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Review

Reversal, maintenance or progression: What happens to the liver after a virologic cure of hepatitis C?

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ABSTRACT

A sustained virological response (SVR) from HCV (synonymous with virological cure) leads to decreased mortality, morbidity and improved quality of life, as well as a reduced incidence of liver disease progression, including liver failure, cirrhosis and hepatocellular carcinoma. Large clinical trials comparing pre- and post-treatment liver biopsies demonstrate improvements in inflammation as well as fibrosis score following SVR. However, a small subset of patients display persistent hepatic inflammation and/or progress to cirrhosis despite SVR. In addition to conferring a risk of fibrosis progression, advanced fibrosis pre-treatment is a major risk factor for post-SVR hepatocellular carcinoma. In this review, we discuss the mechanisms of fibrosis regression uncovered using experimental fibrosis models and highlight potential mechanisms in those few patients with fibrosis progression despite SVR. We also introduce current concepts of fibrosis-dependent tumorigenesis post-SVR in patients with advanced disease. This article forms part of a symposium in *Antiviral Research* on "Hepatitis C: next steps toward global eradication."

Contents

1. Introduction

Hepatitis C virus (HCV) infection is a worldwide pandemic with an estimated 150–200 million people infected globally, and three to four million people newly infected each year as estimated by the WHO (World Health Organization). Its worldwide prevalence is ~1.6–2% (Armstrong et al., 2006). Approximately 75–85% of all patients with acute HCV infection will develop chronic hepatitis C, which is defined as the persistence of HCV RNA >6 months (Thomas and Seeff, 2005). Because 2–30% of all patients with HCV progress to severe liver disease with fibrosis, cirrhosis or hepatocellular carcinoma over ~30 years, HCV has been the leading cause for liver transplantation in the US (Thomas and Seeff, 2005).

Both host- as well as viral-factors determine the pace of disease progression. Host risk factors for advanced liver disease include duration of infection, age at infection (especially if acquired at >40 years of age), co-infection with HIV or HBV, obesity and alcohol consumption (Benhamou et al., 1999). Also, multiple host-spe-

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cific genetic determinants associated with increased fibrosis progression have been identified, which include variants in the *IFNGR2* (Nalpas et al., 2010), and *PNPLA3* genes (Trepo et al., 2011; Valenti et al., 2011) among others (Patin et al., 2012; Huang et al., 2007, 2006). IL28B polymorphisms specifically have been associated not only with lower fibrosis progression rate in patients with non-1 HCV genotype (Bochud et al., 2012), but also as determinants in spontaneous or treatment-induced HCV clearance (see Balagopal et al., 2010 for review). Recently, there is also growing body of epidemiologic evidence demonstrating that coffee may attenuate fibrosis and HCC risk in HCV, however underlying mechanisms are not known (Freedman et al., 2009).

With the most recent approval of direct-acting antivirals (DAAs), for example simeprevir and sofosbuvir and others in the pipeline, the rates of a sustained virological response (SVR), and thus virological cure, have vastly increased across all HCV genotypes for treatment-naïve patients, as well as for treatment-experienced patients. This development is especially heartening for patients with cirrhosis, where cure has been difficult, and where side effects to interferon-based regimens were difficult to tolerate and often precipitated decompensation. Thus, interferon-free regimens now offer the prospect of treating this high-risk patient group while still achieving high SVR rates across all genotypes.

Clinical studies clearly establish that patients benefit from an SVR independent of their fibrosis stage. SVR leads to reduced mortality, morbidity, improved quality of life (Bernstein et al., 2002; John-Baptiste et al., 2009), and diminished risk of end-stage liver disease-associated complications (e.g., hepatic decompensation, HCC, bleeding, ascites) (Veldt et al., 2007; Cardoso et al., 2010; van der Meer et al., 2012); see also for excellent reviews (Thomas, 2013; Pearlman and Traub, 2011). Most patients demonstrate marked improvements in inflammation and fibrosis following SVR; however, in large clinical trials a minority of patients (7–13%) maintain their level of fibrosis or even progress to cirrhosis despite achieving SVR (Poynard et al., 2013, 2002; Maylin et al., 2008) (see also Table 1). It is also vital to recognize that patients with advanced fibrosis remain at risk for HCC at least 8–10 years later despite virologic cure (Aleman et al., 2013).

