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Review

Understanding the molecular mechanism(s) of hepatitis C virus (HCV) induced interferon resistance

Hanadi Qashqari [a], Amany Al-Mars [a], Adeel Chaudhary [a,b], Adel Abuzenadah [b,c], Ghazi Damanhouri [a], Mohammed Alqahtani [b], Maged Mahmoud [a], Maysaa El Sayed Zaki [d], Kaneez Fatima [e], Ishtiaq Qadri [a,*]

[a] King Fahd Medical Research Center, King Abdulaziz University, P.O. Box 80216, Jeddah 21589, Saudi Arabia
[b] Center of Excellence in Genomic Medicine, King Abdulaziz University, P.O. Box 80216, Jeddah 21589, Saudi Arabia
[c] Faculty of Applied Medical Sciences, King Abdulaziz University, P.O. Box 80216, Jeddah 21589, Saudi Arabia
[d] Faculty of Medicine, Mansoura University, Egypt
[e] IQ Institute of Infection and Immunity, Lahore, Pakistan

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ABSTRACT

Hepatitis C virus (HCV) is one of the foremost causes of chronic liver disease affecting over 300 million globally. HCV contains a positive-stranded RNA of ~9600 nt and is surrounded by the 5′ and 3′untranslated regions (UTR). The only successful treatment regimen includes interferon (IFN) and ribavirin. Like many other viruses, HCV has also evolved various mechanisms to circumvent the IFN response by blocking (1) downstream signaling actions via STAT1, STAT2, IRF9 and JAK-STAT pathways and (2) repertoire of IFN Stimulatory Genes (ISGs). Several studies have identified complex host demographic and genetic factors as well as viral genetic heterogeneity associated with outcomes of IFN therapy. The genetic predispositions of over 2000 ISGS may render the patients to become resistant, thus identification of such parameters within a subset of population are necessary for management corollary. The ability of various HCV genotypes to diminish IFN antiviral responses plays critical role in the establishment of chronic infection at the acute stage of infection, thus highlighting importance of the resistance in HCV treated groups. The recently defined role of viral protein such as C, E2, NS3/NS4 and NS5A proteins in inducing the IFN resistance are discussed in this article. How the viral and host genetic composition and epistatic connectivity among polymorphic genomic sites synchronizes the evolutionary IFN resistance trend remains under investigation. However, these signals may have the potential to be employed for accurate prediction of therapeutic outcomes. In this review article, we accentuate the significance of host and viral components in IFN resistance with the aim to determine the successful outcome in patients.

Contents

Abbreviations: HCV, hepatitis C virus; UTR, untranslated regions; IFN, interferon; ISG, interferon stimulatory genes; IRES, internal ribosome entry site; IFNAR, IFN-α receptor; STAT, signal transducer and activator of transcription; TLR3, toll like receptor 3; DsRNA, double stranded RNA viruses; IRF3, interferon regulatory factor 3; NF-κb, nuclear factor kappa-light-chain-enhancer of activated B cells; IL, interleukin; REST, repressor element-1 silencing transcription factor; RE-1, repressor element-1; ISRE, IFN-stimulated response elements; GAS, gamma interferon activation site; GT, genotype; SOCS, suppressor of cytokine signaling; Th, T-helper; CD, cluster of differentiation; SVR, sustained virological response; PI, protease inhibitor; BOC, boceprevir; TVR, telaprevir; IR, insulin resistance; ISDR, interferon sensitivity determining region; IRRDR, interferon and ribavirin resistance determining region; PKR, protein kinase R; eIF2α, eukaryotic initiation factor 2α; PePHD, phosphorylation homology domain; ER, endoplasmic reticulum; UPR, unfolded protein response; SNP, single nucleotide polymorphisms.

* Corresponding author. Tel.: +966 535168434; fax: +966 26952076.
E-mail address: ishtiaq80262@yahoo.com (I. Qadri).

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1. Introduction

Chronic Liver Disease (CLD) is a foremost clinical burden and hepatitis C virus (HCV) is one of the major causes of liver diseases. HCV infection with the disease severity varies from asymptomatic chronic infection to life threatening cirrhosis and hepatocellular carcinoma (Saito et al., 1990). *HCV* is a *single*-stranded *RNA virus* that encodes a distinct polypeptide through internal ribosome entry site (IRES)-mediated translation. Translation product is processed by a combination of cellular and viral proteases to settle into at least 10 components; structural proteins (Core, E1, E2, and p7) and nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Bartenschlager and Lohmann, 2000; Kato, 2000; Moradpour et al., 2007; Penin et al., 2004). Using a variety of methods, six genotypes of HCV and various subtypes has been identified (Simmonds et al., 2005). Treatment options are limited and resistance associated with treatment is a major problem and in most of the cases the end stage liver disease often requires liver transplantation. Therefore, the existing treatment option with maximum outcome results is urgently required to alleviate the suffering of millions of individuals with chronic hepatitis C.

Interferon alpha (IFN-α) or Interferon gamma (IFN-γ) in combination with other antiviral drugs are routinely used to treat HCV infection (Cooksley, 2004). More than 50 years ago, IFNs were identified and are classified into three major types IFN-I, II and III which bind to specific IFN receptors. Type I bind to a cell surface receptor complex called IFN-α receptor (IFNAR) comprising of IFNAR1 and IFNAR2 chains. Type I is present in humans as IFN-α, IFN-β and IFN-ω. Type II IFN (IFN-γ) interacts to IFNGR that consists of IFNGR1 and IFNGR2 chains. Type III IFN bind to a receptor complex consisting of IL10R2 (CRF2-4) and IFNLR1 (CRF2-12) (De Weerd et al., 2007; Liu, 2005). While all three classes of IFN bind to specific receptors they mediate their actions through common denominator such as STAT1/2. The widespread use of IFNs in viral infected individuals have led to serious resistance problems and understanding these resistance mechanisms is imperative to develop an alternative therapeutic strategy to clear the infection.

