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Angiocrine functions of organ-specific endothelial cells

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Preface

Endothelial cells lining blood vessel capillaries are not just passive conduits for delivering blood. Tissue-specific endothelium establish specialized vascular niches that deploy specific sets of growth factors, known as angiocrine factors, which actively participate in inducing, specifying, patterning, and guiding organ regeneration and maintaining homeostasis and metabolism. Angiocrine factors upregulated in response to injury orchestrates self-renewal and differentiation of tissue-specific repopulating resident stem and progenitor cells into functional organs. Uncovering the precise mechanisms whereby physiological-levels of angiocrine factors are spatially and temporally produced, and distributed by organotypic endothelium to repopulating cells, will lay the foundation for driving organ repair without scarring.

Introduction

The microvascular circulation comprises a vast network of capillary endothelial cells (ECs) that connects the arteries to veins. These vascular beds, which are distinct from lymphatic vessels, were perceived as passive conduits with a responsibility for delivering oxygen and nutrients, modulating the coagulation of blood, regulating the transportation of inflammatory cells and serving as gatekeepers of cellular metabolism[1, 2]. However, these cells also perform other necessary physiological tasks: sustaining the homeostasis of resident stem cells and guiding the regeneration and repair of adult organs without provoking fibrosis.

This new paradigm emerged from microanatomical findings that epithelial, hematopoietic, mesenchymal and neuronal cells, along with their corresponding repopulating stem and progenitor cells, reside in close physical proximity to capillary ECs. Genetic and biochemical studies have shown that ECs serve as a fertile, instructive niche that plays key roles in sustaining homeostasis, metabolism and directing organ regeneration in a "perfusion-independent" manner. Tissue-specific ECs mastermind these complex tasks by supplying the repopulating cells with stimulatory and inhibitory growth factors, morphogens, extracellular matrix and chemokines. These EC-derived paracrine factors are collectively defined as angiocrine factors[3,4] (Box 1).

The tissue-specific instructive functions of ECs have been demonstrated in studies showing that the deletion of angiocrine factors in adult ECs disrupts stem-cell homeostasis and impairs organ repair without compromising blood supply. Notably, intravenous

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transplantation and engraftment of tissue-specific ECs following injury augment organ reconstitution and function without instigating maladaptive fibrosis. On the basis of these observations, organotypic capillary ECs are now recognized as specialized niche cells that, through balanced physiological expression of angiocrine factors, maintain stem cells' capacity for quiescence and self-renewal. Spatially and temporally coordinated production of angiocrine factors after organ injury initiates and completes organ regeneration. This transformative model has opened a fresh chapter in translational vascular medicine. It has also raised the possibility that the inherent pro-regenerative potential of tissue-specific endothelium could be used therapeutically to orchestrate fibrosis-free healing and to restore homeostasis in tissues.

Although the angiocrine signals that guide the formation of the liver[5] and pancreas[6] in the fetus have been defined, the contribution of angiocrine signalling to the modulation of homeostasis and regeneration in adult organs has not been well studied until now. In this Review, we describe the instructive and inductive contributions of adult tissue-specific ECs to the homeostatic and regenerative functions of repopulating stem and progenitor cells.

Instructive interactions of capillary ECs

The adult human body contains 10 trillion–60 trillion ECs that cover a vast surface area[7]. Tightly intertwined monolayers of ECs form the lumen of the blood circulatory system, which consists of large arteries, veins and extensively branched capillaries. Lymphatic vessels run parallel to capillaries and exist as an independent and open circulatory system. The surface area of capillaries represents more than 95% of the total circulatory surface area and arborizes into almost every cellular component of organs.

Tissue-specific stem and progenitor cells are strategically positioned in close proximity to homotypic capillary ECs (Fig. 1a–d). This intimate cellular interaction facilitates the delivery of membrane-bound and soluble angiocrine factors from specialized ECs to the recipient cells, which are located on the basolateral surface of blood vessels. Moreover, the luminal surface of ECs can serve as a signalling platform for stem and immune cells that navigate through the circulation. Tissue-resident parenchymal and stem cells regulate the activation state and response of ECs to regenerative stimuli through the production of angiogenic factors such as vascular endothelial growth factor (VEGF)-A, fibroblast growth factor (FGF)-2, stromal-cell-derived factor (SDF-1; also known as CXCL12), angiopoietins and thrombospondin-1 (TSP-1) (Fig. 1e). Thus, the capillary network — without the influence of pericytes and mesenchymal cells — provides an adaptive platform that has the functional plasticity to integrate and relay these intravascular and extravascular cues to both resting and regenerating organs.

Angiocrine-mediated self-renewal and differentiation

The formation of new blood vessels through angiogenesis is crucial to meet the metabolic demands of organs[1, 2]. Accumulating evidence indicates that ECs regulate organ homeostasis and repair through the production of angiocrine factors in an angiogenesis-independent manner (Box 1). The Greek philosopher and scientist Aristotle, who is widely

considered to be the founder of classical biology, proposed that blood vessels direct the configuration of organs[8]. On pathophysiological stress (exposure to ionizing radiation, chemical injury or hypoxic conditions, for example) or loss of tissue mass, defined angiocrine factors emanate from activated ECs (Table 1). The activated ECs relay inflammatory and injury-induced angiocrine signals to quiescent tissue-specific stem cells, which drives regeneration and enforces developmental set points to re-establish homeostatic conditions. Microvascular ECs therefore fulfil the criteria for professional niche cells that choreograph tissue regeneration by cradling and nurturing stem cells with physiological levels and proper stoichiometry of angiocrine factors. The contribution of the endothelial niche to mediating stem-cell homeostasis and function has been studied in depth in neural stem cells (NSCs), spermatogonial stem cells and haematopoietic stem and progenitor cells (HSPCs).

Neural stem cells

The adult brain contains two regions in which NSCs undergo neurogenesis: the ventricular subventricular zone (V-SVZ) and the subgranular zone (SGZ). In the V-SVZ, type B1 quiescent and activated NSCs give rise to type C transit amplifying cells and type A mature neuroblastic cell progenies, which are positioned in the proximity of capillary ECs[9, 10, 11, 12] (Fig. 2a). Similarly, in the SGZ, which is located in the dentate gyrus of the hippocampus, NSCs and their progenies reside near capillaries[13]. Brain capillaries are lined with ECs that are positive for VEGF receptor (VEGFR)-2 and vascular endothelial (VE)-cadherin, positive or negative for the CD133 antigen, negative for or express only low levels of thrombomodulin, and that display zones of variable permeability[4, 10, 12] (Figs 1d, 2a). Subsets of V-SVZ and SGZ blood vessels have a specialized planar morphology in which NSCs extend their endfeet to contact ECs. This close proximity supports the possibility that angiocrine factors regulate neurogenesis.

In vitro studies of neuronal cells that were co-cultured with heterotypic-derived ECs support a model in which ECs regulate NSC homeostasis and differentiation. Primary human umbilical vein ECs have been shown to produce brain-derived nerve growth factor (BDNF), which fosters the expansion of neuroblasts[14]. Bovine pulmonary artery ECs and polyoma-middle-T-immortalized mouse brain capillary ECs, but not smooth-muscle cells, trigger Notch signalling by secreting soluble factors that increase the self-renewal of NSCs and drive neurogenesis[9]. Follow-up studies showed that pigment epithelium-derived factor (PEDF) was one of the secreted angiocrine factors that stimulates Notch-dependent self-renewing symmetric divisions of NSCs[15].