The mechanisms of HCV-mediated liver injury and fibrosis during ongoing infection have been characterized in great detail in the past ~25 years (see Schuppan et al., 2003; Teixeira et al., 2007; Mengshol et al., 2007). However, the mechanisms underlying improvement in these features *after* SVR are less well understood. Below we summarize the data from clinical trials that explore the outcomes and hepatic changes after SVR and data from pre-clinical (rodent) models where fibrosis regression has been studied. We also summarize what is currently known about the liver's regenerative adaptation after SVR, which can return histology to normal in this setting.

2. What is SVR?

Sustained virologic response (SVR) is commonly used to estimate the success of HCV treatment and is defined as the proportion of patients achieving aviremia 24 weeks after completion of therapy. Although there is some support for the identification of SVR as early as 12 weeks after treatment (Zeuzem et al., 2003; Martinot-Peignoux et al., 2010), SVR after 24 weeks has remained the gold standard for therapeutic success, and translates into durable loss of viremia in the vast majority of patients (Ghany et al., 2009). Since relapses beyond a few months after treatment are very rare (<1%) (Pearlman and Traub, 2011) SVR is interchangeably used to indicate virologic cure.

SVR as a virologic cure has been repeatedly challenged, however. With the emergence of sensitive assays such as RT-PCR nucleic acid hybridization (RT-PCR-NAH), the presence of virus can be detected in plasma, lymphocytes and macrophages, as well as in liver tissue of patients with confirmed SVR (Maylin et al., 2008). Also, the observation that protective immunity can be overcome with immunosuppression has fueled controversy in the field (Mehta et al., 2002; Lin et al., 2008), raising the concept of 'occult HCV', and underscoring its clinical relevance to explain relapse after discontinuation of therapy. Occult HCV might also explain the persistence or even progression of liver disease in the minority of patients who fail to improve after conventional SVR, although evidence for this possibility has not yet emerged.

Part of the difficulty in comparing features and risk of HCV persistence despite SVR across studies has been the differences in viral genotype, ethnicity, age, gender and fibrosis status at baseline, as well as dose and duration of treatment, and duration of follow up. All these variables may affect the sensitivity of assays used to detect the presence of HCV RNA as well as the source of the sample analyzed (e.g, plasma, tissue or PBMCs). However, data from several multicenter trials using interferon-based therapy show persistent low re-detection of serum HCV RNA with rates of 0–1% (reviewed in Pearlman and Traub, 2011; Manns et al., 2013). Moreover, as noted above, durable long term viral suppression has been documented in several large patient cohorts followed up to 18 years (Maylin et al., 2008; Pradat et al., 2007). In those cases in which late relapses (defined as occurring after 24 weeks of SVR) were observed, most were associated with additional risk factors (e.g., immune suppression due to chemotherapy or organ transplantation, or re-infection due to high risk behavior) (Lin et al., 2008; Thomopoulos et al., 2008; Everson et al., 2005). Also, as no adverse liver related clinical outcomes have been described in patients with occult HCV infections post SVR, it is unclear if HCV can survive and replicate in extra-hepatic tissue, thus the differentiation between a true relapse or a re-infection has made it difficult to generate conclusive data.

Special mechanisms of re-infection might apply in cases of HCV recurrence after liver transplantation. For example, transmission of HCV via exosomes from Huh7.5.1 cells has been reported (Ramakrishnaiah et al., 2013), which could represent an immune circulation strategy of the virus that allows these exosomes to persist in the host blood and re-infect the liver graft following transplantation.