2. Induction and activation of interferon

The initiation of IFNs production occurs in response to infection which then activates signal transducer and activator of transcription (STAT) complexes for initiation of the classical Janus kinase-STAT (JAK-STAT) signaling pathway (Platanias, 2005). Furthermore, Toll Like Receptor 3 (TLR3) is an important feature for IFNs induction in response to the occurrence of double-stranded RNA viruses (dsRNA) (Dunlevy et al., 2010). The TLR3 will then activate interferon regulatory factor 3 (IRF3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which bind to response elements IFN type I and III promoters (Heim, 2012). A complex array of cytokines, such as interleukins (IL-1, IL-2, IL-12), TNF are also shown to augment interferon production (Haller et al., 2007).

The binding of IFNs to its receptor induces a cascade of signaling event resulting in the activation of STAT1 and STAT2 which together with IRF-9 form the particular ISGF3 complexes. It is well established that a putative ISRE sequence in the STAT1 promoter is inducible by type I IFN and binds the IFN-α/β-induced complex, ISGF3. Several studies have described distinct promoter, enhancer and repressor regions in the regulatory fragment of hSTAT1 gene extending in the second intron. Intracellular amplification of STAT1 expression is shown to be dispensable for increasing cell responsiveness to the IFNs. How HCVmay repress transcription from the STAT1 gene regulatory region remains to be investigated. Upon activation, the newly formed ISGF3 complexes then translocate to the nucleus where they bind to IFN-stimulated response elements (ISRE) in various IFN stimulated genes (ISGs) such as IRF7, MX1, and OAS1 (Donnelly and Kotenko, 2010) and failure to do so lead into resistance. The response to IFN may depend on various STAT homodimers and/or heterodimers during IFN signaling and these STAT dimers launch transcription by binding to Gamma Interferon Activation Site (GAS) elements in gene promoters. Type I IFN can induce gene activation with both ISRE or GAS elements, while type II IFN dependent activation only recruits GAS element (Platanias, 2005). The interplay of viral proteins with such regulatory elements has not been characterized.

3. HCV genotypes (GT) and the development of IFN resistance

HCV is classified into six genotypes (GT-1 to GT-6) and each GT behaves differently during IFN treatment (Table 1), however, the molecular basis of differential responses to treatment in HCV genotypes is not fully understood. HCV genotypes diverge in their nucleotide sequence by ∼30–35%, and furthermore each genotype has a class of different subtypes that differ in their nucleotide sequence by ∼20–25% (Simmonds, 2004). The heterogeneous populations of HCV genomes that coincide in an infected individual due to replication errors are termed as quasispecies (Heim, 2012), thus making it difficult in the predicament of IFN resistance. Therefore viral genetic diversity and its association with induction of chronic liver diseases and treatment response determination still remains to be completely understood.

Several lines of evidence support the notion that IFN resistance plays a role in the establishment of chronic infection at the acute stage of infection (Meier and Ramadori, 2009). In recent years, the mechanisms of viral RNA recognition and RNA virus-triggered sig-

Table 1
Representative percentage of HCV genotypes associated IFN non responders.

HCV genotype	% IFN non-responders
1	61
2	10
3	20–30
4	40

Jamal et al. (2008); NIH consensus statement (2002); Sharieff et al. (2002).

naling pathways have been well studied, however the major challenges remains with the resistance associated with IFN treatment. HCV escape immune recognition or inhibit the host immune response in order to persist. The host cytokine responses to infection play an important role in the pathogenesis, progression, and IFN treatment outcome. The infection of many viruses including HCV triggers a series of signaling events leading to transcriptional induction of type I IFN and pro-inflammatory cytokines. After prolonged persistent HCV infection IFN response is circumvented in which downstream signaling events prevent further IFN production (Lin et al., 2004). This is primarily initiated by the modulated expression levels of the suppressor of cytokine signaling (SOCS) proteins such as SOCS-3 (Bode et al., 2003), thereby inhibiting cytokine signaling and escape from the immune response (Walsh et al., 2006; Huang et al., 2007). Cytokines, such as IL-10 level in HCV-1 patient serum and a stretch of 70 amino acids within the core region have shown to be correlated with Pegylated interferon (PEG/IFN) ribavirin response coupled with correlation with amino acid substitutions within the ISDR. High level of IL-12, IL-18 are shown to be correlated with patients SVR (Yoneda et al., 2011). Moreover, T-helper 1 and 2 (Th1/Th2) response determine the outcome of antiviral therapy and it has been shown that IL-18 is a critical mediator of Th1/Th2 balance. Finally, cluster of differentiation, CD4 and CD8 positive cellular immune responses can also determine HCV persistence (Pawlotsky, 2003).

Pegylated interferon (PEG-IFN) and ribavirin combination therapy is a standard care for HCV treatment which either determine the sustained virological response (SVR) or IFN resistance (Bowden and Berzsenyi, 2006). The duration of treatment is 48 weeks for GT-1 and 4, 24 weeks for GT-2 and 3. This treatment is 75% effective in patients with HCV GT-2 and GT-3, 60% in HCV GT-4, and 50% in HCV GT-1 (Jamal et al., 2008; NIH consensus Statement 2002; Sharieff et al., 2002) as shown in Table 1. Broad understanding of HCV-mediated ailment progression in each GT of patients and viral adaption is necessary toward discovery of novel therapeutic objectives and the improvement of additional intervention strategy at the levels of IFNresistance.

In the presence of ever rising resistance phenotypes, alternative drug development is on rapid rise. Recently, small molecule compounds have been developed that inhibit the viral life cycle, including inhibitors of the non-structural (NS) 3/4A protease, NS5B polymerase and NS5A protein (Vermehren and Sarrazin, 2012). In many clinical studies, the standard care of patients with HCV GT-1 include combination of PEG-IFN plus ribavirin and protease inhibitor (PI), boceprevir (BOC) or telaprevir (TVR) that specifically targets the HCV NS3/4A protease. The triple therapies are shown to improve response in HCV GT- 1 by 20–30% to cure 80% of the patients (Heim, 2012; Sarrazin et al., 2012). However, selection of IFN resistant HCV variant may have adverse effect on patients as such variants occurring at very low frequencies will accumulate (Manns et al., 2007).

