Review

Molecular genesis of drug-resistant and vaccine-escape HBV mutants

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A high rate of viral turnover, combined with an error-prone polymerase, results in a very high frequency of mutational events during HBV replication. Not surprisingly, particular selection pressures, both endogenous (host immune clearance) and exogenous (vaccines and antivirals), readily select out new ‘escape’ mutants. The introduction of nucleoside/nucleotide analogue (NA) therapy for chronic hepatitis B has witnessed the emergence of antiviral drug resistance as the major factor limiting drug efficacy. Furthermore, because of the overlap of the viral polymerase and envelope reading frames in the HBV DNA genome, NA resistance-associated mutations selected in the catalytic domains of the polymerase frequently result in important changes to the neutralizing antibody-binding domains of the hepatitis B surface antigen, including the emergence of antiviral drug-associated potential vaccine escape mutants (ADAPVEMs). The public health significance of ADAPVEMs is considerable in terms of the global programme for control of hepatitis B via universal infant immunization. Thus, prevention of resistance requires the adoption of strategies that not only effectively control HBV replication, but also prevent the emergence of ADAPVEMs.

Molecular virology of HBV

HBV is a small circular DNA virus and is a member of the Hepadnaviridae family [1]. The intact virion comprises the DNA genome and an endogenous viral polymerase surrounded by structural nucleocapsid and surface proteins. The transcriptional transactivator and immune regulator proteins, as well as the structural and polymerase proteins, are encoded for in four overlapping open reading frames (ORFs) in the compact 3.2-kb partially double-stranded DNA genome. These four are the core/precore, polymerase, envelope and X ORFs. The core/precore ORF encodes both the core protein (hepatitis B core antigen), which forms the nucleocapsid, and the precore protein, also known as the hepatitis B e antigen (HBeAg), which is involved in immune evasion. The polymerase ORF encodes the viral polymerase protein, which contains a reverse transcriptase (rt) that is essential for replication. The envelope ORF encodes the pre-S1, pre-S2 and S proteins (collectively, hepatitis B surface antigen [HBsAg]), which form the mature virion envelope. These proteins are also known as large (L), middle (M) and small (S) HBsAg, respectively. Finally, the X ORF encodes the X protein (hepatitis B X antigen), a transcriptional transactivator [1].

Viral entry into hepatocytes is thought to be receptor-mediated, although the receptors involved are yet to be identified. Following virion entry the surface proteins are shed, releasing the nucleocapsid, which is then translocated to the nucleus, with the genome entering the nucleus through the nuclear pores. The partially double-stranded DNA genome is ‘repaired’ in the nucleus to a highly stable covalently closed circular DNA molecule, which is the major transcriptional template and exists as a viral minichromosome from which pregenomic RNA, precore RNA, HBx and HBsAg messenger RNAs (mRNAs) are made. The pregenomic mRNA is the template for both the polymerase and hepatitis B core antigen. Following translation, the HBV polymerase mediates reverse transcription of the pregenomic mRNA transcript. Essentially, the HBV polymerase interacts with epsilon, a cis acting signal containing a stem loop structure on the RNA transcript prior to its encapsidation and subsequent reverse transcription within the nucleocapsid [1]. The surface proteins are synthesized and processed in the endoplasmic reticulum. Following full genome replication, the nucleocapsid translocates out of the cytosol and is packaged into mature virions that then exit the host cell via the exocytosis pathway.
Antiviral drug resistance and chronic hepatitis B

