Progress in Neurobiology 174 (2019) 36–52

Contents lists available at ScienceDirect

Progress in Neurobiology

journal homepage: www.elsevier.com/locate/pneurobio

Review article

Transcriptional and epigenetic mechanisms underlying astrocyte identity

Maria Angeliki S. Pavlou[a,b], Luc Grandbarbe[a], Noel J. Buckley[c], Simone P. Niclou[b,d], Alessandro Michelucci[b,e,*]

[a] Life Sciences Research Unit, University of Luxembourg, Esch-sur-Alzette, Luxembourg
[b] NORLUX Neuro-Oncology Laboratory, Department of Oncology, Luxembourg Institute of Health, Luxembourg, Luxembourg
[c] Department of Psychiatry, Warneford Hospital, University of Oxford, Oxford, United Kingdom
[d] KG Jebsen Brain Tumour Research Center, Department of Biomedicine, University of Bergen, Bergen, Norway
[e] Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-Belval, Luxembourg

ARTICLE INFO

Keywords:
Transcriptional mechanisms
Epigenetic modifications
Astrocyte differentiation
Reactive astrogliosis

ABSTRACT

Astrocytes play a significant role in coordinating neural development and provide critical support for the function of the CNS. They possess important adaptation capacities that range from their transition towards reactive astrocytes to their ability to undergo reprogramming, thereby revealing their potential to retain latent features of neural progenitor cells. We propose that the mechanisms underlying reactive astrogliosis or astrocyte reprogramming provide an opportunity for initiating neuronal regeneration, a process that is notably reduced in the mammalian nervous system throughout evolution. Conversely, this plasticity may also affect normal astrocytic functions resulting in pathologies ranging from neurodevelopmental disorders to neurodegenerative diseases and brain tumors. We postulate that epigenetic mechanisms linking extrinsic cues and intrinsic transcriptional programs are key factors to maintain astrocyte identity and function, and critically, to control the balance of regenerative and degenerative activity. Here, we will review the main evidences supporting this concept. We propose that unravelling the epigenetic and transcriptional mechanisms underlying the acquisition of astrocyte identity and plasticity, as well as understanding how these processes are modulated by the local microenvironment under specific threatening or pathological conditions, may pave the way to new therapeutic avenues for several neurological disorders including neurodegenerative diseases and brain tumors of astrocytic lineage.

Abbreviations: 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; Aβ, amyloid β; AD, Alzheimer's disease; ASCL1, Achaete-Scute Family BHLH Transcription Factor 1; BBB, blood-brain barrier; BDNF, brain-derived neurotrophic factor; BHLH, basic helix-loop-helix; BMP, bone morphogenetic protein; CSCs, cancer stem-like cells; CNS, central nervous system; CNTF, ciliary neurotrophic factor; Coup-Tfi and Coup-tfII, Chicken ovalbumin upstream promoter-transcription factors I and II; DNMT1, DNA methyltransferase 1; E, embryonic day; EZH2, Enhancer of zeste homolog 2 gene; ESET, ERG-associated protein with SET domain; FGF2, fibroblast growth factor; GASC1, gene amplified in squamous cell carcinoma 1; GBM, Glioblastoma; G-CIMP, glioma-CpG island methylator phenotype; GFAP, glial fibrillary acidic protein; GSC, GBM cancer stem-like cells; H1, Histone 1; H2A, Histone 2A; H2B, Histone 2B; H3, Histone 3; H4, Histone 4; H3K4me, methylation of Lysine 4 of Histone 3; H3K9me, methylation of Lysine 9 of Histone 3; H3K4me2, dimethylation of Lysine 4 of Histone 3; H3K9me2, dimethylation of Lysine 9 of Histone 3; H3K9me3, trimethylation of Lysine 9 of Histone 3; H3K27me3, trimethylation of Lysine 27 of Histone 3; H3K36me3, trimethylation of Lysine 36 of Histone 3; H4K20me, methylation of Lysine 20 of Histone 4; HDACs, histone deacetylases; HDAC3, histone deacetylase 3; hESCs, human embryonic stem cells; HMT, histone methyltransferase; IDH1, isocitrate dehydrogenase 1; IDH2, isocitrate dehydrogenase 2; IFN-γ, interferon-gamma; IL-6, interleukin 6; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; KDM5A, lysine-specific demethylase 5A; LCN2, lipocalin 2; LIF, leukemia inhibitory factor; lncRNA, long non-coding RNA; MAPK, Ras/mitogen-activated protein kinase; MECP2, methyl CpG binding protein 2; MGMT, DNA repair enzyme O^6-methylguanine-DNA methyltransferase; miRNAs, microRNAs; MPTP, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NADPH, nicotinamide adenine dinucleotide phosphate; ncRNA, non-coding RNA; NFIA, nuclear factor I A-type; NFIB, nuclear factor I B-type; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NICD, Notch intracellular domain; NSCs, neural stem cells; RA, retinoic acid; RARs, retinoic acid receptors; RXRs, retinoid X receptors; RBP-J, recombining binding protein suppressor of hairless; SCI, spinal cord injury; SHH, Sonic hedgehog; STAT3, signal transducer and activator of transcription 3; STAT/CBP, signal transducer and activator of transcription/CRE binding protein; SVZ, subventricular zone; TBI, traumatic brain injury; TET, ten-eleven translocation family of enzymes; TGF-β, transforming growth factor-beta; TNF, tumor necrosis factor-alpha; PD, Parkinson's disease; WNT1, wingless-type MMTV integration site1

* Corresponding author at: Luxembourg Institute of Health, Department of Oncology, NORLUX Neuro-Oncology Laboratory, 84, Rue Val Fleuri, L-1526, Luxembourg.
E-mail address: alessandro.michelucci@lih.lu (A. Michelucci).

https://doi.org/10.1016/j.pneurobio.2018.12.007
Received 8 October 2018; Received in revised form 20 November 2018; Accepted 28 December 2018
Available online 29 December 2018

1. Introduction

Astrocytes represent the most abundant cell population in the adult brain. As such, they are involved in several activities, ranging from regulating ion and fluid homeostasis, establishing and maintaining the blood-brain barrier (BBB) (Oberheim et al., 2006; Zhang and Barres, 2010) to providing neurons with nutrients and metabolites (Escartin et al., 2007; Pellerin et al., 2007). Further, they are involved in regulating synaptic transmission through the uptake of the neurotransmitter glutamate (Parpura et al., 1994), they interact with neurons as they actively participate in the formation and functioning of neuronal synapses (Barres, 2008) and they communicate with them through calcium signals (Nedergaard, 1994). The active communication between astrocytes and neurons is involved in sleep homeostasis, breathing, circadian regulation and memory (McIver et al., 2013). Further, astrocytes promote the differentiation of oligodendrocyte progenitor cells into mature myelinating oligodendrocytes and support myelin maintenance (Domingues et al., 2016).

Under CNS insults such as trauma, tumor, infection or neurodegeneration, astrocytes become activated, a process known as reactive astrogliosis, resulting in changes in their morphology and expression of their molecular repertoire (Pekny and Nilsson, 2005; Sofroniew, 2009). Reactive astrocytes release pro- and anti-inflammatory cytokines, thus implying both beneficial and detrimental effects in a context-dependent manner. Under specific conditions, the consequence of the neuroinflammatory response following brain injury is the formation of the glial scar, mainly constituted by reactive astrocytes, which acts as a physical insulator to contain the lesioned area. In mammals, intriguingly, the glial scar acts as a negative regulator of neurogenesis and neurite outgrowth, in comparison to lower vertebrates, which are able to recruit glial progenitors that undergo reactive neurogenesis and replace the neurons lost upon injury (Kroehne et al., 2011). Notably, reactive astrocytes exhibit endogenous NSC hallmarks such as self-renewal, multipotency and expression of immature markers. In the mouse cerebral cortex, only a subset of astrocytes are able to restore their proliferation capacity and enter a reprogramming mode, whereupon they are able to form neurospheres and generate neurons (with firing action potential), oligodendrocytes and astrocytes or be directly reprogrammed into neurons or neuroblasts *in vivo* (Bardehle et al., 2013; Gascon et al., 2017; Gotz et al., 2015; Niu et al., 2013). Could these terminally differentiated astrocytes be considered as immature or even as latent stem cells since they possess the capacity to re-enter a self-renewal process and, eventually, potentially a differentiation process and even acquire an alternate cell identity? It is possible that the mechanisms underlying reactive astrogliosis or astrocyte reprogramming may hold the secret of neuronal regeneration, a process that is lost during evolution of the mammalian CNS.

