Have you seen?

THE EMBO JOURNAL

Jostling for position in angiogenic sprouts: continuous rearrangement of cells explained by differential adhesion dynamics

Colin K Choi[1,2] & Christopher S Chen[1,2]

Endothelial sprouting during angiogenesis is a highly coordinated morphogenetic process that involves polarized tip cells leading stalk cells to form new capillaries. While tip and stalk cells previously were thought to be stable and have static phenotypes within the sprout, it is becoming increasingly clear that endothelial cells undergo dynamic rearrangements. A new study using computer simulations, validated by *in vitro* and *in vivo* experimental data, now provides an explanation for these rearrangements, showing that sprouting cells are in a continuum of migratory states, regulated by differential cell-cell adhesions and protrusive activities to drive proper vascular organization.

See also: **K Bentley *et al*** (May 2014)

During angiogenesis, gradients of pro-angiogenic cues such as vascular endothelial growth factor (VEGF) induce a subset of endothelial cells to polarize and become tips cells, and lateral inhibition via delta-like 4 (Dll4)-Notch signaling converts neighboring cells to stalk cells and leads to differential VEGF receptor (VEGFR) expression in the sprout (Hellstrom *et al*, 2007; Lobov *et al*, 2007; Potente *et al*, 2011). Surprisingly, recent studies have found that tip and stalk cells change positions frequently during sprouting, revealing dynamic changes in Notch activity, tip–stalk fates, and cell motility within developing blood vessels (Bentley *et al*, 2009; Jakobsson *et al*, 2010; Arima *et al*, 2011). However, the mechanisms by which individual cells compete for positions remained unclear. Using an elegant, interdisciplinary approach that combined computer simulation predictions with *in vitro* and *in vivo* experimental validations, Bentley *et al* now report in the journal *Nature Cell Biology* two cooperating mechanisms responsible for generating these endothelial rearrangements (Bentley *et al*, 2014). The authors show a feedback system between VEGF and Notch signaling that regulates vascular endothelial (VE)-cadherin to generate spatial differentials in cell-cell adhesions and polarized junctional protrusions. Both (adhesions and protrusions) in turn cooperated to change the local integrity of vessels, facilitate cellular intercalation and lengthening of the stalk, and enable endothelial cells to compete for the tip cell position (Fig 1).

A recent study reported that VE-cadherin was necessary for cellular organization during anastomosis, indicating a key role of cell-cell junctions in physically rearranging endothelial cells (Lenard *et al*, 2013). Differential adhesion via adherens junctions is well recognized in epithelial systems, but additional mechanisms involving cortical reorganization by Dia and myosin-II have also been reported (Levayer *et al*, 2011). To examine whether the endothelial rearrangements during angiogenic sprouting might be regulated by either differential adhesions or junctional-cortex-mediated processes, the authors tested these mechanisms *in silico*. Differential VE-cadherin engagement and cell-cell adhesion strengths, regulated by Notch and VEGFR-2 activities, predicted that cells with high level of VEGFR-2 (and low level of Notch signaling) will have weak adhesion with neighboring cells. Separately, modulations in notch activity between cells predicted serrated VE-cadherin junctions and differentially polarized cortical protrusions that will facilitate cell movements within the sprout. Testing the contribution of these two mechanisms to endothelial cell rearrangement, the authors found that the computational model matched the *in vitro* observations of cellular rearrangement and tip cell competition (Jakobsson *et al*, 2010) only when both differential adhesions and protrusions were present in the model. That is, the simulations predicted that spatial differentials in both VE-cadherin-mediated junctional adhesion strength and protrusions are required for dynamic cellular shuffling and intercalation, and when all cells either have high or weak adhesion/protrusions (tested with DAPT, high VEGF, and Dll4 or VEGFR antibody blocking), proper tip cell selection and endothelial cell rearrangement are inhibited.

Experimental studies further validated these predictions, in which embryoid body and mouse retina assays showed that endothelial junctions within sprouts exhibit a range of VE-cadherin organization. Relatively straight and immobile VE-cadherin boundaries correlated with "inhibited junctions" of high cell-cell adhesion and low protrusive activity, whereas serrated boundaries with endocytic VE-cadherin

1 Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, USA
2 Department of Biomedical Engineering, Boston University, Boston, MA, USA. E-mail: chencs@bu.edu
DOI 10.1002/embj.201488452 | Published online 7 April 2014