In many parts of the world, five HBV-specific nucleoside/nucleotide analogues (NAs) targeting the viral polymerase are approved for the treatment of chronic hepatitis B (CHB): lamivudine (3TC; a cytidine \( \ldots \)-nucleoside analogue), adefovir (ADV; an alkyl phosphonate), entecavir (ETV; \( \ldots \)-cyclopentane), telbivudine (LdT; a thymidine \( \ldots \)-nucleoside analogue) and tenofovir disoproxil fumarate (TDF; an alkyl phosphonate very similar to ADV). Frequently used as monotherapy, most of these NAs are associated with the evolution of antiviral drug resistance mutations in the rt domain of the HBV polymerase gene (pol). For 3TC, the prevalence of resistance rises rapidly, reaching 14% after 1 year [2] and 80% after 4 years [3] of monotherapy. Resistance develops less rapidly with ADV (5.9% over 144 weeks) [4] and ETV (<1% after 3 years but 5.8% after 1 year in 3TC-treated patients), and there is evidence that previous 3TC therapy accelerates ETV resistance [5,6]. Antiviral drug resistance to these NAs is associated with the evolution of antiviral drug resistance mutations in the rt domain of the HBV polymerase gene (pol). For 3TC, the prevalence of resistance rises rapidly, reaching 14% after 1 year [2] and 80% after 4 years [3] of monotherapy. Resistance develops less rapidly with ADV (5.9% over 144 weeks) [4] and ETV (<1% after 3 years but 5.8% after 1 year in 3TC-treated patients), and there is evidence that previous 3TC therapy accelerates ETV resistance [5,6]. Antiviral drug resistance to these NAs is associated with the selection of primary resistance mutations in the \( \ldots \)-nucleosides (lamivudine [3TC] and telbivudine [LdT]), the acyclic phosphonates (adefovir [ADV] and tenofovir disoproxil fumarate [TDF]) and the \( \ldots \)-cyclopentane group (entecavir [ETV]). Length of the viral polymerase (845 amino acids [aa]) is based on HBV genotype A.

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To date, there are eight codons associated with primary antiviral drug resistance in CHB, which map to four of the functional domains of pol [19]: B-domain at codons rtH169, rtL180, rtA181 and rtT184; C-domain at codons rtS202 and rtM204; D-domain at codon rtN236; and E-domain at codon rtM250.

Recent bioinformatic studies [20] have shown that these eight codons are associated with four major
pathways of antiviral resistance: the l-nucleoside pathway, which is identified by the NA resistance-associated mutation, rtM204V/I, resulting in resistance to 3TC and LdT; the acyclic/alkyl phosphonate sugar pathway, which is identified by the rtN236T substitution, resulting in resistance to ADV and reduced sensitivity to TDF; the ‘shared’ pathway, which is identified by the mutation in pol of rtA181T/V and results in resistance to both the l-nucleosides (3TC and LdT) as well as ADV, and reduced sensitivity to TDF; and the treatment-naive ETV resistance pathway, which is identified by detection of the mutations rtL180M plus rtM204V/I plus at least one substitution in one of the rtT184, rtS202 and rtM250 amino acid positions. Finally, various combinations and permutations of these four major pathways have been described in patients with multidrug-resistant HBV, such as rtA181T plus rtN236T plus rtM250L [21].

Causes of antiviral drug resistance
Antiviral drug resistance reflects the reduced susceptibility of a virus to the inhibitory effect of a drug [22] and results from a process of adaptive mutations under NA therapy. Six important factors combine that account for the timing and pattern of resistance: HBV viral replication rates, low fidelity of pol, selective pressure of the drug, the genetic barrier of the drug, role of replication space (hepatocyte turnover) and fitness of the resistance mutant.

In a chronically infected individual, the extent of HBV replication is considerable, reaching $10^{12}$ virions per day. As pol is an rt that lacks proof-reading capacity, HBV replication is also associated with a high mutational rate of $10^{-5}$ substitutions/base/cycle. Thus, all possible single-base changes in the 3,200-nucleotide genome are generated every day, thereby accounting for the observation that single and double mutations associated with NA resistance existing in patients prior to therapy, whilst triple or quadruple mutations require replication in the presence of NA-imposed selection pressure and so rarely pre-exist. Thus, it is not surprising that NAs that have low potency and a low genetic barrier (one or two primary resistance mutations) to resistance (for example, 3TC and LdT), when used as monotherapy will fail quickly, whereas NAs that are potent (for example, TDF and ETV) and have a high genetic barrier (for example, ETV with at least three mutations) to resistance are associated with extremely low resistance rates after 3–5 years of monotherapy [13].

