Vascular Basement Membrane Components in Angiogenesis — An Act of Balance

Lars Jakobsson^{1,*} and Lena Claesson-Welsh^{2,*}

¹*Vascular Biology Laboratory, London Research Institute, Cancer Research UK, 44 Lincoln's Inn Fields, London WC2A 3PX, U.K.;* ²*Uppsala University, Department of Genetics and Pathology, Rudbeck Laboratory, Dag Hammarskjöldsv. 20, 751 85 Uppsala, Sweden*

E-mail: lena.claesson-welsh@genpat.uu.se; Lars.Jakobsson@cancer.org.uk

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Angiogenesis is crucial in the progression of a number of pathological conditions, such as diabetic retinopathy, rheumatoid arthritis, psoriasis, and cancer[1]. In contrast to vessels in healthy tissues, the vasculature in these pathologies is highly unstable, constantly dissolving and renewing. Characteristically, vessels in pathologies have discontinuous basement membrane (BM) coverage[2]. The consequences of shifts in BM density and composition are still relatively unknown. As discussed below, several studies have illustrated that partial loss of the vascular BM during development results in the widening of vessels. This has been suggested to be a result of reduced mechanical resistance to the force inflicted by the blood pressure. However, recent data indicate that depletion of BM laminins (LMs) leads to enlarged vessels even in the absence of cardiac activity and blood pressure[3].

All endothelial cells (ECs) in the body rest on BMs composed of intricate networks of matrix proteins, such as fibronectins, collagen IV, and LMs. LMs are heterotrimeric glycoproteins composed of α -, β -, and γ -chains. The vascular BM has a unique composition with the presence of LM α -chains 4 and 5 that combine with the β 1- and γ 1-chains to form LM-411 and LM-511 (previously denoted laminin 8 and 10, respectively)[4,5].

The function of the vascular LMs has long been sought and their roles have mainly been addressed by gene inactivation (reviewed by Hallman et al.[6]). Deletion of the LM α 5-chain (encoded by *lama5*) results in lethality between embryonic day 14 and 17.5, with failure of neural tube closure, severe brain and limb defects, and dysmorphogenesis of the placenta[7]. Interestingly, the great majority of the vasculature is largely unaffected in the *lama5*^{-/-} embryos, but the placental vasculature is reduced, less branched, and shows an increase in vessel diameter. The *lama4*^{-/-} (lacking the LM α 4-chain) mice suffer from hemorrhages and display enlarged blood vessel width during embryonic and neonatal stages[8]. The vascular phenotype decreases after birth and is gradually lost. Up-regulation of the α 5-chain or other LMs in the *lama4*^{-/-} animals has not been detected. In a recent study[3], we examined the role of BM LMs in the formation of vascular structures in spheroids of differentiating embryonic stem cells, denoted embryoid bodies[9]. This experimental approach in many cases allows for the study of gene modifications that are incompatible with embryonic survival *in vivo*, or alternatively are clouded *in vivo* due to

*Corresponding authors.

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Since there is no flow in embryoid bodies, we conclude that widening of the vessel lumen in the absence of LM deposition is not a consequence of decreased resistance to shear stress or blood pressure in this model. What causes the widening of the vessel lumen in the absence of LMs? We could show that LM deficiency was accompanied by an increase in fibronectin and a decrease in collagen IV protein levels in the BM in the *lamc1*^{-/-} embryoid bodies. It is possible that endothelial integrins become differentially activated as a consequence of this change in BM composition. Fibronectin is known to affect cellular morphology via its binding to β 1 integrins. In accordance, *lamc1*^{-/-} ECs were more spread out than wild-type ECs and, strikingly, the *lamc1*^{-/-} phenotype was partially rescued by addition of purified LM protein. Also, *lamc1*^{-/-} ECs sprouting into a surrounding collagen I matrix showed a higher rate of proliferation, which may reflect different integrin engagement. Since wide and thin vessels normally have roughly the same EC density (number of ECs/circumference), it is tempting to speculate that the degree of EC proliferation dictates the vessel diameter. It is unclear, however, whether an increased EC proliferative index would correlate with the final vessel diameter *in vivo*.

Apart from the EC proliferative index, migratory properties and differentiation cues in the microenvironment contribute to the width of a developing vessel. Mice exclusively expressing the diffusible vascular endothelial growth factor-A120 isoform display enlarged vessels in the developing hindbrain with increased numbers of ECs/vessel length[11]. Furthermore, induced overexpression of activated Notch4 gives rise to wider vessels both in embryoid bodies and *in vivo*, even though EC proliferation is reduced[12]. Also, the Notch ligand jagged 1 is up-regulated in the presence of LM-111 during differentiation of bovine ECs, suggesting a link between the two molecular systems[13].

It is evident from the embryonic stem cell model, as well as from gene-targeted mouse models, that the vascular BM LMs play a role in establishment of the vessel architecture. However, LMs are not required for development of ECs *per se*[3]. Here we suggest that the balance of BM components plays an important role in regulation of EC proliferation and possibly other EC responses, which in turn dictates the vessel diameter. The vascular LMs may, therefore, in addition to serving as a scaffold and promoting cell attachment to avoid apoptosis, serve to control differentiation and restrict EC proliferation.

LM loss of function (LOF) studies have generated valuable *in vivo* data, but the knowledge is still incomplete. Detailed analysis of angiogenic tissues in the LM LOF mice may provide key information that is currently lacking. Furthermore, inducible LM LOFs would allow for analysis of the role of LMs in adult and pathological tissues. A key question is whether single BM components or fragments thereof play distinct roles in the angiogenic process, or if it is the balance between the different components of the BM that guides the morphology of the new vessel.

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