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Gene silencing approaches for the treatment of hepatitis C virus infection

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Abstract

Hepatitis C virus (HCV) infection is a worldwide concern. Only minor portion of the infected patients can clear the virus whereas the majority (more than 70%) progress to advanced stages of liver deterioration which in most cases lead to liver cirrhosis and cancers if remained untreated. Current standard treatment for hepatitis C is the combination treatment with polyethylene glycol pegylated interferon-α and ribavirin (PEG-IFN-α/RBV). Conventional treatment for hepatitis is reported to show variable degree of success and, most recently, the combination of two or more agents has shown great promise. Conventional therapy for HCV, however, is time consuming and expensive and could considerably reduce the quality of life for most of the patients. Gene therapy is an emerging branch of science aimed at the manipulation of genetic sequences. Gene therapy has been recently introduced to virology as a novel anti-viral class of medicines. The innovative idea is to target the HCV viral genome by clipping or blocking to arrest the viral replication. This can be achieved by gene silencing techniques such as siRNA. This will cause viral degradation by hindering the viral genome functionality pre- or post-translational to offer a specific and promising tool which has not been investigated thoroughly. Results from using siRNA *in vitro* are promising, however, this area of research is still evolving.

Key words
siRNA, RNAi, HCV, gene silencing

1. Genetic variations and selective treatments

Hepatitis C is a health issue worldwide [1]. WHO estimated worldwide prevalence of Hepatitis C virus (HCV) infection at about 3% in 1999 [2]. There are some promising results in recent years that the prevalence of HCV infections is decreasing, however due to high intravenous (IV) drug abuse by drug-dependent [3], there is a greater risk of increased transmission of blood-borne infections including HCV infections.

Current standard treatment of hepatitis C virus (HCV) involves the combination treatment with pegylated interferon-α (PEG INF- α) and Ribavirin (RBV). Treatment duration varies with the genotype of the virus [4]. The duration is particularly important as lengthy treatment periods negatively affect the quality of life in affected patients [5-7]. It is, therefore, critical to understand the reasons for the variability in the treatment so that patients could be possibly classified into potential responders and potential non-responders. Such a pre-set classification of patients would be handy to choose appropriate patients who are more likely to be beneficial for the therapy and to avoid unnecessary treatment expenses for non-responders who may need a different treatment approach. Recently, research findings in developed countries, where hepatitis is still a major challenge, has identified single nucleotide polymorphism (SNP's) in genes related to interleukin 28 B (IL28B), patients with certain genotype reported to respond better for the standard hepatitis C treatment compared to other genotype [8, 9]. These findings were extracted from the genome-wide association studies (GWAS) from the varies parts of the world including one performed in Australian cohort by Suppiah et al. (2009) Few in-vitro studies also pointed towards the role of Human leukocyte antigen (10] in the treatment of hepatitis C [11]. HCV genotype 1 and 4 are among the hard to treat infections and need longer duration of treatment, moreover treatment success known as "sustained viral response" (SVR) with these genotypes of virus is poor and ranges between 40-50%. Of note, HCV genotype 4 infections are most prevalent followed by HCV genotype 1. Recent studies have noted that viral factors, particularly genotype and viral load, as well as young age, female gender, lower stage of hepatic fibrosis and insulin resistance are predictors for achieving a SVR to therapy, there has until recently, been a paucity of data on heritable factors that modulate disease outcomes such as treatment response. In the past year recent studies pointed towards the role of genetic make-up of an individual to affect HCV treatment [8, 9]. Genetic effects can influence the clinical expression of chronic viral diseases such as HIV. Until recently, sparse genome screening data were available for Hepatitis C virus (HCV) infection. Several groups have attempted to study genetic effects on disease progression and treatment response by the candidate gene approach, which is now being replaced by genome wide association studies (GWAS) [8, 9]. Suppiah et al, (2009) published the results of a two-stage whole-genome screen, which has been independently replicated by three international groups [9]. All the four studies identified variants that lie in or near the IL28B gene to be associated with viral clearance during treatment in genotype-1 infected Caucasian, Asian and Afro American patients [9]. These variants are associated with an at least 2-fold increased likelihood of SVR [9]. They explain a large part of the variability observed in treatment response rates related to genetic make-up of an individual as of date. However, most of these studies where performed in developed countries where HCV Genotype 1 is more prevalent and such data is lacking for the genotype 4 patients.