4. The role of host factors in interferon resistance

Several host factors influence IFN response in HCV individuals, including age, gender, body weight, fibrosis, diabetes (obesity), and insulin resistance, shown in Table 2. In recent years, it has been observed with alertness that Insulin resistance (IR) is one prime morbidity factor to predict SVR to PEG-IFN and Ribavirin therapy. It is now widely acknowledged that IR seems to be involved in decreased sensitivity to IFN and could block IFN intracellular signaling. IR promotes severity of liver disease such as steatosis and fibrosis progression and induces the secretion of pro-inflammatory cytokine (Romero-Gómez, 2006). HCV interacts with insulin signaling pathway through different mechanisms mostly through the activation of C/EBP beta, CREB, FOXO1 and rate limiting gluconeogenic enzyme PPECK, all leading to insulin resistance (Qadri et al., 2012). In several clinical studies the morbidity factors for IFN resistance were determined which are outlined in Table 2.

5. The role of HCV viral components to interferon resistance

A number of HCV proteins including Core, E2, NS3, and NS5A have been demonstrated to contribute to the lack of a response to IFN treatment and in most cases the studies were carried out HCV GT-1. (See Fig. 1) These studies are summarized below.

5.1. Core protein

The core component and interferon sensitivity-determining region (ISDR) sequences have shown to exhibit changes during anti-HCV therapy, and affect its response (Kozuka et al., 2012). Some regions associated with sensitivity to IFN-α and ribavirin have been recognized within core region (core a.a. 70), a.a. 2209-2248 (ISDR) and a.a. 2334-2379 (interferon and ribavirin resistance-determining region, IRRDR) (Fukuhara et al., 2009). The amino acids 70 and 91 are a significant predictor of poor responses to PEG/IFN-plus-ribavirin therapy, however the exact molecular mechanisms underlying the responses are not known (Akuta et al., 2012). In a recent study, Funaoka et al. (2011) has shown that

Table 2
Host factors association with interferon response.

Host factor	GT	No. of patients	Results	Author
Obesity	1/4	12	Obese: NR (92%) vs. RES (8%)	Walsh et al. (2006)
	2/3	54	Non-obese: NR (46%) vs. RES (54%)	
		11	Obese: NR (36%) vs. RES (46%)	
		68	Non- obese: NR (19%) vs. RES (81%)	
Insulin resistance (IR)	1	2732	increased HOMA-IR in NR patients with IR have a 20% lower SVR than patients without IR	Deltenre et al. (2011)
Steototis%	3		SVR reduced steatosis. Increased steatosis in HCV patients with phenylalanine at position 164 of core	Kumar et al. (2002); Hourioux et al. (2007)
Fibrosis		309	NR: 42.7% chirosis and 57.3% fibrosis	Morgan et al. (2010)
Genetic polymorphism (IL-28)	1	111	62% of subjects failed to achieve SVR 26% relapsers IL28B CC vs. CT/TT	Mccarthy et al. (2010)

SVR: sustained virological responder; NR: non-responses.

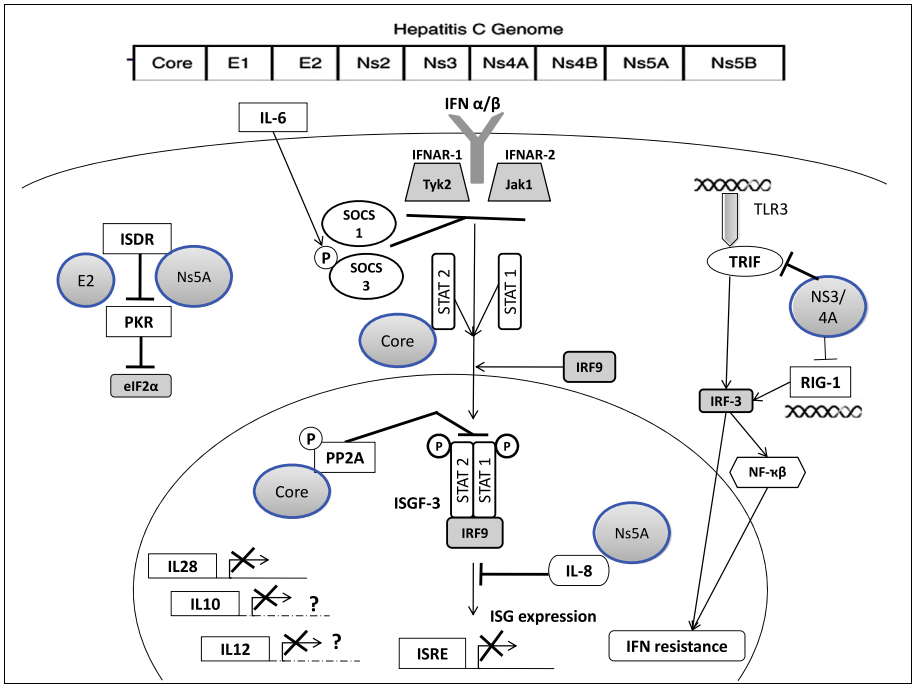

Fig. 1. Schematic depiction of the footsteps by which IFN resistant phenotype is achieved by hepatitis C virus encoded C, E2, NS3/4A NS5A and by various cellular pathways. Abbreviations used in Fig. 1: Protein Phosphatase 2A (PP2A); Viral Nonstructural Protein 5A (NS5A); Viral Core (C); Viral Envelope Protein 2 (E2); Interferon Stimulatory Genes (ISG); Interferon Alpha Receptor (IFNAR); Interferon Responsive Factor (IRF); IFN-Stimulated Response Elements (ISRE); Toll-Like Receptor (TLR); Suppressor of Cell Signaling (SOCS); Interleukin (IL); Interferon Sensitivity Determining Region (ISDR); eukaryotic initiation factor 2 alpha (eI2F-α); TIR-domain-containing adapter-inducing interferon-beta (TRIF); Nuclear factor-kappa B (NFκB); Protein Kinase R (PKR).

HCV R70 and L91 core mutants are resistant to interferon *in vitro*, and the resistance may be driven by IL-6-mediated activation of SOCS3. Previous studies have investigated the response to IFN- α by using HCV cell culture based Core substitutions expression systems. IFN treatment followed by post-transfection with R70Q, R70H, and L91M mutant or infected with the wild type clones showed that the R70Q,R70H, and L91M core mutants have higher levels of resistance than the wild type (Funaoka et al., 2011).

