Mechanosensory Pathways in Angiocrine Mediated Tissue Regeneration

Sina Y. Rabbany, Bi-Sen Ding, Clemence Larroche and Shahin Rafii

Abstract Endothelial cells not only form the vascular networks that deliver nutrients and oxygen throughout the body, they also establish instructive niches that stimulate organ regeneration through elaboration of paracrine trophogens. Priming of the vascular niche promotes repair and regeneration of damaged tissues by establishing an inductive vascular network that temporally precedes new tissue formation. This induction of endothelial cells provides a platform for essential instructive cues. Tissue regeneration in certain organs such as the liver, involves cell mitosis and expansion, which is orchestrated by a dynamic interplay between cytokines, growth factors, and metabolic pathways. Although the intrinsic events of cell mitosis have been thoroughly studied, the extrinsic triggers for initiation and termination of liver regeneration, especially the set points rendered by the original liver size, are unknown. Furthermore, the gatekeepers that control organ size remain unidentified. The prevailing dogma states that liver regeneration involves the proliferation of parenchymal hepatocytes and nonparenchymal cells such as biliary epithelial cells. However, recent findings also implicate hepatic sinusoidal endothelial cells (SECs) as drivers of this process. In the classic liver regeneration model, in which 70 % partial hepatectomy induces regeneration, the abrupt increase in blood flow into the sinusoidal vasculature of the liver's remaining lobes correlates with initiation of the regeneration cascade. As such, the shear stress and mechanical stretch exerted on the endothelial cells may activate mechanosensory mediated molecular programs, and may be involved in the elaboration of endothelial cell-derived angiocrine growth cues that support

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C. A. Reinhart-King (ed.), *Mechanical and Chemical Signaling in Angiogenesis*,
Studies in Mechanobiology, Tissue Engineering and Biomaterials 12,
DOI: 10.1007/978-3-642-30856-7_2,

hepatocyte proliferation. Physiological liver regeneration would therefore depend on the proper inductive and proliferative functioning of liver SECs. Thus, uncovering the cellular mechanisms by which organisms recognize and respond to tissue damage remains an important step towards developing therapeutic strategies to promote organ regeneration. In this chapter, we demonstrate the mechanism by which tissue-specific subsets of endothelial cells promote organ regeneration, and further discuss the roles of physical forces and molecular signals in initiating and terminating angiocrine-mediated tissue regeneration.

1 Introduction

Mammalian cells are able to sense, decode, and respond to physical stimuli in a manner specific to their type. Physical stimuli play a role in virtually all stages of cell differentiation, as early as embryogenesis—where stiffness and contractility provide mechanosensory cues to dictate cell proliferation and differentiation (reviewed in [1])—to post-natal cranial development [2]. They also play an integral role in tissue engineering [3, 4] and regeneration [5, 6]. For instance, frogs (*Xenopus laevis)* during development can only undergo gastrulation, convergence and extension movements if the mesoderm and notochord maintain their stiffness and avoid buckling [7, 8]. Throughout this rearrangement, the marginal zone becomes stiffer in order to avoid tissue deformation and collapse during gastrulation [9]. This increased stiffness further provides the mechanical strength that initiates the following developmental events. Whether these changes also serve as cues within the mechanosensory pathway to trigger other related cellular processes is unknown.

Cell contractility is another significant source of mechanical cues. Contraction of the actomyosin cytoskeleton triggers cellular responses in the contracting cell as well as external forces on the surrounding cells. The embryogenesis of *Xenopus laevis* also appropriately illustrates cellular responses to cell-generated contractile forces. It has been shown that during gastrulation, cultured explants of the dorsal involuting and non-involuting marginal zones of frog embryo, neither of which are subjected to external forces, converge and expand. This study suggests that tissue movement related to gastrulation is dictated by the tissue itself, rather than by surrounding external forces [10].

Mechanotransduction plays an important role in the signaling of endothelial cells because of their unique position in the blood vessel lumen. Endothelial cells have direct contact with blood and so can first sense changes in shear stress caused by alterations in flow rate. It is well known that endothelial cells perceive and respond to blood flow by undergoing a series of molecular changes, including expression of various transcription factors. These molecular alterations guide cell differentiation and may drive tissue regeneration. Nevertheless, the role of biomechanical forces in orchestration of tissue regeneration and subsequent

restoration of the function of damaged or diseased organs remains to be elucidated. This chapter focuses on the role of paracrine (angiocrine) factors derived from sinusoidal endothelial cells (SECs) on initiating hepatic regeneration, and the impending role of biomechanical forces in triggering this angiocrine-mediated regeneration process.

2 Organ Regeneration

Restoration of tissue upon injury or physical loss occurs through the processes of repair, remodeling, and regeneration. While repair and remodeling generally restore tissue continuity by synthesizing extracellular matrix (ECM) proteins and forming scar tissue, regeneration resolves the same issue by recovering the missing organ mass at the original anatomical site.

Three different strategies potentially lead to organ regeneration. Non-dividing cells from the injured organ begin to multiply and grow to resynthesize the lost tissue. Specialized cells dedifferentiate into multipotent cells that replicate, re-specialize and replace the missing tissue. Lastly, pools of stem cells can divide and regenerate the lost tissue. The molecular and cellular pathways that regulate regeneration of adult organs remain largely unknown.

2.1 Liver Regeneration

The liver has a remarkable regenerative capacity and is able to rapidly restore its original weight and size after surgical resection. Liver regeneration is orchestrated by a complex interplay of cytokines, growth factors, and metabolic pathways (reviewed by [11, 12]). The "blood flow" theory, proposed over 50 years ago, suggested increased mechanosensory cues caused by increased blood flow to the liver post-partial hepatectomy (PH) triggered liver regeneration [13].

Another theory suggests that following liver damage factors such as Lipopolysaccharides (LPSs) are produced by the gut and delivered to the liver via the portal vein. LPS then activates the Complement system, leading to the production of anaphylatoxins, complement component 3 (C3a), and complement component 5 (C5a). Both C3a and C5a play a central role in the activation of the Complement system, a part of the innate immune system, which aids antibodies and phagocytic cells in clearing pathogens. LPS, C3a, and C5a all participate in the activation of Kupffer cells, mononuclear phagocytic cells found on the hepatic sinusoids, through Toll-like receptor 4 (TLR4), C3a receptor (C3aR), and C5a receptor (C5aR). The activation of Kupffer cells is indispensable in directing liver regeneration, namely through the production of TNF-α and IL-6, both of which are involved in priming the hepatocytes from the quiescent G0 to the G1 phase [14]. The transition of hepatocytes from G1 to M phases of the cell cycle is promoted by

other factors, such as insulin, growth hormone (GH), bile acids, vasopressin (AVP), platelet-derived serotonin, and endothelial growth factor (EGF) from the blood. These factors cooperate with prostaglandins, heparin-binding EGF-like growth factor, hepatic growth factor, as well as insulin-like growth factor-1. Tumor growth factor and amphiregulin from different hepatic cells block the G1 to M transition of the cell cycle.

Hepatic Stem Cells (HSCs) have the ability to inhibit G1 to S transition [15, 16]. TGF-beta signaling is thus blocked during cell proliferation. Pro-Nerve Growth Factor (Pro-NGF) and NGF, produced by regenerating hepatocytes, are able to trigger apoptosis in activated TGF-producing HSCs. Once the liver has reached its original mass, several factors (including activin A) are responsible for triggering the end of liver regeneration.

The liver sinusoidal vasculature is lined with endothelial cells (SECs) that distribute hepatic blood flow into each lobe. It is well known that occlusion of one particular branch of the portal vein system results in an atrophy of its corresponding lobes and a compensatory hypertrophy of the remaining liver tissue [17]. Given that hepatocytes make up most of the liver volume, this observed atrophy and loss of volume is mainly caused by apoptosis and necrosis of hepatocytes [18]. The exact mechanism of hypertrophy unique to liver regeneration is not completely known. However, considering the speed of regeneration, a very early trigger—possibly an abrupt change in a physiological parameter—may be responsible.

Similar to blood flow changes upon portal vein occlusion, one of the most obvious events in liver regeneration following partial hepatectomy is the subsequent increase in portal blood flow to the remaining lobes. Shear stress resulting from such increased blood flow on vascular lining could represent a first-line trigger for liver regeneration after PH. Cell location dictates the specific cells involved in mechanotransduction of hemodynamic forces in liver vasculature.

