Editorial Commentary

Commentary on "*Fibrogenic signals persist in DAA-treated HCV patients after sustained virological response*"

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A combination of direct-acting antivirals (DAAs) now cures hepatitis C virus (HCV) infection in most patients. The HCV cure will decrease the overall incidence of liver disease progress, liver cirrhosis, hepatocellular carcinoma (HCC) (1,2). After viral treatment, the risk of persistent liver disease remains among those with advanced liver fibrosis stages (3-5). There is a continued discussion about the risk of HCC occurrence and recurrence after HCV cure among cirrhotic patients. It is a significant health concern since the cured cirrhotic patient population will be at a risk of cancer development. Many viral cure patients will have overlapping liver diseases related to non-viral etiologies. These patients require long-term life surveillance since the mechanisms of liver disease progression after HCV cure remains elusive. The development of non-invasive serum biomarkers for early detection and treatment of HCC is needed. In this scenario, understanding the mechanism of virus and host interaction that dictate the reversible and irreversible changes during the advanced stage of liver disease will allow better surveillance after DAA treatment.

Hepatitis C virus is an RNA virus. This virus replicates exclusively in the cytoplasm of hepatocytes. The sustained viral replication in the hepatocytes generates a multifaceted stress response called integrated stress response (ISR) (6). The inflammation process initiated during persistent virus infection helps cell survival and protects against disease. Excessive inflammation results in tissue damage and contributes to the HCV fibrogenesis process. This ISR is a cellular signaling pathway that helps infected hepatocytes to adapt to multifaceted stress. That includes the unfolded protein response, innate immune anxiety, metabolic and oxidative stress. A cellular adaptive program is used by all eukaryotic cells to respond to short-term or long-term chronic stress. That results in both reversible and irreversible shutdown of cellular proteomes and transcriptomes. The adaptive process induces distinct cell programming at the level of mRNA splicing, protein and RNA transport, cytoskeleton, metabolism, stress granule factors, protein biosynthesis (7). Reversible adaptive programming after short-term stress [heat shock, oxidative stress, osmotic stress, ultraviolet (UV) stress] induces cell programming at the level of mRNA and protein transport. Likewise, the cellular stress support pathway operates through cellular reprogramming during chronic HCV infection also occurs at the level of translation, RNA splicing, metabolisms, epigenetics changes. This irreversible adaptive plasticity leads to neoplastic changes and HCC development in the cirrhotic liver (8). The ISR down-regulates normal protein synthesis while promoting the translation of genes essential for cell survival. The hepatic adaptive plasticity in the microenvironment in the cirrhotic liver enables HCC development. This could result in more mRNA and miRNA downregulation than upregulation.

It will be considered a physiological adaptation if the adaptive plasticity is reversible after the HCV cure. In this scenario, the patients will heal the injury and recover from the liver damage over time. If the adaptive plasticity is irreversible after HCV cure, we call it pathological

adaptation. Liver cirrhosis is a pathological adaptation to HCV microbial stress since the robust expression of stress chaperones is seen in the cirrhotic liver (9). The HCV-induced stress changes in the hepatocyte's plasticity in the cirrhotic liver resulted in impaired collagen deposition clearance. HCV cure by DAA treatment does not always restore that stress response. Extracellular vesicles (EVs) could mirror the remarkable molecular reprogramming of infected hepatocytes in the cirrhotic liver; therefore can predict the degree of cellular plasticity and hepatic dysfunction after HCV cure (10).

The article published by Montaldo *et al.* in the Journal of hepatology (11) attempted to address this outstanding question using a group of chronic HCV patients who received DAA treatment. They select 39 chronic HCV-infected patients in a longitudinal study to study the reversible and irreversible changes after DAA treatment. The study included 32 healthy donors. EVs were affinity purified from plasma using microbeads coupled with antibodies to CD9, CD63, and CD81 proteins. The authors have purified EVs from 15 healthy donors; and 16 HCV- infected patients before and after 6 months of viral cure by DAA treatment. The study determined the success of EVs-based biomarkers as an early assessment of fibrosis progression or regression after DAA treatment. This paper presents convincing data showing that fibrogenic signal persists up to 6 months after HCV cure.

