The stem cell potential of glia: lessons from reactive gliosis

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Abstract | Astrocyte-like cells, which act as stem cells in the adult brain, reside in a few restricted stem cell niches. However, following brain injury, glia outside these niches acquire or reactivate stem cell potential as part of reactive gliosis. Recent studies have begun to uncover the molecular pathways involved in this process. A comparison of molecular pathways activated after injury with those involved in the normal neural stem cell niches highlights strategies that could overcome the inhibition of neurogenesis outside the stem cell niche and instruct parenchymal glia towards a neurogenic fate. This new view on reactive glia therefore suggests a widespread endogenous source of cells with stem cell potential, which might potentially be harnessed for local repair strategies.

The potential of glia to act as stem or progenitor cells has only been recognized for about a decade[1–6]. Previously, glia had been viewed as differentiated support cells in the adult brain. However, adult neural stem cells (NSCs) in the two major regions for adult neurogenesis — the subependymal zone (SEZ) and the subgranular zone (SGZ) of the dentate gyrus — were surprisingly identified as cells of glial identity, as discussed further below[2,6–8]. Subsequently, it was shown that radial glia — the ubiquitous glial cell type during development of all vertebrate brains — also act as stem and progenitor cells, and are at the source of many, if not most, neurons in the mouse brain[1,4,5].

Whereas radial glia are largely absent from the adult mammalian brain[8], they persist into mature stages in many other vertebrates as ependymoglia or tanycytes[9–13]. This may explain why neurogenesis continues in a more widespread manner in other vertebrates, but is restricted to a few niches in most adult mammalian brains that have been examined so far. Interestingly, adult NSCs located in the two major niches in the adult brain closely resemble radial glia or tanycytes — radial glia cells lining the ventricle in the adult brain (for recent reviews, see REFS 8,14). These cells seem to be unique as they possess the hallmarks of stem cells, such as the possibility to self-renew over multiple passages, and multipotency — the capacity to generate neurons and all macroglial cells of the adult brain.

These exciting data, that glia that exist in neurogenic niches in the adult brain and during development can act as neuronal progenitors and even stem cells, prompts the question as to whether other glial cells also possess such potential. To evaluate these issues, we first need to examine glia outside the neurogenic niches and then ask to what extent these parenchymal glia share common properties and signalling pathways with stem cells of glial identity.

Glia in the adult brain parenchyma

Across ontogeny and phylogeny, glial cells increase in number and diversity[15,16]. Bony fish and most amphibians retain radial glia from development into adult stages. In mammals and most birds, specialized cuboidal ependymal cells take over to line the ventricles, and other glial cells — the star-shaped parenchymal or protoplasmic astrocytes — multiply and settle in the parenchyma throughout the brain (BOX 1). Another class of glial cells that seem to be newly evolved in the vertebrate lineage — the NG2 glia (also known as synantocytes) — are dispersed throughout the adult brain parenchyma[17]. During development, cells expressing similar marker proteins as the NG2 glia act as oligodendrocyte progenitor cells (OPCs), and most of the NG2 glial cells continue this role into adulthood, in increasing numbers from teleosts to mammals[17].

Markers shared by astrocytes, radial glia and NSCs. Astrocytes are primarily involved in the regulation of neural network activity, through modulation of extracellular transmitter and ion concentrations, and direct signalling at the tripartite synapse and at sites of neurovascular coupling[15]. Astrocytes regulate transmitter levels through the expression of the astrocyte-specific glutamate transporters excitatory amino acid transporter 1 (also known as GLAST) (BOX 1) and excitatory amino acid transporter 2 (also known as GLT1), which enables

Ependymoglia or tanycytes
Radial glia-like cells lining the ventricular surface found in most bony fish and many amphibians that lack differentiated astrocytes and ependymal cells.

Macroglial cells
Glia of neuroectodermal origin, including astrocytes, NG2 cells, oligodendrocytes and ependymal cells.

Oligodendrocyte progenitor cell
A progenitor of the oligodendroglial lineage giving rise to mature oligodendrocytes and NG2 glia.

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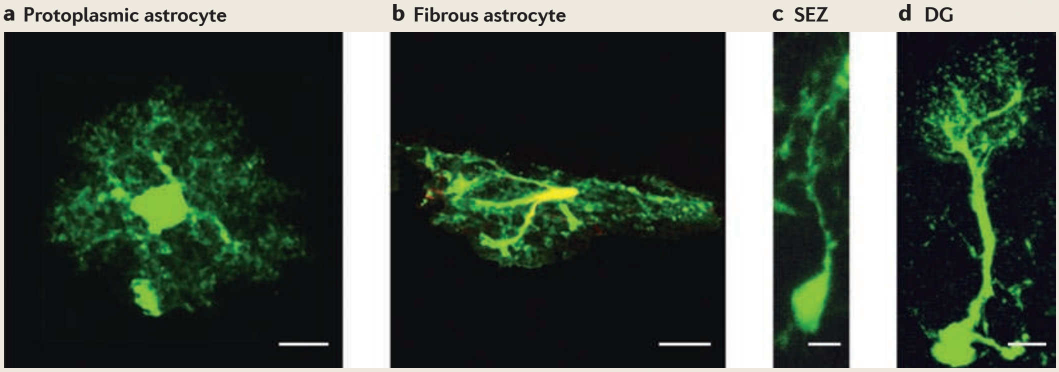
doi:10.1038/nrn2978

them to take up glutamate and convert it using the enzyme glutamine synthetase into glutamine, which is shuttled back to the neurons[18–21]. These proteins are also expressed by radial glia in the developing CNS and by NSCs in the adult mammalian brain. In addition, astrocytes express proteins, such as glial fibrillary acidic protein (GFAP), S100β and aldehyde dehydrogenase 1 family, member L1 (ALDL1H1), that are also expressed by adult NSCs in the SEZ and SGZ[22–25].

Moreover, astrocytes are characterized by their ultrastructural hallmarks, including glycogen granules, which are also shared by adult NSCs (reviewed in REF. 8). In the SEZ, the ventricular contact of the NSCs distinguishes them from normal astrocytes. However, NSCs in the dentate gyrus, the other prominent adult NSC niche, do not have ventricular contact, but resemble 'radial astrocytes' owing to their radial morphology. The NSCs in the dentate gyrus also possess astrocyte hallmarks at the ultrastructural level and share the expression of GFAP, GLAST, S100β, glutamine synthetase and other proteins with prototypic astrocytes[26,27] (BOX 1). Thus, adult NSCs share many hallmarks with genuine astrocytes.

Functional differences between mature and progenitor astrocytes. There are major functional differences between the astrocyte-like subtypes acting as NSCs and prototypic astrocytes in the brain parenchyma. Parenchymal astrocytes do not divide in the healthy brain. Moreover, when isolated *in vitro*, they do not exhibit stem cell potential such as the formation of self-renewing, multipotent neurospheres — at least when isolated from the healthy mouse brain[28]. Neurospheres form from isolated stem cells that proliferate *in vitro* in high doses of growth factors. They can be propagated for many passages and can differentiate into neurons, astrocytes and oligodendrocytes. *In vivo*, however, NSCs divide at rather low frequency and constantly generate neurons[8,25,29]. *In vitro*, NSCs reveal their multipotent nature[8,14], whereas it still remains to be shown whether a single adult NSC can generate both neurons and glia *in vivo*. Given the stem cell hallmarks of self-renewal and multipotency, some have suggested that these NSCs may be a class of their own[22], even if they share functions in homeostasis and gliotransmission with genuine astrocytes[30].

Box 1 | Astrocyte diversity in the brain


a Protoplasmic astrocyte **b** Fibrous astrocyte **c** SEZ **d** DG

Common glial markers
- Glycogen granules
- Glutamine synthetase
- S100β
- GLAST
- GLT1

Immature markers
- GFAP
- Vimentin
- Nestin
- TNC
- BLBP
- DSD1

In the healthy brain, astrocytes differ in morphology (see the figure; visualized by the use of a green fluorescent protein (GFP) reporter). GFP expression has been activated by inducible CRE recombination under the astrocyte-specific promoter of the gene encoding the excitatory amino acid transporter 1 (also known as GLAST) in adult mice. Grey matter astrocytes, the protoplasmic astrocytes, have highly branched fine processes (see the figure, part **a**) that contact the basement membrane surrounding the blood vessels and enwrap synaptic connections. Astrocytes in the white matter, the fibrous astrocytes, have thicker and less branched processes that contact axons — for example, at the nodes of Ranvier (see the figure, part **b**). Some astrocytes have a radial morphology and include the Bergmann glia in the cerebellum and the Müller glia in the retina (not shown). Similar to protoplasmic astrocytes, radial astrocytes[15] extend finely branched processes enwrapping synapses and are in contact with the basement membrane. Despite the diversity of astrocytes in their morphology, some functional hallmarks are shared, such as neurovascular coupling[175], regulation of metabolic functions including ion and glutamate homeostasis[176], and active control of synaptic functions at the tripartite synapse[177]. However, some subsets of astrocytes also perform highly specialized functions, such as pH sensing in the respiratory nuclei in the brain stem[15] or even acting as stem cells in the neurogenic niches of the adult brain. Parts **c** and **d** of the figure depict the radial glia-like stem cells in the subependymal zone (SEZ) at the lateral wall of the lateral ventricle and in the dentate gyrus (DG) of the hippocampus, respectively. These express not only the common astrocyte markers (listed on the left side), but also proteins that are normally present in immature glia and radial glia (listed on the right side). The scale bars represent 10 μm. BLBP, brain lipid-binding protein; GFAP, glial fibrillary acidic protein; GLT1, excitatory amino acid transporter 2; TNC, tenascin C.

Tripartite synapse
A concept of synapse physiology appreciating the role of astrocytes in synaptic transmission. It is composed of the pre- and postsynaptic neuronal terminal, as well as astrocytic processes enwrapping these structures.

