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LETTER TO THE EDITOR

Sofosbuvir directly promotes the clonogenic capability of human hepatocellular carcinoma cells

Dear editors,

The recent launch of several types of direct-acting antiviral agents (DAAs) has opened a new scenario for treating chronic hepatitis C virus (HCV) infection. Extremely satisfactory sustained virological response (SVR) rates have been achieved in DAA treated HCV patients, leading to the extensive use of these regimens worldwide [1]. Because HCV is one of the leading causes of hepatocellular carcinoma (HCC), successful eradication of the infection is expected to dramatically reduce the risk of HCC development in these patients. Counterintuitively, several recent studies have reported an unexpected high rate of HCC development after DAA treatment [2,3]; whereas others did not observe such a risk [4]. However, it remains challenge to make definitive conclusion on this issue because of the heterogeneous populations and methodologies applied in the different studies.

Regardless of this ongoing debate, a popular hypothesis has emerged that tumor development is likely attributed to the indirect effect of DAA treatment by disrupting cancer immunosurveillance, for instance through decreasing natural killer (NK) cell activation and inhibiting its cytotoxic function [5]. These immunological changes could be responsible for reduced immunosurveillance of neoplastic clone growing and spreading. Interestingly, a recent study [6] has profiled the levels of immune mediators including cytokines, growth factors and apoptosis markers in serum of HCV patients treated with DAA and studied the association with the development of HCC. They observed that the indirect effect of immune modulation by DAAs may have little impact on HCC development, although the immune background before treatment could already have a potential effect. In contrast, we have investigated whether DAAs have direct effect on HCC cells.

Sofosbuvir (SOF), targeting the HCV RNA-dependent RNA polymerase, is widely used DAAs for HCV treatment. Importantly, most reported cases with HCC development were treated with SOF-based regimens. To evaluate the direct effect, four human HCC cell lines including Huh7, Huh6, HepG2 and SNU449 were treated with serial concentrations of SOF (0, 0.01, 0.1, 1 μM), which are clinically relevant.

As expected, SOF potently inhibited HCV replication in Huh7-based subgenomic replicon (Supplementary Figure 1). No major effect was observed on the growth of bulk of HCC cells by SOF treatment for 48 or 72 hours determined by MTT and Alamar blue assays (Supplementary Figure 2 and 3). But, surprisingly, SOF increased single cell-based clonogenic capability in all four HCC cell lines. This is reflected by the significantly increased number and size of formed colonies (Fig. 1). In contrast, placebo treatment has no such effect (Supplementary Figure 4).

Thus, we have clearly demonstrated a direct promoting effect of SOF on single HCC cell-based clonogenic initiation and expansion, but not on the growth of the bulk of HCC cells. We interpret that these unexpected results may bear important implications in explaining the clinical observations. In fact, higher risk of HCC development has been mostly observed from HCV patients with advanced diseases (e.g. cirrhosis), [3] or previously treated for HCC (e.g. ablation, resection, chemoembolization [2] or liver transplantation [7]). Although these studies are often blamed for the bias in selection of these particular patient groups, our results however may indicate a direct promoting effect of DAAs on the rare preexisting transformed tumor cells in the cirrhotic liver, the residual HCC cells that are not completely eradicated by treatment, or the circulating tumor cells in the transplant patients. Despite the low number of these tumor cells, they are likely resemble the so called cancer stem cells that are resistance to chemo- or radiotherapy but responsible for tumor initiation, treatment relapse and recurrence after surgical operation [8]. Thus, DAAs are likely not to have a universal but rather specific effects on particular patients in respect to the risk of HCC development; whereas the current clinical studies are unable to fully resolve the ongoing debate. Nevertheless, neither our findings nor the previous study6 shall exclude the possibility of an indirect effect of DAAs on triggering HCC development, as tumor micro-environment was a complicated and evolving field. We believe that the joint efforts of future experimental and clinical research are necessary to clarify this alarming issue and to gain mechanistic insight.

