MECHANOBIOLOGY

Blood flow forces liver growth

Increases in biomechanical forces in the liver's blood vessels have now been shown to activate two mechanosensitive proteins. The proteins trigger blood-vessel cells to deploy regenerative factors that drive liver growth. SEE LETTER P.128

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The molecular pathways that initiate and sustain liver growth during development and after injury are orchestrated in part by a balanced supply of stimulatory and inhibitory factors secreted from specialized liver sinusoidal endothelial cells (LSECs), which line the organ's blood vessels[1–4]. But it is unclear how the liver vasculature senses the need to produce these endothelial-cell-derived (angiocrine) growth factors, such as hepatocyte growth factor (HGF) and Wnt proteins, to guide proper organ growth[4]. On page 128, Lorenz *et al.*[5] show how mechanical forces created by the passage of blood through the liver activate signalling pathways that promote the production of angiocrine factors and the proliferation of the organ's main cell type, hepatocytes, in mice.

Mechanosensing in the liver depends on the amount of blood delivered by the portal vein and the hepatic artery, and on the tensile strength of blood-vessel walls, which is imparted by collagen fibres. The net blood flow (perfusion) subjects LSECs to two major forces[6]. First, mechanical distortion and tension of the vessel wall owing to blood pressure results in cyclic stretch in the cells. Second, friction arising from viscous blood flow over the vessel wall causes fluid shear stress. These synergistic biomechanical forces lead to upregulation of various mechanosensing proteins, inducing LSECs to produce angiocrine factors such as nitric oxide and reactive oxygen species that act to modulate the vasculature, together with 'stronger' angiocrine factors that stimulate hepatic regeneration. However, the mechanism(s) by which biomechanical forces activate the strong angiocrine function of LSECs to choreograph hepatic proliferation have not been elucidated[7].

Lorenz *et al.* set out to investigate these mechanisms using mouse embryos removed from mothers and cultured *ex vivo*. They first observed that an increase in the liver's growth rate over different developmental stages correlated with enhanced blood perfusion through the organ. Most proliferating hepatic cells in the developing liver were localized to regions that had been perfused, and the researchers found that the level of vascular perfusion correlated with the level of activation of two receptor proteins on LSECs that sense and respond to force — integrin β1 and vascular endothelial growth factor receptor 3 (VEGFR3). In turn, these proteins promoted secretion of the key angiocrine factor HGF (Fig. 1).