Cross-resistance
Cross-resistance is defined as resistance to drug(s) that a virus has never been exposed to. The drug resistance-associated mutations selected by particular groups of NA (for example, l-nucleosides, acyclic phosphonates or cyclopentanes) could diminish the antiviral activity of other drugs [23]. This should be considered before any antiviral drug is prescribed and the physician should plan for eventual treatment failure. The initial selection and subsequent rescue therapies should be based on knowledge of cross-resistance [23], so that the second agent used will not induce cross-resistance with the failing agent [24]. Preferably by using the add-on/combination approach [24]. The advantage of using combinations of NA with complementary cross-resistance profiles has recently been highlighted [23] and a summary of cross-resistance profiles based on the viral resistance ‘pathways’ approach is shown in Table 1.

<table>
<thead>
<tr>
<th>Mutation effect</th>
<th>Adefovir-resistant (N236T)</th>
<th>Adefovir-resistant (A181T/V)</th>
<th>Entecavir-resistant</th>
<th>Teloavudine-resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confers complete resistance</td>
<td>Telbivudine</td>
<td>–</td>
<td>–</td>
<td>Lamivudine, telbivudine</td>
</tr>
<tr>
<td>Confers some degree of reduced sensitivity to listed drugs [18,23]</td>
<td>Entecavir</td>
<td>Tenoforv disoprol fumarate</td>
<td>Lamivudine, telbivudine, tenoforv disoprol fumarate</td>
<td>–</td>
</tr>
<tr>
<td>Drugs remaining fully active [23,61]</td>
<td>Adefovir, tenoforv disoprol fumarate</td>
<td>Entecavir, lamivudine, telbivudine</td>
<td>Entecavir</td>
<td>Adefovir, tenoforv disoprol fumarate</td>
</tr>
</tbody>
</table>

Several drug resistance mutations affect sensitivity to other drugs in laboratory analyses, but their effect on treatment outcomes is unclear. First virological breakthrough should be managed with an add-on strategy (combination), instead of switch (sequential) monotherapy. Entecavir-resistant mutations are rtM204I/V with or without rtL180M, plus one or more substitutions at rtT184, rtS202 or rtM250, with or without an rtI169 substitution in the HBV polymerase. Telbivudine-resistant mutations are rtM204I/V plus rtL180M. The effect on sensitivity is variable as results are according to laboratory analyses and not patient studies. Tenoforv disoprol fumarate resistance was not determined because of treatment intensification after week 72 [16,17].
Consequences of antiviral drug resistance in chronic hepatitis B

The consequences of emergence of NA-resistant HBV in treated patients include virological breakthrough and rebound in viral load, biochemical breakthrough – including hepatic flares, histological progression of liver disease, clinical manifestations including hepatic decompensation, increased viral recurrence post liver transplantation and even possible increased tumourigenicity [25]. Several public health issues have been identified including an alteration in the HBsAg antigenicity [26], transmission of drug-resistant HBV [27,28] and a possible threat to the various immunization programmes designed to control hepatitis B [29]. This includes the emergence of antiviral drug-associated potential vaccine-escape mutants (ADAPVEMs) [27]. The remainder of this article will focus on these public health issues.

Public health significance of the pol/S gene overlap

The envelope ORF of HBV encodes three co-carboxyl-terminal proteins, the shortest of which is S, the principal component of the external envelope of the 42 nm virion. The S protein carries the major target of neutralizing antibody, the ‘a’ determinant. Although it is well known that resistance mutations result in non-synonymous changes in the S of HBsAg [30–34], the first study to demonstrate that these substitutions might affect HBsAg protein conformation, and more importantly its antigenicity, was by Torresi et al. [35]. These investigators showed that the common 3TC-resistant substitutions (rtM204V/sI195M, rtM204I/sW196S, rtM204I/sW196L and rtV173L/sE164D plus rtL180M plus rtM204V/sI195M) resulted in reductions in the reactivity of the altered HBsAg with vaccine-induced antibody against HBsAg (anti-HBs). In addition, the converse has been shown in that changes in the S gene introduce changes in pol that correspond with 3TC-resistant compensatory-type mutations [36]. These studies have now been independently confirmed using different in vitro models, including mammalian cell culture transfection and epitope ‘density’ mapping [37]. These latter studies used fully glycosylated HBsAg recombinant proteins, whereas the study of Torresi et al. [35] used yeast-expressed recombinant HBsAg proteins that were not glycosylated. The key findings are summarized in Figure 2.

