REGENERATION

Endothelial cell adaptation in regeneration

Tissue-specific endothelial cells maintain organ homeostasis and instruct regeneration

By **Jesus M. Gomez-Salinero** and **Shahin Rafii**

Endothelial cells (ECs) cover the inner wall of blood and lymph vasculature in normal and malignant tissues. It is widely appreciated that ECs are endowed with unique phenotypic, structural, functional, and angiocrine secretory attributes, generating specialized vascular subpopulations with organotypic and diseased-tissue signatures (*1, 2*). To achieve this high level of organ and tumor heterogeneity, ECs have acquired malleable cellular features that allow them to adapt to normal physiological stressors and to promote tissue homeostasis and regeneration. This is exemplified during liver regeneration in which defined angiocrine (meaning EC-derived) signals from liver sinusoidal ECs initiate and resolve liver regeneration through paracrine signaling to hepatocytes. By contrast, stressed and irritated ECs maladapt to a pathological microenvironment, such as inflamed or chronically injured tissues, favoring fibrosis and tumorigenesis. Thus, EC adaptive functions have beneficial or detrimental effects in organ physiology. Understanding the molecular determinants of EC adaptability could reveal therapeutic targets to facilitate wound healing without fibrosis, combat tumorigenesis, or develop efficacious strategies for organ regeneration, long-term engraftment of bioartificial organs, and tissue transplantation.

Endothelial cells manifest at least two modes of plasticity: cellular and functional. Cellular plasticity encompasses their capacity to generate different EC types (in arteries, veins, or capillaries) and even nonvascular cell types, including hematopoietic cells and parenchymal cells (*3*). During development and in adulthood, this plasticity is regulated in part by the induction of organotypic transcription factors (TFs) that instruct ECs to transition into defined cellular states. Discovery of the molecular pathways that mastermind the cellular plasticity of ECs has paved the way for designing strategies to generate adult hematopoietic stem cells in vitro. For example, identification of four TFs, collectively termed FGRS TFs, has enabled in vitro reprogramming of adult human and mouse ECs by transducing them with FGRS TFs into engraftable hematopoietic stem cells for the treatment of blood disorders (*4*). Identifying other pioneer TFs that direct conversion of ECs to nonvascular cell types will advance the field of direct reprogramming for therapeutic organ regeneration.

Functional plasticity refers to the capacity of ECs to tailor and adapt cellular processes to completely different tissue-specific chores, such as sustaining the blood-brain barrier or adjusting the dynamic filtration function of liver sinusoids and kidney glomeruli. The tissue-specific specialization of ECs orchestrates organogenesis during development, stem cell homeostasis, and regeneration throughout adulthood. The precise mechanism by which ECs acquire this level of heterogeneity is unknown and could be mediated by as yet unrecognized intrinsic genetic and epigenetic regulators governed by signals relayed from the extrinsic microenvironment. These signals emanate from the immediate surrounding microenvironment, including nonvascular cells, extracellular matrix, metabolic signals, and biomechanical forces. Chronic stressors, such as inflammatory and injury signals, elicit an aberrant vascular response, setting the stage for fibrosis, organ dysfunction, and tumorigenesis (*3*).

On the contrary, during regeneration, angiocrine cues supplied from activated ECs in response to injury of surrounding tissue orchestrate scar-free regeneration. For example, release of angiogenic factors by neighboring parenchymal cells induces liver ECs to deploy angiocrine factors, such as angiopoietin-2 (ANG2), R-spondin-3 (RSPO3), and WNT9B, that sustain hepatic homeostasis; or angiocrine secretion of hepatocyte growth factor (HGF) and WNT2 from liver ECs that mediate mouse liver regeneration after partial hepatectomy (*1, 5*). In response to injury, lung epithelial cells produce vascular endothelial growth factor A (VEGF-A) and fibroblast growth factors (FGFs) that activate lung ECs to supply matrix metalloprotease–14 (MMP14) and bone morphogenetic protein 4 (BMP4) and increase the bioavailability of epidermal growth factor (EGF) ligands and thrombospondins, thereby igniting lung epithelial regeneration (*1, 6*) (see the figure). Similarly, tissue-specific ECs supply defined angiocrine factors that contribute to organogenesis and metabolic homeostasis of other organs, including pancreas, myocardium, central nervous system, and even reproductive organs, including testes and ovaries (*1*). Notably, at steady state and during organ regeneration, microenvironmental cues program ECs to establish a nurturing vascular niche to choreograph self-renewal and differentiation of tissue-specific stem cells, including hematopoietic, spermatogonial, and neural repopulating cells (*1, 7*). Similarly, excessive release of tumor vascular niche–derived angiocrine factors supports the emergence of tumor-initiating cells (which have stem cell–like properties) that promote chemoresistance (*8*).

