HBeAg-negative individuals when compared with seropositive cases (68,69), and the degree of mutation of the HBV DNA was significantly associated with the extent of fibrosis (68). This may have been due to the fact that HBeAg-negative patients had undergone HBeAg seroconversion, resulting in an already elevated immune response (70). Although mutations in A3 that result in altered editing can be highly deleterious, lightly edited genomes may facilitate the virus to evolve and even escape from the immune responses (21). Non-resolving inflammation has been shown to be indispensable for immune-selection of the AID/APOBECs-dependent HBV mutations (6).

5. Inhibition of HBV by AID/APOBEC proteins

A3s are effective inhibitors of HBV DNA replication. A3s are effective restrictive factors for various types of virus with ssDNA, including HBV. It has been shown that A3 catalyzes cytosine deamination of HBV DNA produced during reverse transcription as a template, resulting in the presence of uracil in cDNA. Uracils in DNA (including cccDNA) are recognized and excised by cellular uracil DNA glycosylases (UNGs) leading to formation of apurinic/apyrimidinic (AP) sites, which are then recognized by cellular AP endonucleases resulting in DNA digestion (Fig. 1) (4,71-73). Certain studies describe contradictory results and report that the loss of UNG does not restore the levels of viral reverse transcripts, which had been decreased by A3G (74). A previous study found that HBV replication was inhibited by A3G (75). However, the G-to-A hypermutation was not observed in HBV DNA. Further studies using a 3D-polymerase chain reaction (PCR) method showed that A3B, A3C, A3F and A3G extensively deaminated cytidine residues in minus strand DNA. Unexpectedly, three of the four enzymes (A3B, A3F and A3G) deaminated HBV plus strand DNA in human HCC cell lines; however, the underlying mechanism remained undetermined (16). In

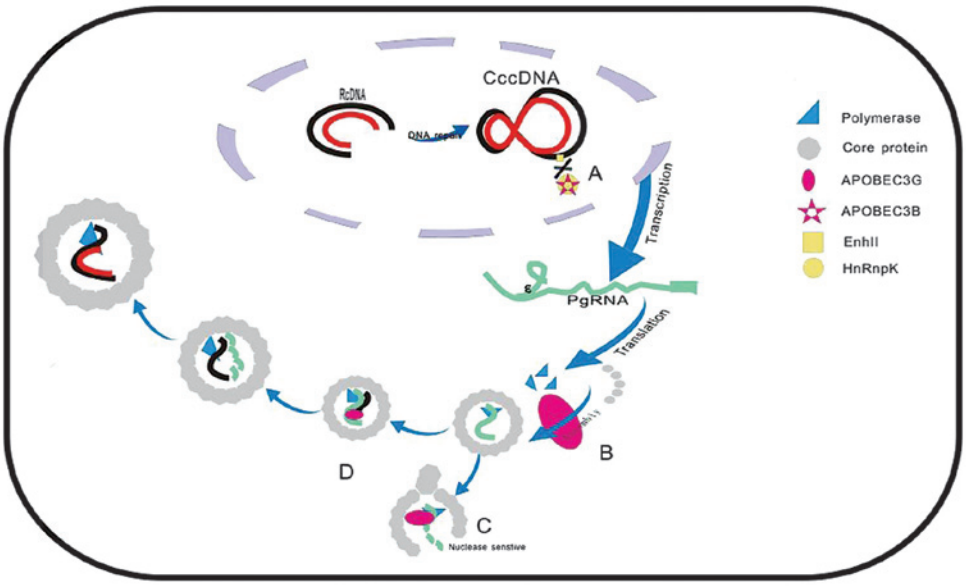

Figure 2. The rcDNA genome is converted into cccDNA by cellular repair factors. Then, the cccDNA is transcribed to the pgRNA and subgenomic mRNAs (not shown). The mRNAs are transported to the cytoplasm. The pgRNA is translated in the cytosol to form HBV core protein and the viral polymerase. These three components assemble to form the core particle. The first (minus) DNA strand forms within the core particles via reverse transcription of the pgRNA to DNA; the pgRNA is degraded by viral RNase H as the plus strand is synthesized. (A) A3B inhibits the binding of HnRnp K to the Enh II of HBV; (B) A3G may inhibit pgRNA packaging; (C) A3G renders HBV core protein-associated full-length pgRNA nuclease-sensitive; (D) A3G blocks DNA strand elongation and targets a DNA-RNA hybrid. rcDNA, relaxed circular DNA; cccDNA, covalently closed circular DNA; pgRNA, pregenomic RNA; HBV, hepatitis B virus; APOBEC, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; HnRnp K, heterogeneous nuclear ribonucleoprotein K; Enh II, enhancer II.

