Tie2 lineage deletion of α6 integrin: endothelial and haematopoietic cells in neovascularization

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This editorial refers to ‘Tie2-dependent knockout of α6 integrin subunit in mice reduces post-ischaemic angiogenesis’ by C. Bouvard et al., pp. 39–47, this issue.

The metabolic demand of a mature tissue determines the degree of blood and oxygen supply, which normally is controlled by vasoregulation at the level of arteriolar resistance vessels. However, in specific conditions such as inflammation and excessive tissue growth including tumours, the metabolic demand exceeds the supply by autoregulation and therefore requires neovascularization. The need for additional blood supply and neovascularization becomes urgent after an acute vascular occlusion. Rapid formation of collaterals is then required, while the distal hypoxic tissue simultaneously shifts to the production of pro-angiogenic factors, in particular vascular endothelial growth factor, which subsequently induces sprouting angiogenesis in an attempt to connect to other perfused vessels and to restore oxygen and nutrient supply to the ischaemic tissue.

In addition, circulating monocytes and endothelial progenitor cells (EPCs) are recruited to the sites of structural arterial widening (arteriogenesis) and sprouting angiogenesis. Monocytes and circulating angiogenic cells (monocyte-lineage cells that acquire several endothelial markers, also called early outgrowth EPCs) support the vascular adaptation in a paracrine manner. Furthermore, endothelial colony-forming cells (ECFCs, also called late outgrowth EPCs), which are present in small numbers in blood but have a high proliferative potential, can contribute to and integrate into the lining of sprouting vessels. In contrast to the monocyte populations, which are generated in the bone marrow, these latter true circulating endothelial progenitors are likely recruited from other sites of the vascular bed.

Much information about neovascularization and sprouting angiogenesis has been obtained from studies on the development of the zebrafish and murine vasculature, in particular after specific gene deletions and mutations. Many of the recognized factors also act in the expansion and viability of tumour blood vessels and have provided new insights in the regulation of sprouting angiogenesis. However, in addition to these pivotal factors, a number of factors are becoming recognized that are involved only after a specific challenge but do not affect normal vascular development. One of these factors is the α6 integrin subunit, which is encountered in the laminin-binding integrins α6β1 and α6β4. Deletion of the α6 integrin subunit is lethal because of epidermal blistering but does not affect normal vascular development. However, when neovascularization is needed after a challenge later in life, such as vessel occlusion or tumour expansion, the required angiogenic response is decreased after tissue-specific α6 gene deletion or inactivation of the α6 protein. This latter finding was challenged by a study that showed an increased angiogenic response in mice in which the α6 gene was deleted under the control of the endothelial Tie1 promoter.

Bouvard et al. deleted the α6 gene specifically in Tie-2 lineage cells, which include endothelial cells and a subset of Tie-2-expressing monocytes/macrophages. They found that after interruption of the arterial blood supply in the mouse hindleg, not only was sprouting angiogenesis reduced but also collateralization and the recruitment of Tie2 macrophages in the ischaemic tissue. The collateralization aspect is of particular interest, because this involves remodelling of the smooth muscle cells rather than endothelial cells. Work of Schaper’s group has demonstrated an important regulatory role of monocytes in this process, while other leucocytes such as lymphocytes and NK cells may play additional roles. Responding to the markedly increased shear forces on the endothelium of collateral arteries induces intracellular signalling and vasodilation and is presently seen as the major contribution of the endothelium to arteriogenesis. Activated endothelial cells also release the proinflammatory mediator angiopoietin-2, which regulates gene expression in Tie2-expressing macrophages and stimulates arteriogenesis. It is possible that the α6 integrins play a role in the endothelial response, but an alternative may be that the absence of α6 integrins affects the contribution of leucocytes in arteriogenesis.

Direct involvement of Tie2-expressing macrophages in arteriogenesis, as shown convincingly in sprouting angiogenesis, has not yet been proven but is plausible given the important contribution of monocytes. In addition to these cells that are recognized by their actual Tie2 expression, one has to consider that Tie2 is important for haematopoietic development and homing of haematopoietic stem cells, and therefore the absence of α6 subunit in Tie2 lineage cells may affect not only Tie2 macrophages but also other members of the haematopoietic system if the α6 integrin–laminin interaction is involved in their development. Indeed, α6β4 integrin-dependent homing of haematopoietic stem cells and participation of α6A in lymphocyte migration have been demonstrated.
Whether the contribution of different cell types underlies the reported difference between Tie and Tie2 lineage-dependent deletions of α6 integrins remains uncertain and requires further studies. In an earlier study, Bouvard et al.\(^6\) showed that when they deleted α6 in human ECFCs, these ECFCs were no longer able to stimulate neovascularization. A reduced homing of these deficient ECFCs probably underlies this observation. A laminin-containing matrix can facilitate the migration of α6 integrin-expressing endothelial cells, pointing to a contribution via endothelial cells.\(^5,8\) At the same time, endothelial sprouting may be counteracted by the fact that α6β4 integrin–laminin interaction can induce Notch expression, favouring further stabilization of vessels.\(^15\)

A final underpinning for the direct involvement of endothelial cells is provided by the observed reduction of the outgrowth of capillary sprouts from the adventitia of α6-deficient aortic rings into a laminin-containing extracellular matrix. Still, the arterial adventitia is also known to harbour progenitor cells and monocytes. A careful comparison of circulating and accumulating haematopoietic cells may be required before Tie2-dependent deletion of genes can be used to unequivocally demonstrate the sole endothelial involvement of that gene.

Bouvard et al.\(^6\) made an important contribution by showing that α6 integrin is also important for ischaemia-induced arteriogenesis and that Tie2 macrophages indeed do accumulate in the ischaemic tissue depending on the presence of α6 integrin. This places the contribution of α6 integrins to neovascularization in a wider perspective, still leaving a Tie-break for the involvement of endothelial and haematopoietic cells.

**Conflict of interest:** none declared.

**References**


