Nature Reviews Molecular Cell Biology | AOP, published online 13 February 2013; doi:10.1038/nrm3533

DEVELOPMENT

Growing a blood vessel network

> aPKC-dependent inactivation of VEGFR internalization and signalling only occurs in the more established vessels

During angiogenesis, endothelial cell proliferation and the activity of sprouting endothelial cells at the tip of growing vessels promote the expansion of the blood vessel network. Angiogenesis also requires the gradual stabilization of growing vessels into a mature tubular network, in which endothelial cells become quiescent. Nakayama *et al.* now show that sprouting endothelial cells, unlike mature stable vessels, display high rates of vascular endothelial growth factor (VEGF) uptake, VEGF receptor (VEGFR) endocytosis and turnover.

The authors investigated the molecular mechanisms that regulate angiogenesis in the mouse retina. Surprisingly, although strong VEGFR activity had previously been associated with the angiogenic front of the growing retinal vasculature, VEGFR2 and VEGFR3 immunostaining did not predominantly label sprouting endothelial cells. Instead, strong staining was observed in the more established vasculature. The authors hypothesized that such a weak signal in sprouting vessels might be due to rapid local turnover and thus low steady-state levels of VEGFRs. Indeed, inhibition of protein degradation, combined with inhibition of protein synthesis, resulted in the accumulation of VEGFR2 and VEGFR3 in angiogenic sprouts, confirming that VEGFRs are rapidly turned over.

VEGFR turnover requires its internalization, and Nakayama *et al.* observed that dynamin-mediated endocytosis of VEGF–VEGFR occurred primarily at the angiogenic front and not in more mature vessels. Thus, spatial differences in VEGFR turnover, dependent on receptor endocytosis, degradation and synthesis, are important regulators of angiogenesis.

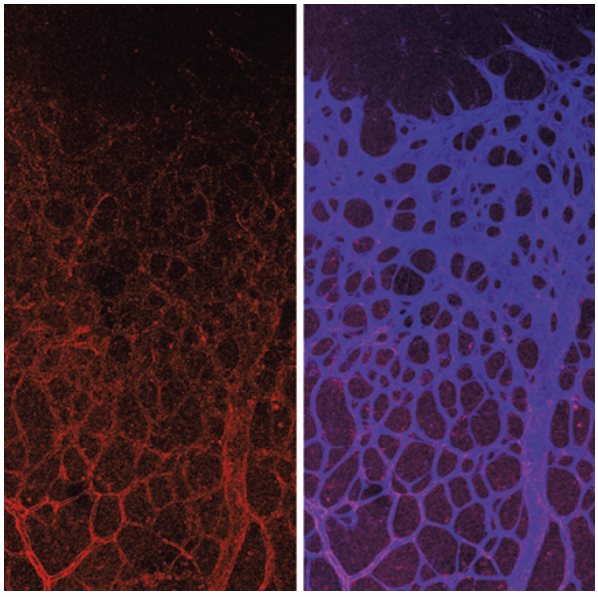
Ephrin B2, a ligand for the EPH receptor family, had previously been shown to regulate VEGFR-mediated signalling. Thus, to further analyse VEGFR endocytosis regulation, the authors isolated interactors of ephrin B2. Among the proteins identified was Disabled 2 (DAB2), which is a clathrin-associated sorting protein that has a role in cargo selection during endocytosis. DAB2 was shown to interact with ephrin B2 indirectly via the polarity protein PAR3. Importantly, VEGFR2 and VEGFR3 interact with both PAR3 and DAB2, suggesting that VEGFR endocytosis is regulated through association with ephrin B2–PAR3–DAB2.

To assess the function of DAB2 and PAR3, the authors knocked down *Dab2* and *Pard3* in cultured endothelial cells. This impaired internalization of both VEGF–VEGFR2 and VEGF–VEGFR3 and decreased signalling downstream of these receptors. Furthermore, endothelial cell-specific gene deletion of *Dab2* and *Pard3* in mice led to pronounced defects in endothelial network development.

How are VEGFR internalization and VEGFR-mediated signalling spatially confined to the angiogenic front? The authors found that atypical protein kinase C (aPKC) phosphorylates DAB2 and that such phosphorylation negatively regulates VEGFR–DAB2 interaction, VEGFR internalization and the downstream signalling cascade that promotes endothelial cell proliferation. Moreover, the authors observed that whereas aPKC is present both at the angiogenic front and in more established vessels, the active form of this kinase is absent in endothelial cells at the angiogenic front. This indicates that aPKC-dependent inactivation of VEGFR endocytosis only occurs in the more established vessels.

Together, these results suggest a model in which high rates of receptor turnover and signalling (mediated by ephrin B2, PAR3 and DAB2) promote sprouting of endothelial vessels, and this activity is inhibited by aPKC-dependent DAB2 phosphorylation in maturing vessels. The signals that control aPKC activation remain elusive. Interestingly, ephrin family members and DAB2 are expressed in many cell types and tissues, which suggests they might regulate receptor pathways and morphogenetic processes in other organs.

Kim Baumann



Mouse retinal vasculature showing weak activated aPKC (red) at the angiogenic front. The endothelium is shown in blue. Image courtesy of Masanori Nakayama, Max Planck Institute for Molecular Biomedicine, Muenster, Germany.

ORIGINAL RESEARCH PAPER Nakayama, M. *et al.* Spatial regulation of VEGF receptor endocytosis in angiogenesis. *Nature Cell Biol.* 27 Jan 2013 (doi:10.1038/ncb2679)

FURTHER READING Herbert, S. P. & Stainier, D. Y. R. Molecular control of endothelial cell behaviour during blood vessel morphogenesis. *Nature Rev. Mol. Cell Biol.* **12**, 551–564 (2011)