Overall, however, there is strong clinical data supporting the contention that long-term viral eradication (SVR) in patients represents a virological cure from hepatitis C. Even after SVR, however, patients with or without advanced liver disease remain at increased risk for health complications – it is unknown how severe and for how long this risk persists, but such data is likely to emerge as a larger fraction of patients are cured by the newest generation of interferon-free regimens. In a retrospective study, HCV patients without advanced fibrosis had an adjusted standardized morbidity ratio of up to 5.9 in comparison to the general population (Innes et al., 2011). Other studies report that the outcome after SVR depends primarily on the fibrosis status at treatment onset, as well as host-related and concomitant risk factors, which are reviewed below.

2.1. What are the clinical outcomes after SVR? – evidence from clinical studies

Successful treatment of HCV with SVR leads to decreased mortality and morbidity, and as well as an improved quality of life. Patients who achieve SVR also have a lower incidence of liver related complications (hepatic encephalopathy, ascites, variceal bleeding and HCC) (Pradat et al., 2007; Braks et al., 2007; Bruno et al., 2007). Patients with advanced fibrosis at treatment especially benefit from SVR, with lower rates of liver failure, transplan-

Table 1
Histological outcomes of sustained virological responders with regard to inflammation and fibrosis.

Reference	Number of patients	Time to biopsy	Therapy	Staging system	Biopsy length	Improved inflammation (%)	Maintained inflammation (%)	Progressive inflammation (%)	Fibrosis regression (%)	Fibrosis maintenance (%)	Fibrosis progression (%)	Cirrhosis regression (%)
Marcellin et al. (1997)	48	2.2 years (mean)	IFN	Knodell	10 mm	94	2	4				
Shiratori et al. (2000)	183	3.7 years	IFN	Metavir/ Desmet	>10 mm	89	10	1	59	40	1	na
Manns et al. (2001)	1034	24 months	PEG/ RBV, IFN/ RBV	Knodell	na	90	na	na	21–26	na	na	na
Poynard et al. (2002)	1094	20 months (mean)	IFN/ RBV, PEG, PEG/ RBV	Metavir	30 mm	86	12	2	25	68	7	67
Toccaceli et al. (2003)	87	29.5 months (median)	IFN	Knodell	na	87	10	2	33	64	3	na
Maylin et al. (2008)	126	6 months (median)	IFN, IFN/ RBV, PEG/ RBV	Metavir	15 mm	57	39	4	56	32	12	64
George et al. (2009)	49	62 months (mean)	IFN/ RBV, PEG/ RBV	Ishak	na	82	12	6	82	na	na	na
Balart et al. (2010)	195	24 weeks	PEG/ RBV	Ishak	10 mm or > 4 PT	na	na	na	48.20	37	14	53
Mallet et al. (2008)	35 (F4 cirrhosis at baseline)	17 months (median)	IFN, IFN/ RBV, PEG/ RBV	Metavir	15 mm	na	na	na		51	na (already F4 cirrhosis)	49
D'Ambrosio et al. (2012)	38 (F4 cirrhosis at baseline)	61 months (median)	IFN/ RBV, PEG/ RBV	Metavir	30 mm (median)	na	na	na		39	na (already F4 cirrhosis)	61
				Morphometry	10 mm or >12 PT	na	na	na		3	8	89
				Necroinflammation (0–3)	10 mm or >12 PT	84	16	0				
				Ishak (portal inflammation)	10 mm or >12 PT	34	66	0				
				Ishak (lobular/ interface inflammation)	10 mm or >12 PT	87/97	13/3	0				

Abbreviations: PT, portal tracts; na, not available or non-applicable.

tation and development of HCC (Veldt et al., 2007). Improvement also extends to extra-hepatic manifestations of chronic hepatitis C, including neurologic, renal, dermatologic and metabolic improvements.

