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Multiscale biomechanics and mechanotransduction from liver fibrosis to cancer

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ABSTRACT

A growing body of multiscale biomechanical studies has been proposed to highlight the mechanical cues in the development of hepatic fibrosis and cancer. At the cellular level, changes in mechanical microenvironment induce phenotypic and functional alterations of hepatic cells, initiating a positive feedback loop that promotes liver fibrogenesis and hepatocarcinogenesis. Tumor mechanical microenvironment of hepatocellular carcinoma facilitates tumor cell growth and metastasis, and hinders the drug delivery and immunotherapy. At the molecular level, mechanical forces are sensed and transmitted into hepatic cells *via* allosteric activation of mechanoreceptors on the cell membrane, leading to the activation of various mechanotransduction pathways including integrin and YAP signaling and then regulating cell function. Thus, the application of mechanomedicine concept in the treatment of liver diseases is promising for rational design and cell-specific delivery of therapeutic drugs. This review mainly discusses the correlation between biomechanical cues and liver diseases from the viewpoint of mechanobiology.

Contents

Abbreviations: HCC, hepatocellular carcinoma; LSEC, liver sinusoidal endothelial cell; HSC, hepatic stellate cell; PV, portal vein; HA, hepatic artery; CV, central vein; ECM, extracellular matrix; MRE, magnetic resonance elastography; AFM, atomic force microscopy; MRI, magnetic resonance imaging; CFD, computational fluid dynamics; IFP, interstitial fluid pressure; CYP, cytochrome P450; HNF4α, nuclear factor 4 alpha; KC, Kupffer cell; YAP, Yes-associated protein; F-actin, actin filaments; KLF2, Kruppel-like factor 2; α-SMA, α-smooth muscle actin; CAF, cancer-associated fibroblast; EMT, epithelial to mesenchymal transition; LOX-L2, lysyl oxidase-like 2; BDL, bile duct ligation; NASH, nonalcoholic steatohepatitis.

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1. Introduction

Liver fibrosis is an abnormal wound healing process during chronic liver injury caused by various pathogenic factors, including alcohol abuse, viral infection, and autoimmune or metabolic disorders [1]. Failure to eliminate these pathogenic factors leads this fibrosis to progress into liver cirrhosis, or hepatocellular carcinoma (HCC), eventually becoming a major cause of mortality worldwide [2]. Globally, liver cirrhosis together with HCC causes approximately 2 million deaths annually, which accounts for 3.5% of all deaths [3]. This highlights the importance of understanding cellular and molecular mechanisms involved in liver fibrosis and tumor progression, as the identification of new treatments against this process is critical to improve clinical outcomes [2,4].

Recently, a growing body of studies has been proposed to target mechanical cues as new approaches for liver fibrosis or HCC treatment [5–9]. The liver is located in a complicated mechanical microenvironment that is crucial for maintaining physiological homeostasis. Dramatic mechanical changes, particularly in tissue stiffness, shear flow and hydrostatic pressure, occur in hepatic interstitial space and sinusoids during hepatic fibrogenesis and hepatocarcinogenesis [6,10,11]. Meanwhile, liver resident cells, especially hepatocytes, liver sinusoidal endothelial cells (LSECs), and hepatic stellate cells (HSCs), are all sensitive to mechanical forces, and able to alter their behaviors and functions through mechanotransduction pathways [5,6]. Thus, mechanical cues remarkably contribute to the pathogenesis of liver fibrosis and cancer.

In this review, tissue-level mechanical features of normal and pathological livers were first introduced. Cell mechanics inside the liver and relevant mechanotransduction pathways during pathological progression were discussed next. Molecular biomechanics of mechanoreceptors involved in liver fibrosis and HCC was then summarized to uncover mechanical regulation of molecular interactions for cellular functions. Drug screening applied to modify hepatic microenvironment or alter mechanotransduction of hepatic cells was finally underlined, and computer-aided drug design and liver-specific drug delivery rationale was also proposed.

2. Mechanical microenvironment of liver tissues

Liver-specific functions such as synthesis, metabolism, detoxification, and host defense are tightly dependent on the unique features of hepatic microstructures and adequate mechanical microenvironment (Fig. 1) [12]. The liver receives dual blood supply from portal vein (PV) and hepatic artery (HA) and delivers blood flow to thousands of hexagonal units so-called lobules, each of which is mainly composed of hepatocytes arranged radially in plates and sinusoids between them (Fig. 2) [13]. The sinusoids are discontinuous capillaries presenting with a length of $\sim$ 250 μm and a diameter ranging from 7 to 15 μm, lined by fenestrated LSECs, and connecting blood flow from the portal tracts (PTs, branches of the HA, PV, and bile duct) to the central veins (CVs) [12,14]. Between the hepatocytes and the sinusoids is the space of Disse, containing minimal extracellular matrix (ECM) and quiescent HSCs in a healthy liver [10]. During the development of liver fibrosis and HCC, these hepatic cells are responsive to biochemical stimuli, which in turn induces changes in mechanical microenvironment and further promotes the pathological progression [5,6,15]. Therefore, quantifying these mechanical cues can bring a new perspective for elucidating the pathological mechanisms of liver diseases.

2.1. Liver tissue stiffness

Liver tissue stiffness is a major feature of *in vivo* mechanical microenvironment, usually measured as the elastic modulus (E) or shear modulus (G), which are defined as [15],

$$E = \sigma/\varepsilon, \text{ and} \tag{1}$$

$$G = \tau/\gamma \tag{2}$$

where σ stands for stress, ε for strain, τ for shear stress, and γ for shear strain.

During liver fibrotic progression, quiescent HSCs are activated and able to synthesize a large amount of ECM, accompanied by increased matrix cross-linking and insufficient ECM degradation, eventually leading to matrix stiffening [16]. Thus, liver tissue stiffness becomes an excellent diagnostic marker for pathological staging of hepatic fibrosis. Two noninvasive techniques, ultrasound transient elastography and magnetic resonance elastography (MRE), are currently applied in clinic to measure liver stiffness as elastic modulus (E) and shear modulus (G), respectively [9,16,17]. The relation between the two moduli is given blow with a Poisson's ratio of 0.5 [5]:

$$E = 3G \tag{3}$$

Despite some variability, the elastic modulus E reads usually < 6 kPa for normal liver, 6–8 kPa for mild or moderate hepatic fibrosis (F1-F2 according to the METAVIR scoring system), 8–12.5 kPa for severe hepatic fibrosis (F3), and > 12.5 kPa for cirrhotic liver (F4) [17]. Liver tissue stiffness can serve as a high-accuracy physical biomarker for the diagnosis of advanced fibrosis and cirrhosis (F3-F4), with superior performance to common serum fibrosis algorithms [17–19]. However, it is also noticed that hepatic inflammation may also cause increases in tissue stiffness and affect the fibrosis assessment by liver tissue stiffness [20]. For patients with autoimmune hepatitis, the baseline elastic modulus is 15.5 ±

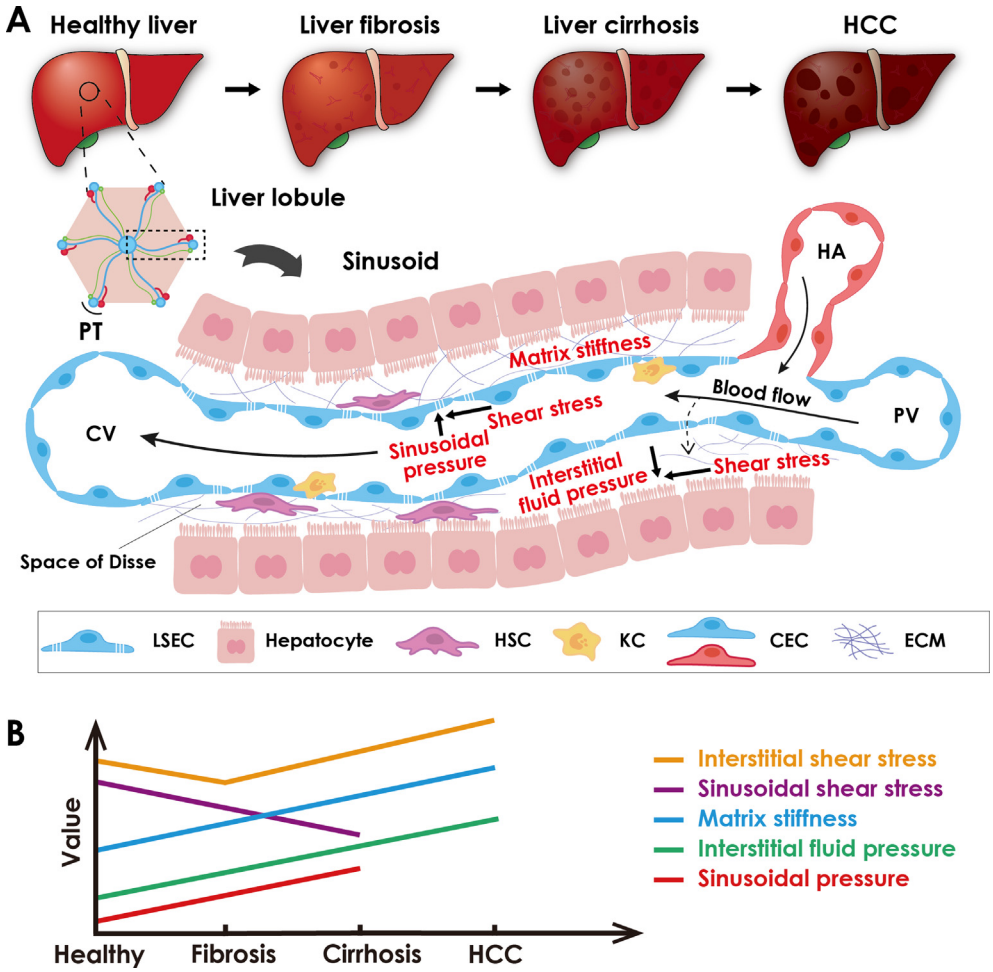

Fig. 1. Alteration of mechanical microenvironment in the development of hepatic fibrosis, cirrhosis, and cancer. *A*) Hepatic microstructures and mechanical microenvironment. The liver is organized into basic functional units termed as lobules, which is mainly composed of sinusoids arranged radially and hepatocytes in plates. The sinusoids are hepatic capillaries lined by fenestrated LSECs and sporadically distributed KCs, connecting blood flows from the branches of HA and PV to CV. All of those vascular tracts are lined with continuous endothelial cells (CECs). Between the hepatocytes and the sinusoids is the space of Disse, containing minimal ECM and quiescent HSCs in a healthy liver. Those mechanical cues such as matrix stiffness, shear stress, and IFP constitute a complicated mechanical niche that is crucial for liver homeostasis and pathogenesis. *B*) A dramatic change in mechanical microenvironment occurs during the development of liver fibrosis, cirrhosis, and HCC, as summarized in Table 1.

9.6 kPa in various liver fibrosis stages (F1-F3), which might be misdiagnosed as liver cirrhosis. After steroid treatment, the stiffness is decreased to 7.2 ± 2.3 kPa without change in fibrotic stage, demonstrating the significant effect of inflammation on liver tissue stiffness values [21].

