Tumor Vessels Are Eph-ing Complicated

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Two new studies have shown that there is considerable crosstalk and cross-regulation of the Ephrin/VEGF pathways in endothelial cells. These findings illustrate how EphrinB2 signaling and VEGF, in a cooperative manner, induce VEGFR activation in endothelial cells and give new insight into how endothelial cell-mediated construction of vessels is accomplished.

Eph-ephrin signaling is well established as the major regulator of cellular topography during embryogenesis, vasculogenesis, and angiogenesis. This pathway is involved in regulating a number of critical processes, including axon guidance, and in many cases determining cell direction, shape, and orientation. Several components of this family of ligands and receptors have been associated with cancer progression (Foo et al., 2006; Kullander and Klein, 2002). Both ligands (ephrins) and receptors (Eph) are classified into two groups, A and B, and A- or B-type receptors have preference for A- or B-type ligands, respectively, with a certain degree of promiscuity and a few known exceptions; e.g., EphA4 receptor can bind both A- and B-type ligands, and ephrin-A5 can bind to EphB2 in addition to all A-type receptors (Murai and Pasquale, 2003).

Eph receptors are the largest family of transmembrane tyrosine kinase receptors, with a single transmembrane domain, a ligand recognition site in the extracellular region, and a cytoplasmic domain that has a juxtamembrane short sequence containing two conserved tyrosines, a tyrosine kinase domain, a sterile α-motif (SAM), and a PDZ binding domain. Ephrin ligands are also membrane-bound proteins: A-type ephrins are GPI-linked proteins, whereas B-type ephrins are transmembrane proteins that contain a cytoplasmic region that also includes conserved tyrosine residues and a PDZ domain (Kullander and Klein, 2002).

A couple of features distinguish this otherwise conventional receptor kinase-driven signaling pathway: one is that both ligand and receptor are membrane-bound, which imposes a requirement for cell-cell interaction for the signal to take place, instead of the long-range action observed for soluble/secreted ligands; and the other is that this engagement often results in the activation of both receptor (forward signaling) and ligand (reverse signaling), with distinct implications for each cell. Meeting of ligand and receptor results in activation of signaling cascades that mostly result in repulsion stimuli between adjacent cells (Murai and Pasquale, 2003), fundamental for boundary formation and maintenance of cell position. However, it can also occasionally result in cell-cell attraction; this dual role is dependent on the cell type as well as the Eph:ephrin ratio gradient (Hansen et al., 2004; Murai and Pasquale, 2003).

This Eph-ephrin bidirectional signaling has been the focus of studies in the past two decades, and two noteworthy recent publications in *Nature* examine more closely the role of ephrin-B2 in angio- and lymphangiogenesis (Wang, et al., 2010; Sawamiphak, et al., 2010). Ephrin-B2 is specific to arterial endothelium and considered a marker for vessel identity from early embryogenesis; it is involved in vascular remodeling and is upregulated at sites of neovascularization, such as tumors and wounds (Foo et al., 2006; Wang et al., 1998). The activation of the PDZ motif of ephrin-B2 has been shown to be required for individual endothelial cell contraction and expansion episodes, and ephrin-B2 expression has been associated with the progression and spread of many human cancers. Upregulation of ephrin-B2 alone also results in increased motility of endothelial cells and has been proposed to be in turn regulated by VEGF (Bochenek et al., 2010). Furthermore, Vihanto and colleagues have shown that ephrin-B2 expression is induced in hypoxic skin, potentially in a HIF-1α-dependent fashion (Vihanto et al., 2005). Ephrin-B2 has long been recognized as a key component of the signaling leading to endothelial cell migration and new vessel formation, probably in coordination with VEGF signaling. However, tangible links between these two pathways have been missing. Some of this void has just been filled with these two independent findings, mechanistically connecting ephrin-B2 and VEGF signaling during angio- and lymphangiogenesis.

