**Fibrinogen triggered signaling pathways modify stem cell behavior in central nervous system disease**

Jia-Di Lin, Yu-Hsuan Chu, Suvra Nath, Christian Schachtrup*

Neural stem/precursor cells (NSPCs) hold great promise in improving central nervous system (CNS) repair, either by triggering endogenous NSPC sources of the CNS or by transplantation of NSPCs. The molecular mechanisms of NSPC survival and integration as well as their cell fate determination are still insufficiently understood, yet will be instrumental for harnessing these cells for brain repair. In our recent *Nature Communications* manuscript entitled “Fibrinogen induces neural stem cell differentiation into astrocytes in the subventricular zone via BMP signaling” (Pous et al., 2020), we advanced towards understanding how CSF signaling directs the brain subventricular zone (SVZ) stem cell niche environment by the disease-triggered deposition of blood-derived factors and how these factors regulate NSPC fate and brain repair. Here, we summarize the relevance of our original findings for NSPC biology in CNS disease as well as possible implications for other CNS stem cell niches and other CNS diseases. Also, we discuss how current knowledge can be applied to control NSPC fate and functions tailored to promote CNS repair.

**Fibrinogen invades neural stem cell niche environment:** In the healthy rodent brain, astrocyte-like type B stem cells from the SVZ stem cell niche gives rise to immature, doublecortin+ young neurons that migrate long-distance to the olfactory bulb and contribute to fine odor discrimination and odor-reward association (Obernier et al., 2018). CNS injury or diseases, such as stroke, multiple sclerosis and trauma trigger the endogenous adult NSPCs originating from the SVZ to proliferate, redifferentiate and migrate towards the lesion area and preferentially differentiate into glial cells or remain in a precursor state contributing to brain repair (Bennet et al., 2013; Bohrer et al., 2015; Pous et al., 2020). The identity of extracellular factors that trigger astroglial differentiation over neurogenesis both within and outside the stem cell niche during CNS disease was unknown. The SVZ contains an extensive planar vascular plexus with NSPCs poised to receive spatial cues and regulatory signals from the vascular system. B1 cells contact blood vessels and receive multiple signals from the vascular plexus that regulate their renewal and differentiation. Indeed, the SVZ vascular system is already permeable for circulating small molecules (sodium fluorescein, 376 Da) under homeostatic conditions (Takazoe et al., 2008). Surprisingly, we showed that the blood-derived coagulation factor fibrinogen, a 340-kDa protein secreted by hepatocytes in the liver and present in the blood circulation at 3–5 mg/mL, deposited massively inside the entire SVZ stem cell niche after a distant cortical injury and altered the stem cell niche environment (Figure 1). Extravascular fibrinogen surrounding CD31+ blood vessels in the SVZ stem cell niche after cortical photobleb or injury (a mouse model for ischemia) or after stroke/thrombosis or injury (a mouse model for mild traumatic cortical injury), suggesting increased leakiness of the specialized SVZ vasculature after brain injury. We also detected extracellular fibrinogen in the human SVZ stem cell niche after stroke, suggesting that increased SVZ vascular leakage is a general feature in stroke. The stem/precursor cell-vasculature communication is also a hallmark of NSPCs in the subgranular zone of the hippocampus, which generate new excitatory neurons important for learning, memory and pattern separation, as well as oligodendrocyte precursor cells (OPCs), which generate new oligodendrocytes important for successful myelin regeneration in multiple sclerosis. Our data suggest that increased vascular permeability with fibrinogen extravasation into the niche environment is a critical event modulating SVZ stem cell behavior, which might pertain for different stem cell and progenitor cell types outside of the SVZ at sites of vascular extravasation.