2.2 Sensing and Regulation of Blood Flow by the Vasculature

The endothelium is the innermost layer of blood vessels, made up of cells that had largely been assumed to function primarily as a delivery conduit for oxygen and nutrients. It has been increasingly appreciated that endothelial cells not only form vascular networks that deliver nutrients and oxygen throughout the body, but also establish instructive niches that stimulate organ regeneration through elaboration of paracrine trophogens. Endothelial cells distributed in different organs exhibit various morphological and functional attributes. Arteries and veins have distinct structures, with arteries comprised of three layers (tunica intima, tunica media and tunica adventitia), while veins consist of only two. The tunica intima is a single layer of simple squamous endothelium attached to a sub-endothelial connective tissue layer; the tunica media, mostly present in arteries, contains circularly arranged elastic fiber, connective tissue, and polysaccharides; the tunica adventitia is made of connective tissue and nerves. In the case of arteries, the tunica media

layer is rich in vascular smooth muscle, which serves to control the caliber of the vessel. Taken together, these cells experience cyclic stretch when they serve to modulate vasodilation and vasoconstriction as well as control blood rheology [19].

By virtue of its location, the endothelium has an important role as the primary interface between circulating blood and the vascular wall. Endothelial cells in particular sense blood flow and shear stress. The role of biomechanical forces in the ability to regenerate tissues and thus restore the function of damaged or diseased organs requires clarification.

3 Biomechanical Forces

Traditionally, it has been thought that biochemical signals serve as the main method in which signaling pathways are activated in endothelial cells. However, new research has determined that mechanical forces experienced by endothelial cells have a significant impact on cell phenotype and function. Biomechanical forces play essential roles in tissue and organ development [20]. Recent studies have demonstrated that mechanical forces can regulate endothelial cell proliferation, survival, migration [21], and ECM remodeling, and hence influence angiogenesis [22]. Mechanosensitivity describes the ability of cells to perceive mechanical stimuli and interpret them into biological signals at the molecular level [23]. As such, mechanotransduction is a set of fundamental physiological mechanisms that allow cells to react to physical forces. Mechanical stretch resulting from pulsatile blood flow can modulate differentiation, proliferation and production of paracrine (angiocrine) factors of vascular cell. In this mechanotransduction-based response of the arterial system, numerous endothelial cellular components such as proteoglycans have a crucial role [24, 25].

3.1 Effect of Mechanical Stress on Endothelial Cells

Cells in the vascular system are subjected to mechanical forces due to the pulsatile nature of blood flow. Endothelial cells and smooth muscle cells in the vessel wall experience these mechanical forces the most. Mechanical stretch, resulting from changes in blood pressure, can modulate several different cellular functions in vascular smooth muscle cells. Our data provides a new framework in which to consider how physical forces and molecular signals synchronize during the program of liver regeneration (Fig. 1).

The effect of shear stress on vascular endothelial cells has been extensively analyzed. There is a large body of evidence showing that it directly influences the expression of many adhesive molecules like VCAM-1 [30] and ICAM-1 [31] and mediator systems such as TGF-β [32] and nitric oxide synthase [33]. Several groups of molecules have been shown to play a role in the detection of flow shear

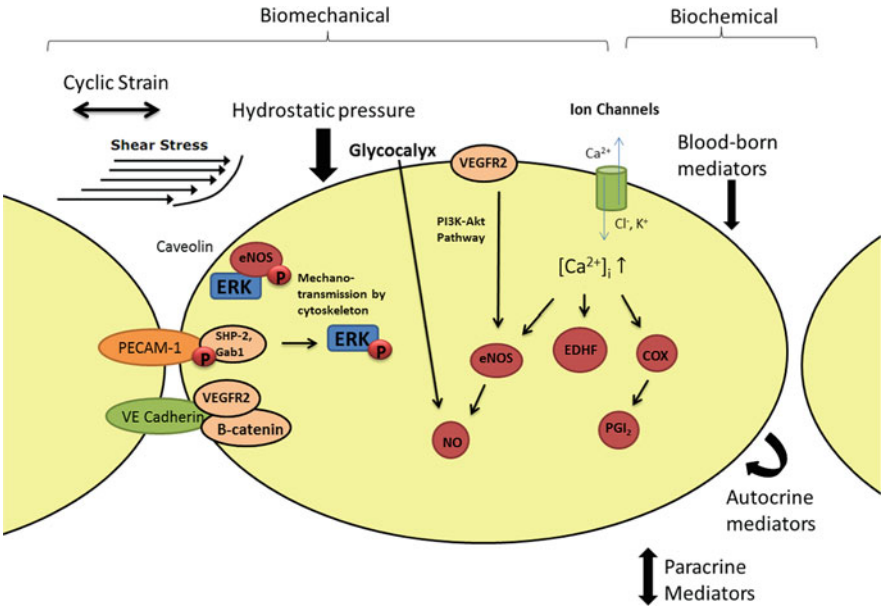

Fig. 1 Early molecular response of endothelial cells to shear stress. Endothelial phenotype is constantly modulated by both biomechanical and biochemical stimuli. Biochemical stimuli include hormones, growth factors, cytokines, etc. that are delivered to the cell via autocrine or paracrine mechanisms. Hemodynamic forces result in hydrostatic pressure, cyclic strain, and shear stress. Different mechanosensors play specialized roles in interpreting this stress in endothelial cells. These sensors include ion channels (TRP, P2X4, K^+, Cl^- channels) [26], integrins, platelet endothelial cell adhesion molecule-1 (*PECAM*-1), VE-cadherin, caveolae, G proteins, glycocalyx, and the endothelial cytoskeleton [27]. The activity of ion channels leads to an influx in Ca^{2+}, which results in vasodilator production (namely nitric oxide), prostacyclin, and Endothelium-Derived Hyperpolarizing Factor (*EDHF*). Shear stress travels through the cytoskeleton to the endothelial surface, where either integrins or PECAM-1 and VE-cadherin are activated sequentially. Activated integrins bind to adaptor protein Shc as well as numerous kinases, including focal adhesion kinase (*FAK*). Shear stress also triggers tyrosine phosphorylation of PECAM-1 and localization of SHP-2 and Gab1 near the cell junction. This will subsequently results in ERK phosphorylation. Shear activation of (VEGF)-A receptor-2 (VEGFR2) at the luminal surface leads to the recruitment of phosphatidylinositol 3 kinase (*PI3 K*), which itself leads to the activation of Akt and eNOS. The activation of ERK and the phosphorylation of serine 1179 of eNOS at caveolae by shear have also been documented. Shear stress is rapidly followed by G protein activation and results in the activation of Ras and extracellular signal-regulated kinases-1/2 (ERK1/2). Deformation of the luminal surface will result in direct shear stress-induced signaling through the glycocalyx (via NO production) (adapted from [28, 29])

force sensation, namely ion channels, cell–matrix and cell–cell junction molecules, Tyrosine Kinase Receptors [(VEGF)-A receptor-2 (VEGFR2)], caveolae, G-protein coupled receptors/G-proteins, glycocalyx, and the endothelial cytoskeleton (reviewed in [20, 27]). Mechanotransduction of hemodynamic forces plays a central role in regulation of arterial diameter. This process is orchestrated

by the production and release of autacoids by the endothelial cells affected. Nitric oxide (NO), prostacyclin (PGI2), and endothelin-1 (ET-1) are the best-characterized endothelium-derived autacoids, the first two being vasodilators, and the last being a vasoconstrictor. Two other factors have been suggested to play a significant role in vasodilation: endothelium-derived hyperpolarizing factor (EDHF) and reactive oxygen species (ROS) [27, 34].

3.2 Integrin-Mediated Mechanotransduction

Integrins, as the main receptors that mediate the connection of the cytoskeleton to the ECM, have been implicated in sensing mechanical forces [35]. When the apical surface of an endothelial cell experiences shear stress due to blood flow, the tension experienced by the cell is transferred to transmembrane integrins, which undergo conformational changes and alter the phenotype of the cell. In order to maximize adhesion, the cell needs to increase its surface area in contact with the ECM so it flattens in a manner consistent with the blood flow. Tension at the upstream end of the cell with then be greater than the tension at the downstream end [35]. In integrin-mediated cell binding to the ECM, proteins in the ECM bind to integrins attached to the cell's cytoskeleton. This linkage bears much of the mechanical force inflicted on the cells, and as such may serve as a candidate mechanotransducer.

Glycoproteins within the extracellular matrix can be displaced by both cyclic stretching and shear stress. These interact with integrins that transmit the mechanical signal intracellularly. Shear stress can cause endothelial cell integrins to form clusters [36], associate with adaptor proteins such as Shc [37], and bind to WOW-1, an antibody that specifically binds to a particular activated intergrin [38].

The cytoplasmic domains of integrins are often linked to intracellular proteins that constitute the cytoskeleton as well as kinases, including focal adhesion kinase (FAK), a key regulator of biochemical cascades triggered by mechanical forces. Therefore, integrins serve as a signal transmitter between the cell and the ECM [27].

