Short Communication

Persistence of Fibrogenic Signals in DAA-Treated HCV Patients Inspite of SVR

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DESCRIPTION

Long-term replication of Hepatitis C Virus (HCV) causing inflammation and tissue damage contributes to trigger a fibrogenic process, eventually leading to hepatic cirrhosis and Hepato Cellular Carcinoma (HCC). The interferone-free Direct-Acting Antiviral (DAA)-based therapy efficiently allows to reach the SVR (Sustained Virologic Response) in above 90% in clinical trials and provides a therapeutic opportunity for people in clinical practice [1,2]. Emerging clinical data from cirrhotic patients treated with DAA stirred a heated debate about the risk of HCC occurrence and recurrence after viral cure [3]; evidence suggests that virus eradication reduces the risk of De Compensated Cirrhosis (DCC) in non-cirrhotic patients and of HCC in cirrhotic one [4-8]. It is, on the other hand, clear that HCV clearance does not always result in healing of liver disease and that cancer risk persists even after viral clearance; several risk-predictive host and comorbidity factors (e.g. older age, advanced liver fibrosis, diabetes, and hepatic inflammation) have indeed been suggested [7].

Concerning liver fibrosis, recent studies, as yet limited, reported that DAA ameliorates the clinical picture in the long-term (48-144 weeks of treatment); this shall encourage further studies to elucidate the mechanism underlying the regression. Indeed, the dissection of molecular mechanisms and pathways involved in this process, now limited to matrix metalloproteinases produced by macrophages [9] or by Specialized Pro-Resolving Mediators (SPMs) [10], represent an exciting challenge in the field to be pursued [11].

Improving patients clinical management implies the identification of prognostic and diagnostic markers as predictive or even causal biomarkers whose quantification can be directly related to fibrogenesis or to its reversal. In particular, new prognostic tools could also clarify the contribution of cell-cell interactions in fibrosis onset and development could highlight etiology-specific elements, thus allowing patient long-term proper surveillance.

Noninvasive fibrosis diagnostic approaches that include liquid biopsy are rapidly increasing with the intent to supplant the more invasive liver biopsy. In this scenario, the characterization of Extracellular Vesicle (EV) cargo in relation to normal and pathological conditions appears instrumental. This was applied to the identification of specific microRNAs present in EVs from HCV patients' samples and modulated by the new DAA-based therapy [12]. Notably, a different EV content in terms of microRNAs correlates with the EV-mediated natural killer cell degranulation capability.

In our recent paper, we coupled functional and structural analysis of plasma-derived Extra Cellular Vesicles (EVs) isolated from Healthy Donors (HD) to HCV-infected patients before (T0) and after DAA-mediated SVR (T6) in a longitudinal analysis. The persistent identified differences appear most likely causal to fibrosis progression.

We found (i) functional differences: in measuring fibrogenic markers of receiving cells, we highlighted as the HD-derived EVs clearly display different effects from patient-derived EVs. Moreover, we found (ii) structural differences in miRNA cargoes: Antifibrogenic miRNAs were found to be significantly under-represented in both T0 and T6-derived EVs with respect to HD.

The functional EV analysis was performed on LX2 cells, a hepatic stellate cell line that, during chronic HCV infection, Trans differentiate into highly proliferative myofibroblast-like cells expressing inflammatory and fibrogenic mediators responsible for Extra Cellular Matrix (ECM) accumulation and contributing to the fibrotic process that leads to cirrhosis and liver failure in advanced stages. In parallel, EV structural analysis showed that EVs derived from HCV-infected patients, with respect to EVs derived from healthy donors, up regulate stellate cell activators (e.g. of DIAPH1) and downregulate antifibrogenic miRNAs (e.g., miR204-5p, miR93-5p, miR143-3p, miR181a-5p, and miR122-5p). Notably, longitudinal analysis highlighted the persistent pro-fibrogenic activity of EVs despite DAA-mediated HCV eradication; this is in correlation with the EV informational content [13].

Regarding miRNAs, their causal mechanistic anti-fibrotic role has been verified by their overexpression by mimic approach. Of note, since our result highlighted their lack in both T0 and T6-derived EVs with respect to HD, efforts in line with other RNA-based therapies can be suggested.

Regarding structural differences in protein cargoes, we found that among 393 mass-spectrometry detected proteins, 74 were found differentially expressed between HD and HCV-derived EVs. Notably, the top overexpressed proteins in T0 and T6-derived EVs include several known liver fibrogenic/pathogenic inducers (e.g. diaphanous homolog 1, DIAPH1, validated by western blot). Overall, employing the combination of structural and functional analysis, this study highlighted early noninvasive prognostic markers that assess long-term post-DAA clinical outcomes. The significance of other here identified markers is as yet not fully addressed. As usual for big data approaches, the causal significance of single identifications is completed only for a few of them. For instance, in our case, the list of the identified proteins found up regulated in T0/T6 versus HD includes several ones that literature data mining suggests of potential implications in pathological processes (Table 1) and that demand further studies; we remain in the hope that researchers working on this issue could explore their role, thus giving more significance to our initial observation. More in general, studies focusing on cellular plasticity driving cell/tissue changes in epithelial tissues [14] and maintaining cell survival shall be extended in addressing the molecular basis of the reversibility/irreversibility of liver fibrosis and to the balance between fibrosis regression and liver regeneration.

Gene names	Protein names	Pathophysiological function	References
		Highly expressed in Hepato Cellular Carcinoma (HCC); it contributes to Cancer Cell Growth and predicts a negative prognosis In HCC	PMID: 34500119
CCT6A	T-complex protein 1 subunit zeta		PMID: 31819524
			PMID: 33897772
WDR1	WD repeat-containing protein 1	Involved in chemotactic cell migration, it predicts poor prognosis and promotes cancer	PMID: 31949654
		progression in HCC	
CAND1	Cullin-associated NEDD8-dissociated protein 1	Overexpressed in HCC and associated with poor overall survival	PMID: 29887951
CAP1	Adenylyl cyclase-associated protein 1	Involved in filament dynamics and cell polarity and associated with tumor migration and metastasis in HCC	PMID: 24359721
CRKL	Crk-like protein	Essential roles in fibroblast structure, motility ,transformation and HCC development	PMID: 24166500
			PMID: 33523562

Table 1: Up regulated proteins identified in HCV-T0/T6 (with respect to HD).

CONCLUSION

On the basis of observations indicating functional differences (i.e. modulation of *FN-1, ACTA2* phosphorylation, collagen deposition) of plasma-derived EVs from HDs, T0 and T6, we performed structural analysis of EVs. These results highlight structural EV modifications that are conceivably causal for long-term liver disease progression in patients with HCV despite DAA-mediated SVR.

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