MYTH: ENDOTHELIUM - PASSIVE PLUMBING WITH POOR POTENTIAL? FINDING: BUSTED

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Endothelium was initially thought to be a modulated form of mesenchyme with function limited to establishing the inner lining of blood vessels ("keeping blood where it belongs"), preventing untoward intravascular thrombosis and participating in selective permeability in tissues. Over several decades the realization emerged that endothelium is a "legitimate" differentiated cell type performing many characteristic and essential functions during development and in adults. Strikingly, recent evidence reveals an unexpectedly diverse developmental plasticity and potential of endothelium in both physiologic and pathologic settings; differentiated endothelium can and does form not only cardiac valves and blood but cartilage, bone and perhaps other tissues as well. These insights suggest new opportunities for understanding the roles of vascular cells and tissues during normal and abnormal development and for discovering new vascular contributions to physiologic functions and disease. The results may lead to new vascular-based prognostic, diagnostic and therapeutic interventions in a variety of genetic and non-genetic diseases, some of which are not currently understood as vascular in origin.

Endothelium – not just for vessels anymore

Early dogma stated that endothelial cells could not be cultured *in vitro* (and therefore could not be studied) because, removed from their normal tissue context, they would inevitably revert to their "true" fibroblast-like identity. However, this hypothesis was contradicted by several demonstrations that a distinct endothelial phenotype could be maintained in cultured cells (1,2). In addition, functional differences (responses to inflammation, basal and induced permeability, patterns of gene expression, etc.) between endothelium resident in different tissues were documented. However, an underlying bias against a stable endothelial phenotype remained especially among scientists studying topics tangential to vascular biology. Experiments also frequently overlooked endothelial heterogeneity: differences between arterial and venous endothelium; unique properties of capillary, cardiac microvascular, and pulmonary microvascular endothelium; capillary and postcapillary venular endothelium, etc. In addition, although vascular biologists appreciated the anatomic and functional diversity of endothelium, they initially shared with their non-vascular colleagues the notion that endothelium represents a terminally differentiated state – "once an endothelial cell, always an endothelial cell" (unless, of course, it became a fibroblast). However, several recent lines of evidence establish a much more expansive view of the functional and developmental plasticity of endothelium.

Endothelium makes valves and (even) fibroblasts

An early challenge to the concept of endothelial terminal differentiation was the demonstration that the transition of endothelium to form mesenchymal tissue is an essential step during endocardial cushion formation and is thus required for the normal development of cardiac valves (3,4). During this process of so-called endothelial-to-mesenchymal transition (EndMT), certain endothelial cells sever their lateral cell-cell contacts, breach the vascular basement membrane, migrate into the surrounding mesenchyme, and assume a fibroblast-like pattern of gene expression and morphology. Similar processes regulated by TGFß, BMPs and the Notch pathway subsequently were shown to contribute to development of cardiac, pulmonary and renal fibroblasts and fibrosis, to produce carcinoma-associated fibroblasts and to modulate metastasis, and to occur in atherosclerosis and wound healing (reviewed in (5)). Because endothelium is a specialized form of epithelium, EndMT is also a specialized form of epithelial-to-mesenchymal transition (EMT). EMT involving non-endothelial cells plays important roles in several additional aspects of development and in wound healing, fibrosis and cancer metastasis (reviewed in (6)). The discovery of EndMT thus integrated endothelial development into a broader biologic context and established that endothelial differentiation to other cell types plays important roles in development and disease.

Endothelium makes blood

The developmental origins of endothelium and circulating peripheral blood cells have been debated and investigated for several decades. Early studies of the mouse yolk sac and chick blastoderm revealed cells apparently destined for the circulation that seemed to arise coincident with, and perhaps from, endothelium (7-10). Notwithstanding the definition and historical ebb-and-flow of the "hemangioblast controversy," there is now general agreement that a population of mesenchyme-derived cells gives rise to both endothelium and blood, and that hematopoietic stem cells arise from such tissue *via* a specialized intermediate progenitor cell type termed "hemogenic endothelium" (reviewed in (11) and elsewhere). This process has been studied in the embryonic dorsal aorta (in the aorta-gonad-mesonephros (AGM) region), yolk sac, vitelline and umbilical arteries, the placenta and using *in vitro* models of differentiation (12-18). Single hemogenic endothelial cells from both differentiated murine embryonic stem cells and from murine embryos can form hematopoietic stem cells when cultured *in vitro*. In addition, the transcription factor Runx1 must be expressed specifically in endothelium to allow hematopoietic stem cell formation from hemogenic endothelium *in vivo*. Fate tracing using lineage-restricted transgenes in mice also demonstrates that hematopoietic stem cells can originate from endothelium. Direct visualization of these processes in both zebrafish and murine embryos further supports the concept that hematopoietic stem cells derive directly from hemogenic endothelium (19-21). In zebrafish a population of such cells undergoes a stereotypical contraction and bending process that brings their neighbors into mutual contact and releases the initiating cells to migrate within the sub-aortic space and give rise to hematopoietic stem cells. The endothelial layer of the dorsal aorta remains intact throughout. A similar process of endothelial transition to extravascular blood island-like structures has also been demonstrated in mice (18). It is not clear precisely how these extravascular hematopoietic precursors in fish and mice seed their corresponding hematopoietic organs (fish appear to use a venous route) or whether the seeding mechanism is the same in both species. However, both intravascular and extravascular transition of hemogenic endothelium to hematopoietic stem cells may occur in both.