Vascular endothelial growth factor (VEGF) is a homodimeric glycoprotein and a sub-family of the cysteine-knot growth factor superfamily, a platelet-derived growth factor family [39, 40]. They have profound involvement in signaling for both vasculogenesis (the de novo formation of blood vessels) and angiogenesis (the growth of blood vessels from pre-existing vasculature). VEGF promotes vasculogenesis during embryonic development, angiogenesis after injury and within muscles following exercise, as well as new vessels that serve to bypass older, blocked vessels.

Overexpression of VEGF can contribute to disease. Solid tumors require sufficient blood supply after they reach a certain size. Tumor cells that have the ability to express VEGF can therefore further expand and metastasize. VEGF overexpression may also cause vascular diseases of the retina. Anti-VEGF therapies are used to control or slow down the progression of diseases facilitated by VEGF

Table 1 Vascular endothelial growth factor function

Receptor	Ligand	Function
VEGFR1 (Flt-1)	VEGF-A, VEGF-B	Decoy receptor of VEGF-R2, unknown
VEGFR2 (KDR/Flk-1)	VEGF-A,VEGF-C, VEGF-E, VEGF-D	Varied
VEGFR3	VEGF-C, VEGF-D	Lymphangiogenesis

overexpression. An outline of VEGF isomers and receptors they interact with is outlined in Table 1.

VEGF-A binds to VEGFR1 (Flt-1) and VEGFR2 (KDR/Flk-1). VEGFR2 seems to mediate most of the known cellular responses to VEGF [41]. The function of VEGFR1 is less well-defined; it is thought to modulate VEGFR2 signaling. VEGFR1 may also act as a decoy receptor, sequestering VEGF from VEGFR2 binding (particularly during embryonic vasculogenesis). VEGF-C and VEGF-D are ligands for VEGFR3, which plays a role in lymphangiogenesis.

3.3 Parallel-Plate Flow System

To understand how shear stress is measured in vivo in small vessels, it is first necessary to have an understanding of how shear stress can be measured in vitro. The parallel-plate flow chamber is a commonly used platform for subjecting monolayers of anchorage-dependent cells, such as endothelial cells, to relatively uniform laminar shear stress. This approach has allowed scientists to recreate the in vivo environment in which endothelial cells are exposed to shear stresses due to blood flow.

A cell monolayer attached to one internal plate surface is subjected to fluid flow by creating a pressure gradient across the chamber. In order to calculate the shear stress on the cells, a Newtonian fluid is assumed. For steady flow between infinitely wide parallel plates, shear stress is determined according to fluid flow, viscosity of the media, and the depth of the chamber.

There are many advantages to using parallel-plate flow chambers. They promote uniform shear stress in a controlled environment. They can also simulate a wide variety of flow rates, which associatively varies shear stress levels. This enables simulation of blood vessels of many different sizes.

A parallel-plate flow chamber with a channel height of 1 mm was used to subject Human Umbilical Vein Endothelial Cells (HUVECs) to shear stress (Stovall Life Sciences, Inc). This chamber is optically transparent, allowing for direct visualization of the cell behavior under flow on a microscope stage and real-time monitoring. A high-precision peristaltic pump (Ismatec IPC, Model #ISM931C) is connected with tygon tubing (1/16 in ID) to the flow cell. The pump can impart a range of fluid shear stress by varying the flow rate. Cells are plated

into the flow cell 24 h before the experiment to allow the cells to grow to confluence. The experiment is performed at 37 °C and 5 % CO_2. The peristaltic pump is placed inside the incubator to minimize the tubing length and as a result the flow path is considerably reduced. At the completion of the experiment, the flow cell can be disassembled by stopping the pump and fixing the cells for visualization or lysing for assaying.

As fluid flows through the parallel-plate flow chamber there are several variables that are used to calculate the shear stress imparted on the cell monolayer on the internal plate of the chamber. The magnitude of shear stress (τ) can be determined by quantifying the following variables: fluid viscosity (μ), chamber width (b), distance between plates (h), and volumetric flow rate (Q). The shear stress acting on the cell monolayer adhering to the plate is expressed in the following mathematical form:

$$\tau_w = \frac{6\mu Q}{bh^2} \tag{1}$$

In order to derive the shear stress model, the Navier–Stokes equations can be used. In the following Navier–Stokes equation for a fluid, ρ is density, u is fluid velocity, μ is viscosity, P is pressure, and t is time.

$$\rho\left(\frac{\partial u}{\partial t} + u \bullet \nabla u\right) = -\nabla P + \mu\nabla^2 u \tag{2}$$

Since it is assumed that the flow is fully developed, there is no change in flow velocity with respect to time, and as a result, the entire left side of the equation is neglected.

$$0 = -\nabla P + \mu\nabla^2 u \tag{3}$$

Rearranging and simplifying the terms, the equation shows the relation between the pressure gradient in the flow direction and the change in shear stress in the direction normal to the plate:

$$\frac{d^2u}{dy^2} = \frac{1}{\mu}\frac{dP}{dx} \tag{4}$$

Since it is assumed that the no-slip boundary condition applies, the following boundary conditions exist for this second order differential equation, where $h =$ the height of flow chamber.

(1) $\frac{du}{dy} = 0$ at $y = 0$ due to the shear rate being zero at the axis of symmetry
(2) $u = 0$ at $y = \pm\frac{h}{2}$ due to the no-slip condition at the top and bottom

integrating Eq. (4) once gives:

$$\frac{du}{dy} = \left(\frac{1}{\mu}\frac{dP}{dx}\right)y + c_1 \tag{5}$$

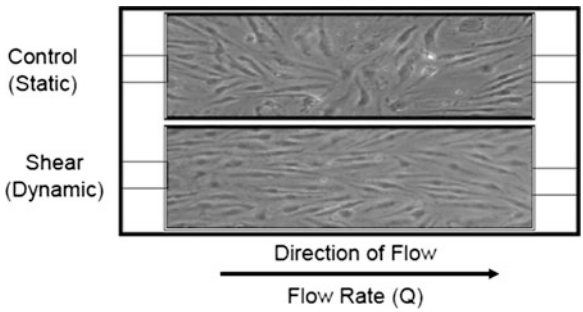

Fig. 2 Transformation of Endothelial Cell Morphology by Shear Stress. Alignment of endothelial cells by shear stress in a parallel-plate flow chamber. HUVECs were exposed to physiological stress of approximately 6 dynes/cm^2 for 12 h aligned with the directions of blood flow

Apply the first boundary condition to eliminate c, and then integrate again, when the second boundary condition is used, the result is:

$$u = \frac{1}{2\mu}\left(\frac{-dP}{dx}\right)\left[\left(\frac{h}{2}\right)^2 - y^2\right] \tag{6}$$

By integrating Eq. (6) over the height, the volumetric flow rate per unit width can be solved.

$$q = \frac{1}{2\mu}\left(\frac{-dP}{dx}\right)\left(\frac{h^3}{6}\right) = \frac{-h^3}{12\mu}\frac{dP}{dx} \tag{7}$$

Assuming two-dimensional flow (since the side walls are much further apart than the parallel plates, or $b \gg h$):

$$Q = -\frac{h^3 b}{12\mu}\left(\frac{dP}{dx}\right) \tag{8}$$

An expression for shear stress can be obtained by using Eq. (5) with $c_1 = 0$, noting that $\tau = [\mu du/dy]_{y=-h/2}$, and substituting for dP/dx from Eq. (8)

$$\tau = -\frac{dP}{dx}\frac{h}{2} = \frac{6\mu Q}{bh^2} \tag{9}$$

It is assumed that flow is laminar, which is true for the low Reynolds number flow being investigated. This laminar flow is also steady state, which helps to simplify the modeling of the shear stress. Another assumption is that the fluid is Newtonian and incompressible, which for the case of blood flow is acceptable (Fig. 2).

3.4 VEGFR2 and Mechanotransduction

VEGFR2 is important for normal endothelial cell proliferation, migration, and angiogenesis. VEGFR2 was established as having a role in the mechanosensory steps preceding integrin activation in integrin-mediated mechanotransduction [42].

VEGFR2 is part of a mechanosensory complex, comprised of Platelet Endothelial Cell Adhesion Molecule 1 (PECAM-1) (which transmits mechanical force), vascular endothelial cell cadherin (VE-cad), beta-catenin (which acts as an adaptor), and VEGFR2 (which activates phosphatidylinositol-3-OH kinase) [42]. This complex is sufficient to confer responsiveness to shear stress induced by flow in heterologous cells. Mechanical activation of VEGFR2 enables it to recruit PI3-kinase, and therefore mediates the activation of Akt and endothelial nitric oxide synthase (eNOS). Blocking the receptor activity in these complexes leads to a significantly diminished response to mechanical stress in endothelial cells. Therefore, a correctly assembled complex is indispensable to form a sensing mechanotransduction mechanism.

