

Viral Factors Associated with Response to Antiviral Therapy for Chronic Hepatitis C Virus Infection

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Abstract

A first-generation protease inhibitor (PI) against HCV non-structural 3/4A serine protease was approved worldwide at the end of 2011. We are now facing a revolutionary change in therapeutic strategies for chronic HCV infection, from interferon (IFN)-based therapies to direct-acting antivirals (DAAs). The efficacy of antiviral therapy varies with HCV genotype. The most intractable and most common HCV worldwide is HCV genotype 1 (G1). Viral and host factors participating in the virological outcome of IFN-based therapy have been extensively examined. However, in the era of DAAs, the significance of these factors will gradually decrease. Instead, viral factors related to resistance against DAAs are becoming the main focus. In this review, the viral factors participating in the response to IFN-based therapies are summarized, and the issue of viral resistance to DAAs is discussed.

Keywords: Pegylated-interferon (PEG-IFN); Ribavirin (RBV); Direct-acting antivirals (DAAs); HCV genotype; Interferon susceptibility-determining region (ISDR); Interferon ribavirin resistance-determining region (IRRDR); Protease inhibitor (PI)

Introduction

The last decade has been the era of pegylated-interferon (PEG-IFN) plus ribavirin (RBV) combination therapy for chronic HCV infection. However, at the end of 2011, first-generation nonstructural 3/4A (NS3/4A) serine protease inhibitors (PIs), such as terapeutic [1] and boceprevir [2], were approved for the treatment of chronic HCV genotype 1 (G1) infection, and triple therapy with PEG-IFN and RBV was widely introduced worldwide. Although other drugs that indirectly suppress the life cycle of HCV (i.e., Nieman-Pick C1-like protein 1 cholesterol absorption receptor antagonist, which interferes with cell entry by HCV [3]) may be developed in the future, the main focus of anti-viral therapies will be DAAs along with IFN plus RBV, or a combination of DAAs without IFN/RBV. Therefore, we are now at the starting point of an era of DAAs for treatment of HCV infection.

The efficacy of PEG-IFN plus RBV combination therapy has been examined extensively. However, the sustained viral response (SVR: serum HCV RNA negative at 6 months after treatment) rate in chronic HCV G1 infection is 50% or less. Surprisingly, the SVR rate of triple therapy in prior non-responders is about 40% and may be up to 70% in relapsers, and may be higher than 70% after introducing second-generation, macrocyclic PIs [4]. Within the next few years, combinations of different DAAs (PI with NS5A inhibitor and/or NS5B inhibitor) excluding PEG-IFN and/or RBV will be widely applied to the anti-viral therapy for all HCV genotypes.

The response to PEG-IFN plus RBV therapy is affected by HCV genotype, serum viral load and amino acid substitutions in a particular region of HCV. In 2009, genetic polymorphisms near the IL28B gene were determined as a tremendously potent host factor affecting the outcome of PEG-IFN plus RBV therapy for HCV G1 patients [5,6] and even after the discovery of IL28B, additional viral factors may be independent factors influencing the response to PEG-IFN plus RBV therapy.

In the era of PEG-IFN plus RBV therapy, the issue of emerging resistant virus was not generally taken into consideration [7]. However, this issue will be the focus of much attention in the early stages of the

DAA era [8,9]. The rapid replication rate of HCV, along with the low fidelity of RNA polymerase, may result in the natural generation of resistant mutants at baseline (before usage of DAA). Resistance mutations may then be selected for and connected to unfavorable outcomes of DAA-based antiviral therapy [10]. In addition, emergence of cross-resistant mutants will result in the failure of newly introduced DAA-based therapies. Detection of HCV genotypes/subtypes will remain indispensable in the DAA era, because the sensitivity to DAA and the extent of genetic barriers in the generation of resistant mutants may differ in various genotypes/subtypes.

In this review, viral factors affecting the response to IFN monotherapy and/or PEG-IFN plus RBV combination therapy are summarized. In addition, viral factors that may be related to DAA-based anti-viral therapy are discussed.

HCV Genotype

Nucleotide sequence data for HCV isolates obtained in several studies have been deposited in the EMBL/DDBJ/ GenBank databases, and a phylogenetic tree was constructed by the neighbor-joining method based on the partial nucleotide sequence of the NS5B region (1,087 nt) of HCV [11]. HCV has been classified into at least six major genotypes (G1-G6) and within each genotype, closely related variants are grouped into numerous subtypes (1a, 1b, 2a, 2b, etc.) [12]. HCV genotypes 1a, 1b, 2a, 2b and 3a are distributed globally. Other genotypes have geographical restrictions; HCV G4 is more common in the Middle East and Egypt than in other areas of the world, G5a is common in South Africa, and G6 is reported in Southeast Asia [13-15]. In Japan, approximately 70% of patients with HCV have G1 (almost

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exclusively subtype 1b), and 30% have G2 (subtype 2a or 2b) [16], with very small number of patients infected with other genotypes.

