Does Multicolor Lineage Tracing of Endothelial Cells Provide a Black and White Answer on Clonal Expansion in Post-Natal Angiogenesis?

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Postnatal angiogenesis occurs in physiological (ie, blood vessel growth in muscle in response to exercise and with cyclic changes within menstrual tissue) and pathological conditions (eg, diabetic retinopathy). Many attempts to promote postnatal angiogenesis have been attempted to drive revascularization of ischemic tissues. Surprisingly, we continue to lack fundamental information on which endothelial cells possess replicative potential in blood vessels. Prior work established that infusion of H^3 thymidine into young mammals revealed endothelial cell proliferation in small and large vessels that is high at birth, varies within and among organ vasculature, and declines rapidly over the first few weeks of life. In adult animals, the rate of homeostatic endothelial cell turnover has been quantified as <1% in rodents. Despite these pieces of information, we do not know specifics about the individual proliferative capacities of endothelial cells, whether this activity is restricted to a small subset or is a feature ubiquitously present within all endothelial cells in a blood vessel.[1]

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Manavski et al[2] in this issue have used inducible endothelial cell–restricted multicolor reporter (Confetti) transgenic mice to interrogate the clonal expansion of individual endothelial cells within normally developing murine tissue vasculature or within tissues challenged by ischemia and hypoxia. This system has permitted quantification of all the endothelial cells within a fixed sampling volume of the tissue vasculature to detect an increase in local distribution of a singular color of endothelial cells within areas of clonal expansion compared with near equivalent levels of random color distribution in areas lacking clonal expansion. In brief, the authors failed to detect significant color enrichment (<10% clonal expansion) in the developing retinal vasculature at postnatal day 5, consistent with prior work in zebrafish that had identified random integration or cell mixing accounting for endothelial cell distributions in developmental angiogenesis. In contrast, in several different models of neonatal and adult hypoxic and ischemic tissue injury, clonal expansion of endothelial cells was observed in 69 to 28% to 35%, respectively, of the vascular endothelial cells post-injury. Laser capture of clonally expanded versus nonclonally expanded endothelial cells in injured tissues permitted transcriptome comparisons and an unexpected increase in gene transcripts involved in endothelial-to-mesenchymal transition (EndMT) in the clonally expanded cells. In vitro exposure of endothelial cells to hypoxia also enhanced expression of mesenchymal cell phenotypic markers and altered cell morphology consistent with EndMT changes. In sum, these results were interpreted by the authors to indicate that clonal expansion of endothelial cells contributes to growing vascular endothelium after hypoxia and ischemia and this process involves partial induction of EndMT.

It is interesting that clonal endothelial expansion in blood vessels did not occur during developmental angiogenesis. How can we explain this? Could this be because of the choice of tissue for study? It is known that the entire retinal vascular bed develops between postnatal days 1 and 10 in the mouse. This is without question the most rapid vascular bed to develop and mature during development of any organ system. It is also a significantly smaller vascular bed compared with the sheer volume of vasculature in many other organs like the heart, lung, liver, or skeletal muscle. Would clonal endothelial expansion be more evident in a tissue with a more prolonged period and extensive contribution from developmental angiogenesis? Use of the methods described in the present paper[2] may permit such comparisons, and we may learn that there are tissue- and organ-specific patterns of endothelial replication with varying contributions from clonal endothelial expansion.

The greatest strength of the present work is the definitive demonstration that clonal endothelial expansion contributes to angiogenic vessel growth after hypoxic and ischemic tissue injury. This point had been suggested decades ago by investigators who infused H^3 thymidine into living rodents and determined that endothelial replication in young animals or adult animals stressed with hypertension or endotoxin infusion was enriched in distinct clusters of cells in the aorta, whereas most other areas of the aorta were devoid of endothelial replication.[3] In those studies, one could not be sure that all the nuclear-labeled cells belonged to the endothelial lineage. In addition, it was not apparent whether the clusters of replicating cells represented unique sites of endothelial cells that possessed growth potential, whereas the nonproliferative areas contained mature cells lacking expansion potential.[3] The present work extends those early observations to illustrate

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that there are indeed distinct regions with clonal expansion of resident endothelial replication in blood vessels that generate large numbers of progeny on certain injurious stressors (hypoxia and ischemia) but did not determine how these specific clonal precursors could be prospectively identified or what metabolic or transcriptional differences might be seen from other resident neighboring endothelial cells that did not possess proliferative potential.

