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Editorial

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Autophagy and hepatic stellate cell activation – Partners in crime?

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Following liver injury, hepatic stellate cells (HSCs) lose their characteristic lipid droplets to differentiate into extracellular matrix producing myofibroblasts. Activation of HSCs is considered one of the main mechanisms contributing to the development of hepatic fibrosis in chronic liver diseases [1]. Although several key pathways for HSC activation, such as TGFβ and PDGF have been identified, there is still a lack of clinically applicable approaches to target HSCs for anti-fibrotic therapies. Identification of additional pathways involved in HSC activation may reveal more suitable anti-fibrotic targets than those already identified. The study by Thoen *et al.* in this issue of the *Journal of Hepatology* investigates the role of autophagy in HSC activation and the loss of HSC lipid stores, one of the most characteristic features of the HSC activation process [2].

Like most biological systems, cells need to degrade many of their components thus allowing for constant turnover and renewal of proteins and organelles, and adaptation to changing conditions. Degradation occurs through two specific machineries – the proteasome and the lysosome [3]. The proteasome degrades proteins that are specifically tagged by markers such as ubiquitin, allowing this precise proteolytic machinery to recognize proteins destined for degradation. The lysosome is able to degrade various cellular components through different processes termed macroautophagy, microautophagy, and chaperone-mediated autophagy [4]. These three types of autophagy differ from each other in terms of the delivery method of the "cargo" to the lysosome, and their selectivity for specific types of cargo. The study by Thoen *et al.* focuses on macroautophagy, a catabolic process in which the cargo is first sequestered inside double-membrane vesicles called autophagosomes and then fused to lysosomes [4]. After degradation, resulting amino acids or other small molecules are released back into the cytoplasm and can be used for various purposes including energy harvest [4,5]. Accordingly, macroautophagy (referred hereafter as "autophagy") is increased in nutrient poor states, or stress conditions [4,5]. Increased autophagy has also been described in cell death and may, therefore, play a dual role in cell survival depending on conditions and stimuli. However, in many cases this is probably "cell death with autophagy rather than cell death by autophagy" [6] suggesting that the autophagic response is largely a cell-protective response in mammalian cells. Accordingly, defective autophagy has been linked to common human diseases, such as neurodegenerative conditions including Alzheimer's disease, Parkinson's disease, metabolic disorders, such as diabetes and obesity, and aging [3].

Thoen *et al.* explore the relationship between autophagy and HSC activation, and introduce the idea of targeting autophagy for the prevention of HSC activation [2]. Several key findings of the study support the idea that autophagy promotes HSC activation: (i) Fibrotic livers from CCl_4-treated mice displayed increased expression of LC3-II, one of the proteins involved in elongation of autophagosomes, and a useful indirect measure of autophagosomes. (ii) Autophagic flux is increased in mouse HSCs after *in vitro* activation as demonstrated by DsRed-GFP-LC3B transfection. With this method red punctae from DsRed (stable in an acidic lysosomal milieu) mark autophagolysosomes and yellow punctae from mixed DsRed, and GFP (whose fluorescence is quenched in the acidic lysosomal milieu) fluorescence label autophagosomes. The authors found a significant increase in red punctae during HSC activation indicating increased autophagic flux. (iii) Autophagy inhibitors bafilomycin A1, 3-methyladenine, and hydroxychloroquine efficiently suppress *in vitro* activation of mouse and human HSCs as evidenced by decreased expression of activation makers, such as platelet-derived growth factor receptor, *Acta2*, and *Col1a1* mRNA expression, and α-SMA protein. Notably, disrupting bafilomycin treatment allowed HSCs to resume activation suggesting that toxic effects of bafilomycin are unlikely to be involved in the suppression of HSC activation.

Despite the appealing hypothesis and the strong evidence for autophagy contributing to HSC *in vitro* activation, the study by Thoen *et al.* leaves a number of open questions. One area in which this study falls short, is the investigation of underlying mechanisms. Thoen *et al.* observed that bafilomycin treated cells had increased large lipid droplets, which are more characteristic of quiescent rather than activated HSCs. Additionally, treatment of HSCs with PDGF induced co-localization of lipid droplets and LC3-B fluorescence suggesting that autophagy is responsible for the metabolism lipid droplet metabolism. These findings are similar to observations in hepatocytes where autophagy negatively

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regulates lipid stores through a process called macrolipophagy [7]. However, there is no solid evidence that the decrease in HSC lipid droplets promotes their activation in liver fibrosis. One study addressing this issue found that HSCs that do not contain any lipid droplets do not activate spontaneously nor do they show increased activation in response to CCl_4 treatment or bile duct ligation [8]. Additionally, mTOR contributes to the activation of HSCs and promotion of liver fibrosis [9–11] but inhibits autophagy [12]. The data from Thoen *et al.* seem to contradict the HSC-activating yet autophagy-inhibiting effect of mTOR. Therefore, it would be important to further delineate the relationship between mTOR, autophagy, and HSC activation. A second concern is the complete reliance of Thoen *et al.* on chemical inhibitors of autophagy. It cannot be excluded that these inhibitors exert non-specific effects and that the observed decrease in HSC activation is not entirely mediated by autophagy inhibition. Elegant genetic approaches such as the conditional deletion of *Atg7* have been employed to study autophagy in the liver and other organs [7], and should be used to confirm pharmacological approaches. Finally, the study on HSC activation is entirely based on *in vitro* models of HSC activation. The only *in vivo* part of this study is on whole liver extracts from CCl_4-treated mice and lacks an investigation of autophagy regulation in HSCs and other cell types in the fibrotic liver. *In vitro* activation of mouse and human HSCs differs considerably from *in vivo* activation due to the absence of various cell–cell interactions and soluble mediators that are typically present in the injured liver [13,14]. To exclude that the observed induction of autophagic flux is merely a result of cell culture conditions, confirmatory *in vivo* studies are needed that compare autophagic flux in quiescent and activated HSCs directly in the liver, or between HSCs isolated from normal or fibrotic livers. Along this line, it should be pointed out that the comparison of autophagic flux in quiescent and culture-activated HSCs by DsRed and GFP fluorescence is challenging due to different time intervals between transfection and analysis under these conditions.

When considering the potential therapeutic implications of the study by Thoen *et al.*, one needs not only to consider the often protective role of autophagy in human physiology and pathophysiology [3], but also its functions in hepatic cell populations besides HSCs [15]. In the liver, autophagy appears to predominantly exert protective functions including the promotion of hepatic function during aging, protection from hepatocellular carcinoma, protection from liver disease due to α1-antitrypsin deficiency, and protection from Mallory-Denk body formation and liver injury in alcoholic liver disease [16–19]. However, under some conditions the autophagic machinery may also promote liver disease – e.g. in patients with viral hepatitis where HBV and HCV hijack this machinery for their own benefit [15]. In conclusion, targeting autophagy for anti-fibrotic therapies is likely to have broad and many unwanted effects in the liver and other organs, and currently does not appear to be an attractive target for anti-fibrotic therapies. Further *in vitro* and *in vivo* studies are required to confirm and understand the role and targets of autophagy in HSCs in the fibrotic liver, and may reveal novel players in the HSC activation process.

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