3.5 Regulation of MicroRNA's by Biomechanical Forces

MicroRNAs (miRNAs) are small noncoding RNA molecules (21–25 nucleotides) that function as post-transcriptional regulators by binding to complementary sequences on target mRNA transcripts [43, 44]. This usually results in translational repression, target degradation, or gene silencing [45]. Approximately 30 % of human protein coding genes are estimated to be regulated by miRNAs. The role of miRNAs in various biological processes, in both health and disease, is becoming more evident. These processes include cell proliferation and differentiation, angiogenesis [46–48], and cardiovascular homeostasis [49, 50]. The role of miRNA in endothelial cell (EC) biology was shown by studying the various knockdowns of key molecules in miRNA biogenesis (such as Dicer, and Drosha). EC Dicer knockouts studied in vitro showed changes in the expression of genes involved in EC biology, as well as reduced EC proliferation and angiogenesis [51]. ECs with knockdown of both Dicer and Drosha had significant reduction in capillary sprouting and tube forming activity [52]. Therefore, an overall reduction of miRNAs (via knockdown of Dicer and/or Drosha) significantly affects EC functions in vitro and in vivo, which indicates miRNAs might have an important role in regulating angiogenesis and vascular function [43, 44, 52, 53]. EC gene expression in response to pulsatile shear flow indicated that miRNAs (specifically miR-12b) play an important regulatory role in the inhibition of EC proliferation by pulsatile shear stress [54]. Additionally, when the influence of shear stress on aortic arch vessels was investigated, it was found that a flow-induced genetic pathway was necessary for angiogenic sprouting. This pathway requires the production of mechanosensitive zinc finger transcription factor (klf2a) that induces an EC-specific miRNA (mir-126) to activate VEGFR signaling [55]. This is yet another example of miRNAs facilitating integration of a physiological stimulus using growth factor signaling in ECs to guide angiogenesis.

4 Hemodynamics and the Liver

4.1 Blood Flow in the Liver

The liver is the body's most important metabolic organ and plays a central role in detoxifying blood-transported components. The liver has a unique dual circulation system, receiving 75 % of its oxygenated blood from the hepatic portal vein and 25 % from the hepatic artery. Each of these two vessels provides about half of the liver's oxygen requirement. The hepatic artery provides arterial blood, whereas the hepatic portal vein is responsible for transporting the venous blood from the spleen and the gastrointestinal tract, and its associated organs. In humans, both hepatic lobes are composed of about a million small lobules that are a maximum of 1–2 mm in size. Blood entering the liver flows through morphologically sinusoidal microvessels surrounded by hepatocytes and drains into a centrally located vein. This exceptional architecture ensures that the blood is brought into best possible contact with the hepatocytes when it flows through the organ. These veins then merge into the hepatic vein, which drains the blood out of the liver.

Blood flow subjects the endothelial cells to two major complex hemodynamic forces. The cyclic strain that results from mechanical distortion and tension of a vessel's wall, caused by the pumping action of the heart, affects the vascular cells. The shear stress, due to the frictional force that results from viscous blood flow over the vessel lumen, is imparted onto the endothelial cells lining the vessel's lumen. Blood pressure is the major determinant of cyclic stretch, while shear stress is determined by the blood flow, viscosity, and the diameter of the blood vessel. The liver's capacity to withstand vascular stress is illustrated through its management of blood flow following PH. Following a 2/3 PH, all blood provided by the hepatic portal vein is shunted to the remaining one-third of the liver, effectively tripling the incoming blood flow rate and volume, causing acute portal hypertension. This increase in blood flow is achieved by circumferential vessel wall expansion resulting in the stretching of endothelial cells and other cell types in the vessel wall. The liver lacks the ability to control the volume of portal blood flow despite it comprising up to 75 % of total liver intake. It is reasonable to postulate that the increase in blood volume entering the liver following partial hepatectomy may play a role in triggering the rapid regeneration of the liver. The cellular response to this stress may be mediated through mechanotransduction. Interestingly, when portal blood flow is experimentally deprived, a compensatory increase of arterial blood supply, known as the hepatic arterial buffer response (HABR), occurs. This phenomenon implicates that the abrupt shift in blood flow distribution after partial hepatectomy is a first-line trigger to provoke the regeneration process, while regeneration is halted following the gradually normalized blood flow.

4.2 Liver Regeneration

Liver regeneration has also been linked to the proliferation of hepatocytes post partial hepatectomy, which coincides with the secretion of a variety of growth factors and cytokines, such as interleukin-6 (IL-6), hepatocyte growth factor (HGF), and tumor necrosis factor (TNF). It has previously been shown that growth factors and cytokines produced by hepatocytes play an important role in orchestrating the process of liver regeneration. In fact, it seems both parenchymal cells (particularly hepatocytes) and nonparenchymal cells (Kupffer cells, SECs, hepatic stellate cells, and billiary epithelial cells) are involved in producing cytokines for liver regeneration [56]. Hepatic SECs are the second most common liver cells after hepatocytes [57]. They compose a functionally unique capillary network that vascularizes organs such as bone marrow and liver. Hepatic SECs play a vital role in providing nutrients and growth factors to proliferating hepatocytes via the formation of new vessels during regeneration. Bone marrow SECs in adult mice support hematopoietic regeneration through expression of specific angiocrine trophogens, such as Notch ligands [58, 59]. The hepatic circulation is also largely lined by LSECs [60, 61], with each hepatocyte dwelling in cellular proximity to ECs. However, the lack of a suitable definition of liver ECs and scarcity of relevant mouse angiogenic genetic models [14, 16] have hampered studies of the role of LSECs in regulation of hepatic regeneration [62–64].

As early as 1965, portal venous blood flow has been identified as an important factor in liver regeneration [13]. Liver mass is significantly reduced post-PH (due to liver resection), and the remaining hepatic segments are subjected to increased portal blood flow and pressure. The earliest and most notable change following PH is the drastic increase in liver blood flow, with an increase in liver blood flow of up to 10-fold. Considering that hepatic SECs respond to shear stress and radical increases in blood flow, hepatic SECs must play a role in the initiation of liver regeneration.

5 Partial Hepatectomy Model

Recently, we have used a PH model to examine the instructive role of LSECs in hepatic regeneration (Fig. 3) [65]. In this model, resection of 70 % of the liver mass without perturbing the integrity of the residual liver vasculature activates hepatocyte regeneration [11, 63, 66]. This method is in contrast to the administration of hepatotoxic chemicals, which impair the organization of LSECs and causes tissue hypoxia, cell death, and inflammation [16, 60, 67]. This approach offers an instructive model for examining the role of structurally and functionally intact LSECs in supporting liver regeneration.

Growth factors such as those in the VEGF family play a significant role in the regeneration of bone marrow SECs [68]. As such, we hypothesized that two VEGF

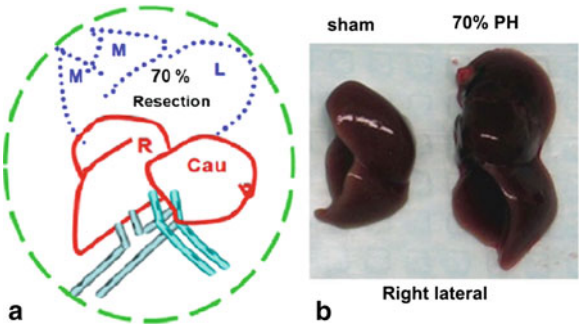

Fig. 3 Liver Hepatectomy. **a** Schematic diagram of partial hepatectomy model in mouse. **b** Picture of sham and 70 % partial hepatectomy (*PH*)

receptors, VEGFR2 or VEGFR3, also modulate liver SEC function. Using VEGFR2-GFP mice in which the expression of green fluorescent protein (GFP) is driven by the native promoter of VEGFR2, we have demonstrated that VEGFR2 and VEGFR3 are exclusively expressed in the liver endothelial cells but not in other liver cell types, including hepatocyte nuclear factor 4α (HNF4A)$^+$ hepatocytes (Fig. 4a). As shown in Fig. 4b, the distribution of VEGFR3 expression is restricted to VEGFR2$^+$ LSECs that branch out from CD34$^+$VEGFR3$^-$ large vessels.