Subsequent in vivo experiments demonstrated that angiocrine factors derived from brain ECs regulate the homeostasis and regeneration of NSCs both through direct cellular contact and in a paracrine manner[13, 16, 17, 18]. Under steady-state conditions, angiocrine expression of the membrane-bound proteins EphrinB2 and Jagged-1 (refs 19, 20) sustains the dormancy of quiescent NSCs. Direct contact of EC-derived EphrinB2 and Jagged-1 with the endfeet of these cells suppresses their entry into the cell cycle and keeps them in an undifferentiated state. Moreover, neurotrophin-3 (NT-3), which is selectively produced by ECs in the brain and choroid plexus, maintains NSC quiescence, in part, through the induction of endothelial

nitric-oxide synthase and the production of nitric oxide[21, 22]. Although NSCs could also supply endothelial nitric-oxide synthase, the angiocrine release of NT-3 in the V-SVZ and cerebrospinal fluid dictates nitric oxide production that sustains stem-cell quiescence. Conditional deletion of NT-3 in adult mouse brain ECs depletes NT-3 in both cerebrospinal fluid and the V-SVZ, which leads to an increase in dividing activated NSCs that express glial fibrillary acidic protein (GFAP) and accelerates the exhaustion of the NSC pool. Thus, angiocrine factors actively enforce the quiescence that is crucial for the long-term maintenance of the NSC population.

During regenerative processes, irrigation of the V-SVZ by soluble angiocrine factors such as BDNF[14], PEDF[23], betacellulin[24] and placental growth factor-2 (PlGF-2)[25], and of the SGZ by VEGF-C[26, 27], orchestrates proliferation and differentiation of both quiescent and activated NSCs into transit amplifying cells and neuroblasts. Notably, graded angiocrine deposition of SDF-1 (ref. 28) and BDNF[29] by blood vessels that run along the rostral migratory stream in the mouse brain guides the proliferation of transit amplifying cells and their migration to the olfactory bulb[30]. Therefore, brain capillary ECs not only supply the V-SVZ and SGZ with region-specific regenerative and path-finding cues, but also secrete angiocrine factors into cerebrospinal fluid to potentially modulate neuronal homeostasis throughout the brain.

Crosstalk between neuronal cells and angiogenic ECs allows the endothelial niche to adapt to regenerative neurogenesis (Fig. 1e). During vascular sprouting, cross-activation of ECs by neuronal-derived angiogenic factors regulates the differential production of angiocrine factors (Fig. 2a). After hypoxic injury, upregulation of VEGF-A through the activation of VEGFR-2 enhances the production of nitric oxide, which induces BDNF in brain capillary ECs to drive the expansion and maturation of transit amplifying cells[16]. Growth differentiation factor (GDF)-11 also enhances neurogenesis by remodelling the blood vessels[31]. Thus, endothelial niche cells in the brain possess a remarkable angiocrine plasticity that can adapt to the physiological demands of NSCs to initiate, execute and finalize neurogenic programmes.

Spermatogonial stem cells

Undifferentiated type A spermatogonial stem cells from mice reside in the vicinity of interstitial capillaries within the seminiferous tubules of the testes[32]. After perturbation of the testicular microenvironment, transplanted donor-derived spermatogonial stem cells localize to zones that are enriched in capillaries. In vitro studies have shown that spermatogonial stem cells that express the G-protein-coupled receptor GPR125 can directly convert to multipotent progenitor cells. Incubation of such spermatogonial stem cells with vascular-like stromal cells that carry the CD34 antigen is essential for the conversion of spermatogonial stem cells to pluripotent stem cells[33, 34, 35], and indicates that angiocrine factors play an important part in regulating the maintenance and self-renewal of spermatogonial stem cells. Indeed, transcriptional analysis of testicular endothelium suggests that ECs could be a rich source of glial-cell-line-derived neurotrophic factor (GDNF)[4]. Further analysis of the phenotypic and functional properties of testicular ECs is necessary to determine the degree to which ECs influence spermatogonial stem-cell

homeostasis by deploying angiocrine factors and depositing peritubular extracellular matrix components.

Hematopoietic stem cells

The first evidence that ECs establish an instructive niche for haematopoietic cells (Fig. 2b) was the demonstration that homotypic human bone-marrow-derived ECs expand human umbilical cord blood-derived $CD34^+$ cells ex vivo[36, 37]. Furthermore, heterotypic primary ECs isolated from brain, heart and fetal tissues have since been shown to promote the proliferation of mouse[38, 39], human[40, 41] and non-human primate HSPCs[42]. However, these co-culture studies were performed in media supplemented by serum that contained supraphysiological doses of growth factors and under ambient oxygen tension, which masked the full potential of ECs to regulate the function of the cells.

The development of techniques for serum-free and xenobiotic-free culture of primary human or mouse homotypic EC monolayers (Box 2) has facilitated the identification of angiocrine factors that support the self-renewal and differentiation of HSPCs in such co-culture studies[43, 44]. Co-culture studies have also been used to demonstrate that bone-marrow sinusoidal ECs that are positive for VEGFR-3, VEGFR-2, VE-cadherin and CD31 stimulate the self-renewal of HSPCs by expressing soluble and membrane-bound angiocrine factors[45, 46, 47], including bone morphogenic protein (BMP)2 and BMP4, insulin growth factor binding protein (IGFBP)2, SDF-1, Desert hedgehog (Dhh) protein, Notch ligands, Wingless-type MMTV integration site (Wnt)5a, and Kit ligand (Fig. 2b). Bone-marrow sinusoidal ECs also drive the lineage-specific differentiation of HSPCs by producing granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-6, IL-8, granulocyte colony-stimulating factor (G-CSF), IL-1, tumour necrosis factor (TNF), chemokines and metalloproteinases[45]. Notably, ECs that are transitioning through various activation states also produce inhibitory factors, such as transforming growth factor (TGF)-β1 (ref. 48), dickkopf-related protein (DKK)1 and DKK3, which block WNT signalling, and Noggin, which interferes with BMP signalling[45] (Fig. 2b, Table 1). Thus, ECs express inhibitory and stimulatory angiocrine factors that regulate the quiescence and proliferation of HSPCs.

ECs cultured under serum-free conditions were shown to supply angiocrine factors at physiological levels that increase the self-renewal of repopulating authentic mouse haematopoietic stem cells by 150-fold[46] and of human cord blood severe combined immunodeficiency repopulating cells by 8-fold[49]. Direct contact between haematopoietic cells and ECs is essential for the self-renewal and differentiation of HSPCs[45, 46, 47]. Compared with mesenchymal cells, ECs are more efficient at expanding umbilical cord blood-derived HSPCs[50]. Other angiocrine factors, such as prostaglandin E2 (PGE2) (refs 51, 52), pleiotrophin[53] and epidermal growth factor (EGF)[54], drive haematopoietic reconstitution, which establishes ECs as a physiological repository of HSPC-supportive factors.

The first *in vivo* evidence to support the role of the endothelial niche in haematopoiesis came from a study of mice that are unable to produce soluble Kit ligand, an essential

regulator of haematopoietic stem-cell biology[55]. It demonstrated that compartmentalized — yet interactive — stromal and endothelial niche cells regulate the regeneration of HSPCs. In response to physiological stress, the activation of matrix metalloproteinase (MMP)-9 leads to the release of soluble Kit ligand from cells in the niche, which stimulates the regeneration and proper transportation of HSPCs. Follow-up studies showed that phenotypically marked stem cells reside in close proximity to the endothelial niche[56]. Further evidence indicated that haematopoietic regeneration and thrombopoiesis after chemotherapy or irradiation is impaired by the conditional deletion of VEGFR-2 in ECs of adult mice[47] and by the targeting of VE-cadherin to disrupt reconstitution of the endothelial niche[57, 58]. Therefore, the endothelial niche is essential not only for sustaining the self-renewal of haematopoietic stem cells, but also for multi-lineage reconstitution (Fig. 2b, Table 1).