HCV genotype has been determined based on the sequence of the 5'-non-coding region (5'NC) [17] or core region [16] using the polymerase chain reaction (PCR) technique. Genotyping is highly sensitive and specific, but occasionally, genotype cannot be distinguished because of amplification failure. The cost of genotyping is expensive, and requires proper handling and storage of samples. Therefore, genotype-specific synthetic oligopeptides derived from the NS4 region have often been used in enzyme-linked immunosorbent assay (ELISA) to detect the presence of type-specific antibodies in the sera of infected patients [18]. Such "serological typing" is considered to have comparable specificity to genotyping [19].

HCV genotype is the most significant predictor of the response to IFN- α and RBV combination therapy [20]. SVR rate is lower in patients infected with HCV G1 or G4 than in those infected with HCV G2 or G3 [21], being 42-52% and 81-84% [22,23], respectively, for HCV G1 (48 weeks of therapy) and G2 (24 weeks of therapy). In addition, determination of HCV genotype/subtype is considered to be important even after the introduction of DAAs because the extent of genetic barrier and sensitivity to DAAs is thought to vary with genotype.

Viral Load

Pre-treatment serum HCV RNA levels have been implicated as an important predictor of the response to IFN mono-therapy and combination therapy with IFN- α and RBV [24]. "High viral load" has been defined as ≥ 5 log IU/ml in Japan, and the rate of SVR has been determined to be lowest among HCV G1 patients with "high viral load" [25]. However, the significance of viral load is diminishing with the progress of response-guided therapy [26], in which the optimal duration of PEG-IFN plus RBV combination therapy is set based on the virological response to therapy to achieve higher SVR rates.

Real-time reverse transcriptase PCR (RT-PCR)-based assays that offer amplification over a broad dynamic range have been introduced in routine diagnostics, showing detection limits of ≤ 10 IU/ml and linear quantification up to 10^7 to 10^8 IU/ml without the need for pre-dilution. Therefore, this method is suitable for monitoring virological response to PEG-IFN and RBV combination therapy. The extent of reductions in HCV RNA during the initial treatment phase is closely associated with the achievement of SVR [27]. Thus, evaluation of rapid virological response (RVR; reduction of HCV RNA at 4 weeks of therapy) or early virological response (EVR; reduction of HCV RNA at 12 weeks of therapy) has become more important than pretreatment HCV RNA levels [28].

Amino Acid Substitutions in NS5A and Response to IFN-Based Therapies

Interferon susceptibility-determining region (ISDR)

In 1996, Enomoto et al. [29] reported that the number of substitutions in the region spanning amino acid (aa) residues 2209-2248 (237-276 of NS5A), designated the interferon sensitivity-determining region (ISDR) of HCV G1b, as compared with the HCV-J sequence, was a predictor of response to IFN therapy. The number of aa substitutions in ISDR was determined by direct sequencing and was clarified as follows: wild type, sequence is identical to that of HCV-J; intermediate type, 1 to 3 changes from HCV-J; mutant type, 4 to 11 changes from HCV-J. They concluded that mutant type is significantly associated with SVR. The number of aa substitutions in ISDR is an

independent factor associated with the outcome of IFN therapy. As the NS5A protein was thought to suppress IFN-induced protein kinase, PKR, a primary mediator of the IFN-induced antiviral response [30], the significance of NS5A in IFN resistance was widely recognized.

Numerous papers have been published on the significance of ISDR as a predictor of outcome in PEG-IFN plus RBV combination therapy against HCV G1b, even after the discovery of a potent host genetic factor (genetic variation near the IL28B gene) [31,32]. However, the results vary among different geographic regions, probably due to differences in HCV G1b subtype [33]. On meta-analysis focusing on geographical differences, the number of substitutions differed between Japan and Europe, and the likelihood of SVR with each additional mutation within the ISDR was more marked in Japanese. SVR rate with mutant type was only about half in European patients vs. 96% in Japanese [34]. The number of aa mutations was inversely correlated with viral load and hyper variable region-1 (HVR-1) complexity that could be associated with response to IFN-based therapy [35].

The sequence of ISDR was further examined in HCV G1a, G2 and G3a [36]. Mutation number in ISDR had no significant effect on response to IFN-based therapy in G1a [37,38] and G3a [36]. However, mutations in the region corresponding to the ISDR of G1b (2126-2228) was reported to be associated with outcome of therapy in HCV G2a, and more than two mutations in ISDR were associated with good response to PEG-IFN plus RBV [39]. Although there was a report on a particular aa substitutions in ISDR in relation to the response to IFN [40], data were unclear.

