Editorial

Signal transducer and activator of transcriptions (STATs)—at the crossroads of obesity-linked non-alcoholic steatohepatitis and hepatocellular carcinoma

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The obesity pandemic is widely regarded as major health challenge for modern society. This multi-organ disorder is linked to the development of a number of prevalent diseases, such as type 2 diabetes, cardiovascular and liver diseases, including primary liver cancer (1). The hepatic manifestations of obesity are collectively called non-alcoholic fatty liver disease (NAFLD) (2). The increased prevalence of NAFLD parallels the global obesity pandemic and is predicted to be the largest cause of liver transplantations (1). The term NAFLD encompasses different disease states, ranging from simple steatosis (NAFL) to more severe forms of liver disease such as non-alcoholic steatohepatitis (NASH), with various degree of fibrosis (3). NASH may progress to cirrhosis and hepatocellular carcinoma (HCC) (4). Although NASH arising from fatty liver disease seems to be an underlying lesion for some HCC cases, the extent to which the presence or the severity of NASH explains obesity-related HCC remains unclear (5). Moreover, HCC is increasingly diagnosed among obese individuals independent of NASH and cirrhosis (6), suggesting that cirrhosis is not necessary for the development of HCC in obesity. However, the precise mechanisms that give rise to HCC in some NASH patients and not in others remain largely unknown. A number of molecular mechanisms have been linked to obesity and its associated abnormalities that may facilitate the development of NASH and HCC, such as adipose tissue and hepatic inflammation, lipotoxicity and oxidative stress, and insulin resistance and ensuing hyperinsulinemia (7). These, and other pathological events in obesity, have complex interactions, so their relative contribution to the development of HCC in various stages of NAFLD progression remains to be determined. For example, the molecular mechanisms driving the recruitment and activation of immune cells contributing to NASH pathologies have remained unclear. Similarly, we have a poor understanding of the mechanisms involved in lipotoxicity and oxidative stress-induced NASH and HCC.

The dynamic regulation of protein phosphorylation is a fundamental mechanism in regulating the functions of various cell-signaling proteins and their dysregulation (an imbalance of phosphorylation and dephosphorylation) often associated with the development of various diseases (8). The members of the signal transducer and activator of transcription (STAT) protein family play important roles in obesity-related liver pathologies. For instance, the upregulation of STAT-3 signaling has been reported as a factor in the development of HCC in both rodent models and humans (9,10). The transcriptional functions of STATs are dynamically regulated through their phosphorylation and dephosphorylation at specific tyrosine residues, which is mediated through non-receptor intracellular tyrosine kinases (Janus-activated kinases or JAKs) and phospho tyrosine phosphatases (PTPs). The phosphorylation of

STATs induces their transcriptional function, whereas their dephosphorylation has the opposite effect, which is often intrinsic to the activation process. Because the negative regulation of STATs is so crucial to controlling their transcriptional functions, it is reasonable to assume that the loss of negative regulation is a part of their persistently activated states that are associated with NASH and HCC.