In addition, several studies elaborate the relationship between matrix stiffness and liver fibrosis. Compared with those bulk stiffness values measured by ultrasound transient elastography and MRE *in vivo*, the liver tissue stiffness obtained by rheometry or atomic force microscopy (AFM) *in vitro* is much lower (Table 1). For example, the shear storage modulus of fresh liver tissue determined by rheometry is about 400 Pa for healthy rats and increased three or four-fold in carbon tetrachloride (CCl_4)-induced rat model of fibrosis [22]. Actually, increased liver tissue stiffness by lysyl oxidase (LOX)-mediated collagen cross-linking occurs in early stages of liver fibrosis, even prior to significant collagen deposition. Treatment with LOX inhibitors partially prevents early tissue stiffening, which, in turn, slows down HSC activation and liver fibrosis. More interestingly, local matrix stiffness quantified using AFM reveals mechanical heterogeneity of normal and fibrotic liver tissues [23]. The elastic modulus of normal mouse livers is around 150 Pa, with slightly stiffer periportal zones than pericentral zones. In contrast, the presence of fibrotic tracts dramatically increases local stiffness up to 1–6 kPa in fibrotic livers, except for the distant zones away from collagen deposition. These findings demonstrate that local matrix stiffness is highly region-specific and may induce various responses in nearby cells.

It is noticed that majority of above stiffness measurements applies a linear elastic model to describe mechanical properties of liver tissue and estimate the tissue elasticity alone [24]. Unfortunately, liver tissue viscosity, serving as another fundamental mechanical feature and determined by stress relaxation and strain creeping, is usually excluded from those studies, potentiating the bias in the estimation of tissue stiffness and even the misdiagnosis [25]. To address this issue, several nonlinearly viscoelastic models, such as Voigt model, modified Mooney-Rivlin model, and Prony series-based model, are employed to assess both the elasticity and viscosity of liver tissues based on the data obtained from ultrasound elastography, MRE, or rheometer [26–30]. Evidently, both elasticity and viscosity are well correlated with liver fibrosis, although viscosity is less effective than elasticity for fibrotic staging [26]. In some cases, neglecting viscosity does not significantly reduce the performance of elasticity measurements in liver fibrosis assessment. Nevertheless, a typical fat accumulation still affects

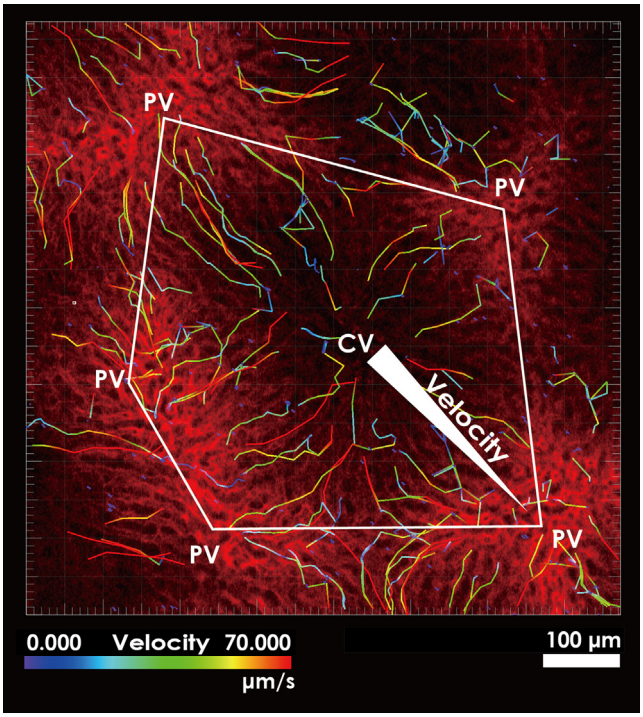

Fig. 2. Global hemodynamics distribution determined by particle tracking velocimetry in liver tissue after tissue clearing. Representative fluorescent image of C57BL/6 mice hepatic sinusoidal networks, which was recorded at λExc/λEm: 655 nm to 670 nm and labelled by tomato lectin. Velocity streamlines of fluorescent particles were generated from PV to CV through sinusoidal networks, with increasing velocity from periportal to pericentral zones indicated. The diameter of particles was 2 μm, and particle motion was recorded in an interval of 0.25 ms at λExc/λEm: 365 nm to 445 nm.

the viscosity of liver tissue in early stage NAFLD, but not liver elasticity, suggesting that viscosity may provide particularly useful information for early diagnosis of NAFLD [27].

The development of HCC is often accompanied with a transition from the premalignant to the tumorous microenvironment and highly correlated with liver cirrhosis and tissue stiffening [8]. Liver tissue stiffness measurements are used for risk assessment of HCC development, post-hepatectomy liver failure, and liver-related death [31,32]. For example, elastic modulus of the liver measured by ultrasound transient elastography is significantly greater in patients with HCC, and liver tissue stiffness value > 12.0 kPa is defined as an independent risk factor for HCC concurrence in hepatitis C virus (HCV) patients [32]. Notably, the liver stiffness of HCC tumors is even higher than the surrounding parenchymal regions in compensated cirrhotic livers [33]. Meanwhile, local viscosity of malignant hepatic tumors is significantly higher than that in benign tumors, making the viscosity as the better discriminator for tumor characterization than the elasticity [34].

Collectively, although liver tissue stiffness serves as the indicator in the diagnosis and prognosis of liver fibrosis, cirrhosis and even HCC development, global and local tissue stiffness measurements provide distinct readouts. Moreover, it is evident that elasticity-based test is just a simplified mechanical approach, asking for viscoelasticity-based analysis when necessary.

2.2. Flow shear stress

In the liver, shear stress is defined as the frictional force applied by blood or interstitial fluid flowing on the surface of hepatic cells. The blood flow shear stress (τ) is usually calculated based on the Hagen-Poiseuille formula,

$$\tau = \frac{4\mu Q}{\pi r^3} \tag{4}$$

where Q is the blood volume flow-rate, μ is the blood dynamic viscosity, and r is the radius of blood vessel [35]. Noting that Eq. (4) is based on the parabolic velocity profile of steady Poisseuille flow, where the blood is considered as a Newtonian fluid with constant dynamic viscosity. Since these assumptions do not always hold in the hepatic microcirculations, the estimation of shear stress using Eq. (4) is only approximate, especially in the sinusoids. Meanwhile, interstitial flow in the space of Disse originates from blood plasma extravasating from sinusoids through fenestrae and gaps in LSECs, and the shear stress caused by interstitial flow has never been directly measured yet [36].

Hepatic hemodynamics in the large vessels is usually measured for clinical evaluation, using Doppler ultrasonography or magnetic

Table 1
Alteration of mechanical microenvironment in the development of hepatic fibrosis, cirrhosis, and HCC.

Mechanical microenvironment	Species	Healthy	Non-cirrhotic stage of fibrosis (F1-F3)	Cirrhosis (F4)	HCC	Method	Ref.
Liver tissue stiffness (elastic modulus)	Human	<6 kPa	6–12.5 kPa	>12.5 kPa	>12.0 kPa	Ultrasound transient elastography	[17,32]
Liver tissue stiffness (shear modulus)	Human	2 kPa (90 Hz)		5 kPa (90 Hz)		MRE	[17]
Liver tissue stiffness (shear modulus)	Human			6.1 ± 2.3 kPa (60 Hz)	12 kPa in tumors, 6.2 ± 2.1 kPa in normal regions (60 Hz)	MRE	[33]
Liver tissue stiffness (shear storage modulus)	Rat	400 Pa	1.2–1.6 kPa			Rheometry	[22]
Matrix stiffness (elastic modulus)	Mouse	150 Pa	1–6 kPa			AFM	[23]
Sinusoidal shear stress	Mouse	17.9 dyne/cm^2	12.9 dyne/cm^2			Intravital microscopy	[45]
Sinusoidal shear stress	Human	2.8–4.2 dyne/cm^2		0.21–2.2 dyne/cm^2		Vascular corrosion casting, micro-CT imaging and CFD modeling	[56,57]
IFP	Human	13.19 ± 8.32 mmHg		18.34 ± 8.82 mmHg	Encapsulated: 49 ± 24 mmHg, Nonencapsulated: 23 ± 15 mmHg	Insertion of needle for pressure measurements	[81,107]

resonance imaging (MRI) [37,38]. The total hepatic flow rate of healthy humans determined by MRI is 19.7 ± 4.6 mL/(min·kg), with PV and HA providing 80.9% and 19.1% of total hepatic blood flow entering the liver, respectively [38]. The portal flow rate is decreased in cirrhotic livers with increased intrahepatic resistance and portal pressure, but the arterial flow is inversely increased to maintain total liver perfusion to adequate levels [39]. In addition to these basic flow parameters such as flow rate or flow velocity, shear stress in PV and HA is also detected by time-resolved four-dimensional (4D) flow MRI, which shows 3D blood flow patterns along three respective flow directions [40,41]. Animal studies further unravel the effect of shear stress in the pathogenesis of liver diseases. Decreased PV shear stress is observed in rat cirrhotic livers with portal hypertension, which is closely associated with abnormal ultrastructural changes of the PV [42].

To date, *in vivo* noninvasive measurements of intrahepatic microcirculation are not possible yet for human subjects due to quite narrow hepatic sinusoids. Shear stress is estimated to be about 0.1–0.5 dyne/cm^2 within human sinusoidal vessels in early studies [43]. Recently, the sinusoidal volumetric blood flow and diameter are observed by intravital microscopy and the sinusoidal shear stress is calculated in the range of 10–20 dyne/cm^2 for healthy mice or rats [44–46]. During liver fibrogenesis, vascular structures are changed within the intrahepatic circulation including sinusoidal capillarization, angiogenesis and vasoconstriction, leading to increased intrahepatic resistance and significant hemodynamics alterations [47]. A case in point is the marginally-reduced sinusoidal shear stress in mild fibrotic livers observed in a murine steatosis-inflammation-tumor model, consistent with those data from PV [42,45]. Alternatively, ultrasound localization microscopy (ULM) with clinically approved contrast microbubbles offers new possibilities for noninvasive imaging and hemodynamics quantification of microvessels at capillary level (>15 μm in diameter) [48]. After further improvement of spatial resolution, ULM could be helpful for sinusoidal blood flow measurements and liver fibrosis staging in the future [49]. In addition to the flow shear inside the sinusoids, it seems reasonable to speculate that interstitial shear stress exerted on the hepatocytes decreases correspondingly in mild fibrotic livers, especially with the lining of defenestrated LSECs. In cirrhotic livers, however, vascular leakage and elevated sinusoidal pressure may augment interstitial flow [50]. Unfortunately, it is still short of measured data for interstitial shear stress due to the technical difficulties for the very narrow space of Disse (1–2 μm).