Haematopoietic regeneration is orchestrated by the differential production of angiocrine factors that are induced by signalling pathways activated within ECs[45](Fig. 2b). After myeloablative stress, angiogenic factors such as VEGF-A, VEGF-C, FGF-2 and the angiopoietins upregulate other angiocrine factors, including Jagged-1, through activation of AKT (also known as protein kinase B). Conditional deletion of Jagged-1 in ECs impairs haematopoietic recovery[59], which suggests that Notch activation prevents the exhaustion of HSPCs. During the angiogenic phase of regeneration, AKT phosphorylation is accompanied by the activation of p42/p44 mitogen-activated protein kinase (MAPK). This triggers the secretion of G-CSF, macrophage colony-stimulating factor (M-CSF), GM-CSF and IL-6 to expand populations of myeloid, megakaryocytic and lymphoid progenitor cells and aid haematopoietic reconstitution[45] (Fig. 2b). In turn, maturing haematopoietic cells produce inhibitory angiogenic factors that prevent excessive sprouting of regenerating sinusoidal vessels. For example, mature megakaryocytes produce TSP-1, which decelerates angiogenesis and shuts off the production of activating angiocrine factors to restore homeostasis[4, 60]. Notably, AKT-activated bone marrow ECs, which emulate some of the functions of in vivo angiogenic ECs, expand long-term repopulating haematopoietic stem cells under serum-free culture conditions, whereas bone-marrow-derived stromal cells direct stem-cell attrition[44]. Moreover, protection of the haematopoietic microenvironment through transplantation of AKT-activated bone marrow ECs, but not mesenchymal ones, accelerates haematopoietic recovery after lethal irradiation[44]. Therefore, the equilibrium between AKT and MAPK activation regulates multi-lineage haematopoietic recovery.

The contribution of the endothelial niche to steady-state haematopoiesis was unravelled by studies in which selective deletion in ECs of SDF-1, Kit ligand or Jagged-1 impaired the maintenance of HSPCs[44, 61, 62, 63, 64]. Several studies have also scrutinized the relative contribution of bone marrow perivascular cells to the homeostasis of HSPCs[65, 66, 67]. Because the functions and structural stability of endothelial and non-vascular cells is mutually dependent, the deletion of factors in one niche has the potential to perturb the constituents of the neighbouring one. Therefore, genetic manipulations within the intimately associated endothelial niche and accompanying perivascular cells could have off-target effects, which must be taken into consideration. Nonetheless, the findings of these in vivo and reductionist in vitro studies suggest that, irrespective of the localization of HSPCs, angiocrine factors that are presented by either arteriolar or sinusoidal endothelial niches have executive functions and serve as 'rheostats' that choreograph haematopoietic stem-cell self-

renewal and differentiation during homeostasis and recovery after haematopoietic suppression. Furthermore, these studies demonstrate that some, but not all, heterotypic ECs can support HSPC expansion, which confirms that each organotypic vascular bed is endowed with unique angiocrine attributes that are suitable for stem-cell homeostasis and reconstitution[4, 44, 46, 47].

During fetal development, inductive signals from ECs[68] specify the development of haemogenic ECs[69, 70]. Thus, an endothelial niche could induce the direct conversion of all ECs into haemogenic ones, which give rise to definitive haematopoietic stem cells. Notably, endothelial niche-derived angiocrine signals are essential for the direct conversion of adult ECs into haematopoietic cells. In this approach, adult ECs were transduced with the transcription factors FosB, Gfi1, Runx1 and Spi1 (collectively termed FGRS). However, FGRS-transduced ECs failed to convert to engraftable haematopoietic cells unless they were co-cultured in direct contact with ECs[71]. Moreover, co-culture of haematopoietic cells that were derived from mouse and non-human primate pluripotent stem cells and an endothelial niche enhanced the engraftment of putative haematopoietic cells, in part through the deployment of Notch ligands[72, 73]. Thus, angiocrine signals from ECs participate in the specification, development, homeostasis, self-renewal and differentiation of haematopoietic stem cells.

Angiogenesis and osteogenesis

The skeletal system is constantly being remodelled, yet the ratio of skeletal mass to haematopoietic volume remains constant. Although penetrating bone marrow arteries and sinusoidal vessels provide instructive signals for maintenance of the haematopoietic mass, specialized type H and type L bone capillaries modulate osteogenesis without compromising haematopoiesis[74, 75, 76]. Type H vessels that are positive for CD31 and Endomucin regulate osteoblasts and the proliferation of chondrocytes[74, 75]. Type L vessels are an extension of type H vessels, and form sinusoidal vessels within the haematopoietic bone cavity. The angiocrine production of Noggin modulates skeletal patterning and ossification (Table 1). Notably, the expression of Noggin is downregulated by Notch signalling, which suggests that crosstalk between osteogenesis and haematopoiesis is conferred through the angiocrine expression of Jagged-1 (ref. 59). Hence, the balanced production of stimulatory and inhibitory angiocrine factors negotiates the allocation of space to bone and haematopoietic compartments.

Regeneration and fibrosis in the liver

Liver sinusoidal ECs distribute the intrahepatic blood flow between the hepatic artery, the hepatic vein and the portal vein[77]. The tightly regulated hepatic blood flow requires these cells to perform as an adaptable plexus network. Notably, non-lymphatic liver sinusoidal ECs form fenestrated ECs that lack a basement membrane and are negative for CD34, positive for VEGFR-1, VEGFR-2, VEGFR-3, VE-cadherin and the chemokine receptor CXCR7, have low levels or a lack of CD31 and express factor VIII. They also express stabilin-2, CD32b, CD209L and lymphatic vessel endothelial hyaluronic acid receptor 1

(Lyve1) (refs 78–81). A combination of these markers has been used to isolate, characterize and cultivate non-lymphatic liver sinusoidal ECs81.

Under homeostatic conditions, angiocrine signals modulate the expansion of hepatocytes by enabling the proliferation of diploid Axin2- and T-box transcription factor 3 (Tbx3)-positive cells that repopulate the liver[82]. These cells are located next to ECs in the central vein of the liver. Angiocrine production of Wnt2 and Wnt9b from these specialized ECs maintains Axin2 and Tbx3 double-positive hepatocytes that ultimately give rise to distal non-pericentral hepatocytes (Fig. 3a, Table 1). Deletion in ECs from adult mice of the gene Wntless, which encodes a specific transporter for Wnt-ligand secretion, depletes hepatic repopulating cells. Furthermore, specific angiocrine expression of the Wnt agonist Rspondin3 by ECs of the central vein of the liver — but not portal-vein ECs — establishes a β-catenin-dependent metabolic zonation[83]. Therefore, the repopulating potential and metabolic zonation of hepatocytes are established by extrinsic angiocrine signals that are derived from an 'inductive' central-vein-of-liver endothelial niche.

The angiocrine contribution of liver sinusoidal ECs to liver regeneration can be studied through the surgical resection of up to 70% of the liver's mass, known as a partial hepatectomy, which induces regeneration throughout the remaining liver. During the initial angiogenesis-independent inductive phase, which occurs 1–4 days after partial hepatectomy, VEGFR-2–AKT-dependent upregulation of transcription factor Id1 in non-proliferating liver sinusoidal ECs stimulates the expression of Wnt2 and hepatocyte growth factor (HGF) (Fig. 3a). Activation of VEGFR-1 on non-angiogenic liver sinusoidal ECs also induces the production of HGF and other pro-hepatic factors, such as heparin-binding EGF (HB-EGF), TGF-α and connective tissue growth factor (CTGF), to drive liver regeneration[79]. On days 4–12 after partial hepatectomy, liver sinusoidal ECs promote proliferative angiogenesis to meet the metabolic demands of the enlarging liver (Fig. 3a). Putative bone-marrow-derived ECs that co-express CD133, CD45 and CD31 and have the capacity to produce HGF can also engraft into populations of regenerating liver sinusoidal ECs, which helps to boost regeneration of the liver[84].

Liver regeneration has a programmed set point to ensure restoration of the original hepatic mass that is modulated by liver sinusoidal ECs in an angiocrine-dependent manner[85]. During the inductive phase of liver regeneration, Angiopoietin-2 is downregulated in liver sinusoidal ECs, which diminishes the expression of the inhibitory factor TGF-β1. In the angiogenic phase of liver regeneration (4–8 days after partial hepatectomy), Angiopoietin-2 expression returns to normal levels to facilitate VEGFR-2-dependent angiogenesis (Fig. 3a). Liver regeneration is delayed in adult mice in which Angiopoietin-2–Tie2 signalling is disrupted, which underscores the angiocrine function of Angiopoietin-2 in hepatic regeneration (Table 1).

