Virology & Mycology

ISSN: 2161-0517

Abhishek and Gadepalli, Virol Mycol 2018, 7:1
DOI: 10.4172/2161-0517.1000175

Letter to the editor Open Access

Current Challenges in Hepatitis C Virus

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Received date: Jan 25, 2018; **Accepted date:** 12 Feb, 2018; **Published date:** 19 Feb, 2018

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Abstract

HCV is an enveloped, positive-strand RNA virus and belongs to the genus *Hepacivirus* within the family *Flaviviridae*. Hepatitis C virus is characterized by a high genomic diversity resulting from the high mutation rate . The genome of this virus is approximately 9.6 Kb long and encodes a single poly-protein. The encoded poly-protein is co- and post-translationally processed to produce a total of 10 viral proteins by both viral and host proteases (N terminus-Core-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-C terminus). The structural proteins at the N terminus (Core, E1 and E2) are directly involved in the formation of new virions while the rest are non-structural protein involved in various aspects of HCV life cycle. P7 protein is known as an ion channel protein (Viroporin) and NS2 protein is a cysteine autoproteases catalysing the cleavage between NS2 and NS3 protein. Both p^7 and NS2 proteins are also known to involve in virus assembly. NS3 to NS5B (NS3-NS4A-NS4B-NS5A-NS5B) are indispensable for viral RNA replication as the major component of the replication complex. NS3 protein has both serine protease and helicase activity. Protease activity cleaves the viral protein from NS3 to NS5B while helicase activity plays an important role in viral assembly. NS4A protein is co-factor of NS3 protein. NS4B provides the platform for the viral RNA replication to occur by generating membranous web structures near the ER membrane of the host cell. NS5A protein does not have any known enzymatic activity but is important for both RNA replication and assembly. Finally NS5B protein is an RNA dependent RNA polymerase synthesizing plus- and minus-strand of viral RNA. Amongst these NS3, NS5A and NS5B proteins are the major targets of currently available Direct-acting Antiviral agents (DAAs).

Treatment and Resistance

In late 1990s, the addition of Ribavirin (nucleotide analog) increased the Sustained viral response (SVR) by 20% as compared to the Interferon monotherapy [1-4], later pegylation of interferon (pegINF) increased the SVR further by 10-15% [5,6]. Currently administration of pegINFα and Ribavirin for 6-12 months before starting DAAs have increased the SVR over 90% compared to 15-20% by INFα monotherapy.

As already mentioned NS3 protein has two enzymatic activities: Serine protease and Helicase. Most DAAs targeting this protein acts as protease inhibitors. Boceprevir and Telaprevir were first approved DAAs for clinical use in 2011 followed by Simeprevir, Asunaprevir and Paritaprevir. Several mutations are frequently observed at V36, V55, R155 and D168 of NS3 protein associated with antiviral drug resistance (Table 1).

NS5A protein with no known enzymatic activity but is important for the viral replication and assembly. Resistance against the first DAAs against NS5A protein (Daclatasvir) was observed due to mutations at Y93H and L31V.

NS3 Inhibitors	Genotype 1a	Genotype 1b
Boceprevir	V36M, T54S, R155K	T54A/S, V55A, A156S, V170A
Telaprevir	V36M, R155K	V36A, T54A, A156S
Simeprevir	R155K, D168E/V	Q80R, D168E/V
Asunaprevir	R155K, D168E	D168E/V/Y
Paritaprevir	D168A/V/Y	Y56H, D168V
NS5A Inhibitors		
Daclatasvir	M28T, Q30E/H/R, L31M, H58D, Y93H/N	L31M/V, Y93H
Ledipasvir	Q30E/R, L31M, Y93C/H/N	Y93H
Ombitasvir	M28T, Q30R	Y93H
NS5B Inhibitors		
Dasabuvir	M414T, S556G	S556G
Baclabuvir	A21V, P495L/S	None

Table 1: Most commonly observed resistance mutations [7].

NS5B protein which directly catalysing the synthesis of plus- and minus-strands of viral RNA through its RNA dependent RNA polymerase activity is highly error prone enzyme due to lack of proofreading capability. Thus the virus likely exists as quasispecies, or a group of genomes forming a structured ‘cloud’ in sequence space with replication near the maximum error rate allowed before genomic integrity is lost [8,9] NS5B inhibitors are classified into either nucleotide analog (Sofosbuvir) or non-nucleoside analog (Dasabuvir and Beclabuvir). Sofosbuvir became the first choice of DAA for several all oral regimens for treatment of chronic hepatitis C as it showed excellent SVRs in numerous clinical trials with high genetic barrier to resistance development unlike other DAAs against NS3 and NS5A. Commonly developed resistance mutations observed in NS5B protein are summarized in Table 1, but interestingly due to different

mechanism and sites of interaction with NS5B protein no cross resistance was observed between nucleotide and non-nucleoside inhibitors.

Resistance Testing and Challenges

Unlike satisfactory functioning for HIV screening and treatment Global networks, lack of such network for active, passive and sentinel surveillance and screening for HCV have made estimation of prevalence and also people with antiviral drug resistance HCV infection a challenging task. Moreover unavailability of routine resistance testing have further raised the problem. Antiviral susceptibility can be determined by Phenotypic and Genotypic methods. Phenotypic testing is based on the calculation of inhibitory viral growth concentration for the antiviral drug. The main advantage of phenotypic testing is that it provides genotypic and phenotypic correlation of the associated mutational changes. The major limitations of phenotypic testing are time consumption, expensive, and less sensitive in order to detect minority variants or archived resistance mutations.

On the other hand genotypic testing is based on PCR amplification and sequencing of viral drug target genes associated with mutations is much faster. Most of the currently available diagnostic genotypic resistance tests uses Sanger Sequencing method and if another quasispecies of virus is present at more than 25% of the total then majority or consensus sequence is displayed with some secondary traces. Unlike this Next generation sequencing (NGS) Technology uses PCR-amplified single-molecule sequencing, means all quasispices are individually sequenced and displayed which further enables to pick up minority variants even as low as 1% of the total. Whole genome sequencing (WGS) is sequencing of nearly full length viral genome using NGS technology with advantage of providing additional data other than resistance gene sequencing [10]. The main limitation is that NGS is currently used in research settings and its validation for routine diagnostic use is still in process. Secondly, the unprecedented resolution of the technology can be hampered by Reverse transcriptase and PCR errors, as well as sequence related errors which obscure the presence of true low-frequency minority variants [11].

Conclusion

Although progresses in the development of DAAs and combination therapies has increased the SVR and now more than 90% of the chronic hepatitis C patients are expected to be cured but due to lack of proper network for screening of HCV and also limited availability of resistance diagnostic tools and antiviral drugs is still challenging task. Moreover rising antiviral drug resistance are being reported and treating those patients who have failed in prior antiviral treatment have to be addressed. A deep understanding of the viral replication biology is needed for the development of newer classes of antiviral agents targeting different viral protein.

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