Figure 2. Polymerase and envelope link of HBV: relevance to antiviral drug resistance and vaccine escape

<table>
<thead>
<tr>
<th>Env</th>
<th>‘a’ determinant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-S1</td>
<td>Pre-S2</td>
</tr>
<tr>
<td>Terminal protein</td>
<td>Spacer</td>
</tr>
<tr>
<td>Pol</td>
<td>rt domains</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Envelope mutants</th>
<th>Polymerase mutants*</th>
<th>Ag–Ab binding IC₅₀ μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Wild type</td>
<td>1.09</td>
</tr>
<tr>
<td>HBIG escape</td>
<td>rtW153Q</td>
<td>&gt;55.0</td>
</tr>
<tr>
<td>sD144E/G145R</td>
<td>rtG153E</td>
<td>&gt;55.0</td>
</tr>
<tr>
<td>Drug-resistant</td>
<td>rtF166L</td>
<td>1.86</td>
</tr>
<tr>
<td>sF158Y</td>
<td>rtV173L</td>
<td>14.86</td>
</tr>
<tr>
<td>sE164D</td>
<td>rtV173S</td>
<td>8.29</td>
</tr>
<tr>
<td>sW196S</td>
<td>rtM204I</td>
<td>5.26</td>
</tr>
<tr>
<td>sI195M</td>
<td>rtM204V</td>
<td>12.5</td>
</tr>
<tr>
<td>sM198I</td>
<td>rtV207I</td>
<td>54.53</td>
</tr>
</tbody>
</table>

Adapted from [35]. *rtL180M in polymerase (Pol) causes no change in envelope (Env). Ab, antibody; Ag, antigen; HBIG, hepatitis B immunoglobulin; IC₅₀, 50% inhibitory concentration; rt, reverse transcriptase.
chimpanzees that carried high titres of circulating anti-HBs pre-challenge [27]. This chimpanzee study also established the genetic stability of the rtV173L plus rtL180M plus rtM204V variant in a non-immunized chimpanzee, in whom no revertants to wild type (WT) were detected over time compared with infection with the sG145R vaccine-escape mutant, which quickly back-reverted to WT [27]. This latter observation reveals the important role of compensatory mutations in ‘fixing the genetic archive’, especially in the setting of transmission of NA resistance.

Hepatitis B immunization programme
Since its widespread introduction, the hepatitis B vaccine has become an essential part of global infant immunization programmes. The vaccine has been particularly important for countries where the incidence of HBV-related hepatocellular carcinoma (HCC) is high. The hepatitis B vaccine is an effective means of preventing HBV infection, producing protective levels of antibodies in up to 95% of recipients [38]. The current commercially available hepatitis B vaccine used in most programmes is a yeast-derived recombinant HBsAg, containing only the small S protein. As discussed above, neutralizing antibodies (anti-HBs) elicited by hepatitis B vaccines specifically target the ‘a’ determinant of HBsAg.

It has been recognized that the administration of hepatitis B vaccine can result in the selection of antibody escape variants such as sG145R [39]. In high-prevalence countries, such as China, Thailand and Taiwan, long-term monitoring has shown that hepatitis B immunization programmes have increased the incidence of HBV variants with a number of ‘signature’ substitutions associated with vaccine failure in the HBsAg protein [39,40], although the vaccine programme reduces the overall burden of hepatitis B [41]. Mutations in and around the ‘a’ determinant might lead to an alteration in the antigenicity of the HBsAg protein so that anti-HBs might fail to neutralize the virus [42–50]. Infection of immunized individuals with these vaccine-escape mutants (VEMs) [45] is therefore possible. VEMs are typically characterized by the presence of particular single amino acid changes in the S protein, for example, sG145R [46,48]. Some of these variants have been found in individuals for at least a decade, suggesting they are stable and potentially transmissible variants of HBV. In Taiwan, up to 28% of children with CHB also harbour HBsAg mutants. Not surprisingly then, VEMs capable of causing infection in fully immunized individuals are common in countries with high rates of endemic HBV infection and universal hepatitis B infant immunization programmes [45,48]. To date, the emergence of VEMs has not had a known negative effect on the immunization programme of any particular country [51], but many of these programmes do not include the use of a birth-dose of hepatitis B immunoglobulin, which is known to accelerate VEM selection [44,45,47,51].