The onset of astrogenesis relies upon extracellular signaling and intrinsic epigenetic modifications, such as DNA methylation and histone modifications. Disruption in any of these mechanisms causes abnormal astrocyte differentiation and leads to neurodevelopmental disorders (Molofsky et al., 2012; Sloan and Barres, 2014). The importance of astrocytes for neuronal integrity and survival is also prominent in the adult brain. Conditional ablation of astrocytes in adult mice results in neuronal loss and severe motor deficits (Schreiner et al., 2015). On the other hand, their contribution to neuroinflammation and the loss of normal homeostatic functions, may corroborate their implication in the onset and progression of neurodegenerative diseases, including Parkinson's and Alzheimer's diseases (PD and AD) (Niranjan, 2014). Brain cancer is another neurological disease where astrocytes are currently implicated both as tumor initiating cells and modulators of the tumor microenvironment (Ahmed et al., 2013). Supporting the notion that brain tumors can arise from differentiated cells, transduction of murine astrocytes *in vivo* with oncogenic lentiviral vectors results in tumor formation. Mature astrocytes were shown to be able to dedifferentiate and gain expression of progenitor/stem cell markers or even transdifferentiate into neurons during tumorigenesis (Friedmann-Morvinski et al., 2012). Transformed astrocytes can be reprogrammed to re-acquire stem cell features, but also give rise to more malignant phenotypes, thereby generating a heterogeneous cell population within the malignant tumor (Friedmann-Morvinski et al., 2012; Friedmann-Morvinski and Verma, 2014).

Clearly, astrocytes are crucial players in the developing and adult CNS in both health and disease. Consequently, it is critical to elucidate the transcriptional and epigenetic mechanisms underlying acquisition of astrocyte identity and plasticity, as well as understanding how these processes are modulated under pathological conditions. The comprehension of these mechanisms will pave the way to novel therapeutic approaches aimed, for example, at restoring the homeostatic astrocytic functions in brain tumors or at enhancing their neurogenic properties in neurodegenerative diseases. This review focuses on those transcriptional and epigenetic mechanisms underlying the physiological differentiation and activation of astrocytes that contribute to our understanding of aberrant phenotypes identified under pathological conditions leading to neurological disorders.

2. Epigenetic modifications: basic mechanisms and their role in CNS development

Epigenetics generally refers to any heritable alteration occurring in a cell that has an immediate effect on gene expression, without modifying the DNA sequence. This includes DNA methylation and histone modifications, which are able to alter DNA accessibility and chromatin structure, and RNA-mediated epigenetic mechanisms that mainly act at the transcriptional and post-transcriptional level (Pavlou et al., 2017).

2.1. DNA methylation

DNA methylation entails the transfer of a methyl group from *S*-adenosyl methionine to the 5′ position of cytosines, resulting in the formation of 5-methylcytosine (5mC) (Mehler, 2008). The methylation process occurs throughout the entire genome, although it is enriched in CpG islands. These sites are usually found in promoter regions and, therefore, methylation generally leads to reduced gene transcription. This is mediated by two mechanisms: i) DNA methylation prevents the association of DNA binding factors, such as transcription factors, to their cognate DNA sequence or ii) the methylated CpGs can recruit proteins involved in gene repression, such as co-repressors (Fig. 1) (Klose and Bird, 2006). 5mC can be converted to 5-hydroxymethylcytosine (5hmC) *via* an oxidation reaction catalyzed by the ten-eleven translocation family of enzymes (TET). Although its function still remains enigmatic, 5hmC was thought to exist both as an intermediate product in the process of active DNA demethylation and to represent an epigenetic modification regulating chromatin or transcriptional factors (Kriaucionis and Heintz, 2009; Tahiliani et al., 2009). Total 5hmC levels increase during development of the human cerebellum and chromosomal regions positive for this mark are linked with neurodevelopmental genes (Wang et al., 2012).

In the mammalian genome, DNA methylation is mediated by DNA methyltransferases 1, 3a and 3b (DNMT1, 3a, 3b). However, only DNMT3a and DNMT3b are able to exert *de novo* methylation, while DNMT1 is responsible for the maintenance of DNA methylation after replication (Klose and Bird, 2006). DNMTs are essential for embryonic development, and their loss interferes with tissue homeostasis (Bird, 2002). DNMT3a and DNMT3b are expressed in NSCs and have a crucial function in neurogenesis and neuronal function (Okano et al., 1999; Wu et al., 2010).

2.2. Histone modifications

DNA methylation and demethylation are critical regulatory mechanisms underlying CNS development. Nevertheless, the ability of

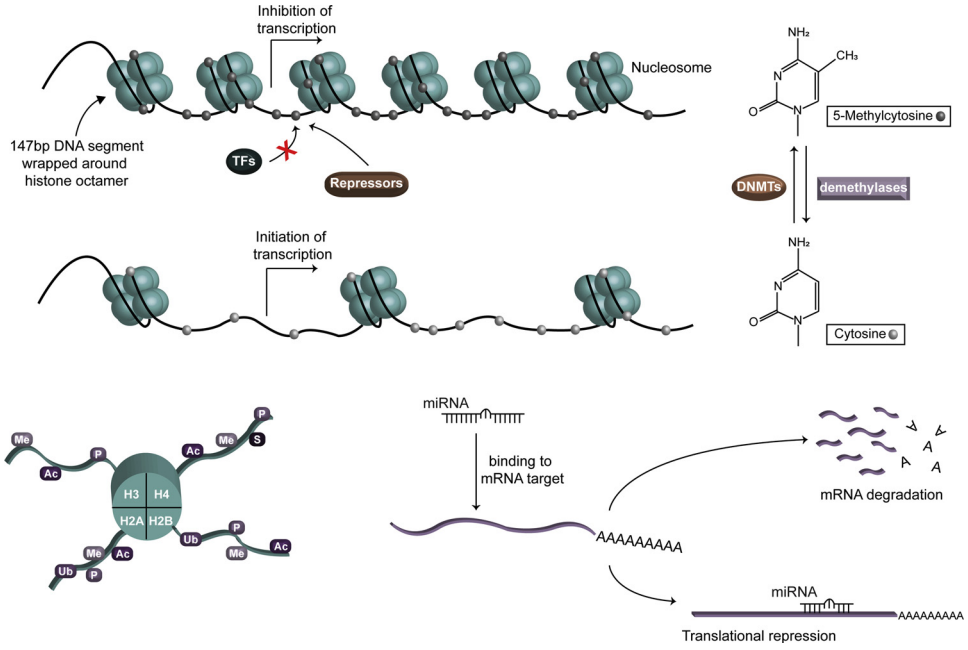

Fig. 1. Depiction of key epigenetic mechanisms. DNA methylation conducted by DNMTs results in the inability of transcription factors to bind to gene promoter regions or in the recruitment of repressor proteins. Both events contribute to gene repression. Conversely, DNA demethylation catalyzed by demethylases allows gene transcription. The major histone modifications include methylation (Me), acetylation (Ac), phosphorylation (P), ubiquitination (Ub) and sumoylation (S), influencing chromatin condensation and gene transcription both positively and negatively depending on the histone marks. Targeted mRNAs are recognized by miRNAs *via* sequence complementarity. The final result of this interaction involves the degradation of the mRNA target or the repression of its translation.

various regulatory factors to access their target genes is also influenced by chromatin modifications occurring at the histones level. Histones are essential proteins enabling the packaging of DNA into chromatin. They are responsible for the first level of chromosome condensation, the nucleosome. This protein-DNA complex is comprised of an octamer of core histone proteins and a 147bp long DNA segment wrapped around the histones (Fig. 1). Every nucleosome core is composed of two molecules of each histone 2 A (H2 A), histone 2B (H2B), histone 3 (H3) and histone 4 (H4), and is separated from the next by a ˜80bp DNA sequence, known as linker DNA. It is in this region that histone 1 (H1) binds, enabling the stabilization of the structure. All histones are subjected to post-translational modifications such as acetylation, methylation, phosphorylation, ubiquitination or sumoylation that primarily occur at the N-terminal tail of the proteins (Fig. 1). The tagging of histones with specific modifications shapes the condensation of chromatin in different manners, modulating the ability of DNA to associate with the transcriptional machinery and thereby regulating gene expression (Graff and Mansuy, 2008; Kouzarides, 2007; Tessarz and Kouzarides, 2014). Histone acetylation is catalyzed by histone acetyl transferases (HATs) and is, in principle, linked to transcriptional activation. On the other hand, histone deacetylation is catalyzed by histone deacetylases (HDACs) and is associated with transcriptional repression. Histone methylation is related to both transcriptional activation and repression, depending on the amino acid residue that is modified. For instance, lysine methylation of histone tails is associated with both activation and repression of gene expression; hence the effect of histone methylation on gene expression differs according to the position in the histone tail and the number of methylation in the lysine residues. For example, H3 methylation at lysine 4 (K4me), K36, and K79 leads to transcriptional activation, whereas H3 methylation at K9 and K27 is associated with transcriptional silencing. Several of these histone marks are essential during CNS development as they enable the sequential activation and deactivation of neurogenic and gliogenic gene promoters, resulting in the production of neuronal and glial cells at the appropriate developmental stages (Murao et al., 2016).