Glial fibrillary acidic protein
(Often abbreviated to GFAP.) An intermediate filament, which is expressed in astroglia — depending on the subtype and developmental stage — and is strongly upregulated during astrogliosis.

Neurosphere
A clonal aggregate derived from a single cell. It can be propagated for several passages giving rise to further neurospheres, indicative of stem cell self-renewal, and can be differentiated into the main neural lineages, such as neurons, astroglia and oligodendroglia, indicative of multipotency.

Box 2 | Using GFAP$^{-/-}$vimentin$^{-/-}$ mice to study intermediate filaments and reactive astrocytes

Intermediate filaments are components of the cytoskeleton, and their composition is specific for individual cell types, developmental or (patho)physiological stages. Upregulation of the intermediate filaments glial fibrillary acidic protein (GFAP), vimentin, nestin and the so far much less investigated synemin, is a hallmark of reactive astrocytes.

Genetic deletion of intermediate filaments often has no obvious phenotypic effect but, in the case of injury, astrocytes lacking intermediate filaments exhibit profound alterations. Deletion of both genes encoding GFAP and vimentin[61] prevents filament formation and attenuates reactive astrogliosis, resulting in a striking array of initially negative and later often positive effects on injury size and functional recovery.

These proteins are therefore more than simply marker molecules, but have important functions in wound closure and scar formation. In the middle cerebral artery occlusion model of stroke, GFAP$^{-/-}$vimentin$^{-/-}$ mice exhibit a profound increase in infarct volume[178] . This may be due to reduced glutamate uptake, accumulation of glutamine, reduced release of tissue plasminogen activator inhibitor and a reduced capacity to elicit blood–brain barrier formation[178]. Interestingly, these defects are at least partially due to impairments in appropriate targeting of proteins. Analysis of brain injury in these mice further revealed initial beneficial functions and later partially harmful roles of intermediate filament upregulation[61]. For example, synaptic regeneration is much improved at later stages in GFAP$^{-/-}$vimentin$^{-/-}$ mice[42]. Further, they develop less dense scars and show improved post-traumatic regeneration after hemisection of the spinal cord, as well as improved survival and differentiation of transplanted stem cells[60,61,178]. Thus, reactive astrocytes contribute to the inhibition of neurogenesis of transplanted stem cells as well as axonal regeneration.

NG2 glia. A different set of glia that is dispersed throughout the adult brain parenchyma — the NG2 glia (for a recent review, see REF. 17) — proliferates even outside the stem cell niches in the adult brain. These cells, of which almost all also express alpha-type platelet-derived growth factor receptor (PDGFRα), constantly divide with a long cell cycle length of more than 1 month[31,32] and generate differentiated, myelinating oligodendrocytes as well as further NG2 glia[33,34]. In addition, *in vivo* fate mapping studies have suggested that they may also generate other glia, such as astrocytes, or neurons in specific brain regions[34,35]. However, other fate mapping studies have shown that the generation of astrocytes is limited to embryonic development[35–37], and neurogenesis from this population of glial progenitors could not be confirmed by these other studies[33,37]. However, although specialized to gliogenesis *in vivo*, these cells may exhibit a broader potential *in vitro*[36,38]. Indeed, when NG2 cells were isolated at postnatal stages they could give rise to multipotent neurospheres[36], whereas the very few neurospheres derived from the adult brain and CNS have not been traced back to NG2- or PDGFRα-expressing glial progenitors[28,39]. Thus, these data seem to suggest that glial cells outside the neurogenic niches indeed differ profoundly from adult NSCs, both in their behaviour *in vivo* and in their capacity to self-renew and reveal multipotency *in vitro*.

The glial response to injury

The behaviour of glial cells and their progenitors may change after brain injury — one of the most potent stimuli for macroglia as well as microglia. Microglia are the first cells to be activated, rapidly migrating to the injury site and initiating much of the further glial reaction and communication with the immune system[40]. NG2 glia also react at early stages by increasing their proliferation[32,37], whereas astrocytes are the last population to react by multiple and complex changes in morphology, gene expression and function, a process referred to as 'astrogliosis'.

Microglia
Glia of mesodermal origin, and the resident macrophages of the CNS.

Astrogliosis occurs in many injury conditions, ranging from neurodegenerative or inflammatory conditions to acute invasive brain injury, such as trauma or stroke[24,41]. In all of these conditions, astrocytes become hypertrophic and upregulate most prominently the intermediate filaments GFAP and vimentin (BOX 2). These reactive astrocytes share hallmarks with NSCs and developmental radial glia, such as expression of nestin, vimentin and brain lipid-binding protein (BLBP)[24,42] (discussed below). In addition, in more severe injuries such as severe trauma, hypoxia or stroke, a proportion of reactive astrocytes also proliferates, as shown by incorporation of the thymidine analogue bromodeoxyuridine (BrdU), accounting in part for the increased number of astrocytes surrounding the injury site[22,43]. Some of these cells even reveal *in vitro* stem cell potential with self-renewal and multipotency when grown under neurosphere conditions[28,44].

The source of reactive astrocytes

In the context of the above discussion, the key question is from where these reactive astrocytes with NSC characteristics originate. Are they indeed a dedifferentiated subset of formerly mature astrocytes, as has been proposed[44]? Or are they derived from the NG2 progenitor cells present also throughout the brain parenchyma, as suggested by other studies[34]? Finally, stem or progenitor cells may migrate from the known neurogenic niches — for example, the SEZ — towards the sites of brain injury (BOX 3).

Protoplasmic astrocytes. A major source of reactive astrocytes are the mature and highly branched protoplasmic astrocytes typically found in the grey matter (BOX 1), as determined by genetic lineage tracing[28]. After stab wound injury in mice, genetically traced astrocytes become hypertrophic (FIG. 1a), change their morphology (FIG. 1b) and upregulate the expression of GFAP (FIG. 1c) and vimentin (FIG. 1d)[28], all hallmarks of reactive astrocytes (BOX 2)[24]. Within 3–5 days, these reactive astrocytes upregulate proteins that are characteristic of NSCs, radial glia or progenitors, such as the intermediate filament nestin, the DSD1 proteoglycan, CD15 and in some cases

GLAST::CREERT2
A genetic fate mapping construct for targeting of the tamoxifen-inducible form of the CRE recombinase (CREERT2) to the locus of the astrocyte-specific glutamate transporter GLAST.

musashi[28,44–46] (FIG. 1). Furthermore, half of the entire pool of genetically labelled astrocytes also re-enters the cell cycle within 1 week after stab wound injury[28,32], consistent with dedifferentiation (FIG. 1). This high proliferation rate does not seem to be specific to a potential subset of astrocytes labelled by GLAST::CREERT2-mediated recombination, as this rate was also observed without any genetic labelling in the entire astrocytic pool[32]. At any given time point, however, only a few astrocytes are proliferating, with their proportion increasing mostly at later stages (5–7 days) after injury. Thus, proliferation of astrocytes is delayed compared to microglia or NG2 glia (see also REFS 37,47 for the fast activation of NG2 glia) and within a week after injury half of all astrocytes have undergone cell division[28,32].

This prompts the question of subtype heterogeneity among reactive astrocytes. Are the proliferating reactive astrocytes a distinct subset with less mature properties than the remainder, or is this a quantitative response that depends on the strength of the stimuli from the lesion? The observation that smaller injuries cause less activation of astrocyte proliferation suggests that the latter scenario might be the case[24,48]. These are key issues to be addressed in future work that also highlight the urgent need to better define astrocytes, especially their subtypes with distinct functions, at the molecular level[23,25,49].

Thus, protoplasmic astrocytes have the capacity to resume cell proliferation and re-express proteins present in radial glia at earlier developmental stages or adult NSCs. Moreover, as determined by genetic lineage tracing, a fraction of reactive astrocytes also shows long-term self-renewal and multipotency in the neurosphere assay, whereas astrocytes isolated from a non-injured brain region generate virtually no neurospheres[28]. As this study could also exclude a contribution of stem cells migrating from the SEZ towards the injury site from which neurosphere-forming astroglia could be isolated, this indicates that a subset of reactive astrocytes within the cerebral cortex gain the potential of exerting self-renewal and multipotency when exposed to the appropriate conditions. Thus, reactive astrocytes are a novel source of cells with stem cell potential within the injury site. This is of particular importance for approaches to brain repair, as these cells are present in the damaged nervous tissue, where they are needed. Using these cells for repair may therefore avoid the complications associated with transplantation.

Other possible sources. There may be additional sources of glial cells with increased phenotype plasticity within the site of brain injury. It has been suggested that the NG2- or PDGFRα-expressing glial progenitors described above might broaden their progeny after injury, generating reactive astrocytes or even Schwann cells[47,50,51], but other studies could not confirm the generation of astrocytes after injury[33,37,52]. So far, no direct evidence has been obtained that derivatives of this lineage in the adult CNS can give rise to multipotent and self-renewing neurospheres — that is, they do not reveal NSC potential *in vitro*. Nevertheless, as actively proliferating cells throughout the CNS, NG2 glia could be a further promising set of glia for potential use in neural repair strategies.

A further population of glial cells — the ependymal cells lining the ventricle — has been shown to have remarkable stem cell potential after injury[52,53]. Notably, the behaviour of ependymal cells in the forebrain and spinal cord differs profoundly after injury, highlighting regionalization as a further important aspect to consider in regard to reactive gliosis and brain repair. Thus, an exciting new concept is that differentiated glial cells local to the injury site, such as ependymal cells and astrocytes, re-acquire multilineage potential, which they can enact at least *in vitro*[28,52].