In those populations where 3TC has been widely used to treat patients continuously for periods of several years, viruses with alterations in HBsAg are likely to occur relatively frequently, and some will have the potential to be ADAPVEMs [29]. The emergence of such ADAPVEMs in 3TC-treated patients does not readily translate to a significant imminent threat to the global hepatitis B immunization programme [29]. For a new viral species to pose a threat in this setting, Clements et al. [29] have proposed that an ADAPVEM would need to possess the following four characteristics: it must be a stable mutant; it must have undergone sufficient changes in antigenicity such that anti-HBs generated by the current vaccine no longer neutralizes it; it must be transmissible and cause infection in immunized individuals, and so have the opportunity for ongoing spread; and it must cause disease (acute or chronic) in infected individuals. Of these four characteristics, there is evidence to date that the first three have been acquired; it is not known if ADAPVEMs have the same propensity to cause disease as do current circulating strains of HBV [29], although one case of primary infection with a 3TC-resistant HBV was associated with acute hepatitis [28]. Clearly, further studies are needed to fully elucidate the clinical, pathological and epidemiological significance of these emerging ADAPVEMs.

Evolution of HBsAg during antiviral therapy
In order to study the effects of long-term antiviral therapy on the HBsAg of HBV, we have performed an analysis of the pol/rt sequences and, therefore, the S gene, obtained from serum samples of 2,136 patients with either HBV monoinfection or HIV–HBV coinfection that were submitted for drug resistance testing from 1991 to 2009 to the Victorian Infectious Diseases Reference Laboratory, North Melbourne, Australia. These sequences have been analysed using the SeqHepB programme [52]. A function of this programme is to identify and differentiate polymorphic and reportable amino acid substitutions within pol and S gene, taking into account the HBV genotypes. Based on whether the pol/rt sequences have amino acid substitutions at any of the eight rt codons (Figure 1) that are associated with drug resistance (rt169, rt180, rt181, rt184, rt202, rt204, rt236 and/or rt250), the patients were categorized as either having drug-resistant (DR; n=737) or drug-susceptible (DS; n=1,399) HBV infections.

The frequency of reportable amino acid substitutions within the HBsAg for these two types of HBV isolates (DR or DS for the total cohort) is shown in Figure 3. As highlighted in this figure, a number of HBsAg codons in the DR isolates have significantly higher rates of amino acid substitutions compared with equivalent codons in
the DS isolates \((P<0.05; \text{determined by Fisher’s exact and Holm’s method of} P\text{-value correction})\). The HBsAg codons were s47 (rt55), s75 (rt84), s134 (rt142), s164 (rt173), s172 (rt181), s173 (rt181), s175 (rt184), s192 (rt200), s195 (rt204), s196 (rt204), s199 (rt207), s220 (rt229) and s221 (rt229; Figure 3). Importantly, no HBsAg codons were identified by statistical analysis to have higher rates of reportable amino acid substitutions in the DS isolates compared with equivalent regions in the DR isolates. Figure 3 also includes a plot of the location of the rt domains, the major B-cell epitopes of HBsAg and the CTL-T helper epitopes.