5.1 Flow Cytometry Analysis

Flow cytometric analysis on non-parenchymal cells demonstrates the expression of endothelial-specific marker VE-cadherin on non-hematopoietic VEGFR3$^+$ VEGFR2$^+$CD45$^-$ LSECs, 97.6 % of which are non-lymphatic endothelial cells expressing coagulation factor VIII (Fig. 5a, b). We have designated a unique phenotypic and operational signature for LSECs and other cells of adult mice as shown in Table 2. Identification of LSECs as VEGFR3$^+$CD34$^-$ and non-sinusoidal endothelial cells such as VEGFR3$^-$CD34$^+$ is sufficient for quantification, purification, and molecular profiling of LSECs.

In order to determine the mechanism by which LSECs regulate hepatic proliferation, we examined the regenerative kinetics of hepatocytes and LSECs after partial hepatectomy. Two days after partial hepatectomy, staining with VE-cadherin, hepatocyte marker epithelial (E)-cadherin, and mitotic marker phosphorylated-histone-3 (P-H3) revealed that P-H3$^+$E-cadherin$^+$ mitotic hepatocytes were positioned in the proximity of non-proliferating LSECs (Fig. 5c).

Proliferation of LSECs starts at day 4 and plateaus by day 8 after PH (Fig. 6a). In comparison, quantification of P-H3$^+$HNF4A$^+$ hepatocytes showed that the rate of hepatocyte proliferation peaks during the first four days, leveling off by day 8 (Fig. 6b). The roles of the inductive signals in liver regeneration is schematized (Fig. 6c).

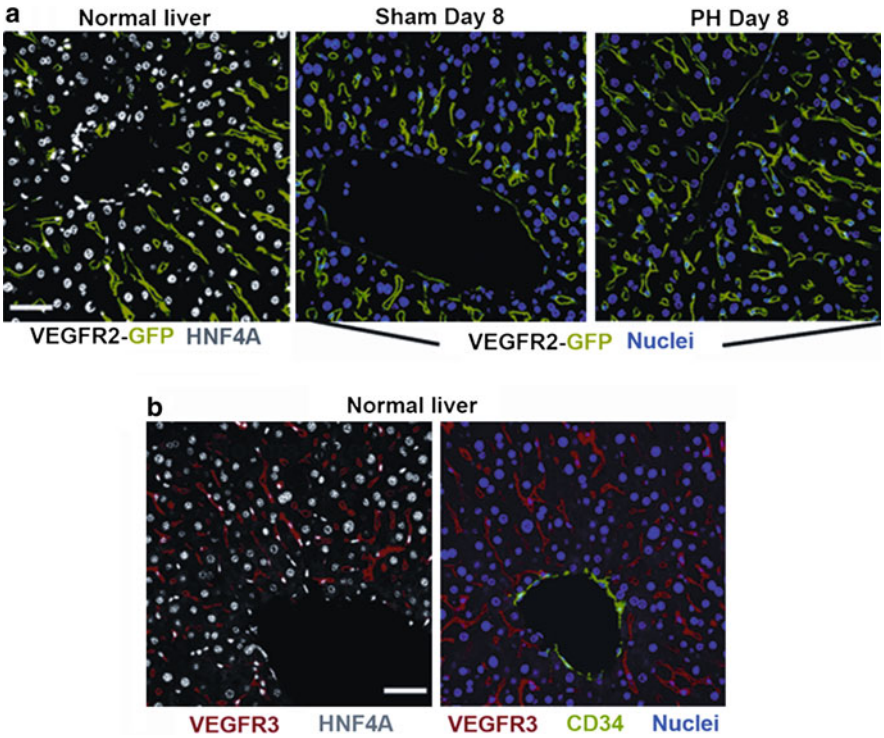

Fig. 4 Phenotypic signature and contribution of LSECs to physiological liver regeneration induced by 70 % partial hepatectomy. **a** Liver sections obtained from VEGFR2-GFP reporter mice. During liver regeneration VEGFR2 is exclusively expressed on the liver endothelial cells. **b** Restricted expression of VEGFR3 on LSECs, but not $CD34^+$ large vessels or hepatocytes. Scale bars, 50 μm (Adapted from [65])

Table 2 Signatures of various cell types in adult liver mice

Cell Type	Markers
Liver-sinusoidal ECs	**$VEGFR3^+CD34^-$**$VEGFR2^+$VE-cadherin$^+$Factor$VIII^+$Prox-1^-CD45^-
Non-sinusoidal ECs	**$VEGFR3^-CD34^+$**$VEGFR2^+$VE-cadherin$^+$ $CD45^-$
Lymphatic ECs	$VEGFR3^+CD34^+$Prox- 1^+Factor$VIII^-CD45^-$

These results suggest a two-step contribution of LSECs in mediating hepatic reconstitution. At the early phases of PH (days 1–3 after partial hepatectomy), inductive angiogenesis in the non-proliferative LSECs stimulates hepatic regeneration, possibly by releasing angiocrine factors. In contrast, 4 days after partial hepatectomy, the increased demand of blood supply for the regenerating liver is met by proliferative angiogenesis of LSECs.

To investigate the significance of VEGF receptors during liver SEC driven hepatic regeneration, the VEGFR2 gene was conditionally deleted by crossing $VEGFR2^{loxP/loxP}$ mice with ROSA-$CreER^{T2}$ mice, generating inducible VEGFR2-

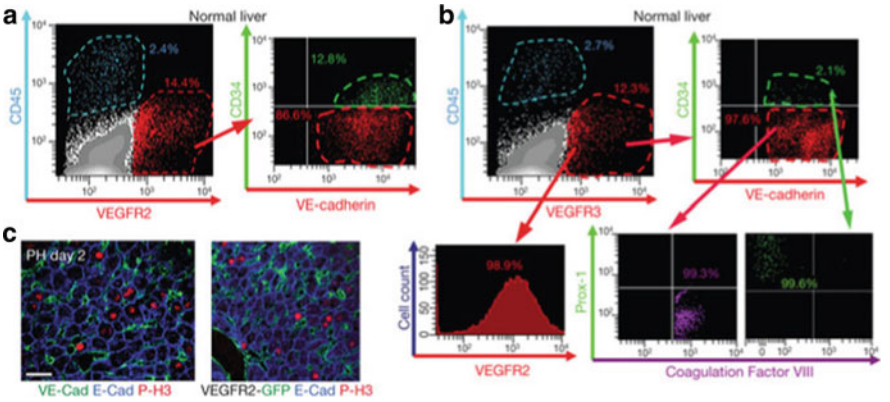

Fig. 5 **a** Flow cytometric analysis of the liver nonparenchymal cells. VEGFR2^{+} cells that are CD45^{-}, express endothelial-specific VE-cadherin. **b** Specific expression of VEGFR3 on VEGFR2^{+}VE-cadherin^{+}CD45^{-} LSECs, with a predominant fraction being CD34^{-} Factor-VIII^{+}Prox-1^{-}. **c** Forty-eight hours after partial hepatectomy, E-cadherin^{+}P-H3^{+} mitotic hepatocytes are localized adjacent to VE-cadherin^{+} and VEGFR2^{+} endothelial cells. Scale bars, 50 μm (Adapted from [65])

deficient, VEGFR2$^{flox/flox}$ (VEGFR2$^{fl/fl}$) mice [68]. Owing to the endothelial-cell specific expression of VEGFR2 in the liver, in VEGFR2$^{fl/fl}$ mice only liver ECs, but not non-endothelial cells, will manifest functional defects. As control, we used mice with heterozygous deletion of the VEGFR2 gene (VEGFR2$^{fl/+}$). Forty-eight hours after partial hepatectomy, bromodeoxyuridine^{+} hepatocyte proliferation (BrdU^{+}HNF4A^{+} cell number) was decreased by 67 % in VEGFR2$^{fl/fl}$ mice (Fig. 7a, b). Despite the patency of the VE-cadherin^{+}isolectin^{+} perfused vessels at this early phase, the regeneration of liver mass was attenuated in VEGFR2$^{fl/fl}$ mice (Fig. 7c). The result demonstrates that in the early phases (PH days 1–3) of liver regeneration, targeting VEGFR2 primarily impairs the effect of endothelial-derived angiocrine factors to induce hepatocyte regeneration, but not vascular perfusion capacity.

However, in VEGFR2$^{fl/fl}$ mice at the later stages of liver regeneration (PH days 4–8), proliferative angiogenesis was also defective (Fig. 7c), interfering with the assembly of patent VE-cadherin^{+}isolectin^{+} vasculature (Fig. 7d, e), thereby diminishing restoration of the liver mass for at least 28 days. Furthermore, in VEGFR2$^{fl/fl}$ mice, liver function after PH was abnormal, as manifested by elevated plasma bilirubin levels. To confirm the endothelial-specific VEGFR2 function in mediating liver regeneration, VEGFR2$^{loxP/loxP}$ mice were also crossed with VE-cadherin-CreER^{T2} mice to induce endothelial-selective deletion of VEGFR2.

