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Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration

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During embryogenesis, endothelial cells (ECs) induce organogenesis before the development of circulation[1-4]. These findings suggest that ECs not only form passive conduits to deliver nutrients and oxygen, but also establish an instructive vascular niche, which through elaboration of paracrine trophogens stimulate organ regeneration, in a manner similar to EC-derived angiocrine factors that support hematopoiesis[5-7]. However, the precise mechanism by which tissue-specific subsets of ECs promote organogenesis in the adults is unknown. Here, we demonstrate that liver sinusoidal endothelial cells (LSECs) constitute a unique population of phenotypically and functionally defined $VEGFR3^{+}CD34^{-}VEGFR2^{+}VE\text{-}cadherin^{+}FactorVIII^{+}CD45^{-}$ ECs, which through the release of angiocrine trophogens initiate and sustain liver regeneration induced by 70% partial hepatectomy (PH). After PH, residual liver vasculature remains intact without experiencing hypoxia or structural damage, which allows for studying physiological liver regeneration. Employing this model, we show that inducible genetic ablation of VEGF-A receptor-2 (VEGFR2) in the LSECs impairs the initial burst of hepatocyte proliferation (days 1-3 after

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PH) and subsequent reconstitution of the hepato-vascular mass (days 4-8 after PH) by inhibiting upregulation of the EC-specific transcription factor *Id1*. Accordingly, *Id1*-deficient mice also manifest defects throughout liver regeneration, due to diminished expression of LSEC-derived angiocrine factors, including hepatocyte growth factor (HGF) and Wnt2. Notably, in *in vitro* co-cultures, VEGFR2-Id1 activation in LSECs stimulates hepatocyte proliferation. Indeed, intrasplenic transplantation of $Id1^{+/+}$ or $Id1^{-/-}$ LSECs transduced with Wnt2 and HGF ($Id1^{-/-}Wnt2^{+}HGF^{+}$ LSECs) re-establishes an inductive vascular niche in the liver sinusoids of the $Id1^{-/-}$ mice, initiating and restoring hepato-vascular regeneration. Therefore, in the early phases of physiological liver regeneration, VEGFR2-Id1-mediated inductive angiogenesis in LSECs through release of angiocrine factors Wnt2 and HGF provokes hepatic proliferation. Subsequently, VEGFR2-Id1 dependent proliferative angiogenesis reconstitutes liver mass. Therapeutic co-transplantation of inductive $VEGFR2^{+}Id1^{+}Wnt2^{+}HGF^{+}$ LSECs with hepatocytes provides for an effective strategy to achieve durable liver regeneration.

Sinusoidal ECs (SECs) compose a structurally and functionally unique capillary network that vascularizes specific organs, including bone marrow (BM) and liver. In adult mice, BM SECs, via expression of specific angiocrine trophogens, such as Notch ligands, support hematopoietic regeneration[5-7]. Similarly, the hepatic circulation is predominantly lined by liver SECs (LSECs)[8-10], with each hepatocyte residing in cellular proximity to LSECs. However, the lack of phenotypic and operational definition of liver ECs and paucity of relevant mouse angiogenic genetic models[11-13] have handicapped studies of the role of LSECs in regulation of hepatic regeneration[14-18].

Here, we use a physiologically relevant PH model to elucidate the instructive role of LSECs in mediating hepatic regeneration (supplementary Fig. 1). In contrast to the administration of hepatotoxic chemicals, which impairs the organization of LSECs and causes tissue hypoxia, cell death, and inflammation (supplementary Fig. 2)[8,13,19], in the PH model, resection of 70% of the liver mass without perturbing the integrity of the residual liver vasculature[11] activates hepatocyte regeneration[15-17]. As such, this model provides an instructive model for interrogating the role of structurally and functionally intact LSECs in supporting liver regeneration.