**Fibrinogen instructing NSPC differentiation into astrocytes:** Neuropathological effects of fibrinogen in the CNS are numerous, such as triggering microglial activation, induction of astrocyte scar formation, inhibition of neurite outgrowth and blocking OPC differentiation and remyelination (Petersen et al., 2018); however, how fibrinogen orchestrates NSPC-niche cell communication remains unclear. Our data showed that fibrinogen induces NSPC differentiation into astrocytes via BMP receptor signaling. BMP signaling in the adult SVZ and subgranular zone is neurogenic at basal levels, while an increased magnitude of BMP signaling promotes astrogenesis and completely blocks OPC differentiation (Petersen et al., 2017). Fibrinogen acts as a multi-faceted signaling molecule by interacting with integrins and non-integrin receptors and by functioning as a carrier of growth factors and regulating their bioavailability. We showed that fibrinogen-triggered activation of the BMP signaling pathway in NSPCs and OPCs occurred independently of fibrinogen-bound BMP. Fibrinogen through its αC domain-β1-integrin binding enhances BMP type I receptor (BMPR I) association in lipid rafts to activate BMP signaling in a ligand independent manner and directs lineage specification of SVZ NSPCs. BMPR I subtype activation has been demonstrated to define reactive astrocyte functionality. Yet, we have not defined the BMPR I subtype that fibrinogen utilizes to induce SVZ NSPC differentiation into astrocytes, but our data indicated that fibrinogen activates the ACVR1 receptor for the formation of astrocyte-like cells from NG2+ OPCs (Petersen et al., 2017). Thus, fibrinogen might inhibit neurogenesis and remyelination via a BMP-dependent cell fate switch of neuronal and oligodendrocyte progenitors, respectively. Future studies will further elucidate the identity of the fibrinogen-activated BMPR I subtype in NSPC populations and the functionality of fibrinogen-activated newborn astrocytes. In the hippocampal stem cell niche, BMPR signaling is required to balance NSPC quiescence/proliferation and to prevent loss of the stem cell activity that supports continuous neurogenesis. Analysis of vascular permeability in aging and in patients with mild cognitive impairment revealed that progressive blood-brain barrier breakdown in the hippocampus may contribute to early stages of dementia associated with Alzheimer’s disease (AD). Fibrinogen is deposited in the AD brain and its depletion protects from cognitive decline in animal models of AD (Sweeney et al., 2018). It will be important to test whether fibrinogen is, as our study showed for SVZ NSPCs, an important modulator of hippocampal NSPC fate via activating the BMPR signaling pathway in AD. Together, it will be necessary to biochemically describe the fibrinogen-BMPR I subtype interaction in different NSPC populations, to enable targeted manipulation of fibrinogen-induced signaling pathways in neural stem cells.

**Transcriptional control of adult SVZ NSPC differentiation into astrocytes:** Id proteins (Id1–4) are helix-loop-helix proteins that function as dominant-negative regulators of basic helix-loop-helix (bHLH) transcription factors. The Id-bHLH association has been implicated in several physiological processes including cell proliferation, differentiation and apoptosis in various cell types (Ruzinova and Benezra, 2003). Id protein expression is triggered and stabilized by different extracellular stimuli, such as by the TGF-β superfamily, cytokines and growth factors and thus Id proteins are central in transiting environmental changes into cellular responses. In the developing brain, Id proteins and their interacting bHLH factors are tightly regulated and play crucial roles for neural stem cell self-renewal and differentiation into neurons, astrocytes, and oligodendrocytes (Imayoshi and Kageyama, 2014). In the adult brain, high levels of Id proteins are found in the neurogenic niche and define stemness while their functions during CNS injury and diseases are poorly characterized. We showed that Id3 expression in the SVZ NSPC niche is strongly elevated by fibrinogen deposition after injury. Depleting Id3 or blocking the BMPR I inhibit the fibrinogen-induced NSPC differentiation into astrocytes, indicating the fibrinogen-mediated astrogligenesis is dependent on the BMP-Id5 axis (Pous et al., 2020). In our previous study, we showed that Id3 expression is regulated by multiple signaling pathways in astrocytes. In our recent work, we demonstrate the transcriptional regulation of astrocyte-specific genes, including GAFAP and GLAST (also known as S1c1a3) by the bHLH protein E47 (Bohrer et al., 2015). Intriguingly, besides GAFAP and GLAST, we also found the Id-E47-dependent expression of several solute carrier (SLC) family members increased in NSPCs after BMP treatment (S1c1a2, S1c2a18, S1c38a3, S1c39a14, S1c7a11) (Additional Figure 1) (Bohrer et al., 2015), or after MOG33-35 induced experimental autoimmune encephalomyelitis (S1c12a5, S1c39a2, S1c8a2) (Chu et al., unpublished data). The involvement of these SLC family members in glutamate transport, intracellular ion balance, as well as vesicle transportation suggests another potential role of Id proteins in regulating cellular homeostasis and metabolism under inflammatory conditions. In addition, studies in tumor cells have indicated that Id proteins induce the release of VEGF, GROα (also known as CXCL1) and IL-8, thus promote tumor neo-angiogenesis and contribute to the dissemination of brain tumor malignancy. Therefore, environmental stimulation modifying Id protein expression in NSPCs may result in an altered...
Fibrinogen drives the axonal and neuronal regeneration mechanisms in CNS diseases.

**References**

Bennet DJ, Luciano D, Jo R, Abd K, Paez-Gonzalez P, Sheng H, Benner DS, Lu C, Englova C, Iuc CT (2013) Protective autostimulation from the SV2 niche after injury is controlled by Notch modulator Thylid. Nature 497:369-373.

Bohr H, Pflir S, Mamdahzada K, Schildge S, Riede S, Herbert L, Hils M, Pou S, Rauch KS, Durmit I, Pfeifer D, Dengel J, Kirsch M, Schachtur C, Schachtur C (2015) The balance of Id3 and E47 determines neural stem/progenitor cell differentiation into astrocytes. EMBO J 34:2804-2819.