2.2. What happens to the liver after SVR? – maintenance, progression or reversal of fibrosis?

Several large trials analyzing progression of liver disease by liver biopsies pre- and post-treatment show stabilization or a decrease in hepatic fibrosis, including a regression of cirrhosis in patients that achieve SVR (Poynard et al., 1995) (see Table 1). However, 1–14% of patients who achieved SVR (Table 1) had progression of fibrosis.

A study from Poynard et al. (2013) assessed a total of 993 patients with Fibrotest (a panel of serum markers) and transient elastography as biomarkers for fibrosis and reported a decrease of cirrhosis with SVR in 49% (24/42 patients), but also described 15 new cirrhosis cases among 128 patients with SVR (12%) and advanced fibrosis (F0–F3) at inclusion. Cumulative time of analysis was 10 years, which limited the prevalence rate of cirrhosis regression to only 5% after ten years. Importantly, in this and another study, younger age and higher platelet count were important factors associated with likely fibrosis regression/reversal (Poynard et al., 2002), suggesting that early cirrhosis might be more likely to regress than established cirrhosis, and that an absence of portal hypertension may be a determinant of reversibility. Likewise, patients with biopsy proven HCV cirrhosis achieving SVR post treatment, showed a significant decrease in hepatic venous pres-

sure gradient (HVPG) levels compared to non-responders (−2.1 ± 4.8 vs. 0.6 ± 2.8 mmHg, $p < 0.05$), which was also significantly associated with both histological improvement and SVR (Roberts et al., 2007). HVPG (HVPG < 10 mmHg) is a good negative predictor of clinical decompensation in patients with compensated cirrhosis (Ripoll et al., 2007).

Specific features of the extracellular matrix (ECM) might explain the differences in fibrosis reversibility after SVR. With a longer duration of liver fibrosis and cirrhosis there is increasing accumulation of high-density fibrillar collagens (e.g., collagens I and III) as well as proteoglycans and other ECM constituents (Friedman, 2004; Schuppan et al., 2001). Rodent models of fibrosis reversibility have examined ECM regression after cessation of toxic or obstructive liver injury (thioacetamide, CCl_4 or bile duct ligation) (Issa et al., 2004). Similar to the reversal of HCV-associated cirrhosis, experimental cirrhosis in these models evolves from a micronodular to a macrondular cirrhosis as it reverses (Wanless et al., 2000; Issa et al., 2004). Moreover, older fibrotic septae are more resistant to degradation than more recently deposited ones. Those fibrotic septae that persisted for more than a year were characterized by pauci-cellularity and increased ECM cross-linking (Issa et al., 2004). Collagen cross-linking enhances the resistance of collagen to degradation, and is a critical determinant of fibrosis irreversibility. For example, in a transgenic mouse model in which the collagen I harbors a mutation that renders it resistant to degradation by collagenase, fibrosis fails to regress even after cessation of the injurious agent (Issa et al., 2003).

Elastin, another non-collagenous matrix component, may also contribute to the resistance to fibrosis reversion after SVR. Elastin accumulates in mature cirrhosis and is dependent on macrophage-derived MMP12 for degradation (Pellicoro et al., 2012a,b). In cirrhosis, its synthesis is enhanced while its degradation is decreased, leading to elastin accumulation. Elastin's contribution to fibrosis persistence may be as a result of cross-linking of its pre-form, tropoelastin by the enzymes lysyl-oxidase (LOX) or tissue transglutaminase (tTG), which also renders the cirrhotic tissue more resistant to degradation and therefore less likely to regress (for reviews see (Schuppan et al., 2001; Friedman, 2008a,b; Pellicoro et al., 2012a,b). Interestingly, recent clinical studies that incorporate serum elastin into algorithms for fibrosis assessment are more accurate in diagnosing cirrhosis; for example, the Elasto-Fibro-Test out performs Fibroscan or Fibrotest alone (Poynard et al., 2012). Similarly, there are new MRI contrast agents that detect elastin, which might improve our ability to assess the maturity and potential reversibility of cirrhosis, which in turn could identify those patients at higher risk for persistent cirrhosis and complications including HCC or liver failure.