5.2. E2 protein

An intriguing observation was made by Michael Lai and colleagues where resistance to interferon was attributed to a 12 amino acid sequence within E2 containing an identical site of protein kinase R (PKR) phosphorylation, both on itself and on eukaryotic initiation factor 2 α (eIF2α) (Taylor et al., 1999). They found that HCV E2 blocks PKR phosphorylation in cells coupled with PKR's ability to inhibit protein synthesis. The interaction of E2 and PKR may be one mechanism by which HCV circumvents the antiviral effect of interferon (Chayama et al., 2000) and additional mechanism may exist by which a double-stranded RNA-activated protein kinase-eukaryotic initiation factor 2alpha (PKR-eIF2α) phosphorylation homology domain (PePHD) is located within E2 protein. It has been demonstrated that "RGQQ" motif in HCV- GT-2a and 2b PePHD domain showed a close correlation with IFN resistance. It has also been proposed that serine phosphorylation of HCV E2 gene in HCV-GT-1a have a role in interferon resistance (Afzal et al., 2011). It is important to note that E2 protein is the first viral components that interact with the liver cell surface receptors via the external loop of CD81 (Pileri et al., 1998), thus making the vaccine efforts challenging due to the PePHD acquisition site within the E2.

5.3. NS3/4A protein

NS3 protein NS*4A* comprises the viral serine *protease* (*NS3/4A Protease*) and is an essential component for HCV translation, replication and eventual life cycle (Chevaliez and Pawlotsky, 2006). It also cleaves the cellular Trif and Cardiff which are involved in IFN response mediated by TLR3 and NF-κB. NS3/4A mediates the cleavage of this adaptor protein, leading to affect HCV antiviral response (Li et al., 2005). In some studies NS3/4A protease inhibitors indicated a low genetic impediment to resistance and different amino acid substitutions confer resistance to protease inhibitors are shown to pre-exist in HCV patients (Pawlotsky, 2012). Second-generation NS3/4A protease inhibitors, such as MK-5172 or ACH-2684, are projected to require wider genotypic coverage with a higher impediment to IFN resistance (Clark et al., 2013).

5.4. NS5A/B proteins

HCV NS5A/B proteins are involved in viral replication and interact with the interferon and other anti-viral pathways (Le Guillou-Guillemette et al., 2007). HCV NS5A sequence comparison in SVR and IFN resistant scenarios has identified specific domains that exhibit sequence variation associated with IFN therapy (Gale and Foy, 2005). It has been found that the Interferon Sensitivity-Determining Region (ISDR) of HCV-1b NS5A is resistant to IFN-α (Enomoto et al., 1995; 1996). NS5A quasispecies of HCV-1b-infected patients have been studied to determine the relationships between pre- and post-treatment NS5A quasispecies mutations and the IFN-α sensitivity and it was observed that serine residues involved in phosphorylation of NS5A protein were highly conserved. Pawlotsky et al. (1998) have proposed that the association between HCV RNA clearance, low viral load and low nucleotide sequence entropy may play a critical role in the sensitivity of HCV-1b to IFN-α that could be patient specific and located at different positions of the viral genome. This could allow escape variants to be selected by IFN-α-stimulated immune responses.

A recent study has reported that GT-1 NS5A overexpression resulted in less IFN responsiveness than in GT-3 through stronger binding to STAT1 (Kumthip et al., 2012). The binding of C-terminal

region of NS5A to STAT1 leads to decreased P-STAT1 levels, ISRE signaling, ISG transcription and, ultimately, to preferential GT-1 resistance to IFN treatment (Kumthip et al., 2012). The association between ISDR mutations and IFN treatment response is observed by Kobayashi et al. (2002). Vermehren and Sarrazin (2011) have shown that various nucleoside or nucleotide inhibitors of NS5B polymerase possess inconsistent antiviral activity in different HCV genotypes but interestingly with higher impediment to IFN resistance.

6. The role of cellular responses induced interferon resistance

An evolving line of evidence support the hypothesis that HCV infection, replication and/or individual protein expression triggers a wide range of cellular adaptive responses, including reactive oxidative stress, increased hepatic gluconeogenesis, intrahepatic lipid accumulation, cell cycle arrest, apoptosis, mitochondrial stress and endoplasmic reticulum (ER) stress. In addition, several studies suggested that HCV induced cellular responses may contribute to chronicity by superfluous modulating tumor suppressor function, cell proliferation, and various oncogenic pathways (Qadri et al., 2002; Sheikh et al., 2008; Syed et al., 2010).

PKR: During HCV replication the positive and negative stranded RNA are produced (ds-RNAs) which then bind to PKR resulting in its auto-phosphorylation. In the subsequent reaction activated PKR phosphorylates the eukaryotic initiation factor 2 (eIF2α) in response such viral infections. The phosphorylated eIF2α forms an inactive complex with eIF2B resulting in substantial decrease in protein synthesis. It has been shown that HCV GT E2 protein inhibits PKR kinase activity both in vitro and in human cells (De Rueda et al., 2008). The ISDR region NS5A protein with additional 26 amino acids has defined consequence on NS5A/PKR interaction. Other mechanisms that may involve the eventual phosphorylation event of PKR may be affected by the viral induced ROS; MRP2 and fatty acids (Qadri et al., 2004, 2009, 2012). In a recent study, palmitate, a saturated fatty acid, was shown to bind PKR and consequently inhibiting its autophosphorylation at Thr451 and Thr446 (Cho et al., 2011). Palmitate synthesis is induced during HCV sub-genome replication, thus this could offer another mechanism by which IFN resistance is achieved by viral infection. It has been suggested that mutation of Lys296 and Asp432 in the ATP binding site of PKR are important for palmitate binding. The deeper understanding of palmitate+PKR interaction could explain the mechanisms by which palmitate mediates kinase signaling pathways that could have implication on the efficacy of HCV antiviral therapies.

