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

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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 CNS disease alters 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, its 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, redirect their migration path towards the lesion area and preferentially differentiate into glial cells or remain in a precursor state contributing to brain repair (Benner et al., 2013; Bohrer et al., 2015; Pous et al., 2020). The identity of extracellular factors that trigger astrogliogenesis 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 (Tavazoie 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 surrounded CD31$^+$ blood vessels in the SVZ stem cell niche after cortical photothrombosis (a mouse model for ischemic stroke) as well as after stab wound 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 of 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-induced NSPC-derived 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 begins in the hippocampus and 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 cortical injury. Depleting Id3 or blocking the BMPR I inhibit the fibrinogen-induced NSPC differentiation into astrocytes, indicating the fibrinogen-mediated astrogliogenesis is dependent on the BMP-Id3 axis (Pous et al., 2020). In our previous study, we showed that Id3 prevents the transcriptional regulation of astrocyte-specific genes, including GFAP and GLAST (also known as Slc1a3) by the bHLH protein E47 (Bohrer et al., 2015). Intriguingly, besides GFAP and GLAST, we also found the Id-E47-dependent expression of several solute carrier (SLC) family members increased in NSPCs after BMP treatment (Slc1a2, Slc25a18, Slc38a3, Slc39a14, Slc7a11) (**Additional Figure 1**) (Bohrer et al., 2015), or after MOG33-35 induced experimental autoimmune encephalomyelitis (Slc12a5, Slc39a2, Slc8a2) (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 upon environmental alteration. 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 disease malignancy. Therefore, under variant environmental stimulation modifying Id protein expression in NSPCs may result in an altered

cellular physiology, metabolic processes or cytokine/extracellular vesicle secretion.

Therapeutic usage of modified NSPCs for brain repair: Inflammation is a hallmark of different CNS pathologies and the hostile inflammatory microenvironment of transplanted NSPCs severely affects their survival and integration, leading to a poor outcome in tissue repair. Importantly NSPCs, beside their regenerative and restorative capacity, regulate the functional aspects of microglial cells. NSPCs of the SVZ revealed a secretory protein profile distinct from other brain cells modulating microglial activation, proliferation and phagocytosis. NSPC-derived VEGF was necessary and sufficient to exert at least some of these effects in mice (Mosher et al., 2012). Recently, it was recognized that NSPCs secrete extracellular vesicles, which encase proteins and microRNAs, which act as morphogen to preferentially target microglia regulating their morphology and immune responses (Morton et al., 2018). Additionally, under chronic inflammatory condition, NSPCs ameliorate neuroinflammation by detecting the extracellular succinate released by mononuclear phagocytes. The uptake of extracellular succinate, which is released by the inflammatory mononuclear phagocyte, upregulates the succinate co-transporter of the SLC family in the NSPCs and triggers the secretion of prostaglandin E2 by the NSPCs with consequential anti-inflammatory effects (Peruzzotti-Jametti et al., 2018). Compelling evidence from experimental animal disease models and early-phase clinical trials identifies the transplantation of NSPCs as a viable path towards the development of clinically applicable exogenous stem cell therapies. The contemporary view is that transplanted NSPCs act as local 'source' capable of producing and secreting a wide array of immune and neurotrophic factors (Martino and Pluchino, 2006). Therefore, focusing on reciprocal interaction between differentially activated states of NSPCs and microglia under homeostatic and pathological condition will lead to a better understanding of interactive cellular and molecular pathways involved aiming to identify novel therapeutic targets for mobilizing, tailoring and modulating NSPC based stem cell therapy, while suppressing the aberrant inflammatory effects of microglia.

Concluding remarks: The discovery that the blood-derived coagulation protein fibrinogen is key in regulating the differentiation of NSPCs into astrocytes following cortical brain injury has potential implications for multiple CNS disease processes in different stem cell niches. The transplantation of induced pluripotent stem cells (iPSCs), which can be generated from adult somatic cells and differentiated into NSPCs, provides therapeutic effects by replacement of lost neurons and severed axons and via creation of a permissive microenvironment to promote CNS tissue repair. However, the unfriendly environment of the lesioned CNS may cause an unfavorable cell differentiation of transplanted human iPSC-derived precursor cells. We showed a robust and sustained fibrinogen deposition at the injury site in mouse and rat animal models of traumatic injury (Schachtrup et al., 2007) and we showed a fibrinogen-induced NSPC differentiation into reactive astrocytes (Pous et al., 2020). Accordingly, improved protocols for manipulating human iPSC differentiation and functionality into desired cell types resistant to the unfriendly environment and with an identity ameliorating inflammation and promoting regeneration are needed. To this end, using small molecule inhibitors and CRISPR/Cas9 technology to interfere with BMP signaling by blocking or deleting Id proteins may help address this issue without affecting important functions of the BMP signaling pathway in stem cell proliferation and differentiation (**Additional Figure 1**). Indeed, our studies showed that the BMP–Id3 axis regulates members of the SLC family and targeting Id proteins in iPSC-derived precursor cell grafts might prohibit their differentiation into reactive astrocytes and modulate their cytokine/extracellular vesicle secretion reducing neuroinflammation and neurodegeneration (**Additional Figure 1**). Our study suggests that manipulation of the fibrinogen-induced transcriptional regulator Id3 in human iPSCs could control their fate and functions tailored to promote CNS repair.