Liver sinusoidal ECs are capable of either guiding the regeneration of the liver or engendering its pathological recovery by fibrosis, depending on differentially activated signalling pathways. After an acute insult to the liver, upregulation and activation of the SDF-1 receptor CXCR7 induces the angiocrine factors apelin and follistatin-like-1, which promote fibrosis-free repair[86] (Fig. 3a, Table 1). By contrast, chronic injury to the liver by

repeated injection of carbon tetrachloride or bile-duct ligation leads to the upregulation of another SDF-1 receptor, CXCR4, and the suppression of CXCR7, which shifts the balance to angiocrine secretion of pro-fibrotic TGF-β1 and BMP2, and leads to scarring. Thus, liver sinusoidal ECs are a cellular node that governs the regeneration, homeostasis and pathology of the liver.

Regeneration of the lung epithelium

The pulmonary circulation has an expansive capillary surface area that is covered by a thin layer of ECs. A delicate alveolar–capillary membrane is formed by the juxtaposition of these cells and alveolar epithelial cells to mediate gas exchange[87]. This close association facilitates cellular crosstalk to modulate diverse pulmonary physiological processes. Using purification methods to eliminate lymphatic ECs (Box 2), pulmonary capillary ECs are identified by their expression of CD31, CD34, FGF receptor (FGFR)-1, VEGFR-1, and VEGFR-2, and their lack of CD45 (ref. 88). The contribution of pulmonary capillary ECs to lung regeneration can be demonstrated by removal of the left lung in mammals. Known as a pneumonectomy, the procedure leads to a compensatory growth of lung mass that is driven by the expansion of alveolar epithelial stem and progenitor cells, which include alveolar type (AT)2 epithelial cells[89]. After the pneumonectomy, the propagation of AT2 cells is elicited by MMP-14, which is induced in pulmonary capillary ECs[88]. MMP-14 activates the EGF receptor (EGFR) on alveolar epithelial progenitor cells through its unmasking of the cryptic EGF-like motif in HB-EGF and the γ2 chain of Laminin-5, and the proliferation of these cells achieves neo-alveologenesis (Fig. 3b, Table 1). Selective conditional deletion of FGFR-1 or VEGFR-2 from adult ECs impairs regeneration of the lung[88] (Table 1). Following the pneumonectomy, recruitment of platelets and delivery of SDF-1 to its receptors on pulmonary capillary ECs could also lead to the upregulation of MMP-14 in ECs[90]. And selective conditional deletion of MMP-14 in adult ECs abrogates regeneration of the alveolar epithelium with negligible impact on vascular perfusion (Table 1). The progenitors of airway basal cells[91] could also proliferate in response to angiocrine MMP-14 induction. FGF-2 and FGF-5 derived from cultured human basal cells stimulate the FGFR-1-dependent production of MMP-14 by pulmonary capillary ECs, which in turn supports the expansion and differentiation of basal cells[92]. Therefore, selective angiocrine upregulation of MMP-14 in pulmonary capillary ECs ignites the propagation of alveolar epithelial cells and basal cell progenitors.

Angiocrine signals also modulate the fate of other types of lung progenitor cell. BMP4 binds the BMP receptor BMPR-1 on ECs, which leads to NFATc1 transcriptional activation of TSP-1 and the differentiation of lung epithelial stem and progenitor cells[93] (Fig. 3b, Table 1). In 3D-homotypic co-cultures, angiocrine-derived TSP-1 stimulates the differentiation of lung epithelial progenitor cells, and in *Tsp1*-deficient mice, the differentiation of progenitor cells into AT1 and AT2 cells is impaired. Collectively, these studies demonstrate that the activation of VEGFR-2, FGFR-1 and BMPR-1 on pulmonary capillary ECs instructs neo-alveologenesis to restore gas-exchange function in the regenerating lungs, which sets the stage for the treatment of emphysema-like disorders with EC transplantation or angiocrine factors[94].

The modulation of metabolism

Molecular interactions between ECs and islet cells regulate pancreatic function. During the recuperation of islet cells from chemical injury, angiocrine signals drive regeneration of the pancreas and the resolution of diabetes[95, 96]. An angiocrine supply of BMP2 and BMP4 is crucial for islet-cell homeostasis[96]. Notably, the deposition of Laminin-α4 chain by ECs stimulates insulin production by islet cells[97]. Co-culture of islet cells with pancreatic ECs improves the survival of transplanted islet cells[98]. Angiocrine delivery of TSP-1, through the modulation of TGF-β1, promotes islet-cell proliferation[99]. Angiocrine signals from ECs also guide the stage-specific differentiation of pluripotent stem cells into islet cells. For example, EC-derived EGF-like 7 (EGFL7) specifies the fate and proliferation of pancreas and duodenum homeobox (Pdx)1-positive islet-cell progenitors[100]. Therefore, identification of the phenotypic and functional attributes of islet-specific ECs could unravel the angiocrine heterogeneity within pancreatic tissues and lead to new therapeutic strategies for islet-cell production.

Angiogenesis and transdifferentiation of ECs to adipogenic cells modulate thermogenesis and the remodelling of adipose tissue[2, 101]. Remarkably, ECs can also adjust metabolism in an angiogenesis-independent manner by deploying certain angiocrine factors. Adipose tissue regulates its mass by monitoring the differentiation of adipocyte stem cells into mature adipocytes. For example, in peroxisome proliferator-activated receptor-γ reporter mice, adipose stem cells were shown to embrace the white-fat vasculature, but not the vasculature of other tissues[102]. During adipogenesis, angiocrine signals such as TNF, IL-6, insulin growth factor (IGF)1 and IGFBPs modulate the expansion and differentiation of adipocyte stem cells. In turn, adipocytes produce angiogenic factors that fine-tune the induction of angiocrine factors. Deciphering the molecular details involved in the crosstalk between ECs and adipocyte stem cells has potential for the treatment of obesity, diabetes and metabolic syndromes.

Myogenic homeostasis

The idea that an endothelial niche modulates myogenesis stems from studies in which ECs were shown to facilitate the formation of striated muscle that was derived from pluripotent cells[103]. Moreover, satellite striated-muscle precursor cells that express Pax7 and Myf5 are positioned in close proximity to ECs[104]. Co-culture studies showed that ECs in heterotypic cultures support cycling of these satellite cells through the angiocrine production of IGF1, HGF, FGF-2, homodimers of platelet-derived growth factor B-chains (PDGF-BB) and VEGF-A (Table 1). Thus, homeostasis and regeneration of striated-muscle cells could also be coordinated by angiocrine signals.

Likewise, cardiac myocytes are surrounded by the unique capillary and specialized ECs that comprise the endocardium. Cardiac contractility and survival are controlled by EC-derived angiocrine factors, which include Neuregulin-1, endothelin-1 and nitric oxide[105]. Neuregulin-1 is induced and released in response to hypoxic stress by the microvascular endocardial and subendocardial coronary arteries[106] (Table 1). Notably, deletion in adult ECs of the gene that encodes Neuregulin-1 decreases angiocrine-mediated protection of the

ischaemic myocardium. Thus, after ischaemic injury, angiocrine factors execute regenerative programs that evoke cardiac repair and sustain myogenic-cell contractility.

Angiocrine functions as morphogens

Certain angiocrine factors, produced mainly by angiogenic ECs, consist of morphogens and chemokines that provide signals for proper tissue patterning and direct repopulating cells to their predetermined anatomical destinations[107]. During lung development, sprouting capillary ECs guide the patterning of alveolar epithelial cells[108]. Similarly, after hepatotoxin-induced liver injury, the remaining liver sinusoidal ECs provide the cellular roadmap that steers the proper assembly of the hepatic architecture[109].