The patients were then further categorized into HBV monoinfections (DS \(n=1,229\) and DR \(n=615\)) or HIV–HBV coinfection (DS \(n=181\) and DR \(n=111\)). Interestingly, the same HBsAg codons as seen in the total cohort was found to have higher rates of reportable amino acid substitutions in the DR isolates of patients with HBV monoinfections, and these were statistically significant \((P<0.05)\). Only the HBsAg codons s164 (rt173), s195 (rt204) and s196 (rt204) showed similar differences between the DR and DS isolates of patients coinfected with HIV and HBV. This difference in the number of HBsAg codons observed between the two patient groups might be explained by the small number of patients with HIV–HBV coinfection in the study. Nonetheless, it was interesting to note that the rates of amino acid substitutions in s164 and s195 were substantially higher in the DR isolates of HIV–HBV coinfected patients (39% for s164 and 96% for s195) compared with HBV-monoinfected patients (7% for s164 and 40% for s195), whereas the converse was observed for s196 (coinfected 13% and monoinfected 46%). These observations reflect the higher rate of detection for the rtV173L (sE164D) plus rtL180M (silent in S) plus rtM204V (sI195M) 3TC resistance mutational profile in coinfected patients compared with the rtM204I (sW196stop/S/L) profile. The ADAPVEM potential of rtV173L plus rtL180M plus rtM204V (sE164D plus sI195M) has already been discussed above and is shown in Figure 2.

**Figure 3.** The HBsAg amino acid mutation frequency from the SeqHepB database from 1991 to 2009

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**Table 1.** Frequency of reportable amino acid substitutions within each hepatitis B surface antigen (HBsAg) codon position \((n=2,136)\). The length of HBsAg varied between sequences, and this has been taken into account during proportion calculation. The locations of the functional domains in the overlapping pol are shown, coplotted with the major B-cell and T-cell epitopes. CTL, cytotoxic T-lymphocyte; HLA, human leukocyte antigen; M, mini-loop; rt, reverse transcriptase; 1, loop 1; 2, loop 2.

<table>
<thead>
<tr>
<th>Proportion, %</th>
<th>HBsAg codon positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant isolates ((n=737))</td>
<td></td>
</tr>
<tr>
<td>Sensitive isolates ((n=1,399))</td>
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</table>
Of the reportable amino acid substitutions detected in HBsAg codons that had significantly higher substitution rates in DR isolates than in DS isolates, not all were the result of an overlap with the known drug resistance-associated mutations within pol. Nonetheless, most of them are located in a specific rt catalytic domain rather than an interdomain [19]: s75 in domain A; s164, s172 and s173 in domain B; and s192, s195, s196 and s199 in domain C. It is possible that the rt of DR HBV isolates is under greater positive selective pressure than the DS isolates. In the context of envelope functional domains, s134 (loop 1) and s164 (downstream of loop 2) are both located in the ‘a’ determinant of HBsAg [42,48]. Importantly, the common 3TC-associated HBsAg mutational profile (rtV173L/sE164D plus rtL180M plus rtM204V/sI195M) has already been shown to cause a reduction in the reactivity of HBsAg with antiHBs in vitro [35] and in vivo [27]. Finally, s175 is located in an important HLA class-I-restricted epitope initially described by Chisari and Ferrari [53] and classified in their paper as ENV 338–347 (assigned s175–s184 in Figure 3), implying an immunological rather than an NA-driven selection pressure.

As well as non-synonymous substitutions, antiviral therapy also resulted in HBsAg truncation that occurred more frequently in HBV isolates that are DR compared with those that are DS; in particular, the presence of stop codons in the C-terminal (s172, s196 and s199) end of HBsAg (Figure 4). It is noteworthy that the occurrence of sW182stop (rtV191I) was substantially higher in HBV isolated from HIV–HBV-coinfected patients. Investigation into the clinical effects and significance of these C-terminal truncations is ongoing.

Figure 4. Frequency of HBsAg stop codon mutations in HBV-monoinfected and HIV–HBV-coinfected patients

The hepatitis B surface antigen (HBsAg) stop codon mutation frequency in HBV-monoinfected patients (n=1,844) was compared with HIV–HBV-coinfected patients (n=292) in either the nucleoside/nucleotide drug-sensitive (DS) or drug-resistant (DR) group. The length of HBsAg varied between sequences, and this has been taken into account during proportion calculation.
in the progression to HCC as they possess transactivational activity, revealed by increased nuclear factor κB or activator protein 1 promoter activity [56].