Interferon ribavirin resistance-determining region (IRRDR) and HS5A region

As NS5A is a key protein in IFN resistance, extended aa sequences have been examined in association with response to IFN-based therapies. The extended ISDR, including PKRBD (2209-2274) and V3 (2356-2379), has therefore been tested. In 2007, IFN RBV resistance-determining region (IRRDR) (2334-2376) was identified as a factor involved in response to PEG-IFN plus RBV therapy for HCV G1b [41]. A high degree (≥ 6) of sequence variation in IRRDR was thought to be a useful marker for predicting SVR, whereas a less diverse (≤ 5) IRRDR sequence predicted non-SVR. In addition, the significance of part of the ISDR plus its carboxy-flanking region (2232-2262) and IRRDR on therapeutic outcome of G2a along with G2b patients has been reported [42].

Sequence variations in IRRDR may be correlated with those in ISDR and may be related to core aa 91 mutations, while they may be inversely correlated with serum viral load. Another group suggested that IRRDR was unlikely to be an independent factor affecting the outcome of PEG-IFN plus RBV therapy [43]. Substitution of aa 2356 in HCV G1b and 2368 in HCV G1a were noted as markers of prognosis in IFN-based therapy on meta-analysis [44]. In contrast, aa substitution at particular positions within IRRDR was reported not to affect the outcome of PEG-IFN plus RBV therapy [41]. Therefore, the significance of IRRDR is uncertain. More information is needed to confirm the significance of this region.

Substitutions in regions other than NS5A in association with response to IFN-based therapies

E2 region: As the double-stranded RNA-dependent protein kinase (PKR)-phosphorylation homology domain (PePHD) within the E2 protein may inhibit the function of IFN-induced antiviral effector

PKR, mutation of the E2 region including the sequence of the PKR/eIF-2 α phosphorylation homology domain (E2-PePHD) (659-670) was examined and compared based on response to IFN-based therapy. However, this region was highly conserved and was not related to SVR in HCV G1 [45].

Substitution of core aa70/91: In 2006, Akuta et al. [46] first reported that substitution of R70Q/H and L91M was related to non-responsiveness of IFN plus RBV therapy in Japanese HCV G1b patients. The significance of core 70 substitution has since been confirmed in large-scale studies [47,48]. No substitution at core 70 (70R) was found to be an independent predictive factor for SVR in a large-scale Japanese study into HCV G1b, treated by PEG-IFN plus RBV [49]. In addition, a Spanish group confirmed that the absence of both 70R and 91L in the core region was significantly associated with treatment failure in G1b patients [50]. These findings suggested that core 70/91, particularly substitution at 70, is a key factor for predicting therapeutic efficacy in HCV G1b, regardless of geographic differences and irrespective of genetic variations in IL28B.

More importantly, substitution at aa 70/91 may affect the efficacy of triple therapy of terapeutic, PEG-IFN and RBV. According to the study of Akuta et al. [51] genetic variations near the IL28B gene and aa substitutions in the core region were identified as predictors of viral dynamics during triple therapy.

Direct acting antivirals (DAAs) and resistance mutations

The era of DAAs for treatment of HCV infection is now beginning. Recent advances in HCV cell culture systems, recognition of the HCV life cycle with progress in molecular biology have led to develop numerous novel drugs against specific viral proteins critical for the HCV life cycle. These drugs are termed DAAs. Among these DAAs, which target NS3/4A serine protease, NS5A protein and NS5B RNA polymerase will be possible targets in the near future [4]. Among these, usage of first-generation NS3/4A PI, terapeutic or boceprevir, as a triple therapy along with IFN and RBV has been approved in Asia and Western countries.

The significance of viral factors focusing on resistance to IFN-based therapies, as described previously, will gradually fade away in the future. However, these factors will remain important until IFN and RBV are excluded from standard HCV regimens. In the future, combinations of different DAAs without IFN and/or RBV will become the main focus of anti-viral therapy. Therefore, the importance of resistance mutations against DAAs will be of greater importance than factors against IFN-based therapy.

HCV is a highly reproductive virus with 10^{10} - 10^{12} new virions being produced daily [52]. In addition, due to the relatively low fidelity of HCV RNA-dependent RNA polymerase and the lack of an exonucleolytic proofreading mechanism, numerous variants are continuously produced during reproduction. Concomitance of various, but closely related mutants (quasi-species), is a characteristic of chronic HCV infection, and these include variants with altered conformation of the DAA binding site. Therefore, DAA-resistant variants are constantly being generated, even before the widespread use of DAA. Drug-resistant mutants usually have very low frequencies due to their lower replication competence than the corresponding wild-type viruses. However, fitness advantages to pre-existing drug-resistant variants during DAA therapy favor the selection of resistant mutants in major populations, thereby contributing to treatment failure. In

addition, compensatory mutations selected in the same genome during DAA-based therapy may participate in the acquisition of comparable reproduction efficiency as the wild-type virus.