2.3. Mechanisms of fibrosis reversal – what happens to the liver after SVR? – evidence from rodent models

Although rodent models of experimental fibrosis have yielded a better understanding of mechanisms underlying fibrosis reversal (Friedman, 2007), the mechanisms of fibrosis reversion after HCV SVR have not been directly investigated, but are likely to be similar to rodent models. Recent humanized mouse models that support HCV replication and liver injury (Washburn et al., 2011; de Jong et al., 2010; Dorner et al., 2013) also display collagen accumulation and activated stellate cells (myofibroblasts). Therefore, these models may offer new opportunities to uncover mechanisms of fibrosis regression that are HCV-specific (Washburn et al., 2011) (for reviews see Shlomai et al., 2014; Lerat et al., 2011).

Hepatic stellate cells (HSCs) comprise the major fibrogenic cell population in liver (Friedman, 2008a,b). In addition to their fibrogenic contribution, stellate cells regulate the balance between the synthesis and degradation of ECM. Upon liver injury, HSCs activate and proliferate to myofibroblasts producing collagen and other ECM components. Importantly, they also produce tissue inhibitor of metalloproteinase 1 (TIMP-1) which inhibits the ECM-degrading MMPs, thereby tipping the balance toward ECM accumulation. TIMP-1's importance is illustrated by a TIMP-1 over-expressing mouse model (Yoshiji et al., 2000), in which these transgenic mice fail to degrade/reverse fibrosis to the same extent as control animals. Moreover, ECM components, especially collagen I, can activate and sustain HSC survival, thereby enlarging the fibrogenic cell population. Importantly, TIMP-1 also directly promotes survival of activated HSCs, so its induction during fibrosis progression helps sustain these fibrogenic cells and prevent their apoptosis.

Reduction in the number of activated HSCs is critical to reversibility of fibrosis. Three major pathways help clear fibrogenic, activated HSCs: (1) apoptosis (Iredale et al., 1998); (2) senescence (Krizhanovsky et al., 2008); and (3) reversion to quiescence (Friedman, 2012; Kisseleva et al., 2012; Troeger et al., 2012). Apoptosis of activated stellate cells has been documented in rodent experimental fibrosis model (BDL and CCl_4). In these models, cessation of the liver injury either by biliojejunal anastomosis (for BDL) or by stopping hepatotoxin administration (for CCl_4) leads to rapid clearance of activated HSCs by apoptosis (Elsharkawy et al., 2005). Interestingly, a recent GWA study in a combined cohort of 2342 HCV-infected patients also identified several susceptibility loci for HCV-induced liver fibrosis progression which were linked to genes that regulate apoptosis (*RNF7 and MERTK, TULP1*) further implicating apoptotic control in liver fibrosis progression/regression in humans (Patin et al., 2012).

Cellular senescence is a genetically controlled program preventing cell division once cells exceed a finite proliferative capacity. Seminal work by Krizhanovsky et al. (2008) has demonstrated that HSCs undergo senescence and then accumulate in experimental hepatic fibrosis. Transgenic mice lacking key senescence regulators (p53−/− or INK4a/ARF−/− or p53−/−/INK4a/ARF−/− double knockout mice) have enhanced hepatic fibrosis compared to controls with increased HSC proliferation. The p53 and the p16/Rb pathways drive senescence in activated HSCs during resolution of experimental hepatic fibrosis. Senescent HSCs are also targeted by NK cells for clearance in vitro and in vivo, thereby additionally contributing to fibrosis resolution.