6.1. Single nucleotide polymorphisms (SNPs)

Several studies from across the glove has indicated that host genetic polymorphisms can predict IFN therapy outcome. These independent genome-wide association studies in HCV-infected patients have identified numerous single-nucleotide polymorphisms (SNPs) near IL28B gene (Slev, 2012). (Bellanti et al., 2012) have shown that upstream sequence of IL28B gene that encodes IFN-λ3, predict the outcome of treatment, but their impact on viral kinetics and relation to other predictors remains to be investigated. IFN-λ3 *(IL-28B)* may inhibit HCV replication via the JAK-STAT pathway, leading to expression of ISGs (Zhang et al., 2011). During treatment of 110 patients with HCV genotype 1 infection two SNPs, rs12979860 and rs8099917 were also related to early viral kinetics (Lindh et al., 2011). In one Middle Eastern study, the SNP of rs12979860 were strongly associated with sustained virological response (SVR) in patients infected with HCV GT-4, but not with liver disease severity (Asselah et al., 2012). This pattern of IL28B SNP analysis with specific HCV genotype may be used to guide during the IFN treatment.

7. Transcript gene expression

Interferon(s) induce the production ofinterferon-stimulated genes (ISGs) that have defined roles in eradicating the viruses through the natural course of infection (Fensterl and Sen, 2009; De Veer et al., 2001). ISG expressions have a direct correlation on the mechanism(s) of PEG-IFN and ribavirin responses and this expression profile may explain the variation in the treatment efficiency. HCV infection has been shown to persist for years in spite of the expression of hundreds of ISGs, (Chen et al., 2005; Sarasin-Filipowicz et al., 2008). In contrast, Bellecave et al. (2010) have shown that there is no significant correlation between serums or intrahepatic HCV loads with ISG expression levels. A failure to boost the ISGs in response to HCV infection has led to attempts to use external IFN- α as therapy in cases of chronic HCV infection. In the study by Chen et al. (2005) the expression profiling of pretreatment with chronic HCV infection identified 18 important genes whose expression differed significantly between responders and non-responders. These findings showed that IFN-stimulated genes were highly expressed in non-responders, indicating that IFN preactivation in patients would limit the effect of IFN antiviral therapy however, the linkage between ISGs and SNP is not well established (Asselah et al., 2010).

8. Suppressor of cytokine signaling 1 (SOCS1)

Type I interferon (IFN) signaling is blocked by Suppressor of cytokine signaling 1 (SOCS1) and suppressor of cytokine signaling 3 (SOCS3). In order to inhibit cytokine signaling and to escape the immune response, HCV core protein is known to induce the SOCSs expression, thus circumventing the IFN responsive pathways and rendering the cells not to response to IFN. SOCS3 were significantly higher in HCV infected individuals, particularly in nonresponders to IFN treatment, than in healthy individuals (Shao et al., 2010). With the upregulation of SOCS-3 expression of interferon stimulated genes (ISGs) and protein kinase receptor (PKR) is inhibited through inactivation of the JAK-STAT pathway. Downregulation of PPAR-γ and an upregulation of SOCS-7 were also observed upon expression of the HCV GT 3 core protein (Pazienza et al., 2010). The IFN resistance has been shown to be induced by IL-6-mediated upregulation of SOCS3. These mechanisms may explain some of the aspects of clinical IFN resistance, however there are multitude of other factors that also determine the outcome (Funaoka et al., 2011). In a separate study, it was observed that SOCS3 gene does not seem to be implicated in therapy resistance (Hamdi et al., 2012), however, p53 and ISGs association in their expression was involved in HCV-infected patients. In addition, HCV infection is known to affect the human immune response through SOCS protein modulation, and is thought to be linked intimately with the development of insulin resistance and metabolic syndrome through activation of rate limiting gluconeogenesis enzyme PEPCK (Sheikh et al., 2008; Qadri et al., 2012). It remains to be investigated how increased gluconeogenesis (hyperglycemia) and insulin resistance conditions may be linked with IFN resistance during HCV infection in a genotype specific manner.

References

Afzal, S., Idrees, M., Ali, M., Ilyas, M., Hussain, A., Akram, M., Butt, S., Saleem, S., Rehman, I.U., Ali, L., Shahid, M., 2011. Envelope 2 protein phosphorylation sites S75 & 277 of hepatitis C virus genotype 1a and interferon resistance: a sequence alignment approach. Virol J. 15 (8), 71.

Akuta, N., Suzuki, F., Hirakawa, M., Kawamura, Y., Sezaki, H., Suzuki, Y., Hosaka, T., Kobayashi, M., Kobayashi, M., Saitoh, S., Arase, Y., Ikeda, K., Chayama, K.,