another study, Henry *et al* (17) compared the HBV editing by all seven enzymes in a quail cell line, which did not produce any endogenous DNA cytidine deaminase activity. The study identified that all of the A3s, not including A3DE, deaminated the HBV DNA levels from 10^{-2} to 10^{-5} *in vitro*, with A3A proving to be the most efficient editor (17). The hyperediting ability of A3A was confirmed by Lucifora *et al* (4). Although A3C was weaker at inhibiting HBV replication compared with A3G, its capacity for DNA hyperediting was higher than A3G and A3H (18,20). A3G demonstrated a strong preference for CpC, whereas A3H demonstrated a preference for TpC (19). These findings indicate that human A3 enzymes impact HBV replication by cytidine deamination. However, the induction of hypermutations is not sufficient for full inhibition of HBV replication.

A cytidine deaminase null mutant of A3B (E255A) was also found to inhibit HBV RNA production, although it was unable to edit HBV (28). It was proposed that only the carboxy (C)-terminal deaminase domain of A3B catalyzes HBV hypermutations (24,26). By investigating a series of A3B point mutants, it was found that the N- and C-terminal cytosine deaminase domains of A3B (24) and A3G (76) exerted inhibitory effects on HBV replication. By contrast, a truncated splice variant of A3B that lacked the C-terminal deaminase domain exerted no effect on HBV replication (26). However, A3G, which was defective for deaminase activity, was found to inhibit HBV replication (75). From the above-mentioned reports, it is hypothesized that the inhibition of HBV may be mediated by additional, different mechanisms.

Possible editing-independent mechanisms. In principle, a decrease in HBV DNA may result from either accelerated DNA degradation or decreased synthesis. A previous report suggested that A3G decreased the viral DNA levels by inhibiting pgRNA packaging (75). Subsequently, Rösler *et al* (15) revealed that the early stages of HBV DNA morphogenesis, including RNA and protein synthesis, binding of pgRNA to the core protein, and self-assembly of the viral core protein, were unaffected. However, A3G rendered HBV core protein-associated full-length pgRNA nuclease sensitive. Nguyen *et al* (27) analyzed the mechanisms of deamination-independent suppression of HBV replication by A3G and did not identify enhanced DNA degradation by A3G, either *in vivo* or *in vitro*. A3G appeared to inhibit the very early steps in viral reverse transcription and blocked DNA strand elongation. Furthermore, the deamination-independent antiviral function of A3G targets DNA-RNA hybrids (27). The A3B protein inhibits the binding of heterogeneous nuclear ribonucleoprotein K to the enhancer II of HBV and represses the activity of HBV. In addition, A3B directly suppresses HBV S-promoter activity (23). The process is presented schematically in Fig. 2.

Inhibition of HBV by additional APOBEC proteins. AID and A1 also inhibit HBV replication. AID was significantly upregulated in ~50% of HBV and HCV cirrhotic samples indicating that ectopic expression of AID was also a feature of HBV liver disease. A1 was markedly expressed in one sample (although normalization of the expression levels was not conducted), A2 transcripts were detected in certain samples, but A4 was not detected in any sample. AID was identified to be as effective as A3G at hyperediting HBV DNA (21). Mutation matrices and editing frequencies in the HBV minus strand DNA were comparable, for example, the editing frequency for A1 was 32%, and for A3G was 34% (18). C-to-T (U) hypermutations were observed in rcDNA and nucleocapsid viral RNA indicating that AID deaminates viral RNA and ssDNA (77). A1

Table II. Summary of linkage studies between clinical indicators and AID/APOBEC.