Compared to individual sinusoids, the global hemodynamics distribution in an entire hepatic lobule, that plays a central role by linking macro- and micro-scale blood flow, is more difficult to be quantified experimentally or theoretically due to its complicated structure. Various mathematical and computational approaches are used for predicting the hepatic blood flow and related liver functions at lobule level [13]. For instance, virtual lobules are constructed algorithmically based on graphical approach using morphologic parameters to elicit portal-to-centrilobular blood flow, mass transfer, and cell-level chemical distribution in the hepatic lobules [51]. Porous media theory is also adopted to construct 3D live lobule models for deciphering blood and interstitial flow dynamics, glucose metabolism, and bile production in physiological and pathological conditions [52–55]. Meanwhile, medical image-based flow simulations provide elaborative information of hepatic microcirculation. After vascular corrosion casting, high-resolution (2.6 μm) micro-computed tomography (CT) scanning and computational fluid dynamics (CFD) simulations are applied to construct 3D numerical microcirculation models to explore microperfusion and permeability in normal and cirrhotic livers [56,57]. Generally, flow shear stress remains under 10 dyne/cm^2 and presents lower values in the cirrhotic microcirculation compared to a normal liver, which is consistent with those data measured by intravital microscopy [45]. Moreover, CFD models of liver sinusoidal network are also constructed based on 3D tissue reconstruction of high-resolution confocal images [58,59]. Adding new fluid transportation information obtained from intravital microscopy may improve the accuracy of fluid dynamic modeling, as demonstrated in bile flow analysis [60]. In order to raise the imaging depth, we recently developed an *in situ* model of the hepatic lobular microcirculation network using tissue clearing and high-resolution confocal imaging techniques, and quantified the global velocity distribution in the lobules using micro-scale particle tracking velocimetry (Fig. 2). A significant increase of flow velocity was observed from periportal to pericentral zones, consistent with zonal gradient of leukocyte velocity in the liver sinusoids visualized by intravital microscopy [61]. Deep learning neural network employed for data analysis in this model would favor to extract the spatial–temporal profiles of lobular hemodynamics in the pathogenesis of liver fibrosis.

HCC is a well-vascularized tumor with unpaired feeding arteries instead of normal PTs [62,63]. All arterial branches supplying a tumor are interconnected with each other throughout the tumor vasculature, playing an important role in hepatic tumor development, invasion, and metastasis [64]. This abnormal tumor angiogenesis leads to the increased supply from hepatic arteries and the decreased supply from portal flow, serving as an indicator in the diagnosis of HCC [39,65]. Moreover, liver is also a predilection site for metastatic tumors, and the low shear stress and high tissue stiffness in a cirrhotic liver can increase the possibility of cancer cell arrest and migration. Recently, a detailed poroviscoelastic model of a liver lobule is applied to decipher the dynamics of tumor-parenchyma interactions (adhesive, repulsive, and elastic forces) during metastatic seeding and growth, demonstrating how a micrometastase disrupts the normal interstitial flow and mechanical pressure [66]. Meanwhile, increased local tissue stiffness induces a “fingering” tumor invasion pattern along paths of mechanical least resistance, giving insight into the effect of tissue mechanics on promoting liver metastatic growth.

Taken together, hepatic blood flow velocity or rate varies significantly from normal to pathological livers, yielding different patterns at distinct sinusoid and lobule scales. Flow-induced shear stress can be estimated based on flow velocity data and mechanical modeling, which could be improved through high-resolution imaging and multi-scale modeling.

2.3. Hydrostatic pressure

In the liver, hydrostatic pressure is defined as the pressure exerted by fluid due to gravity against the capillary wall or surrounding tissue, including sinusoidal pressure and interstitial fluid pressure (IFP). Both can be evaluated by portal hypertension, a frequently presenting clinical syndrome in cirrhotic patients [67,68].

Portal hypertension is defined by increased pressure gradient between the PV and the inferior vena cava (above 5 mmHg) due to the raised intrahepatic resistance [69]. The hepatic venous pressure gradient (HVPG), which is calculated as the difference between the wedged hepatic venous pressure (WHVP) and the free hepatic venous pressure (FHVP) either with a wedge catheter or a balloon catheter, serves as the gold standard approach for estimating the degree of portal hypertension in liver diseases [70]. The HVPG measurement can be used in the diagnosis of liver cirrhosis, the risk prediction of complications and HCC, and the monitoring of the efficacy of medical treatment [69,70]. Most patients with non-cirrhotic stage of fibrosis (F1-F3) yield an HVPG score within normal range (1–5 mmHg), while cirrhotic patients (F4) have an HVPG of 6 mmHg or greater [70]. HVPG > 10 mmHg is termed as clinically significant portal hypertension, indicating the increased

risk of developing gastroesophageal varices and HCC [69,71]. In decompensated cirrhosis, defined by the development of ascites, variceal hemorrhage, encephalopathy, and jaundice, an HVPG of >16 mmHg is an independent predictor of poor outcome [70].

Portal hypertension is the direct or indirect driver of almost all the complications of liver cirrhosis, except for jaundice [72]. For instance, development of gastroesophageal varices and associated variceal bleeding is the most direct consequence of increasing portal pressure. Portal hypertension also induces portosystemic shunts, thereby increasing the amount of vasodilators bypassing the liver metabolism and degradation and leading to systemic and splanchnic vasodilatation [73]. This triggers the activation of homeostatic mechanisms such as the sympathetic nervous system and renin-angiotensin-aldosterone system to increase the levels of circulating vasoconstrictors, which in turn induce renal vasoconstriction, reduce renal blood flow and glomerular filtration rate, and lead to the development of hepatorenal syndrome [74]. On the other hand, various surgical procedures, such as transjugular intrahepatic portal-systemic shunt (TIPS), splenectomy and pericardial devascularization, are developed to relieve portal hypertension. These procedures induce a marked decrease in portal blood flow thereby helping lower the portal pressure [75]. Despite the increased HA flow in compensation, the total hepatic blood flow is usually reduced after operation, leading to decreased sinusoidal shear stress which may facilitate LSEC dysfunction and thrombus formation [75–77]. Therefore, the hemodynamic analysis is required to assess the risk of postoperative sequela.

In cirrhotic livers, portal hypertension gives rise to elevated sinusoidal pressure, which accelerates transsinusoidal fluid flux and increases intrahepatic IFP [78]. The elevated IFP may induce the activation and proliferation of HSCs to accelerate the progression of liver cirrhosis and the related complications [79]. In case of HCC, IFP is elevated throughout the tumor because of the collapsed lymphatics and abnormally leaky capillaries, only decreasing at the tumor periphery where functional lymphatics exist [80,81]. Elevated tumor IFP generates a fluid flow toward the surrounding normal tissue, exposing HCC cells in the tumor margin to the increased interstitial shear stress [82]. The high IFP inside the tumor and the sharp drop in IFP at the tumor-normal tissue interface impedes the chemo- and immuno-therapy by preventing drug penetration and motility of immune cells [83]. Therefore, measurements of IFP could be used as a physical biomarker to predict and assess the efficacy of cancer therapy. Unfortunately, the traditional measurements of IFP are often performed with invasive methods such as insertion of wick-in needles or piezoelectric pressure catheters, limiting their clinical application [84]. Encouragingly, noninvasive estimation of tumor IFP has been actualized based on dynamic contrast enhanced (DCE)-CT or DCE-MRI images in preclinical studies of HCC, which is promising to assist in the clinical decision-making process [85].

In short, elevated hydrostatic pressure contributes to the development and progression of serious complications in liver cirrhosis, and impedes the drug delivery and immunotherapy for HCC. It is expected that noninvasive assessment of IFP may provide valuable information for the management of patients with liver cirrhosis or HCC in the foreseeable future.

2.4. Other mechanical stimuli in livers

This review mainly focuses on the alterations of mechanical microenvironment above mentioned during liver fibrogenesis and cancerization and summarizes their related cellular responses and mechanotransduction pathways. As exemplified in Table 1 and illustrated in Fig. 1B, the liver tissue stiffness is increasing with fibrosis progression, and displays even higher values in tumor regions of HCC patients. Decreased sinusoidal shear stress is observed in both mild fibrotic and cirrhotic livers, while elevated intrahepatic IFP is stemmed from cirrhosis-related portal hypertension and increased sinusoidal pressure and further enhanced in the tumor microenvironment of HCC. Presumably, the interstitial shear stress may go down and up in mild fibrotic and cirrhotic livers, respectively, and rise in the margin of HCC tumors. Significantly, the matrix stiffness, IFP, and interstitial shear stress are elevated even more in the tumor microenvironment of HCC, compared with those in liver cirrhosis.

In fact, other mechanical factors such as mechanical stretch and osmotic pressure also have impacts on liver diseases, although these factors have not been measured clinically yet [6,15,67]. For instance, mechanical stretch of LSECs contributes to fibrogenesis and portal hypertension by inducing Notch-dependent C-X-C chemokine ligand 1 (CXCL1) release and neutrophil recruitment [86]. Peritoneal lavage with distilled water during surgery is reported to improve the survival rate of patients with spontaneously ruptured HCC, showing the cytocidal effect of hypotonic stress in cancer cells [87]. More details can be referred in the related reviews [6,7,87,88].

3. Mechanoregulation and mechanotransduction of hepatic cells

During development and progress of liver fibrosis and HCC, the changes in mechanical microenvironment can induce the dedifferentiation and functional loss of hepatocytes, promote the capillarization and angiogenesis of LSECs, accelerate the myofibroblastic activation of HSCs, and facilitate the proliferation, migration and invasion of HCC cells (Fig. 3 and Supplementary Table 1). Technically, the effect of matrix stiffness on hepatic cells is investigated by 2D or 3D culture *in vitro*, utilizing compatible biomaterials with varied elastic moduli. The impact of shear stress is usually studied in a flow chamber or microfluidic chip, while *in vitro* models of hydrostatic pressure is produced by fluid gravity or compressed gas. Mechanistically, mechanical regulations on hepatic functions can be corroborated by those mechanotransduction pathways for various types of hepatic cells, as discussed below.