The size and activation state of the endothelial niche could also dictate the stem-cell mass and the extent to which an organ achieves its final volume and shape. Injury to ECs can decrease the surface area of the endothelial niche and deplete the stem-cell pool. This raises the possibility that an obligatory expansion in the mass of stem cells demands an equivalent increase in the surface area of the endothelial niche. Indeed, overexpression of VEGF-A in the V-SVZ of the brain increases the size of the endothelial niche, which augmentes neurogenesis[17, 110]. Whether the size of the endothelial niche in other organs is the main rheostat by which the mass of stem cells is set is an intriguing question that deserves further scrutiny.

Molecular determinants of angiocrine heterogeneity

In response to as yet unknown signals, organotypic ECs acquire unique structural features, such as fenestrations, and are induced to release specific sets of angiocrine factors[4, 111]. Notably, their response to various systemic endocrine factors, such as progesterone, is restricted to specific vascular boundaries — namely the uterine vascular bed[112]. The mechanisms through which ECs acquire and maintain tissue-specific attributes could be regulated by extrinsic biophysical cues as well as by the expression of intrinsic cues that include certain transcription factors.

Specific groups of transcription factors, such as those that are encoded by the homeobox, or Hox, genes, are expressed in organotypic ECs[4]. The differential expression of Hox genes provides positional identities to vascular zones at defined anatomic sites. In mice, Hoxa3 and Hoxc11, which are associated with anterior and posterior domains, respectively, are differentially expressed in ECs along the body axis[113]. Hox gene products might also contribute to organotypic angiocrine expression. For example, Hoxa9 triggers the expression of MMP-14 and EphrinB4, whereas Hoxb5, Hoxb3 and Hoxd3 control the positional expression of EphrinA1, VEGFR-2 and type I collagen[114]. It is plausible that extravascular cues, such as the biophysical forces that are imparted by shear stress and matrix elasticity, as well as crosstalk between parenchymal cells and ECs, enforce the expression of tissue-specific transcription factors in microvascular ECs and determine angiocrine heterogeneity.

Future directions

The emerging idea that organ homeostasis and regeneration are directed by tissue-specific microvascular ECs that function as instructive endothelial niches could be groundbreaking. This is because it focuses future work on what was conceived to be only a passive 'plumbing' system. These observations set the stage for capitalizing on the potential of specialized homotypic ECs as therapeutic drivers that can shepherd the regeneration of functional organs. However, the angiocrine profile of different organ-specific ECs is widely divergent, and a specific angiocrine profile must be established to elicit the desired effect on the local stem-cell population. Notably, the angiogenic and inflammatory states of ECs also play an important part in directing the proper production of angiocrine factors. Moreover, activation of signalling pathways other than AKT and MAPK — specifically, the Jak–Stat pathway — could switch on the expression of unique angiocrine factors. Therefore, to translate the potential of homotypic ECs to the clinical setting, the mechanism by which generic heterotypic ECs adopt tissue-specific angiocrine functions needs to be elucidated. The development of serum-free and xenobiotic-free co-culture platforms, such as E4ORF1^{+} ECs (Box 2), enables researchers to molecularly eavesdrop on the crosstalk between non-lymphatic homotypic or heterotypic ECs and tissue-repopulating stem cells. This could uncover the pathways that regulate the expression of tissue-specific angiocrine factors that are transcriptionally and biophysically induced.

In each organ, ECs perform the role of professional niche cells that elaborate a panoply of distinct sets of angiocrine factors that are distributed and delivered with precision to stem cells. Notably, ECs prevent stem cells from becoming exhausted or undergoing excessive self-renewal that could provoke the emergence of detrimental mutations. Our understanding of how stem cells integrate and interpret these myriad endothelial-derived signals to perform their organotypic functions is poor. Nonetheless, published data indicate that the coordinated presentation of a mixture of these angiocrine signals constitutes the long-sought holy grail of stem-cell self-renewal. Notably, the oscillatory release of inhibitory and stimulatory angiocrine factors might be crucial for the safe physiological self-renewal of engraftable stem cells, a process that is unlikely to be replicated by current approaches, in which stem cells are randomly pulsed with a cocktail of growth factors or small molecules. Thus, it is possible to translate the therapeutic potential of ECs for reconstituting pools of stem cells by recreating the appropriate endothelial niche, possibly through the transplantation of organotypic ECs.

The feasibility of this approach has been demonstrated by the intrajugular transplantation of pulmonary capillary ECs into the regenerating alveoli of mice. By supplying the proper doses of angiocrine factors, these engrafted cells drive repopulation of epithelial cells and improve respiratory function[88, 90]. Infusion of human cord blood-derived ECs into neonatal mice with hyperoxia-induced alveolar injury also enhances the repair of alveoli[115]. The intravascular transplantation of liver sinusoidal ECs rescues fibrosis-free regeneration of the liver in Id1-deficient mice, which are refractory to liver regeneration[81]. In mice, transplantation of ECs also restores liver function after chemical injury[77, 84] and it corrects the phenotype of haemophilia A mice[116]. Administration of mouse brain capillary ECs[117, 118, 119], lung ECs[119], bone marrow ECs[44] or other heterotypic ECs[117, 118, 119, 120]

enhances haematopoietic recovery after irradiation. Remarkably, senescent pancreatic islet cells regain their function[121] when transplanted into young diabetic mice, which suggests that the infusion of young ECs could rejuvenate islet-cell function. Engineering clinical-grade tissue-specific or generic ECs[43, 122, 123] that are capable of engrafting and adapting to an injured microenvironment, as well as producing the proper stoichiometry of angiocrine factors, will augment tissue regeneration and provide guidance for morphogenesis without instigating fibrosis.

It is plausible that the homeostasis and regenerative potential of almost every organ in mammals are regulated by angiocrine factors. Indeed, angiocrine signals might regulate the regenerative potential of stem cells in the hair-follicle bulge, $Lgr5^+$ gastrointestinal repopulating cells, ovarian follicular cells, uterine syncytial cells, chondrocytes and tenocytes, as well as repopulating cells in endocrine organs and epithelial cells in the thymus and urogenital tissues. The development of technologies for isolating non-lymphatic capillary ECs (Box 2) from such sites will enable the angiocrine potential of ECs within these organs to be identified.

Although most of the studies described in this Review focused on angiocrine-derived growth factors and chemokines, the release of EC-derived exosomes and deposition of specific components of the extracellular matrix into the EC basal lamina, as well as the expression of adhesion molecules, such as E-selectin[124], could also modulate homeostatic and reparative processes. Two notable examples are the release of Laminin-α4 by pancreatic ECs, which induces the production of insulin by islet cells[125], and MMP-14 generated by lung ECs through unmasking of the cryptic EGF-like motif in HB-EGF that is embedded within the Laminin-5 γ2 chain[88]. Similarly, extracellular matrix components that are laid down by organotypic ECs could also modulate tissue homeostasis by refining the affinity of adhesion molecules or by exposing growth factors that are embedded in the extracellular matrix.

Aberrant production of angiocrine factors could constitute the underlying pathogenesis of various conditions, such as cardiovascular or cerebrovascular diseases and the ageing process. Indeed, it is not known whether senescent ECs develop dysfunction in angiocrine production. An important topic not addressed in this Review is the mechanism by which inflammatory and immune cells could alter the normal angiocrine profile of ECs and thereby influence organ repair and scarring. The isolation and molecular profiling of ECs from the tissues of people with various disorders, such as individuals who are experiencing the complications of age-related and inflammatory diseases, could reveal the contribution of perturbed angiocrine signalling to these disorders. Restoration of the angiocrine function of maladapted or senescent vasculature, possibly through the transplantation of engineered ECs, could set the stage for treatments and help to diminish the economic burden of such disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary of angiocrine factors

Ang	Angiopoietin
BDNF	Brain derived nerve growth factor
BMP2, BMP4	Bone morphogenic proteins
BTC	Betacellulin
CTGF	Connective tissue growth factor
DKK1, DKK3	Dickkopf WNT Signaling Pathway Inhibitor 1 and 3
Dhh	Desert hedgehog
EGFL7	Epidermal growth factor like-7
EFNB2	EphrinB2
FGF2	Fibroblast growth factor-2
GDF11	Growth differentiation factor-11
GDNF	Glial cell line-derived neurotrophic factor
HB-EGF	Heparin binding-epidermal growth factor
HGF	Hepatocyte growth factor
IGFBP	Insulin growth factor binding protein
Jag1	Jagged-1
IL1	Interleukin-1
IL6	Interleukin-6
KL	Kit-ligand

