Editorial

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Mechanical stimuli control liver homeostasis

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Mechanical cues are known to control cell proliferation, differentiation, migration and malignant transformation. Such cues include compression, shear stress from blood flow, and stretch generated by cell-cell or cell-extracellular matrix interactions. Physical stimuli of the microenvironment are sensed by cell membrane-associated proteins such as integrins, ion channels, or growth factor receptors. They are then propagated to the actomyosin cytoskeleton whose contractility regulates intracellular signalling pathways, eventually leading to changes in gene expression and determination of the appropriate cellular response.[1,2]

In this issue of the *Journal of Hepatology*, Pocaterra and coworkers investigate how the mechanical properties of hepatocytes maintain adult liver homeostasis.[3] They address the issue by focusing on the role of CAPZ, an actin capping protein. Contraction of the actomyosin cytoskeleton occurs by sliding of actin fibres on myosin motor proteins. Actin self-assembles into filaments of which one extremity is called the pointed end, while the other extremity is called the barbed end. This terminology was inspired by the arrowhead-like shape created by the decoration of polymerized actin by myosin. Capping proteins bind the barbed end of polymerized actin and regulate the dynamics of actin assembly.[4] Therefore, CAPZ is an excellent entry point to dissect the role of actin dynamics, mechanotransduction and cell behaviour in hepatocytes.

Pocaterra initiated their work by implementing a classical loss-of-function approach, and first demonstrated that the response to geometrical constraints differs between wild-type and CAPZ-deficient primary fibroblasts. The technical approach consisted in growing the cells on small or large fibronectin-coated adhesive areas, and comparing their behaviour on the two substrates. Wild-type fibroblasts proliferate on large adhesive areas, but display nearly no proliferation on small adhesive areas. In contrast, CAPZ knockout cells maintain a more significant level of proliferation both on large and small adhesive areas. Moreover, CAPZ inactivation was associated with increased contractility, and CAPZ-deficient cells exerted higher tension forces on their substratum, thereby validating CAPZ inactivation as a biologically significant approach for investigating the cells' response to mechanical cues.

Yes associated protein (YAP) is a known mediator of mechanical stimuli, and, importantly, CAPZ knockout fibroblasts grown on small fibronectin-coated areas retain nuclear YAP, unlike wild-type cells. In other terms, CAPZ-deficient cells combine YAP activation with increased actomyosin contractility.

Building further on these initial observations, Pocaterra and coworkers turned their attention to the liver, and generated a mouse line with liver-specific inactivation of CAPZ. Increased nuclear localization of YAP and activation of typical YAP target genes were monitored, in line with the findings on cultured fibroblasts. More spectacular, CAPZ knockout livers were twice the size of normal liver, and this was caused by intense hepatocyte overproliferation. There was also massive induction of progenitor-like cells, as a result from transdifferentiation of hepatocytes. Therefore, CAPZ regulates YAP activation and controls liver growth and hepatocyte differentiation.

Metabolism in the liver is zonated, which means that hepatocytes display heterogeneous metabolic activities depending on their location along the porto-central axis. YAP signalling was previously shown to control zonation,[5] and this prompted Pocaterra and coworkers to investigate the topography of gene expression in the lobules. They found reduced expression and function of pericentral genes in CAPZ knockouts. In addition, and in line with earlier work on the repressive role of YAP on gluconeogenic genes,[6] CAPZ knockout livers displayed low expression of these genes, and this was functionally related to decreased steady-state blood glucose levels. Very elegantly, Pocaterra and coworkers also demonstrated that the CAPZ knockout liver phenotype was dependent on YAP. So, CAPZ and actin dynamics exert a tight control on hepatocyte proliferation, fate maintenance and metabolism via YAP.

