Platelet-Derived Growth Factors (PDGFs) and Perivascular Cells


Mats Hellstrom

AngioGenetics Sweden AB, Gotenborg, Sweden

The characterization of pdgfB knockout mice demonstrated that PDGF-BB plays a critical role in recruitment of perivascular cells by endothelial cells of blood vessels. The knockout mice die during embryonic development with severe vascular defects and almost complete lack of pericytes surrounding vessels. Blood vessels are dilated, variable in size, and there are many microaneurysms. Undifferentiated mesenchymal cells are induced to differentiate into pericytes during early embryonic life, and then PDGF-BB produced by vascular endothelial cells promotes proliferation and migration of pericytes and smooth muscle cells (SMCs) so that they surround blood vessels.

In order to assess the role of PDGF-BB in adult animals, conditional pdgfB knockouts were generated by engineering loxP sites around the pdgfB gene and using a Tie1 promoter to express cre recombinase in endothelial cells. The efficiency of recombination was assessed by quantitative RT-PCR for pdgfB mRNA on isolated capillary fragments. The recombination rate was found to vary, and in some mice was fairly low, while in others was as high as 90%. Even mice with very low levels of pdgfB mRNA in capillary fragments survived into adulthood. Mice with the conditional pdgfB allele were crossed with a LacZ reporter strain (XlacZ4) in which the pericytes are labeled. In control mice, there was heavy staining in arteries and veins, while in mice with low pdgfB mRNA in capillary fragments, there was marked reduction in coverage of arteries and veins with SMC. Some of these mice developed retinopathy in which some part of the retina were normal, some areas showed dilated vessels and capillary dropout, and some regions showed retinal neovascularization breaking through the internal limiting membrane into the vitreous cavity. Areas of retina showing proliferative changes in the inner retina often showed evidence of traction and rosette formation in photoreceptors and NV growing into the vitreous. This phenotype is similar to that seen in patients with proliferative diabetic retinopathy. The severity of retinopathy correlated with the amount of pericyte loss. The threshold for development of retinopathy appeared to be reduction of pericytes coverage by about 50% or less.

Angiogenic sprouts contain specialized endothelial cells called tip cells. They are migratory, nonproliferative, and have numerous long filopodia and a distinct pattern of gene expression. Adjacent to tip cells are stalk cells, which proliferate and form the lumen of the vessel. VEGF is the cue that guides tip cells to migrate into the tissue.

The γ-secretase protease complex is best known in relation to APP in Alzheimer’s disease, but it is also essential for notch signaling. When postnatal day (P3)-P5 mice were treated with a γ-secretase inhibitor (DAPT), there was a distinct increase in microvascular density in the peripheral retina. Mice treated at P2-P6 showed numerous filopodia extensions and sprouting from the vascular network giving the appearance of branching and fusion of
vessels. There was a doubling of the number of tip cells per unit area. This suggests that γ-secretase inhibits the development of tip cells.

To test whether the effect of DAPT was due to a direct effect on endothelial cells, its effect was tested on HUVECs cultured in collagen gels. When HUVECs are placed in collagen gels, they start to sprout within 24h and the sprouts can be quantified. The number of sprouts are increased by addition of VEGF or DAPT, and the effects of VEGF and DAPT appeared additive.

In DAPT-treated neonatal mice, pericytes recruitment appeared normal, although in regions of very high vessel density, interactions between pericytes and endothelial cells appeared disturbed. The astrocyte networks in front of the developing vascular network didn’t seem to be affected. VEGF-A protein levels are not altered. In the mouse model of oxygen-induced ischemic retinopathy, new vessel tufts sprout from veins in regions bordering avascular zones. Treatment with DAPT resulted in a reduction in the size of avascular zones, doubling vascular density and reducing vascular tuft formation by one-third of that in controls.

In summary, DAPT treatment promotes tip cell formation, endothelial cell proliferation, and branching. A hypothesis would be that a signal from the tip cell to the stalk cell, possibly a Delta4/notch signal, could be important for notch-mediated lateral inhibition of the stalk cells. The combination of the presented and published data supports this hypothesis. (1) γ-secretase is essential for notch signaling, (2) γ-secretase inhibitors can promote endothelial cell proliferation by notch inhibition, (3) the tip cells express Delta4, (4) cell autonomous notch signaling regulates endothelial cell branching during tubule formation, and the number of tip cells are increased when notch is inhibited.

The VEGF gradient is important for maintaining a tip cell phenotype, and it is known that VEGF signaling can trigger Delta4 expression via notches 1 and 4. Notch signaling can suppress VEGFR2 signaling. In tip cells there is high Delta 4 and VEGFR2 expression, while in stalk cells there is no Delta4-like expression and low VEGFR2 expression. This suggests that lateral inhibition could take place. Similar cells are exposed to VEGF, but a future tip cell is closer to the VEGF source, which would cause it by the mechanism described above to suppress the tip cell phenotype in the neighboring cell so that it becomes a stalk cell. Both VEGF-A and Delta4-like knockouts are haploinsufficient; they are extremely dose sensitive. In Drosophila trachea development, FGF2 induced tip cell and notch signaling is important for differentiation of stalk cells.

VEGFR2 signaling is important for chemotaxis. There are important repulsive signals mediated by netrin and semaphorins, and as suggested above, there may also be notch-mediated lateral inhibition from tip cells to stalk cells.