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Macro- and micro-heterogeneity of lung endothelial cells: they may not be smooth, but they got the moves

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It has long been recognized that there is tremendous heterogeneity in endothelial cells (ECs) from different vascular beds[1] and that organ-specific differences in EC phenotype have important physiological and pathophysiological implications. It has also been suggested that within the vasculature, there are "niches" of highly proliferative, progenitor-like ECs that likely play an important role in vascular repair and regeneration.[2] The lung vascular bed is particularly intriguing because of its many unique features. It has the largest endothelial surface area of any organ and is the only vascular bed that needs to accommodate the entire cardiac output. Moreover, it can do this at rest using only a fraction of the available microvasculature area, at arterial pressures that are little more than venous. As cardiac output increases, the lung can recruit additional unused microvascular area to maintain low arterial pressure. Also, there is a paucity of smooth muscle and other supportive mural cells in its small arterioles, possibly because they operate at such low pressures, which makes these structures particularly fragile.

Thus, the observation by the Stevens group some years ago[3] that a significant proportion of pulmonary microvascular ECs (PMVECs) showed high proliferation potential may provide insights into how homeostasis of the lung microvasculature may be maintained, namely by endowing the lung with a uniquely high reparative capacity. The importance of being endowed with high regenerative ability is underlined by the evolving understanding of the role of EC injury and apoptosis in the pathogenesis of pulmonary arterial hypertension (PAH). EC apoptosis has been implicated as a critical trigger in the pathogenesis of PAH, leading to both direct and indirect consequences. Although the "angioproliferative" sequelae have received far more attention, it is not hard to imagine that apoptosis of ECs of fragile distal lung arterioles, consisting of little more than endothelial tubes literally "hanging in the breeze," could lead to loss of these structures.[4] Therefore, an ability to efficiently replace damaged ECs may be an important mechanism by which the lung maintains vascular homeostasis after exposure to endothelial toxins or other environmental stress.

The recent work published in this issue of *Pulmonary Circulation*[5] takes EC heterogeneity to another level by demonstrating differences in proliferative, angiogenic, and bioenergetic profiles of rat pulmonary arterial ECs (PAECs) derived from the main pulmonary artery and its main branches. This suggests a significant degree of micro-heterogeneity even within an EC population derived from one level (i.e. conductance arteries) of the same bed. One can only speculate about the implication of this observation, though it is consistent with the idea of progenitor cell "niches" within the pulmonary vasculature, presumably for the purpose of effecting rapid repair after endothelial injury. The authors also show that the parental proliferative, angiogenic, and bioenergetic characteristics are preserved in subsequent generation PAEC colonies derived by single-cell cloning. They show that single-cell colonies derived from PAECs maintain parental growth profiles and glycolytic rates, despite a decrease in basal oxygen consumption, indicative of a lower bioenergetic demand. Second-generation single-cell clones, which produced a more morphologically homogeneous population, exhibited a similar bioenergetics profile to first-generation clones, and were otherwise identical to parental and first-generation populations. Of note, second-generation clones, selected from proliferating first-generation single-cell colonies, appeared to produce more colonies than first-generation clones. The authors conclude that single-cell cloning might be useful for the expansion of cells with limited proliferation potential by selectively choosing proliferation competent clones, which also produces a more homogeneous culture while maintaining very strong resemblance to the parent population.

Another intriguing finding in this report is the difference in relative use of aerobic glycolysis between different pulmonary EC populations. While PAECs use oxidative phosphorylation as their primary metabolic pathway, PMVECs rely largely on glycolysis for adenosine triphosphate (ATP) production. Recent studies have shown that, unlike other cell types with much higher energetic demands (i.e. myocytes), ECs from many different beds primarily use aerobic glycolysis as the primary mechanism for ATP generation.[6]

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Therefore, the macro-heterogeneity in bioenergetics between PAECs and PMVECs is surprising and suggests that PAECs may be metabolically distinct from ECs from other regions, as summarized in Table 1 of their report.[5] The reasons for such a stark metabolic difference is puzzling, especially since PAECs would normally be exposed to desaturated arterial blood, with relatively low levels of oxygen. In contrast, PMVECs of the distal arteriolar and capillary beds are exposed to high oxygen concentrations through their proximity to the inhaled air and yet use predominantly aerobic glycolysis. Perhaps this may be explained by the importance of maximizing oxygen delivery to the alveolar capillary blood by minimizing losses due to metabolism.[7] It may also be related to other potential advantages of the glycolytic pathway of metabolism for ECs, including decreased production reactive oxygen species, as previously suggested by Carmeliet et al.[6]

It is also interesting to speculate about some of the other implications of this work in our evolving understanding of the EC growth and survival in lung vascular health and disease. Over the last few decades, there has been increasing interest in the role of growth dysregulation of vascular cells as a major mechanism of microvascular occlusion and obliteration in PAH, which has gained great momentum with the more recent focus on metabolism. However, the work by the Stevens group suggests that high proliferative potential is a normal characteristic of a high proportion of microvascular as well as some more proximal PAECs, associated with dependence on aerobic glycolysis for the generation of ATP under normal conditions. Thus, rather than being a pathological phenomenon driving obliterative arterial remodeling, we must assume that this feature has physiological and adaptive consequences, possibly (as suggested earlier) by conferring a particularly high vascular reparative capacity.[7] If this is indeed the case, then rather than being maladaptive, the presence of highly proliferative, glycolytic ECs in the lung microvasculature may play a key role in maintaining lung vascular homeostasis, conferring the ability to rapidly repair damage to the endothelium in the distal lung vasculature after a toxic insult. A corollary of this premise is that incomplete repair of the lung vasculature would have pathological consequences, resulting in the loss of lung microcirculation, which could have therapeutic implications, e.g. the use of angiogenic strategies to enhance the endogenous reparative capacity in the lung microvasculature.[8]

The findings reported in this issue of *Pulmonary Circulation* may also have implications regarding the underlying cellular processes that drive uncontrolled growth of microvascular ECs in PAH. Accepting that aerobic glycolysis is the predominant metabolic pathway used by normal PMVECs, then it follows that mechanisms other than, or in addition to, a shift in glucose metabolism must underlie the emergence of growth dysregulated microvascular ECs in PAH. Thus, while a dependence on aerobic glycolysis may be necessary for appearance of growth dysregulation, in the case of PMVECs it is clearly not sufficient. Certainly, a plethora of potential pathways have been suggested in recent years;[9] however, the precise mechanisms that link EC apoptosis, which is widely recognized as the trigger for EC growth dysregulation, to the appearance of hyperproliferative microvascular ECs remain unclear. In this regard, it is of interest that a mediator implicated in the malignant transformation of cancer cells, namely translationally controlled tumor protein (TCTP), has been shown to be selectively released from apoptotic ECs in exosomes, and is markedly upregulated in proliferative ECs in complex arterial lesions both in experimental models of PAH as well as the human disease.[10]

The report by Lee et al. underlines the importance of heterogeneity in EC growth, bioenergetic, and angiogenic properties from different vascular regions or even from within the same region of the pulmonary vascular bed. While these findings may be open to a number of interpretations, we believe that they support an important role for PMVECs, and possibly PAECs as well, in the maintenance of the integrity of the lung vascular bed, especially in the context of an insult resulting in lung EC injury and apoptosis. The implications of these results for lung vascular disease, in particular the appearance of growth dysregulated ECs, are less clear and more work is needed to better define the molecular and bioenergetic mechanisms that underlie this phenomenon.

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