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Reply to: "Arginase 1: a potential marker of a common pattern of liver steatosis in HCV and NAFLD children"

Arginase, NASH and HCC: What role for macrophages?

To the Editor,

We thank Alisi and colleagues for their comments [1] on our recent report characterizing the effects of the hepatitis C virus (HCV) proteins on metabolic liver zonation and steatosis [2]. In a transgenic mouse model with hepato-specific expression of the full complement of HCV proteins we found that fatty acid synthase, a major lipogenic enzyme, was redistributed to the midzone of the lobule, coinciding with zonated accumulation of lipids. Based on these and additional results, we hypothesized that low levels of viral proteins are sufficient to drive striking alterations of hepatic metabolic zonation. However, not all genes that display zonated expression in the liver were altered in the animal model studied. Notably, we found no change in the pattern of expression of E-cadherin or arginase 1. In contrast to the results reported by Alisi *et al.* we did not study the global level of expression of the latter.

Alisi *et al.* compared arginase 1 expression in liver tissues from children with no hepatic pathologies or suffering from non-alcoholic fatty liver disease (NAFLD) or hepatitis C [1]. The quantification of the immunhistochemical staining showed identical mean intensity of arginase 1 in healthy livers and in HCV-positive patients without steatosis. This is consistent with our results showing no difference of arginase 1 expression in hepatocytes from control and HCV-transgenic mice [2]. In our work, appearance of steatosis did not alter arginase distribution. In contrast, Alisi *et al.* observed increased arginase 1 expression in HCV patients with steatosis and in NAFLD. Their results highlight the link between lipid accumulation and arginase expression, as well as the effect of HCV infection on arginase 1 accumulation. Noteworthy, Alisi *et al.* give no information on the HCV genotype, while our study focused solely on genotype 1. It would be of interest to determine whether the reported observations are genotype specific or more generalizable.

As Alisi and colleagues correctly pointed out, the role of arginase 1 in steatosis is not well understood and possibly underestimated. Interestingly, its increased expression has been described in HCV infection and it was suggested that it might participate in promoting cell growth and proliferation [3]. Indeed, arginase 1 converts arginine to ornithine, which is further metabolized to polyamines that promote cellular proliferation. Thus, elevated arginase 1 expression may be hepatoprotective during chronic infection by promoting survival and proliferation of HCV-infected hepatocytes. In consequence, the inflammatory immune response that contributes to liver damage would be inefficient in eliminating virus-infected cells.

Elevated arginase 1 expression is associated with many tumor types [4] and therapeutic strategies aiming at inhibiting this metabolic pathway are being tested in the clinic. Interestingly, in HCC, the predominant peri-tumoral distribution of arginase 1 expression is in agreement with proteomic analyses of samples from HCV infected HCC and non-tumoral liver [3,5].

Recent evidence indicates that interactions between tumor cells and the host microenvironment have a major role in driving cancer progression and metastasis [6,7]. The abundance of macrophages, pivotal members of tumor stroma, strongly correlates with poor prognosis in different types of solid tumors, including HCC [8]. It has been proposed that tumor-associated macrophages (M2-polarized) play an important role in generating overall immunosuppressive milieu within the tumor microenvironment that suppresses anti-tumor immunity and promotes tumor progression [6]. Interestingly, the differential metabolism of L-arginine provides a means of distinguishing the two macrophage activation states. M1, or classically activated macrophages, upregulate iNOS to catabolize L-arginine to nitric oxide and citrulline, while M2, or alternatively activated macrophages, induce arginase 1, the enzyme upstream of polyamines production, and thus increase collagen synthesis and cellular proliferation [9].

In HCC, high numbers of peri-tumoral M2-polarized TAMs are associated with poor patient prognosis, and while no analyses of arginase 1 expression have yet been conducted in this context, it is likely that they express high levels of this enzyme along with the other M2 genes described. In this context, TAM-derived arginase 1 might promote tumorigenesis in a non-cell autonomous manner. Furthermore, several TAM-derived factors, such as

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TGF-β, IL-10, and arginase 1, make a significant contribution to tumoral immunosuppression [7].