2.3. Non-coding RNAs

Non-coding RNA (ncRNA) molecules are transcribed from DNA, but are not translated into proteins. Among these, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) play a variety of roles in fine-tuning gene expression by transcriptional and post-transcriptional regulation. Many studies have shown that, in addition to histone modifications and DNA methylation, ncRNAs also participate in the mechanisms that ensure the sequential production of distinct neural cell types from NSCs during development and, generally, in multiple aspects of brain development and connectivity (Follert et al., 2014). MiRNAs represent one of the best characterized groups of ncRNAs. They are approximately 21–23 nucleotides long and are known for binding to the 3′ untranslated region (UTR) of their mRNA targets and either degrading them or inhibiting protein translation, thus contributing to post-transcriptional repression of gene expression (Fig. 1) (Mehler, 2008). Certain miRNAs have been described to be restricted in a particular organ suggesting a tissue-specific function. Likewise, several miRNAs are brain specific and play an important role in neurogenesis and in the development of glial cells (Fiore et al., 2011), with some being preferentially expressed in neurons or in astrocytes and others being equally distributed (Smirnova et al., 2005). These small ncRNAs seem to be regulated upon brain development. For example, a group of brain-expressed miRNAs was reported to be upregulated during neuronal differentiation, suggesting a potential contribution in controlling the timing of neuronal fate specification (Sempere et al., 2004). Another study documented a sequential increase of specific miRNA clusters at different developmental stages of the murine brain (Miska et al., 2004).

Taken together, it is evident that epigenetic modifiers, such as DNA methylation, histone modifications and RNA-mediated processes, accurately drive the development of the brain influencing lineage commitment of CNS cells, including neurons, oligodendrocytes and astrocytes.

3. Differentiation of NSCs into astrocytes

NSCs have the ability to self-renew and to differentiate into neurons, oligodendrocytes and astrocytes. Throughout mammalian brain development, the process of differentiation towards a neurogenic or gliogenic fate is finely timed and regulated. During the fetal expansion phase, NSCs self-replicate *via* symmetric cell division, whilst later, during mid-gestation, they undergo asymmetric cell division and receive cues to differentiate into neurons. Lastly, in late-gestation to perinatal periods they enter the gliogenic phase and differentiate into astrocytes and oligodendrocytes (Hirabayashi and Gotoh, 2005;

Takouda et al., 2017).

3.1. Key extracellular signals and transcriptional activators

3.1.1. The Notch pathway

The choice of NSCs between a neuronal and a glial cell fate is tightly regulated by environmental and intrinsic factors. Numerous studies have shown that specific signals, such as the neurogenic basic helix-loop-helix (bHLH) transcription factors, are involved in fate determination of the three neural lineages. They can, for example, promote the production of neurons whilst concurrently inhibiting the acquisition of glial fate. For instance, the pro-neural genes *Achaete-Scute Family BHLH Transcription Factor 1 (ASCL1*, *MASH1*), *Neurogenin 1* (*NEUROG1)*, or *Neurogenin 2* (*NEUROG2)* all promote neuronal fate determination while suppressing expression of astrocytic genes (Nieto et al., 2001; Sun et al., 2001). Inhibition of neurogenesis induced by pro-neural genes requires expression of transcriptional repressors such as HES1 and HES5, which are the downstream targets and effectors of the Notch pathway. The Notch signaling is highly conserved (for review see: (Louvi and Artavanis-Tsakonas, 2006)) and comprises four transmembrane Notch receptors (Notch1-4) and several ligands (Jagged1-2 and Delta-like1-4). Binding of the ligands to Notch receptors induces a proteolytic cleavage of the receptor resulting in the release of the Notch intracellular domain (NICD). Once released, the NICD fragment is translocated to the nucleus where it acts as a transcriptional coactivator. NICD cannot bind directly to DNA, but instead interacts with the DNA binding protein, suppressor of hairless (RBP-*J*) to initiate transcription of Notch target genes, such as the *HES* gene family (Andersson et al., 2011; Borggrefe and Oswald, 2009). Notch signaling is required to maintain a balance between the progenitor cell pool and its differentiating progeny (Borggrefe and Oswald, 2009; Imayoshi et al., 2010). In addition, Notch signaling promotes a glial cell fate while neuronal identity is repressed (Gaiano et al., 2000; Louvi and Artavanis-Tsakonas, 2006; Taylor et al., 2007). Later, Notch inhibits generation of both neurons and oligodendrocytes and promotes the differentiation of astrocytes (Grandbarbe et al., 2003).

3.1.2. The JAK-STAT signaling pathway

Following astrocyte specification, the first step of astrocytic differentiation is loss of neurogenic competence and expression of astrocytic genes. Members of the interleukin (IL)-6 family of cytokines, including leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF) and cardiotrophin-1, bind to their cognate receptors inducing their dimerization. This enables the receptor-associated Janus kinase (JAK) to autophosphorylate and become activated. JAKs in turn phosphorylate and activate signal transducer and activator of transcription 3 (STAT3) enabling formation of homodimers. STAT3 homodimers subsequently translocate to the nucleus where they bind to the promoters of astrocytic genes, including *GFAP*, permitting its expression resulting in astrocytic differentiation (Bonni et al., 1997). The JAK-STAT signaling pathway regulates several distinct cellular events including stem cell self-renewal and pluripotency capacity (Niwa et al., 1998; Raz et al., 1999), proliferation in *Drosophila* germ (Tulina and Matunis, 2001) and intestinal lines (Beebe et al., 2010). In all these cases, STAT3 governs expression of genes that determine cell identity. It might be that this common pathway essential for astrocyte differentiation and stem cell self-renewal reinforces the similarities between these cells and reflects a potentially evolutionary remnant of plasticity.

3.1.3. The BMP pathway

The bone morphogenetic protein (BMP) pathway also plays a role in astrocyte differentiation. BMP signaling activates the transcription factors SMAD1, 5 and 8 to form a complex which translocates to the nucleus to activate its target genes. BMPs have an important role in NSCs fate determination. Notably, SMADs inhibit oligodendrocyte development, but promote both neurogenesis and astrogliogenesis as well as astrocytic differentiation and maturation (Mabie et al., 1999; Setoguchi et al., 2004; Xiao et al., 2010). For example, BMP signaling promotes astrocytes maturation through phosphorylation of SMAD1/5/8 pathway leading to acquisition of a process-bearing morphology and expression of the most common astrocytic markers *GFAP*, *Aquaporin 4* (*AQP4)*, and *S100B* (Scholze et al., 2014). Moreover, *in vivo*, in a BMP receptor double knockout mouse (*Bmpr1a* and *Bmpr1b*), the number of astrocytes was decreased between 25–40 % (See et al., 2007). Astrocyte number is also reduced *in vitro*, following inhibition of BMP by noggin, while the oligodendrocyte number is increased (Cate et al., 2010). Notably, both STAT3 and SMAD1 form a complex that is bridged by the coactivator p300 leading to induction of astrocytic genes in a synergistic manner (Nakashima et al., 1999).

Both the levels and timing of expression of pro-astrogenic genes is critical for successful orchestration of initiation, maintenance and termination of differentiation. However, some of these components are also involved in the maintenance of stem cell identity, thus emphasizing their fine-tuned regulation of NSCs and astrocyte uniqueness. This duality must be accommodated in any forward translational strategy.

The signaling pathways responsible for the astrocytic differentiation represent the essential players in physiological astrogenesis, but these mechanisms are not sufficient to define the spectrum of gene activity that occurs upon astrocytic differentiation.

3.2. Importance of DNA methylation in the acquisition of the astrocytic fate

In the CNS, cell intrinsic factors and extracellular cues influence the onset of astrogliogenesis. Concomitantly, dramatic changes in DNA methylation of astrocyte-specific promoters contribute to the neurogenic to gliogenic switch. This process explains why the responsiveness of NSCs to the astrogenic signaling pathways varies according to developmental stages. For example, early-derived cortical precursor cells are not able to undergo astrocytic differentiation in the presence of LIF, even though they express functional LIF receptors (Molne et al., 2000). In contrast, cultures of murine neuroepithelial cells derived from embryonic day (E) 14 differentiate readily into astrocytes upon LIF stimulation. However, *GFAP* is not expressed in cultures from E11.5 neuroepithelial cells even when the STAT3 pathway is induced (Takizawa et al., 2001). These studies reveal that the well-coordinated switch from neurogenic to astrogenic fate is not solely dependent on the presence of extracellular factors, but also requires intrinsic determinants.

