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Inflammatory patterns in plasma associate with hepatocellular carcinoma development in cured hepatitis C cirrhotic patients

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Abstract

Introduction: The risk of hepatocellular carcinoma persists in some patients despite achieving sustained virologic response with current interferon-free direct-acting antiviral therapy for hepatitis C. The subject of an even higher carcinoma risk in this context has been reported and is currently being debated. The quest for understanding this paradox relative to the dynamics of inflammatory biomarkers in cirrhosis patients receiving antiviral therapy thus remains a subject of importance.

Objective: Here, we aimed at evaluating the effects of direct-acting antiviral therapy-induced hepatitis C cure on plasmatic markers of systemic inflammation measured before, during and after treatment. Specifically, soluble immune mediator phenotype associations that impact the odds of hepatocellular carcinoma development and the related changes that arise upon direct-acting antiviral-mediated hepatitis C clearance in cirrhosis patients was investigated.

Methods: Employing multiplex technology that measured up to 91 circulating biomarker proteins, we profiled the plasma soluble immune mediator concentrations of cirrhosis patients who developed posttreatment hepatocellular carcinoma and their respective negative controls, before and after direct-acting antiviral treatment.

Results: Elevated pretherapy concentrations of specific soluble immune mediators including MCP-3, GDNF, CDCP1, IL-17C, IL-17A, signalling lymphocytic activation family 1, CCL11, FGF-5, LIF-R, interleukin 10 (IL-10), IL-10RA, IL-15RA, beta NGF, CCL28, CCL25 and NT-3 distinguished patients who developed posttreatment hepatocellular carcinoma relative to those that did not. Particularly, GDNF, FGF-5 and IL-15RA displayed independent predictive biomarker attributes for delineating carcinoma emergence regardless of de novo or recurrence groupings. Upon successful therapy, the elevated pretherapy soluble immune mediator establishment of the patients who eventually developed hepatocellular carcinoma stayed largely

unperturbed whereas a panel of some 38 soluble immune mediators in the post-therapy carcinoma-free patients experienced significant ameliorations.

Conclusions: These results have considerable implications for delineating potential hepatocellular carcinoma emergence before initiating direct-acting antiviral therapy for hepatitis C in cirrhosis patients. They provide preliminary contribution to unravelling cases where the benefit of direct-acting antiviral therapies would be superior to the risk of developing carcinoma.

KEYWORDS
chemokines, cytokines, direct-acting antivirals, hepatitis C, hepatitis C virus cirrhosis, hepatocellular carcinoma, interferon-free therapy, soluble immune mediators

Key Summary

Established knowledge on this subject

- Current interferon (IFN)-free direct-acting antivirals (DAAs) are effective at eliminating hepatitis C virus (HCV), but risks of residual liver disease and development of hepatocellular carcinoma persists.
- The hepatic inflammation that occurs during chronic hepatitis C causes systemic changes in blood soluble immune mediators (SIMs) that impact carcinogenetic processes involved in the growth, invasion and metastasis of hepatocellular carcinoma (HCC).
- DAA-induced HCV cure does not lead to a complete immunological restitution of the altered soluble inflammatory compartment in chronic hepatitis C.

Significant and/or new findings of this study

- An elevated pre-therapy plasma profile of an extended repertoire of SIMs in cirrhosis was associated with HCC development post-DAA therapy.
- Successful DAA therapy did not alter the baseline elevated plasma SIM profile of cirrhosis patients that developed post-therapy HCC contrary to its effect in those that remained HCC-free.

INTRODUCTION

Hepatocellular carcinoma (HCC) still emerges in some patients despite clearance of hepatitis C virus (HCV) upon successful antiviral therapy. This has been shown not only for interferon (IFN)-based therapies[1] but also for therapies using IFN-free direct-acting antiviral (DAA) regimens.[2] Several studies have demonstrated that HCV cure by IFN-free DAA therapy does not seem to alter the short-term risk of HCC emergence, as it tends to remain still high, particularly in patients with established liver cirrhosis.[3–6] There have been further concerns as to whether the risk of HCC recurrence may even be increased following IFN-free HCV therapy with DAAs.[7] A need thus exists to evaluate whether, and to what extent, HCC development is impacted by hepatic immune events in cirrhosis patients who receive IFN-free DAA therapy.