Further implications. The above discussion of the glial response to injury has both positive and negative implications. The good news is that there are cells with NSC potential within the injury site, and this response seems to occur in various injury conditions. The bad news is that the brain environment *in vivo* does not allow

Box 3 | **Neuroblast recruitment after injury — overcoming antineurogenic signals**

Reactive astrocytes only enact their neurogenic potential *in vitro*, whereas neuroblasts residing in the endogenous sites of adult neurogenesis are attracted to the site of injury and can overcome antineurogenic signals[179]. For example, after stroke injury in the rodent striatum, immature neurons migrate along the vasculature towards the injury site[103,180]. Ablating these neuroblasts in mice after focal cerebral ischaemia increased the infarct volume and exacerbated sensorimotor behavioural deficits[181]. Some of the recruited neuroblasts progress towards a mature neuron state, expressing the neuronal markers NeuN and dopamine- and cyclic AMP-regulated neuronal phosphoprotein (DARPP32), and even innervate appropriate target regions, acquiring the hallmarks of medium spiny neurons in the striatum[179]. Similarly, after injury in the mouse cerebral cortex, neuroblasts close to the injury site generate glutamatergic projection neurons that project to appropriate and distant target sites[182,183].

The exact origin of these cells is not yet certain, but is likely to be the endogenous sites of neurogenesis[160,184], as has also been observed in the rat hippocampus[185]. This would represent a remarkable plasticity in terms of neuronal subtype specification, as subependymal zone (SEZ) cells normally generate GABAergic and glutamatergic olfactory bulb interneurons[160,186] rather than striatal medium spiny neurons and glutamatergic cortical projection neurons. Given the distinct subtypes of GABAergic and glutamatergic progenitors in the SEZ[160,187], it is intriguing to speculate that SEZ progenitors may be restricted in their main neuronal subclass — to generate inhibitory or excitatory neurons — but may be able to transgress restrictions in further subtype specification under certain conditions. Most importantly, these examples provide compelling evidence that some endogenous stem or progenitor cells can overcome the endogenous antineurogenic signalling, and hence may provide important clues as to how to achieve this in other sets of progenitors.

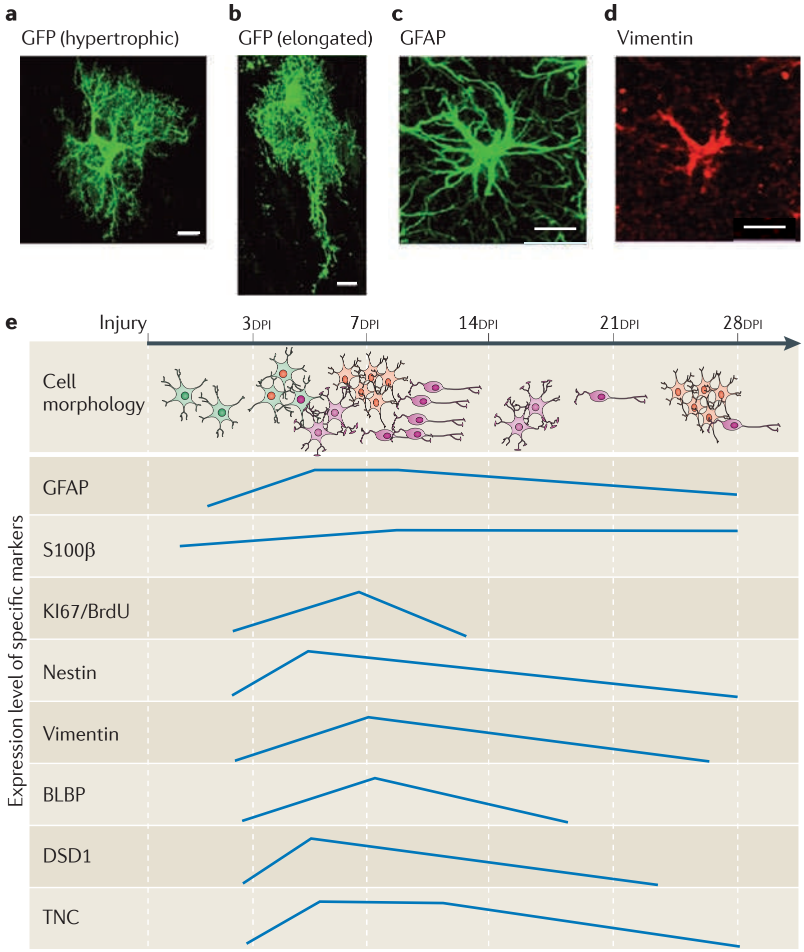


Figure 1 | **Astrogliosis after brain injury.** **a** | After brain injury, astrocytes — labelled here with green fluorescent protein (GFP) — become hypertrophic, with swelling of the cell body and main processes. **b** | Astrocytes in the perilesion area elongate some processes into the injury core. **c**,**d** | These reactive astrocytes also upregulate expression of the intermediate filaments glial fibrillary acidic protein (GFAP) and vimentin, which are involved in the response of astrocytes to injury. **e** | A schematic summary of the time course of the morphological changes of astrocytes after injury (top panel) and changes in gene expression indicative of dedifferentiation and proliferation (bottom panel). Astrocytes start to become hypertrophic at 3 days post injury (DPI) and upregulate the expression of the GFAP and nestin, as well as proteoglycans such as the DSD1 proteoglycan and tenascin C (TNC). The intermediate filaments are crucial for the reactive response (BOX 2) and DSD1 proteoglycan and TNC are indicative of the partial dedifferentiation of reactive astrocytes. One week after stab wound injury, astrocytes at the injury border are clearly hypertrophic and elongated, and strongly express vimentin and brain lipid-binding protein (BLBP). This correlates with the peak of proliferation of reactive astrocytes, as indicated by levels of KI67 expression and bromodeoxyuridine (BrdU) incorporation. Accordingly, the total number of astrocytes (identified by S100β labelling) increases over time. When proliferation stops, the expression of the proteins characteristic for immature glia (the DSD1 proteoglycan, TNC, nestin and vimentin) decline, whereas the absolute number of GFAP-positive astrocytes remains increased. The scale bars represent 10 μm.

Hypertrophy
Reaction of glia to injury characterized by swelling of the cell body and the main processes.

these cells to enact their neurogenic potential. Genetic fate mapping has demonstrated only glial derivatives from the glial cells reacting to injury *in vivo*, despite their multilineage potential *in vitro*. Astrocytes proliferate and increase their own number, whereas OPCs and ependymal cells generate oligodendrocytes and astrocytes in some injury paradigms, but no neurogenesis has been observed from these cells outside the neurogenic niches after injury[28,33,37,51,52]. To use these cells for repair, we must therefore pursue three central questions which will be discussed in the remainder of this Review. First, what are the functions of reactive astrocytes after brain injury, beyond their progenitor roles? Second, what are the signals that initiate and allow the changes in fully mature astrocytes towards proliferation and the potential to form self-renewing and multipotent neurospheres? Third, to develop this knowledge into functional repair, what are the environmental signals preventing neurogenesis from endogenous or transplanted cells?

Functional consequences of reactive astrogliosis

Beneficial effects. Experimentally ablating all reactive astrocytes that proliferate in response to injury highlights the beneficial role of reactive gliosis. Specifically, it resulted in a pronounced increase in tissue damage, lesion size and neuronal loss in various mouse models of injury[22,24]. The negative outcome is correlated and possibly due to disrupted repair of the blood–brain barrier (BBB) and a pronounced increase in entry, spread and persistence of inflammatory cells at the injury site[22,24]. The metabolic support that astrocytes provide for neurons during ischaemia[54,55] may further contribute to the increased death of neurons upon injury after ablating proliferating astrocytes[42].

One of the major hallmarks of reactive astrogliosis is the hypertrophy and upregulation of the intermediate filaments GFAP and vimentin. The functional consequences of these morphological changes have been studied through genetic deletion of these intermediate filaments (BOX 2) or their upstream regulators, such as the transcription factor signal transducer and activator of transcription 3 (STAT3)[56,57]. Worsening of the effects of injury was observed when STAT3 was genetically deleted in the mouse CNS[56,57]. As STAT3 is mostly active in reactive astrocytes, these data together suggest that reactive astrocytes have important functions in preventing extensive bleeding and tissue loss after acute injury of the mammalian brain[42].

Genetic manipulations in mouse models of demyelinating injury or chronic amyloidosis have further revealed the role of reactive astrocytes in restricting damage in chronic injury conditions, by forming a protective barrier, limiting inflammation and contributing to removal of aberrant deposits of amyloid and extracellular debris[58,59]. Thus, the functions of reactive astrocytes are partially overlapping with those of reactive microglia, the main glial subtype in the CNS that is specialized in phagocytosis and integrating inflammatory responses[40].

Detrimental effects. Interestingly, however, the long-term consequences of reactive gliosis may be less advantageous. For example, mice with mild reactive gliosis owing to the complete lack of intermediate filaments develop less dense scars around the injury site and consequently have improved post-traumatic axon regeneration and survival, as well as improved differentiation of transplanted stem cells[60] (BOX 2). Thus, reactive astrocytes seem to contribute to the inhibition of neurogenesis from transplanted stem cells and inhibit axonal regeneration. The general concept evolving is that in early stages of injury, reactive astrocytes are needed to limit tissue damage including scar formation, which provides beneficial effects initially in shielding the brain, whereas the consequences of persistent reactive astrocytosis can be harmful[24,61,62].

Selecting the best. The diverse, opposing roles of reactive gliosis mean that this process cannot be labelled 'good' or 'bad'. For example, scar formation is often considered a negative outcome of reactive gliosis as it contributes to the inhibition of axonal regeneration through the secretion of chondroitin sulphate proteoglycans (CSPGs) and other molecules inhibitory to axonal outgrowth[63]. Conversely, the scar tissue also has beneficial aspects (at least initially) — by forming a dense physical barrier it shields the intact brain regions from injured tissue. A better molecular understanding of reactive astrogliosis may allow for selective blockade of molecules that inhibit axonal outgrowth but otherwise permit reactive astrocytes to form a protective scar. This will require a more complete characterization of the signalling events that influence the different functions of reactive astrocytes after injury *in vivo*.

