Editorial Commentary

Squeezed into defection?—nuclear displacement by steatosis activates yes-associated protein (YAP) linked to oncogenic pathways in hepatocytes

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Nonalcoholic fatty liver disease (NAFLD) has become the most prevalent liver disorder of our times, estimated to affect at least a quarter of the world's population (1). Histologically, NAFLD ranges from steatosis to steatohepatitis with an increasing risk to progress into cirrhosis and hepatocellular carcinoma (2). Most cases of NAFLD-associated HCC develop in cirrhosis, but HCC may complicate non-cirrhotic disease, representing a major logistical challenge as our current understanding of the drivers of liver tumorigenesis at early stages of NAFLD remains insufficient to guide risk assessment and personalized prediction (3).

Genetic aberrations promoting the onset and progression of HCC are notoriously diverse, making screening, treatment and prognostication difficult (4,5). Cellular and molecular mechanisms that contribute to the development of HCC in NAFLD involve DNA damage responses, inflammatory changes and interactions with the gut microbiome (6). Recent research indicates that various developmental pathways including Notch, Wnt/beta-catenin, Hedgehog and Hippo/yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) substantially contribute to this process (7). In advanced liver disease, these regulatory cascades are chronically activated and create a microenvironment conducive to uncontrolled hepatocellular growth and genomic instability, consistent with a high risk of HCC in cirrhosis (7). While altered hepatocellular lipid metabolism, insulin resistance and some other mechanisms have been implicated in the development of non-cirrhotic HCC, much less is known about liver tumorigenesis in the absence of significant inflammation and fibrosis (8,9).

One of the many molecular signaling cascades implicated in HCC development, Hippo/YAP/TAZ is an evolutionarily conserved pathway that regulates organ development and regeneration, acting through multiple intracellular kinases to control the activity of YAP and the paralog TAZ, which may otherwise translocate to the nucleus and induce the expression of genes involved in cell growth and proliferation (10,11). Activation of YAP/TAZ as a gene regulatory tool is shared by several developmental pathways including G protein-coupled receptors and the transforming growth factor-β (TGFβ) and Wnt/β-catenin cascades (12). In the liver, YAP activation has been linked to hepatocyte growth factor/MET signaling and glypican 3-mediated tumorigenesis (13,14). Sustained activation of YAP/TAZ is therefore a major oncogenic force identified in many tissues including the liver (15,16).

Hippo/YAP/TAZ signaling has a key role in mechanotransduction, which is an overarching term for the cellular responses to physical stimuli that are translated into biochemical signals (17). Mechanical stress in biological tissues may result from shear, stretch and compression forces that activate a variety of cellular mechanosensors found in the cytoskeleton (actin, microtubules, and intermediate filaments) and on the cell surface where integrins, focal adhesions and ion channels detect conformational changes related to cell-cell and cell-substrate interactions (18). Rather than being committed to a specific extracellular ligand/receptor, YAP/TAZ is activated in response to many of these physical signals relevant to cell-cell contact and positional changes (17,19,20). Sustained activation of YAP/TAZ has been described in parenchymal hepatocytes,

liver sinusoidal endothelial cells (LSECs), Kupffer cells and hepatic stellate cells (HSCs) in response to increased tissue stiffness due to inflammation, fibrosis or elevated sinusoidal pressure (21-24). However, we know little about the potential role of intrahepatic lipid accumulation in generating mechanocrine signals in NAFLD.

In a recent work, Chin *et al.* (25) focused on the earliest steps of NAFLD pathophysiology to investigate the impact of steatosis on YAP signaling, based on the hypothesis that lipid droplets in hepatocytes may disrupt mechanosensing, similar to what we see in response to increased stiffness due to fibrosis or inflammation. To answer this intriguing question, the authors first analyzed if there is a correlation between tissue stiffness and lipid content in liver specimens obtained from patients who underwent liver resection or transplantation with NAFLD-associated and non-NAFLD cirrhosis. By using parallel plate rheometer, they found that neither the etiology of cirrhosis nor the amount of intrahepatic lipid affected liver tissue stiffness (25). This is not very surprising since stiffness in cirrhosis is predominantly determined by an excessive degree of fibrosis, and the presence of intracellular lipids (which is often diminishing in NAFLD-cirrhosis anyway) may not have a significant additional impact (26). Further analysis of liver specimens derived from NAFLD-cirrhosis revealed that individual hepatocytes have heterogeneous distribution of intracellular fat, consisting of cells with large lipid droplets displacing the nucleus and cells with small lipid droplets and their nucleus left in place. When intracellular distribution of YAP was analyzed, the relative number of cells with YAP-positive nuclei positively correlated with the size of lipid droplets, indicating that YAP translocation was more likely to occur in nuclei squeezed to the side of hepatocytes (25).