Initially, the authors performed a functional analysis *in vitro* cell culture using LX2 stellate cells. Activated stellate cells (HSC) play a critical role in the fibrogenesis process in liver cirrhosis. The co-culture of the hepatocyte-HSC model used for EV functional studies. The data presented in this paper show when hepatocyte-HSC cells co-culture treated with EVs from healthy individuals decreased the expression of fibrogenic markers (fibronectin 1, ACTA2, COL1α1, and TGFβ1) at the mRNA and protein levels. A similarly reduced fibrogenic genes expression was not observed with EVs derived from HCV patients. They found the face of these fibrogenic markers is induced and not restored after HCV cure by DAA treatment. Notably, the generated expression levels of FN1 and pSMAD 2/3. The authors showed that the expression of this fibrogenesis is not restored for 6 months after DAA treatment even after these patients showed decreased expression of liver enzymes (ALT, AST, and gGT). These results suggest that the fibrogenic response was induced by HCV infection not restored after DAA treatment.

The authors performed a more detailed functional analysis of exosome cargoes derived from regular healthy and HCV-infected patients before and after DAA treatment. They examined whether upregulated expression of these fibrogenic factors is due to gene silencing. They looked at the expression level of 12 miRNA in the EV preparation derived from HCV-infected patients. They found explicitly miRNA-204-5p, miRNA-181a-5p, miRNA-143-3p, miRNA-93-5p, and miRNA-122 levels are decreased significantly in the EVs from HCV-infected patients. These results are consistent with prior reports by other groups and us indicating expression of miRNA-122 depleted due to stress. The expression levels of miRNA-204-5p and miRNA-143-3p were not restored. To understand the cause-effect relationship, they showed that overexpression of miRNA restored the mRNA expression. They presented data indicating that when the miRNA mimics were transfected to LX2 cells resulted in decreased expression of fibrogenic mRNA (FN-1, ACTA2, CoL1α1, TGFβ1, TgFβR1, CTGF). These data suggest lack of restoration of expression of selected miRNA after HCV cure by DAA treatment may have a role in fibrosis progression.

In the final step, the authors have performed the proteomics of EV-cargoes by liquid chromatography-mass spectroscopy (LC-MS) to identify whether specific proteins expression can reflect the potential biomarker for liver disease progression after HCV cure. They found 74 proteins induced in HCV-infected patients as compared to controls. Cell membrane and cytosolic proteins are enclosed more in the exosomes. Among those, DIAPH1 is expression remained high in HCV infected, and its levels remained elevated 6 months after DAA treatment.

A recent study reported by De Battista *et al.* at the NIH (12) shows that the development of HCV-induced HCC resulted in significant gene downregulation. More than 74% of genes are down-regulated in HCV-induced HCC, suggesting many of the genes expression needed for terminal differentiation of hepatocytes are lost during HCC development in liver cirrhosis. The article did not look for the loss of specific proteins or mRNA genes in DAA-treated EV compared to healthy control. It may provide another set of tumor suppressor biomarkers that are lost during HCV infection. The expression levels of those genes after viral cure may provide more accurate information about the extent to which genes needed for optimal hepatic function are restored after the HCV core. Nevertheless, this is an outstanding work that opens the potential for a novel biomarker platform for predicting persistent liver cirrhosis and HCC risk after HCV cure.

In conclusion, this paper provides evidence suggesting

that EVs and their cargo hold a strong potential for biomarker development to assess liver fibrosis progression after DAA treatment. This work provides a step forward path for discovering novel EVs-based prognostic and diagnostic biomarkers to monitor liver disease related to viral and non-viral etiologies. This study shows a proof-of-principle concept of EVs-based surveillance that can be utilized in the liver clinic for early detection of HCC among patients with liver cirrhosis.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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