Hepatic damage, including metabolic disorders, is linked to activation of Kupffer cells (the tissue resident macrophages in the liver) and other stromal cells, which sustain inflammatory cytokine secretion [10]. These resident cells are also responsive to inflammatory factors and adipokines secreted by adipocytes. Thus, synergic events can perpetuate a vicious cycle that amplifies inflammatory processes, which will sustain both steatosis and inflammation while maintaining macrophage activation and the associated expression of arginase-1. Altogether, these events might favour disease progression, worsen hepatic damage and increase the risk of tumorigenesis.

In this context, we fully agree that a better understanding of cell autonomous and non-autonomous effects of arginase 1 and/or macrophage polarization could open new therapeutic horizons for liver diseases, particularly in the setting of NAFLD and HCC.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] Alisi A, Comparcola D, De Stefanis C, Nobili V. Arginase 1: a potential marker of a common pattern of liver steatosis in HCV and NAFLD children. J Hepatol 2015;62:1207–1208.
[2] Moreau M, Riviere B, Vegna S, Aoun M, Gard C, Ramos J, et al. Hepatitis C viral proteins perturb metabolic liver zonation. J Hepatol 2015;62:278–285.
[3] Cao W, Sun B, Feitelson MA, Wu T, Tur-Kaspa R, Fan Q. Hepatitis C virus targets over-expression of arginase I in hepatocarcinogenesis. Int J Cancer 2009;124:2886–2892.
[4] Rodriguez PC, Ochoa AC. Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. Immunol Rev 2008;222:180–191.
[5] Kuramitsu Y, Nakamura K. Current progress in proteomic study of hepatitis C virus-related human hepatocellular carcinoma. Expert Rev Proteomics 2005;2:589–601.
[6] Hao NB, Lu MH, Fan YH, Cao YL, Zhang ZR, Yang SM. Macrophages in tumor microenvironments and the progression of tumors. Clin Dev Immunol 2012;2012:948098.
[7] Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013;19:1423–1437.
[8] Zhu W, Guo L, Zhang B, Lou L, Lin Z, Zhu X, et al. Combination of osteopontin with peritumoral infiltrating macrophages is associated with poor prognosis of early-stage hepatocellular carcinoma after curative resection. Ann Surg Oncol 2014;21:1304–1313.
[9] Rath M, Muller I, Kropf P, Closs EI, Munder M. Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages. Front Immunol 2014;5:532.
[10] Bieghs V, Trautwein C. The innate immune response during liver inflammation and metabolic disease. Trends Immunol 2013;34:446–452.

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Protective effects of moderate alcohol consumption on fatty liver: A spurious association?

To the Editor:

We read with interest Moriya and colleagues' [1] paper on the longitudinal role of alcohol intake in relation to fatty liver. We were somewhat surprised by their findings and overall conclusion that light-to-moderate and even higher alcohol intake protects against developing fatty liver over time. They point out that several other studies also allege that moderate consumption is associated with a lower risk of non-alcoholic fatty liver disease (NAFLD) but acknowledge in their introduction that the apparent protective effects may be spurious associations induced by confounding factors. However, their analysis is also prone to a number of biases that have been well documented in the field of alcohol epidemiology [2,3].

Firstly, their choice of past week non-drinkers as a referent group is unsound. Past week non-drinkers include a mixture of lifelong abstainers, former drinkers (who may have quit for multiple reasons, including poor health) and infrequent drinkers. This misclassification error is known to negatively bias the health status of the aggregate group of non-drinkers and has been demonstrated to produce protective effects; studies that actively separate these distinctive non-drinking groups show none [3]. There is evidence that even if lifetime abstainers are separated from other non-drinkers they remain a poor choice of referent category for several reasons [3,4], including misclassification bias and that lifetime non-drinking may itself be indicative of poor health earlier in the life-course. Additionally, declines in consumption (including reductions to infrequent drinking) are more common amongst former heavy drinkers [3]. This is a potential source of bias, even in studies that are able to disentangle less than