Both the liver mass and formation of perfused vessels in the VE-cadherin-CreER^{T2}VEGFR2$^{fl/fl}$ mice were decreased after PH, which emphasizes the significance of VEGFR2 in mediating liver regeneration. In fact, if the VEGF-A/VEGFR2 pathway supports SEC-driven hepatic regeneration, then VEGF-A should enhance liver regeneration. We compared the effect of VEGF-A$_{164}$ with

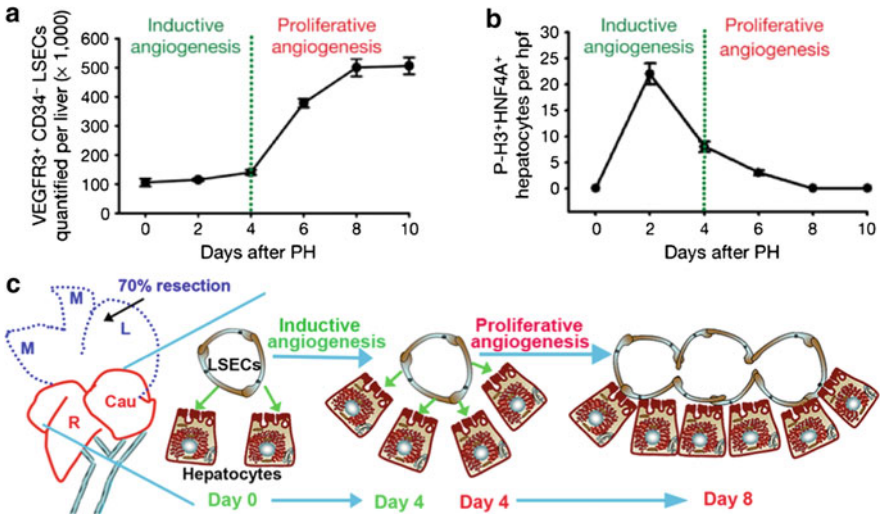

Fig. 6 **a** Kinetics of liver SEC expansion and **b** hepatocyte mitosis during liver regeneration ($n = 4$). **c** Proposed schema describing the roles of SECs in liver regeneration. Upon partial hepatectomy (day 0), LSECs initiate the proliferation of proximal hepatocytes by exerting "inductive signals", possibly by releasing angiocrine factors. Day 4 post-PH, there is a significant liver SEC expansion (proliferative angiogenesis) to sustain hepatic reconstitution. Scale bars, 50 μm. Error bars, SEM

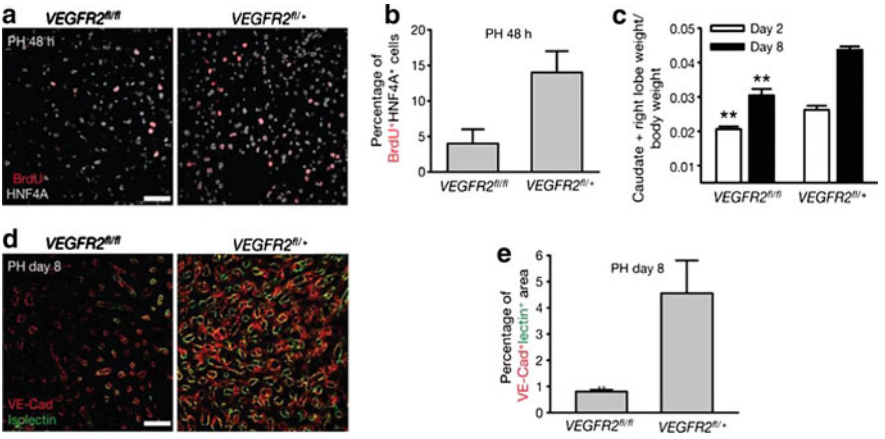

Fig. 7 Partial hepatectomy induced VEGFR2-Id1 activation in LSECs mediates liver regeneration. **a**, **b** Hepatocyte proliferation after partial hepatectomy is impaired in VEGFR2$^{fl/fl}$ mice ($n = 5$). **c**–**e** Inhibition of liver mass regeneration **c** and functional VE-cadherin^{+}isolectin^{+} vessel formation **d**, **e** in VEGFR2$^{fl/fl}$ mice after partial hepatectomy ($n = 4$–6). $^{**}P < 0.001$. Scale bar, 50 μm. Error bars, SEM (Adapted from [65])

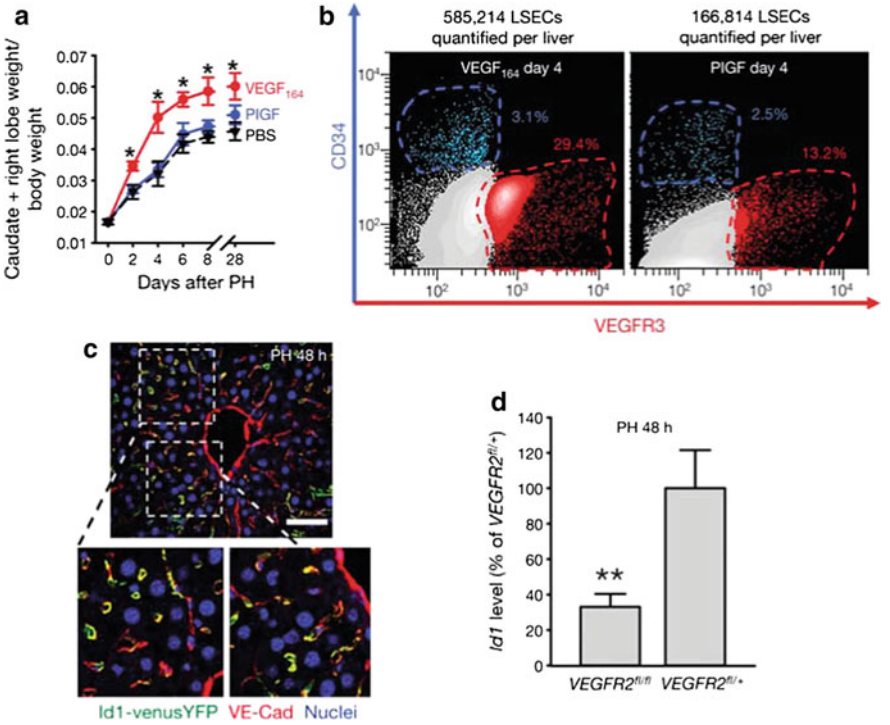

Fig. 8 Injection of VEGF-A_{164}, but not VEGFR1-specific ligand PlGF, accelerates the regeneration of liver mass (**a**), associated with an incremental increase in $VEGFR^{+}CD34^{-}$ liver SEC number (**b**) (n = 4). **c** Regenerative liver section of $Id1^{VenusYFP}$ mouse. Id1 is selectively upregulated by partial hepatectomy in VE-cadherin^{+} vessels. **d** VEGFR2 deletion diminishes Id1 upregulation in the regenerative liver **d** ($n = 5$).; $^{*}P < 0.05$, $^{**}P < 0.01$. Scale bars, 50 μm

placental growth factor (PlGF), as the latter selectively activates VEGFR1 [69]. After partial PH, $VEGF_{164}$, but not PlGF, accelerated the regeneration of both liver mass and the number of $VEGFR3^{+}CD34^{-}$ LSECs, which were sustained for at least 28 days (Fig. 8a, b). Therefore, after partial hepatectomy, the activation of VEGF-A/VEGFR2, but not PlGF/VEGFR1, is crucial for priming LSECs to initiate and maintain hepatic proliferation.

5.2 Microarray Analysis

To identify the angiocrine signals that stimulate liver regeneration, we used microarray analysis (Fig. 9). Among the endothelial-specific genes, the transcription factor Id1 was specifically upregulated in the endothelial cells activated by PH [70]. Using $Id1^{venusYFP}$ reporter mice in which the venus YFP expression is

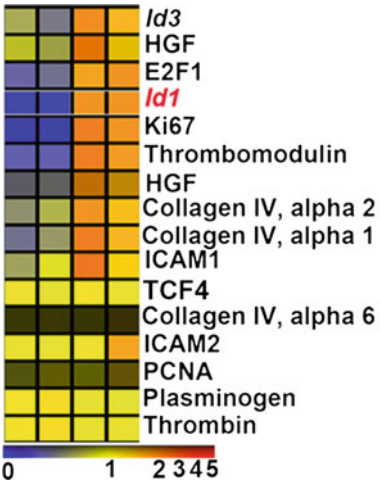

Fig. 9 Heatmap comparing transcriptional profiles. Tree view of the representative Affymetrix gene expression in the regenerative liver 48 h after partial hepatectomy (PH 48 h) compared to that of sham-operated mice (Sham 48 h). Note that the upregulation of angiogenic transcription factor Id1 is comparable to Ki67 and higher than E2F1, both of which are closely associated with hepatocyte proliferation

driven by the Id1 promoter [71], we found Id1 upregulation in LSECs 48 h after PH (Fig. 8c) which was significantly blunted in VEGFR2$^{fl/fl}$ mice (Fig. 8d).