During neuronal differentiation, astrocyte-specific gene promoters are methylated and transcription is silenced. However, in late-gestation many astrocytic genes become demethylated enabling NSCs to acquire gliogenic competences (Hatada et al., 2008). Recently, directly isolated murine neural stem and progenitor cells from different developmental stages (E11.5, 14.5 and 18.5) were used to determine the DNA methylome of each stage, revealing distinct and successive waves of global DNA methylation/demethylation that regulate the sequential generation of neurons, astrocytes and oligodendrocytes in the developing brain (Sanosaka et al., 2017). Notch ligands expressed in neuroblasts and immature neurons during mid-gestation activate their neighboring NSCs by inducing expression of the transcription factor nuclear factor I A (*NFIA*). NFIA in turn performs a dual act: it induces demethylation of the *GFAP* promoter and promotes dissociation of DNMT1 from the *GFAP* promoter, unveiling its STAT binding site (Namihira et al., 2009) (Fig. 2). Deletion of the *Dnmt1* gene results in demethylation of astrocytic genes and of genes involved in the JAK-STAT signaling pathway. Consequently, increased STAT activity and expression of astrocyte-related genes leads to precocious astroglial differentiation (Fan et al., 2005). Further, specific deletion of *Dnmt1* in NSCs increased the generation of astrocytes in the dentate gyrus (Noguchi et al., 2016b). Ablation of *Dnmt1* during late embryonic development did not impact astrogliogenesis *per se*, but *Gfap* expression was upregulated in the existing astrocytes of adult mice (Noguchi et al., 2016a). In addition, DNA

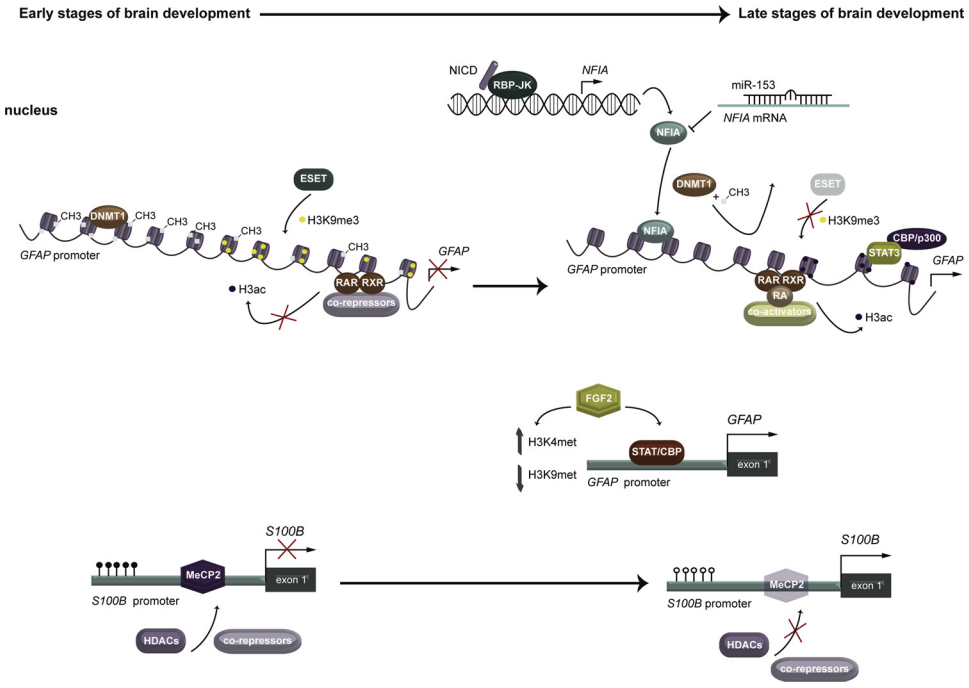

Fig. 2. Examples of major transcriptional and epigenetic events driving gene expression of two main astrocytic markers, *GFAP* and *S100b*. In NSCs, *GFAP* promoter is highly methylated due to DNMT1 activity. In addition, ESET protein is responsible for the elevated trimethylation marks of histone H3K9. Also, the dimer RAR/RXR forms a complex with various co-repressors recruiting HDACs which maintain a closed chromatin structure, thus leading to the repression of *GFAP* expression. During late-gestation, Notch ligands expressed in cells determined to become neurons bind to Notch protein of their neighboring NSCs. This binding induces proteolytic cleavage and release of the NICD, which enters the nucleus and binds to RBP-JK. The NICD/RBP-JK complex induces the expression of *NFIA*. MiR-153 targets *NFIA* mRNA inhibiting its translation. NFIA is able to bind to the *GFAP* promoter resulting in the removal of methyl groups (CH3) and in the dissociation of DNMT1. Further, ESET protein levels are reduced leading to decreased H3K9me3. Lastly, binding of RA to RAR/RXR dimer attracts transcriptional co-activators, allowing the chromatin to obtain a relaxed structure through acetylation of histone H3. Upon these conformational changes, STAT3 is able to bind to the *GFAP* promoter recruiting the transcriptional co-activators CBP/p300. FGF2 additionally facilitates access of the STAT/CBP to the *GFAP* promoter, while also increases and decreases H3K4 methylation (H3K4met) and H3K9 methylation (H3K9met) respectively. As a result, *GFAP* expression is induced and astroglial differentiation commences. Similarly to *GFAP*, the expression *S100B* is also repressed in early brain development. This is due to promoter methylation and to the binding of MeCP2, which recruits HDACs and co-repressors to the promoter. In later stages, along astrocytic differentiation, promoter demethylation and decreased MeCP2 levels lead to the induction of *S100B* expression.

methylation has an important role in the control of expression of the immature astrocytic marker S100B. Methyl CpG binding protein 2 (MECP2), a protein linked to gene silencing which recruits histone deacetylases (HDACs) and corepressors, is bound on the promoter region of *S100b* inactivating gene expression. However, at E14 a specific cytosine residue of a CpG site within the promoter region is demethylated and MECP2 can no longer bind to this site leading to the initiation of murine *S100b* expression (Namihira et al., 2004) (Fig. 2).

Overall, it appears that demethylation of astrocyte-specific gene promoters is crucial for the timely activation of gene expression and therefore for the regulation of astrocyte differentiation in the developing brain.

3.3. Histone modifications associated to astrocytic differentiation

During neural development, both neurogenic and gliogenic gene promoters undergo various histone modifications, which ensure the sequential production of each cell type. In the neural tube at E14, exposure of neuroepithelial cells to the astrocyte inducing cytokine BMP2 resulted in a significant increase of histone H3 acetylation in the *S100B* gene promoter (Namihira et al., 2004). During astrocyte differentiation, LIF acts synergistically with retinoic acid (RA) to induce an open chromatin conformation through histone H3 acetylation, resulting in activation of the *GFAP* promoter in NSCs (Asano et al., 2009). RA receptors (RARs) form complexes with retinoid X receptors (RXRs) and bind to RA response elements in the promoter regions of target genes. When the RA ligand is absent, RAR/RXR associates with transcriptional repressors leading to gene silencing by recruitment of HDACs (Fig. 2). Conversely, binding of RA enables the release of HDACs from the RAR/RXR complex and the recruitment of histone acetyltransferase (HAT) co-activators. Thus, RA-bound RAR/RXR associates with the *GFAP* promoter allowing the chromatin to adopt an open conformation through histone H3 acetylation, specifically in the STAT site-containing region. This facilitates efficient binding of STAT3 to the promoter, therefore inducing *GFAP* expression (Asano et al., 2009) (Fig. 2). In addition, activated STAT3 is able to recruit the transcriptional co-activators CBP/p300, resulting in acetylation of H3K9 and H3K14 at the *GFAP* promoter (Fig. 2). These histone modifications further lead to enhanced H3K4me3 and recruitment of RNA polymerase II and activation of gene transcription (Cheng et al., 2011). Intriguingly, histone deacetylase 3 (HDAC3) was reported to regulate the switch between oligodendrocyte and astrocyte fate; deletion of HDAC3 induces robust astrocyte differentiation with concomitant loss of oligodendrocytes (Zhang et al., 2016). Similarly, inhibition of HDACs either with trichostatin A or sodium butyrate reduced *GFAP* expression in human astrocytes and reorganized the intermediate filament network of the cells (Kanski et al., 2014). Further histone modifications are for the expression of *GFAP*. Fibroblast growth factor 2 (FGF2), which regulates the competence of rodent cortical progenitors to differentiate into astrocytes in response to CNTF, facilitates access of the signal transducer and activator of transcription/CRE binding protein (STAT/CBP) to the *GFAP* promoter by strongly increasing H3K4me while blocking H3K9me around STAT binding site of the *GFAP* promoter (Fig. 2). Since H3K4me and HeK9me are linked to transcriptional activation and repression respectively, FGF2 alters H3 methylation in a way that favors the activation of the *Gfap* promoter (Song and Ghosh, 2004).