A, APOBEC3 polymorphism studies

Study (ref.)	Cohort description	Association with APOBEC3 polymorphisms
Abe *et al*, 2009 (85)	724 patients with chronic HBV infection and 469 healthy control subjects.	No significant association between A3B deletion polymorphism and chronic HBV carrier state. A3B gene deletion homozygosity was associated with mild liver fibrosis.
Zhang *et al*, 2013 (28)	1,124 patients with HBV-associated HCC, 510 individuals with persistent HBV infection and 826 healthy control subjects. Population, Han Chinese.	At least one A3B deletion allele increased the risk for persistent HBV infection and HCC development.
Ezzikouri *et al*, 2013 (86)	179 HBV chronic carriers and 216 healthy control subjects. Population, Moroccan.	No significant difference in the frequencies of deleted A3B alleles or genotypes between the two groups. Patients with deleted genotypes experienced a faster progression of liver disease than those with insertion genotypes. A3B deletion exhibited significantly lower viral loads than patients with the wild-type. A3G H186R polymorphism R/R genotype frequencies were not significantly different in the HBV-infected patients and healthy subjects.

B, Hypermutation studies

Study (ref.)	Cohort description	Association with hypermutation
Beggel *et al*, 2013 (68)	80 treatment-naïve HBV infection patients (47 HBeAg-positive and 33 HBeAg-negative).	Hypermutation rates for HBeAg-negative patients were >10-fold higher than those of HBeAg-positive patients. HBeAg-negative patients higher hypermutation rates were significantly associated with the degree of fibrosis.
Noguchi *et al*, 2005 (69)	8 Japanese adult patients with acute HBV infection, 10 patients were chronic carriers.	Hypermutated HBV DNA was detected in 1/8 patients with acute HBV infection and 4/10 patients with chronic HBV infection. In the latter group, hypermutated genomes were found only in eAb-positive patients.

C, APOBEC3 expression studies

Study (ref.)	Cohort description	Association with APOBEC3 expression
Vartanian *et al*, 2010 (21)	41 cirrhotic samples (10 alcoholic cirrhosis, 10 HBV^+, 11 HBV^+HCV^+, 10 HCV^+, 4 normal livers).	5/7 A3 genes were significantly upregulated in the order: HCV ± HBV > HBV > alcoholic cirrhosis. A3C and A3D were up regulated for all groups. Interferon inducible A3G was overexpressed in virus-associated cirrhosis, as was AID in 50% of these HBV/HCV samples.
Xu *et al*, 2007 (37)	29 pairs of HCC and surrounding non-cancerous tissue samples.	A3B transcripts were significantly elevated in 24/29 HCC.
Kou *et al*, 2007 (46)	51 HCC patients (14 HBV^+, 30 HCV^+) with 25 CH and 26 LC patients, and 6 normal livers.	AID was significantly upregulated in HCC and surrounding non-cancerous liver tissues underlying CH or LC.

AID, activation-induced cytidine deaminase; APOBEC, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; HBV, hepatitis B virus; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; HBeAg, hepatitis B e antigen.

exhibited a strong preference for TpC and a strong aversion to GpC (19) and AID editing of the HBV target was concentrated at the GpC and ApC sites (21). Furthermore, the low expression levels of A1 and AID in Huh7 cells was consistently associated with decreased levels of core-associated HBV DNA by quantitative PCR. However, A2 did not exert any effect on HBV replication (25).

AID/APOBECs incorporation into viral particles. Research on HBV reveals that the antiviral activity of A3G requires incorporation into assembling viral particles to inhibit reverse transcription. Factors required for incorporation of the antiviral deaminase protein, A3G into HBV nucleocapsids continues to be investigated. It has been demonstrated that A3G and A3C bind to the HBV core protein in immunoprecipitation experiments (78,79). Such binding is essential for reverse transcription following infection. The binding of A3G to the HBV core protein was only indirectly demonstrated with coexpression of RT and pgRNA, however, not with core protein alone (15,18). The results are consistent with the findings of Nguyen *et al* (80) that A3G was specifically incorporated into replication-competent HBV nucleocapsids by interacting with viral RT and RNA packaging signals. However, by fluorescence resonance energy transfer (FRET) and acceptor

Table III. Summary of linkage studies between HCC and AID/APOBECs.