Preliminary reports provided an indication of a possible association between serum levels of distinct cytokines and the development of HCC.[8,9] Specifically, HCV clearance by IFN-free DAA therapy was reported to coincide with the induction of a rapid reduction in inflammation but increase in key HCC angiogenesis drivers such as the vascular endothelial growth factor (VEGF); an immune balance modification that may affect the anti-tumour surveillance machinery.[9]

Further, a potential pretreatment modification of a repertoire of soluble immune mediators in the serum of patients who eventually developed de novo HCC upon DAA therapy compared to controls was reported.[8] This gave an early indication that a skewed balance of mediators within the inflammatory milieu existent before DAA therapy may contribute to the post-DAA therapy emergence of HCC. The basis for this observation could perhaps be grounded in findings from a previous report that implicated specific soluble immune mediators (SIMs) as being involved in carcinogenetic processes that impact growth, invasion and metastasis of HCC; the cancer which occurs almost exclusively in inflamed livers.[10] Further to this, we recently showed that SIM-mediated immune surveillance of HCC may be important for HCC development.[11] Based on this background, we here aimed to address specific SIM-phenotype associations that impact the odds of HCC development and the related changes that ensue upon IFN-free therapy-mediated HCV clearance in cirrhosis patients.

MATERIALS AND METHODS

Patient population

In this study, we enrolled a total of 31 patients with baseline HCV-related liver cirrhosis who had undergone IFN-free hepatitis C therapy with DAAs at the liver outpatient clinic of Hannover Medical School (MHH). The criteria of cirrhosis diagnosis were based on liver histology (F5 and F6 according to ISHAK [modified Knodell score]), transient elastography (>14.5 kPa), and definite morphological signs in ultrasound, magnetic resonance imaging (MRI) and computed tomography (CT) as detailed previously.[3] All patients had no coexistent chronic inflammatory disease(s) or any known cancers other than HCC. Of these patients, 15 were those who had developed posttreatment HCC at some point during treatment or within a 24-weeks follow-up period (herein referred to as Cirrh-to-HCC). Control groups including 16 age-matched patients who remained HCC-free upon therapy (herein referred to as Cirrh-to-NoHCC) and 10 healthy individuals were also recruited. All patients were HCC-free by ultrasound, CT or MRI technology before therapy start. In furtherance, an HCC emergence surveillance schedule was routinely done upon therapy initiation for all patients during antiviral therapy and during a 6-month follow-up period. This surveillance schedule involved ultrasound imaging routinely performed at 3-month intervals during antiviral therapy and 6 months after therapy. This surveillance schedule is borne out of routine clinical practice at our centre. The Cirrh-to-HCC patients included four recurrences and 11 de novo cases. Overall, no analysed clinical parameter statistically differentiated the two stratified cohorts of HCC or HCC-free from each other. While the Cirrh-to-HCC patients had a median albumin, platelets, bilirubin, aspartate aminotransferase, alanine aminotransferase and fibroscan of 32 g/L, 102 tsd/il, 16 imol/l, 99 U/L, 59 U/L, 27.40 kPa, respectively, the Cirrh-to-NoHCC had these measures at 32 g/L, 90 tsd/il, 18 imol/L, 75 U/L, 87 U/L, 21.50 kPa, respectively.

Characteristics of patient cohorts and a description of the different DAA regimens for treatment according to international guidelines (HCV guidelines, European Association for the Study of the Liver, American Association for the Study of Liver Diseases) have been detailed in Table 1.

Measurement of plasma SIM concentrations

We performed multianalyte plasma SIM profiling employing a multiplex technology (Olink's Proseek Multiplex Inflammation), which simultaneously measured the expression of 91 multiple biomarker proteins (Table S1), based on a proximity extension assay. The precision, reproducibility and scalability of this SIM measurement technology have been previously described.[12–14] Plasma SIM concentrations of patients were analysed at baseline, and longitudinally at the end of therapy and follow-up of therapy as detailed in the Supporting Information Material. The precise time-points at which plasma were sampled and used for SIM assessments is detailed in Table S2. Based on a standardised limit of detection, a total of 17 proteins that had a missing data frequency of more than 45% were excluded from the analysis.

Statistical analyses

Data were analysed using the GraphPad Prism software (GraphPad Software) or Microsoft Excel (for spider graphs). Quantitative analyses were done using the Student' *t* test or the Mann–Whitney test according to the distribution of data. For multiple comparisons, one-way analysis of variance with a posttest correction was used. Multiple *t* tests were controlled using the false-discovery rate (FDR) correction approach, with a desired FDR (Q) of 10%. In general, $p < 0.05$ were considered to be statistically significant. The statistical test used for each analysis is detailed in the respective figure legends.