MMP14	Metalloproteinase 14
NRG	Neuregulin
NO	Nitric oxide
NT-3	Neurotrophin-3
PEDF	Pigment epithelium-derived factor
PGE2	Prostaglandin-E2
PlGF1	PlGF2, Placental growth factor-1 or 2
SDF1	Stromal-derived factor-1 (Cxcl12)
TSP1	Thrombospondin-1
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor
VEGFR1	Vascular endothelial growth factor receptor-1 (Flt1)
VEGFR2	Vascular endothelial growth factor receptor-2 (KDR, Flk1)
Wls	Wntless
Wnt2, Wnt9B	Wingless-type MMTV integration site family

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Box 1

Physiology of the angiocrine factors

The paracrine factors produced by ECs that maintain organ homeostasis, balance the self-renewal and differentiation of stem cells and orchestrate organ regeneration and tumour growth are known as angiocrine factors. The term 'angiocrine' was created to emphasize the biological significance of the instructive factors produced by the ECs that influence the homeostasis of healthy and malignant tissues[3]. Angiocrine factors comprise secreted and membrane-bound inhibitory and stimulatory growth factors, trophogens, chemokines, cytokines, extracellular matrix components, exosomes and other cellular products that are supplied by tissue-specific ECs to help regulate homeostatic and regenerative processes in a paracrine or juxtacrine manner. These factors also play a part in adaptive healing and fibrotic remodelling. Subsets of angiocrine factors can act as morphogens to determine the shape, architecture, size and patterning of regenerating organs. The angiocrine profile of each tissue-specific bed of ECs is different and reflects the diversity of cell types found adjacent to ECs in organs (Fig. 1a–e). Although subsets of angiocrine factors are produced constitutively, some angiogenic factors can modulate the production of other tissue-specific angiocrine factors. For example, VEGF-A induces the expression of defined angiocrine factors through interaction with VEGFR-1 and VEGFR-2 (Fig. 1e). Similarly, FGF-2 (through the activation of FGFR-1) and the angiopoietins (through their interaction with the receptor Tie2) drive the expression of unique clusters of angiocrine factors. TSP-1 functions in a complex manner and can act as an inhibitory angiogenic factor as well as directly influence the differentiation of stem and progenitor cells. The molecular programmes that govern the production of context-dependent angiocrine factors from organ-specific ECs remain undefined.

Box 2

In vitro endothelial niche platform

To propagate pure populations of organ-specific capillary ECs and to avoid their contamination with lymphatic ECs, monoclonal antibodies to EC-specific surface markers are infused intravenously into mice. The animals are killed after 10 minutes to avoid leakage of antibodies into the lymphatic vessels. Next, the intravitally labelled ECs are isolated through enzymatic digestion of tissues and subsequent flow sorting to ensure the removal of pericytes and smooth-muscle cells[4, 43]. Purified ECs are then plated with angiogenic factors in the absence of pituitary extracts and serum to avoid the loss of tissue-specific signatures and to block the transition from endothelial to mesenchymal cells. However, these conditions can support the expansion of angiocrine-competent ECs only in the short term. To remove this hurdle, purified ECs can be transduced with lentiviral vectors that express myristoylated AKT or the non-oncogenic adenoviral gene E4ORF1 (ref. 43). E4ORF1 sustains the survival of ECs through specific activation of the AKT–mTOR pathway. ECs that express E4ORF1 are not transformed and behave like primary ECs that ultimately undergo replicative senescence. This approach enables their long-term cultivation, with high viability in serum-free and xenobiotic-free conditions, which establishes an ex vivo platform for endothelial niches that recapitulates organotypic niches[4]. Monolayers of E4ORF1$^+$ ECs sustain their organotypic pro-stem-cell functions, making them superior to ECs that have been immortalized by simian virus 40 large T antigen or polyomavirus middle T antigen, which fail to expand stem cells[45]. Co-culture studies using E4ORF1$^+$ ECs have helped to identify angiocrine pathways that foster the expansion and proliferation of parenchymal and stem cells. So far, E4ORF1$^+$ ECs have not demonstrated any malignant potential and could be adapted to comply with regulatory clinical guidelines. Thus, these engineered ECs provide an ideal platform for interrogating the angiocrine function of ECs in models of organ regeneration and for translating the pro-regenerative therapeutic potential of these cells to the clinical setting.

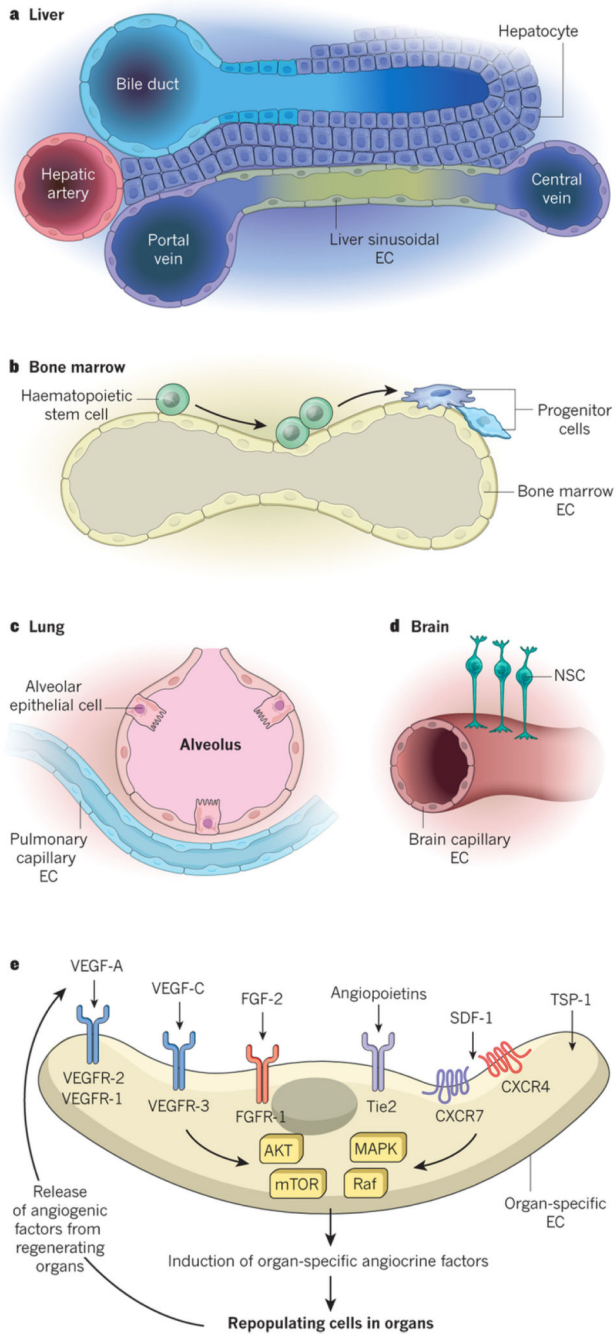

Figure 1. Cross-talk of specialized capillary ECs with organ-specific parenchymal cells and their corresponding stem cells modulate homeostatic and regenerative processes