During organ repair, aberrant signals might corrupt the regenerative functions of ECs, leading to fibrosis and tumorigenesis. Persistent stress-induced stimulation of ECs could alter the organ-specific function of ECs, favoring scarring through a maladaptation process, including endothelial-to-mesenchymal transition (*3*), whereby ECs acquire fibroblastic features. An association between several chronic diseases and deregulated endothelium has recently been established, for instance, in dementia or cardiovascular diseases (*9, 10*). Mechanistically, differential signaling pathway activation in ECs can favor fibrosis over regeneration. For example, in the liver, differential activation of the C-X-C chemokine ligand 12 (CXCL12) cognate receptors, with CXCR7 predominantly expressed over CXCR4, on ECs induces expression of angiocrine factors that promote healing without fibrosis (*7*). By contrast, chronic inflammation caused by the ligation of biliary ducts results in dominant CXCR4 activation over CXCR7, which promotes healing with profibrotic changes. These dichotomous responses of liver ECs indicate that differential expression and activation of specific inflammatory receptors on ECs could relay external cues in favor of regeneration instead of fibrosis and vice versa.

Stress-induced responses of ECs also play important roles in promoting cancer at the expense of regeneration. The malleability of ECs forces them to participate in two-way cross-talk with their disrupted microenvironment, inducing the release of abnormal angiocrine factors and thereby setting the stage for tumorigenesis and tumor growth. For example, the excessive and dysregulated release of FGF4 by tumor cells triggers the expression of the E26 transformation-specific TF ETS2

Division of Regenerative Medicine, Ansary Stem Cell Institute, Department of Medicine, Weill Cornell Medicine, New York, NY, USA. Email: srafii@med.cornell.edu

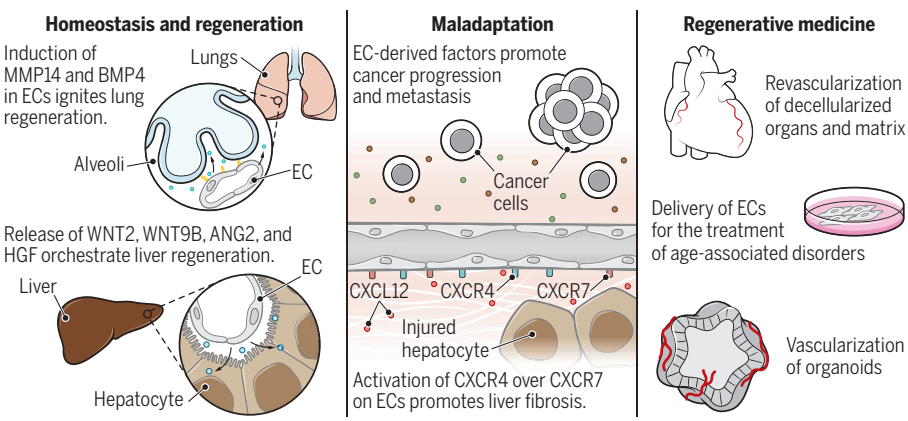
and the cell surface receptor Jagged-1 in the tumor endothelium. This maladaptive EC response activates the angiocrine release from ECs of protumorigenic insulin-like growth factor 1 (IGF1) and decreases expression of antitumorigenic IGF binding protein IGFBP7, reinforcing aggressive and chemoresistant tumor growth (*8*). Moreover, ECs in distant, noncancerous organs can respond to the signals supplied by tumor cells, thereby facilitating metastasis through the activation of Notch and the expression of vascular cell adhesion molecule 1 (VCAM1) on the distal endothelium (*11*). These tumor-associated changes in the endothelium favor the persistence of invasiveness and treatment-refractory cancer stem cells. Therefore, tumor EC–derived angiocrine factors or their triggers are potential druggable anticancer targets.

set the stage for the treatment of aging-associated maladies.

Uncovering the molecular determinants of EC adaptability could enable deconvolution of the intricate pathways that drive adult organ repair that has defied developmental biologists for decades. For example, although development of in vitro techniques, such as tissue-specific organoids and organ-on-a-chip models, have enhanced our understanding of tissue morphogenesis, these models do not fully explain the mechanism for the resistance of certain tissues such as lung, heart, intestines, and kidneys to self-repair. The development of vascularized organoids will not only allow deciphering of the pathways that choreograph tissue repair, but also enable manufacturing of mini-organs for regenerative medicine and facilitate their

Endothelial cells in organ regeneration

Endothelial cells (ECs) are key mediators in the regulation of organ physiology and malignant cell pathophysiology. They form vascular conduits and also supply angiocrine factors (such as WNTs, HGF, and MMPs) that regulate organ homeostasis. Under stress conditions, ECs can maladapt, supplying factors that promote the development of fibrosis, cancer, and other maladies. The use of tissue-specific ECs will be essential to choreograph functional organ regeneration and repair.