3.1. Hepatocytes

Hepatocytes are hepatic parenchyma cells comprising 80% of liver mass, and perform numerous vital functions, including metabolism, synthesis, and detoxification [89,90]. The phenotype and function of primary hepatocytes are highly regulated by substrate stiffness, spanning over a wide range of 1 Pa-116 kPa [5,91–95]. Hepatocytes cultured on a soft substrate remain a relative differentiated epithelial phenotype for a long duration compared to a stiff substrate [93]. Increased stiffness induces downregulation of albumin and urea synthesis, cytochrome P450 (CYP) activity, as well as drug metabolism [93,94]. Gene profiling of human hepatocytes shows that high stiffness increases the expression of those genes involved in the regulation of actin cytoskeleton and ECM-receptor interaction, indicating a mechanotransduction from ECM to cytoskeleton [90]. Several cytoskeleton-related mechanotransduction pathways participate in this stiffness-induced responses of hepatocytes. For example, fibrotic levels of matrix stiffness, as recapitulated as the *in vitro* hydrogel stiffness of 1–60 kPa, inhibit the hepatocyte nuclear factor 4 alpha (HNF4α)-targeted, liver-specific gene expressions, mainly through the activations of β_1 integrin, focal adhesion kinase (FAK), and Rho/Rho-associated protein kinase (ROCK) pathway [23]. High stiffness also modulates the phenotypes of hepatocytes and other hepatic cells by inducing nuclear deformation directly [94]. Disconnecting the cytoskeletal-nuclear links by cytoskeleton disruption or nesprin disconnection

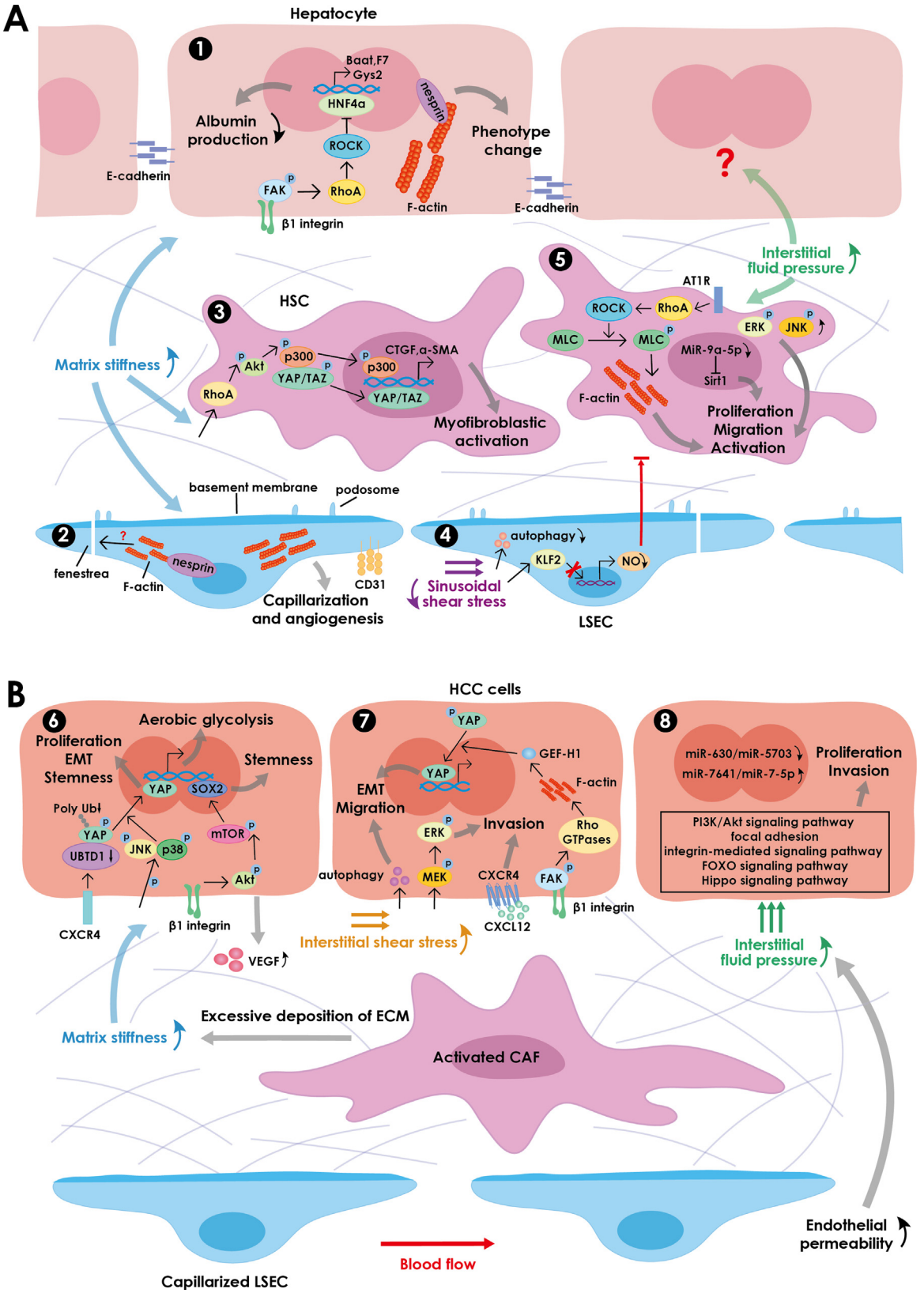

Fig. 3. Schematic representing mechanotransduction pathways in liver fibrosis and HCC. *A*) During liver fibrosis progression, the increased matrix stiffness attenuates liver-specific functions of hepatocytes *via* β_1 integrin signaling-mediated HNF4α inhibition (①), promotes capillarization and angiogenesis of LSECs *via* cytoskeleton remodeling or nuclear deformation (②), and accelerates myofibroblastic activation of HSCs *via* p300 or YAP signaling (③). In cirrhotic livers, the decreased sinusoidal shear stress leads to LSEC dysfunction (④), while the increased IFP promotes HSC activation *via* ERK-, JNK-, MiR-9a-5p- or AT1R-dependent pathways (⑤). *B*) During HCC development, the increased matrix stiffness (⑥), enhanced peripheral interstitial flow (⑦) and elevated IFP (⑧) in the tumor microenvironment promote growth, invasion and metastasis of HCC cells *via* β1 integrin, YAP, and PI3K/Akt signaling.

restores healthy cell phenotype and morphology. Additionally, nuclear deformation squeezed by large lipid droplets in fatty acid-treated hepatocytes increases the nuclear translocation of a specific mechanotransducer, Yes-associated protein (YAP), and prevents hepatocytes from sensing proper matrix stiffness by disrupting stress fibers and focal adhesions [95]. Noting that this is a special case for fibrotic NAFLD, but not liver fibrosis caused by other pathogenic factors, which is thus not included into those mechanotransduction pathways in Fig. 3.

Flow shear stress is another key factor in modulating the phenotype and function of hepatocytes, as demonstrated by several microfluidic liver-on-a-chips that are developed to mimic the liver structure and microenvironment [96]. The effects of shear flow on hepatocytes are biphasic, promoting live-specific functions at low shear stress and damaging hepatocytes at high shear stress [97]. The porous membrane or micropillar array is usually placed inside the liver chips to prevent direct flow exertion onto the hepatocytes and reduce the shear stress level on the surface of hepatocytes, similar to that of the space of Disse *in vivo* [98–101]. An increase of liver-specific outcomes, such as urea and albumin production and CYP enzyme activities, is also observed, no matter if the hepatocytes are cultured in 2D, 3D or spheroid structures. For example, a two-layered microfluidic chip separated by a porous polyester (PE) membrane is developed in our lab to mimic liver sinusoidal structure and functions [102,103]. The primary murine LSECs and Kupffer cells (KCs, resident liver macrophages) are cultured in the upper channel connected to a syringe pump for applying direct shear flow, and the hepatocytes and HSCs are cultured in the lower channel. A shear stress of 0.1–1 dyne/cm^2 is exerted on the upper channel substrate, and the fluid dynamics inside liver chip is quantified by CFD or immersed boundary method (IBM) simulations [102,104–108]. The shear stress on the surface of hepatocytes in the lower channel is much lower than that of the upper channel, and the alteration of shear stress exhibits a biphasic pattern with varying endothelial permeability, collagen layer thickness or lower channel height [104,105]. When the hepatocytes are cultured alone, shear flow induces an increase in albumin secretion, and enhances the activities of CYP1A2 and CYP2D6. Moreover, the combination of non-parenchymal cells (NPCs) co-culture and shear flow yields even higher albumin secretion, since NPC-derived hepatocyte growth factor (HGF) production is increased by shear stress and contributes to liver-specific functions [102]. Numerical simulations suggest that the interstitial shear stress on the surface of hepatocytes decreases with increased thickness of collagen layer at low endothelial permeability in mild fibrotic livers, but increases with the increase of the thickness when the endothelial permeability is high in cirrhotic livers [105]. Thus, the alteration of shear stress could be one of the reasons for hepatocytes to lose their functions during fibrogenesis.

Moreover, applying hydrostatic pressure on hepatocytes is reported to enhance albumin production and upregulate the expression and activity of CYP1A2 in an aryl hydrocarbon receptors (AhR)-dependent manner [106]. It should be noted that the pressure applied (10 cm$H_2O \approx$ 7.5 mmHg) is right at the normal range of IFP in healthy livers (13.19 ± 8.32 mmHg), hence it remains uncertain how evaluated IFP (18.34 ± 8.82 mmHg) affects the phenotype and function of hepatocytes in cirrhotic livers [107].

3.2. LSECs

LSECs are highly specialized endothelial cells forming the wall of the hepatic sinusoids and play a crucial role in maintaining intrahepatic homeostasis [11]. LSECs display unique phenotype with non-diaphragmed fenestrae and a lack of basement membrane, and act as a selective barrier between blood flow and hepatocytes [109]. They contribute to the regulation of the vascular tone and maintain HSC quiescence in physiological conditions, but become rapidly capillarized by losing their fenestrae and forming a basement membrane during the early stages of liver fibrosis [11]. The capillarization of LSECs promotes angiogenesis, vasoconstriction, and fibrosis development. Thus, the visualization and recognition of LSEC fenestrae are of great interest to understand the pathological processes of liver fibrosis.

Specialized fenestra structure serves as a standard phenotypic indicator of LSECs, ranging from 50 to 300 nm in diameter and usually organized in clusters termed sieve plates [15,110]. While these fenestrae can be visualized routine by scanning electron microscopy (SEM), other super-resolution fluorescence techniques and AFM, together with the multiple algorithms based on neural networks, are also proposed to quantify fenestra morphologies [111,112]. Meanwhile, the actin filaments (F-actin) appear to surround the fenestrae and the depolymerization of F-actin results in the increase in fenestra number and the decrease in cell elastic modulus [113,114]. Therefore, mechanical remodeling of F-actin may affect the LSEC phenotype directly.

Matrix stiffening is known to promote LSEC capillarization and angiogenesis. LSECs placed on stiffer substrates present higher numbers of actin stress fibers and podosomes, and undergo a functional loss in hyaluronan endocytosis [115,116]. The capillarization of LSECs on stiff substrate is further confirmed by the decreased porosity of fenestrae and increased expression of CD31 on cell surface [94,117]. More interestingly, LSECs cultured on 200–610 Pa substrates migrate collectively to form capillary-like structures, simulating angiogenesis during the early-stage of liver fibrosis, which could in turn generate mechanical forces leading to HSC activation through condensation of collagen fibrils [6,118]. By contrast, stiffer substrates (>1.2 kPa) promote random migration of LSECs, mimicking the leaky vessels as observed in the late-staged fibrotic or cirrhotic livers, which induces fewer collagen fibril remodeling. To date, the matrix stiffness-induced mechanotransduction of LSECs are not fully understood, in which the cytoskeleton remodeling and nuclear deformation are probably involved in this process [94,117].