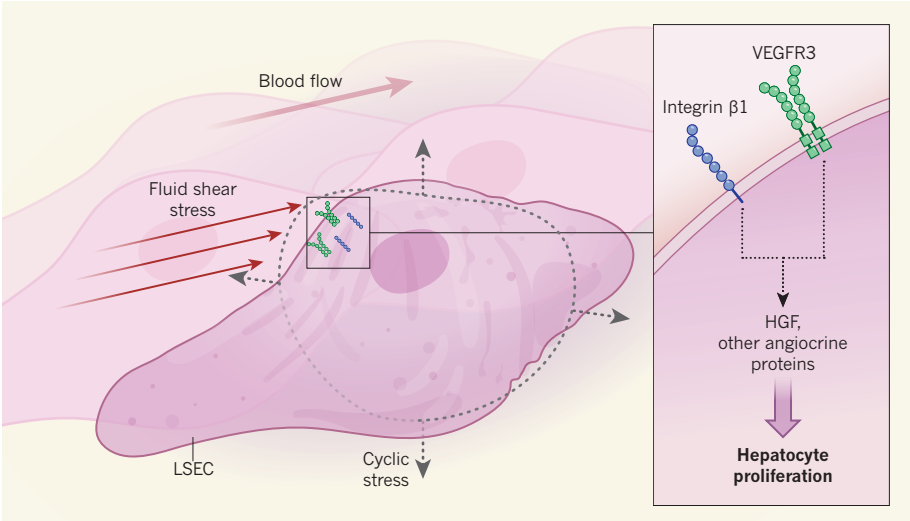

Figure 1 | Biomechanical forces mediate proliferation of liver cells. Blood flow exposes liver sinusoidal endothelial cells (LSECs), which line blood vessels in the liver, to two types of force — fluid shear stress, caused by friction across the cells, and cyclic stretch, caused by dilation of LSECs as the vessels expand. Lorenz *et al.*[5] report that these forces activate two mechanosensing receptor proteins on LSECs: integrin β1 and VEGFR3. Through as-yet-unknown mechanisms, activation of these proteins promotes expression of the gene encoding hepatocyte growth factor (HGF), along with other LESC-derived (angiocrine) proteins. HGF is secreted by LSECs, and promotes proliferation of the liver's main cell type, hepatocytes.

The authors then modified perfusion rates in cultured embryos by using drugs to halt or increase the fetal heartbeat. Blocking liver perfusion reduced HGF secretion and resulted in diminished hepatic growth — as did deleting the genes that encode integrin β1 or VEGFR3 in LSECs in embryos *in vivo*. By contrast, enhancing the rate of blood perfusion induced the secretion of HGF, and this was again mediated by integrin β1 and VEGFR3.

Lorenz *et al.* then turned to livers removed from adult mice and cultured *ex vivo*. They increased perfusion in the livers by injecting a buffer solution or by removing 70% of the liver, which redirects a large volume of fluid to the organ's remaining lobes at high pressure. They measured the perfusion rate using an imaging technique called contrast-enhanced ultrasonography. Enhanced perfusion led to increased LSEC diameter, increased blood volume and flow and so increased shear stress, leading to higher activation of integrin β1 and VEGFR3.

Together, these experiments provide evidence that, in mice, activation of mechanosensors in blood vessels in both the fetal and adult liver triggers angiocrine signalling to promote hepatocyte proliferation — presumably, to enable liver growth during embryonic development and maintenance and regeneration in adults. Next, Lorenz *et al.* turned to human cells cultured *in vitro*. Here, too, mechanical stretching of LSEC-like cells or antibody-dependent activation of integrin β1 led to a robust increase in secretion not only of HGF, but also of other angiocrine factors. These secreted factors promoted the proliferation and survival of human hepatocytes grown *in vitro*. Finally, the authors showed that, in metabolically healthy people, increases in systemic blood pressure correlated with significantly larger livers.

Lorenz and colleagues have used sophisticated approaches to link mechanical forces to the induction of angiocrine-mediated liver development and growth. However, several

issues remain unresolved. For instance, the cyclic stretch that LSECs undergo *in vivo* when the vessel widens after exposure to accelerated perfusion is biaxial — that is, the cell is stretched both along the direction of the vessel and sideways. By contrast, Lorenz and colleagues' *ex vivo* and *in vitro* experiments imparted only uniaxial cyclic stretch[8]. This difference might bias the signalling and angiocrine outputs the group observed. Whether other vascular mechanosensor receptors have a role in the induction of angiocrine factors also needs to be elucidated[9].

In addition, the role of this biomechanically responsive pathway during injury remains to be dissected. Excessive increases in shear stress (for example, as a result of acute loss of liver mass) could be detrimental, leading to suboptimal liver regeneration. Lorenz *et al.* also did not directly assess whether lack of biomechanical activation of integrin β1 and VEGFR3, as might occur in diseases such as diabetes, would lead to decreases in the liver's regenerative potential[1,2].

In future, the ideal magnitude of cyclic stretch or shear stress required to initiate the physiological induction of angiocrine factors should be studied. The recruitment of circulating endothelial progenitor cells (EPCs), which are thought to supply the liver with HGF, could also be affected by shear-dependent activation of LSECs, further altering the liver's supply of angiocrine factors[10]. Indeed, how increased biomechanical forces alter the delivery of regenerative modulators to the liver, including circulating EPCs, inflammatory cells and platelets, to drive liver growth without encouraging scarring, needs further investigation.