Each organ is arborized by an extensive network of specialized capillaries **(A)**. Within each organ the capillaries assume unique structural, phenotypic, functional and angiocrine attributes. In the liver, hepatocytes are juxtaposed to fenestrated liver sinusoidal endothelial cells (LSECs) marked by the unique phenotype CD34-VEGFR1^{+}VEGFR2^{+}VEGFR3^{+}VEcad^{+}CXCR7^{+}CD31$^{low/}$-FactorVIII^{+} **(B)**. In the hematopoietic organs such as bone marrow, the stem and progenitor cells are in direct

cellular contact with arterial and fenestrated specialized sinusoidal vessels demarcated by $VEGFR3^{+}VEGFR2^{+}VEcad^{+}CD31^{+}$ ECs **(C)**. In the lungs, the alveolar epithelial cells and their progenitors reside in the vicinity of continuous nonfenestrated pulmonary capillary endothelial cells (PCECs) defined by the signature $VEGFR2^{+}FGFR1^{+}VEcad^{+}CD31^{+}$ CD45 ECs **(D)**. In the brain the majority of capillaries compose of tightly connected vessels with a common core phenotype of $VEGFR2^{+}VEcad^{+}CD133^{+}$ $thrombomodulin^{-/lo}w$ ECs **(E)**. At steady state conditions or during an angiogenic state upregulation of angiogenic factors, including VEGF-A through activation of its cognate tyrosine kinase receptors VEGFR2 and VEGFR1, FGF2 through FGFR1, angiopoietins through Tie2 not only modulate angiogenic processes, but also trigger or resolve the expression of tissue-specific angiocrine factors. Thrombospondins not only temper angiogenic response but also directly influence the proliferation and differentiation of the pancreatic islet and lung epithelial cells. Recruitment of pAkt-mTOR and MAPK/Raf signaling most likely play a role in choreographing the expression of the organotypic angiocrine factors **(E)**.

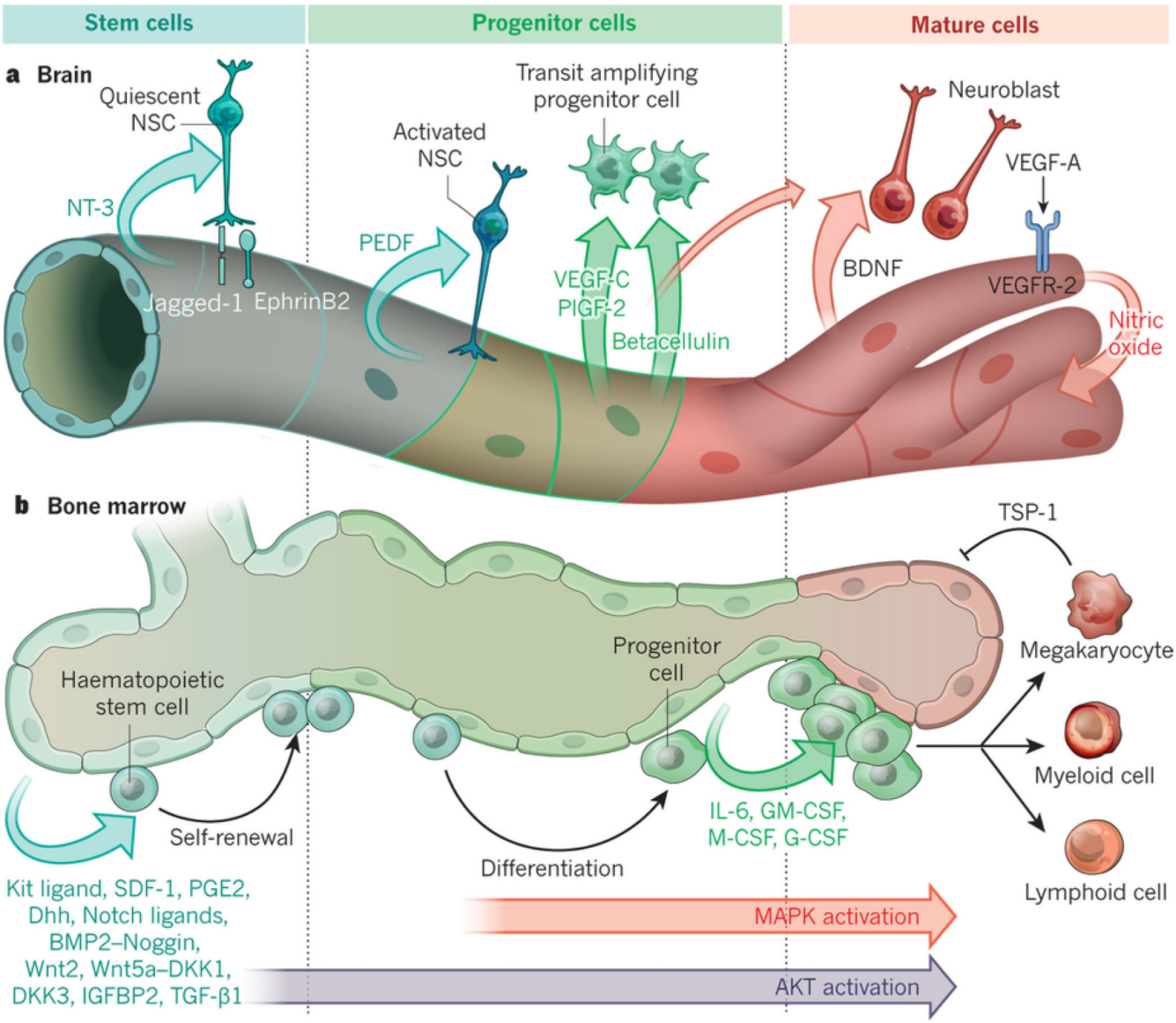

Figure 2. Tissue-specific ECs by supplying membrane-bound and secreted angiocrine factors orchestrate self-renewal and regeneration of stem and progenitors cells

(A) Brain capillary ECs are strategically localized to the neural stem and progenitors cells. These ECs elaborate specific angiocrine factors, including membrane-bound Jagged-1 and EphrinB2 and NT-3 that establish the quiescence and survival of the Type-B1 quiescent NSCs (qNSCs) within the V-SVZ. ECs also deploy angiocrine factors, including PEDF, VEGF-C (mostly in SGZ) and PLGF2 that foster proliferation of Type-B1 activated NSCs (aNSCs). Angiocrine secretion of Betacellulin stimulates the amplification of Type-C progenitors (TAC). During angiogenesis, upregulation of VEGF-A through activation of VEGFR2 and induction of NO upregulates BDNF, which in conjunction with Betacellulin encourage the differentiation into neuroblast and mature neurons, leading to completion of neurogenesis.

(B) Bone marrow arterial and sinusoidal ECs produce angiocrine factors that support the maintenance and regeneration of hematopoietic stem and progenitor cells (HSPCs)

following myeloablative insult. At steady state conditions low levels of AKT activation in ECs stimulate production of Kit-ligand, Cxcl12, Notch-ligands, IGFBP2, Wnts, BMP2, Dhh and BMP4, which maintain and promote the self-renewal of hematopoietic stem cells. After myeloablative stress, inflicted by chemotherapy or irradiation, co-activation of AKT and MAPK initiates expression of progenitor active angiocrine factors, including IL6, GM-CSF, G-CSF, M-CSF, Metalloproteinases, chemokines and other factors forcing balanced differentiation of stem cells into lineage-committed progenitors. EC-derived Notch-ligands, (i.e. Jagged-1) prevent exhaustion of HSPCs. Induction of thrombospondin1 (TSP1) expression by maturing megakaryocytes puts a brake on ongoing hemangiogenesis finalizing the regeneration process.