Sawamiphak and colleagues used two ephrin-B2 mutants in their study, one with five tyrosine residues in the cytoplasmic signaling domain changed to phenylalanine, which thus impaired phosphotyrosine-mediated signaling, and the other with a deletion of a single Valine residue in the PDZ binding region, which restricted its interaction with PDZ-binding proteins and inhibited PDZ reverse signaling in the endothelium (Sawamiphak et al., 2010). This latter modification results in decreased filipodial extensions in tip cells, shown to be a consequence of failure to internalize and activate VEGFR2. Their results show ephrin-B2 as a direct activator of VEGFR2, even in the absence of VEGF-A, and possibly as a cooperating partner with VEGF in the signaling leading to tip cell extension and vessel sprouting. This team also used an orthotopic glioma model to appraise the effect of ephrin-B2 PDZ domain inhibition in tumor angiogenesis and observed a striking difference in tumor growth. Tumor volume in mutant mice was less than 25% of that seen in wild-type littermates, a phenotype directly correlated with a decreased, normalized tumor vascularization and an accompanying lower rate of sprouting and filipodia formation. Moreover, they show that this role of ephrin-B2 appears to be specific to endothelial cells.

The work described by Wang et al. similarly bridges the reverse activation of ephrin-B2 with the VEGF-dependent activation of VEGFR3 (Wang et al., 2010). The developmental defects observed in animals lacking endothelial ephrin-B2 are consistent with defective endothelial cell sprouting: embryos show edema and hemorrhagic skin vasculature and smaller and less complex retinal vasculature; endothelial cells devoid of this ligand fail to form protrusions and networks in culture. These results were nicely complemented with an endothelial gain-of-function model, which showed, in essence, the opposite phenotype. Similarly to what was observed for VEGFR2, the VEGF-dependent activation and internalization of VEGFR3 is compromised in the absence of a functional endothelial ephrin-B2. VEGFR3 is the primary receptor for VEGF-C, which is itself a critical factor in lymphangiogenesis. Induction of either ephrin-B2 or its preferred receptor EphB4 is sufficient to induce VEGFR3 internalization, even though they fail to induce its activation.

These discoveries are important, in that they elucidate how these two signaling pathways crosstalk during angiogenesis and lymphangiogenesis, and make room for the identification of other players in what promises to be an intricate association. Even though there is a manifest cooperation between endothelial ephrin-B2 and VEGF required for VEGFR activation, more work is needed to clarify what ephrin-B2 effector (probably PDZ-binding) protein(s) facilitate this functional interaction.

Antiangiogenic therapies have been explored as cancer treatments for some time now, and although the "starvation" of tumors via direct VEGF signaling antagonism can result in tumor vasculature regression, significant recent evidence shows that these beneficial effects can be merely transient, and may even result in drug resistance, elevated invasion, and increased metastasis (e.g., Pàez-Ribes et al., 2009), with a much less than desirable impact on patient survival. The identification of other components of this evidently very complex network of ligands, receptors, and downstream activated cascades is thus crucial to provide alternative or complementary strategies in the development of antiangiogenic therapies.

REFERENCES

Bochenek, M.L., Dickinson, S., Astin, J.W., Adams, R.H., and Nobes, C.D. (2010). J. Cell Sci. *123*, 1235–1246.

Foo, S.S., Turner, C.J., Adams, S., Compagni, A., Aubyn, D., Kogata, N., Lindblom, P., Shani, M., Zicha, D., and Adams, R.H. (2006). Cell *124*, 161–173.

Hansen, M.J., Dallal, G.E., and Flanagan, J.G. (2004). Neuron *42*, 717–730.

Kullander, K., and Klein, R. (2002). Natl. Rev. *3*, 475–486.

Murai, K.K., and Pasquale, E.B. (2003). J. Cell Sci. *116*, 2823–2832.

Pàez-Ribes, M., Allen, E., Huddock, J., Takeda, T., Hiroaki, O., Vinals, F., Inoue, M., Bergers, G., Hanahan, D., and Casanovas, O. (2009). Cancer Cell *15*, 220–231.

Sawamiphak, S., Seidel, S., Essmann, C.L., Wilkinson, G.A., Pitulescu, M.E., Acker, T., and Acker-Palmer, A. (2010). Nature *465*, 487–491.

Vihanto, M.M., Plock, J., Erni, D., Frey, B.M., and Huynh-Do, U. (2005). FASEB J. *19*, 1689–1691.

Wang, H.U., Cheng, Z.-F., and Anderson, D.J. (1998). Cell *93*, 741–753.

Wang, Y., Nakayama, M., Pitulescu, M.E., Schmidt, T.S., Bochenek, M.L., Sakakibara, A., Adams, S., Davy, A., Deutsch, U., Lüthi, U., et al. (2010). Nature *465*, 483–486.