Nakamura, Kumada, H., 2012. Amino acid substitution in HCV core/NS5A region and genetic variation near IL28B gene affect treatment efficacy to interferon plus ribavirin combination therapy. Intervirology 55 (3), 231–241.
Asselah, T., Estrabaud, E., Bieche, I., Lapalus, M., De Muynck, S., Vidaud, M., Saadoun, D., Soumelis, V., Marcellin, P., 2010. Hepatitis C: viral and host factors associated with non-response to pegylated interferon plus ribavirin. Liver Int. 30 (9), 1259–1269.
Asselah, T., De Muynck, S., Broët, P., Masliah-Planchon, J., Blanluet, M., Bièche, I., Lapalus, M., Martinot-Peignoux, M., Lada, O., Estrabaud, E., Zhang, Q., El Ray, A., Vidaud, D., Ripault, M.P., Boyer, N., Bedossa, P., Valla, D., Vidaud, M., Marcellin, P., 2012. IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C. J. Hepatol. 56 (3), 527–532.
Bartenschlager, R., Lohmann, V., 2000. Replication of hepatitis C virus. J. Gen. Virol. 81 (Pt 7), 1631–1648.
Bellanti, F., Vendemiale, G., Altomare, E., Serviddio, G., 2012. The impact of interferon lambda 3 gene polymorphism on natural course and treatment of hepatitis C. Clin. Dev. Immunol. 849373, 9.
Bellecave, P., Sarasin-Filipowicz, M., Donzé, O., Kennel, A., Gouttenoire, J., Meylan, E., Terracciano, L., Tschopp, J., Sarrazin, C., Berg, T., Moradpour, D., Heim, M.H., 2010. Cleavage of mitochondrial antiviral signaling protein in the liver of patients with chronic hepatitis C correlates with a reduced activation of the endogenous interferon system. Hepatology 51 (4), 1127–1136.
Bode, J.G., Ludwig, S., Ehrhardt, C., Albrecht, U., Erhardt, A., Schaper, F., Heinrich, P.C., Häussinger, D., 2003. IFN-α antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. FASEB J. 17, 488–490.
Bowden, D.S., Berzsenyi, M.D., 2006. Chronic *hepatitis C virus* infection: genotyping and its clinical role. Future Microbiol. 1 (1), 103–112.
Chayama, K., Suzuki, F., Tsubota, A., Kobayashi, M., Arase, Y., Saitoh, S., Suzuki, Y., Murashima, N., Ikeda, K., Takahashi, N., Kinoshita, M., Kumada, H., 2000. Association of amino acid sequence in the PKR-eIF2 phosphorylation homology domain and response to interferon therapy. Hepatology 32 (5), 1138–1144.
Chen, L., Borozan, I., Feld, J., Sun, J., Tannis, L.L., Coltescu, C., Heathcote, J., Edwards, A.M., McGilvray, I.D., 2005. Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. Gastroenterology 128, 1437–1444.
Chevaliez, S., Pawlotsky, J.M., 2006. HCV genome and life cycle. In: Tan, S.L. (Ed.), Hepatitis C Viruses: Genomes and Molecular Biology. Horizon Bioscience, UK, Chap. 1.
Cho, H., Mukherjee, S., Palasuberniam, P., Pillow, L., Bilgin, B., Nezich, C., Walton, S.P., Feig, M., Chan, C., 2011. Molecular mechanism by which palmitate inhibits PKR autophosphorylation. Biochemistry 50 (6), 1110–1119.
Clark, V.C., Peter, J.A., Nelson, D.R., 2013. New therapeutic strategies in HCV: second-generation protease inhibitors. Liver Int. 33 (1), 80–84.
Cooksley, W.G., 2004. The role of interferon therapy in hepatitis B. MedGenMed: Medscape General Medicine 6 (1), 16.
De Rueda, P.M., Casado, J., Paton, R., Quintero, D., Palacios, A., Gila, A., Quiles, R., León, J., Ruiz-Extremera, A., Salmerón, J., 2008. Mutations in E2-PePHD, NS5A-PKRBD, NS5A-ISDR and NS5A-V3 of hepatitis C virus genotype 1 and their relationships to pegylated interferon-ribavirin treatment responses. J. Virol. 82 (13), 6644–6653.
De Veer, M.J., Holko, M., Frevel, M., Walker, E., Der, S., Paranjape, J.M., Silverman, R.H., Williams, B.R., 2001. Functional classification of interferon-stimulated genes identified using microarrays. J. Leukocyte Biol. 69 (6), 912–920.
De Weerd, N.A., Samarajiwa, S.A., Hertzog, P.J., 2007. Type I interferon receptors: biochemistry and biological functions. J. Biol. Chem. 282 (28), 20053–20057.
Deltenre, P., Louvet, A., Lemoine, M., Mourad, A., Fartoux, L., Moreno, C., Henrion, J., Mathurin, P., Serfaty, L., 2011. Impact of insulin resistance on sustained response in HCV patients treated with pegylated interferon and ribavirin: a meta-analysis. J. Hepatol. 55 (6), 1187–1194.
Donnelly, R.P., Kotenko, S.V., 2010. Interferon-lambda: a new addition to an old family. J. Interferon Cytokine Res. 30 (8), 555–564.
Dunlevy, F., McElvaney, N.G., Greene, C.M., 2010. TLR3 Sensing of viral infection. Open Infect. Dis. J. 4, 1–10.
Enomoto, N., Sakuma, I., Asahina, Y., Kurosaki, M., Murakami, T., Yamamoto, C., Izumi, N., Marumo, F., Sato, C., 1995. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J. Clin. Invest. 96, 224–230.
Enomoto, N., Sakuma, I., Asahina, Y., Kurosaki, M., Murakami, T., Yamamoto, C., Ogura, Y., Izumi, N., Marumo, F., Sato, C., 1996. Mutations in the nonstructural protein 5a gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N. Engl. J. Med. 334, 77–81.
Fensterl, V., Sen, G.C., 2009. Interferons and viral infections. BioFactors 35 (1), 14–20.
Fukuhara, T., Taketomi, A., Okano, S., Ikegami, T., Soejima, Y., Shirabe, K., Maehara, Y., 2009. Mutations in hepatitis C virus genotype 1b and the sensitivity of interferon-ribavirin therapy after liver transplantation. J. Hepatol. 52 (5), 672–680.
Funaoka, Y., Sakamoto, N., Suda, G., Itsui, Y., Nakagawa, M., Kakinuma, S., Watanabe, T., Mishima, K., Ueyama, M., Onozuka, I., Nitta, S., Kitazume, A., Kiyohashi, K., Murakawa, M., Azuma, S., Tsuchiya, K., Watanabe, M., 2011. Analysis of interferon signaling by infectious hepatitis C virus clones with substitutions of core amino acids 70 and 91. J. Virol. 85 (12), 5986–5994.
Gale, M.J., Foy, E.M., 2005. Evasion of intracellular host defence by hepatitis C virus. Nature 436 (7053), 939–945.