RESULTS

Pretherapy-elevated plasma SIM profiles characterised cirrhosis patients that developed HCC upon DAA therapy

Circumventing the low throughput of conventional methods, we here applied the innovative Proseek multiplex technology to profile multiple SIMs ($n = 91$) in the plasma of cirrhosis patients who either developed HCC (Cirrh-to-HCC) or remained HCC-free (Cirrh-to-NoHCC) following HCV treatment with current IFN-free DAAs. Upon a comparison of SIM concentrations at baseline, we observed a similar pattern of expression between Cirrh-to-HCC and Cirrh-to-NoHCC patients relative to normal values. Thus, compared to healthy individuals, SIMs that were upregulated, downregulated or remained unaltered occurred in a similar manner between the two cohorts (Figure 1a). Generally, most (i.e., over 50%) of the SIMs analysed here displayed superior mean plasma concentrations at this time-point in the patients with baseline cirrhosis irrespective of their HCC status following treatment. Interestingly however, we identified a set of 16 SIMs including MCP-3, GDNF, CDCP1, interleukin (IL) 17C (IL-17C), IL-17A, signalling lymphocytic activation family 1 (SLAMF1), CCL11, fibroblast growth factor-5 (FGF-5), LIF-R, IL10, IL-10RA, IL-15RA, beta NGF, CCL28, CCL25 and NT-3 that were present at significantly higher concentrations in the Cirrh-to-HCC patients after correcting for multiple comparisons.

(Figure 1a,b). In addition, FGF-23, FGF-19, MMP-10 and Flt3L displayed a trend of superior pretherapy plasma concentration in the Cirrh-to-HCC cohort. We further discovered IL-12B (an upregulated SIM) and the stem cell factor (SCF) (a downregulated SIM) as the only two SIMs that were rather significantly lower in the Cirrh-to-HCC compared to the Cirrh-to-NoHCC cohort (Figure 1c).

Further calculating the area under the receiver operating characteristic curves (AUROCs), three of the 16 elevated SIMs showed

TABLE 1 Individual patient characteristics

Patient	Sex	Age	HCV gen.	Baseline MELD score	Child	DAA regimen	Treatment outcome	HCC status	Time from initial HCC diagnosis to DAA therapy start (months)	HCC treatment prior DAA therapy	Time to HCC after DAA therapy (months)	Characteristics of current HCC	HCC treatment after DAA
HCC 1	M	80	1b	9	A6	SOF/LDV	SVR	Recurrence	23	RFA, PEI	1	1 Nodule, 30 mm, BCLC A2	Atypical liver resection
HCC 2	M	65	1a	9	A6	SOF/LDV/RBV	SVR	De novo	n/a	n/a	1.5	1 Nodule, 23 mm, BCLC A2	RFA
HCC 3	F	47	1b	7	B7	SOF/SIM	SVR	De novo	n/a	n/a	2	1 Nodule, 24.6 mm, BCLC A4	Liver segment resection
HCC 4	M	63	1a	10	A5	SOF/RBV	SVR	De novo	n/a	n/a	3	1 Nodule, 26.3 mm, BCLC A3	Microwave ablation
HCC 5	M	61	3a	14	B7	SOF/RBV	Relapse	De novo	n/a	n/a	4	1 nodule, 24 mm, BCLC A3	TACE, RFA
HCC 6	M	77	1b	10	B7	Abb3D/RBV	SVR	Recurrence	23	RFA	10	1 Nodule, 6.7 mm, BCLC A2	RFA
HCC 7	F	51	1a	8	A5	SOF/RBV	Relapse	Recurrence	4	RFA, TACE	12	4+ Nodules, >30 mm, BCLC B	TACE, RFA 4 months later
HCC 8	M	50	3a	20	C12	SOF/RBV	Relapse	De novo	n/a	n/a	22	2 Nodules, 12.2 mm, BCLC A4	RFA
HCC 9	F	54	1a	11	B8	SOF/LDV/RBV	SVR	De novo	n/a	n/a	9	1 Nodule, 42 mm, BCLC A2	TACE
HCC 10	M	62	1b	7	A6	SOF/LDV	SVR	De novo	n/a	n/a	0.5	1 Nodule, 28 mm, BCLC Al	Atypical liver resection
HCC 11	M	51	1a	8	A6	SOF/RBV	SVR	De novo	n/a	n/a	23	1 Nodule, 87 mm, BCLC C	None
HCC 12	F	74	1b	8	A5	SOF/DAC	SVR	De novo	n/a	MWA	n/a	1 Nodule, 15 mm, BCLC 0	PEI
HCC 13	M	71	1a	8	A6	SOF/LDV	SVR	Recurrence	8	TACE	n/a	Multilocular, BCLC B	TACE
HCC 14	M	61	1b	7	A5	Abb3D/RBV	Partial-response	De novo	n/a	n/a	4	1 Nodule, 12 mm, BCLC 0	Atypical liver resection
HCC 15	M	46	3a	15	B8	SOF/RBV	Relapse	De novo	n/a	n/a	24	2 Nodules, max. 20 mm, BCLC A4	TACE

(Continues)

TABLE 1 (Continued)