Endothelium makes cartilage and bone

Recent studies of a rare genetic disease suggest that the developmental plasticity of endothelium extends beyond mesenchyme, fibroblasts and blood. The results reveal a vascular-based pathologic process that combines aspects of both endothelial "de-differentiation" and "stemness," with significant implications for development, tissue repair and disease. Patients with fibrodysplasia ossificans progressiva (FOP) bear a heterozygous activating mutation of AKL2 (activin-like-kinase 2) and develop heterotopic ossifications that result from an EndMT-like transition of endothelium to cells that in many ways mimic mesenchymal stem cells (22). In the presence of inflammation, these multipotent, endothelium-derived cells undergo chondrogenesis and ossification leading to the clinical manifestations of the disease. These observations in FOP patients were confirmed using genetically engineered mice and cultured endothelial cells both expressing a version of ALK2 that incorporated the same activating mutation. This endothelial-to-multipotent mesenchyme transition is recapitulated by treatment of genetically normal endothelial cells *in vitro* with TGF-β2 and BMP4, requires ALK2 expression and is inhibited by BMP7 and VEGF. Similar both to earlier descriptions of EndMT and to the formation of hematopoietic stem cells by hemogenic endothelium, this process involves an extravascular "transition" apparently distinct from the asymmetric cell division (yielding both self-renewal and differentiation) that is the *sine qua non* of "authentic" stem cells. Indeed, the authors carefully make this distinction by describing these cells as "stem-like" rather than stem cells. In addition, these "multipotent mesenchymal stem-like cells" continue to express genes (VE-cadherin, vWF, Tie1, Tie 2) that reflect their endothelial origin and that are not expressed by *bona fide* mesenchymal stem cells (from bone marrow, for example).

These studies relied in part on transgene expression in endothelium driven by the Tie2 promoter, which also shows significant expression in hematopoietic cells. In contrast, studies of hematopoietic stem cell formation by hemogenic endothelium included the VE cadherin promoter that produces less extra-endothelial expression. While this raises the possibility that

hematopoietic cells themselves might contribute to ALK2-dependent lesions in FOP other experiments are not consistent with this as a predominate pathogenic mechanism. It therefore seems clear that differentiated endothelium expressing activated ALK2 undergoes EndMT to produce multipotent stem-like cells which, in the presence of inflammation, give rise directly to the characteristic cartilaginous and bony lesions in FOP patients.

What doesn't (or can't) endothelium make?

The concept of endothelium as both a functionally and developmentally plastic tissue has thus developed over nearly a century and has accelerated significantly during the past decade. What is different about the most recent discoveries is their unexpected intersection with the fundamental concept of terminal differentiation. Ironically, while the occurrence of EndMT in some ways reprises the early prediction that endothelium reverts to a less specialized state *in vitro,* endothelium's ability to produce blood and bone disproves the implied corollary hypothesis that this tissue is irreversibly committed developmentally *in vivo*. Perhaps this is not surprising given that fibroblasts, neurons (reviewed in (23)) and endothelial cells themselves (24) can be manipulated experimentally to form induced pluripotent stem (iPS) cells that share many properties with embryonic stem cells including the ability to form most if not all cell lineages found in normal adults. Interestingly, both iPS cells and ALK2-dependent mesenchymal stem-like cells show subtle but significant and consistent gene expression and phenotypic differences compared to their corresponding normal counterparts (ES cells and mesenchymal stem cells, respectively). However, endothelium is one of only a few current examples of cells that progress to a phenotype with a significantly expanded developmental potential. In addition, the discovery of ALK2-dependent EndMT and heterotopic ossification in FOP is unique because it explains the pathogenesis of a naturally occurring human disease and is not limited to specific conditions of experimental manipulation.

That the developmental potential of endothelium can be so perturbed in disease suggests that endothelial cells may also be manipulated in the laboratory and the clinic for prognostic, diagnostic and therapeutic benefit in ways that were not conceivable only a few years ago. Studies of FOP (22) do, in fact, demonstrate that this is feasible. Thus, investigating and manipulating further the developmental potential of endothelium may yield significant advances in a broad range of research related and clinically relevant areas.

Note: Unfortunately the short format of this article precluded describing many other studies that have made important contributions to these fields. As always, such advances owe their very existence to the directly and indirectly antecedent work of a much larger number of investigators.

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