Reply to "*Follistatin-like protein 1 and chronic liver disease progression: a novel pro-inflammatory and pro-fibrogenic mediator?*"

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In response to the editorial comments by Maurizio Parola regarding "*Follistatin-like protein 1 and chronic liver disease progression: a novel pro-inflammatory and pro-fibrogenic mediator?*", which was an insightful commentary on our work published in *Gut* 2022 (1). We offer the following reply.

Risk factors of developing chronic liver diseases and subsequent hepatic fibrosis are widely recognized, but it is still not quite clear about the intrinsic mechanisms, cellular kinetics and dynamic changing courses during disease progression. In our study, we mainly focused on macrophages' roles during fibrotic liver diseases. It is widely acknowledged that macrophages can be both pro- and anti-inflammatory, depending on time course, cellular origin or micro-environmental factors, etc. For example, in human disease, macrophages harbor both pro-inflammatory roles fueling disease progression, and anti-inflammatory roles leading to injury resolution, tissue repairing and even fibrosis regression (2).

In rodent models, infiltrated macrophages, characterized by Ly6C^{hi} CD11b^{+} F4/80^{+} population and recruited after liver injury, are generally regarded as pro-fibrotic, whereas liver-resident cells may harbor 'restorative' function. In widely-adopted murine models of liver fibrosis using hepatoxicities such as CCl4 or bile duct ligation, fibrosis developed rapidly after continuous or waves of liver injuries, and mice were sacrificed when disease progressed to certain level (3). In our study, we roughly concluded 'liver macrophages' as pro-inflammatory and profibrotic based on the notion that, under these animal models, severalty of liver fibrosis depends on levels of liver inflammation controlled by infiltrated macrophages. These models are way too simplistic to completely emulate the clinical and pathologic features of human disease. Actual roles of macrophages in human settings could be far more complicated. Could these FSTL1^{+} macrophages also participated in fibrosis regression? How about their cell fate? Shall FSTL1^{+} infiltrated macrophages delineate to Ly6C^{lo} 'restorative' cells? These questions may be answered using improved or carefully designed animal models (4).

The editors mentioned that methionine-choline deficient (MCD) diet may not be a fair animal model to stimulate human non-alcoholic steatohepatitis (NASH). We acknowledged this problem and it was a general consensus that MCD-induced NASH can only be regarded as a form of diet-induced chronic liver injury that badly resembled human NASH (5). Human NASH can be stimulated by feeding mice with 'junk food' diet, with excessive fat, sugar (especially fructose) and cholesterol. Mice fed this these kinds of diet readily developed steatohepatitis with underlying systematic metabolic shifts or metabolic syndromes such as obesity, elevated blood glucose, impaired insulin sensitivity, accumulation of visceral fat and ascending systematic inflammation. However, these models took longer time and can hardly progress to severe fibrosis, therefore not suitable for our project. Compared with high-fat and -cholesterol diets (HFD) and Western diets,

steatohepatitis and advanced fibrosis (≥F3) are more rapidly induced by the MCD diet, which is an appealing feature of this model (6). Therefore, we found it feasible to adopt this model, merely as a different cause of liver injury, to evaluate macrophages roles in injury-initiated inflammation and inflammation-generated fibrosis. We will explore the function of FSTL1 in the other dietary protocol (HFD and Western diets) in the next future.

Previous literatures reported that hepatic stellate cells (HSCs) or fibroblasts (FBs) functioned as privileged source of FSTL1 (7,8). In our work, we found that FSTL1 was also expressed on macrophages in fibrotic liver tissues. Though Lyz2-Cre guided deletion of FSTL1 in macrophages, we substantiate FSTL1's pro-inflammatory and pro-fibrotic roles. The editors found that, it seems that FSTL1 was mainly expressed by macrophages. This phenomenon may not be accurate as it can only be indirectly interpreted from immunofluorescence staining. As our work mainly focused on characterize macrophage FSTL1's role during hepatic inflammation and fibrosis, we did not spare our efforts on the proportion of macrophages among total $FSTL1^+$ cells. Future researches could focus on this problem using techniques like flow cytometry.

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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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