As the VEGF family plays a critical role in the regeneration of the BM SECs[6], we hypothesized that VEGF-receptors[20-22], including VEGFR2 or VEGFR3 also modulate LSEC function. Using *VEGFR2-GFP* mice in which the GFP expression is driven by the native promoter of VEGFR2, we demonstrate that VEGFR2 and VEGFR3 are exclusively expressed in the liver ECs but not other liver cell types, including hepatocyte nuclear factor 4α (HNF4A)$^{+}$ hepatocytes (Fig. 1a, supplementary Fig. 3). Notably, distribution of VEGFR3 expression is restricted to VEGFR2^{+} LSECs that branch out from CD34^{+}VEGFR3^{-} large vessels (Fig. 1b). Polyvariate flow cytometric analysis on nonparenchymal cells (NPCs) demonstrates the expression of EC-specific marker VE-cadherin on non-hematopoietic VEGFR3^{+}VEGFR2^{+}CD45^{-} LSECs, 97.6% of which are non-lymphatic (Prox1^{-}CD34^{-})[22] ECs expressing Coagulation Factor VIII (Fig. 1c, d). Thus, we have designated a unique phenotypic and operational signature for LSECs of the adult mice as VEGFR3^{+}CD34^{-}VEGFR2^{+}VE-cadherin^{+}FactorVIII^{+}Prox-1^{-}CD45^{-} vessels,

distinguishing them from VEGFR3^{-}CD34^{+}VEGFR2^{+}VE-cadherin^{+}CD45^{-} non-sinusoidal ECs and VEGFR3^{+}CD34^{+}Prox-1^{+}FactorVIII^{-}CD45^{-} lymphatic ECs. Identification of LSECs as VEGFR3^{+}CD34^{-} and non-sinusoidal ECs as VEGFR3^{-}CD34^{+} is sufficient for quantification, purification and molecular profiling of LSECs.

To determine the mechanism by which LSECs regulate hepatic proliferation, we studied the regenerative kinetics of hepatocytes and LSECs after PH. Two days after PH, 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. 1e). However, proliferation of LSECs starts at day 4 and plateaus by day 8 after PH (Fig. 1f, supplementary Fig. 4). In comparison, quantification of P-H3^{+}HNF4A^{+} hepatocytes showed that the rate of hepatocyte proliferation peaks during the first 4 days, while leveling off by day 8 (Fig. 1g). These results suggest a chronologically biphasic contribution of LSECs in mediating hepatic reconstitution. At the early phases of PH (days 1-3 after PH), inductive angiogenesis in the non-proliferative LSECs stimulates hepatic regeneration possibly by releasing angiocrine factors, while 4 days after PH, the increased demand of blood supply for the regenerating liver is met via proliferative angiogenesis of LSECs.

To investigate the significance of VEGF-receptors during LSEC-driven hepatic regeneration, we designed experiments to conditionally delete the *VEGFR2* gene by crossing *VEGFR2*$^{loxP/loxP}$ mice with*ROSA-CreER*^{T2} mice, generating inducible VEGFR2-deficient, *VEGFR2*$^{flox/flox}$ (*VEGFR2*$^{fl/fl}$) mice (supplementary Fig. 5)[6]. Due to the EC-specific expression of VEGFR2 in the liver, in *VEGFR2*$^{fl/fl}$ mice only liver ECs but not non-EC cells, will manifest functional defects. As control, we used mice with heterozygous deletion of the *VEGFR2* gene (*VEGFR2*$^{fl/+}$). Forty eight hours after PH, bromodeoxyuridine^{+} hepatocyte proliferation (BrdU^{+}HNF4A^{+} cell number) was decreased by 67% in *VEGFR2*$^{fl/fl}$ mice (Fig. 2a, b). Notably, 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. 2c). Therefore, in the early phases (PH days 1-3) of the liver regeneration, targeting VEGFR2 primarily impairs the effect of EC-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. 2c), interfering with the assembly of patent VE-cadherin^{+}isolectin^{+} vasculature (Fig. 2d, e), thereby blunting restoration of the liver mass for at least 28 days (supplementary Fig. 5). Furthermore, in *VEGFR2*$^{fl/fl}$ mice, liver function after PH was abnormal as manifested by elevated plasma bilirubin levels. To corroborate the EC-specific VEGFR2 function in mediating liver regeneration, *VEGFR2*$^{loxP/loxP}$ mice were also crossed with *VE-cadherin-CreER*^{T2} mice to induce EC-selective deletion of VEGFR2 (supplementary Fig. 5). Both the liver mass and formation of perfused vessels in the *VE-cadherin-CreER*^{T2}*VEGFR2*$^{fl/fl}$ mice were decreased after PH, underlining the significance of VEGFR2 in mediating liver regeneration. Indeed, if the VEGF-A/VEGFR2 pathway promotes the LSEC-driven hepatic regeneration, then VEGF-A should enhance liver regeneration. Hence, we compared the effect of VEGF-A$_{164}$, to placental growth factor (PlGF), with the latter selectively activates only VEGFR1[21]. After