But how was YAP activation promoted in the absence of CAPZ? YAP nuclear localization is classically determined by Hippo pathway activity, where phosphorylation of YAP by Large tumor suppressor kinases 1/2 (LATS1/2) prevents YAP nuclear localization. Following their finding that CAPZ does not function exclusively via LATS1/2-mediated regulation, Pocaterra and coworkers looked again at the actomyosin skeleton and noticed increased myosin light chain phosphorylation in CAPZ-deficient livers. This suggested the involvement of the Rho-kinase ROCK, a regulator of myosin light chain phosphorylation. Administration of a ROCK inhibitor to mice with liver-specific inactivation of CAPZ confirmed that ROCK activity is required for all the YAP-induced effects. Taken together, Pocaterra and coworkers conclude that actomyosin contractility signals to YAP using LATS1/2- and ROCK-dependent mechanisms, in order to stimulate

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YAP-induced hepatocyte proliferation, metabolic patterning and differentiation.

Regulation of liver functions by mechanical cues is attracting ever increasing attention because several pathological conditions such as fibrosis or non-alcoholic fatty liver disease induce physical changes that impact pathophysiology. Also, regenerative therapy of liver disease by administration of stem cell-derived hepatic tissue is coping with difficulties related to the optimization of the physical properties of substrates on which the cells should be grown for production of therapeutically effective tissue. In that context, mechanical stimuli acting indirectly on hepatocytes were recently highlighted by the group of Eckhard Lammert. This team demonstrated that blood flow-mediated stretching of endothelial cells stimulates production of angiocrine signals that promote hepatocyte proliferation.[7] In parallel, physical forces which act directly on hepatocytes are interpreted by membrane-located mechanosensors that modulate actomyosin contractility and eventually control signalling pathway activity.

The Hippo-YAP pathway pervades many studies that connect mechanical stimuli with liver functions.[8] The study or Pocaterra and coworkers raises questions on the target genes involved in hepatocytes' response to the actomyosin/ROCK-YAP cascade. The reduced expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase monitored in CAPZ knockout livers likely results from repression of peroxisome proliferator–activated receptor gamma coactivator 1 (PGC1α), a transcriptional regulator which was shown earlier to mediate YAP's inhibitory effects on gluconeogenesis.[6] Interactions with β-catenin signalling cannot be excluded in this context: this pathway is essential for proper metabolic zonation, and modulates Hippo signalling.[9] Transdifferentiation of hepatocytes to progenitor-like cells was found by Pocaterra and coworkers to be associated with enhanced Notch activity. This is in agreement with other studies where YAP overexpression in hepatocytes promoted their conversion to a progenitor-like phenotype by stimulating the Notch pathway; in that context, YAP/TEA-domain transcription factor 4 (TEAD4) complexes were shown to bind to the *Notch2* and *Sox9* genes.[10]

YAP activity needs to be maintained within strict limits to allow hepatocyte differentiation,[11] and this is well illustrated in studies where stem cell-derived hepatocytes (iHep cells) were grown as aggregates, a culture condition where the cells are subjected to mechanical stimuli that are likely closer to physiology than when cells are grown on coated plastic dishes: in aggregates, Hippo signalling inactivates YAP and induces hepatocyte differentiation.[12] Likewise, YAP and hepatocyte nuclear factor (HNF) 4, a transcription factor driving hepatocyte differentiation, compete for binding to TEAD4.[13] Therefore, the cells can form YAP/TEAD4 complexes which promote dedifferentiation to a progenitor-like state, or HNF4/TEAD4 complexes, which maintain hepatocyte differentiation. In parallel, high F-actin dependent mechanical stress favours formation of YAP/TEAD complexes at the expense of the antiproliferative YAP-ARID1A-SWI/SNF complexes.[14] Taken together, one can conclude that mechanical cues determine which proteins associate with YAP to induce the most appropriate cellular response. Rendering YAP available in the nucleus is an essential step in this process. Pocaterra and coworkers nicely tell us how F-actin dynamics and ROCK control YAP nuclear localization and how this impacts liver homeostasis. Implementing the new knowledge in the analysis of disease conditions is the next challenge; we anticipate that the CAPZ-deficient liver model developed by Pocaterra and coworkers will provide much help for future studies.

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Conflict of interest

The author declares no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure form for further details.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.jhep.2019.04.002.

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Author names in bold designate shared co-first authorship.

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