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BLOOD FLOW SUPPRESSES NOTCH SIGNALLING VIA DLL4 AND IS REQUIRED FOR ANGIOGENESIS IN RESPONSE TO HYPOXIC SIGNALLING

T Chico, O Watson, F van Eeden *University of Sheffield*

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Aims The role of blood flow in angiogenesis is complex. In some situations angiogenesis proceeds in the absence of blood flow, while in others blood flow is required for vessel formation. Transgenic zebrafish embryos allow detailed in vivo visualisation of vascular development by confocal microscopy and developing embryos survive in the absence of blood flow via diffusion. This allows investigation of the interaction between blood flow and angiogenesis in a manner impossible in other models.

We therefore sought to investigate how blood flow influences Notch signalling (a key regulator of angiogenesis) and its contribution to vessel development.

Methods and Results We induced complete cessation of blood flow in developing zebrafish embryos by two methods; morpholino knockdown of cardiac troponin T2 (*tnnt2*) or chemical cardiac cessation by incubation with the myosin ATPase inhibitor BDM. Using a transgenic (*CSL:Venus*) that expresses a reporter at sites of Notch signalling we found absence of blood flow by either method increased vascular Notch signalling (arrowed in Figure 1A). Rt-PCR for a panel of candidate genes showed absent blood flow specifically upregulated

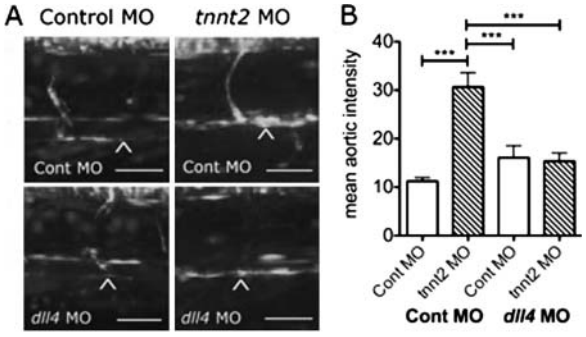

Figure 1

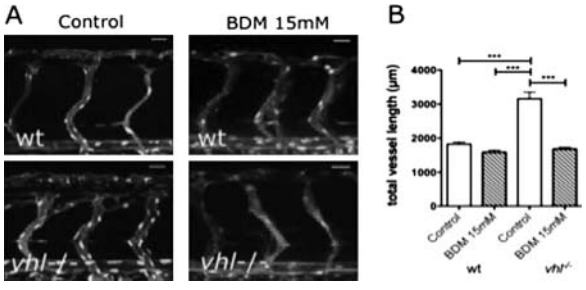

Figure 2

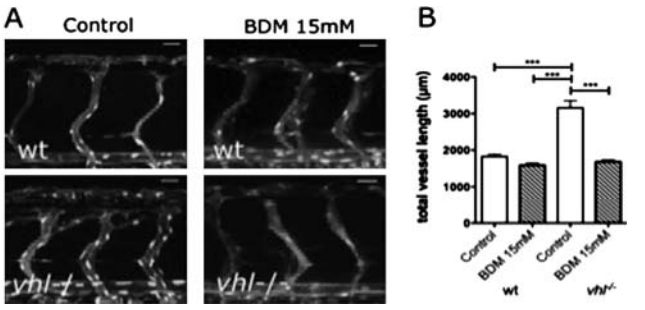

Figure 3

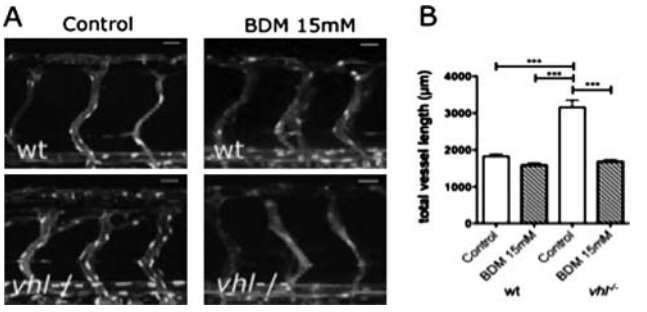

Figure 4

the Notch ligand *dll4* ($p<0.05$), but not other Notch ligands, receptors, or VEGF pathway members. Morpholino knockdown of *dll4* prevented absent blood flow from upregulating Notch signalling in *CSL:Venus* transgenics (Figure 1A and B), confirming blood flow supresses vascular Notch signalling via suppression of *dll4*.

Despite finding that blood flow suppresses Notch signalling, absent blood flow did not affect formation of the trunk vasculature in developing embryos. We therefore examined von hippel lindau (*vhl*) mutants that have constitutively upregulated hypoxic signalling. We generated *vhl* mutants in a double transgenic background (*kdrl:HRAS-mCherry* labels endothelial membrane, *flk1:EGFP-nls*labels endothelial nuclei). Compared with wildtypes, homozygous *vhl* mutants displayed excessive and aberrant angiogenesis with significantly increased endothelial number, vessel diameter and length (Figure 2A). Absence of blood flow by either *tnnt2* knockdown or BDM treatment abolished these pro-angiogenic effects, though normal vessel patterning was preserved. Indicating that the additional vessels of the $vhl^{-/-}$ mutant are flow dependent (Figure 2B).

Conclusions Blood flow suppresses vascular Notch signalling via downregulation of *dll4*. Blood flow is not required for normal vessel patterning but is required for angiogenesis induced by upregulation of hypoxic signalling. These data indicate important differences in hypoxia-driven versus developmental angiogenesis and suggest ways that hypoxia-driven angiogenesis could be manipulated therapeutically.