Haller, O., Kochs, G., Weber, F., 2007. Interferon, Mx, and viral countermeasures. Cytokine Growth Factor Rev. 18 (5–6), 425–433.
Hamdi, N., El-Akel, W., El-Serafy, M., Esmat, G., Sarrazin, C., Abdelaziz, A.I., 2012. Transcriptional response of MxA, PKR and SOCS3 to interferon-based therapy in HCV genotype 4-infected patients and contribution of p53 to host antiviral response. Intervirology 55 (3), 210–218.
Heim, M., 2012. Interferons and hepatitis C virus. Swiss Med. Wkly. 142, w13586.
Hourioux, C., Patient, R., Morin, A., Blanchard, E., Moreau, A., Trassard, S., Giraudeau, B., Roingeard, P., 2007. The genotype 3-specific hepatitis C virus core protein residue phenylalanine 164 increases steatosis in an in vitro cellular model. Gut 56 (9), 1302–1308.
Huang, Y., Feld, J.J., Sapp, R.K., Nanda, S., Lin, J.H., Blatt, L.M., Fried, M.W., Murthy, K., Liang, T.J., 2007. Defective hepatic response to interferon and activation of suppressor of cytokine signaling 3 in chronic hepatitis C. Gastroenterology 132, 733–744.
Jamal, I.S., Yusuf, S., Azhar, M., Jamall, S., 2008. Is pegylated interferon superior to interferon, with ribavarin, in chronic hepatitis C genotypes 2/3? World J. Gastroenterol. 14 (43), 6627–6631.
Kato, N., 2000. Genome of human hepatitis C virus (HCV): gene organization, sequence diversity, and variation. Microb. Comp. Genomics 5 (3), 129–151.
Kobayashi, M., Watanabe, K., Ishigami, M., Murase, K., Ito, H., Ukai, K., Yano, M., Takagi, K., Hattori, M., Kakumu, S., Yoshioka, K., 2002. Amino acid substitutions in the nonstructural region 5A of hepatitis C virus geno- types 2a and 2b and its relation to viral load and response to interferon. Am. J. Gastroenterol. 97, 988–998.
Kozuka, R., Enomoto, M., Hai, H., Ogawa, T., Nakaya, M., Hagihara, A., Fujii, H., Kobayashi, S., Iwai, S., Morikawa, H., Tamori, A., Kawada, N., 2012. Changes in sequences of core region, interferon sensitivity-determining region and interferon and ribavirin resistance-determining region of hepatitis C virus genotype 1 during interferon-alpha and ribavirin therapy, and efficacy of retreatment. Hepatol Res. 42 (12), 1157–1167.
Kumar, D., Farrell, G.C., Fung, C., George, J., 2002. Hepatitis C virus genotype 3 is cytopathic to hepatocytes: reversal of hepatic steatosis after sustained therapeutic response. Epatology 36 (5), 1266.
Kumthip, K., Chusri, P., Jilg, N., Zhao, L., Fusco, D.N., Zhao, H., Goto, K., Cheng, D., Schaefer, E.A., Zhang, L., Pantip, C., Thongsawat, S., O'Brien, A., Peng, L.F., Maneekarn, N., Chung, R.T., Lin, W., 2012. Hepatitis C virus NS5A disrupts STAT1 phosphorylation and suppresses type I interferon signaling. J. Virol. 86 (16), 8581–8591.
Le Guillou-Guillemette, H., Vallet, S., Gaudy-Graffin, C., Payan, C., Pivert, A., Goudeau, A., Lunel-Fabiani, F., 2007. Genetic diversity of the hepatitis C virus: impact and issues in the antiviral therapy. World J. Gastroenterol. 13 (17), 2416–2426.
Li, K., Foy, E., Ferreon, J.C., Nakamura, M., Ferreon, A.C., Ikeda, M., Ray, S.C., Gale Jr., M., Lemon, S.M., 2005. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the toll-like receptor 3 adaptor protein TRIF. Proc. Natl. Acad. Sci. USA 102 (8), 2992–2997.
Lin, R.J., Liao, C.L., Lin, E., Lin, Y.L., 2004. Blocking of the alpha interferon-induced Jak-stat signaling pathway by Japanese encephalitis virus infection. J. Virol. 78 (17), 9285–9294.
Lindh, M., Lagging, M., Arnholm, B., Eilard, A., Nilsson, S., Norkrans, G., Söderholm, J., Wahlberg, T., Wejstål, R., Westin, J., Hellstrand, K., 2011. IL28B polymorphisms determine early viral kinetics and treatment outcome in patients receiving peginterferon/ribavirin for chronic hepatitis C genotype 1. J Viral Hepat. 18 (7), 325–331.
Liu, Y.J., 2005. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu. Rev. Immunol. 23, 275–306.
Manns, M.P., Foster, G.R., Rockstroh, J.K., Zeuzem, S., Zoulim, F., Houghton, M., 2007. The way forward in HCV treatment: finding the right path. Nat. Rev. Drug Discov. 6 (12), 991–1000.
Meier, V., Ramadori, G., 2009. Hepatitis C virus virology and new treatment targets. Expert Rev. Anti Infect. Ther. 7 (3), 329–350.
Moradpour, D., Penin, F., Rice, C.M., 2007. Replication of hepatitis C virus. Nat. Rev. Microbiol. 5 (6), 453–463.
Morgan, T.R., Ghany, M.G., Kim, H.Y., Snow, K.K., Shiffman, M.L., De Santo, J.L., Lee, W.M., Di Bisceglie, A.M., Bonkovsky, H.L., Dienstag, J.L., Morishima, C., Lindsay, K.L., Lok, A.S.HALT-C Trial Group, 2010. Outcome of sustained virological responders with histologically advanced chronic hepatitis C. Hepatology 52 (3), 833–844.
NIH Consensus Statement on Management of hepatitis C, 2002. NIH Consens State Sci Statements. 19 (3), pp. 1–46.
Pawlotsky, J.M., 2003. Hepatitis C virus genetic variability: pathogenic and clinical implications. Clin. Liver Dis. 7, 45–66.
Pawlotsky, J.M., 2012. New antiviral agents for hepatitis C. F1000. In: Biol. Rep. 4, 5.
Pawlotsky, J.M., Pellerin, M., Bouvier, M., Roudot-Thoraval, F., Germanidis, G., Bastie, A., Darthuy, F., Rémiré, J., Soussy, C.J., Dhumeaux, D., 1998. Genetic complexity of the hypervariable region 1 (HVR1) of hepatitis C virus (HCV): influence on the characteristics of the infection and responses to interferon alfa therapy in patients with chronic hepatitis C. J. Med. Virol. 54 (4), 256–264.
Pazienza, V., Vinciguerra, M., Andriulli, A., Mangia, A., 2010. Hepatitis C virus core protein genotype 3a increases SOCS-7 expression through PPAR-{gamma} in Huh-7 cells. J. Gen. Virol. 91 (Pt 7), 1678–1686.
Penin, F., Dubuisson, J., Rey, F.A., Moradpour, D., Pawlotsky, J.M., 2004. Structural biology of hepatitis C virus. Hepatology 39, 5–19.