Therefore, aimless continuation of DAAs will induce development of “monster viruses” that may form the major population of HCV, even after discontinuation of therapy, showing efficient reproduction and resistance to DAA. In addition, usage of DAAs as mono-therapy should be avoided, as the risk of emerging cross-resistant mutants with other DAAs may develop.

NS3/4A PIs

NS3/4A PIs are designed to inhibit the serine protease structure of NS3/4A. For first-generation PIs (linear type), five aa positions (36, 54, 155, 156 and 170) have been reported to participate drug resistance [8]. After introduction of first-generation PIs (terapeutic or boceprevir) as mono-therapy, resistant mutants appeared very quickly (within 15 days). Therefore, combination with IFN and RBV is a standard regimen to suppress the proliferation of resistant mutants. The role of IFN and RBV in triple therapy is therefore somewhat important. Triple therapy is considered to be inapplicable in patients who did not respond at all to prior IFN and RBV combination therapy (complete non-responder; less than 2 log drop in serum HCV RNA during treatment). In patients who have HCV core 70/91 mutations, triple therapy should be carefully introduced. Genetic barriers are considered to be higher in G1b than in G1a [8,53]. Therefore, determination of HCV G1 subtype is essential for predicting the emergence of resistant mutants.

Evaluation of the pre-treatment existence of resistant viruses may provide useful information. However, as the population of resistant mutants is usually very low (1% or less), highly sensitive methods (ultra-deep sequencing, etc.) are required to identify pre-existing resistant mutants [54].

During triple therapy, the quantity of serum HCV RNA must be monitored serially, and if virological relapse or persistence of HCV is recognized, triple therapy should be discontinued as soon as possible. This may be the best way to prevent further development of “monster viruses” [55].

Second-generation (macrocytic type) PIs (TMC435 [56], MK7009 [57], etc.) will be soon applied to HCV G1 patients. Three major aa positions (155, 156 and 168) have been reported to be involved in drug resistance [8]. R155K/T/Q is considered to be a major cross-resistance site in first-generation PIs. Second-generation PIs are superior to first-generation PIs due to the reduced onset of severe adverse effects.

NS5A inhibitors

Five positional mutations in NS5A (28, 30, 31, 58 and 93) have been reported *in vitro* and *in vivo* to be associated with different levels of resistance. Many NS5A inhibitors are now undergoing clinical trials. A trial of combination therapy with NS5A inhibitor (BMS-790052) and NS3/4A PI (BMS-650032) in Japanese HCV G1b patients with prior null response to PEG-IFN plus RBV therapy revealed extremely high SVR rates [58]. In contrast, the efficacy of this combination is discouraged in American HCV G1a patients that were previously null responders. The SVR rate of this combination treatment in naïve patients is similar to that of triple therapy (terapeutic, PEG-IFN and RBV). Therefore, this combination may be useful for HCV G1b patients who cannot receive IFN or RBV. Detection of HCV G1 subtype may therefore be critical for introduction of this combination therapy.

NS5B polymerase inhibitors

NS5B polymerase inhibitors are divided into two groups (non-nucleoside type and nucleoside type). The anti-viral potency of the non-nucleoside type is markedly lower when compared to that of PI, and efficacy may be HCV genotype specific [59]. Because of the lower potency of antiviral effects with high risk of developing resistant mutants, non-nucleoside-type DAAs may not be promising.

Instead, clinical trials of nucleoside-type DAAs are now underway, in combination with other types of DAA, in combination with RBV without IFN or in combination with PEG-IFN plus RBV. Emergence of resistant mutants is not a practical issue. PSI-7977 (nucleoside type NS5B polymerase inhibitor) is reported to be promising for chronic HCV G2 and G3 infection without usage of IFN [60].

Conclusion

Viral factors participating in the response to IFN-based therapy were summarized. These factors may be still important in the early stages of DAA (first generation NS3/4A PI)-based therapy (triple therapy with PEG-IFN and RBV), because the efficacy of this type of therapy depends largely on susceptibility to PEG-IFN and RBV. With the addition of most PI agents, drug resistance mutations are selected for, but these resistance mutations are typically associated with reduced replication fitness and retained sensitivity to PEG-IFN/RBV.

However, if viral breakthrough is confirmed during therapy, the application of early stopping rules may reduce the enrichment of drug-resistant viruses, probably allowing the chance to respond to second-generation PIs, which will be approved in the near future.

In the next stage of DAA therapy, most chronic HCV infection will be treated by combinations of different class DAAs without PEG-IFN and RBV. Therefore, the issue of drug resistance mutations will be the subject of much discussion.

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