What triggers reactive gliosis?

Signalling at the astrocyte endfeet in contact with the basement membrane surrounding blood vessels, seems to act as a sensor for brain injury eliciting most of the programme of astrocyte reactivity, except their dedifferentiation towards stem or progenitor cells[45].

Integrins are a major class of basement membrane receptors, and glia-specific deletion of β1 integrin in the postnatal mouse brain is sufficient to elicit astrocyte reactivity in the otherwise healthy brain[45]. This results in alterations in the localization of members of the dystrophin-associated glycoprotein complex (DAG) and the water channel aquaporin 4 at the interface between the astrocyte endfeet and the basement membrane[45]. As a consequence, these β1 integrin-deficient astrocytes acquire almost all the hallmarks of reactive astrocytes after brain injury — namely, hypertrophy and upregulation of GFAP, vimentin and proteoglycans including the DSD1 proteoglycan and tenascin C (TNC).

However, although DSD1 proteoglycan and TNC are radial glia hallmarks, β1 integrin-deficient astrocytes do not re-enter the cell cycle[45] and do not initiate neurosphere formation, indicative of their failure to acquire stem or progenitor hallmarks. As proliferation of reactive astrocytes occurs only after severe invasive injury[24], this implies that signals eliciting this response may only be released following other incoming signals provided by severe insults. Conversely, this study together with work on the astrocyte–basement membrane interface after injury or aquaporin 4 and dystroglycan deletion[64–66] support the concept that signalling at this interface may regulate the astrogliosis hallmarks of hypertrophy and intermediate filament and proteoglycan upregulation, and hence possibly also scar formation.

Basement membrane
A specialized sheet-like structure of the extracellular matrix around blood vessels, capillaries and underneath the meninges. Astrocytes, meningeal cells and/or endothelial cells are involved in the generation of the basement membrane within the brain parenchyma.

Shared signals in the NSC niche and injury

To determine how to selectively activate one of the beneficial aspects of reactive astrogliosis — namely the acquisition of some degree of NSC potential — it is possible to gain insights by comparing signalling after injury with signals that are active in the endogenous NSC niches. As our Review focuses on this comparison, please refer to previous reviews for a comprehensive discussion of signalling after brain injury[22,24,41–43,46,48]. Indeed, the comparison between signals in the NSC niche and after injury reveals a striking degree of overlap in signalling pathways, such as fibroblast growth factor (FGF), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF), which all converge on the mammalian target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK) pathways (FIG. 2).

Growth factor signalling during injury. FGF2, EGF and VEGF are released after invasive brain injury and ischaemia by various cell types, and reactive astrocytes in turn upregulate the respective receptors (FIG. 2). This receptor upregulation allows reactive astrocytes to grow in neurosphere culture conditions containing FGF2 and EGF, in contrast to astrocytes isolated from the healthy adult brain[28,46,67]. *In vivo*, glial cell proliferation is reduced in FGF2 knockout mice or after blocking FGF2 upregulation in traumatic brain injury[68,69]. Similarly, the EGF receptor (EGFR) ligands EGF and transforming growth factor alpha (TGFα) both induce astrocyte proliferation *in vitro*[70]. Overexpression of TGFα *in vivo* triggers an increase in the expression of its receptor (EGFR)[71,72], upregulation of intermediate filaments and hypertrophy of GFAP-positive cells. However, astrocytes did not re-enter the cell cycle in one of these studies[71]. Conversely, the TGFα-deficient mouse mutant waved-1 shows a reduction in GFAP-expressing postnatal astrocytes[73], and astrocyte proliferation is impaired in EGFR-deficient mice *in vivo*[74]. Activation of EGFR also upregulates CSPG proteins including phosphacan and neurocan, which are key components of the glial scar[75], demonstrating the important role of EGFR-mediated signalling in activating astrocytes after injury.

VEGF is mostly expressed in the choroid plexus in the healthy brain, but is prominently upregulated in the course of various pathological events in the CNS (FIG. 2c). VEGF infusion into the rat brain increased proliferation of reactive astrocytes[76], and neutralizing antibodies against VEGF or vascular endothelial growth factor receptor 1 (also known as FLT1) interfered with this mitogenic effect[76,77]. Besides causing astrocyte proliferation, VEGF also plays an important part in post-traumatic angiogenesis (for a review, see REF. 78), thereby possibly orchestrating wound closure and BBB repair by astrocytes through vascular remodelling and regeneration.

EGF-, FGF- and VEGF-receptor signalling all activate the serine/threonine kinase mTOR[79–81], and mTOR signalling is accordingly activated in reactive astrocytes upon ischaemic spinal cord injury[79]. Further support for a potent role of this pathway in the injured brain is based on the decrease in GFAP and vimentin levels upon treatment of injured rats with the mTOR inhibitor

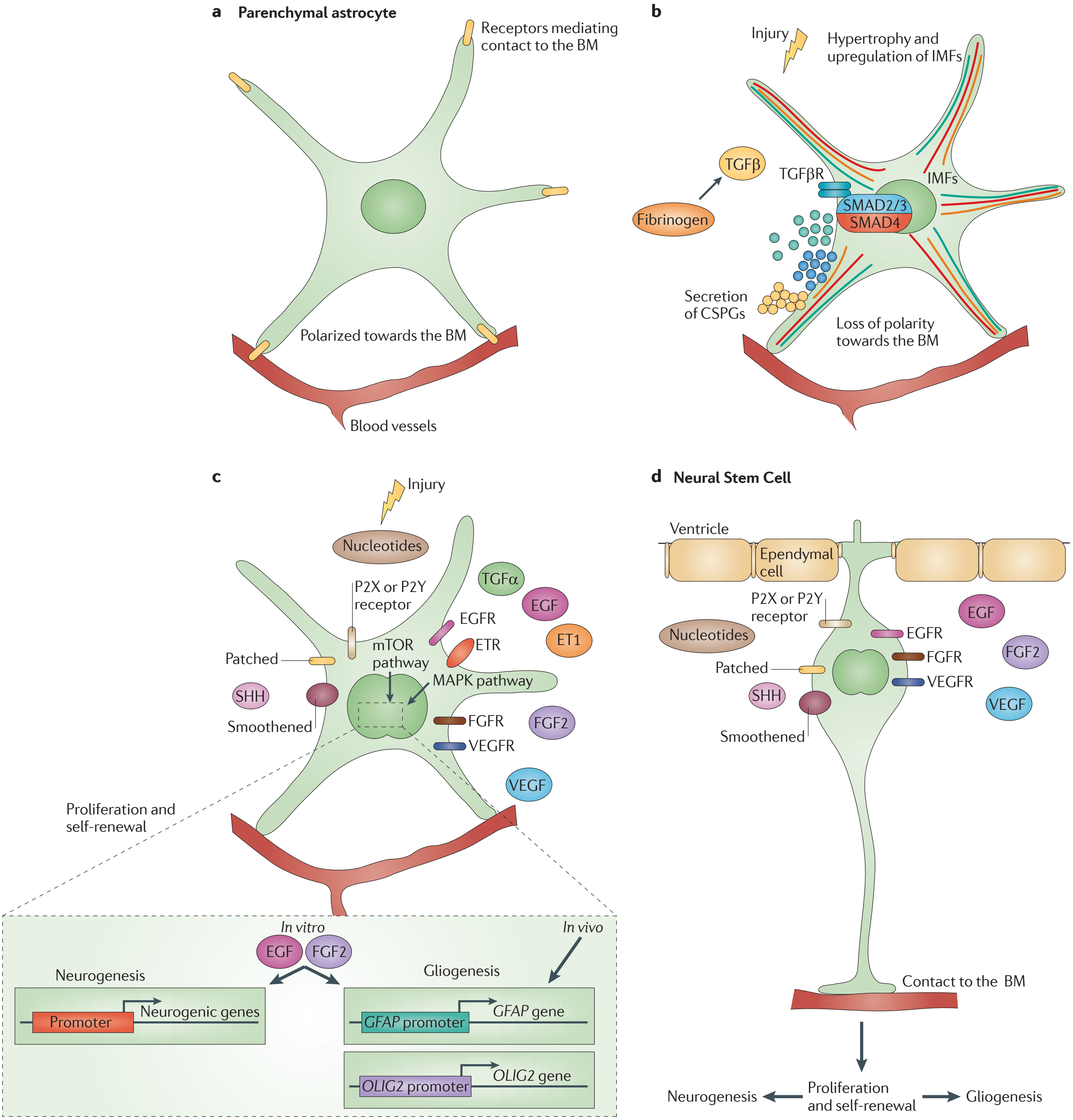


Figure 2 | **Signals shared after brain injury and in the NSC niche.** **a** | Mature astrocytes in the healthy brain are polarized towards, and make contact with, the basement membrane (BM) surrounding blood vessels and do not divide. **b** | Loss of signalling from the BM as seen after deletion of β1 integrin or as a possible result of brain injury leads to reactive astrogliosis, including loss of the polarized expression of endfeet proteins, hypertrophy and upregulation of intermediate filaments (IMFs), and secretion of chondroitin sulphate proteoglycans (CSPGs)[45,66]. Upon brain injury, secretion of CSPGs is triggered by fibrinogen, which activates transforming growth factor beta (TGFβ) signalling. This pathway also promotes upregulation of glial fibrillary acidic protein (GFAP) and neurocan and may thereby inhibit axonal regeneration. **c** | Signals triggering astrocyte proliferation after injury are fibroblast growth factor (FGF), epidermal growth factor (EGF) and transforming growth factor alpha (TGFα), which act via the mitogen-activated protein kinase (MAPK) or the mammalian target of rapamycin (mTOR) pathway. The latter is also activated by purinergic signalling following the release of ATP after brain injury. Proliferating reactive astrocytes do not generate other glia or neurons *in vivo*. However, exposure to further EGF and FGF2 signalling *in vitro* confers the capacity to generate neurons, astrocytes and oligodendrocytes *in vitro*. **d** | These factors, as well as vascular endothelial growth factor (VEGF) and sonic hedgehog (SHH), are also active in the adult neurogenic niches *in vivo*, where they also promote proliferation of neural stem and progenitor cells. EGFR, EGF receptor; ET1, endothelin 1; ETR, ET receptor; FGFR, FGF receptor; OLIG2, oligodendrocyte transcription factor 2; TGFβR, TGFβ receptor; VEGFR, VEGF receptor.

rapamycin[79] and the astrocyte hypertrophy and proliferation that follows overactivation of mTOR signalling by deletion of its inhibitor phosphatase and tensin homologue (PTEN), even in the absence of injury[82].