The intrinsic malleability of ECs makes them ideal for regenerative medicine, as does their localization in vessel walls, allowing easy access to infused drugs, and their expression of organ-specific markers allows the development of strategies to modulate the function of specific EC types. Furthermore, EC regeneration has potential for the treatment of aged organs. For example, oxidative stress leads to attrition of ECs, which increasingly occurs with age, altering hematopoietic stem cell activity and perturbing lineage differentiation. Notably, intravenous transplantation of ECs from young mice can revert this phenotype in aged mice (*12, 13*). Moreover, epigenetic manipulation of human blood-circulating EC progenitors transiently augments vasculogenesis after ischemia (*14*). Rejuvenation of the senescent EC state, by the infusion of specific as yet unrecognized cytokines or the infusion of young ECs, could long-term in vivo engraftment. Additionally, vascularized tumoroids (tumor organoids) could be developed to evaluate the influence of different drugs in the emergence of cancer during organ regeneration processes, as ECs have a primary role in tumor development and expansion (*8*). Clearly, innovating techniques to generate adaptable autologous ECs that can arborize organoids may lead to transformative therapeutic paths in the near future. However, engineering of long-lived, responsive, and malleable organotypic ECs from, preferably, patient ECs is necessary and a main challenge to overcome.

Decellularized human organ scaffolds are another area of active regenerative medicine investigation to enable organ replacements. This approach has been hampered by the lack of proper seeding of adaptable ECs within the narrow confines of decellularized capillaries. This is a major problem because placement of current decellularized scaffolds in patients often results in poor functional recovery due to poor vascularization. Populating the decellularized matrix with durable and adaptable ECs will enhance the life span and improve the survival of these potentially lifesaving implants (*15*).

Translation of these concepts to the clinical setting poses a herculean task and is compounded by two major obstacles: social and scientific. From a social standpoint, one of the main obstacles to overcome is the rampant proliferation of pseudoscientific clinics and stem cell tourism that promise false hope to desperate patients (*16*). Their unsupervised approach without regulatory oversight could adversely affect the health of patients because nonvalidated treatments may delay the approval of evidence-based treatments. From a scientific perspective, capitalizing on the regenerative and rejuvenating functions of ECs by employing tissue-specific TFs, the conversion of autologous adult ECs into organotypic ECs, and vascularizing organ models in vitro so that they reach human size will lead to innovative therapeutic strategies. For example, these approaches could open the door to the development of artificial organs for transplantation, as well as the manufacture of human organs in donor animals. Importantly, one of the main limitations yet to be circumvented is the development of nonimmunogenic organs.

Notwithstanding these caveats, translation to the regeneration field could be augmented by formulating standard procedures to incorporate vascular networks within implants that can sustain their long-term engraftment and provide the proper tissue-specific angiocrine signals to drive organ repair without scarring and tumorigenesis. Achievement of these goals will bring us closer to fulfilling the promise of regenerative medicine to treat millions of disabled patients worldwide who need organ replacement. ■

REFERENCES AND NOTES

1. S. Rafii *et al.*, *Nature* **529**, 316 (2016).
2. D. J. Nolan *et al.*, *Dev. Cell* **26**, 204 (2013).
3. E. Dejana *et al.*, *Nat. Commun.* **8**, 14361 (2017).
4. R. Lis *et al.*, *Nature* **545**, 439 (2017).
5. H. G. Augustin *et al.*, *Science* **357**, eaal2379 (2017).
6. J.-H. Lee *et al.*, *Cell* **156**, 440 (2014).
7. B.-S. Ding *et al.*, *Nature* **505**, 97 (2014).
8. Z. Cao *et al.*, *Cancer Cell* **31**, 110 (2017).
9. C. Iadecola, *Neuron* **80**, 844 (2013).
10. M. J. Gimbrone Jr. *et al.*, *Circ. Res.* **118**, 620 (2016).
11. E. Wieland *et al.*, *Cancer Cell* **31**, 355 (2017).
12. A. P. Kusumbe *et al.*, *Nature* **532**, 380 (2016).
13. M. G. Poulos *et al.*, *J. Clin. Invest.* **127**, 4163 (2017).
14. S. Fraineau *et al.*, *Stem Cell Rep.* **9**, 1573 (2017).
15. A. F. Pellegata *et al.*, *Front. Bioeng. Biotechnol.* **6**, 56 (2018).
16. D. Sipp *et al.*, *Nature* **561**, 455 (2018).

ACKNOWLEDGMENTS

S.R. is the founder of, and an unpaid consultant to, Angiocrine Bioscience, San Diego, California.

10.1126/science.aar4800

GRAPHIC: JOSHUA BIRD/*SCIENCE*

Endothelial cell adaptation in regeneration

Jesus M. Gomez-Salinero and Shahin Rafii

Science **362** (6419), 1116-1117.
DOI: 10.1126/science.aar4800