The liver mass recovery in Id1-deficient (Id1$^{-/-}$) mice after PH was impaired for 28 days and remained unchanged upon VEGF-A_{164} administration (Fig. 10a). Furthermore, after partial hepatectomy, Id1$^{-/-}$ mice exhibited significant decrease in mitotic BrdU^{+}HNF4A^{+} hepatocyte number, disrupted formation of functional VE-cadherin^{+}isolectin^{+} vessels, diminished proliferation of VEGFR3^{+}CD34^{-} LSECs, and abnormal liver function, as evidenced by an increase in plasma bilirubin levels. Thus activation of the VEGF-A/VEGFR2 pathway through upregulation of Id1 drives liver regeneration [65].

The role of Id1 upregulation in mediating the angiocrine function of LSECs on hepatocyte proliferation was also examined by a liver SEC–hepatocyte co-culture system. Co-incubation of isolated hepatocytes with primary LSECs led to a ninefold increase in hepatocyte number, which was selectively inhibited by knockdown of Id1 in LSECs (Fig. 10b). Conditioned medium from LSECs failed to support hepatocyte growth, underlining the importance of cell–cell contact in liver SEC-derived angiocrine function. Therefore, lack of Id1 results in defective inductive function of LSECs, impairing hepatocyte regeneration.

To determine whether in vivo angiocrine effects of Id1$^{+/+}$ LSECs could initiate hepatocyte regeneration in Id1$^{-/-}$ mice, we used the intra-splenic transplantation approach on day 2 after PH to engraft LSECs into the Id1$^{-/-}$ liver vasculature [72].

GFP-marked Id1$^{+/+}$ LSECs selectively incorporated into the VEGFR3^{+}sinusoidal vascular lumen and restored the regeneration of liver mass and SEC expansion (Fig. 10c). In contrast, the transplanted Id1$^{+/+}$ LSECs failed to restore the regeneration of the Id$^{+/+}$ liver. Partial vascular chimaerism afforded by the incorporation of Id1-competent LSECs generates sufficient endothelial cell-derived inductive signals to initiate hepatic proliferation in the Id1$^{+/+}$ liver.

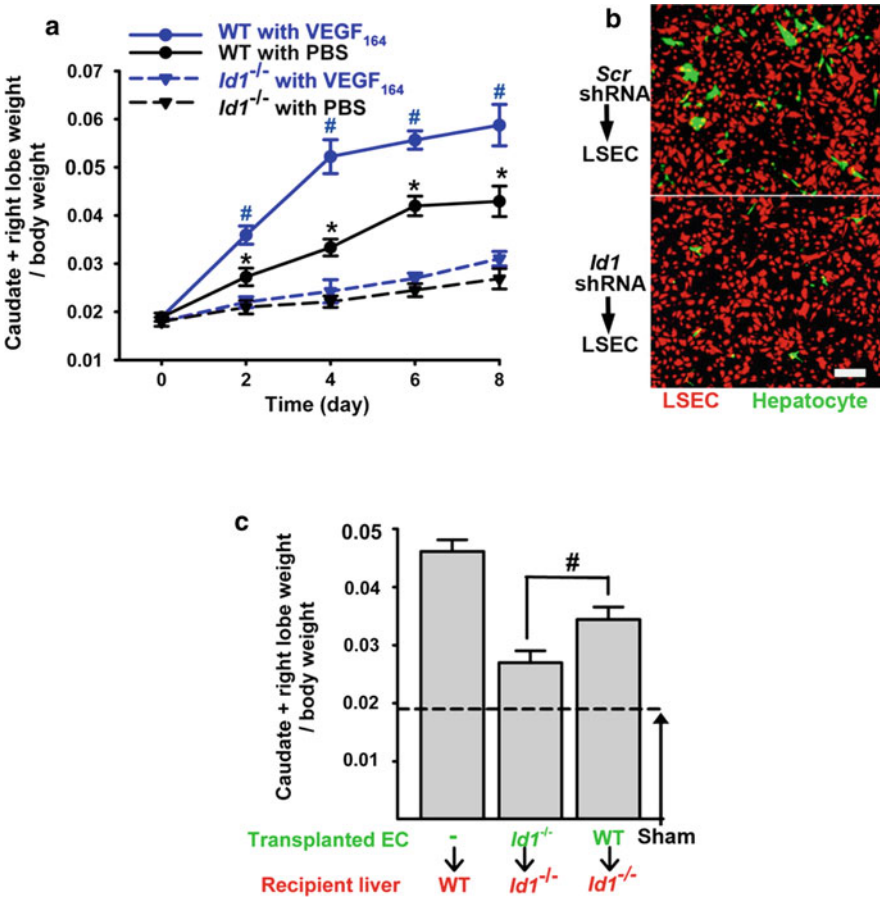

Fig. 10 Id1 upregulation in LSECs is essential for liver regeneration. **a** Compared with their wild-type (*WT*) littermates, $Id1^{-/-}$ mice manifest impaired regeneration in liver mass, which fails to be rescued by VEGF-A_{164} administration ($n = 5$). **b** The liver SEC-dependent stimulation of hepatocyte proliferation was specifically inhibited by Id1 gene knockdown. Scr, scrambled liver SEC-conditioned medium ($n = 4$). **c** Transplantation of $Id1^{+/+}$ LSECs restores the regeneration of mass in the $Id^{-/-}$ liver ($n = 4$). *Dashed line*, level of $Id1^{-/-}$ liver without endothelial cell transplantation. *P,0.05, versus $Id1^{-/-}$ **a**; **P,0.01, versus $Id1^{-/-}$ with $VEGF_{164}$ **a**. Scale bars, 50 mm **b** and 20 mm. Error bars, SEM

To identify endothelial-derived angiocrine factors that induce liver regeneration, we analyzed LSECs purified from the wild-type and $Id1^{+/+}$ mice 48 h after partial hepatectomy. The expression of Wnt2 and HGF, but not other hepatic trophogens expressed by LSECs, such as Wnt9B and thrombomodulin, was drastically diminished in $Id^{+/+}$ LSECs (Fig. 11a). These results suggest that Id1 upregulation in LSECs initiates hepatocyte proliferation through inducing Wnt2 and HGF expression. To test this hypothesis, on day 2 after partial hepatectomy, we engrafted $Id1^{-/-}$ LSECs transduced with Wnt2, HGF or both into the $Id1^{-/-}$

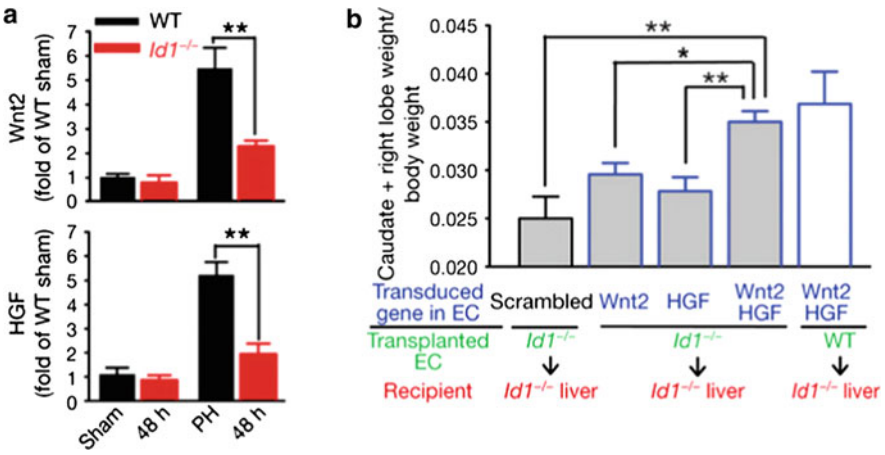

Fig. 11 Id-1-mediated induction of Wnt2 and HGF in LSECs stimulates hepatic regeneration. **a** Upregulation of HGF and Wnt2 is impaired in $Id1^{-/-}$ LSECs after partial hepatectomy ($n = 5$). **b** Intrasplenic transplantation of GFP-marked $Id1^{-/-}$ LSECs carrying both Wnt2 and HGF ($Id1^{-/-}Wnt2^{+}HGF^{+}GFP^{+}$) rescues the regeneration of $Id1^{-/-}$ liver mass ($n = 4$). $^{*}P < 0.05$; $^{**}P < 0.01$. Error bars, SEM

liver vasculature by intra-splenic transplantation. Only $Id1^{-/-}$ LSECs carrying both Wnt2 and HGF ($Id1^{-/-}Wnt2^{+}HGF^{+}$) restored the regeneration of mass and liver SEC expansion in the $Id1^{-/-}$ liver (Fig. 11b), which suggests a collaborative effect between HGF and Wnt2. Therefore, Id1-activated LSECs through Wnt2 and HGF synthesis induce proliferation of juxtaposed hepatocytes (Fig. 12).

