Fetal neurogenesis: breathe HIF you can

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Microvascular circulation creates a supporting niche for neurogenesis through the secretion of angiocrine factors. The emerging concept that energy balance and metabolic status play a role in the modulation of stem cells suggests that oxygen delivery by nearby capillary vascular beds could also regulate neurogenesis. Blood vessel formation and neuron production proceed in a coordinated fashion in the developing cerebral cortex, providing a unique opportunity to test the possibility that oxygen supply regulates cell fate decisions in neurogenic niches. The interesting study by the Carmeliet laboratory yields evidence that this is indeed the case and identifies HIF-1 α as the central element.

See also: C Lange *et al* (May 2016)

All neurons in the central nervous system (CNS) derive from a pseudostratified neuroepithelium that lines what will later become the cerebral ventricles and the central canal of the spinal cord. In the telencephalon, the onset of neurogenesis at around embryonic (E) days E9.5–E10.5 coincides with the acquisition of glial features by neuroepithelial cells. Radial glia (RG) are elongated cells with thin cytoplasmic extensions spanning the entire thickness of the developing brain parenchyma; however, their cell bodies are retained next to the ventricular lumen, where they divide. As neurogenesis progresses RG gradually switch from expansion to asymmetrical differentiative divisions to self-renew and generate neuronally committed progeny, which includes either a neuron that migrates away to populate the growing cortical plate or a basal progenitor (BP), which allocates to the immediately adjacent subventricular

zone to act as a restricted neuronal progenitor (Florio & Huttner, 2014). Proper cortical development requires controlled proliferation of the RG population and a timely switch from RG expansion to the production of neurons and BP.

The development of a cortical vascular network is also a complex process that occurs coincidentally with neurogenesis (Quaegebeur *et al*, 2011). Vasculogenesis of a primitive perineural plexus around the neural tube by mesoderm-derived angioblasts is followed by angiogenic sprouting of new vessels that invade the brain parenchyma. Surface pial vessels surround the entire brain by E9.5. In contrast, periventricular vessels form a lattice situated half way between the ventricular and pial surfaces. The ventro-lateral to dorso-medial gradient of periventricular vessel formation in the cortex matches that of the neurogenic gradient, but blood vessel formation precedes neurogenesis by about a day (Vasudevan *et al*, 2008).

The study presented by Lange and colleagues initially draws the attention to the intriguing temporal and spatial correlation between the ingrowth of blood vessels in the developing cortex and the switch from RG expansion to neuronal production (Lange *et al*, 2016). The authors provide visual congruence of angiogenic progression along the lateral-to-dorsal gradient with parallel induction of RG neurogenic activity between E10.5 and E12.5. Establishment of the intraparenchymal vascular bed by E12.5 is accompanied by expansion of the BP population and colonization of the vascularized region by newly generated neurons (i.e., Ki67-negative cells that had incorporated EdU at E11.5) basically absent in avascular cortical areas of earlier embryos. Moreover, the authors show the same spatial-temporal

correlation in ferrets, an indication that this relationship could also apply to gyrencephalic mammals like humans.

Lange and colleagues then turn to a genetic model in which blood vessel formation is selectively impaired in the CNS to functionally investigate the potential cross talk between angiogenesis and neurogenesis. Gpr124 (tumor endothelial marker 5, TEM5), a wnt7a/b-specific co-activator of wnt signaling selectively expressed by endothelial cells and pericytes in the developing brain, is required for prenatal CNS angiogenesis. Gpr124 mutants (GPR124^{KO}) fail to develop a periventricular vascular plexus of luminized vessels and die at midgestation. At E13.5, absence of Gpr124 results in disorganized vascular tufts, unable to supply enough oxygen as monitored with the hypoxia marker pimonidazole. In the hypoxic mutant environment, the RG population is abnormally expanded and lower proportions of neurogenic BP and newborn neurons (i.e. Ki67-negative cells that had incorporated EdU at E12.5) are detected. Similar results are obtained after selective deletion of Gpr124 in endothelial cells following exposure of PDGFB-Cre^{ERT2};Gpr124^{KO/lox} E10.5 embryos to tamoxifen. Rather than caused by a change in BP fate or in neuronal survival, the lack of neurons is explained by proliferating RG cells failing to commit to the production of neurogenic BP. These results show that suppression of angiogenesis and/or hypoxia increases RG expansion and reduces neurogenesis.

To unveil the underlying mechanisms, Lange and colleagues compare the global expression profile of sorted Gpr124 wild-type and mutant RG. Gene ontology and Ingenuity analyses on RNA-Seq data reveal a significant and robust upregulation of genes

Figure 1. Implication of hypoxia and HIF-1 α in angiogenesis and the switch from RG expansion to neurogenesis during cortical development.