These growth factors, as well as the vasoactive peptide endothelin 1 (ET1), also activate extracellular signal-regulated protein kinases (ERK) and Jun N-terminal kinase (JNK)-dependent MAPK pathways in astrocytes after injury. These signalling pathways act synergistically to phosphorylate the immediate early gene products c-FOS and c-JUN, which increase astrocyte proliferation and GFAP expression[83]. Accordingly, phosphorylation of ERK is specifically detected in reactive astrocytes after injury[83,84]. A particularly intriguing aspect of these signalling pathways is their co-regulation of extracellular matrix (ECM) components. For example, EGF regulates neurocan and phosphacan (see above), and endothelin-mediated signalling promotes expression of matrix metalloprotease 9 (MMP9)[85]. Thus, it is interesting to consider a specific link between the proliferative response of reactive astrocytes and a particular expression profile of ECM components (for a review of ECM after injury, see REF. 86) with obvious relevance for axonal regeneration. Notably, ECM components expressed by radial glia or young astrocytes are more growth-permissive than those of mature astrocytes. Hence, promoting the astrocyte dedifferentiation response may simultaneously have beneficial side effects on axonal regeneration.

Conversely, components of the ECM potently influence the effects of the above-mentioned growth factors, such that FGF signalling requires heparin sulphate proteoglycans and EGFR activation is influenced by the ECM component TNC[87]. Moreover, the cell surface expression of DSD1 proteoglycan is upregulated in reactive astrocytes[45] and influences both FGF2-dependent neurogenesis and EGF-dependent gliogenesis from NSCs[46,88]. These data therefore not only highlight the complex feedback and feedforward loops in signalling cascades after brain injury, but also suggest a link to the NSC niche in which DSD1 proteoglycan and TNC are characteristic components[89–91].

Growth factor signalling in the NSC niche. The growth factors that act after injury, discussed above, also act in the stem cell niches *in vivo*[92–95] (FIG. 2d) and have been instrumental in the discovery of NSCs, as they stimulate the expansion of adult NSCs *in vitro*[14]. For example, FGF2 is released from astrocytes in the SEZ in the uninjured adult brain and stimulates proliferation of FGFR1-expressing NSCs in the adult rat brain[93]. Intraventricular infusion or subcutaneous injection of FGF2 increases proliferation and neurogenesis in the adult rat SEZ[96,97], whereas injection of neutralizing antibodies to FGF2 substantially inhibits proliferation in this region[95]. Intracerebroventricular administration of EGF also expanded the pool of proliferative progenitors in the adult rodent SEZ[94,96,98]. Notably, in contrast to FGF2, EGF infusion is accompanied by a strong astrogliosis in the SEZ extending into the neighbouring striatum[92]. Thus, EGF seems to divert NSCs towards a highly proliferative astrocyte lineage. This is reminiscent of NSCs with high levels of EGFR, which have been proposed to represent an activated NSC state[92]. VEGF also supports NSC proliferation and survival, and specifically enhances neurogenesis upon intracerebroventricular infusion into the rat SEZ and SGZ[99,100]. NSCs are thought to reside in a 'vascular niche' in which signalling factors co-regulate angiogenesis and NSC proliferation or differentiation[101]. Given the role of VEGF in angiogenesis following injury (described above), this suggests the intriguing possibility that the concept of the vascular niche may also apply to the injured brain, with reactive astrocytes developing NSC potential in specific vascular niches through signals that normally act on endothelial cells, such as VEGF, stromal cell-derived factor 1 (SDF1) and angiopoietin 1 (REFS 102,103).

SHH signalling. A key regulator of adult NSCs in the SEZ and SGZ that has recently also been observed after CNS injury is the morphogen sonic hedgehog (SHH). SHH is an important mitogen for maintaining the proliferation of rodent adult NSCs in neurogenic niches[104,105]. Reactive astrocytes also upregulate SHH upon brain injury[106,107], and local or systemic administration of SHH after injury stimulates proliferation of mouse reactive astrocytes[106,108] (FIG. 2c). SHH injection promotes proliferation and neurosphere formation of cells from outside the neurogenic niche[108], making it a prime candidate — together with EGF, FGF2 and VEGF — for mediating the dedifferentiation response of reactive astrocytes.

Notably, a key target of SHH-mediated signalling is the transcription factor oligodendrocyte transcription factor 2 (OLIG2), which is accordingly also highly expressed in neurosphere cells[109,110]. However, for neuronal differentiation to occur, OLIG2 must be downregulated: its maintained expression is a potent inhibitor of neurogenesis from neurosphere cells *in vitro* as well as in the SEZ *in vivo*[110,111]. Thus, the potent upregulation of OLIG2 in reactive glia after brain injury may interfere with progression of these glial cells towards neurogenesis.

WNT signalling. A morphogen of similar importance during development is WNT, which acts by several signalling cascades including the canonical pathway involving β-catenin (for a review, see REF. 112). WNT and β-catenin signalling is also required for neurogenesis in the adult SGZ and has an important role in proliferation and neurogenesis in this region during development[113,114]. Overexpression of WNT3 in the rat dentate gyrus is sufficient to increase neurogenesis from these progenitors, whereas blocking WNT signalling abolishes neurogenesis *in vitro* and *in vivo*[113]. WNT7A and β-catenin promote NSC self-renewal in both adult neurogenic niches, whereas deletion of WNT7A or inhibition of β-catenin *in vivo* result in a decrease in stem cell numbers[114]. In this regard, it is interesting to note that WNT and β-catenin signalling increases in proliferating astrocytes and in NG2 glia after traumatic brain injury[115]. However, cell type-specific gain- or loss-of-function studies are required to assess whether this correlation is of functional importance for reactive glia to re-enter the cell cycle.

Purinergic signalling. Purinergic signalling is a further signalling pathway shared between the NSC niche and the injured brain parenchyma. NSCs express the ecto-ATPase ectonucleoside triphosphate diphosphohydrolase 2 (NTPDase2)[116], a cell surface enzyme that hydrolyzes extracellular nucleotides. Knock down of NTPDase2 results in impaired NSC proliferation and differentiation *in vitro*[117]. Nucleotides and EGF induce converging, but also differential, intracellular signalling pathways and can act synergistically in the control of cell proliferation and cell survival in adult neurogenesis[118,119]. Similarly, purinergic signals are one of the first and most potently activated after injury, as ATP is immediately released by damaged cells (for a review, see REF. 120) and may also interact with EGF signalling in this context.

In vitro *models.* The above discussion highlights that many of the key signalling pathways that are active in the NSC niches are also present after brain injury, providing candidate pathways to mediate the activation and partial dedifferentiation of reactive glia. Interestingly, scratch wound-injured rat spinal cord astrocytes *in vitro* also dedifferentiate into multipotent neurosphere cells[121], suggesting that at least some signals promoting astrocyte dedifferentiation may derive from injured astrocytes themselves[108]. Conversely, diffusible signals obtained from cortical or hippocampal astrocytes exposed to scratch wound injury *in vitro* act antineurogenically on co-cultured NSCs from the rat dentate gyrus, favouring the astrocytic fate[122]. This effect may be due to ciliary neurotrophic factor (CNTF) and leukaemia inhibitory factor (LIF) in the culture medium collected from wounded astrocytes, as these factors largely promote differentiation to the astrocyte fate[122] (discussed below). Experiments were carried out in different medium conditions, thereby explaining some of the differences between these two studies. A further *in vitro* model of reactive astrocytes is the co-culture with fibroblasts, which will also provide an interesting tool to examine potential dedifferentiation of astrocytes[123].

Distinct signals in the NSC niche and after injury

The fact that a given signalling pathway is active in both the NSC niche and after injury does not necessarily mean that it will also exert the same effects. A prominent example of this is signalling exerted by the bone morphogenic proteins (BMPs) (FIG. 3). This apparent discrepancy reveals the importance of the concerted action of various signalling pathways and highlights the distinct context of BMP activity under normal conditions and in response to injury.

BMP signalling. Outside the neurogenic niches and especially after injury, BMP signalling promotes astrocyte fate[124,125]. Inside one of the neurogenic niches of the adult brain, the SEZ, BMP signalling is proneurogenic at endogenous levels[126], whereas it inhibits neurogenesis at higher levels[127,128]. Infusion of the BMP inhibitor noggin or genetic deletion of the core mediator of TGFβ and BMP signalling, the transcription factor SMAD4, abrogated neurogenesis in the mouse SEZ[126]. As manipulation of the TGFβ signalling pathway had no effect on adult SEZ neurogenesis[126], both noggin infusion and SMAD4 deletion in this region reveal the proneurogenic role of BMP in this context. Interestingly, this is achieved by a well-known role of BMP signalling, namely antagonizing the expression and function of the transcription factor OLIG2 (REF. 126). OLIG2 promotes gliogenesis in the adult mouse brain both within and outside the stem cell niches[111,126,129,130] (FIG. 3b). *In vivo* experiments revealed that inhibition of BMP signalling in the SEZ by infusion of the BMP inhibitor noggin, by genetic deletion of SMAD4 (REF. 126) or by endogenous upregulation of BMP inhibitors, such as chordin, after lysolecithin-induced demyelination[131] resulted in upregulation of OLIG2 expression and fate conversion towards the oligodendrocyte lineage at the expense of neurogenesis in the adult mouse SEZ. Indeed, neurogenesis could be rescued in the absence of BMP signalling by antagonizing the aberrantly elevated OLIG2-mediated transcription[126], thereby directly demonstrating *in vivo* that BMP promotes neurogenesis in the SEZ by inhibiting OLIG2 expression and transcription.