Of clinical significance is the recent observation that NA therapy selects for HBV mutants that encode truncated surface proteins and therefore could theoretically accelerate the progression to HCC [25,57]. Treatment of CHB with all the NAs can result in the selection of HBV variants with point mutations in pol that not only confer NA resistance but also result in changes to HBsAg (Figure 5) [26,58]. In particular, the point mutation that causes the rtA181T change in pol also encodes a stop codon (sW172*) in the overlapping surface proteins (Figure 5), resulting in truncation of the last 55 amino acids of the C-terminal hydrophilic region of the HBsAg. In vitro analysis of rtA181T/sW172* HBV has shown that it is defective in secretion of viral particles resulting in intracellular retention of surface proteins and has a dominant negative effect on WT virion secretion resulting in lower viral loads extracellularly [58]. Two recent reports [25,57] have now provided evidence for involvement of HBV encoding the rtA181T/sW172* mutation in the pathogenesis of and progression to HCC. Analyses of HBV DNA from patients who developed HCC despite 3TC therapy revealed stop codon mutations in the envelope gene in seven of eight patients compared with the control group, in which no patients developed HCC. Using expression constructs encoding the HBV surface proteins, these investigators demonstrated that surface proteins truncated at amino acids sL21, sW156 or sW172 (the last of which corresponds to the surface proteins expressed from rtA181T/

Figure 5. HBsAg mutations selected during NA therapy

Adapted from [42] and [48]. The hepatitis B surface antigen (HBsAg) mutations selected during nucleoside/nucleotide analogue (NA) treatment with β-nucleosides (such as lamivudine [3TC] or telbivudine [LdT]), acyclic phosphonates (such as adefovir [ADV] or tenofovir disoproxil fumarate [TDF]) or α-cyclopentane (such as entecavir [ETV]). The mini-loop (codons 120–124), and loop 1 and loop 2 regions of the ‘a’ determinant are shown.

in the progression to HCC as they possess transactivational activity, revealed by increased nuclear factor κB or activator protein 1 promoter activity [56].
C-terminally truncated HBsAg HBV variants. Of drug resistance and therefore the emergence of these effectively inhibit HBV replication eliminating the risk as well as the development of treatment strategies that do not select for potentially oncogenic DR HBV, future challenges in the treatment of carcinogenesis, the main long-term goal of antiviral HBV variants that are potentially oncogenic, negates the overall efficacy of NAs in preventing hepatocarcinogenesis, the main long-term goal of antiviral therapy in CHB. Future challenges in the treatment of CHB involve the development of antiviral therapies that do not select for potentially oncogenic DR HBV, as well as the development of treatment strategies that effectively inhibit HBV replication eliminating the risk of drug resistance and therefore the emergence of these C-terminally truncated HBsAg HBV variants.

Conclusions

The current patterns of antiviral drug resistance in CHB are complex. However, four major pathways can be defined in most cases, based on rtM204V/L, rtN236T, rtA181T/V and ETV-associated mutations (rtL180M plus rtM204V plus one of rtT184, rtS202 or rtM250), with the emergence of multidrug resistance a clear cause for concern in the longer term. Furthermore, broad clusters of compensatory mutations during 3TC therapy will compromise future rescue therapy options with the newer more potent drugs, such as ETV. The best cost-effective strategy is to prevent or avoid the emergence of antiviral drug resistance in the first place [22]. This is especially relevant from a public health perspective because most antiviral-resistant HBVs have an HBsAg and have the potential to behave as vaccine escape mutants, be transmitted and infect immunized individuals. Furthermore, there is preliminary evidence that these viruses can be associated with increased oncogenicity. The global programme for control of hepatitis B is built on the foundation of universal infant immunization and will continue to reduce new incident infections of hepatitis B [60]. The challenge of drug resistance to this highly successful campaign requires timely and effective involvement of public health groups cooperatively working with treating physicians to ensure that successful and appropriate therapy guidelines for hepatitis B are achieved and implemented, and that this strategy minimizes or eliminates antiviral drug resistance [23].

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Disclosure statement

The authors declare no competing interests.

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