Shear flow is recognized as a main regulator of LSEC homeostasis. Under physiological conditions, LSECs can sense the changes in shear stress and response to it rapidly [6]. Shear stress-induced increase of transcription factor Kruppel-like factor 2 (KLF2) promotes the secretion of vasodilating agents, which cause the relaxation of blood vessels, inhibit the activation and constriction of HSCs, and eventually reduce the shear stress of blood flow [6,11,119,120]. In fibrotic or cirrhotic livers, however, the capillarized LSECs undergo endothelial dysfunction, losing their abilities to generate vasodilator agents in response to shear stress, which can be reversely improved by statin administration [119]. Meanwhile, shear stress also presents a vasoprotective effect by activating autophagic flux in LSECs, and the deficiency in endothelial autophagy promotes the development of liver fibrosis [121]. After partial hepatectomy (PHx), a common surgery for patients with liver cirrhosis or HCC, the shear stress on LSECs increases immediately and modulates the release of paracrine factors that contribute to liver regeneration [5]. For example, the production of hepatocyte growth inhibitor, transforming growth factor-beta 1 (TGFβ1), is drastically decreased *via* G protein-coupled receptors (GPCRs) in response to increased shear stress following liver resection [14]. Increased perfusion on LSECs activates β_1-integrin and vascular endothelial growth factors receptor 3 (VEGFR3) that promote the secretion of angiocrine signals such as HGF, interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) [122]. On the other hand, disturbed shear stress is responsible for LSEC senescence driven by Notch activation, which retards liver regeneration on day 14 of post-PHx [123]. Moreover, excessive shear stress could damage

LSECs and induce hemorrhagic necrosis [11,124]. These findings demonstrate a biphasic role of shear stress on LSEC functions.

To date, it is still unavailable to address the regulation of hydrostatic pressure on LSECs. Elevated microvessel pressure can enhance pulmonary vascular permeability and promote tube formation of human umbilical vein endothelial cells (HUVECs), providing some clues for the possible responses of LSECs in cirrhotic livers [67].

3.3. HSCs

HSCs, a mesenchymal cell type localized in the space of Disse, yield a quiescent phenotype and store vitamin A as lipid droplets in healthy livers [125]. During liver injury, HSCs lose vitamin A and differentiate into migratory, proliferative, and contractile myofibroblasts, which are responsible for the excessive ECM deposition in fibrosis and cirrhosis [16].

The myofibroblastic differentiation of HSCs is a critical event in liver fibrosis [125]. Applying a stiff substrate alone is able to induce myofibroblastic activation of HSCs, characterized by increased expression of α-smooth muscle actin (α-SMA) and excessive deposition of ECM [8,125,126]. Stiff substrate is known to activate protein kinase B (Akt) signaling *via* Ras homolog family member A (RhoA) to induce p300 phosphorylation and its nuclear translocation, leading to transcription of α-SMA and HSC activation [127]. Substrate stiffening promotes YAP/Transcriptional coactivator with PDZ-binding motif (TAZ) nuclear translocation and traction force generation in HSCs [128]. Noting that p300 is identified as a cytoplasm-to-nucleus shuttle for TAZ, the stiffness-induced YAP/TAZ nuclear translocation may be also facilitated by p300 shuttle [129]. Moreover, HSCs are able to differentiate into cancer-associated fibroblasts (CAFs) during the development of HCC, and play a tumor-promoting role by establishing a stiff tumor niche and cytokine secretion [8,127].

Increased hydrostatic pressure is demonstrated to upregulate myofibroblastic activation of HSCs. Partial ligation of inferior vena cava *in vivo* or pressure-loaded *in vitro* enhances the expression of angiotensin II type 1 receptor (AT1R), upregulates the RhoA, ROCK1 and the phosphorylation of myosin light chain 2 (MLC2), increases the formation of actin stress fibers, and promotes the HSC activation [79]. In addition, other pathways are also involved in the pressure-induced HSC activation, such as the retinoid metabolic pathway, the extracellular signal-regulated kinase (ERK) 1/2 and c-Jun N-terminal kinase (JNK) signaling pathways, or the overexpression of microRNA miR-9a-5p-induced Sirt 1 inhibition [130–132]. Contraction of activated HSCs could further increase the intrahepatic resistance and portal pressure, forming a positive feedback loop to exacerbate liver cirrhosis and portal hypertension.

To date, it is still unknown how interstitial flow affects the phenotype and function of HSCs. While technical difficulties exist in experimental measurements of flow velocity profiles in such the narrow space of Disse, it seems possible to conduct numerical simulations and physical model tests *in vitro* to predict the shear stress exposed to HSCs and test shear-induced cell responses using a typical flow chamber assay.

3.4. HCC cells

More than 80% of HCC cases occur in advanced fibrotic or cirrhotic livers, and the increased tissue stiffness and IFP are crucial for HCC oncogenesis and progression [8]. Matrix stiffening-induced nuclear deformation is hypothesized to increase chromosome missegregation, genetic instability, and DNA damage, which may contribute to hepatocarcinogenesis [9,94]. Mechanical compression may induce activation of tumorigenic Wnt/β-catenin pathway [133,134], contributing to hepatocarcinogenesis. An additional effect of the mechanically-activated HSCs in cirrhotic livers is the production of oxygen species (ROS), which promotes DNA damage and mutations in hepatocytes, therefore favoring tumorigenesis [135].

Besides suppressing liver-specific functions, increasing matrix stiffness is able to promote the proliferation, migration, invasion, and drug resistance of HCC cells [136–139]. High stiffness also contributes to tumor heterogeneity, which is considered as the major cause of drug resistance, enhancing the epithelial to mesenchymal transition (EMT) and the stemness characteristics of HCC cells [137,140–143]. Additionally, HCC cells tend to spread and migrate faster on viscoelastic substrates than on purely-elastic substrates with same stiffness, whereas the opposite effect occurs with hepatocytes [144]. Typically, YAP- and β_1 integrin-related signaling pathways are involved in stiffness-dependent mechanotransduction in HCC cells. For instance, increasing matrix stiffness remarkably up-regulates the expression of C-X-C chemokine receptor type 4 (CXCR4), decreases the levels of ubiquitin domain-containing protein 1 (UBTD1), reduces the YAP ubiquitylation, and activates the transcription of YAP-targeted genes that are involved in the proliferation, EMT, and stemness of HCC cells [141]. Meanwhile, stiffer ECM is known to promote HCC migration through enhanced glycolysis *via* JNK and p38 mitogen-activated protein kinase (MAPK)-activated YAP signaling [145]. On the other hand, matrix stiffening could drive β_1 integrin/Akt/mechanistic target of rapamycin (mTOR)/sex determining region Y box 2 (SOX2) signaling in stemness regulation of HCC cells [142]. Matrix stiffness is positively correlated with angiogenesis in liver tumor tissues, where increased matrix stiffness promotes the expression of vascular endothelial growth factor (VEGF) through β_1 integrin/phosphoinositide 3 kinase (PI3K)/Akt pathway and further facilitates HCC angiogenesis [146–148].

In the tumor microenvironment of HCC, the interstitial shear stress is increased in the peripheral zone of the tumors [82]. The elevated interstitial shear flow could amplify those stiffness-induced responses of HCC cells, leading to the dedifferentiation demonstrated by the loss of liver-specific functions [9,108]. It is known that interstitial flow has significant impact on proliferation, migration, and invasion of HCC cells [149,150]. Shear stress tends to endow HCC cells with high migration ability *via* integrin-FAK-Rho GTPases signaling-induced F-actin reorganization in a time-dependent manner [151]. Shear stress-induced remodeling of F-actin could transmit those biomechanical signals from β1 integrin to YAP through guanine nucleotide exchange factor (GEF)-H1, promote the nuclear translocation of YAP, and induce EMT in HCC cells [152]. Additionally, autophagy is also reported to play a crucial role in shear stress-induced EMT and migration of HCC cells [153]. On the other hand, increased tumor IFP may induce an aggressive cancer phenotype, promoting proliferation, migration and invasion of HCC cells. Under a pressure loading of 15 mmHg, downregulated miR 630 and miR 5703 and upregulated miR 7641 and miR 7 5p are identified to be associated with several pathways involved in the development of HCC, including PI3K/Akt signaling pathway, focal adhesion, integrin mediated signaling pathway, forkhead box O (FOXO) signaling pathway and Hippo signaling pathway [154].

Moreover, mechanical properties of HCC cells themselves are closely related with their invasion and metastasis. Despite high liver tissue stiffness in HCC patients, most of HCC cells are softer than normal cells [155]. The stiffness of multiple HCC cell lines is measured by AFM or micropipette aspiration using elastic or viscoelastic models, indicating a negative correlation between the stiffness of HCC cells and their invasive capabilities [156,157]. Low stiffness of HCC cells could hamper T-cell-mediated cancer cell killing due to the reduced membrane pore formation caused by impaired perforin-generated forces, which may provide a

mechanical checkpoint for immunotherapy [158]. Also confirmed recently is that cancer cell stiffening by membrane cholesterol depletion could improve the efficacy of tumor immunotherapy [159].

Collectively, the oncogenesis and metastasis of HCC are highly correlated with increased ECM stiffness, interstitial shear stress and IFP in tumor microenvironment, while the mechanical properties of HCC cells govern tumor immune evasion. Therefore, mechanical modulation represents a promising strategy for antitumor therapy of HCC.

3.5. Immune cells

Multiple types of immune cells are residing or circulating in the liver. KCs are liver-resident macrophages enriched in the periportal regions, providing the first line of defense in hepatic immune system [109,160]. During chronic liver injury, KCs are activated to produce a series of proinflammatory and profibrogenic cytokines, induce the recruitment of other immune cells, and promote HSC activation and liver fibrosis [161]. KCs also participate in the development of HCC by modulating C-C motif chemokine 22 (CCL22) production and recruitment of regulatory T cells (Tregs) [162]. To date, the effect of mechanical microenvironment on KCs is poorly understood, although disturbed sinusoidal blood flow in cirrhotic livers was hypothesized to tame KCs to M2-like tumor-associated macrophages a decade ago [163]. Moreover, KC elimination limits the PHx-associated hyperperfusion and liver regeneration, indicating a regulatory role of KCs in maintaining intrahepatic shear stress [46].

Neutrophils are the most abundant innate immune cell type that plays an essential role in defense against infection and tissue damage [161,164,165]. Hepatic neutrophil infiltration is a critical pathologic feature in various types of liver diseases [166]. Aberrant accumulation of neutrophils in liver can exacerbate acute liver injury, such as ischemia/reperfusion and drug-induced liver injury [164,167]. However, neutrophils also display anti-fibrotic and pro-resolutive functions during development and resolution of liver fibrosis [167,168]. They can play dual roles in HCC development and progression, exhibiting either anti-tumorigenic or pro-tumorigenic phenotypes. Meanwhile, the elevated neutrophil-to-lymphocyte ratio (NLR) is identified as an inflammatory marker associated with worse prognosis in patients with HCC [169,170]. The expression of anti-inflammatory microRNA-223 in neutrophils hinders HCC progression by targeting multiple inflammatory and oncogenic genes in hepatocytes [171]. On the other hand, CAFs activate the expression of programmed cell death 1 ligand 1 (PD-L1) in neutrophils, exerting a pro-tumor effect in HCC by suppressing T cell function through PD1/PD-L1 signaling pathway [172]. In addition, neutrophils could act as a bridge to facilitate liver metastasis *via* a "two-step adhesion" where neutrophils are captured by endothelial cells prior to their capture of those circulating tumor cells [173–175]. Evidently, the above observations highlight potential therapeutic strategies targeting neutrophils for the treatment of liver fibrosis or HCC. Moreover, the regulatory effect of mechanical microenvironment on neutrophils must be taken into consideration. Stiff substrates enhance neutrophil activation through integrin/FAK signaling, demonstrated by increased neutrophil extracellular trap (NET) formation and higher secretion of pro-inflammatory cytokines [176]. Fluid shear stress promotes cluster formation of high affinity lymphocyte function-associated antigen-1 (LFA-1, $\alpha_L\beta_2$ integrin) bonds on arrested neutrophils, initiating Ca^{2+} flux and transmigration [177]. Thus, the increased matrix stiffness and altered shear stress in the liver microenvironment may modulate the recruitment and activation of neutrophils, which in turn affect the development and therapy of liver fibrosis and HCC.