Imayoshi I, Kageyama R (2014) BHLH factors in self-renewal, multipotency, and fate choice of neural progenitor cells. Neuron 82:9-23.

Martino G, Pluchino S (2006) The therapeutic potential of stem cells in neurodegenerative diseases. Nat Rev Neurosci 7: 395-406.

Morton MC, Neoklezi VN, Seluzicki CM, Holberg JC, Feliciano DM (2018) Neonatal subventricular zone neural stem cells release extracellular vesicles that act as a microglial morphogen. Cell Rep 23:79-89.

Mosher L, Anders RH, Fukushima T, Bieri G, Hasegawa-Moriyama M, Hey G, Guzman R, Wyss-Coray T (2012) Neural progenitor cells regulate microglial function and activity. Nat Neurosci 15: 1485-1493.

Obenier N, Cebran-Silla A, Thomson M, Parraggia J, Anderson R, Guinto C, Rodas Rodriguez J, Garcia-Verdugo JM, Alvarez-Buylla A (2018) Adult neurogenesis is sustained by symmetric self-renewal and differentiation. Cell Stem Cell 22:221-234.

Peruzzotti-Jametti L, Bernardst JD, Vicario N, Costa AS, Kwok CK, Leonardi T, Bloyt LM, Bico I, Balazrtz B, Volpe G, Mallucci G, Manfettet K, Hallek J, Westendorf J, Fry IG, Hallenbeck JM, Murphy MP, Edenhofe F, Frezza C, Pluchino S (2018) Macrophage-derived extracellular vesicles direct oligodendrocyte differentiation to support CNS neuroinflammation. Cell Stem Cell 22:355-368.

Petersen MA, Ryu JK, Chang KJ, Etessabyrin A, Barchele L, Mendola AS, Kamau-Devers W, Fancy SP, Thor A, Bushong EA, Baeza-Raja B, Syme CA, Wu MD, Rios Coronado PE, Meyer-Franke A, Yahn S, Pou S, Lee JK, Schachtur C, Lassmann H, et al. (2017) Fibrinogen activates BMP signaling in oligodendrocyte progenitor cells and inhibits inflammation after vascular damage. Nature 546:1009-1012.

Pous S, Deschande SS, Nath S, Meexy S, Malik SC, Sch indictment L, Bohrer C, Topp K, Pfeifer D, Fernández-Klett F, Drost-Burts S, Gallekts D, Dikker V, Akassoglou K, Schachtur C (2010) Fibrinogen induces immature neural stem cell differentiation into astrocytes in the subventricular zone via BMP signaling. Nat Commun 11:630.

Ruziñova MB, Ben Ezra R (2003) Id proteins in development, cell cycle, and cancer. Trends Cell Biol 13:410-418.

Schachtur C, Lu P, Jones L, Lee RE, Lu J, Lucas BD, Zhong B, Akassoglou K (2007) Fibrinogen activates integrin alpha-v beta 3 in mesencephalic rat and human neural progenitors. J Neurosci Res 85:1739-1748.

Tavares M, Van der Velden L, Silva-Vargas V, Louisaint M, Colonna L, Zaidi B, Garcia-Verdugo JM, Doetsch F (2008) A specialized vascular niche for adult neural stem cells. Cell Stem Cell 2:279-288.
Additional Figure 1: Proposed model for tailored NSPCs ameliorating inflammation and promoting neuronal regeneration in CNS disease.

(A) In the healthy brain, NSPCs of the SVZ continuously generate mobile DCX+ neuroblasts that migrate through the rostral migratory stream to the olfactory bulb to become newborn interneurons. Cortical injury results in increased SVZ vasculature permeability and fibrinogen deposition into the SVZ stem cell niche environment. Fibrinogen induces astrogliogenesis via the BMP–Id3 axis, regulating a group of genes belonging to the SLC family, including SLCA3 (GLAST), and Slc1a2 (GLUT1). Local provisional fibrinogen thus activates BMP signaling in NSPCs inducing their differentiation into astrocytes at sites of vascular permeability in the CNS.

(B) Fibrinogen is massively deposited in the brain or spinal cord parenchyma in CNS disease with BBB opening. In our working model we propose that fibrinogen induces human iPSC-derived glial precursor cell differentiation into reactive astrocytes via activation of the BMPRI – Id3 axis. We propose to manipulate fibrinogen-induced signaling pathways in human iPSC-derived glial precursor cells to control their differentiation and their cytokine/extracellular vesicle secretion reducing neuroinflammation and neurodegeneration. BBB: Blood-brain barrier; BMP: bone morphogenetic protein; BMPRI: BMP receptor type I; CNS: central nervous system; iPSC: Induced pluripotent stem cell; Id: Inhibitor of DNA binding protein; NSPCs: neural stem/precursor cells; SLC: the solute carrier; SVZ: subventricular zone.