Patient	Sex	Age	HCV gen.	Baseline MELD score	Child	DAA regimen	Treatment outcome	HCC status	Time from initial HCC diagnosis to DAA therapy start (months)	HCC treatment prior DAA therapy	Time to HCC after DAA therapy (months)	Characteristics of current HCC	HCC treatment after DAA
Control Cirrh-to-NoHCC Patients													
Cirrh 1	M	59	Ib	13	B9	SOF/SIM	SVR						
Cirrh 2	M	68	Ib	11	A5	SOF/RBV	SVR						
Cirrh 3	F	68	1b	7	A5	SOF/RBV	SVR						
Cirrh 4	M	48	1a	9	A5	SOF/SIM	SVR						
Cirrh 5	F	53	1b	11	A6	SOF/SIM	SVR						
Cirrh 6	M	53	3a	12	A5	SOF/RBV	SVR						
Cirrh 7	M	59	1a	11	A6	HAR + RBV	SVR						
Cirrh 8	M	54	1b	9	A5	SOF/DAC	SVR						
Cirrh 9	F	46	3a	10	A6	SOF/RBV	SVR						
Cirrh 10	F	61	1b	9	B7	SOF/RBV	SVR						
Cirrh 11	F	64	1b	10	A6	Abb3D/ RBV	SVR						
Cirrh 12	F	63	1b	8	B7	Abb3D/ RBV	SVR						
Cirrh 13	M	63	1b	7	A5	HAR + RBV	SVR						
Cirrh 14	M	42	1b	8	A5	HAR + RBV	SVR						
Cirrh 15	M	41	1a	8	A6	HAR	SVR						
Cirrh 16	F	56	3	-	B	SOF/RBV/ pIFN	SVR						

Abbreviations: BCLC, Barcelona clinic liver cancer; Cirrh-to-NoHCC, cirrhosis not developing HCC; DAA, direct-acting antiviral; DAC, daclatasvir; gen., genotype; HAR, harvoni; HCC, hepatocellular carcinoma; LDV, ledipasvir; MELD, Model for End-stage Liver disease; MWA, microwave ablation; n/a, not applicable; PEI, percutaneous ethanol injection; RBV, ribavirin; RFA, radiofrequency ablation; SIM, simeprevir; SOF, sofosbuvir; SVR, sustained virologie response; TACE, transcatheter arterial chemoembolization.

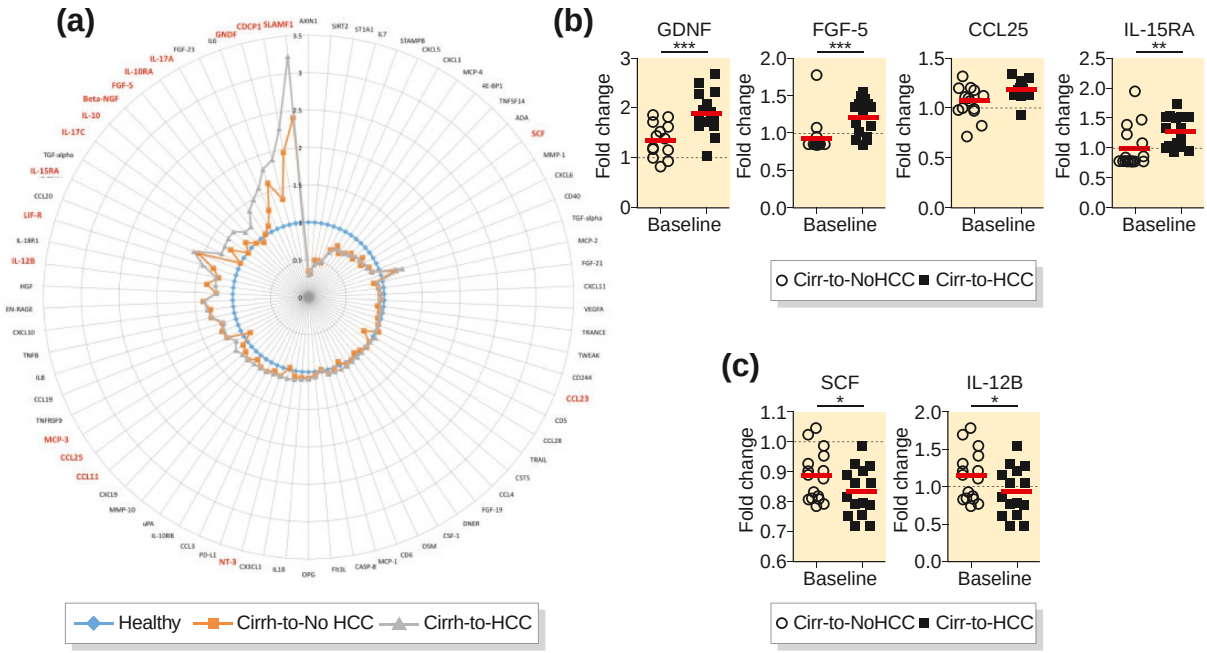

FIGURE 1 Plasma concentrations of soluble immune mediators (SIMs) at baseline. (a) Comparative pretherapy SIM concentrations of cirrhosis developing hepatocellular carcinoma (Cirrh-to-HCC) and cirrhosis not developing hepatocellular carcinoma (Cirrh-to-NoHCC) patients relative to healthy controls. Significant variables are indicated in red. (b) Representative graphs of individual SIMs with increased concentrations at baseline in Cirrh-to-HCC patients with statistical $p \geq 0.005$. (c) Individual SIMs that displayed significantly lower concentrations at baseline in the Cirrh-to-HCC compared to Cirrh-to-NoHCC cohort