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. 2f, g). Therefore, after PH, the activation of VEGF-A/VEGFR2, but not PlGF/VEGFR1, is crucial for priming LSECs to initiate and maintain hepatic proliferation.

To identify the angiocrine signals that stimulate liver regeneration, we employed microarray analysis (supplementary Fig. 6, supplementary table 1). Among the EC-specific genes, the transcription factor *Id1* was specifically upregulated in the PH-activated ECs[23]. Using $Id1^{venusYFP}$ reporter mice in which the venusYFP expression is driven by the *Id1* promoter[24], we found exclusive *Id1* upregulation in LSECs 48 hours after PH (Fig. 2h), which was significantly blunted in $VEGFR2^{fl/fl}$ mice (Fig. 2i). Remarkably, the liver mass recovery in *Id1*-deficient ($Id1^{-/-}$) mice after PH was impaired for 28 days and remained unchanged upon $VEGF\text{-}A_{164}$ administration (Fig. 3a, supplementary Fig. 7). Furthermore, after PH, $Id1^{-/-}$ 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 (Fig. 3b, c, supplementary Fig. 7). Thus, activation of the VEGF-A/VEGFR2 pathway through upregulation of Id1 drives liver regeneration.

The role of Id1 upregulation in mediating the angiocrine function of LSECs on hepatocyte proliferation was also examined by a LSEC-hepatocyte coculture system. Co-incubation of isolated hepatocytes with primary LSECs led to a 9-fold increase in hepatocyte number, which was selectively abolished by knockdown of *Id1* in LSECs (Fig. 3d, e, supplementary Fig. 8). Conditioned medium (CM) from LSECs failed to support hepatocyte growth, underlining the importance of cell-cell contact in LSEC-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 intrasplenic transplantation approach on day 2 after PH to engraft LSECs into the $Id1^{-/-}$ liver vasculature (Fig. 3f)[25]. GFP-marked $Id1^{+/+}$ LSECs selectively incorporated into the $VEGFR3^+$ sinusoidal vascular lumen and restored the regeneration of liver mass and LSEC expansion (Fig. 3g, supplementary Fig. 9). In contrast, the transplanted $Id1^{-/-}$ LSECs failed to restore the regeneration of the $Id1^{-/-}$ liver. Moreover, in the $Id1^{-/-}$ liver, transplantation of $GFP^+Id1^{+/+}$ LSECs at day 2 after PH initiated the proliferation of the hepatocytes in their immediate proximity (Fig. 3h, i). Thus, partial vascular chimerism afforded by the incorporation of *Id1*-competent LSECs generates sufficient EC-derived inductive signals to initiate hepatic proliferation in the $Id1^{-/-}$ liver.