Deposition of repressive histone marks is also evident during astrocyte differentiation. ERG-associated protein with SET domain (ESET) protein, a H3K9 histone methyltransferase (HMT), is highly expressed during the early stages of brain development, but it is strongly downregulated during the transition from neurogenesis to astrogenesis. The *GFAP* promoter is a direct target of ESET and its expression at early stages is repressed *via* elevated H3K9me3 marks (Tan et al., 2012). At later stages of brain development where ESET levels are reduced, H3K9me3 is decreased and *GFAP* is activated (Fig. 2). Similarly, the histone demethylase known as gene amplified in squamous cell carcinoma 1 (GASC1) is important for the developmental stage-dependent differentiation of astrocytes. *GASC1* hypomorphic mutant mice exhibit

an increased amount of GFAP$^+$ cells in the forebrain together with abnormal behaviors and synaptic activity (Sudo et al., 2016). The roles of astrocytes on synaptic plasticity may partially explain why the mutant mice present such phenotypes and further help to understand the abnormal behaviors. Moreover, deletion of *Enhancer of zeste homolog 2* gene (*EZH2*), encoding a polycomb group protein which is a H3K27 HMT, on cortical progenitor cells leads to accelerated astrocyte differentiation (Pereira et al., 2010).

On the other hand, the H3K4, lysine-specific demethylase 5 A (KDM5 A) is pivotal for the repression of astrocyte differentiation in NSCs. Knockdown of *KDM5 A* in NSCs increases the generation of astrocytes, while *KDM5 A* overexpression reduces transcriptional activity of the *GFAP* promoter. Induction of astrocytic differentiation reduced recruitment of KDM5 A to the *GFAP* promoter and increased H3K4me, thus maintaining NSCs in an undifferentiated state by suppression of astrogliogenesis (Kong et al., 2017).

3.4. Essential miRNAs for the generation of astrocytes

Several studies unveiled the important regulatory role that miRNAs play in astrocyte differentiation. During glial specification, miR-153 targets *NFIA* and *NFIB* mRNAs, an essential step for the initiation of astrocyte differentiation (Fig. 2). Overexpression of miR-153 delayed the onset of astrogenesis favoring an undifferentiated state, while inhibition of miR-153 induced premature gliogenesis (Tsuyama et al., 2015). In early developmental stages of the forebrain, miR-153 is highly expressed. As CNS development progresses, the levels of miR-153 are reduced followed by increased *NFIA/B* expression, revealing its role in astrocyte fate specification.

MiR-31 was shown to be necessary for the specification and differentiation of astrocytes. In NSCs, miR-31 is suppressed by multiple stem cell factors, such as LIN28, C-Myc, SOX2 and OCT4. However, upon astrogliogenesis, miR-31 is upregulated *via* the STAT3 and SMAD1/5/8 signaling pathways contributing to the promotion of astrocyte differentiation, in part by targeting and reducing *Lin28* mRNA levels. Indeed, in the absence of miR-31, astrocytes fail to properly differentiate, while overexpression of miR-31 is able to partially induce astrocyte differentiation (Meares et al., 2018).

Investigations into the role of *Chicken ovalbumin upstream promoter-transcription factors I* and *II* (*Coup-tfI* and *Coup-tfII*) during CNS development have identified miR17/106 as another important regulator of the neurogenic to gliogenic switch. COUP-TFI/II is transiently expressed in the ventricular zone of early embryonic CNS. Double knockdown of *Coup-tfI/II* in NSCs resulted in greater silencing of the STAT3-binding site of the *Gfap* promoter due to reduction of acetylated histone H3 and dimethylated H3K4 (H3K4me2) and increase of H3K9me2. In the developing mouse forebrain, double knockdown of the transcription factors inhibited the initiation of astrogenesis (Naka et al., 2008). Later on, the same group identified miR17/106 as a downstream regulator of COUP-TFI/II. The mRNA of *p38*, which is pivotal for the physiological process of astrogenesis, was found to be a direct target of miR17/106 (Naka-Kaneda et al., 2014). Therefore, it seems that miR17/106 is an important regulator of the neurogenic to gliogenic switch.

4. Spinal cord/traumatic brain injuries and neurodegenerative disorders

Apart from their importance and multifunctionality in the healthy CNS, astrocytes respond to brain damage, infection or disease through a process known as reactive astrogliosis. The first description of astrocyte reactivity was documented by Virchow, showing that the spinal cord tissue was more fibrillary in neurosyphilis patients than in healthy individuals (Barcia et al., 2016). The concept of astrocyte reactivity emerged with the discovery of the intermediate filament GFAP (Eng et al., 1971) and strong GFAP expression in astrocytes became a sign of reactivity (Bignami and Dahl, 1976). Another distinctive attribute of reactive astrocytes reported by early neuropathologists was hypertrophy as evidenced by enlarged cell body and processes (Wilhelmsson et al., 2006). During astrogliosis, the glial scar formation is accompanied with the appearance of newly proliferated astrocytes. Human specimens showed evidence of astrocytic proliferation in response to a variety of insults, such as infection and acute demyelinating lesions (Colodner et al., 2005). However, if these newly divided astrocytes have been originated either from mature astrocytes that re-entered the cell cycle or from local progenitor cells is still a matter of investigation (Buffo et al., 2008; Carlen et al., 2009).

Astrogliosis is primarily associated with neuroprotection. For example, during ischemia, reactive astrocytes (i) protect neurons from oxidative stress through a glutathione dependent mechanism (Chen et al., 2001; Iwata-Ichikawa et al., 1999), (ii) offer protection from $NH4^+$ toxicity (Rao et al., 2005), (iii) contribute to BBB repair (Tian et al., 2011), (iv) participate in the water brain homeostasis (Zador et al., 2009) and (v) are involved in the clearance of excitotoxic glutamate (Brown, 1999; Rothstein et al., 1996). The subsequent formation of the glial scar acts as a physical insulator to contain the lesioned area. Selective ablation of reactive astrocytes in adult mice expressing a herpes simplex virus-thymidine kinase from the *Gfap* promoter by treatment with ganciclovir, led to pronounced inflammatory responses, leukocyte infiltration, extensive tissue disruption, increased demyelination, neuronal degeneration, and failure of the BBB repair (Faulkner et al., 2004; Myer et al., 2006; Voskuhl et al., 2009).

4.1. The molecular repertoire of reactive astrocytes

At the molecular and functional levels, astrogliosis is highly diverse (Anderson et al., 2014); even in the same region of the mouse cerebral cortex, astrocytes respond heterogeneously to stab wound injury. In fact, although it was shown that almost all astrocytes become hypertrophic and overexpress GFAP following injury, some had their processes polarized towards the lesion, others proliferated and others remained static (Bardehle et al., 2013). In addition, activated astrocytes acquire NSC properties and modify not only their phenotypic profile, but they also undergo major gene expression changes. The transition from a normal to a reactive astrocytic phenotype is induced by extra- and intra-cellular signaling and epigenetic mechanisms that triggers, resolves or maintains astroglial reactivity. The machinery that will promote the “conversion” of astrocytes depends on the specific injury or degeneration event. Several epigenetic factors and signaling pathways have been described to play a role in astrogliosis, including the JAK/STAT pathway, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, and the MAPK pathway (Ben Haim et al., 2015).

4.1.1. Reactive astrocytes in spinal cord and traumatic brain injuries

Following CNS injury, activated microglia react rapidly and recruit astrocytes by secreting several pro-inflammatory mediators including tumor necrosis factor-alpha (TNF), IL-1β, IL-6, and interferon-gamma (IFN-γ) (Sofroniew and Vinters, 2010). Reactive astrocytes can proliferate, become hypertrophic and migrate to the site of the injury leading to the formation of a physical glial barrier that impedes axonal regeneration in chronic spinal cord injury (SCI). Nevertheless, ablation of reactive astrocytes or interference with their activation mechanism upon injury, results in exacerbated tissue degeneration and spread of inflammatory cells indicating that the glial scar has a potential function (Karimi-Abdolrezaee and Billakanti, 2012). The transcription factor NF-κB is responsible for triggering gene expression of several inflammatory mediators during inflammation (Fig. 3). Astrocyte-specific inactivation of NF-κB resulted in improved functional recovery following SCI (Brambilla et al., 2005). Notably, NF-κB activation of astrocytic cultures by TNF was unable to induce a reactive phenotype, but seemed to convert astrocytes into neural progenitor-like cells (Gabel et al., 2016).