However, the level of BMP is important, as is well known from studies on gradients of BMP levels during development. When BMP levels are abnormally raised, either by BMP infusion[128] or lack of low-density lipoprotein receptor 2 (LRP2), which helps to clear BMPs[127], cell cycle progression is inhibited and cell death increased, thereby also abrogating neurogenesis. Interestingly, in the SGZ — the neurogenic niche of the hippocampus — BMP signalling seems to primarily affect self-renewal of NSCs by attenuating proliferation and thereby mediating the slow, self-renewing proliferation of NSCs[132].

After brain injury, BMPs are upregulated and promote astrogliogenesis[125,133] (FIG. 3a). Infusing antibodies against noggin promotes BMP-mediated signalling and increases the number of GFAP-positive astrocytes. Notably, these co-express the proteoglycan NG2, normally characteristic of the OPCs, suggesting that these astrocytes may derive from local NG2/OLIG2$^+$ cells. Thus, these NG2/OLIG2$^+$ cells may generate astrocytes in the presence of high BMP levels[50,124]. This distinct effect of BMP within and outside the neurogenic niche may be due to differences in activity of the STAT signalling pathway, which is considerably higher outside than inside the neurogenic niches[126,134]. The cooperative effect of BMP and STAT signalling, discussed below, is only one example highlighting the important role of interaction between signalling pathways. Other cooperative effects — with, for example, notch and other signalling pathways — are likely to further influence the difference in BMP signalling observed between inside and outside the SEZ.

BMP–STAT signalling. BMP signalling interacts with STAT-mediated transcription possibly through interaction with the transcriptional coactivator p300. Upon BMP signalling, SMAD1 translocates into the nucleus and binds to the GFAP promoter close to the STAT3 binding site and interacts with STAT3 through p300 (REFS 135,136) (FIG. 3a). This interaction promotes expression of GFAP and acquisition of an astrocyte fate even

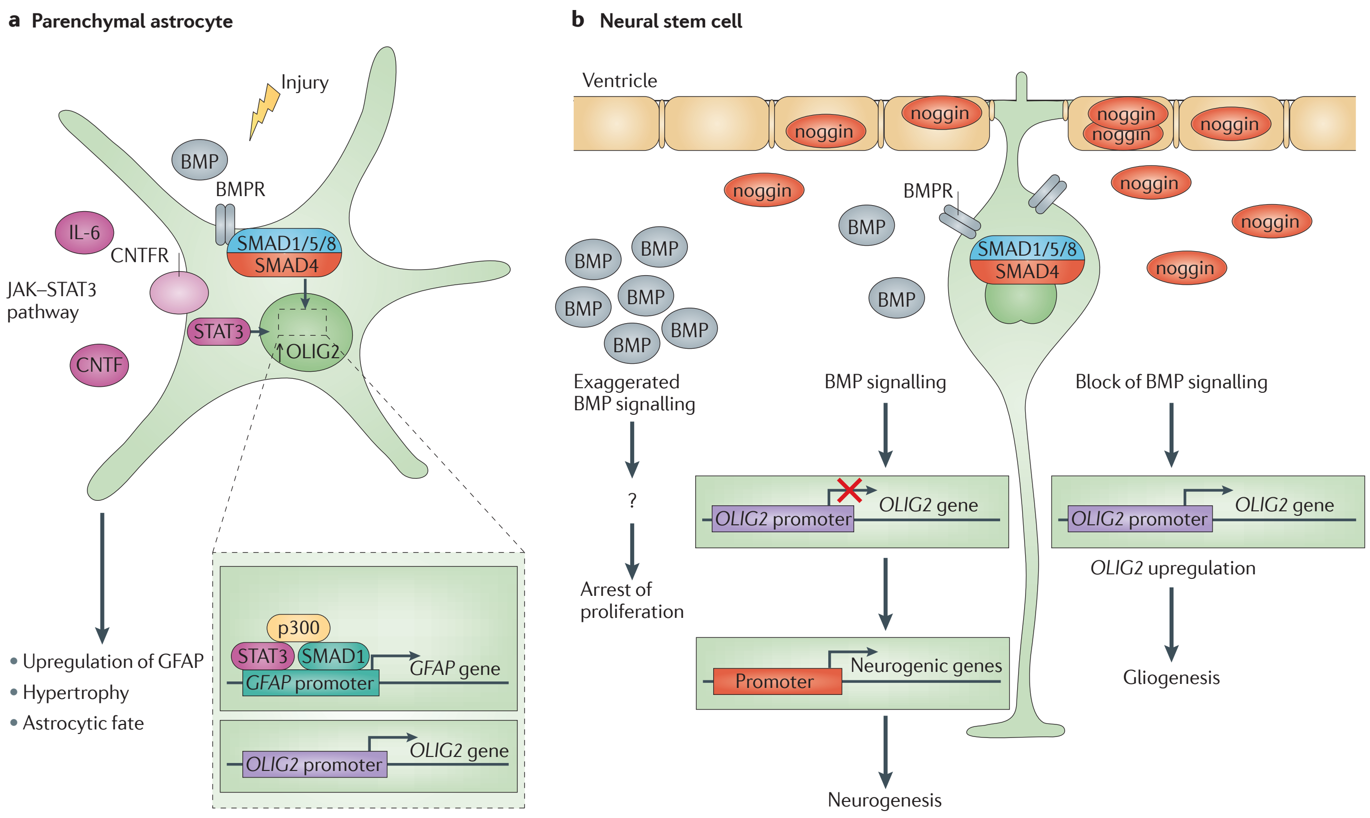


Figure 3 | **Distinct signalling after brain injury and in the NSC niche. a** | Bone morphogenic protein (BMP) signalling in reactive astrocytes promotes a gliogenic fate[124,125], which seems to depend on the high levels of signal transducer and activator of transcription (STAT) signalling in reactive astrocytes[126,134]. Downstream of BMPs, the transcription factor SMAD1 and activated STAT3, bind to the glial fibrillary acidic protein (GFAP) promoter at distinct sites and interact with the transcriptional coactivator p300, inducing GFAP expression and thereby stabilizing the astrocytic fate[135,136]. **b** | In the adult subependymal zone (SEZ), BMP signalling seems to antagonize the expression of oligodendrocyte transcription factor 2 (OLIG2), a transcription factor that promotes gliogenesis in the adult brain and is also upregulated in reactive astrocytes after injury[51,129,188]. This differential effect of BMP signalling in the SEZ might be due to the low levels of STAT signalling in this location[134] compared with in reactive astrocytes[56]. In the presence of BMP signalling and low levels of OLIG2 expression, neurogenic genes are expressed. Inhibition of BMP signalling in the SEZ — for example, by infusion of the BMP inhibitor noggin — results in upregulation of OLIG2 expression and fate conversion towards the oligodendrocyte lineage at the expense of neurogenesis in the adult mouse SEZ[126]. When BMP levels are abnormally raised[127,128], cell cycle progression is inhibited, thereby also abrogating neurogenesis. Notably, ependymal cells play a crucial part in controlling the appropriate BMP levels, as they express BMP inhibitors such as noggin. BMPR, BMP receptor; CNTFR, ciliary neurotrophic factor receptor; IL-6, interleukin-6.

more potently than STAT signalling alone[137], and acts as a key pathway to initiate and maintain astrocyte fate during development[136-139].

After injury, interaction of BMP and STAT signalling provides a powerful cooperative pathway to restrict reactive astrocytes in their glial lineage, as STAT signalling is potently activated by release of the cytokines interleukin-6 (IL-6), ciliary neurotrophic factor (CNTF) and cardiotrophin-1 (FIG. 3a) by astrocytes themselves, microglia and possibly other cell types upon brain injury[43]. Receptor activation by these ligands triggers activation of the Janus tyrosine kinase (JAK)–STAT pathway[140], leading to translocation of STAT1 and/or STAT3 into the nucleus. Of relevance here, STAT- and SMAD-mediated transcription both promote astroglial traits (discussed below). During development, CNTF and cardiotrophin-1 promote differentiation of cerebral cortical precursor cells into astrocytes by activation of the CNTF receptor, which leads to phosphorylation of STATs. These shuttle to the nucleus and bind to the promoter region of genes encoding astrocytic proteins (for example, GFAP and S100β) to promote transcription of these genes. While promoting astrocyte gene expression this causes simultaneous inhibition of the neuronal lineage in culture[141,142].

After injury, STATs increase the expression of GFAP and components of the JAK–STAT signalling cascade itself, including interleukin-6 receptor subunit beta (also known as GP130), JAK1, STAT1 and STAT3. This creates a positive-feedback loop that seemingly stabilizes the astrocytic phenotype. This function is further supported by the response to injury of mice with conditional deletion of STAT3. This was achieved by deleting the floxed STAT3 gene using different CRE-expressing mouse lines (nestin-CRE or GFAP-CRE). Whereas mice only expressing CRE had no change in phenotype, deletion of STAT3 attenuated the upregulation of GFAP, reduced astrocyte hypertrophy, limited the migration of astrocytes into the injury site and led to more widespread infiltration of inflammatory cells. This resulted in an increase in injury volume and impaired recovery of motor functions several weeks after the injury[56,57].

As these effects resemble those observed after deletion of GFAP and vimentin, the transcriptional regulation of these genes by STAT3 may be fundamental to the observed phenotypes. Conversely, conditional knockout of the protein suppressor of cytokine signalling 3 (SOCS3), which inhibits STAT signalling, is associated with an increase in astrocytic migration into the lesion and enhanced contraction of the lesioned area, as well as improvement of functional recovery after spinal cord injury in mice. Notably, however, deletion of SOCS3 had no effect on astrocyte proliferation[57].