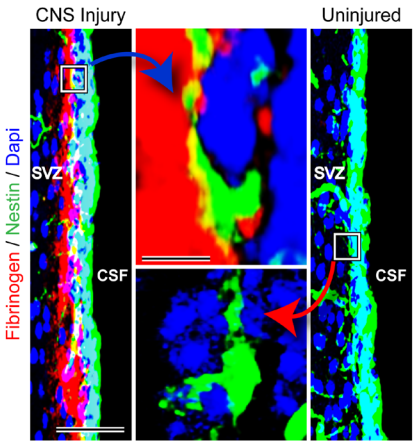

Figure 1 | Fibrinogen invades neural stem cell niche environment.
The blood-clotting protein fibrinogen (red) is deposited in the stem cell niche 5 hours after photothrombotic ischemia (PT day 0) and regulates the contribution of stem cells (Nestin^{+}, green) to repair mechanisms in central nervous system diseases. Scale bars: 36 µm, left and right and 5 µm, enlargements (middle). CNS: Central nervous system; CSF: cerebrospial fluid; SVZ: subventricular zone. Reprint from Pous et al. (2020).

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Additional file:
Additional Figure 1: *Proposed model for tailored NSPCs ameliorating imflammation and promoting neuronal regeneration in CNS disease.*

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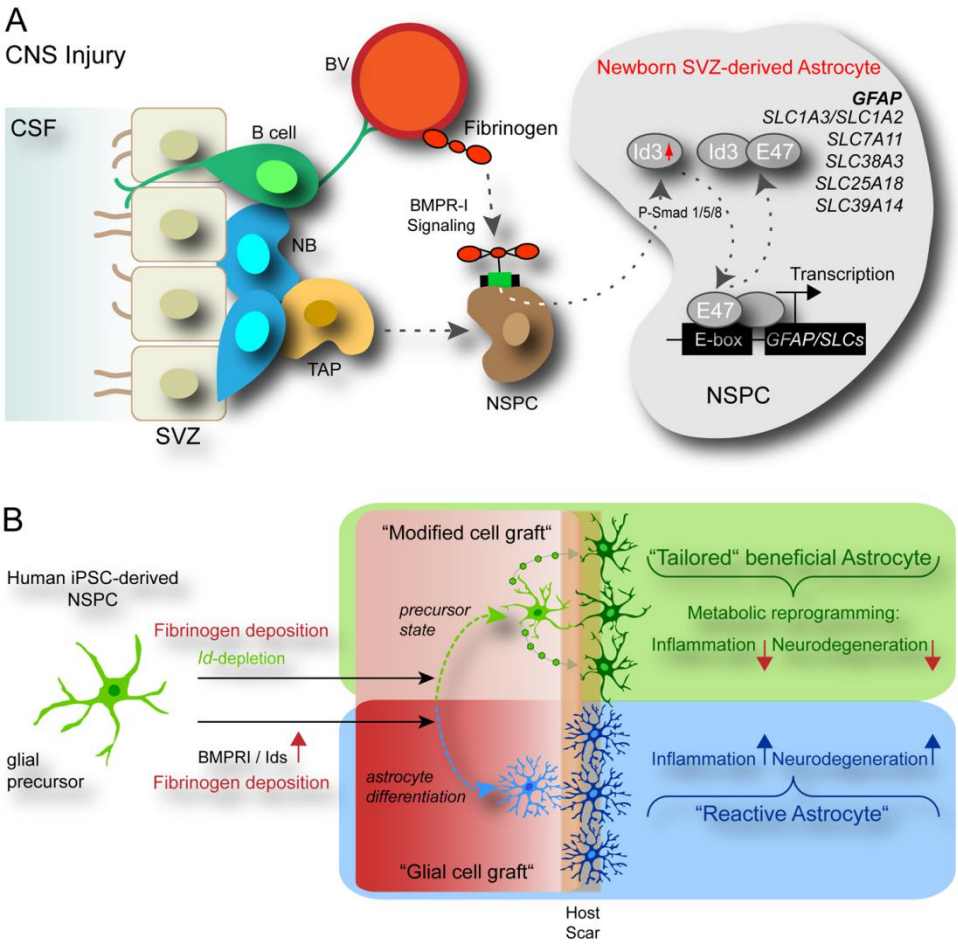

Additional Figure 1: Proposed model for tailored NSPCs ameliorating imflammation and promoting neuronal regeneration in CNS disease.

(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.