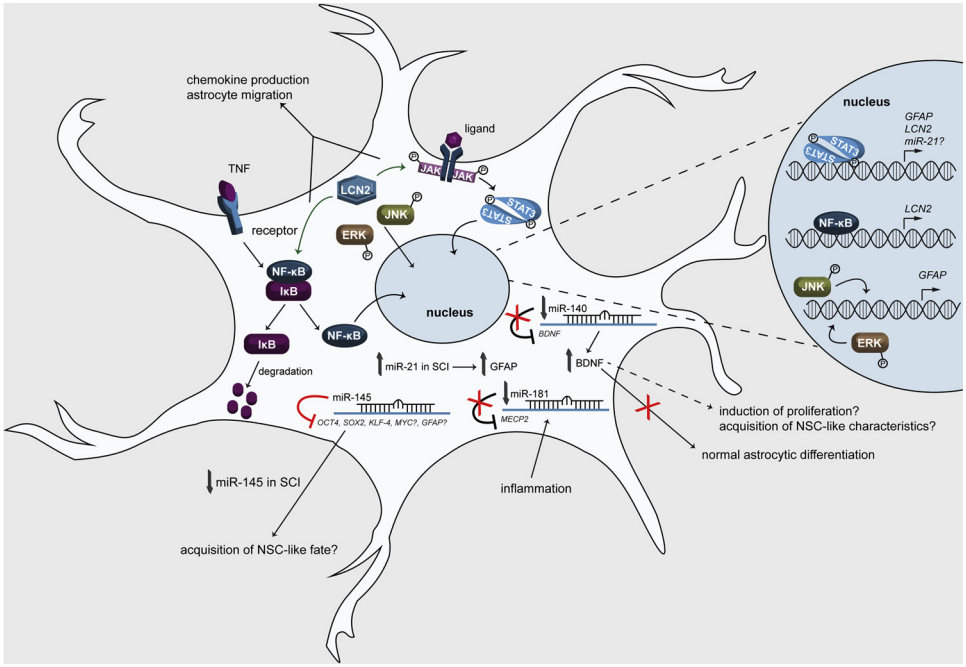

Fig. 3. Schematic illustration of specific transcriptional and epigenetic alterations during reactive astrogliosis. Under inflammatory conditions or in the presence of a lesion, several signaling pathways are activated. Binding of TNF to the cognate receptor allows the dissociation and degradation of IκB from the IκB/NF-κB complex. NF-κB is then released and translocates into the nucleus, where it will activate the transcription of key reactive astrocytic markers, such as *LCN2*. Activation of the JAK/STAT pathway enables the phosphorylation of STAT3 and its subsequent translocation to the nucleus, which will induce the transcription of *GFAP*, *LCN2* and potentially of *miR-21* as well. In turn, LCN2 is able to further activate the JAK/STAT and NF-κB signaling pathways stimulating chemokine production and astrocyte migration. In addition, the JNK and MAPK/ERK pathways are activated and implicated in the induction of *GFAP* expression. MiR-21 levels are elevated in SCI and positively influence GFAP protein levels. In differentiated cells, the pluripotency factors OCT4, SOX2 and KLF-4 are not present due to miR-145 activity. However, reduced miR-145 levels in SCI may potentially reactivate the expression of these proteins, together with the translational activation of the suggested *GFAP* and *MYC* mRNA targets. In this way, reactive astrocytes obtain stem cell hallmarks. In addition, miR-181 is decreased upon inflammation and is no longer able to target and control the translation of *MECP2* mRNA. Under physiological conditions, miR-140 inhibits BDNF translation and cell proliferation participating in astrocyte differentiation. However, inflammation results in miR-140 decrease and is associated with cell proliferation as well as acquisition of NSC characteristics.

After SCI, JAK-STAT signaling is also activated (Fig. 3). Reactive astrocytes in STAT3 conditional knockout mice showed limited migration to the lesion site, failure of astrocyte hypertrophy and *GFAP* upregulation, widespread leukocyte infiltration, neural disruption and demyelination (Herrmann et al., 2008; Okada et al., 2006). Furthermore, the investigation of astrocyte-related signaling responses resulting from diverse neurotoxic insults, revealed the implication of STAT3 pathway as a broadly triggered signaling pathway for astrogliosis (O'Callaghan et al., 2014).

It is evident that in response to different injury or inflammatory stimuli, distinct pathways are stimulated, all leading to astrocytic activation. Fibrinogen, a blood protein that leaks into the CNS following BBB disruption, was discovered to activate transforming growth factor-beta (TGF-β) signaling pathway after traumatic CNS injury. Activation of TGF-β results in neurite outgrowth inhibition, while inhibition of TGF-β signaling attenuated glial scar formation (Schachtrup et al., 2010). Toxin-induced reactive gliosis in the corpus callosum showed that endothelin-1 induces astrocyte proliferation and *GFAP* expression through activation of ERK- and c-Jun N-terminal kinase (JNK)-dependent pathways (Gadea et al., 2008) (Fig. 3). Similarly, traumatic scratch injury in astrocytes triggered a calcium influx from the extracellular compartment and activated the JNK pathway to activate *GFAP* expression (Gao et al., 2013).

Transcriptomic analysis of reactive astrogliosis showed that gliosis consists of a rapid induction of gene expression after insult and identified lipocalin 2 (*LCN2*) and Serpina3 as robust markers of reactive astrocytes (Zamanian et al., 2012). Initially described as iron-trafficking protein involved in multiple processes such as apoptosis, innate immunity and renal development, LCN2 is secreted in several brain injury conditions such as inflammation (Jin et al., 2014) and stroke (Elneihoum et al., 1996), or in response to neurodegeneration (Bi et al., 2013). It also promotes apoptosis, morphological changes, and migration of astrocytes both *in vitro* and *in vivo*. In all cases, it has been detected in high amounts predominantly in astrocytes and to be selectively toxic to neurons. In fact, LCN2 deficiency reduced astroglial-induced neurotoxicity *in vitro* (Jin et al., 2014) and resulted in a significant attenuation of hippocampal neuronal loss, white matter damage, BBB permeability and cognitive decline in a mouse model of cerebral ischemia (Kim et al., 2017). NF-κB and STAT3 are able to regulate the expression of *LCN2* (Fig. 3). Intriguingly, LCN2 is capable of initiating the activation of JAK2/STAT3 and NF-κB signaling pathways to induce the production of chemokines and astrocytic cell migration (Lee et al., 2011) (Fig. 3), thus establishing a positive feedback loop which maintains astrocytes in a reactive state.

Interestingly, a recent study using a SCI mouse model defined three distinct astrocytic populations based on their marker genes: naive, reactive and scar-forming astrocytes (Hara et al., 2017). When reactive astrocytes were transplanted into naive or injured spinal cord models, they were converted to naive astrocytes or formed astrocytic scars respectively, unveiling their environment-dependent plasticity. Upregulation of genes associated with type I collagen in the lesioned area following SCI is responsible for the conversion of reactive into scar-forming astrocytes. Pharmacological blockage of reactive astrocyte–type I collagen interaction was sufficient to prevent scar formation strengthening the beneficial effect of reactive, but not scar-forming astrocytes (Hara et al., 2017).

Traumatic brain injury (TBI) is an acute injury of the brain resulting in a direct neuronal loss followed by a neuroinflammatory phase. In the acute stage, the neuroinflammatory "cascade" attempts to respond against brain damage. However, in the chronic stage, this reaction leads to neurodegenerative-like symptoms, including diminished or altered state of consciousness, impaired motor and cognitive skills (Lozano et al., 2015). In an effort to treat TBI, the hematopoietic growth factor granulocyte colony-stimulating factor was used in a TBI mouse model resulting in behavioral recovery associated with increased astrogliosis and hippocampal neurogenesis. Activated astrocytes participated together with microglia in the release of neurotrophic factors to mediate repair and enhance survival of injured neurons (Song et al., 2016). In a TBI rat model, the increase of $GFAP^+$ cells under brain injury revealed the possible involvement of astrocytes in perivascular caspase-3-mediated apoptosis (Glushakova et al., 2018).

4.1.2. Reactive astrocytes in neurodegenerative diseases

Given the multitude of vital roles that astrocytes play in the regulation of brain physiology, it is not surprising that reactive astrocytes are involved in the initiation and progression of age-related neurodegenerative diseases including AD, PD, and amyotrophic lateral sclerosis.

The most prevalent neurodegenerative disease is AD, which is accompanied by a progressive accumulation of senile plaques, mainly composed of amyloid β (Aβ), and neurofibrillary tangles composed of tau protein. Tissue samples from AD patients present an overabundance of reactive astrocytes that are closely associated with amyloid plaques in the cerebral cortex. A remarkable and stereotypical spatial organization of glial cells around Aβ plaques has been recently characterized, with an inner shell of amoeboid/activated microglia and an outer shell of reactive and polarized astrocytes, a structure described as "reactive glial net" (Bouvier et al., 2016). Reactive astrocytes endocytose Aβ peptides released from degenerating neurons, resulting in the toxic accumulation of Aβ, followed by astrocytic death and subsequent release of Aβ (John Lin and Deneen, 2013; Maragakis and Rothstein, 2006).