values above 0.80 (Figure 2a). We further evaluated the potential correlation between baseline plasma SIM levels and the emergence of recurrence and de novo HCC upon DAA treatment initiation. Overall, nine of the 16 SIMs (i.e., GDNF, IL-17A, IL-15RA, SLAMF1, CCL11, IL10-RA, LIF-R, IL10, CCL28) that showed elevated concentrations in the Cirrh-to-HCC patients together with other SIMs such as CCL4 and the delta and Notch-like epidermal growth factor-related receptor were particularly present at higher concentrations at baseline in the patients that developed de novo HCC (Figure 2b). Here too, the three SIMs that displayed an AUROC above 0.8 were GDNF (0.9156), IL-10RA (0.8071) and IL-15RA (0.8121) (data not shown). Notably, while SIMs such as CCL25, CDCP1 and IL-17C were significantly higher in both recurrence and de novo HCC (Figure 2c), the plasma concentrations of IL-17C and the FGF-5 and FGF-23 were particularly higher in recurrence HCC with FGF-5 in particular having an AUROC of 9333 (Figure 2d).

Successful DAA therapy did not alter the baseline plasma SIM profiles in cirrhosis patients that developed posttherapy HCC

To further assess the possible existence of a differential regulation of distinct SIMs during IFN-free treatment of hepatitis C in relation to HCC emergence, we studied the plasma SIM kinetics in the two Cirrh-to-HCC and Cirrh-to-NoHCC patient cohorts (Figure 3). Plasma were longitudinally sampled at baseline (therapy start), end-of-therapy and follow-up (i.e., at least 12 weeks postsustained virologic response [SVR]) and the concentrations of the described SIMs measured. Patient Cirrh-16 who had received IFN-based therapy was exclude from the analysis. Patients Cirrh-15, HCC-1, HCC-9 and HCC-14 for whom no plasma samples were available at end-of-therapy and/or follow-up were also excluded from the analysis. We observed that while the HCC-free control cohort experienced significant therapy-mediated SIM reductions of some $n = 38$ SIMs at end-of-therapy and/or follow-up (Figure 3b), those in the eventual HCC developers remained fairly stable with only 16 SIMs experiencing relatively partial but significant reductions (Figure 3a,b). But for GDNF which dipped at end-of-therapy and even restored at follow-up, the SIMs that showed comparatively elevated pretreatment concentrations in the Cirrh-to-HCC patients remained stable all through the treatment period as well as follow-up (Figure 3a). In both cohorts, SCF, FGF-21 and FGF-23 showed significant increments upon therapy. Aside from the relatively reduced reductions in the plasma SIM concentrations of the Cirrh-to-HCC patients following DAA therapy, there were a considerable number of other SIMs (CCL25, TGF-alpha, CCL23, CST5, IL15RA, beta-NGF and FGF-5) that rather trended upwards compared to their counterparts in the Cirrh-to-NoHCC patients.

The few SIMs that appeared normalised in the Cirrh-to-HCC cohort seemed to emanate from the de novo rather than the recurrence HCC subgroup (data not shown). These results highlight an overall nonperturbation of the soluble immune compartment in