To identify EC-derived angiocrine factors that induce liver regeneration, we analyzed LSECs purified from the wild-type and $Id1^{-/-}$ mice 48 hours after PH. Among the known hepatic trophogens[10,18,26-28], the expression of Wnt2 and HGF, but not other trophogens expressed by LSECs, such as Wnt9B and thrombomodulin, were drastically diminished in $Id1^{-/-}$ LSECs (Fig. 4a, supplementary Fig. 10). 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 PH, we engrafted $Id1^{-/-}$ LSECs transduced with Wnt2,

HGF, or both Wnt2 and HGF into the *Id1*$^{-/-}$ liver vasculature via intrasplenic transplantation. Only *Id1*$^{-/-}$ LSECs carrying both Wnt2 and HGF (*Id1*$^{-/-}$*Wnt2*$^{+}$*HGF*$^{+}$) restored the regeneration of mass and LSEC expansion in the *Id1*$^{-/-}$ liver (Fig. 4b), suggesting a collaborative effect between HGF and Wnt2. Notably, transplantation of *Id1*$^{-/-}$*Wnt2*$^{+}$*HGF*$^{+}$ LSECs into *Id1*$^{-/-}$ mice increased the mitotic BrdU^{+}HNF4A^{+} hepatocyte number to a similar degree achieved by *Id1*$^{+/+}$ LSEC transplantation (Fig. 4c). The mitotic hepatocytes were also found to be positioned adjacent to the transplanted *Id1*$^{-/-}$*Wnt2*$^{+}$*HGF*$^{+}$GFP^{+} LSECs (Fig. 4d). Therefore, Id1-activated LSECs through elaboration of Wnt2 and HGF induce proliferation of juxtaposed hepatocytes (Fig. 4e).

Here, we have employed conditional VEGFR2 knockout, *Id1*$^{-/-}$ mice, and an EC transplantation model to identify the essential angiocrine role of a specialized organ-specific vascular niche cell, defined operationally as VEGFR3^{+}CD34^{-}VEGFR2^{+}VE-cadherin^{+}FactorVIII^{+}Prox1^{-}CD45^{-} LSECs, in orchestrating PH-induced physiological liver regeneration. Similar to upregulation of Id1 in the angiogenic tumor vessels[23], Id1 expression is minimal in the normal LSECs, but after PH activation of VEGFR2 induces exclusive upregulation of Id1 in the angiogenic LSECs. We demonstrate that in the first 3 days post-PH, activation of the VEGFR2-Id1 pathway switches on an inductive angiogenesis program in non-proliferative VEGFR3^{+}CD34^{-} VEGFR2^{+}Id1^{+} LSECs, which through production of angiocrine factors Wnt2 and HGF, 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 LSECs support liver regeneration through a biphasic mechanism: at the early phase immediately post-PH, inductive angiogenic LSECs promote organogenesis through release of angiocrine factors, while proliferative angiogenic LSECs vascularize and sustain the expanding liver mass.

We show that transplantation of the *Id1*$^{-/-}$*Wnt2*$^{+}$*HGF*$^{+}$ LSECs into *Id1*$^{-/-}$ mice initiates and restores liver regeneration. This finding along with the observation that hepatic proliferation is severely blunted in the *VEGFR2* and *Id1*-deficient mice, suggest that LSECs are chartered with the responsibility to establish an inductive vascular niche to initiate hepatic proliferation by elaborating angiocrine factors. Since isolation of LSECs for therapeutic liver regeneration might encounter technical difficulties, endothelial progenitor cells (EPCs) derived from non-hepatic tissues may alternatively substitute for LSECs to initiate and restore liver regeneration[29]. Notably, *VEGFR2*$^{+}$*Id1*$^{+}$ EPCs could initiate angiogenesis through release of angiocrine factors rather than structurally incorporating into vessel wall[29]. As such, intrahepatic transplantation of EPCs will open up new avenues of cell therapy to promote liver regeneration.

In PH model employed in our study, the vascular integrity of the residual liver lobes is maintained with minimal inflammatory response (supplementary Fig. 2), thereby establishing an ideal model to study EC-dependent liver regeneration. However, in chemical (CCl_4)-induced liver injury models, severe vascular damage and cell death might require the recruitment of other non-EC cells, including stellate cells[19] and pro-angiogenic hematopoietic cells, such as CXCR4^{+}VEGFR1^{+} hemangiocytes[30], to support liver regeneration.