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2. Pharmacological agents versus gene-based technologies

Polyethylene glycol pegylated interferon-α and ribavirin (PEG-IFN-α/RBV) have been used with great success [4]. Standard treatment for hepatitis is reported to show high degree of variability in terms of efficacy (ranges from 40-70% of treatment success) and is dependent on numerous host and virus related factors [10, 12]. These antiviral drugs are relatively safe, but side effects are also reported. Importantly, the risk of liver cancer was shown to be reduced in patients taking direct acting antiviral drugs [13, 14]. Current treatment for HCV genotype 1 and 4 comprises of combination treatment with 180 μg of PEG IFN-alpha-2a once weekly or with 1.5 μg/kg of PEG IFN-alpha-2b once weekly and 1000 mg to 1200 mg twice daily of ribavirin. The dose of ribavirin is patient weight dependent, higher dose is given for patients who weigh more than 75 kg. Duration of the treatment is 48 weeks subject to the achievement of considerable efficacy at each stage of the treatment [4]. Standard HCV treatment is not easily tolerable and, in some patients, reduces the quality of life to greater extend [5]. However, gene therapy options, defined as is the experimental use of genetic manipulation techniques to correct errors associated with genetic diseases or to modify undesirable deoxyribonucleic acid sequences, can provide an additional tool for combatting the disease. Targeting HCV viral genome can offer a more effective and specific option to eradicate HCV infections. Using a specific silencing RNA (siRNA) which can be considered as a new class of antiviral drugs based on the specific silencing of genetic sequences in the HCV virus.

Recently newer HCV treatment options has emerged with promising improvement in terms of efficacy which would be available for patients use in near future [15]. However, interferon alpha (IFN-alpha) remains to be the mainstream treatment for HCV [15]. IFN-alpha is an indigenous human body compound and hence it is not irrational to assume that host related factors would determine its efficacy. Host related factors such as age, gender, liver disease state, insulin resistance are well known to determine the HCV treatment outcome with IFN-alpha [16]. Most recent studies have explored the role of genetic makeup of an individual to play a significant role in the treatment outcome with IFN-alpha [8, 17]. Genome-wide association studies (GWAS) performed independently in different parts of the globe has pointed specifically the role of IL28B polymorphism in HCV treatment variability which is reported to account for about 15% variability in treatment outcome in HCV genotype 1 infection [8, 9, 18-23]. All of these studies consistently found a link with the favorable treatment outcome and SNPs in the IL28 gene. And Identified a gene-rs8099917, non- responders were twice likely to possess this gene compared to responders whereas patients who cleared virus without treatment were reported to be least likely to possess this so called “risk gene” [9]. It was known earlier that people of African descent were less likely to respond to standard HCV treatment than Europeans and Asians being more likely to respond to the same treatment. Consequently, research findings suggest that rs8099917 gene is more prevalent in African patients compared to Europeans and Asians [23]. However, treatment of HCV infections can be achieved by state-of-the-art gene therapy agents. Gene therapy is an emerging field of study which has been rarely used in virology.

3 Gene silencing technologies and HCV

To target a specific sequence for using RNA interference (RNAi) technology, a double strand RNAs also called silencing RNA (siRNA) can be used. Previously, siRNA has been used for silencing of specific genes specially in in vitro research using cell lines. However, the repurposing of this technology in targeting the genome of microorganisms has been suggested as a novel class of antiviral drugs. This attractive and promising approach will allow for the use of siRNA therapeutically to target genes in the virus. The advantage here is that siRNA offers a sensitive and highly specific way to achieve the antiviral effect. A range of HCV core and structural proteins and proteins involved in viral pathogenesis can be targeted. The knockdown off the C-terminal and N-terminal of the HCV-3a core protein using siRNA has been reported to inhibit viral growth effectively [24-26]. Interestingly, the strategy of using multiple siRNA sequences against several targets can have higher efficiency and prevent viral resistance [27-29]. Moreover, this approach was successful in eliminating the development of resistance [30]. Additionally, the combination conventional therapy and RNAi also showed some promise, where the treatment of RNAi combined with IFN-α was shown to be effective [31]. An additional aspect holding a promise for this approach, is that RNAi can possibly transmits from one hepatic cell to adjacent cells, which further extends the antiviral activity of siRNA sequences. Extending the RNAi targets to specific cellular cofactors involved in HCV replication was also attempted with success [32].

4. Conclusions

Small interfering RNA (siRNA) are promising tools for the gene expression inhibition of viral genome, proposing a valuable option for HCV treatment. The use of multiple RNAi sequences directly targeting HCV were shown to have good efficiency and low toxicity. Using siRNA against HCV genome can propose a more effective and specific solution to combat HCV infections. Gene silencing techniques, including siRNA can represent a new class of antiviral agents and are promising solutions for drug discovery.

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Conflict of interests:

The authors declare no conflicts of interests.

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