At embryonic stage E10.5, the absence of blood vessels results in a hypoxic environment that induces and maintains elevated levels of HIF-1 α in RG cells. In these conditions, there is an expansion of this population caused by predominant symmetrical divisions, while the presence of HIF-1 α prevents their premature commitment to produce differentiated progeny. At E13.5, however, the growth of blood vessels induced by HIF-1 α leads to an increase on tissue oxygen levels. As a result, HIF-1 α is degraded relieving its inhibitory pressure on neurogenesis. Therefore, hypoxia and HIF-1 α create a regulatory loop that ensures initial RG expansion during early cortical development and subsequent induction of angiogenesis promotes a switch to RG asymmetrical neurogenic divisions allowing the generation of neurons.

involved in glycolysis, angiogenesis, and proliferation including several targets of HIF-1 α and HIF-2 α , accompanied by down-regulated expression of neurogenic genes. Subsequent biochemical analyses demonstrate a strong induction of HIF-1 α and some of its targets in the avascular areas. Indeed, the authors demonstrate that the hypoxic environment that precedes angiogenesis maintains elevated levels of HIF-1 α which subsequently decrease as growing blood vessels oxygenate the tissue.

Mechanistic studies are then undertaken to provide conclusive evidence on how oxygen-related HIF-1 α activity regulates neurogenesis. Loss-of-function experiments by HIF-1 α partial deletion in RGs and gain-of-function approaches by *in utero* electroporation of either a wild-type or a transcriptionally dead HIF-1 α reveal a dual role of this transcription factor in cortical development. First, presence of HIF-1 α seems to be required for blood vessel formation, since angiogenesis is severely impaired in its absence. Second, elevated HIF-1 α transcriptional activity maintains RG in a proliferating state preventing their commitment to differentiation. Therefore, this model places HIF-1 α in the center of a self-regulatory

loop where expression of HIF-1 α induced by low oxygen levels ensures the expansion of RG. Then, HIF-1 α itself triggers the initial invasion of blood vessels that oxygenate the tissue causing its own degradation. Subsequent downregulation of HIF-1 α target genes leads to the formation of neurogenic BP and cortical neurons. The model is nicely confirmed since exposing Gpr124 KO embryos to hyperoxia between E10.5 and E13.5 not only reduces HIF-1 α levels, but also restores RG neurogenic differentiation. Finally, the authors show that, in RG, the hypoxia-inducible activator of the rate-limiting glycolytic enzyme phosphofructokinase-1, Pfkfb3 plays a critical role in maintaining their glycolytic metabolism and expansion after HIF-1 α stabilization and how its downregulation accelerates the initiation of neurogenesis.

Evidence accumulated in recent years definitely places vasculature as a major contributor to stem cell niches (Rafii *et al*, 2016). The present study elegantly demonstrates that, in addition to endothelium-derived signaling molecules, blood vessels can regulate cell fate decisions through oxygen supply. Many adult stem cells inhabit relatively hypoxic niches and are

glycolytic, whereas more restricted progenitor cells rely on oxidative phosphorylation for the production of ATP (Mohyeldin *et al*, 2010; Burgess *et al*, 2014). The switch between glycolysis and oxidative phosphorylation could be controlled by differentiation programs. The data by Lange *et al* (2016) support the interesting complementary concept that oxygen availability and related metabolism can regulate cell specification during tissue growth.

References

- Burgess RJ, Agathocleous M, Morrison SJ (2014) Metabolic regulation of stem cell function. *J Intern Med* 276: 12–24
- Florio M, Huttner WB (2014) Neural progenitors, neurogenesis and the evolution of the neocortex. *Development* 141: 2182–2194
- Lange C, Turrero García M, Decimo I, Bifari F, Eelen G, Quaegebeur A, Boon R, Zhao H, Boeckx B, Chang J, Wu C, Le Noble F, Lambrechts D, Dewerchin M, Kuo CJ, Huttner WB, Carmeliet P (2016) Relief of hypoxia by angiogenesis promotes neural stem cell differentiation by targeting glycolysis. *EMBO J* 35: 924–941
- Mohyeldin A, Garzón-Muvdi T, Quiñones-Hinojosa A (2010) Oxygen in stem cell biology: a critical

- component of the stem cell niche. *Cell Stem Cell* 7: 150–161
- Quaegebeur A, Lange C, Carmeliet P (2011) The neurovascular link in health and disease: molecular mechanisms and therapeutic implications. *Neuron* 71: 406–424
- Rafii S, Butler JM, Ding BS (2016) Angiocrine functions of organ-specific endothelial cells. *Nature* 529: 316–325
- Vasudevan A, Long JE, Crandall JE, Rubenstein JL, Bhide PG (2008) Compartment-specific transcription factors orchestrate angiogenesis gradients in the embryonic brain. *Nat Neurosci* 11: 429–439