However, here is one unsolved enigma: How is removal of 70% of the liver sensed by the LSECs in the residual liver to ignite hepatic proliferation[14-17]. Conceivably, the mass of the liver is maintained through continuous release of as yet unrecognized inhibitory factors. Removal of the liver shifts the balance towards the predominance of vascular excitatory factors, which activate LSECs. Likewise, an increase in the mass of the liver 4 days post-PH instigates the release of factors that stimulate sprouting-angiogenesis in LSECs. Subsequently, recovery of the liver to its developmentally pre-determined baseline mass might re-establish as yet unidentified inhibitory signals that terminate the regenerative process. The rapid regeneration of the liver after PH requires collective and global proliferation of a large number of hepatocytes. Indeed, as each hepatocyte resides in close proximity to LSEC, this remarkably harmonious activation of hepatocytes is achieved by switching on angiocrine-dependent regenerative program to induce proliferation of mature hepatocytes throughout the residual liver after PH. Whether, angiocrine factors could also promote the propagation of liver progenitor cells[14], in addition to the mature hepatocytes, remains to be investigated.

In our study, Wnt2 and HGF represent the predominant liver-specific angiocrine factors driving hepatic regeneration. As direct cellular contact between LSECs and hepatocytes was essential for proliferation of hepatocytes, it is conceivable that other angiocrine factors might collaborate with Wnt2 and HGF to modulate liver regeneration. For instance, EC-specific extracellular matrix components, proteases, adhesion molecules and chemokines might also participate in hepatogenesis. Our *in vitro* EC-hepatocyte coculture model and *in vivo* intrasplenic transplantation model provide for ideal model to assess the role of these unknown angiocrine factors in modulating hepatic homeostasis during recovery from chemical or traumatic injury.

Accumulating evidence suggests that in addition to LSECs, other organ-specific vascular niche plays a seminal role in organ-repair and tumorigenesis[5-7]. For example, stress-induced expression of Notch-ligands by the bone marrow SECs was shown to be essential for hematopoietic stem cell reconstitution[5]. Furthermore, elaboration of specific prototypical angiocrine factors, such as BMP2, Nitric oxide, FGF2 and PDGFβ by tumor vessels also directly provoke tumor progression and metastasis[7]. Collectively, these data suggest that tissue-specific expression of defined angiocrine factors may dictate heterogeneity of vasculature in regulating developmental and adult organogenesis.

So far, attempts in liver regeneration by hepatocyte transplantation have culminated in limited success[25]. Our study indicates that co-transplantation of hepatocytes or their progenitor cells[14] with *VEGFR2*$^{+}$*Id1*$^{+}$ LSECs or EPCs might permit designing effective strategies to rescue hepato-vascular function in patients inflicted with traumatic or infectious liver damage. Furthermore, the fact that physiological liver regeneration is dependent on the proper inductive and proliferative functioning of the LSECs, also calls for the assessment of the potential increased risks of anti-angiogenic therapy in clinical trials involving liver regeneration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

Method Summary

Transgenic Reporter, Gene Targeted Animals and Mouse Surgery

C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME). *VEGFR2-GFP* mice were acquired from Dr. Janet Rossant (the Hospital for Sick Children, Canada). *VE-cadherin-CreER*^{T2} mice were kindly provided by Dr. Luisa Iruela-Arispa (University of California, Los Angeles, CA). Inducible VEGFR2 knockout (generated by Dr. Thomas N. Sato) and *Id1*$^{-/-}$ mice were previously described[6,23]. *Id1*^{venusYFP} mice were obtained from Dr. Robert Benezra (Sloan Kettering Institute, New York, NY)[24]. PH was performed by resecting three most anterior lobes. Hepatic engraftment of endothelial cells was adapted as previously reported[25]. All animal experiments were performed under the guidelines set by Institutional Animal Care and Use Committee.