Extracellular cues play important regulatory roles of the expression of astrocyte-specific genes during astroglial development and it is possible that similar mechanisms are responsible for controlling gene expression in neurodegenerative diseases. Activation of JAK2/STAT3 pathway precedes the onset of astrogliosis in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD (Sriram et al., 2004). On the other hand, deletion of astrocytic STAT3 in the MPTP model markedly reduced the number of reactive astrocytes (O'Callaghan et al., 2014). These observations clearly identify a role for activated STAT3 in astrocyte damage, induction of astrogliosis and possibly *GFAP* upregulation. In the MPTP mouse model, increased expression of wingless-type MMTV integration site1 (*WNT1*) was attributed to reactive astrocytes. It was shown that Wnt1, together with other factors derived by these cells, promoted the generation of dopaminergic neurons from adult NSCs *in vitro*, and that pharmacological activation of Wnt/β-catenin pathway *in vivo* enabled the recovery of dopaminergic neurons, revealing a neuroprotective effect of Wnt signaling and reactive astrocytes in PD (L'Episcopo et al., 2011). Wnt signaling regulates the development of midbrain dopaminergic neurons and is required for their differentiation (Arenas, 2014). In addition, Wnt proteins are abundantly present in the adult brain maintaining and protecting neuronal functions, including the dopaminergic neurons of the *substantia nigra* (Inestrosa and Arenas, 2010). On the other hand, canonical Wnt signaling is upregulated in PD and proteins encoded by *PARK* genes, involved in familial PD, have been shown to modify Wnt signaling by regulating β-catenin levels or by interacting with key Wnt signaling components (Berwick and Harvey, 2014). Further exploration of the role of the Wnt pathway in astrocytic function and their subsequent beneficial effect on dopaminergic neurons might be of significant importance in order to fully understand the contribution of reactive astrocytes to both neurodegeneration and neuroprotection during ageing.

4.1.3. Heterogeneity of reactive astrocytes

As it was discussed, in response to threatening events, astrocytes could adopt protective or deleterious relationships towards neural tissue. In fact, gene expression analysis of reactive astrocytes derived from stroke or inflammatory mouse models revealed the existence of two distinct context-dependent populations with distinct phenotypes that relied on the type of the injury (Zamanian et al., 2012). While reactive astrocytes from the different models shared several deregulated genes, at least 50% of the altered genes were unique for each condition. Reactive astrocytes in ischemia (called A2 astrocytes) seem to possess a molecular identity that may be protective, while the ones activated by LPS (namely A1 astrocytes) have a negative effect. This type of astrocyte was found abundantly in several neurodegenerative diseases, including PD, AD and Huntington's disease (Liddelow et al., 2017). Recently, it was shown that aged astrocytes acquired an A1-like identity that depends on the brain region. It was suggested that this type of astrocyte might contribute to cognitive decline in normal aging, while increasing the vulnerability of the aged brain during injury (Clarke et al., 2018). These studies highlight the heterogeneity of astrogliosis, where astrocyte activity depends on the type of inducing injury and constitute an additional hint that epigenetic mechanisms may participate in this astrocytic response since different genes must be activated each time in response to specific stimulations. Accurate coordination of gene expression depending on the type of injury is necessary and it appears that this expression is additionally brain region- and age-specific. It is possible that the same molecular pathways are used in CNS insults, leading to different expression patterns at each time. In this sense, this diverse responsive ability of astrocytes unveils their highly plastic potential and offers an opportunity for this to be exploited in therapeutic strategies.

4.2. MiRNAs' functioning in the process of astrocyte reactivity

Similarly to astrocyte differentiation, epigenetic regulation plays a role in reactive astrogliosis as well. For example, multiple miRNAs have been reported to be modulated upon reactive astrogliosis. MiR-145, enriched in rat spinal neurons and astrocytes, was found to be a negative regulator of astrogliosis. It was downregulated in SCI and astrocyte-specific overexpression of miR-145 decreased the size of astrocytes and their proliferative and migratory abilities. *GFAP* and *MYC* mRNAs were suggested as potential targets of miR-145 (Wang et al., 2015) (Fig. 3). MiR-145 has also been detected at low levels in human embryonic stem cells (hESCs) and increasing levels during differentiation suppress the pluripotency genes OCT4, SOX2 and KLF-4 (Fig. 3) (Xu et al., 2009). An important aspect would be to identify whether miR-145 reduction in SCI is able to activate the neural stem cell genes in astrocytes, enabling them to obtain an immature identity and if miR-145 downregulation contributes to the acquisition of a stem cell-like fate in an attempt to restore the brain damage and regenerate.

In contrast to the down-regulation of miR-145, miR-21 was upregulated in a time-dependent manner after SCI in mouse. Inhibition of miR-21 abrogated the increased expression of reactive markers such as GFAP (Fig. 3), augmented astrocytes' hypertrophic response and increased axon density in the site of the lesion (Bhalala et al., 2012), depicting the detrimental effects of increased miR-21 levels upon injury. This study revealed a novel role of miR-21 in regulation of astrocyte hypertrophy and glial scar progression. Interestingly, BMP signaling has been described to modulate miR-21 levels in cultured astrocytes (Sahni et al., 2010), and a miR-21 regulatory sequence contains two STAT3-binding sites, providing the possibility that astrocytic miR-21 levels may also be regulated by JAK-STAT signaling (Loffler et al., 2007).

MiR-140 was shown to suppress normal astrocytic cell proliferation by binding to the 3′ UTR of brain-derived neurotrophic factor (*BDNF*) mRNA inhibiting its translation (Fig. 3). LPS-induced inflammation resulted in increased production of BDNF, IL-6 and TNF, which were restored with ectopic miR-140 expression (Tu et al., 2017). Glioma patients also present low amount of miR-140, which has been shown to restrict tumor growth and metastasis. The anti-proliferative effect of miR-140 may be used to restrict the detrimental effects of reactive astrogliosis on the damaged tissue and as a potential therapeutic strategy for cancer patients.

The family of miR-181 was reported to be present in the mature CNS but not early in brain development, showing a high expression in astrocytes compared to neurons (Hutchison et al., 2013). Induction of inflammation by treating mice with LPS leads to significant decrease of all miR-181 family members (Fig. 3). Overexpression of miR-181c in astrocytic cultures exposed to LPS increased cell death and changes in the levels of miR-181 resulting in modified expression of several inflammatory cytokines. Interestingly, mRNAs encoding MeCP2 and X-

linked inhibitor of apoptosis were identified as miR-181 targets. Notably, in the hematopoietic system, miR-181 represses LIN28, a well-described pluripotent marker, mediating stem cell differentiation (Li et al., 2012). Further investigation of the possible role of miR-181 towards cell fate specification during brain development would be of great value. These findings unveil the important role of miRNAs in the molecular responses of astrocytes under inflammatory conditions and illustrate how they alter the astrocytic transcriptome under reactive astrogliosis.

Although it has been recently shown that a TNF treatment of murine astrocytes differentiated from neural precursor cells *in vitro* resulted in the modifications in the levels of H3K4me3 and H3K27me3 at the promoters of specific genes (Michelucci et al., 2016), evidences on the extents of DNA methylation and histone modifications in reactive astrocytes are still lacking. Identifying additional epigenetic modifications that influence the process of astrocytic activation will be crucial not only to further understand the underlying mechanisms, but also to intervene and potentially attain functional recovery after a CNS injury or disease. However, it has to be borne in mind that reactive astrogliosis *per se* offers several protective roles to the CNS, such as uptake of potentially excitotoxic glutamate, repair of the BBB and limitation in the spreading of inflammatory cells or infectious elements from the injured or diseased tissue to the healthy parts. Interestingly, it is possible that some clinically approved drugs may act predominantly on astrocytes or on the astrocytic network, although originally designed for other targets (Pekny and Pekna, 2014). In this sense, it is possible that already available drugs are able to act on molecules selective to astrocytes or on epigenetic mechanisms related to reactive astrogliosis offering at least the possibility of adjusting the damage-recovery equilibrium within the CNS.

Overall, the ability of astrocytes to become reactive, undergoing dramatic morphological and molecular changes, highlights their plasticity. Under specific environmental and intrinsic cues, this plasticity may also confer astrocytes the capacity to dedifferentiate, thus re-expressing features of NSCs, or even to be reprogrammed into a different cell lineage.