To further analyze the impact of lipid droplets on hepatocellular mechanics, Chin *et al.* loaded human HCC-derived Huh-7 cells and primary human hepatocytes with oleic acid and linoleic acid at doses not too high to cause cytotoxicity, senescence or apoptosis (25). They then investigated the spreading of lipid-loaded cells on substrates with increasing stiffness, which showed inverse correlation with the amount of intracellular lipids. Moreover, they demonstrated that fatty acid treatment had an adverse effect on focal adhesions and stress fibers, indicating disruption of the cytoskeleton. While the proportion of YAP-positive nuclei did not correlate with substrate stiffness, it increased significantly in response to the addition of insulin (promoting the formation of large lipid droplets), indicating that nuclear displacement has a positive impact on YAP translocation (25). These findings suggest that large lipid droplets and nuclear displacement, frequently observed in macrovesicular steatosis, could become a source of mechanical stress in hepatocytes and promote YAP activation, thus establishing a novel mechanistic link between hepatocellular lipid accumulation and oncogenesis.

It is somewhat surprising that YAP translocation was not affected by substrate stiffness in primary cultured hepatocytes and Huh-7 cells, although it was stimulated by formation of large lipid droplets, disruption of the cytoskeleton, and displacement of the nucleus. This may perhaps indicate that YAP responds more readily to mechanocrine signals emanating from the cytoskeleton than from cell surface mechanosensors, at least in this experimental setting. Specific transcriptional targets of YAP were not looked at by Chin and colleagues, but the broad impact of YAP/TAZ activation is likely to involve cancer-promoting effects when these events occur *in vivo* (16). Notably, while YAP translocation was essentially universal in hepatocytes with large lipid droplets identified in NAFLD-cirrhosis specimens, the proportion of YAP-positive nuclei still reached about 60% in hepatocytes with small lipid droplets or no steatosis at all (25). This indicates that, regardless of the presence or size of lipid droplets, YAP signaling is mostly active in cirrhosis, while persisting steatosis may further enhance YAP activation.

Intriguingly, the association between YAP-positive nuclei and large lipid droplets in human liver samples analyzed by Chin and colleagues is seemingly at variance with a recent report from Japan, in which 154 HCC patients were histologically classified based on the extent of tumor tissue steatosis, and the presence of large lipid droplets predicted better survival (27). Although distribution of YAP was not analyzed in this work, YAP activation, if it occurs in macro-steatotic HCC, is unlikely to have a beneficial effect on clinical outcomes. Of note, YAP and TAZ have rather heterogeneous localization patterns and co-expression profiles in HCC, and the prognostic significance of cytoplasmic and nuclear presence of YAP/TAZ remains to be determined (28).

NAFLD is a highly heterogeneous disease and steatosis has been generally associated with benign liver outcomes. Lipids are primarily stored in hepatocytes as triglycerides, which have been considered relatively harmless with no direct correlation to the amount of lipid derivatives metabolites that drive lipotoxicity in hepatocytes (29). While several questions remain unanswered, the work of Chen and colleagues represents a provocative novel

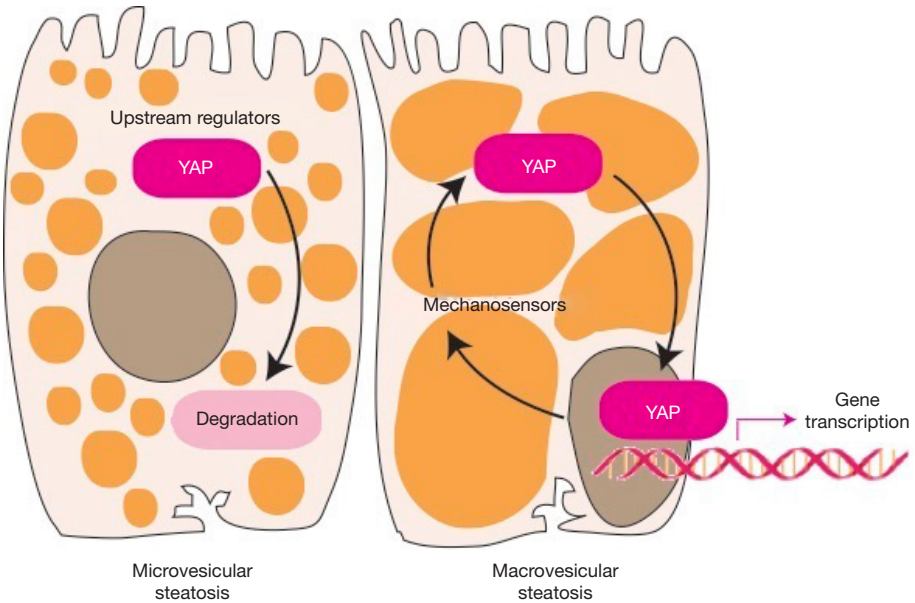

Figure 1 Mechanocrine signaling in steatosis. Association between lipid droplet size in hepatocytes and the fate of YAP, a transcriptional regulator linked to multiple oncogenic pathways, is schematically illustrated. In the absence of cytoskeletal disruption and nuclear displacement (microvesicular steatosis), YAP is phosphorylated by upstream regulators (Hippo kinase cascade) and becomes degraded in the proteasome. Space-occupying effect of large lipid droplets (macrovesicular steatosis) may disrupt the cytoskeleton and displace the nucleus, activating intracellular mechanosensors and translocating YAP to the nucleus where it promotes cell growth and proliferation. YAP, yes-associated protein.

paradigm of how mechanosignaling originating from the physical impact of lipid droplets may potentially contribute to the activation of oncogenic pathways in early-stage NAFLD (*Figure 1*).

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