Recently, in a series of experiments conducted using a different combination of mouse models, Grohmann *et al.* (11) has discovered that independent signaling pathways contribute to the development of obesity-linked NASH and HCC, which is mediated through two distinct members of the STAT protein family—STAT-1 and STAT-3. The former promotes obesity-linked hepatic T cell infiltration, NASH and fibrosis, whereas the latter supports the development of obesity-linked HCC, independent NASH. The authors have shown that an obesity-linked lipotoxic and oxidative hepatic environment inactivates a member of the phospho-tyrosine phosphatase protein family called the T cell protein tyrosine phosphatase (TCPTP), in the hepatocytes of both obese mice and humans. TCPTP is a negative regulator of the phosphorylation-mediated activation of STAT-1 and STAT-3. Importantly, Grohmann *et al.* found that the inactivation of TCPTP correlated with increased levels of tyrosine-phosphorylated forms of STAT-1 and STAT-3, demonstrating that they have a potential link in the development of NASH and HCC. To explore the role of the inactivation of TCPTP in obesity-linked liver pathologies, the authors used a *Cre*-LoxP recombination based method to delete TCPTP in hepatocytes (*Alb-Cre*;*Ptpn2*$^{fl/fl}$) in a mouse model. Subsequently, Grohmann *et al.* demonstrated that the deletion of TCPTP in mice promoted T cell recruitment in the liver resulting in NASH and fibrosis, as well as HCC (1/3rd of mice) in response to a high-fat diet (HFD) in *Alb-Cre*;*Ptpn2*$^{fl/fl}$ male mice but not in *Ptpn2*$^{fl/fl}$ mice. Upon a differential gene expression analysis of liver samples, they found that the majority of the differentially expressed genes (18 out of 24) are predicted targets for interferon signaling and/or are transcriptional targets of STAT-1 and STAT-3, showing that they play a role the development of NASH and HCC. Next, they sought to determine the relative contributions of STAT-1 and STAT-3 signaling in the development of NASH and HCC in some high-fat-fed *Alb-Cre*;*Ptpn2*$^{fl/fl}$ mice by correcting STAT-1 and STAT-3 signaling individually. The authors achieved this by crossing the *Alb-Cre*;*Ptpn2*$^{fl/fl}$ mice onto *Stat-1*$^{fl/+}$ or *Stat-3*$^{fl/+}$ to reduce STAT-1 or STAT-3. Subsequently, Grohmann *et al.* found that STAT-1 heterozygosity partially corrected increased phospho-STAT-1 levels, whereas STAT-3 heterozygosity completely corrected increased phospho-STAT-3 levels. Interestingly, they found that inhibiting phospho-STAT-1 corrected hepatic inflammation, as reflected by the analysis of their marker genes, which was not observed under STAT-3 heterozygosity, suggesting that STAT-1 signaling is responsible for T cell recruitment and the development of NASH and fibrosis. Surprisingly, this did not prevented HCC development in the high-fat-fed *Alb-Cre*;*Ptpn2*$^{fl/fl}$ mice suggesting that tumor development in these mice occurs independent of NASH and fibrosis, and opening the possibility that STAT-3 signaling plays a role. Consistent with this finding, the authors found that STAT-3 heterozygosity completely suppressed tumor development, but did not alter T cell infiltration and the development of NASH and fibrosis. Thus, these findings provided proof that the promotion of STAT-3 signaling is sufficient to drive tumor development in obesity-related NAFLD, independent of NASH and fibrosis. In aggregate, these findings revealed how obesity-related hepatic oxidative stress can independently contribute to the pathogenesis of NASH, fibrosis, and HCC. Thus, the research identified a mechanism of the growing incidence of HCC in NAFLD patients without advanced fibrosis or cirrhosis, a topic of substantial clinical importance that is on rise along with growing pandemic of obesity and type 2 diabetes.

This timely finding is likely to stimulate research in this field and may open new opportunities for the development of therapeutic interventions of obesity-related NASH and HCC, an unmet need in this field. However, there are a number of limitations that should be taken into account when moving forward. For example:

(I) Considering the complex relationship between obesity-related abnormalities and liver pathologies, it would be important to know whether the inhibition of one pathway (e.g., STAT-1) contributes to disease progression through the other pathway (e.g., STAT-3), or *vice versa*. In our opinion, this information is crucial because similar kinases and phosphatases regulate the phosphorylation of STAT-1 and STAT-3, and the deletion of STAT-3 is known to increase STAT-1 phosphorylation (12); therefore, this possibility

also exists when targeting STAT-1 signaling. In addition, it would be interesting to know the consequence of targeting STAT-1 and STAT-3 signaling simultaneously, because adaptive changes are key in pathogenesis and therapeutic resistance. It is likely that there are other drivers for obesity-linked NASH and HCC, which may become upregulated in response to targeting STAT-1 and STAT-3.

(II) A basal blood glucose level of around 10 mM in an insulin tolerance test and >15 mM at a 2 h time point in a glucose tolerance test would suggest that the whole-body insulin sensitivity and glucose homeostasis in *Alb-Cre*;*Ptpn2*$^{fl/fl}$ mice are not normal as stated, which will substantially deteriorate on a HFD for up to 40 weeks. However, information related to glucose homeostasis and insulin sensitivity in *Alb-Cre*;*Ptpn2*$^{fl/fl}$ mice on a HFD as well as the weight they gain compared to age-matched chow-fed mice is missing and not taken into account. Thus, there is a need for caution in the interpretation of this data.

(III) Sex differences exist in liver pathologies in humans, including NASH and HCC (13). This study was performed using only male *Alb-Cre*;*Ptpn2*$^{fl/fl}$ mice. It is unclear whether the female mice also develop NASH and HCC similar to their male counterparts or exhibit sex differences, as can be found in human diseases. Thus, there is a need for caution in the translation of this knowledge human pathophysiology.

(IV) There is limited information to provided on oxidized and inactivated TCPTP, which is the foundation for this study. For example, there is a lack of information about the percentage pool of oxidized TCPTP out of total TCPTP levels. Simple immunoblotting may not be sufficient in this case, as there are a number of other possible interpretations of the observed results, which have not been verified or discussed. For example, TCPTP protein levels appear to be higher in liver samples from patients with NAFLD, and in liver samples from mice being kept on a choline-deficient-HFD. It is unclear whether this means that TCPTP is upregulated in response to oxidative damage as a compensatory response, or if the oxidation of TCPTP prevents its degradation and helps stabilize it. Hence, future studies are needed to clarify responses to these pertinent questions.

Nevertheless, this work substantially advanced our knowledge of an extensive health problem that is on the rise.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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