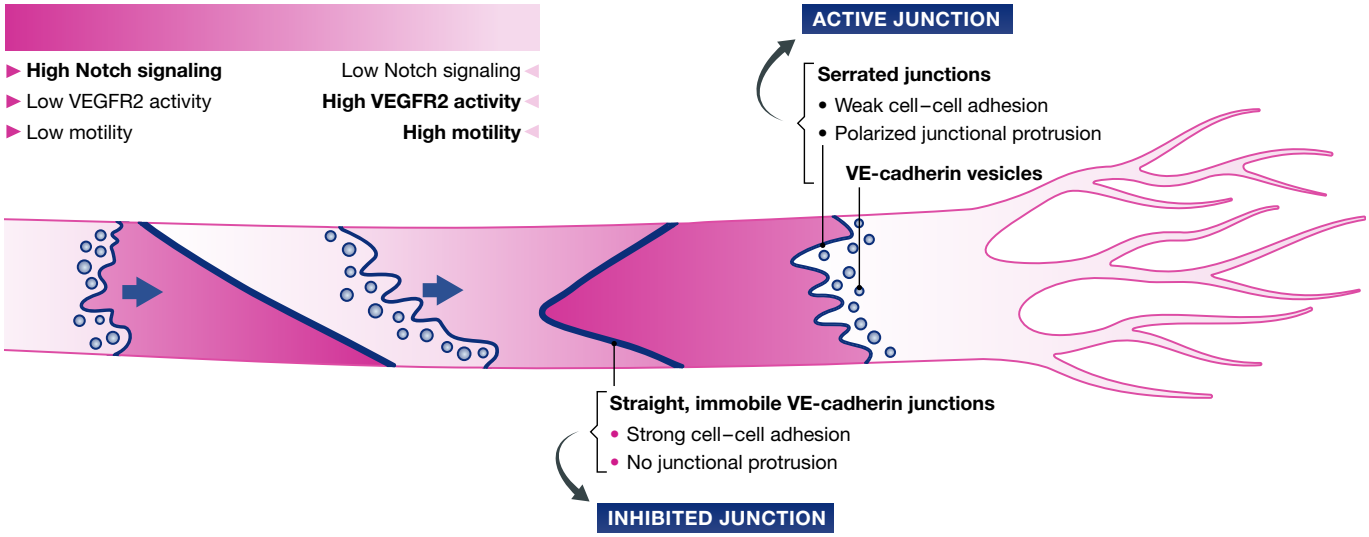

Figure 1. Differential cell–cell adhesions and protrusion drive continuous endothelial rearrangement.
Notch signaling and VEGFR-2 activity throughout angiogenic sprouts are heterogeneous and dynamic, which result in differential VE-cadherin junctional activity and a continuum of intercalated cellular rearrangement. Endothelial cells with active VEGFR-2 signaling and low Notch signaling (no shade) have weak cell–cell adhesion and polarized junctional-cortex protrusions (indicated by serrated VE-cadherin junctions and vesicles). In contrast, cells with high Notch signaling (darker shade) have straight junctions and inhibited protrusions due to immobile VE-cadherin junctions that promote strong cell–cell adhesion. Highly motile endothelial cells are propelled through the sprout via differential adherens junction dynamics between individual cells.

vesicles and higher Dll4 presence correlated with "active junctions" of low adhesion and high protrusion. Furthermore, pathologically high VEGF conditions, such as occurs in glioblastoma and diabetic retinopathy, led to clusters of all-or-none VE-cadherin-mediated junction activities that resulted in minimal cellular rearrangements. Thus, the spatial control and fine-tuning of vessel permeability at the individual cell level, induced by VEGF, appears to be critical to normal sprouting (Siekmann *et al*, 2013).

The implications of this study extend beyond the boundaries of angiogenesis, suggesting both a fundamental mechanism responsible for dynamic rearrangements among cells as well as the importance of such rearrangements in executing multicellular morphogenetic programs. It is easy to imagine how additional variations on these themes could lead to the emergence of other tissue structures. The comprehensive interdisciplinary approach utilized in this study provides a powerful platform for future investigation.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Arima S, Nishiyama K, Ko T, Arima Y, Hakozaki Y, Sugihara K, Koseki H, Uchijima Y, Kurihara Y, Kurihara H (2011) Angiogenic morphogenesis driven by dynamic and heterogeneous collective endothelial cell movement. *Development* 138: 4763 – 4776

Bentley K, Franco CA, Philippides A, Blanco R, Dierkes M, Gebala V, Stanchi F, Jones M, Aspalter IM, Cagna G, Weström S, Claesson-Welsh L, Vestweber D, Gerhardt H (2014) The role of differential VE-cadherin dynamics in cell rearrangement during angiogenesis. *Nat Cell Biol* 16: 309 – 321

Bentley K, Mariggi G, Gerhardt H, Bates PA (2009) Tipping the balance: robustness of tip cell selection, migration and fusion in angiogenesis. *PLoS Comput Biol* 5: e1000549

Hellstrom M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Lindblom P, Alva J, Nilsson AK, Karlsson L, Gaiano N, Yoon K, Rossant J, Iruela-Arispe ML, Kalen M, Gerhardt H, Betsholtz C (2007) Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. *Nature* 445: 776 – 780

Jakobsson L, Franco CA, Bentley K, Collins RT, Ponsioen B, Aspalter IM, Rosewell I, Busse M, Thurston G, Medvinsky A, Schulte-Merker S, Gerhardt H (2010) Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nat Cell Biol* 12: 943 – 953

Lenard A, Ellertsdottir E, Herwig L, Krudewig A, Sauteur L, Belting HG, Affolter M (2013) *In vivo* analysis reveals a highly stereotypic morphogenetic pathway of vascular anastomosis. *Dev Cell* 25: 492 – 506

Levayer R, Pelissier-Monier A, Lecuit T (2011) Spatial regulation of Dia and Myosin-II by RhoGEF2 controls initiation of E-cadherin endocytosis during epithelial morphogenesis. *Nat Cell Biol* 13: 529 – 540

Lobov IB, Renard RA, Papadopoulos N, Gale NW, Thurston G, Yancopoulos GD, Wiegand SJ (2007) Delta-like ligand 4 (Dll4) is induced by VEGF as a negative regulator of angiogenic sprouting. *Proc Natl Acad Sci U S A* 104: 3219 – 3224

Potente M, Gerhardt H, Carmeliet P (2011) Basic and therapeutic aspects of angiogenesis. *Cell* 146: 873 – 887

Siekmann AF, Affolter M, Belting HG (2013) The tip cell concept 10 years after: new players tune in for a common theme. *Exp Cell Res* 319: 1255 – 1263