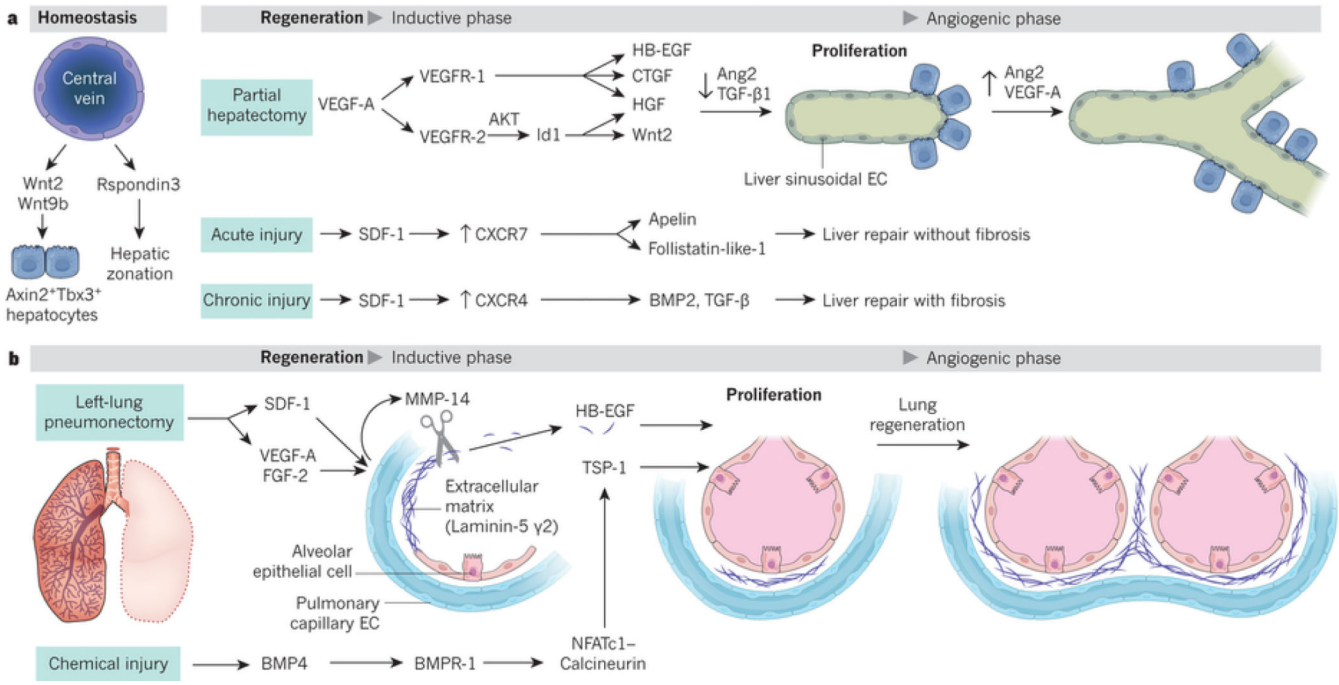

Figure 3. Tissue-specific ECs by supplying membrane-bound and secreted angiocrine factors support regeneration of alveolar epithelial cells and hepatocytes

(A) At steady state Wnt2 and Wnt9b produced by liver central vein ECs sustains liver mass by replenishing the $Axin2^{+}Tbx3^{+}$ hepatic stem cell pools. After 70% partial hepatectomy (PH), VEGF-A via AKT activation induces Id 1 in LSECs upregulating HGF and Wnt2. VEGF-A through activation of VEGFR1 upregulates HGF, HB-EGF and CTGF. These angiocrine factors stimulate hepatocyte proliferation without provoking angiogenesis, (inductive phase). Four days after PH, increase in liver initiates proliferative angiogenesis. Upon PH downregulation of Ang2 in LSECs and TGF-β accelerates hepatic recovery. During resolution phase of liver regeneration (days 4–8 post-PH), VEGF-A and restoration of Ang2 stimulates angiogenesis and finalize hepatic reconstitution. Activation of CXCR7 on LSECs triggers pro-regenerative and anti-fibrotic angiocrine factors, including Apelin, follistatin-1-like that facilitates fibrosis-free healing. Chronic injury by persistent CXCR4 activation and CXCR7 suppression stimulates TGF-β and BMP4 leading to fibrosis. Balance of CXCR4 and CXCR7 in LSECs negotiates liver regeneration and fibrosis.

(B) After removal of mice left lung (pneumonectomy, PNX), the PCECs in right lung express membrane-bound MMP14, which unmasks the cryptic EGF-receptor ligands from HB-EGF and Laminin5 γ-2. This inductive phase orchestrates the angiogenesis-independent compensatory alveolar epithelial regeneration. After chemical injury (i.e. bleomycin), BMP4 through engagement of its receptor Bmpr1 sets up NFATc1/Calcineurin-dependent transcription of TSP1. TSP1 facilitates differentiation of lung epithelial progenitors into functional epithelial cells. After PNX, upregulation of the VEGF-A, FGF2 and deposition of platelets on PCECs by production of SDF1 and CXCR4 activation induces MMP14 initiating alveolar regeneration. Increase in lung mass triggers angiogenic phase of lung regeneration.

Table 1

Heterogeneity of the angiocrine factors at steady state and during regeneration

EC origin	Repopulating cells	Regenerative Model EC Status	Angiocrine Factor(s)	EC-specific gene knock out Emergent Phenotype
Bone Marrow sinusoidal ECs	Hematopoietic stem and progenitor cells (HSPCs)	Steady State Chemotherapy, Irradiation Angiogenic ECs	Kit-ligand, SDF1, IGFBP2 DKK1, BMP2, BMP4, Dhh **AKT-activated** Kit-ligand, Jagged1, Jagged2, Dhh, FGF2, Angiopoietin2, EGF, Pleiotrophin **AKT-MAPK activated** IL6, G-CSF, GM-CSF, Ang2, Jagged1 and Jagged2	*Kit-ligand*[61]; *SDF1*[62]: Stem cell depletion *Jagged1*[59]: Impaired hematopoietic recovery and stem cell depletion *VEGFR2*[47]: Angiocrine dysregulation, Hematopoietic failure *VE-cadherin inhibition*[57]: Hematopoietic recovery impairment
Testicular capillaries	GPR125$^+$ germline stem cells	Chemical-induced sterilization	GDNF, Fgf2	
Brain Capillaries	Neural stem cells (NSCs) Transient amplifying cells Neuroblasts	Steady state Regeneration Angiogenesis	Jagged1, EphrinB2, NT-3 PEDF, Betacellulin, VEGF-C Nitric Oxide, BDNF	*Jagged1*[19], *Ephrinb2*[19]: Exhaustion of neural stem cells *NT-3*[20]: Decrease in NSC cycling
Liver sinusoids Liver central vein ECs	Hepatocytes and stellate cells Axin2$^+$Tbx3$^+$ liver stem cells	70% partial hepatectomy Bile duct ligation Acute and chronic CCl4 Homeostatic conditions	Id1-Wnt2, Id1-HGF, Angiopoietin-2 Anti-Fibrosis: Apelin, Follistatin-like-1, Noggin Pro-fibrosis: TGF-β, BMP4 Wnt2 and Wnt9b	*Tie2, Angiopoietin-2*[85]: Shift in liver recovery set-point *Id1*[81]: Impaired liver regeneration *Cxcr4*[86]: Decrease in liver fibrotic healing *Cxcr7*[86]: Increase in pro-fibrotic healing *Wntless (Wls)*[82]: Defective liver regeneration and self-renewal
Pulmonary capillaries	Lung alveolar epithelial cells Type 2 AECs Epithelial progenitors cells	Left lung Pneumonectomy Bleomycin	MMP14-mediated EGF-Receptor ligand release BMP4-NFAT/c-Thrombospondin1	*MMP14*[90]: Abrogated neo-alveologenesis but intact capillary vasculature *Vegfr2*[88], *Fgfr1*[88] Impaired alveolar regeneration *Thrombospondin1*[93]: Impaired epithelial regeneration
Pancreatic capillaries	Islet cells Pdx1$^+$ islet progenitors	Streptozocin Pluripotent stem cell derivatives	BMP4, BMP2, Thrombospondin1 EGFL7	*EGFL7*[100]: *Delayed Islet cell differentiation*
White fat vessels	Adipose stem cells	Steady state	IGF1, IGFBP, TNF, IL1	
Striated muscle microvessels	Pax7$^+$Myf5$^+$ Satellite cells	Steady state and post-myotoxin	IGF, HGF. FGF, PDGF-BB	
Cardiac myocytes capillaries	Myocytes	Coronary artery ligation	Neuregulin-1, Endothelin-1, Nitric oxide	*Neuregulin-1*[106]: Impaired myocyte regeneration, Loss of cardiac protection
Osteogenesis Type-H and L vessels	Osteoblasts	Steady state	Noggin, BMPs, Jagged-1	*Noggin*[74]: Impaired osteogenesis