This question of the replicative potential of endothelial cells has been a topic of increasing interest in the field of angiogenesis and vascular regeneration. Several laboratories have published evidence over the past 5 years supporting the concept that rare resident vascular endothelial cells possess stem and progenitor cell activity for regenerating or expanding endothelial cells on injury or in response to angiogenic stimuli. For example, the murine lung endothelium has been shown to be comprised of rare resident $CD31^+CD105^+Sca\text{-}1^+CD117^+$ endothelial cells that displayed clonal proliferative potential in vitro, capacity for forming a capillary bed on implantation into a recipient when as few as a single cell was infused, and loss of CD117 expression in lung endothelial cells caused a 10-fold reduction in clonogenic proliferation in vitro.[4] Another group used Hoechst 33342 dye staining of lung endothelial cells to identify a side population (identified by flow cytometry) enriched in clonogenic potential. The side population cells displayed enhanced clonal proliferative potential, formed a capillary bed on transplantation into recipient mice, rescued blood flow into the hindlimb of animals with experimentally induced ischemia, and were found to be enriched in a quiescent state within blood vessels.[5] Recently, a third group has identified ProCR (protein C receptor) expressing endothelial cells that contribute to developmental and stress angiogenic endothelial expansion. The $ProCR^+$ cells display clonal proliferative potential in vitro, can contribute at a single cell level to vascular formation in vivo in both capillary and large-vessel structures, and fate-mapping studies determined that $ProCR^+$ endothelial cells contributed to endothelial cell expansion in blood vessels for up to 10 months in some tissues.[6] Whether the clonal expansion of endothelial cells observed in the present studies is derived from any or all of these various putative resident endothelial stem cells or downstream resident progenitors will require further studies.

The finding that at least partial EndMT may be involved in clonal expansion and new blood vessel formation is interesting. The present study did not demonstrate functional EndMT as the new capillaries were perfused and carried systemically delivered imaging reagents. The process of EndMT is well known to play an important role in cardiac development, where a subset of endothelial cells lining the endocardium undergo EndMT to form the cardiac valves and contribute to several other lineages. Other studies revealed that EndMT participates in organ fibrosis, solid tumor formation, scleroderma, heterotopic ossification, pulmonary arterial hypertension, arteriosclerosis, and cerebral cavernous malformation.[7] In fact, in adult murine vasculature, EndMT disrupts vascular endothelial barrier properties, leading to deterioration of vascular function and plaque formation observed in atherosclerosis and vein graft stenosis. Emerging evidence is suggesting that some proangiogenic and proproliferative molecules such as bFGF (basic fibroblast growth factor) may be essential in protecting normal endothelial cells from undergoing EndMT via TGF-β (transforming growth factor β) signaling pathways. In areas of injury, endothelial heterogeneity may lead to enhanced TGF-β) signaling pathways by the presence of bFGF and greater EndMT, but the same combination in other endothelial cells leads to inhibition of EndMT. Some prior work also relates endothelial heterogeneity in displaying enhanced expression of EndMT gene expression and functions during angiogenic responses to hypoxia.[7] It is clear that additional work will need to be completed to understand what role changes in gene expression favoring partial EndMT play in the process of clonal endothelial expansion after vascular injury.

A caution in the pathological studies performed in the transgenic reporter mice must also be raised. The inbred mouse strains used in the myocardial infarction and hindlimb ischemia studies are one known to display robust angiogenic responses. Determining whether clonal endothelial expansion is a driver of the potent angiogenic response or a result of the response and the genetic backgrounds of the mice used in this study is an unknown. Additional studies in more clinically relevant models (ie, mice with impaired revascularization potential) may help address this question.[8] From a clinical perspective, it is not obvious how the data in the present work may be translated into a therapeutic to promote clonal expansion to promote revascularization, particularly if EndMT, with its limitations delineated in the paragraph above, is the driver of the clonal expansion.[9]

The present work in the Confetti mice by Manavski et al[2] provides black and white evidence that in postnatal angiogenesis in some conditions, some blood vessel–resident endothelial cells display clonal expansion potential. Unraveling the phenotypic, molecular, and functional attributes of this vascular cell subset and how they may harnessed may contribute significantly in our quest to modulate blood vessel growth/repair for therapeutic gain.

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Disclosures

None.

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