In addition to KCs and neutrophils, other immune cell subsets such as T cells, monocytes and macrophages also contribute to the development of liver diseases, and their phenotype and function are modulated by the mechanical microenvironment. For instance, the high stiffness and high IFP in tumors hinder the infiltration of cytotoxic T lymphocytes (CTLs) and reduce the efficacy of immunotherapy [178,179]. Therefore, the therapy targeting and improving mechanical microenvironment of liver tissue has the potential to become the new adjuvant treatment standard in liver fibrosis and HCC.

3.6. Hepatic differentiation of stem cells

Liver transplantation remains the only well-established treatment for liver failure and end-stage cirrhosis, but the shortage of available donors emphasizes the urgent need for alternative therapeutic strategies such as cell-based transplantation and liver tissue engineering [180]. Considering that adult hepatocytes are difficult to proliferate *in vitro*, functional hepatocyte-like cells that are differentiated from various stem cells could serve as novel sources for cell therapy of liver injury, liver organoid generation, and bioartificial liver development [181].

Cellular mechanical microenvironment plays a vital role in the hepatic differentiation of stem cells. Soft substrates with stiffness similar to that of physiological liver enable hepatocytic differentiation of liver progenitor cells, adipose-derived stem cells and pluripotent stem cells, as demonstrated by the upregulation of liver-specific functions [182–184]. On the other hand, higher traction forces generated by liver progenitor cell cultured on stiff substrates promote biliary differentiation through Notch-dependent signaling [185]. Liver organoid growth is controlled by matrix stiffness *via* integrin/Src family of kinases (SFK)/YAP signaling, with the values mimicking physiological liver tissue stiffness that is optimal for organoid growth [186]. Hepatic differentiation of human embryonic stem cells (hESCs) is more complicated, usually undergoing multiple stages favored by distinct mechanical and chemical stimuli. For example, the impacts of substrate stiffness are often coupled with substrate topography, even though substrate stiffness dominates the fate decision of hESCs in hepatic differentiation [187]. Stiff substrates favor stemness maintenance and definitive endoderm differentiation while soft substrates promote progenitor cell differentiation and hepatocyte-like cell maturation. Furthermore, shear stress also promotes hepatic differentiation of both liver progenitor cells and induced pluripotent stem cells (iPSCs), as expected [188–190]. Hydrostatic pressure exposure enlarges nuclear areas of liver progenitor cells, indicating potential differentiation effects [191].

To summarize, increased liver tissue stiffness can induce hepatocyte dedifferentiation, LSEC capillarization, and HSC activation during early stages of liver fibrosis, initiating a positive feedback loop that further enhances matrix stiffening and liver fibrogenesis and eventually leading to liver cirrhosis. In cirrhotic livers, increased intrahepatic resistance, stemmed from dysregulation of LSECs and HSCs, causes portal hypertension and elevated IFP, which further induces HSC activation and matrix stiffening during liver cirrhosis progression. Most of HCC cases occur in cirrhotic livers. The increased matrix stiffness, interstitial shear stress, and IFP tend to promote HCC oncogenesis, progression and metastasis, hinder the drug delivery and immunotherapy, and enhance tumor angiogenesis and activation of CAFs derived from HSCs, which subsequently facilitate the remodeling of tumor mechanical microenvironment. During development of liver fibrosis and HCC, hepatic cells are highly sensitive to mechanical stimuli. They sense the alterations of mechanical microenvironment and adapt their phenotypes and functions through mechanotransduction pathways, which in turn speed up pathological progression. Therefore, the

application of drugs targeting tissue mechanical microenvironment or cellular mechanotransduction pathway could be a potential therapeutic approach to liver diseases, as discussed later.

4. Molecular biomechanics of hepatic mechanoreceptors

In order to further understand the molecular mechanisms of mechanosensing process and screen potential mechanoreceptor-targeted drugs, a series of biomechanical assays are developed to quantify the force-induced conformational change of membrane receptors and the mechanical regulation of receptor-ligand interactions. For briefing those approaches of micropipette aspiration, optical tweezers, AFM, and molecular dynamics simulations, please refer to those related reviews previously published [192–194]. Here we just focused on typical mechanoreceptors involved in the mechanoregulation of hepatic cells, attempting to elucidate the related cell responses from a molecular biomechanical perspective.

4.1. β_1 integrin

β_1 integrins are a large subfamily of heterodimeric transmembrane receptors for ECM proteins including fibronectin, laminin, and collagens. As mentioned above, β_1 integrin signaling is crucial for mechanotransduction of hepatic cells. In response to altered matrix stiffness or shear stress, β_1 integrin senses and transmits external forces into hepatic cells by recruiting F-actin binding proteins such as talin, kindlin, and vinculin, which in turn activate a series of downstream pathways including FAK, PI3K, Src and JNK to regulate cell phenotype and function [5,195].

Mechanical interactions between β_1 integrins and their ligands are determined to reveal the underlying molecular mechanisms for cell-ECM adhesion [196,197]. As an example, integrins have three major conformations: low-affinity bent conformation with closed headpiece, intermediate-affinity extended conformation with closed headpiece, and high-affinity extended conformation with open headpiece [198]. Direct force measurements of $\alpha_5\beta_1$ integrin/fibronectin interactions are conducted with AFM force-ramp protocols and dynamic force spectroscopy analysis is used to identify two energy barriers of the paired complex [199]. The inner barrier only appears at non-physiological high forces, while the outer barrier, which is more important at lower, physiologically relevant forces, is mainly regulated by integrin activation. Mechanical forces can enhance integrin-mediated cell-ECM interactions through integrin clustering or conformational change [5]. Force-assisted activation of the headpiece guarantees the catch bond behavior of integrin-ligand interactions, as the forces prolong bond lifetimes of $\alpha_5\beta_1$ integrin/fibronectin complex in the 10–30 pN range [200]. Although the direct ligand-binding site of $\alpha_5\beta_1$ integrin is located at the β I domain, molecular dynamics simulations and structure-based mutagenesis assays identify a single specific residue (Asp154) of α_5 subunit that is responsible for strong preference of fibronectin binding [201]. Furthermore, an acidic extracellular pH value in the tumor microenvironment is known to induce the activated conformation of $\alpha_5\beta_1$ integrin with open headpiece, promoting actin cytoskeletal rearrangement and cell morphological changes associated with EMT [202]. These findings yield further insights into the molecular mechanisms of force-induced β_1 integrin signaling in liver diseases.

4.2. E-cadherin

E-cadherin is a transmembrane glycoprotein that forms homodimers in Ca^{2+}-dependent adhesion between epithelial cells including hepatocytes [203]. The cytoplasmic tail of E-cadherin binds to β-catenin, which transmits external forces into the cells through F-actin remodeling and is responsible for the contact inhibition of cell proliferation in epithelial cells [5]. In normal liver, homophilic E-cadherin binding also mediates the adhesion between hepatocytes and HSCs, suppressing TAZ expression and HSC activation [204]. During development of liver fibrosis, the dissociation of this binding leads to myofibroblastic differentiation of HSCs. Moreover, E-cadherin may act as a double-edged sword in tumor development and metastasis [205,206]. On the one hand, impaired expression of E-cadherin plays a key role in HCC development and is associated with a worse prognosis of liver cancer patients [207,208]. On the other hand, moderate E-cadherin levels are presented in tumor cells, which play a key role in mediating loose interactions between neighbor cells necessary for collective migration [209,210] and are likely to promote HCC invasion and metastasis.

Structurally, E-cadherin is composed of an extracellular domain with five tandemly-repeated immunoglobulin-like domains (termed EC domains), a transmembrane (TM) domain and a cytoplasmic tail that is linked to F-actin [211]. All of these structures contribute to E-cadherin-mediated cell–cell adhesion. Crystal structures and biomechanical assays indicate the extracellular regions of E-cadherins from two opposing cells bind together in two distinct conformations: X-dimer and strand-swap dimer [212]. During this *trans* dimerization, a kinetic intermediate X-dimer forms first, as the indicative of a catch bond behavior that strengthens the bond in the presence of forces [203]. Over time, the X-dimer matures to form a higher affinity strand-swap dimer with a slip bond behavior which becomes shorter lived under forces. Meanwhile, both N-terminal EC domain (EC1) and TM domain can form *cis* clusters to stabilize E-cadherin-mediated adhesion [211,212]. Additional evidences suggest that both inside-out and outside-in signaling may involve in allosteric regulation of E-cadherin-mediated cell adhesion [213]. External forces applied from the outside of the cell could activate the E-cadherin/β-catenin signaling and promote the release of β-catenin from junctions into the cytosol and nucleus, where β-catenin targets developmental patterning gene transcription [214]. In contrast, binding of p120 catenin to E-cadherin cytoplasmic region regulates both *cis* and *trans* E-cadherin dimerization [215]. Furthermore, steered molecular dynamics (SMD) simulations indicate that mutations in E-cadherin genome reported in different types of cancer could reduce spring constant and mechanical stability of E-cadherin, which further the understanding of the molecular mechanisms involved in cancer progression [216,217].

4.3. β_2 integrin and CD44

β_2 integrin and CD44 are adhesion molecules involved in neutrophil accumulation in liver diseases. More than 85% of hepatic neutrophil infiltration occurs in the narrow sinusoids, where shear stress in human is rather low and rolling of neutrophils seems not necessarily required [43,218]. The mechanisms for neutrophil recruitment in hepatic sinusoids are drastically different from the classical inflammation cascade in normal tissue, and display distinct paradigms in the specific types of liver diseases [219]. For example, macrophage-1 antigen (Mac-1, $\alpha_M\beta_2$ integrin), but not LFA-1, is required for hepatic neutrophil infiltration during sterile thermal injury, local N-Formyl-Met-Leu-Phe (fMLF) stimulation, or virus infection, whereas shedding of Mac-1 *via* IL-10 exposure leads to a CD44-dependent, integrin-independent recruitment during lipopolysaccharide (LPS)-induced systemic inflammation [220–222]. Distinct functions of these adhesion molecules are assumed to be governed by their ligand-binding kinetics and bond strength [192].