Image Acquisition, Image Analysis, and Flow Cytometric Analysis

Fluorescent images were captured on AxioVert LSM510 or 710 confocal microscope (Zeiss). For flow cytometry, antibodies were conjugated to Alexa Fluorescent dyes or Qdots (Invitrogen, CA). Purified liver cells were analyzed on LSRII-SORP (BD Biosciences, CA). Doublets were excluded by FSC-W × FSC-H and SSC-W × SSC-H analysis, and single stained channels were used for compensation.

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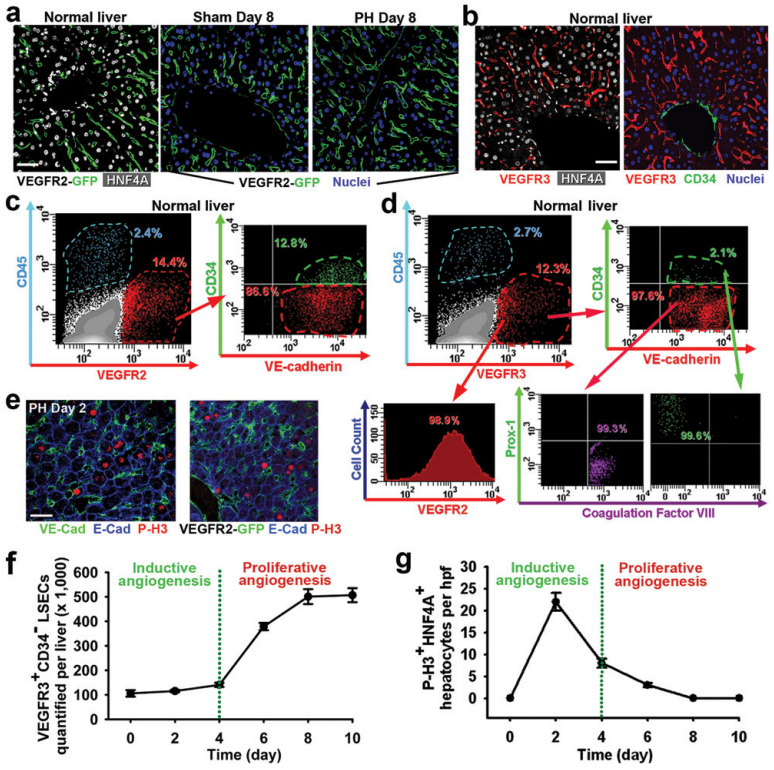

Figure 1. Phenotypic signature and contribution of LSECs to physiological liver regeneration induced by 70% partial hepatectomy (PH)

a) Liver sections obtained from VEGFR2-GFP reporter mice[6]. During liver regeneration VEGFR2 is exclusively expressed on the liver ECs. **b)** Restricted expression of VEGFR3 on LSECs, but not $CD34^+$ large vessels or hepatocytes. **c)** Polyvariate flow cytometric analysis of the liver nonparenchymal cells. $VEGFR2^+$ cells that are $CD45^-$, express EC-specific VE-cadherin. **d**) Specific expression of VEGFR3 on $VEGFR2^+$VE-$cadherin^+CD45^-$ LSECs, with a predominant fraction being $CD34^-$Factor$VIII^+Prox\text{-}1^-$. Thus, LSECs could be identified as $VEGFR3^+CD34^-$ cells. **e)** 48 hours after PH, E-$cadherin^+P\text{-}H3^+$ mitotic hepatocytes are localized adjacent to VE-$cadherin^+$ and $VEGFR2^+$ ECs. **f, g)** Kinetics of LSECs expansion (f) and hepatocyte mitosis (g) during liver regeneration (n = 4). Hpf, high power field. Scale bars, 50 μm. Error bars, s.e.m.

