



Figure 1. Influence of Notch signaling on angiogenesis and VEGF signaling. (A) Patent blood vessels are exposed to proangiogenic stimuli that initiate the formation of new blood vessels through angiogenesis. However, only a limited number of cells respond to these proangiogenic cues, suggesting the existence of mechanisms by which these behaviours can be reduced in neighboring cells. (B) Similar to the initiation of angiogenesis, throughout sprout development the tip cell continues to migrate while the following connector cell must remain attached to the patent vessel. These distinct behaviours are apparent despite the fact that both cells are subjected to similar proangiogenic signals from the surrounding environment (C and D). Model of tip cell specification and angiogenic sprouting in the zebrafish. (C) Notch ligands DeltaC and Dll4 in the patent blood vessel activate Notch receptors, leading to the downregulation of *vegf receptor-3/flt4* after the initiation of sprouting. These endothelial cells display reduced angiogenic behavior and remain in the patent blood vessel. Those cells with less Notch activation retain *flt4* expression and migrate into the developing sprout. (D) In the developing blood vessel sprout, Notch signaling is activated in the connector cell, leading to the downregulation of *flt4*. The migratory tip cell continues to express *flt4* and *kdr*.

zebrafish embryos lacking Notch signalling exhibit ectopic and persistent *flt4* expression within all endothelial cells^{7,12} while Notch activation completely represses its expression.¹² Taken together, these findings indicate that the Notch signalling pathway limits angiogenic behavior of endothelial cells within developing blood vessel sprouts, in part through the modulation of the Vegf signalling pathway. Interestingly, Notch similarly modulates receptor tyrosine kinase signalling pathways in other developmental processes, most notably in the *Drosophila* tracheal system. In this case, Notch negatively regulates expression of the fibroblast growth factor receptor to limit the contribution of cells to new tracheal branches.¹³ Thus, the negative regulation of receptor tyrosine kinase signalling appears to be a general conserved role for the Notch pathway during tissue morphogenesis.

Given the need to regulate angiogenic sprouting at several different steps, it is likely that Notch signaling is used reiteratively throughout this process (Fig. 1C and D). First, when cells within a patent vessel are exposed to a proangiogenic signal (e.g., Vegf), only a restricted number of cells need to initiate an angiogenic program (Fig. 1C). This makes sense physiologically as the emergence of too many cells from the patent vessel would likely compromise the integrity of the vascular system. The extent of the angiogenic response would be limited by the induction of Dll4 that in turn activates Notch in neighboring cells. Accordingly, Vegf is able to induce *dll4* expression in an experimental setting.^{6,11} In turn, Dll4

positive cells would down-regulate Vegf receptors in neighboring cells through activation of Notch thereby preventing their migration into the developing sprout (Fig 1C). Similarly, during the sprouting process itself, the migratory behavior of connector cells must be limited to retain a patent connection to the original blood vessel (Fig. 1D). In this case, the Dll4-positive tip cell would activate Notch in the adjacent trailing cell, thereby reducing its response to Vegf and again preventing excessive migration and proliferation.

Despite these recent insights, many open questions remain. Notably, what are the molecular effects of Notch activation on the Vegf signalling pathway? Is there a complete inhibition of Vegf signalling in these cells, or does Notch act as a switch to determine the proper cellular output? Given the role of Notch as a direct transcriptional activator, the identification of direct target genes that influence Vegf signalling will be of great interest. An additional question of interest is how cell-selective activation of Notch occurs during the sprouting process, especially given the widespread expression of Notch receptor and ligand transcripts in nearly all endothelial cells. Are there post-transcriptional mechanisms that allow for specific expression of receptor and ligand protein? Are proteins known to play a role in asymmetric Notch activation, such as Numb, playing a role as well? Finally, the studies reviewed here focus mainly on the development of arteries. Indeed, the expression of both Notch receptors and ligands is restricted to arterial endothelial cells.^{12,14,15} This raises the question of what signals coordinate sprouting of venous endothelial cell, as well as lymphatic cells. Will there be different pathways to control this process in veins? Future studies that take advantage of both the mouse and zebrafish models will undoubtedly provide answers to these and other questions surrounding the dynamic relationship between Notch and Vegf during blood vessel development.

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