Mac-1 and LFA-1 are two members of β_2 integrin subfamily constitutively expressed on neutrophils, interacting with intercellular cell adhesion molecule 1 (ICAM-1) and other ligands on endothelial cells to mediate neutrophil recruitment. Their structures and allostery are similar to those of β_1 integrins, except that the extra α I domain for β_2 integrin not only serves as the binding pocket of external ligand but also binds to the pocket of β I domain as an internal ligand [198,223,224]. Molecular dynamics simulations indicate that the internal ligand binding is a prerequisite to initiate allosteric transmission and mechanical outside-in activation in a typical β_2 integrin [225]. Besides, two conservative salt bridge interaction pairs within α I domain α_7 helix are related to force-induced ligand binding and shear resistance ability [226]. At molecular level, both binding affinity and bond strength of Mac-1-ICAM-1 complexes are much lower than those of LFA-1-ICAM-1 interactions, likely attributed to their distinct conformations at the interface of Mac-1 or LFA-1 and ICAM-1 [227–230]. Thus, LFA-1 appears to dominate neutrophil adhesion and gives rise to stronger outside-in signaling at post-capillary venules where they are exposed to a high level of shear stress [231,232]. At cellular level, however, ICAM-1 on LSECs binds to Mac-1 with lower association- and dissociation-rates but higher effective binding affinity compared with LFA-1, consistent with Mac-1-dominated neutrophil adhesion and crawling in hepatic sinusoids *in vivo* [233,234]. Numerical calculations and Monte Carlo simulations validate that Mac-1-ICAM-1 interactions are dominant in neutrophil free crawling, since the crawling speed is positively correlated to the effective binding affinity of the complex [233]. It is worth mentioning that the mechanical and physical microenvironment in hepatic sinusoids may amplify the role of Mac-1, since bond strength of Mac-1-ICAM-1 interactions is sufficient enough to support neutrophil adhesion under low shear stress in the narrow sinusoids.

In addition to β_2 integrins, CD44, a type I transmembrane receptor for hyaluronan expressed on the surface of neutrophils, is also able to mediate cell trafficking in multiple organs including liver [235]. The CD44 gene consists of 20 coding exons, which can be alternatively spliced into multiple forms of CD44 including standard (CD44s) and variant isoforms of CD44 (CD44v) [236,237]. Hyaluronan is a linear glycosaminoglycan composed of repeating disaccharide units of N-acetylglucosamine and N-glucuronic acid, anchored on the surface of LSECs by a variety of scavenger receptors, and acts as the key ligand for neutrophil CD44 [235,238]. Since the hyaluronan-binding domain (HABD) of CD44 is common to all of CD44 isoforms, CD44s and CD44v bind to hyaluronan with similar binding kinetics and bond strength [239]. The HABD has two distinctive conformations: low-affinity ordered (O) state and high affinity partially disordered (PD) state [240]. Mechanical force can trigger the allosteric O-to-PD transition and enhance the CD44-HA interactions [240,241]. In addition, the specialized molecular weight or structural modification of hyaluronan seems crucial to the interactions of CD44-HA complexes [238,242]. Nevertheless, the impact of hyaluronan molecular weight is still controversial, as seen in high binding affinity but low bond strength for the interactions between CD44 and high molecular weight hyaluronan [238,242]. Taken together, blockade of hepatic neutrophil infiltration by targeting Mac-1-ICAM-1 or CD44-hyaluronan interactions may provide an anti-inflammatory treatment strategy in liver diseases.

To sum up, multiple conformations and force-induced allosteric activation are common features of the above mechanoreceptors, as reported for other mechanoreceptors such as Notch, VEGFR and GPCR [243–245]. Mechanical regulation of the mechanoreceptors not only offers deep insights into the underlying molecular mechanisms of cell responses, but also provides potential therapeutic targets for drug design related to liver diseases. Antibodies or small inhibitors targeting receptor-ligand interaction or allosteric transition could serve as a therapeutic strategy to hinder the progression of liver fibrosis and HCC.

5. Mechanical cue-targeted drug design and delivery

Multiscale biomechanics studies mentioned above provide useful information for designing novel approaches in treating liver fibrosis and HCC. Application of the so-called mechanobiology-based medicine (mechanomedicine) in this field is a more promising strategy for drug design and delivery compared to those commercially available routine methods provided [246,247].

5.1. Potential drugs targeting tissue mechanical microenvironment

As aforementioned, mechanical microenvironment, especially the increased matrix stiffness and IFP that facilitates hepatic fibrogenesis and hepatocarcinogenesis, and hinder drug delivery and immunotherapy of HCC, could serve as an important, untapped potential target for future adjuvant therapies of liver fibrosis and HCC [6,248]. Below are several examples.

LOX-like 2 (LOXL2), an enzyme involved in the cross-linking of collagen and elastin, is identified as an important factor controlling matrix stiffness and the inhibition of LOXL2 with β-aminopropionitrile (BAPN) results in a marked blunting of early tissue stiffening and a significant decrease in HSC activation in the rat CCl_4 model of fibrosis [8,22]. However, treatment with Simtuzumab, a humanized monoclonal antibody against LOXL2, is ineffective for patients with advanced fibrosis (F3-F4) caused by nonalcoholic steatohepatitis (NASH) or HCV infection in several clinical trials (Table 2) [250–252]. These negative outcomes may be attributed to the delays in timing to initiate drug intervention. In animal model, treatment with LOXL2 inhibitors is started even prior to CCl_4 administration, preventing early liver stiffening and fibrotic progression. In contrast, those clinical trials are conducted with patients having been diagnosed with advanced liver fibrosis, at which Simtuzumab may not have an effect. Thus, it is highly important to test the efficacy of Simtuzumab on the mild-moderate fibrosis in future trials [253]. On the other hand, a higher level of LOXL2 may contribute to tumor progression, promoting EMT and angiogenesis in HCC [254,255]. LOXL2-specific inhibitory antibody AB0023 impedes the formation of tumor microenvironment and reduces tumor sizes in both primary and metastatic xenograft models of cancer [249]. Combination of LOXL2 inhibitor with Sorafenib could serve as an advantageous strategy for HCC therapy.

Sorafenib, an oral multi-kinase inhibitor targeting VEGFR2 and downstream intracellular serine/threonine kinases, is the most common gold-standard treatment for advanced HCC [256]. Recent animal studies also demonstrate that Sorafenib could reduce liver fibrosis by ameliorating intrahepatic vascular resistance, inhibiting angiogenesis, and decreasing collagen deposition [257]. Meanwhile, biomechanical tests indicate that Sorafenib could reverse early-stage fibrosis by inhibiting liver stiffening-induced angiogenesis and collagen condensation [118]. These data indicate that mechanical modulation may play a role in the therapeutic mechanisms of Sorafenib treatment in liver fibrosis and HCC, reducing angiogenesis-induced matrix stiffening and IFP increasing.

Simvastatin is a hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitor that is mainly used for cardiovascular diseases [258]. In cirrhotic livers, LSECs become dysfunctional and lose the ability to regulate vasodilation in respond to shear stress, contributing to HSC activation and portal hypertension. Correspondingly, Simvastatin could enhance the expression of KLF2 in LSECs directly and improve HSC phenotype *via* a NO-dependent pathway in cirrhotic

Table 2
Clinical trials of potential therapies targeting mechanical microenvironment in liver fibrosis or HCC (https://clinicaltrials.gov).

Agent	Action mechanism	Potential mechanical cues	Trail ID	Phase	Population (*n*)	Status	Outcomes	Ref.
Simtuzumab	LOXL2 antibody	Matrix stiffness	NCT01672866	2b	NASH-related liver fibrosis (222)	Terminated	No effect on fibrosis	[251]
Simtuzumab	LOXL2 antibody	Matrix stiffness	NCT01672879	2b	NASH-related liver cirrhosis (259)	Terminated	No effect on fibrosis	[251]
Simtuzumab	LOXL2 antibody	Matrix stiffness	NCT01707472	2a	HIV/HCV-related liver fibrosis (18)	Completed	No effect on fibrosis	[250]
Sorafenib	Multikinase inhibitor	Matrix stiffness, IFP	NCT00105443	3	Advanced HCC (602)	Completed	Survival benefit	[256]
Sorafenib	Multikinase inhibitor	Matrix stiffness, IFP	NCT00492752	3	Advanced HCC (226)	Completed	Survival benefit	[256]
Simvastatin	Induce KLF2 expression	Matrix stiffness, IFP	NCT01095185	3	Liver cirrhosis (150)	Completed	Survival benefit	[260]
Simvastatin	Induce KLF2 expression	Matrix stiffness, IFP	NCT02994485	4	Liver cirrhosis (40)	Completed	Lowering portal hypertension	[261]

HIV, human immunodeficiency virus.

Table 3
Effects of mechanotransduction-targeted drugs in animal models of liver fibrosis or HCC.

Agent	Action mechanism	Target cells	Animal model	Outcomes	Potential mechanical cues	Ref.
Y-27632	Inhibit RhoA/ROCK pathway	unknown	BDL-induced liver fibrosis in rats	Reduce intrahepatic resistance	Stiffness-induced LSEC capillarization and HSC activation	[266]
1,8-naphthalidine	Inhibit PI3K/Akt/Smad or JAK2/STAT3 pathway	HSCs	BDL-induced liver fibrosis in rats	Show antifibrotic effect	Stiffness-induced HSC activation	[267]
Asiatic acid	Inhibit PI3K/Akt/mTOR pathway	HSCs	CCl_4-induced liver fibrosis in rats	Show antifibrotic effect	Stiffness-induced HSC activation	[268]
Pantoprazole	Promote YAP degradation	HSCs	BDL-induced liver fibrosis in rats	Show antifibrotic effect	Stiffness-induced HSC activation	[269]
Morin	Inhibit Hippo/YAP and TGF-β1/Smad pathways	HSCs	DEN-induced liver fibrosis in rats	Show antifibrotic effect	Stiffness-induced HSC activation	[270]
5-fluoro-7H-pyrrolo[2,3-d] pyrimidine	Inhibit FAK/PI3K/Akt/mTOR pathway	HCC cells	Nude mice bearing SMMC7721 xenografts	Inhibit tumor growth	Stiffness or IFP-induced proliferation of HCC cells	[271]
Isoliquiritigenin	Inhibit PI3K/Akt/mTOR pathway	HCC cells	Nude mice bearing SMMC7721 xenografts	Induce apoptosis in HCC through autophagy induction	Stiffness-induced drug resistance of HCC cells	[272]
Metuzumab	Decrease β_1 integrin/FAK/Akt activation *via* CD147 blockade	HCC cells	Nude mice bearing SMMC7721 or K7721 xenografts	Inhibit tumor growth and metastatic potentials	Stiffness, shear stress or IFP-induced proliferation and metastasis of HCC cells	[273]
5-HT	Promote ERK/YAP/TEAD pathway	HCC cells	Nude mice bearing HepG2 xenografts	Promote tumor growth	Stiffness or shear stress-induced proliferation of HCC cells	[274]

JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3; DEN, diethylnitrosamine.

rat livers [11,259]. The deactivation of HSCs and increased sinusoidal vasodilation may lead to a decrease in portal pressure and IFP, and prevent the matrix stiffening. Clinical trials confirm these animal studies with the decreased portal pressure and the increased survival ratio in patients with cirrhosis after Simvastatin treatment [260,261]. Meanwhile, LSEC-targeted delivery of Simvastatin alleviates LSEC capillarization to suppress HCC development, reducing collagen deposition in tumor microenvironment and improving efficacy of anti-PD-L1 immunotherapy [262]. These findings underline the anti-fibrotic and anti-tumor effects of Simvastatin targeting endothelial dysfunction and liver mechanical microenvironment.