Protein	Experimental targets	Indication	Reference
A1	TM, TR	All of the TM and one TR had liver dysplasia. 8/35 TM developed HCC.	95
A1	TM, TR	The aberrant editing markedly reduced levels of protein expression by the tumor suppressor gene, *NAT1*.	96
A2	TM	HCC developed in 2/20 A2 TM at 72 weeks of age. Significantly high frequencies of nucleotide alterations in *EIF4G2* and *PTEN* genes were observed in hepatocytes.	97
A3	CL, HCCT	C-terminally truncated *HBx* mutants generated by A3 enhanced the colony forming ability and proliferative capacity of neoplastic cells. A3B upregulated *HSF1*.	37
A3A	CL	A3A led to induction of cellular DNA breaks and activation of damage responses in a deaminase-dependent manner. A3A expression induced cell cycle arrest.	98
AID	CL, HCCT	The majority of liver tissues with AID upregulation contained multiple genetic changes in the p53 gene. Aberrant activation of AID in hepatocytes resulted in accumulation of multiple genetic alterations in the p53 gene.	46
AID	TM	HCC developed in 27% of tissue-nonspecific alkaline phosphatase-AID TM at the age of 90 weeks. The HCC expressed α-fetoprotein and possessed deleterious mutations in the tumor suppressor gene, *TRP53*.	94

AID, activation-induced cytidine deaminase; APOBEC, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; TM, transgenic mice; TR, transgenic rabbit; CL, cell line; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; HCCT, HCC tissue; *NAT1*, novel A1 target no 1; *EIF4G2*, eukaryotic translation initiation factor 4 γ, 2; *PTEN*, phosphatase and tensin homolog; *HBx*, hepatitis B virus X; *HSF1*, heat shock transcription factor 1; *TRP53*, tumor protein P53.

photobleaching experiments, Zhao *et al* (81) revealed that A3G directly binds to core proteins. Similarly, direct interaction of HBV core protein and A3A was confirmed by proximity ligation assay and FRET analysis. Deletion analysis was used to confirm that the central region of the HBV core protein (between aa 77 and 149) was involved in the interaction with A3A (4). Additionally, the A3B, A3C and A3F enzymes were also found to be associated with the HBV capsid by interaction with the core protein (16). Similar to A3G, AID was co-immunoprecipitated with the nucleocapsid core protein. The assumption was made that AID formed a ribonucleoprotein complex with the HBV core proteins and RNA during nucleocapsid assembly in which AID deaminated cytosines of the viral RNA, including pgRNA and ssDNA (77).

6. APOBEC polymorphisms in HBV infections

The APOBEC family is considered to be significant in innate cellular immunity, particularly in HBV hyperediting and infection inhibition. It was of interest to elucidate whether gene polymorphisms alter host susceptibility to HBV progression of CH. A3B is an efficient editor and inhibitor in the process of HBV infection. A 29.5-kb deletion between exon 5 in A3A and exon 8 in an A3B gene cluster resulted in complete removal of the A3B coding region (82,83). A hybrid gene containing the coding region of A3A and the 3'-untranslated region of A3B was initially discovered by mapping end-sequence pairs from a human fosmid library against the human genome reference sequence assembly (82). This was confirmed by dense single nucleotide polymorphism marker mapping (83). A report on the frequency of the deletion allele conducted using 1,277 diverse human samples revealed that its expression is rare in African and European individuals (frequency of 0.9 and 6%, respectively), more common in East Asian and Amerindian individuals (36.9 and 57.7%, respectively) and almost universal in Oceanic populations (92.9%) (84). Analyzing the association between this polymorphism and chronic HBV infection may clarify the effect of A3B on the establishment of chronic HBV carrier state. Abe *et al* (85) initially investigated the association between this polymorphism and chronic HBV infection. No association was identified between the chronic HBV carrier state and deletion polymorphisms; however, the extent of liver fibrosis was found to be associated with insertion homozygosity (85). Similarly, another report demonstrated that there was no significant difference in the frequencies of deleted A3B alleles or genotypes between patients with CH B and control subjects. By contrast, subjects bearing a deleted genotype experienced a more rapid progression of liver disease when compared with those exhibiting an insertion genotype (86). This result indicated that in the A3B deletion polymorphism, A3 cytidine deaminases do not predispose an individual to chronicity, but may modulate the course of persistent HBV infection. These results are contradictory to a study, which revealed that a 29.5-kb deletion obliterated the production of A3B, and was significantly associated with increased susceptibility to persistent HBV infection in an ethnic Han Chinese population (28).