Pileri, P., Uematsu, Y., Campagnoli, S., Galli, G., Falugi, F., Petracca, R., Weiner, A.J., Houghton, M., Rosa, D., Grandi, G., Abrignani, S., 1998. Binding of hepatitis C virus to CD81. Science 282, 938–941.

Platanias, L.C., 2005. Mechanisms of type-I- and type-II-interferon-mediated signaling. Nat. Rev. Immunol. 5 (5), 375–386.

Qadri, I., Iwahashi, M., Simon, F., 2002. Hepatitis C virus NS5A protein binds TBP and p53, inhibiting their DNA binding and p53 interactions with TBP and ERCC3. Biochim Biophys Acta. 1592, 193–204.

Qadri, I., Iwahashi, M., Cappasso, J., Hopken, M.W., Flores, S., Schaack, J., Simon, F.R., 2004. Induced oxidative stress and activated expression of manganese superoxide dismutase (MnSOD) in HCV-replicating cells: role of JNK, p38MAPK & AP-1. Biochem. J. 10, 416–417.

Qadri, I., Hu, L.J., Iwahashi, M., Al-Zuahbi, S., Quattrochi, L., Simon, F.R., 2009. Interaction of hepatocyte nuclear factors in transcriptional regulation of tissue specific hormonal expression of human multidrug resistance associated protein-2 (ABCC2). Pharmacol. Toxicol. 234 (3), 281–292.

Qadri, I., Choudhury, M., Rahman, S.M., Knotts, T.A., Janssen, R.C., Schaack, J., Iwahashi, M., Puljak, L., Simon, F.R., Kilic, G., Fitz, J.G., Friedman, J.E., 2012. Increased phosphoenolpyruvate carboxykinase gene expression and steatosis during hepatitis C virus subgenome replication: role of nonstructural component 5A and CCAAT/enhancer-binding protein β. J. Biol. Chem. 287 (44), 37340–37351.

Romero-Gómez, M., 2006. Insulin resistance and hepatitis C. World J. Gastroenterol. 12 (44), 7075–7080.

Saito, I., Miyamura, T., Ohbayashi, A., Harada, H., Katayama, T., Kikuchi, S., Watanabe, Y., Koi, S., Onji, M., Ohta, Y., Choo, Q.L., Houghton, M., Kuo, G., 1990. Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. Proc. Natl. Acad. Sci. USA 87, 6547–6549.

Sarasin-Filipowicz, M., Oakeley, E.J., Duong, F.H., Christen, V., Terracciano, L., Filipowicz, W., Heim, M.H., 2008. Interferon signaling and treatment outcome in chronic hepatitis C. Proc. Natl. Acad. Sci. USA 105 (19), 7034–7039.

Sarrazin, C., Hézode, C., Zeuzem, S., Pawlotsky, J.M., 2012. Antiviral strategies in hepatitis C virus infection. J. Hepatol. 56 (Suppl), S88–S100.

Shao, R.X., Zhang, L., Peng, L.F., Sun, E., Chung, W.J., Jang, J.Y., Tsai, W.L., Hyppolite, G., Chung, R.T., 2010. Suppressor of cytokine signaling 3 suppresses hepatitis C virus replication in an mTOR-dependent manner. J. Virol. 84 (12), 6060–6069.

Sharieff, K.A., Duncan, D., Younossi, Z., 2002. Advances in treatment of chronic hepatitis C: 'pegylated' interferons. Cleve. Clin. J. Med. 69 (2), 155–159.

Sheikh, M.Y., Choi, J., Qadri, I., Friedman, J.E., Sanyal, A.J., 2008. Hepatitis C virus infection: molecular pathways to metabolic syndrome. Hepatology 47, 2127–2133.

Simmonds, P., 2004. Genetic diversity and evolution of hepatitis C virus – 15 years on. J. Gen. Virol. 85 (Pt 11), 3173–3188.

Simmonds, P., Bukh, J., Combet, C., Deléage, G., Enomoto, N., Feinstone, S., Halfon, P., Inchauspé, G., Kuiken, C., Maertens, G., Mizokami, M., Murphy, D., Okamoto, H., Pawlotsky, J., Penin, F., Sablon, E., Shin-I, T., Stuyver, L., Thiel, H., Viazov, S., Weiner, A., Widell, A., 2005. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 42 (4), 962–973.

Slev, P., 2012. Host genomics and HCV personalized medicine. Ann. Clin. Lab. Sci. 42 (4), 363–369.

Syed, G.H., Amako, Y., Siddiqui, A., 2010. Hepatitis C virus hijacks host lipid metabolism. Trends Endocrinol. Metab. 21 (1), 33–40.

Taylor, D.R., Shi, S.T., Romano, P.R., Barber, G.N., Lai, M.M., 1999. Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. Science 285 (5424), 107–110.

Vermehren, J., Sarrazin, C., 2011. New hepatitis C therapies in clinical development. Eur. J. Med. Res. 16, 303–314.

Vermehren, J., Sarrazin, C., 2012. The role of resistance in HCV treatment. Best Pract. Res. Clin. Gastroenterol. 26 (4), 487–503.

Walsh, M.J., Jonsson, J.R., Richardson, M.M., Lipka, G.M., Purdie, D.M., Clouston, A.D., Powell, E.E., 2006. Non-response to antiviral therapy is associated with obesity and increased hepatic expression of suppressor of cytokine signalling 3 (SOCS-3) in patients with chronic hepatitis C, viral genotype 1. Gut 55, 529–535.

Yoneda, S., Umemura, T., Katsuyama, Y., Kamijo, A., Joshita, S., Komatsu, M., Ichijo, T., Matsumoto, A., Yoshizawa, K., Ota, M., Tanaka, E.Nagano Interferon Treatment Research Group, 2011. Association of serum cytokine levels with treatment response to pegylated interferon and ribavirin therapy in genotype 1 chronic hepatitis C patients. J. Infect. Dis. 203 (8), 1087–1095.

Zhang, L., Jilg, N., Shao, R.X., Lin, W., Fusco, D.N., Zhao, H., Goto, K., Peng, L.F., Chen, W.C., Chung, R.T., 2011. IL28B inhibits hepatitis C virus replication through the JAK-STAT pathway. J. Hepatol. 55 (2), 289–298.