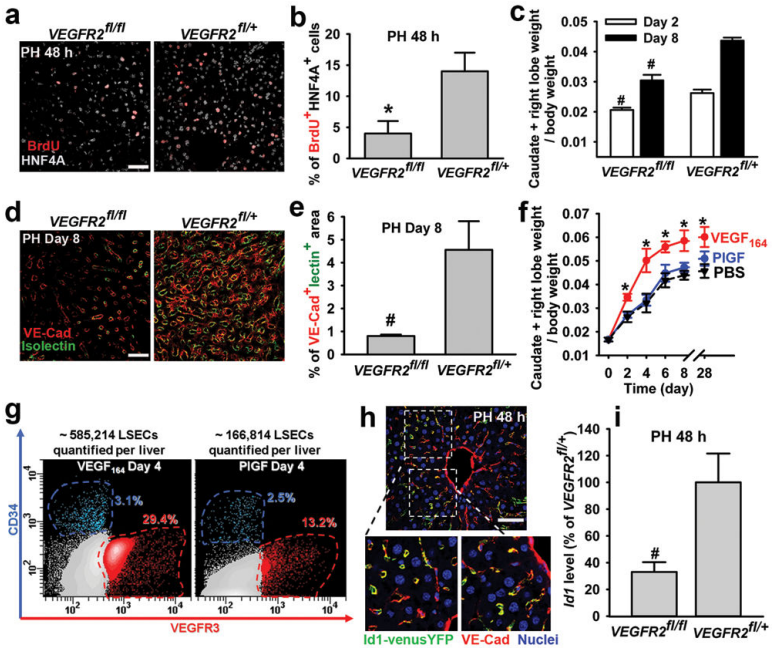

Figure 2. VEGFR2-Id1 activation in LSECs mediates PH-induced liver regeneration

a, b) Hepatocyte proliferation after PH 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 PH (n = 4-6). **f, g)** Injection of VEGF-A$_{164}$, but not VEGFR1-specific ligand PlGF, accelerates the regeneration of liver mass (f), associated with an incremental increase in VEGFR3^{+}CD34^{-} LSEC number (g) (n = 4). **h)** Regenerative liver section of *Id1*^{VenusYFP} mouse[24]. *Id1* is selectively upregulated by PH in VE-cadherin^{+} vessels. **i)** VEGFR2 deletion diminishes *Id1* upregulation in the regenerative liver (n = 5). *P < 0.05; #P < 0.01, versus *VEGFR2*$^{fl/+}$ (b-e, i), versus PlGF-treated group (f). Scale bar, 50 μm. Error bars, s.e.m.