Thus, the JAK–STAT pathway cooperates with BMP signalling in upregulating the expression of astrocyte genes after brain injury, regulating astrocytic fate (FIG. 3a). Moreover, the relatively low STAT signalling in the neurogenic niches[134] may therefore account for some of the differences in signalling between these different locations and possibly also explain how BMP signalling acts differently inside and outside the neurogenic niches.

TGFβ signalling. As mentioned above, TGFβ signalling seems to play no major part in neurogenesis in the adult SEZ[126], whereas it has a major role in reactive gliosis outside the neurogenic niches after brain injury. Reactive astrocytes contribute to scar formation by depositing proteoglycans such as neurocan and phosphacan that inhibit regeneration[63], which is also influenced by TGFβ signalling[143], possibly in combination with activation of EGFR[75] (see above).

Upon injury with BBB breakdown, the plasma component fibrinogen is released into the brain tissue. Fibrinogen regulates the formation of active TGFβ, which leads to phosphorylation of SMAD2. Phosphorylated SMAD2 then forms a complex with the common partner SMAD4 (Co-SMAD4) and shuttles to the nucleus, regulating transcription, which may then cause reactivity of astrocytes and neurocan release in mice after stab wound injury[143] (FIG. 2b). These findings are supported by studies in mice lacking SMAD3, the other SMAD family protein downstream of TGFR signalling, in which there is also a reduction in GFAP-positive cells, microglia and NG2-positive cells after cortical stab wound injury[144]. The inhibition of neurite outgrowth *in vitro* in conditioned medium of fibrinogen-treated astrocytes could be reversed by a TGFβ receptor inhibitor[143]. This study is an exciting example of how detrimental effects of reactive gliosis could possibly be modulated by targeting distinct signalling pathways.

Pathway interactions after injury

The complex interactions between various signalling pathways after injury are only beginning to be understood. For example, IL-6-type cytokines alone are not capable of promoting astrocyte proliferation, but they enhance EGF-stimulated astrocyte proliferation after injury in the rat, suggesting an interaction of the EGFR–MAPK and JAK–STAT pathway[145,146]. Also, ATP released from damaged cells after injury acting via P2Y receptors synergistically enhances the proliferative effects of FGF2 after injury, whereas P2X receptors inhibit the ability of FGF2 to stimulate DNA synthesis in rat cortical astrocyte cultures[147]. These distinct effects are mediated by phosphorylation at different residues of STAT3, as activation of P2Y and P2X receptors by the release of ATP and other purinergic ligands described above can stimulate STAT3 phosphorylation at residue Tyr705, whereas only P2Y mediates the additional phosphorylation at Ser727 (REF. 148). Furthermore, P2 receptors can activate ERK, and interference with this ERK signalling prevents Ser727 STAT3 phosphorylation[148]. As described above, STAT3-mediated transcription is crucial to regulate reactive astrogliosis, and phosphorylation of STATs modulates its nuclear translocation and transcriptional activity. These cooperative effects thereby regulate the response of reactive astrocytes.

Thus, the interaction between multiple pathways may restrict glia from generating neurons after injury, but some of these signals are also needed for the beneficial aspects of reactive gliosis (see above). For example, it may seem desirable to abrogate STAT3 to inhibit the potent bias towards astrogliosis and allow neurogenesis, but this may interfere with the wound closure and scar-forming aspects of the reactive astrocytes as highlighted in mouse lines with conditional STAT3 deletion[56,57]. These considerations demonstrate the urgent need to better understand the molecular pathways regulating distinct aspects of reactive astrogliosis in order to separate the different functions of this process.

Reprogramming astrocytes into neurons

Given the above considerations, the question arises as to whether such a barrage of inhibitory signals can be overcome. Indeed, neuroblasts migrating from neurogenic niches into the sites of brain injury can not only overcome the antineurogenic environment in the adult brain parenchyma[149], but can seemingly even generate the appropriate neuronal subtype affected by the injury (BOX 3). This could provide a starting point in the search for the missing link between the acquisition of a dedifferentiated state and successful neurogenesis. Some insights into the possible nature of this missing link were provided by studies on cultures of postnatal astrocytes, which are not yet fully mature and hence more plastic than astrocytes in the adult parenchyma, discussed below.

Neurogenic fate determination by PAX6. Although reactive astrocytes can generate neurons *in vitro*, they fail to do so *in vivo*, suggesting that it is the lineage progression between an undifferentiated stem cell and a committed neuroblast that fails to become activated in reactive astrocytes *in vivo*. For example, dedifferentiation of reactive astrocytes may not be sufficiently complete to render gene loci encoding neurogenic fate determinants accessible, preventing their reactivation. Intriguingly, like NSCs — which express mRNAs of neurogenic factors, but do not yet possess the proteins[25] — reactive astrocytes in the cerebral cortex seem to upregulate mRNA of the transcription factor PAX6 after focal laser lesion[46], whereas they lack detectable levels of PAX6 protein[129]. In the neurogenic niches, *PAX6* mRNA levels are further upregulated in the progeny of the NSCs, which then possess PAX6 protein[129], playing a crucial role for neurogenesis both during development[150] and in adulthood[111]. Interestingly, reactive Müller glia in the retina, which exhibit a limited degree of spontaneous neurogenesis, also regain PAX6 at the protein level following NMDA-induced damage in mice[151]. Thus, it might be possible to overcome the failure to enter a neurogenic programme by directly expressing neurogenic fate determinants in reactive astrocytes to reach sufficiently high levels of PAX6 protein (FIG. 4a).

The concept that inherently non-neurogenic astrocytes can be instructed to generate neurons has been firmly established *in vitro*. Within the developing cortex, PAX6 is normally present in radial glia, but its expression is drastically reduced in postnatal astrocytes[150]. When astrocytes isolated from the mouse cerebral cortex were forced to re-express PAX6 by means of retroviral vectors, they lost GFAP expression and acquired a neuronal morphology and the early neuronal marker tubulin β3 chain[150]. Further work confirmed that PAX6 and other neurogenic factors can cause a true glia-to-neuron conversion. After losing glial hallmarks, such as GFAP and passive electrical conductance, cells gradually acquire the electrical properties of neurons, such as repetitive action-potential firing following electrical stimulation[152].

Consistent with the above findings, retroviral delivery of neurogenic fate determinants, such as PAX6, into the adult injured mouse cerebral cortex or *Neurog2*, which encodes neurogenin 2 (NGN2), into adult rat injured spinal cord can induce the expression of the early and mature neuronal markers doublecortin (DCX) or NeuN in a subpopulation of transduced cells[129,153]. These studies provide proof-of-principle evidence that proliferating cells can be reprogrammed *in vivo* towards neurogenesis. Given that the retroviral vectors used in these studies incorporate only in proliferating cells and that neurons are postmitotic, it seems that the origin of these new neurons is the glia whose proliferation is enhanced after injury[28,52,129]. However, the exact type of proliferative glial cells infected in these paradigms is not known, which could be important as distinct types of glia may differ in their susceptibility to neurogenic reprogramming. Although reactive astrocytes represent a particularly