Seven haplotypes (designated with Roman numerals, I-VII) were reported to be present in various human populations based on the five single amino acid polymorphisms, N15Δ, R18L, G105R, K121D and E178D (87). The N15Δ and R105G mutations may independently cause a marked decrease in A3H expression. Only the haplotypes II, IV and VII were without these two mutations, and were stably expressed and

found to efficiently inhibit HIV-1 replication (88). In addition, Wang *et al* (87) found that E140K, H54R, E56Q, C85S and C88S, as well as the two mutants, W115A and W115L, disrupted A3H expression. The A3G H186R polymorphisms in HBV-infected patients were found to be different to those of healthy Moroccan individuals, although the difference was not significant (86). The outcomes of a selection of studies are summarized in Table II. The association between AID/APOBEC polymorphisms and liver disease requires further, in-depth investigation.

7. Emerging role of the AID/APOBEC family in the development of cancer

The AID/APOBEC family and their associated editing patterns may be involved in oncogenesis. Recent analyses of the mutations have implicated APOBEC cytidine deaminases as significant factors in the mutagenesis of human cancer genomes (89,90). Within cancer genomes, APOBEC mutagenesis has been found to be pervasive and correlated with APOBEC mRNA levels (89-91). Roberts *et al* (89) identified that tumor samples, from 14 types of cancer, contained hundreds of APOBEC-signature mutations, constituting ≤68% of mutations in the exome. In at least six distinct types of cancer, similar results have revealed that A3B is upregulated, and its preferred target sequence was frequently mutated and clustered (91).

AID is critical in class-switch recombination and SHM of the immunoglobulin gene in B lymphocytes (92). Animal models have demonstrated that the aberrant expression of AID contributes to HCC tumorigenesis (93,94). A positive correlation between somatic mutation frequencies and AID expression levels was observed in the p53 gene in liver tissues with AID upregulation, as downregulation and somatic mutations in the p53 gene have been well characterized in human hepatocarcinogenesis (46). These data indicate that AID/APOBEC-catalyzed deamination may be important in generating somatic mutations during the progression of virus-associated HCC.

Transgenic overexpression of A1 in rabbit and mouse livers resulted in liver dysplasia and HCC through excessive editing of hepatic mRNAs, such as tyrosine kinase (95). Furthermore, hyperediting of the novel A1 target no 1 mRNA that encoded a tumor suppressor gene, created stop codons and truncated protein products, which are linked to liver cancer (96). Aberrant A2 expression resulted in nucleotide alterations in the transcripts of a specific target gene and may be involved in the development of human HCC via hepatic inflammation (97). A3 deaminases generate C-terminally truncated *HBx* mutants, which enhance the colony-forming ability and proliferative capacity of neoplastic cells. Notably, A3B was observed to be widely upregulated in HCC tissues, and promoted the growth of neoplastic human HepG2 liver cells and upregulated heat shock transcription factor 1 expression levels (37). The expression of A3A may lead to induction of DNA breaks and activation of damage responses in a deaminase-dependent manner. Consistent with the above-mentioned observations, A3A expression was found to affect genomic integrity by inducing cell-cycle arrest (98). The outcomes of a selection of studies are summarized in Table III.

8. Conclusion

Innate immunity mechanisms are the first line of defense against invading viruses. The AID/APOBEC family of cytidine deaminases is significant in the regulation of tissue development, responding to metabolic regulation and facilitating innate immunity by restricting numerous types of virus, including HBV. Unresolved issues are the mechanisms of AID/APOBEC-dependent specific recognition of HBV DNA, degradation of cccDNA, and the security and availability of experimental models, which are required for further investigation. Recent analyses of the mutations have indicated that AID/APOBEC cytidine deaminases are significant factors in the mutagenesis of human cancer genomes. A3B, which is only localized to the nucleus, is proposed to be responsible for a large proportion of dispersed and clustered mutations in multiple distinct cancers, including HCC. It is essential to elucidate the mutational processes underlying the development of cancer, and its potential implications on cancer etiology, prevention and therapy.

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