There is now solid evidence of reversion of activated stellate cells to a more quiescent state in rodent models of fibrosis (Kisseleva et al., 2012; Troeger et al., 2012). In these studies, HSCs revert to quiescence and remain in the liver after withdrawal of a hepatotoxin. Troeger et al. (2012) used a tamoxifen-inducible vimentin-CRE-ER while Kisseleva et al. (2012) used a HSC-specific collagen a2(1)-CRE-ER promoter to map HSC fate during fibrosis regression. A sizable fraction of once-activated cells had reverted to a quiescent state. However, these cells remained 'primed', with an enhanced capacity to reactivate upon re-exposure to fibrogenic stimuli. Persistence of these "inactivated stellate cells" could explain why patients with previous liver injury are more susceptible to accelerated fibrosis after re-injury, for example, following repeated bouts of alcohol binging or following recurrent viral infection or re-activation, since their HSCs are largely primed to reactivate more quickly.

Macrophages are a key cellular determinant of the resolution of liver fibrosis. An important study by Iredale and colleagues used a genetic macrophage depletion model in which loss of macrophages during the onset of liver injury led to decreased ECM degradation and clearance of myofibroblasts (Fallowfield et al., 2007; Jiao et al., 2012), whereas their depletion during resolution amplified fibrosis, indicating that there might be different classes of macrophages that contribute to fibrosis progression and regression, respectively. That indeed appears to be the case with the

identification of specific fibrolytic subsets of macrophages that are expanded during fibrosis regression (Ramachandran et al., 2012).

Mechanisms underlying the activity of fibrolytic macrophages are increasingly clarified. Macrophages can produce TRAIL and MMP9 which can promote myofibroblast apoptosis (Elsharkawy et al., 2005). Moreover, macrophages and dendritic cells also promote resolution and produce MMPs (MMP13, MMP9, MMP2, MMP8) that can directly degrade ECM (Jiao et al., 2012; Fallowfield et al., 2007). Apart from directly promoting fibrosis resolution via production of MMPs, macrophages can mediate anti-inflammatory effects, for example by phagocytosing apoptotic hepatocytes which can activate HSCs (Canbay et al., 2003), and by differentiating into regulatory macrophages that produce suppressor cytokines locally. However, macrophages represent a highly heterogenous cell population so they can exert divergent effects depending on their subtype. While monocyte-derived tissue cells, Mreg/M2c-like macrophages, contribute to the resolution of inflammation and fibrosis, a Ly-6C$^+$ (Gr1$^+$) subset of hepatic macrophages derived from recruitment of inflammatory monocytes via CCl2/CCr2 might be responsible for profibrogenic effects (Karlmark et al., 2009).

2.4. Mechanisms of fibrosis reversal – what happens to the liver after SVR? – evidence from clinical data

Clinical evidence documenting the fates of hepatic stellate cells, macrophages and dendritic cells during fibrosis regression in humans is limited in contrast to the extensive evidence in rodent models. Nevertheless, principles of hepatic stellate cell apoptosis, senescence or reversion have been well demonstrated in experimental studies using isolated human hepatic stellate cells (see review Friedman, 2008a,b; Friedman et al., 2013). Similarly, dendritic cell and macrophage subsets share common yet also partly opposing features in mice and men (for review, see Hashimoto et al., 2011; Aloman et al., 2011; Liaskou et al., 2012; Tacke and Zimmermann, 2014).

Histological analysis of liver tissue in HCV patients pre- and post-SVR has been limited to the assessment of inflammation and fibrosis using semi-quantitative scoring (e.g, Metavir and Ishak scores). More quantitative analyses including immunostaining for cell specific markers or morphometric analysis have not been routinely performed, even though they may be more sensitive and quantitative (Manousou et al., 2011; D'Ambrosio et al., 2012). Because there is reluctance to perform liver biopsy, especially after SVR, most analyses of fibrosis regression after SVR have relied upon non-invasive measurement of fibrosis (e.g. transient elastography).