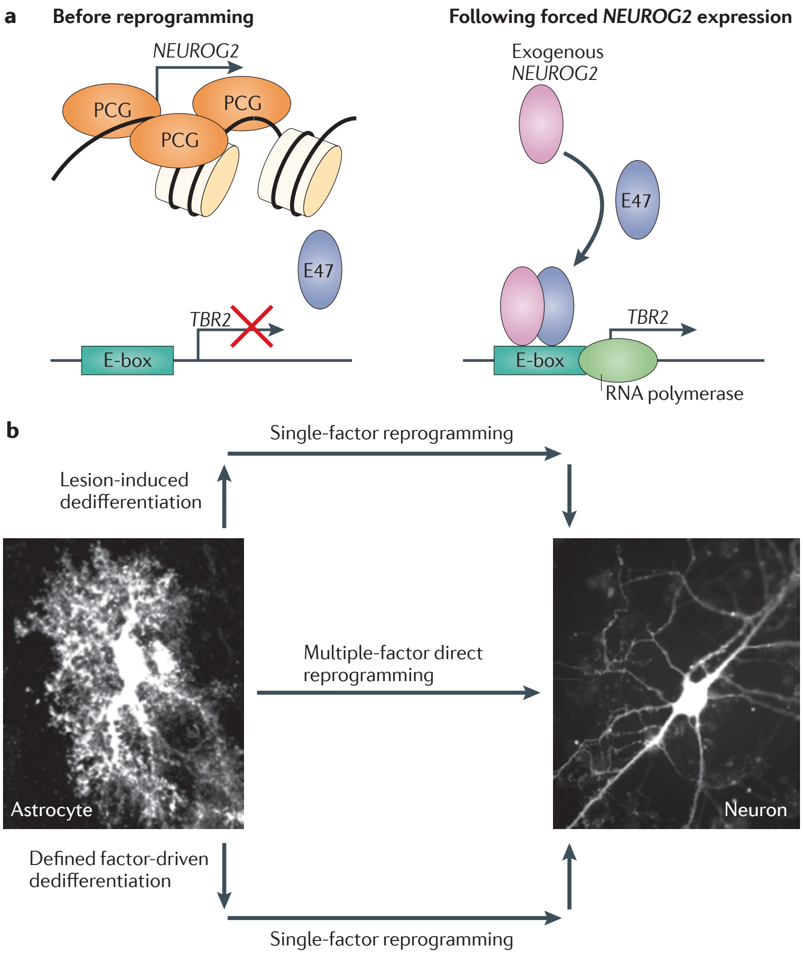


Figure 4 | **Reprogramming parenchymal astrocytes into neurons. a** | Possible molecular mechanism underlying reprogramming of postnatal astroglia. In postnatal astrocytes, the chromatin around gene loci encoding neurogenic fate determinants — for example, *NEUROG2*, which encodes the transcription factor neurogenin 2 (NGN2), is held in a closed structure by the polycomb group repressor complex (PCG)[158] (left panel). Consequently, downstream targets of these fate determinants, such as T-box brain protein 2 (TBR2) in the case of NGN2, are not expressed. Exogenous expression of *NEUROG2* (right panel) can bypass the silenced endogenous gene locus. NGN2 binds together with the ubiquitously expressed E-box binding protein E47 to enhancer regions of its target genes, inducing neurogenesis. **b** | Schematic drawing summarizing possible avenues for generating neurons from astrocytes. Following lesion-induced reactivation, astrocytes dedifferentiate, rendering them akin to astrocyte-like stem cells. *In vitro*, these cells have neurogenic potential, which can then be enhanced and directed towards different subtype identities by single neurogenic transcription factors[155] (shown by the upper pathway). There is evidence that this is also possible *in vivo*, although the cellular identity of the cells generating the reprogrammed neurons is currently unknown[129,153]. Alternatively, rather than relying on injury-induced dedifferentiation, a more controlled dedifferentiation may also be achieved by forced expression of defined factors alone or in combination with growth factor signalling[189] (shown by the lower pathway). Alternatively, direct reprogramming, bypassing a dedifferentiated state, may be achieved by a defined cocktail of factors (shown by the middle pathway). Although this has not yet been achieved experimentally in the nervous system, a similar approach has been successful in reprogramming exocrine cells of the pancreas of adult mice into insulin-secreting β-cells[163].

attractive target for eliciting neurogenesis after injury due to their dedifferentiation as discussed above, there may be considerable differences in responsiveness between reactive astrocytes depending on the degree of reactivation and re-entry into the cell cycle. Furthermore, there may be an optimal time window for induction of neurogenesis given that hallmarks of proliferation and dedifferentiation in reactive glia are transient, as outlined above[28,46,52,53]. Moreover, other proliferating cell types, such as NG2 cells, may be amenable to reprogramming *in vivo*, given the early findings that OPCs from the postnatal rat optic nerve can be reprogrammed towards an NSC-like state involving an astrocyte intermediate[38].

Other neurogenic fate determinants. Other neurogenic fate determinants, such as the proneural genes *NEUROG2* and the mammalian homologue of achaete-scute (MASH1; also known as ASCL1), are even more effective than PAX6 in reprogramming astrocytes *in vitro*[152]. Constant signalling of these factors activates a full neuronal programme from astrocytes *in vitro*, differentiating these cells into fully functional synapse-forming glutamatergic neurons[154,155] (FIG 4a). Moreover, ASCL1 or the transcription factor DLX2 are each sufficient to induce a GABAergic neuron phenotype in astrocytes *in vitro*[155]. A possible explanation is that these neurogenic fate determinants not only induce a generic neuronal fate, but possess neuronal subclass-specifying activity during development and adult neurogenesis[156].

The next step will be to identify strategies to drive astrocytes towards the selective generation of specific subtypes within each subclass, such as the different types of glutamatergic cortical projection neurons (that is, corticofugal and callosal neurons) and the GABAergic interneurons (fast spiking, burst or regular spiking). This may allow for the regeneration of selective populations of neurons that degenerate in conditions such as amyotrophic lateral sclerosis (that is, glutamatergic corticospinal motor neurons) or the modification of hyperactive neural circuits by insertion of distinct inhibitory neurons[157].

Postnatal astrocytes. Postnatal astrocytes represent a transition point between radial glia and mature astrocytes. These cells are still proliferative, but have lost an intrinsic neurogenic capacity, in part owing to the epigenetic silencing of *NEUROG1* and/or *NEUROG2* loci by polycomb group complex-mediated repression during the transition from cortical neurogenesis to astrogliogenesis[158]. *NEUROG1* and *NEUROG2* are basic helix–loop–helix transcription factors directly regulated by PAX6. They play important parts in inducing a glutamatergic neuron identity from NSCs during cortical development by inducing the expression of T-box brain protein 1 (TBR1) and TBR2 (REF. 159). Forced expression of *NEUROG1* and *NEUROG2* may thus allow for bypassing of this epigenetic barrier in postnatal astrocytes (FIG. 4a), as the downstream targets of this proneural gene have not yet been made inaccessible by epigenetic modifications. Reprogramming of postnatal astrocytes *in vitro* into glutamatergic neurons by *NEUROG2* indeed involves the same sequence of expression of TBR2 and TBR1 (REF. 155) as observed in the specification of glutamatergic neurons in the developing and adult brain[160,161]. This suggests that these downstream targets of *NEUROG2* are still accessible in postnatal astrocytes or can be made accessible by recruitment of the chromatin-remodelling machinery by *NEUROG2* (FIG. 4a).

However, further progressive epigenetic silencing during astrocyte differentiation is likely to confer reduced susceptibility to single-factor reprogramming. It is thus important that astrocytes, when activated by injury, dedifferentiate and acquire neurosphere-forming potential. Forced expression of *NEUROG2* or *DLX2* can induce many of these neurosphere cells to generate glutamatergic and GABAergic neurons, respectively[155]. These data provide proof-of-principle evidence that even adult astrocytes can be reprogrammed towards distinct neuronal fates. This seems to require two steps, one promoting dedifferentiation towards a stem cell- or progenitor-like state, followed by a subsequent step of re-specification towards a neuronal fate (FIG. 4b).

Thus, injury-induced reactivation is likely to involve epigenetic modifications that render the nucleus of the reactive astrocytes more susceptible to the activity of neurogenic genes. Reactivation may involve chromatin remodelling in glia, as occurs in the mouse retina *in vivo* after neurotoxic damage. Specifically, Müller glia re-express BRG1-associated factor 60C (BAF60C), a component of the switch/sucrose non-fermentable (SWI/SNF) chromatin remodelling complex that physiologically is only expressed in retinal progenitors during development[162].

Fully understanding the molecular processes underlying the reactivation and dedifferentiation of astrocytes may allow for the definition of a set of transcription factors that render mature astrocytes amenable to subsequent neuronal reprogramming or even permit direct reprogramming without prior dedifferentiation (FIG. 4b), as has been achieved for the conversion of pancreatic α-cells into β-cells *in vivo*[163]. Thus, glial cells reacting to injury may present potential targets for direct reprogramming into neurons *in situ*, given their many similarities with stem cells. Such approaches embody novel alternatives to exogenous sources of neurons for therapeutic intervention.

Outlook and perspectives

There is increasing evidence that adult glia could be a source of new neurons in the context of brain injury. A tight balance will be required to elicit neurogenesis for repair as well as maintain the beneficial aspects of the reactive glia. This not only applies to reactive astrocytes but to ependymal cells and potentially the NG2 glia, of which the beneficial or detrimental effects after brain injury are least well understood so far. Moreover, even if neurogenesis can be elicited *in vivo*, a crucial challenge will be to determine how these regenerated neurons are connected within the pre-existing neuronal network and to what extent they are beneficial or detrimental. This question has been widely ignored given the beneficial outcome of transplantation experiments with young neurons in patients with Parkinson's disease as well as in mouse models, as the synaptic wiring of these young neurons is largely unknown[164,165].

Another key challenge to address in the future lies in determining the extent to which the findings in rodents discussed above apply to the human brain, as there may be specific differences[16]. A related challenge will be whether such phenomena can be used for clinically viable strategies for brain repair. Of note, in postmortem material from patients with stroke, haemorrhage, Huntington's disease or multiple sclerosis, some evidence for a proliferative reaction of neural cells and potential endogenous neurogenesis has been reported[166–170]. However, there is urgent need for more data obtained from human patient material, and standards in regard to fixation and stainings would also greatly advance the reproducibility of such data. Based on the presently available data, injury-induced recruitment of progenitors or neuroblasts as observed in rodents (BOX3) seems also to occur in humans[170]. However, the relatively large distances to be covered in the human brain compared with the rodent brain and potential differences between the migratory behaviour of human and rodent neuroblasts make injury-induced recruitment a complex task.

For regions further away from neurogenic sites that cannot be reached by endogenous neuroblasts, neurons may be locally regenerated from activated glial cells, as described above. Indeed, cells expressing transcription factor SOX2, Musashi, vimentin and nestin at sites of brain haemorrhage[171] or stroke[172] in humans may be taken as first evidence for dedifferentiation of local glia towards a more stem- or progenitor cell-like state at injury sites. Moreover, a population resembling OPCs of the adult rodent brain, which express OLIG2 and the indicator for proliferation KI67, has also been observed in the normal healthy human brain[173]. Interestingly, such cells in the white matter seem to be particularly ready to convert towards neurogenesis *in vitro*[174] and may be the source of neurons close to multiple sclerosis lesions in the white matter of patients *in vivo*[166].

Glia in the human brain therefore provide novel avenues to approach brain repair in patients with diverse neurological disorders and injuries. However, in line with the increased diversity of glia during phylogeny, glial cells in the human brain are indeed more complex and diverse than in the rodent brain[16]. It is therefore of particular importance to better understand this diversity with the final aim of harnessing glia for improved outcome after brain disease.

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Acknowledgements

We apologize to investigators whose work could not be cited owing to space limitations. We are grateful to members of the Götz laboratory for fruitful discussions and are particularly indebted to L. Dimou, A. Grande, C. Heinrich, J. Ninkovic and S. Sirko for their helpful comments on the manuscript. Work in the laboratory is funded by the EU, the Deutsche Forschungsgemeinschaft (including SFB 596, 870 and SPP 1356), the German Federal Ministry of Education and Research (BMBF), the Helmholtz Association (CoReNe; Helma), the Fidelity Foundation and ForNeuroCell of the Bavarian State Ministery of Science, Research and Arts.

Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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