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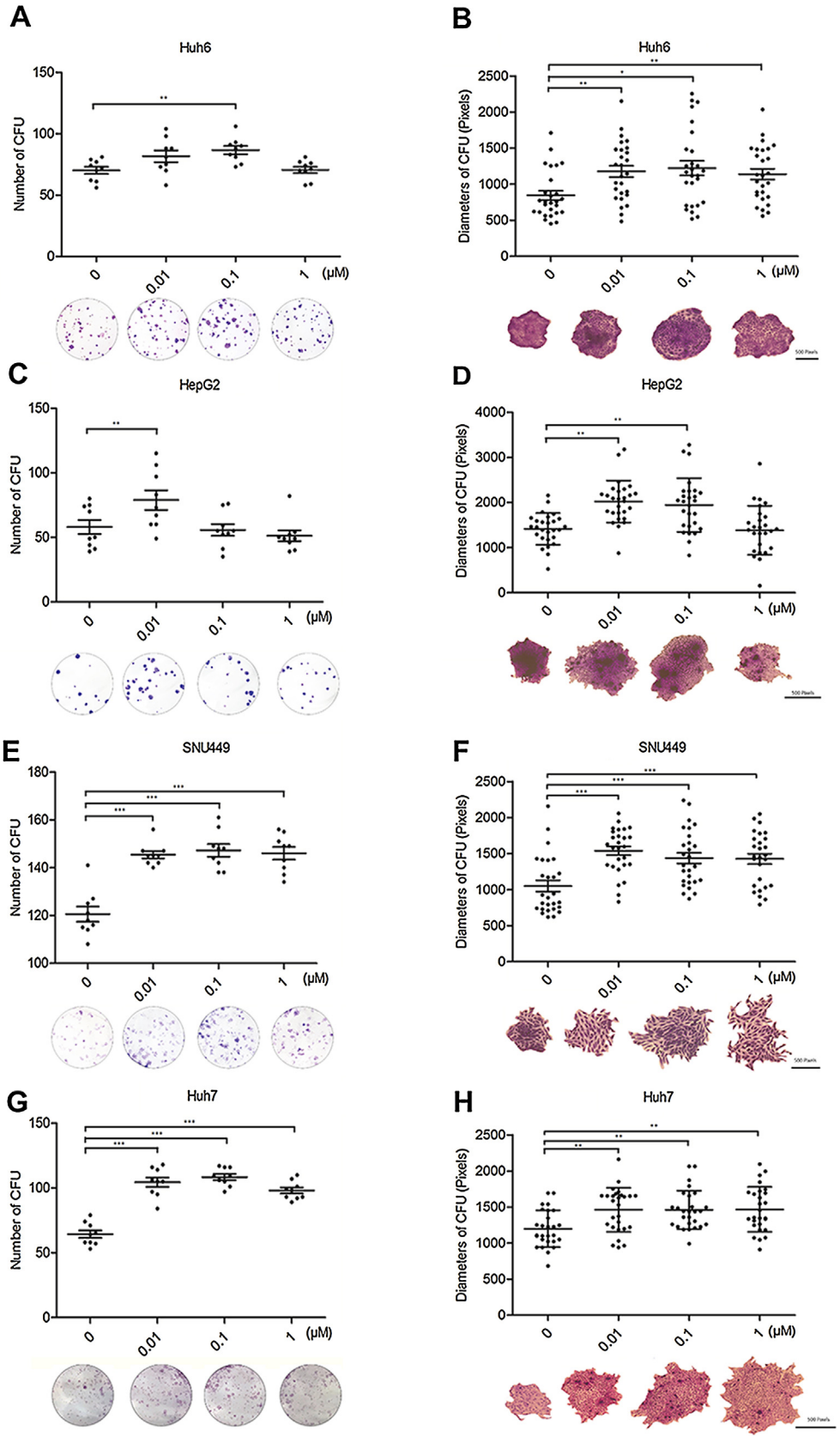

Figure 1 Sofosbuvir (SOF) treatment promotes the clonogenic capability of human hepatocellular carcinoma cells. A, C, E, F. The number of colonies/1000 cells of HepG2, SNU449, Huh6 and Huh7 after SOF treatment (mean ± SEM, $n = 9$); B, D, G, H. The diameters of colony formation units. Every three random colonies from nine independent wells were measured (mean ± SEM, $n = 27$). Mann—Whitney test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.clinre.2018.11.016

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