Exactly how do integrin β1 and VEGFR3 upregulate angiocrine factors? It is plausible that fluid shear stress induces integrin-mediated nuclear localization of specific transcription factors and so promotes the expression of angiocrine-factor genes[2–4]. Furthermore, integrin-mediated modulation of the elasticity of the extracellular matrix around hepatocytes in response to shear stress could also modulate hepatocyte proliferation. But what about VEGFR3? Proteins of the VEGFR family are activated by phosphorylation. Biomechanically independent phosphorylation of VEGFR2 on LSECs activates the protein AKT, which recruits the transcription factor Id1 to DNA, inducing the expression of *Wnt2* and *HGF* genes[2]. But the mechanism by which phosphorylation of VEGFR3 turns on angiocrine factors is unknown.

These questions notwithstanding, Lorenz and colleagues' work takes into consideration the complexity of the biophysical environment to which LSECs are exposed *in vivo*, and so solves a mystery that has puzzled liver biologists for decades. The development of strategies that precisely regulate the magnitude of shear stress and cyclic stretch in the liver vasculature might restore angiocrine-dependent regenerative functions of the liver in pathological conditions, such as in cirrhosis, hepatitis and vascular abnormalities. This could in turn open the door to more-effective therapeutic liver regeneration. ■

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MICROBIOLOGY

The electrifying energy of gut microbes

Some bacteria make energy in a process that is accompanied by transfer of electrons to a mineral. A previously unknown electron-transfer pathway now reveals an energy-generation system used by bacteria in the human gut. SEE LETTER P.140

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The ability of certain bacteria to transfer electrons has been exploited for a variety of energy-generating applications, such as microbial fuel cells[1], because the flow of charge carried by electrons underlies the process that generates electricity. It was thought that the capacity to achieve substantial levels of electron transfer occurred only in a specialized subset of bacteria. These microbes make energy by a mechanism that requires minerals for the electron-transfer process that accompanies energy generation[2]. On page 140, Light *et al.*[3] report the discovery of an electron-transfer pathway in gut bacteria, and reveal that components of this pathway are present in diverse microbial species.

The molecule ATP provides the fundamental energy 'currency' for most cells, and is mainly produced by two mechanisms: fermentation, an anaerobic process in which ATP is generated from a limited repertoire of carbon sources, and respiration, a process that provides a high yield of ATP from a wide array of carbon sources and requires a compound that can accept electrons. In multicellular organisms, respiration involves electron transfer along an electron-transport chain that culminates in electrons being transferred to oxygen[4].

By contrast, microbes can use a number of alternatives to oxygen as electron acceptors that enable respiration in anaerobic environments lacking fermentable energy sources[2,5]. For example, the bacteria *Shewanella oneidensis* and *Geobacter metallireducens* reside in mineral-rich environments, and these highly studied microbes have an anaerobic respiration process that uses minerals, such as iron(III) oxide (Fe_2O_3), as respiratory electron acceptors[2]. However, because insoluble mineral deposits cannot be transported into the cell, mineral-respiring bacteria use a mechanism[2] called extracellular electron transfer (EET), in which electrons are transferred to the exterior of the cell. In the case of these bacteria, this process involves electron transfer from an NADH molecule to components that include a quinone molecule in the lipid membrane and a series of proteins containing haem groups that provide a path for electron transfer. The loss of an electron converts NADH to NAD^+, which is used in the energy-generation process.

The food-borne bacterial pathogen *Listeria monocytogenes* sometimes has a host-associated part of its life cycle. This bacterium can infect humans, and can proliferate in nutrient-rich environments that enable the use of fermentation as a metabolic strategy[6]. However, although *L. monocytogenes* has a life cycle in which neither minerals nor respiration is crucial for survival, Light *et al.* report that, when *L. monocytogenes* was placed in an electrochemical chamber in which an electrode can trap electrons, an electric current was generated, suggesting that this type of bacterium has the capacity for EET. This report now clarifies evidence presented decades ago[7], indicating that this bacterium can change extracellular iron in the Fe^{3+} form to the Fe^{2+}