As noted above, there are very few studies assessing liver histology post SVR. However, one important effort by D'Ambrosio et al. (2012) addressed this question using fibrosis quantification and immunohistochemical markers. They performed a prospective study with 38 patients with Metavir score F4 (ie, cirrhosis) before antiviral treatment and after SVR. After a median of 67 months (54–110 months) they reported a significant decrease in fibrosis as assessed by Metavir score, and collagen content as assessed by sirius red quantification. They also observed reduced ductular reaction and decreased numbers of putative human progenitor cells by Ck7 staining. The authors also documented a return to normal lobular metabolic zonation, as assessed by staining for glutamine synthase (GS) and CYP2E1. Surprisingly, however, the extent of sinusoidal capillarization as assessed by CD34 staining (a marker for sinusoidal endothelium) as well as numbers of myofibroblasts scored by extent of staining for αSMA (alpha smooth muscle actin, a marker for myofibroblasts) did not differ before and after SVR. Moreover, 31% (11 patients) displayed a worsening of αSMA score after SVR, independent of post-treatment fibrosis stage indicating an increased number of potentially fibrogenic myofibroblasts. This study excluded patients with alcohol consumption, co-infection with HBV or HIV as well as patients >75 years in an effort to control for known risk factors for fibrosis progression or limited cirrhosis regression. The extent of hepatic steatosis, another risk factor for limited fibrosis regression, was similar in both patient groups of regressors and non-regressors. Follow up in this patient population and analysis for further underlying risk factors such as fatty liver disease would be helpful to distinguish between the contribution of concomitant risk factors, or post-SVR HCV specific mechanisms that account for this finding. Indeed, 66% of patients had persistent portal inflammation while lobular and interface inflammation had completely resolved (Table 1). Other studies have also reported sustained or even worsening of inflammatory activity up to 5 years later (Maylin et al., 2008) (see also Table 1) despite proven virological clearance. Together, these observations underscore the need for detailed characterization of liver biopsies to elucidate underlying mechanisms in HCV associated fibrosis and liver regeneration post-SVR.

3. Progression of fibrosis to cirrhosis even after SVR?

Overall, progression of liver fibrosis to cirrhosis is rare but can occur despite SVR. Based on a large pooled data set from 3010 naive patients, Poynard has estimated the prevalence at 7% (Poynard et al., 2002). Maylin et al. have analyzed 121 patients with pre and post-treatment liver biopsies and reported fibrosis progression in 12% patients after ruling out de novo infections or the existence of occult HCV via PCR of liver tissue (Maylin et al., 2008).

The presence of significant liver co-morbidities or risk factors such as alcohol consumption or fatty liver disease are likely causes for many of the cases in which progression occurs post-SVR. In general, a combination of more than one liver disease, for example hemochromatosis with alcohol consumption (Britton and Bacon, 2002), or chronic viral hepatitis with alcoholic liver injury, can drive fibrosis progression in HCV patients (Schuppan et al., 2003). Gene expression patterns that correlate with hepatitis C and early progression to fibrosis in liver transplant recipients have been described (Smith et al., 2006). Epidemiological studies and also experimental studies have shown that HCV can alter glucose metabolism and lead to insulin resistance (IR) (Mehta et al., 2000; Aytug et al., 2003; Negro, 2012) which has a strong influence on SVR and fibrosis. Increased HOMA IR scores have been associated with reduced SVR to IFN based therapy (Romero-Gomez et al., 2005; Poustchi et al., 2008; Chu et al., 2009) and accelerated fibrogenesis (Hui et al., 2003; Petta et al., 2008; Moucari et al., 2008; Muzzi et al., 2005).

The evidence in rodent models that activated HSCs which reverting to quiescence are primed to reactivate more briskly, as described above, could contribute to these cases. Additionally, there could be genetic variants that influence the likelihood of regression among those with SVR, just as genetics can influence fibrosis progression. A study by Balart et al. (2010) described significantly decreased rates of fibrosis regression in Latinos (37%) vs. non-Latinos (55%) with SVR and genotype 1 HCV – no studies have yet uncovered variants that influence regression, however.