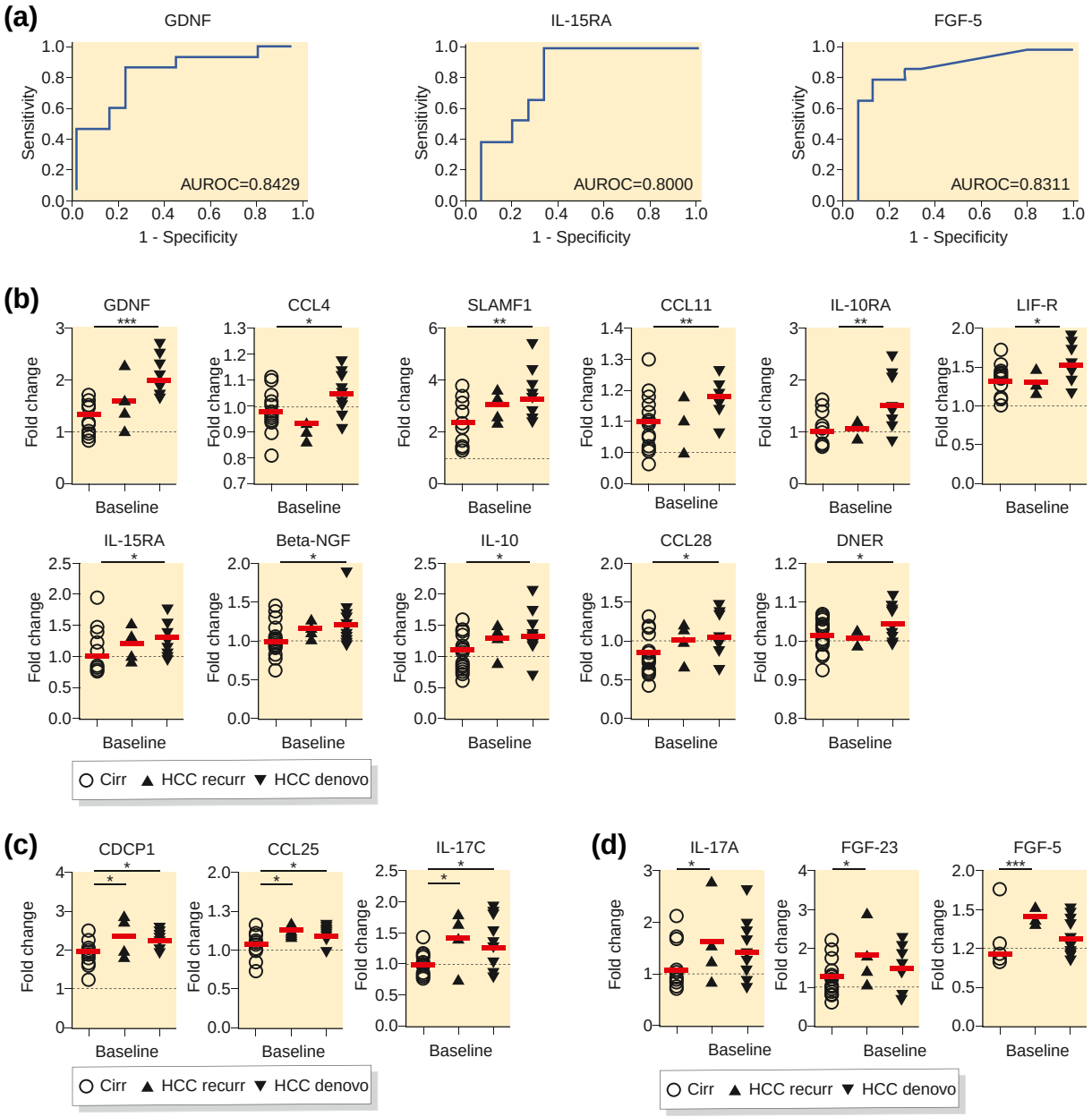

FIGURE 2 Association between baseline plasma soluble immune mediator (SIM) levels and the emergence of posttherapy hepatocellular carcinoma (HCC). (a) Area under the receiver operating characteristic curve (AUROC) of markers of HCC development with values of >0.800 as compared to non-HCC control group. (b) Baseline plasma SIMs with concentrations higher exclusively in de novo HCC. (c) Plasma SIMs at baseline that showed concentrations significantly higher in both recurrence and de novo HCC alike. (d) Baseline SIM concentrations higher exclusively in recurrence HCC

patients who developed HCC contrary to those who remained HCC-free upon DAA treatment.

DISCUSSION

In this plasma screening of a large repertoire of SIMs relative to HCC emergence upon IFN-free therapy for hepatitis C, we showed that a spectrum of pretreatment SIM expressions were highly elevated in patients who later developed HCC and distinguished them from those who did not. Furthermore, pretreatment SIM levels could differentiate de novo from recurrence of HCC emergence. Finally, we demonstrated for the first time that alteration in the inflammatory patterns during IFN-free HCV therapy differ in significant proportions between Cirrh-to-HCC and Cirrh-to- NoHCC patients.

Chronic unresolved inflammation is known to promote and exacerbate malignancies. A clear example is HCC, which has over 90% of its cases arising in the context of liver injury and inflammation. Our study here suggests a pre-existing hyperactivated profile in Cirrh-to-HCC patients as evidenced by elevated baseline