In the PH study, we have used conditional VEGFR2 knockout, $Id1^{-/-}$ mice, and an endothelial cell transplantation model to recognize the essential angiocrine role of a specialized organ-specific vascular niche cell in orchestrating physiological liver regeneration. Similar to upregulation of Id1 in the angiogenic tumor vessels, Id1 expression is minimal in normal LSECs, but after activation of VEGFR2 induces exclusive upregulation of Id1 in the angiogenic LSECs. In the first 3 days after partial hepatectomy, activation of the VEGFR2-Id1 pathway switches on an inductive angiogenesis program in non-proliferative $VEGFR3^{+}CD34^{-}VEGFR2^{+}Id1^{+}$ LSECs. Through production of angiocrine factors Wnt2 and HGF, this program provokes hepatic proliferation. Subsequently, as the regenerating liver demands additional blood supply, VEGFR2-Id1-mediated proliferative angiogenesis of LSECs reconstitutes hepato-vascular mass. Therefore, we introduce the concept that SECs support liver regeneration through a biphasic mechanism: at the early phase immediately after partial hepatectomy, inductive angiogenic LSECs promote organogenesis through release of angiocrine factors, whereas proliferative angiogenic LSECs vascularize and sustain the expanding liver mass.

Our earlier work has shown that transplantation of the $Id1^{-/-}Wnt2^{+}HGF^{+}$ LSECs into $Id1^{-/-}$ mice initiates and restores liver regeneration. These findings, and the observation that hepatic proliferation is severely reduced in the VEGFR2

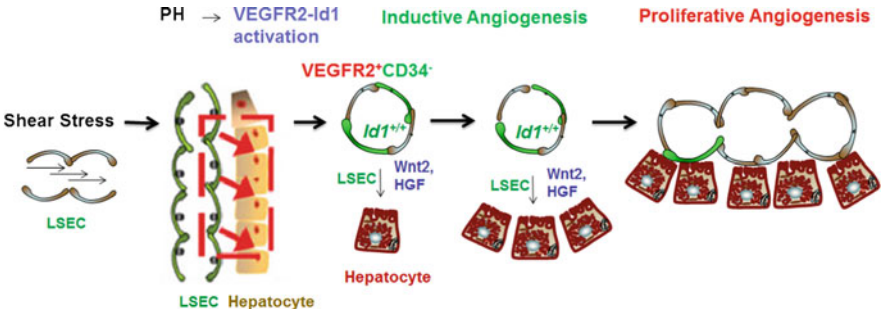

Fig. 12 Requirement for VEGFR2-Id1 pathway in liver SEC-mediated liver regeneration. Intrasplenic transplantation of $Id1^{+/+}$ LSECs into the $Id1^{-/-}$ liver sinusoids restores hepatic-vascular regeneration. Transplanted $Id1^{+/+}$ *or* $Id1^{-/-}Wnt2^{+}HGF^{+}GFP^{+}$ LSECs localize to the vicinity of hepatocytes, promoting inductive and proliferative angiogenesis thereby sustaining physiological liver regeneration

and Id1-deficient mice, suggest that LSECs are responsible for establishing an inductive vascular niche to initiate hepatic proliferation [65].

6 Discussion

Regenerative medicine promises to improve health by repairing or regenerating cells, tissues or organs under different sets of circumstances including acute chemical injury, inflammation, surgical resection, remodeling, and age-related organ degeneration. Developmental biologists have tried to identify extrinsic and intrinsic factors that may potentially act as master regulators of embryonic organ formation. The biomechanical microenvironment also induces ingrowth and differentiation of tissue. For instance, injectable polymer systems have been shown to control the spatiotemporal dynamics of morphogens and in situ programming of cells at sites of injury [73].

Regeneration is a natural process in which the body can restore and repair damage to an existing cell, tissue, or organ. Scientists are interested in organ regeneration due to the possible medical applications of further advances in this field. The liver is one organ that naturally exhibits this ability, which even enables it to regenerate itself after the majority of the organ has been removed. Given that the liver already receives approximately 25 % of an organism's cardiac output, one theory is that an increased blood flow to the liver recorded immediately after PH leads to increased shear stress. Mechanotransduction, the conversion of mechanical forces acting upon a cell into biochemical activity, activates the signaling pathways leading to organ regeneration [74]. Further study of this dynamic between liver blood flow and regeneration could lead to important advances in the field of organ regeneration.

Integrins are receptors that help mediate the connection between the cell and the surrounding matrix. One way in which mechanotransduction is thought to take place is through integrins, which are regarded as mechanosensors due to the conformational changes they adopt when affected by external forces. Two integrins that have been found to exhibit this behavior are the $\alpha 5\beta 1$ and the $\alpha L\beta 2$ integrins [75]. When the apical surface of an endothelial cell experiences shear stress due to the blood flow acting on it, the tension experienced by the cell is transferred to integrins. These integrins, now activated, increase adhesion to the ECM, which alters the phenotype of the cell. In order to maximize adhesion, the cell needs to increase the surface area in contact with the ECM so it flattens in a fashion consistent with the blood flow. This allows the side of the cell facing the oncoming blood flow to resist more tension, while the opposite end of the cell will experience less tension [35]. This would increase the endothelial cell's chance of survival when under the influence of high rates of blood flow and the consequent shear stress.

Human liver can regenerate its original functional mass after hepatectomy and stops this process once the structure of the organ is rebuilt and its normal size restored. The developmental signaling mechanism that serves as the basis for this phenomena requires understanding in order to apply similar regenerative capacity in other organs. Identifying inductive factors that guide stem cells to differentiate into various cell types within different organs can be used as part of a therapeutic strategy.

Interestingly, we have recently shown that after unilateral pneumonectomy (PNX), activation of capillary endothelial cells plays a critical role in initiating and sustaining the regeneration of the lung, another organ constantly subjected to both cell cyclic stretch and changing blood flow [76]. It is plausible that the altered blood flow inside pulmonary vasculature post PNX ignites the activation of VEGFR2, which in turn stimulates the expansion of epithelial progenitor cells. In addition, it is tempting to postulate that changing pulmonary mechanics also plays an essential role in activating epithelial progenitor cells inside alveoli.

Induction of (organ-specific) stem cell differentiation alone may not be sufficient to complete the regeneration process, and creation of the proper local environmental cues and architectural arrangements inherent to the dynamic in vivo setting has been shown to play an integral role. To this end, the physical interactions between cells, ECM, and blood flow, as well as the molecular cross-talk between these constituents play a significant role in tissue regeneration. For example, transplanting hepatocytes in patients with end-stage liver disease results in suboptimal outcomes. With the identified inductive function of LSECs in both initiating and supporting hepatocyte amplification, co-transplantation of properly primed LSECs or pre-conditioning of the host's vasculature would significantly enhance hepatocyte reconstitution, as well as facilitate the engraftment of injected functional hepatocytes via optimized cellular interactions.

The self-regenerative capacity of the liver is frequently hampered by detrimental factors such as extensive resection, hepatotoxins, aging, and tumor metastasis. Thus, end-stage liver disease often requires transplantation therapy. However, lack of available liver tissue consistently curbs successful liver

engraftment in many patients. Development of strategies to expand functional transplantable hepatocytes will significantly increase the success of liver regeneration. We have shown that durable hepatic reconstitution requires cellular interaction between hepatocytes and the liver vascular niche demarcated by specialized liver SECs [65]. Production of liver SECs-derived paracrine growth factors, which we define as "angiocrine factors", has been shown to initiate and maintain hepatocyte regeneration, underscoring the urgency to develop cell therapy approaches to rebuild not only hepatic but also vascular function for liver diseases.

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http://www.springer.com/978-3-642-30855-0

Mechanical and Chemical Signaling in Angiogenesis
Reinhart-King, C. (Ed.)
2013, VI, 282 p., Hardcover
ISBN: 978-3-642-30855-0