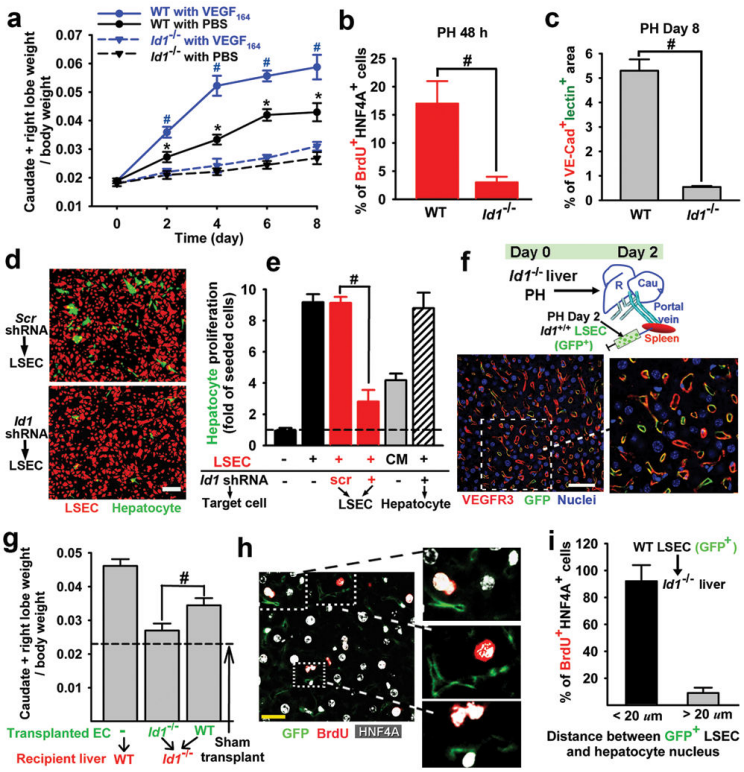

Figure 3. *Id1* upregulation in LSECs is essential for liver regeneration

a) Compared to their wild type littermates (WT), $Id1^{-/-}$ mice manifest impaired regeneration in liver mass, which fails to be rescued by VEGF-A_{164} administration ($n = 5$). **b, c)** Impaired hepatocyte proliferation (b) and assembly of VE-cadherin^{+}isolectin^{+} vessels (c) in the $Id1^{-/-}$ mice after PH ($n = 5$). **d, e)** The LSEC-dependent stimulation of hepatocyte proliferation was specifically inhibited by *Id1* gene knockdown. Scr, scrambled. CM, LSEC-conditioned medium ($n = 4$). **f)** Intrasplenic transplantation of GFP-marked LSECs incorporates into the lumen of VEGFR3^{+} sinusoidal vasculature in the $Id1^{-/-}$ liver[25]. **g, h)** Transplantation of $Id1^{+/+}$ LSECs restores the regeneration of mass (g) and hepatocyte proliferation (h) in the $Id1^{-/-}$ liver ($n = 4$). Dashed line, level of $Id1^{-/-}$ liver without EC transplantation. **k**) Cellular proximity is essential in the stimulation of hepatocyte mitosis by the transplanted GFP$^{+}Id1^{+/+}$ vasculature. *$P < 0.05$, versus $Id1^{-/-}$ (a); #$P < 0.01$, versus $Id1^{-/-}$ with $VEGF_{164}$ (a), versus WT (b, c). Scale bars, 50 (d, f) and 20 (h) μm. Error bars, s.e.m.

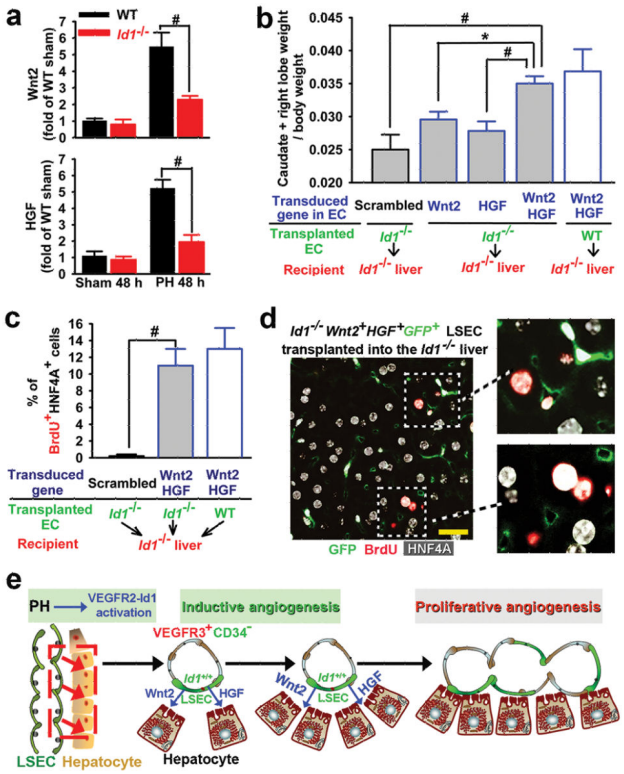

Figure 4. Id1-mediated induction of Wnt2 and HGF in LSECs stimulates hepatic regeneration

a) Upregulation of HGF and Wnt2 is impaired in $Id1^{-/-}$ LSECs after PH (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). **c)** Transplantation of $Id1^{-/-}Wnt2^{+}HGF^{+}$ LSECs restores the impaired hepatocyte proliferation in the $Id1^{-/-}$ liver. (n = 4). **d)** The proximity between the mitotic hepatocytes and the $Id1^{-/-}Wnt2^{+}HGF^{+}$GFP^{+} LSECs in the $Id1^{-/-}$ liver. **e)** Requirement for VEGFR2-Id1 pathway in LSEC-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. *P < 0.05; #P < 0.01. Scale bar, 20 μm. Error bars, s.e.m.