Notably, the mechanical microenvironment of HCC, including the increased matrix stiffness, elevated IFP, and peripheral interstitial flow, forms physical barriers to reduce the efficacy of antitumor therapy by impeding drug penetration and immune cell migration. Thus, it would be useful to downregulate these mechanical barriers for efficient drug delivery. For instance, tumor stroma-targeted nanoliposome (FH-SSL-Nav) can target and eradicate CAFs in HepG2 xenograft nude mouse model, partly reversing chemotherapeutic drug resistance through tumor mechanical microenvironment modulation including downregulation of matrix stiffness and IFP [263]. Phenylboronic acid-decorated soy protein nanoparticles can actively bind to the sialic acid residues overexpressed in HCC cells and reduce tumor IFP simultaneously, leading to the enhanced drug accumulation and antitumor efficiency after loading doxorubicin [264]. Therefore, mechanical microenvironment modulation offers the alternative strategies to further improve tumor treatment outcomes.

5.2. Therapeutic potential for drugs targeting cellular mechanotransduction

Several mechanotransduction pathways, such as integrins, FAK, PI3K/Akt, RhoA/ROCK and YAP/TAZ, are all shown to be functional in hepatic cells during development of live fibrosis or HCC. Thus, targeting these mechanotransduction pathways is promising in drug development of liver diseases [265]. Although no clinical trials have validated the effects of mechanotransduction-targeted drugs in patients with liver fibrosis or HCC, compelling evidences are still existing in animal models (Table 3). For example, Rho-kinase inhibitor Y-27632 reduces portal pressure and intrahepatic vascular resistance in bile duct ligation (BDL) rats, while the inhibition of PI3K/Akt signaling by 1,8-naphthalidine or asiatic acid

dramatically suppresses the activation of HSCs, ECM accumulation, and liver fibrosis *in vivo* [266–268]. In rat fibrosis models, the administration of pantoprazole or morin prevents HSC activation and significantly ameliorates liver fibrosis by inhibiting YAP signaling [269,270]. In xenograft tumor models, downregulation of PI3K/Akt or integrin/FAK pathway significantly inhibits HCC development, while YAP activation promotes hepatoma cell proliferation, invasion, and metastasis [271–274]. These *in vivo* observations further support the anti-fibrotic and anti-cancer effects of mechanotransduction-based manipulation, and highlight their therapeutic potentials for drug development.

5.3. Computer-aided drug design for liver diseases

Computational approaches such as molecular docking, virtual screening, and molecular dynamics simulations are widely applied for structure-based drug design and functional studies [275]. Let's have $\alpha_2\beta_1$ integrin as an example. A biomimetic design strategy is established to develop potent inhibitors for $\alpha_2\beta_1$ integrin-collagen binding, a liver pathology-related therapeutic target [276,277]. Molecular dynamics simulations-coupled molecular mechanics-Poisson-Boltzmann surface area (MM-PBSA) analysis are adopted to examine $\alpha_2\beta_1$-collagen interactions and constructs an affinity binding model consisting of Y157, N154, S155, R288, and L220 residues [276]. A series of heptapeptides mimicking the binding motif of $\alpha_2\beta_1$ are then docked onto a collagen molecule to screen candidates with high binding probabilities [277]. Structural similarity of each candidate to the original structure in the affinity binding model is evaluated using root-mean-square deviation (RMSD) for ensuring a proper binding site. Thereafter, the best candidate is determined by molecular dynamics simulations based on its binding dynamics and inhibitory effect and further validated experimentally by adhesion assay. This typical work provides an excellent example of computer-aided drug design targeting a mechanoreceptor. Similar approaches are also applied in drug discovery of allosteric modulators or biased ligands for Mac-1, E-cadherin and GPCR [275,278–281].

5.4. Hepatic cell-specific drug delivery

Since many of mechanical cues and mechanotransduction pathways are not specific to the livers, a means of delivering therapeutic drugs specifically to a given type of hepatic cells is desirable [7]. Application of nanoparticles has received widespread attention for the efficient delivery of various drugs in liver diseases [282,283]. One of reasoning lies in that KCs and LSECs are major cells involved in the non-specific uptake of nanoparticles due to their potent endocytic capacities, which are responsible for phagocytosis of large- (>200 nm) and small- (100–150 nm) sized nanoparticles, respectively [282,284]. Administered nanoparticles with smaller size than the diameter of LSEC fenestrae (especially 50–150 nm) can filter out into the space of Disse and interact directly with hepatocytes and HSCs [284,285]. Although the LSEC capillarization may reduce the transsinusoidal flux of nanoparticles during the early stage of liver fibrosis, the vascular leakage and elevated sinusoidal pressure facilitate nanoparticle delivery in the late-staged fibrotic or cirrhotic livers. Increased matrix stiffness and IFP also impede the permeation of nanoparticles, and, therefore, improvement of mechanical microenvironment could be necessary for effective nanoparticle delivery in liver cirrhosis therapy. Drug-loaded nanoparticles modified by cell-specific ligands could selectively target the receptors expressed on hepatic cells and improve delivery efficiency. For example, given $\alpha_v\beta_3$ integrin specifically overexpressed on activated HSCs, cyclic RGD peptide (cRGD)-modified nanoparticles can specifically be taken up by activated HSCs *via* their binding affinity with $\alpha_v\beta_3$, establishing a drug delivery system for liver fibrosis and HCC therapy [286,287]. Based on similar mechanisms of specific binding, the nanocarriers modified by galactose, hyaluronan or mannose could also deliver anti-fibrotic drugs to hepatocytes, LSECs or KCs, respectively [282]. In HCC therapy, several overexpressed receptors including asialoglycoprotein receptor (ASGPR), epidermal growth factor receptor (EGFR), transferrin receptor (TfR), and $\alpha_v\beta_3$ are selected to achieve tumor-targeted drug delivery with specific ligand-modified nanocarriers [288,289]. As mentioned above, combining chemo- or immuno-therapy with mechanical microenvironment modulation may further improve the delivery efficiency of these nanocarriers. In addition, tumor-cell-derived microparticles, in the range of 100–1000 nm in diameter, are developed to deliver therapeutic drugs to HCC cells [290]. These extracellular vesicles are released by tumor cells and can be used for drug delivery *via* intravenous injection, where the delivery efficiency is ensured by the increased vessel permeability in tumors. Meanwhile, the softness of microparticles derived from 3D-cultured tumor-repopulating cells is capable of enhancing the drug-delivery efficiency, since the softer particles have stronger ability to cross blood vessels and extravasate into HCC cells due to their higher deformability [291]. Therefore, mechanical cue-targeted drugs can be carried out by these nanoparticles or microparticles for cell type-specific delivery to the liver.

Thus, computer-aided rational drug design targeting mechanical cues, combined with hepatic cell-specific drug delivery, opens new expectable perspectives for therapeutics of liver fibrosis and HCC. Tissue mechanical microenvironment, cell mechanics, and mechanotransduction pathways could serve as potential targets at distinct weighted levels. Future development in multiscale biomechanics and nanomaterials will facilitate the optimized system for drug administration and therapeutic intervention.

6. Conclusions and perspectives

In conclusion, this review highlights the mechanical cues in the development of hepatic fibrosis and HCC. During liver fibrogenesis, altered mechanical microenvironment (*e.g.*, increased matrix stiffness, decreased sinusoidal shear stress, and elevated IFP) leads to phenotypic and functional changes of hepatic cells, including dedifferentiation and functional loss of hepatocytes, capillarization and angiogenesis of LSECs, and activation of HSCs, initiating a positive feedback loop that further modulates mechanical microenvironment, exacerbates liver fibrosis and cirrhosis, and facilitates hepatocarcinogenesis. On the other hand, increased matrix stiffness, elevated IFP and peripheral interstitial flow in the tumor microenvironment promote the growth, invasion and metastasis of HCC tumors, and hinder the drug delivery and immunotherapy simultaneously. At molecular level, mechanical forces induce allosteric activation of mechanoreceptors on cell membrane to convert the forces into biochemical signals, subsequently leading to the activation of downstream mechanotransduction pathways and then impacting cell function. Thus, the application of mechanomedicine concept in liver diseases is promising for drug design and cell-specific delivery.

Nevertheless, major challenges still exist in understanding multiscale biomechanics and mechanotransduction in the liver. First, local mechanical microenvironments such as hepatic interstitial flow and vascular stretch have not been well characterized due to technical difficulties. Meanwhile, the effects of liver ultrastructures (*e.g.*, LSEC fenestrae, hepatocyte microvilli, and bile canaliculi) on mechanical microenvironment remain unclear. Future studies are required to address these issues using high-resolution, real-time imaging platform for living cells or sliced tissues, together with *in situ* mechanical loading and molecular force

measurements. Second, it is still challenging to precisely recapitulate the complicated mechanical microenvironment in human or animal livers at multiscale levels. Existing studies usually investigate the effect of a single type of mechanical stimuli on hepatic cells *in vitro* but those mechanical cues are, however, usually coupled together *in vivo* to modulate the cellular and molecular events [292]. Thus, it is meaningful to replicate the multiple types of mechanical stimuli *in vitro* for globally understanding cellular and molecular responses in the development of liver fibrosis and HCC. Third, it seems attractive to integrate all the quantitative data obtained from multiscale biomechanics measurements and predictions to create a *virtual mechanical liver* for deciphering mechanotransduction-based disease progression and therapeutic efficacy. The development of state-of-the-art biomechanical experimental techniques and computer-based simulation approaches or even artificial intelligence algorithms will provide further information with high spatiotemporal resolution strategies and offer the possibility to understand molecular and cellular mechanisms from a viewpoint of mechanobiology or mechanomedicine. Fourth, it is pre-requisite to conduct clinical tests for those mechanomedicine-based candidate drugs, based on existing evidences from animal models that highlight the importance of targeting the mechanotransduction pathways for curing liver fibrosis and HCC. While the applicability of these candidates needs to be iterated with animal studies and clinical trials, the optimized drug delivery strategy is also critical using bioengineering-based technology such as nano- or micro-particles combined with therapeutic strategies targeting mechanical microenvironment. Fifth, the modulation of tumor mechanical microenvironment or cellular mechanical properties offer alternative strategies to further improve the efficacy of immunotherapy. Development of high-resolution noninvasive measurements and algorithms of intrahepatic mechanical microenvironment may provide individual information for tumor heterogeneity and personalized medicine of HCC in future. Sixth, engineering-based integration of various *in vivo* mechanical cues in livers into a single *in vitro* device is promising by constructing such as microfluidics, organ-on-chips, or organoids. These emerging techniques enable systematic assessment and tests of those mechanically-targeted drug design and drug delivery, bridging from basic research to translational studies from liver fibrosis to HCC.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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