FIGURE 3 Effect of interferon (IFN)-free direct-acting antiviral (DAA) therapy on plasma soluble immune mediator (SIM) levels. Fold changes in plasma SIM concentrations of the cirrhosis patient cohorts that upon DAA therapy either developed HCC (Cirrh-to-HCC) or remained without any HCC (Cirrh-to-NoHCC) were calculated at end-of-therapy (EOT) and follow-up (FU) time-points in reference to baseline values. (a) Plasma SIM kinetics of Cirrh-to-HCC patients from therapy start (TS) through EOT to FU. (b) Plasma SIM kinetics of Cirrh-to-NoHCC patients from TS through EOT to FU. A one-way analysis of variance with the recommended Geisser–Greenhouse correction was used for statistical evaluations. Significant variables are indicated in red

concentrations of a myriad of SIMs. To the best of our knowledge, these findings show for the first time that elevated pretherapy SIM concentrations (such as those of GDNF, FGF-5 and IL-15RA) could serve as independent predictive biomarkers for HCC emergence, and distinguish Cirrh-to-HCC from Cirrh-to- NoHCC patients regardless of de novo or recurrence classification. A prior example suggestive of an association between pretherapy serum SIM levels and the emergence of de novo HCC upon DAA treatment in patients with HCV infection has recently been shown.[15] Our data here drawn from Olink's innovative multiplex technology fairly confirms this observation, although with a different set of SIMs aside eotaxin (CCL11). This study thus extends the spectrum of SIMs whose baseline concentrations and possible networks could be linked with HCC emergence in cirrhosis patients receiving DAA therapy for hepatitis C. Whether the activated immune system is a cause or consequence of therapy-related HCC emergence is an intriguing concern. The results from our data here however seem to suggest the latter as the more plausible option. The elevated pretreatment SIM profile existent before the induction of any therapy-mediated immune changes could indicate a systemic response of already ongoing immune surveillance against early malignant lesions. This systemic inflammation could in turn lead to tumour-specific CD8+ T-cell functional exhaustion, or inhibition as exemplified by the increased pretherapy anti-inflammatory cytokine IL- 10. IL-10 is a critical immune regulatory molecule, which can deregulate cytokine production and T-cell proliferation and has a modulatory effect on hepatic

fibrinogenesis.[16,17] In the midst of the pretherapy hyper-immune profile which characterised the Cirrh-to-HCC patients, the concomitant elevated levels of IL-10 and its receptor IL10RA at baseline was not surprising. In this setting, IL-10 levels may play a role in tuning the prevailing proinflammatory SIM ensemble whiles its engagement with elevated IL10RA may directly inhibit CD8+ T-cell function as shown before[18] and tilts the existing HCC antitumour T-cell surveillance establishment out of balance. Together with the notable reduction in the immuno-stimulatory cytokine IL-12B, this may culminate in the reduced functionality of HCC-specific immune-surveilling T cells, which may favour HCC progression, as we demonstrated recently.[11] This assertion lends credence from evidence suggesting that tumour cells have the capacity to secrete distinct SIMs to foster their growth and metastasis as well as subverting hosts' immune anti-tumour reactions.[19,20]

Aside from IL-10, the SLAM molecules are known for their roles in regulating immune responses, pathophysiology of neoplasm transformations and entry pathways of certain viruses.[21] Recently, SLAM molecules have come to the fore for their potential in diagnosis and therapy of various cancers. The SLAMF1 in particular is the prototype member of the SLAM family of molecules that initiates signal transduction networks in many immune cells including T and B cells, dendritic cells, monocytesmacrophages, natural killer cells and natural killer T cells that constitutively express them, thus modulating their activation and differentiation.[22–24] In patients with colon cancer, for example, upregulation or silencing of SLAMF1 expressed in lymphocytes was reported to be accompanied by increased or reduced lymphocytic cytotoxic activity, respectively.[25] In our Cirrh-to-HCC patient cohort, the elevated SLAMF1 profile existent before DAA therapy initiation was an interesting observation in this regard. On the one hand, it fits very well into our assertion that the elevated SIM profile (as exemplified by SLAMF1) and characteristic systemic inflammation, may be a response to occult HCC in the liver. However, the characteristically higher SLAMF1 level in the patients who developed de novo relative to recurrence HCC and the observation that SLAMF1 levels stayed unaltered upon DAA therapy widen the dimension of plausible conclusions, and warrants further investigation.

Furthermore, assessing the potential immune correlates of IFN-free therapy-mediated HCV clearance with emergence of HCC by analysing longitudinal SIM kinetics, we confirmed our earlier report that innate SIM immunity may not necessarily normalise but experience strong reduction in the levels of specific SIMs in the cirrhosis control cohort that did not develop HCC.[26] Previous studies had reported a similar decline in the mean levels of the SIMs IP-10, MCP-1, MIP-1ß, IL-18[19] and also of CCL-2, CCL-3, CCL-4, CXCL-8, CXCL-10, IL-1b, IL-15, IFN-y, IL-4, IL-10, TGF-b, FGFb and PAI-1[20] following IFN-free antiviral therapy in patients with chronic hepatitis C.

