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RESEARCH HIGHLIGHT

New molecular connections in angiogenesis

Qiling Xu[1], David Wilkinson[1]

[1]*Division of Developmental Neurobiology, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*

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In vertebrates, oxygen and nutrients are delivered to tissues by the circulation of blood through vessels, comprised of a branched network of endothelial tubes termed the vasculature. Crucial for the formation of blood vessels during development is the process of angiogenesis, in which new sprouts form from pre-existing vessels in a complex cascade of cellular events. This involves the activation of an endothelial cell in the vessel to become a highly exploratory 'tip' cell that migrates to invade the surrounding tissues, while remaining tightly connected to the following cells that subsequently generate the tubular structures of a new vessel. In addition to being essential in normal tissues, angiogenesis can contribute to the pathogenesis of diseases such as cancer, in which the formation of new blood vessels enables tumor growth and provides a route for cancer cells to metastasize to other tissues. Elucidation of how angiogenesis is controlled is therefore likely to give important insights into disease mechanisms and to provide new strategies for therapy. Two exciting papers [1, 2] have now significantly advanced our understanding of angiogenesis by revealing a new connection between distinct families of receptors that control endothelial cell migration.

Members of the vascular endothelial growth factor (VEGF) family and their receptors (VEGFRs) are key players in angiogenesis that regulate the proliferation, migration and morphogenesis of endothelial cells [3]. Gradients of VEGFs induce the formation and migration of the tip cell, and through Notch-mediated lateral inhibition, the tip cell prevents its neighbours from adopting the same fate [4]. The interplay between VEGFs and Notch thus generates the nascent blood vessel comprised of a migrating tip cell attached to a stalk that later forms a tube connected to the pre-existing vessel. VEGFRs are receptor tyrosine kinases that are dimerized upon binding of VEGF ligand, leading to phosphorylation and activation of the tyrosine kinase domain. Upon ligand binding, VEGFR is endocytosed and only becomes strongly activated once the receptor has reached the early endosomes, since cell surface VEGFR is associated with membrane phosphatases that antagonize receptor phosphorylation [5]. VEGFR activation is thus dependent upon endocytosis.

Roles of another set of key players – Eph receptor tyrosine kinases and their ephrin ligands – were first uncovered in the context of axon guidance and boundary formation [6, 7]. Ephrins are anchored in the cell surface membrane, either through a GPI linkage (ephrinAs) or a transmembrane domain (ephrinBs), and with some exceptions these bind to EphA and EphB family members, respectively. The clustering of Eph receptor that occurs upon binding to ephrin leads to activation of the tyrosine kinase domain and consequent downstream signaling. Remarkably, the clustered ephrin also transduces signals (termed 'reverse' signaling), such that bi-directional activation occurs upon contact of Eph receptor and ephrin-expressing cells. In the case of ephrinB proteins, reverse signaling is mediated by phosphorylation of specific tyrosine residues that act as docking sites, and by binding of PDZ domain proteins to a C-terminal interaction motif. One of the major effects of signaling through Eph receptors and ephrins is regulation of the actin cytoskeleton that underlies the migration of cells and guidance of neuronal growth cones. Eph receptors and ephrins have a dual personality, in which their activation can trigger actin depolymerization and thus mediate cell repulsion, whereas in other contexts they instead promote cell migration and adhesion [6, 7].

Initial evidence for roles of Eph receptors and ephrins in development of the vasculature came from the results of gene knockouts in mouse of EphB4 or ephrinB2 [8]. EphB4 expression occurs prominently in veins, and ephrinB2 in arteries, and disruption of either of these genes leads to a failure in angiogenic remodeling. However, the molecular and cellular mechanisms

Correspondence: Qiling Xu[a], David Wilkinson[b]
[a]Tel: +44 20 8816 2404; Fax: +44 20 8816 2523
E-mail: qxu@nimr.mrc.ac.uk
[b]E-mail: dwilkin@nimr.mrc.ac.uk

by which Eph-ephrin signaling controls angiogenesis have been unclear. Important new insights have come from recent studies, published in *Nature*, of the role of ephrinB2 in angiogenesis. Wang *et al.* [2] generated a knock-in of GFP reporter into the endogenous ephrinB2 gene and confirmed that expression occurs in arterial endothelial cells, including the tip and stalk cells of the growing vessels. Inducible loss of ephrinB2 function specifically in endothelial cells leads to significant reduction in the formation of blood vessels. Furthermore, endothelial cells derived from the ephrinB2 knockout mice showed greatly reduced cellular protrusions and connections. A parallel study by Sawamiphak *et al.* [1] analyzed the role of ephrinB2 in the vasculature by using a knock-in line defective in PDZ-dependent reverse signaling, due to the absence of the C-terminal valine (ephrinB2 ΔV mutant). Disruption of PDZ-dependent signaling led to a striking decrease in the number of tip cells, vessel branching and filopodial extensions. Conversely, overexpression of ephrinB2 resulted in increased filopodial extensions and disrupted vascular development *in vivo*, and prevented endothelial cells from being included in tubular vessel structures in cell culture [1, 2]. Taken together, these studies reveal that ephrinB2 reverse signaling through PDZ interactions regulates vessel sprouting by promoting tip cell filopodia extension.

Since loss of ephrinB2 function leads to a similar defect in angiogenesis as occurs following disruption of VEGFR activation, these distinct receptors may have synergistic or interdependent roles. Important clues came from the findings that VEGF-induced endocytosis of VEGFR2 and VEGFR3 does not occur in ephrinB2 null mutants [1, 2]. Furthermore, in ephrinB2 mutants there is a great reduction in VEGFR phosphorylation and downstream Akt and Erk1/2 activation [1, 2] – as also occurs after chemical blocking of VEGFR endocytosis – and the chemotaxis of endothelial cells towards VEGF is compromised [2]. Activation of ephrinB2 reverse signaling with EphB4 leads to the internalization of VEGFRs in cultured endothelial cells [1, 2], and can partly rescue the decrease in filopodial extension that occurs following depletion of VEGF activity [1]. As VEGFR2 and VEGFR3 co-localize with ephrinB2 at the cell surface, and VEGFR2 and ephrinB2 co-immunoprecipitate, it is likely that endocytosis involves physical interactions between these proteins. Sawamiphak *et al.* further demonstrated that ephrinB2 reverse signaling is required for VEGFR2 function in tumor angiogenesis, and that tumor growth is impaired in ephrinB2 ΔV mutants compared with wild-type mice [1].

The studies of Wang *et al.* and Sawamiphak *et al.* provide compelling evidence that ephrinB2 is essential for endocytosis and activation of VEGFRs in endothelial cells during physiological and pathological angiogenesis. The interplay between ephrinB2 and VEGFR may provide a mechanism to correctly localize VEGFR activation within endothelial tip cells that is crucial for the formation of new blood vessels. It is striking that many of the same families of guidance molecules are involved in angiogenesis and axon guidance [9], with the endothelial tip cell having an analogous role to the neuronal growth cone in exploratory migration. It will therefore be interesting to uncover whether other examples of cross-talk between Eph-ephrin signaling and distinct receptor systems [10] have roles in the control of cell migration.

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