4. Persisting risk for HCC after achieving SVR

There is clear evidence that patients with advanced fibrosis or cirrhosis who achieve SVR remain at heightened risk for HCC (Hirakawa et al., 2008; Yoshida et al., 1999; Yamashita et al., 2013; Maylin et al., 2008; van der Meer et al., 2012). In a meta-analysis that combined data from 30 studies, the incidence of HCC was 1.05% per person year in those with SVR compared to

3.3% in those patients without SVR (Morgan et al., 2013). In patients with advanced fibrosis (Ishak score 4–6) the cumulative occurrence of HCC after 10 years was 21.8% without SVR and 5.1% with SVR (van der Meer et al., 2012). Risk factors include advanced pretreatment fibrosis, but also, age, steatosis (Ohata et al., 2003), male gender, diabetes, and alcohol consumption (Tanaka et al., 2007). Many tumorigenic pathways for HCV-mediated HCC have been described using in vivo and in vitro models (for review see Tang and Grise, 2009; Shlomai et al., 2014; Lerat et al., 2011), including direct tumorigenic/proliferative effects of HCV particles, but also indirect effects of HCV-associated inflammation, through pathways that include NF-κb signaling (Luedde and Schwabe, 2011) and reactive oxygen species (Valko et al., 2007; Seitz and Stickel, 2006). However, most of these pathways should be eliminated by virological clearance and not persist. Also, histological improvement in inflammation post-SVR has been demonstrated for most patients (see Table 1, also Pearlman and Traub, 2011). However, many studies have shown that HCC can still occur in a low yet persistent number of patients, even several years post-SVR. Pretreatment fibrosis stage has been identified as the major risk factor for post-SVR HCC (Yamashita et al., 2011). This implicates the fibrotic microenvironment as an important determinant of liver tumorigenesis in this subset of patients, however the mechanisms are unclear. Studies have ruled out occult HCV by qPCR in liver tissue biopsies to explain these HCCs that occur post-SVR (Maylin et al., 2008).

Several general mechanisms that are enhanced by fibrosis, could also contribute to tumorigenesis. Fibrillar collagens deposited during fibrosis stimulate integrins to form focal adhesion on the cell surface, leading to enhanced integrin signaling, cell growth and survival via the PI3K and MAPK signaling cascades (Levental et al., 2009). Additional mechanisms include increased migration (Fu et al., 2011) and anti-apoptotic signaling (Zhang et al., 2002) that promote survival of pre-cancerous cells. Additionally, increased matrix stiffness promotes cell proliferation and HSC activation, which generate important mitogenic factors (HGF, interleukin 6, Wnt), creating a positive feedback loop that is conducive to hepatocyte proliferation (Wells, 2008; Friedman, 2008a,b). Growth factors (HGF, FGF) can also be sequestered by the extracellular matrix and signal in an autocrine or paracrine manner, thereby contributing to an increased abundance and reservoir of progrowth stimuli in the extracellular milieu (Schuppan et al., 2001). Indeed, stromal gene expression profiles have been correlated with patient survival in HCC (Hoshida et al., 2008) (see also Zhang and Friedman, 2012 for review).

5. Conclusion

Rapid improvements in HCV therapies will greatly enhance the likelihood of SVR among all patient groups with HCV infection, including those traditionally considered 'hard to treat'. Data from large trials underscore the importance of SVR in leading to improved mortality and reducing adverse outcomes, although fibrosis progression and de novo HCC still occur post-SVR in a small subset of patients. Moreover, HCV-infected patients, despite achieving SVR, remain at a higher health risk in comparison to general population, indicating that SVR is a virological cure but not necessarily a cure from risk of liver disease. Further clarification of mechanisms for liver repair and regeneration after virological cure of HCV is necessary to maximize improvement in long-term outcomes of HCV infected patients.

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