Strikingly, however, no significant changes of such magnitude were observed in the cirrhosis patients who were treated with DAAs and eventually developed HCC. This relatively sustained release of specific SIMs in these patients indicates independent activation of the immune system of the HCV infection in such a setting. It further entrenches the assertion that the systemic inflammation may be a response to occult HCC in the liver as we earlier opined. On another score, it may be indicative of the mechanistic involvement of distinct SIMs in the emergence of HCC. This is of interest given the proven dysregulatory activity pretherapy SIMs such as MCP-3, GDNF, SLAMF1, CCL11, CCL25, beta NGF and NT-3 may have on the mitogen-activated protein kinase pathway. Further studies are thus required to address the paradox of whether the unaltered SIM expression is a cause or consequence of HCC development upon DAA therapy of hepatitis C.

On several scores, our findings in this study confirm and extend previous reports, especially by Debes et al.[8] and Villani et al.[9] However, one notable exception deserves a mention. The kinetics of plasma VEGF levels which were reported to be elevated until end-of-therapy before it normalised at SVR12 in chronic hepatitis C patients receiving IFN-free DAA therapy could not be confirmed. In our observation, VEGFA levels significantly declined in the cirrhosis patients that remained HCC-free posttherapy at both end-of-therapy and follow-up contrary to the unaltered levels in the patients who eventually developed HCC posttherapy, suggesting an association between VEGFA levels and HCC growth. On the contrary, other growth factors such as FGF-21, FGF-23 and SCF significantly increased in both cohorts in coincidence with HCV clearance whilst TGF-alpha, FGF-5, beta- NGF and other mediators such as IL-15RA, CCL25, CCL23 and CST5 all trended upwards exclusively in the Cirr-to-HCC cohort. The differences in observations could stem from the difference in sample size, peculiar cohort characteristics and the contributory role of the other members of the VEGF family that were nondiscriminatorily measured in Villani et al.[9] compared to our assay which measured only VEGFA.

Our study has obvious strengths and limitations. The major strengths include the (a) prospective collection of samples, (b) large panel of SIMs analysed, (c) unbiased approach of SIM profiling and (d) homogeneous treatment cohort and follow-up for screening. Notwithstanding, the number of HCC cases which still remains small and the accompanying small fractions of recurrence and de novo cases are the notable limitations. As a next step in this project, we aim to conduct a large prospective dedicated study, which will address the shortcomings of these findings and thus provide a better insight into the pretherapy HCC immuno-surveillance establishment and the changes it experiences following DAA-induced HCV cure.

In summary, we discovered that the pretreatment activation profile of the soluble immune compartment as measured by highly elevated SIM patterns correlates with posttherapy HCC development. We further demonstrate how the elevated SIM establishment existent before DAA therapy stays unperturbed upon DAA therapy in patients who develop posttherapy HCC contrary to those who remain HCC-free. These findings provide an important basis for a potential build-on to unravel cases where the benefit of DAA therapies would be unequivocally superior to the risk of developing HCC. Attainment of this feat would potentially contribute to improving the management of HCC and the quality of life of patients with chronic hepatitis C.

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CONFLICT OF INTERESTS

Solomon O. Sekyere and Kerstin Port have no conflicts of interest to declare. Katja Deterding has received lecture fees from Gilead, AbbVie and Merck. Markus Cornberg has received lecture fees from AbbVie, Bristol-Myers Squibb, Boehringer Ingelheim Pharma, Gilead, Janssen-Cilag, MSD Sharp & Dohme/Merck, Roche Diagnostic, Roche Pharma and Siemens; advisory board fees from AbbVie, Bristol Myers Squibb, Boehringer Ingelheim Pharma, Gilead, Roche Diagnostic and Roche Pharma; and data safety board fees from Janssen-Cilag. Heiner Wedemeyer has received grants from AbbVie, Gilead, Roche, Roche Diagnostics, Abbott, Myr and Eiger, and consulting fees or honoraria from AbbVie, Abbott, BMS, Boehringer Ingelheim, Eiger, Gilead, Janssen, Novartis, MSD/Merck, Roche, Roche Diagnostics and Transgene. In addition, Heiner Wedemeyer has received money for board memberships from AbbVie, Abbott, BMS, Boehringer, Eiger, Gilead, Myr, Novartis and Roche; honoraria for consultancy or speaking from Eiger, Janssen, Siemens, Abbott, AbbVie, Biolex, BMS, Boehringer Ingelheim, ITS, JJ/Janssen-Cilag, Medgenics, Merck/Schering-Plough, Novartis, Roche, Roche Diagnostics, Siemens, Transgene, ViiV; and for lectures, including service on speakers' bureaus, from the Falk Foundation and OmnisMed.

ETHICS APPROVAL

The study protocol was approved in year 2014 by the ethics committee of Hannover Medical School (Study numbers: 2148-2014 and 2604-2014) and conducted as per the Helsinki declaration. All patients who participated in this study gave their written informed consent. All patients who participated in this study gave their written informed consent.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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