5. Astrocyte plasticity: dedifferentiation and reprogramming

Cellular plasticity refers to the ability of cells to change their fate identity in response to microenvironmental stimuli. Astrocytes exhibit a high degree of morphological and functional plasticity. Recent reports have shown that astrocytes can be converted into functional neurons both *in vitro* (Berninger et al., 2007; Heinrich et al., 2010) and *in vivo* (Liu et al., 2015; Torper et al., 2013). In a mouse model of AD, reactive astrocytes were directly reprogrammed into functional neurons in response to NeuroD1 (Guo et al., 2014). Under physiological conditions, astrocytes generally remain in a quiescent state, but retain their capacity to resume proliferation. Fate mapping of astrocytes in the adult mouse cerebral cortex revealed that, after injury, a subset of mature astrocytes acquired stem cell properties and recapitulated features of early developmental stages (Buffo et al., 2008). This process was originally coined by the authors as “dedifferentiation”. Intriguingly, these cells were able to form neurospheres, to self-renew and to differentiate into neurons and oligodendrocytes *in vitro*. This was later confirmed in models of traumatic or ischemic brain injury (Shimada et al., 2012; Sirko et al., 2013). Furthermore, *in vivo* imaging of astrocytes following acute injury identified a subpopulation of proliferating reactive astrocytes enriched along blood vessels (Bardehle et al., 2013). Notch signaling is crucial for controlling neurosphere formation in the cultures of reactive astrocytes (Shimada et al., 2012). Finding routes to bypass the anti-neurogenic environment in the adult brain, due for example to BMP and Notch signaling, would potentially allow exploitation of the latent multipotency of reactive astrocytes to elicit neuronal repair.

5.1. Influence of external stimuli

Different factors have been identified to induce the conversion of mature astrocytes into neural progenitors. *In vitro*, transforming growth factor alpha promotes sequential conversion of mature astrocytes into neural progenitors and stem cells (Sharif et al., 2007). This study described a novel population of mature astrocytes capable, in response to a single growth factor, to regress progressively into a neural stem-like cell stage *via* an intermediate glial progenitor stage. *In vivo*, Sonic hedgehog (SHH) signaling is a key mediator enabling astrocytes to retain latent stem cell-like properties. Inducible deletion of SHH’s transducer Smoothened followed by stab injury in adult mice resulted in a significant reduction of proliferation in reactive astrocytes and to a decreased ability of these cells to form neurospheres *in vitro* (Sirko et al., 2013). Another study identified elevated SHH levels in astrocytes upon mechanical injury. *In vitro* incubation of astrocytes with conditioned medium derived from injured astrocytes enabled cells to acquire neural progenitor cell characteristics, including self-renewal, multipotency, downregulation of *GFAP* and *S100B* and upregulation of *NES*, *SOX2* and *CD133* (Yang et al., 2012). Notably, in the mouse forebrain, SHH acts as a mitogen in neural progenitor cells of the SVZ enabling them to proliferate and produce new interneurons in the olfactory bulb (Palma et al., 2005). Therefore, SHH is a crucial pathway during neurogenesis and astrocytes dedifferentiation, thus mirroring another important pathway shared by both NSCs and astrocytes.

Further, the role of inflammatory mediators to induce astrocytes dedifferentiation has also been a matter of recent investigation. Exposure to the pro-inflammatory factor TNF of primary astrocytes prepared from newborn mice induces expression of several genes associated with the NF-κB pathway (Birck et al., 2016). More precisely, administration of TNF initiated loss of *Gfap* expression and the decrease of expression of genes related to glycogen metabolism. Moreover, a subset of these cells gained expression of stemness markers, including Oct4, CD44 and Musashi-1 as well as were able to form neurospheres giving rise to neural progenitors and neurons (Gabel et al., 2016). Notably, *in vitro* treatment with TNF or the BMP inhibitor noggin of murine astrocytes differentiated from neural precursor cells resulted in the acquisition of NSC-like properties accompanied by alterations in both the epigenome and transcriptome. Dedifferentiation of astrocytes was associated with modifications in the levels of H3K4me3 and H3K27me3 at the promoters of genes related to cell cycle, stemness or neuronal fate thereby permitting the re-activation of neural progenitor markers (Michelucci et al., 2016).

5.2. Effects of genetic manipulation

As mentioned above, EZH2 is a HMT involved in NSC renewal and maintenance by inducing gene silencing *via* histone methylation and deacetylation. Forced expression of *Ezh2* in postnatal mouse astrocytes resulted in partial, but not complete dedifferentiation of these cells accompanied with the downregulation of Gfap and S100B and the upregulation of NSC markers, including Nes and Sox2 (Sher et al., 2011), revealing the role of Ezh2 in retaining cells in the NSC state and even reverting differentiated cells into a more immature state.

Additional studies suggested that reactive astrocytes share common characteristics with NSCs. Dicer is an indispensable enzyme of the miRNA machinery, responsible for the maturation of miRNAs. Selective deletion of *Dicer* in astrocytes resulted in transgenic mice that exhibited ataxia, profound neuronal degeneration of the cerebellum, seizures, and premature death. Prior to the onset of any neurological symptom, the transcriptome of Dicer-deficient astrocytes was modified in a way that resembled an immature molecular signature. Specifically, hallmark genes of immature/reactive astrocytes were upregulated, while astroglial genes related to mature functions were downregulated contributing to excitotoxicity and a failure to support normal mature brain circuits (Howng et al., 2015; Tao et al., 2011). Collectively, Dicer and

subsequently miRNA production are critically involved in the maintenance of astrocyte identity and function.

These studies reveal that, besides the two neurogenic niches of the adult brain, subpopulations of mature astrocytes in other brain regions maintain their ability to dedifferentiate and acquire NSC-like properties under specific conditions. Nevertheless, the origin of these plastic cells is still a matter of controversy, at least under stroke conditions where it was described that NSCs from the SVZ give rise to a subpopulation of reactive astrocytes in the cortex which contributes to astrogliosis and scar formation (Faiz et al., 2015). This is especially important in cell replacement therapies in order to obtain a desired cell fate and potentially regenerate damaged tissue. Forced expression of the transcription factor *ASCL1* in astrocytes elicits GABAergic neurons, while expression of *NEUROG2* induces glutamatergic neurons, and concomitant expression of *CEND1* and *NEUROG2* induces generation of a mixed GABAergic and dopaminergic neuronal population *in vitro* (Aravantinou-Fatorou et al., 2015; Berninger et al., 2007; Heinrich et al., 2010). In 2013, several research groups managed to reprogram astrocytes into functional neurons *in vivo*. Overexpression of the single transcription factor *SOX2* (Niu et al., 2013) or *NeuroD1* (Guo et al., 2014) or a combination of *ASCL1, BRN2 A*, and *MYT1Ll* (Torper et al., 2013), was sufficient to directly reprogram astrocytes into neurons. Furthermore, it was shown that striatal astrocytes of stroke-induced mice entered a neurogenic program and generated new neurons (Magnusson et al., 2014). In a mouse model of Huntington's disease, some activated striatal astrocytes acted as neural progenitors and gave rise to neurons *in vivo* (Nato et al., 2015). Activated astrocytes directly generated dopaminergic neurons in a Parkinson's disease mouse model (Rivetti di Val Cervo et al., 2017). *In vivo* reprogramming of adult astroglial cells has emerged as a potential therapeutic approach that would avoid cell transplantation and possibly immunosuppression. However, reprogramming is a time consuming process characterized by low efficiency. According to a population shift view of cellular reprogramming, a small population of cells already resides in energetically favorable trajectories that will allow them to respond more readily to reprogramming signals. Consequently, if expression of reprogramming signal is retained over time, stochastic fluctuations in the transcriptome and epigenome will permit other cells to enter this primed state, thereby progressively increasing the efficiency of reprogramming (Del Sol and Buckley, 2014). Unlocking the molecular mechanisms that control cell fate switch may lead to improvements in reprogramming efficiency and provide a regenerative medicine strategy for mitigating neuronal loss in degeneration or trauma.

Taken together, the capacity of astrocytes to become reactive or be reprogrammed provides promise for endogenous brain repair strategies. However, these approaches have to be taken with caution as this may have unwanted side effects, such as tumorigenesis.

6. Brain tumors of astrocytic lineage

Gliomas represent the most common primary tumors of the CNS with an incidence rate of 6.6 per 100.000 individuals in the USA (Ostrom et al., 2016). Adult diffuse gliomas are classified based on histopathological and molecular features in oligodendroglioma, astrocytoma, and Glioblastoma (GBM) (Louis et al., 2016). A fundamental feature of glioma is their cellular diversity, often reflecting pathological analogues of the normal tissue. In this cellular heterogeneity, the question of the cellular origin of gliomas remains largely unresolved. Current evidence indicates that NSCs (Lathia et al., 2015) as well as differentiated cells, such as astrocytes and even neurons, can give rise to gliomas (Friedmann-Morvinski et al., 2012). For the latter, it has been demonstrated that differentiated astrocytes transduced with oncogenic lentiviral vectors are able to give rise to malignant gliomas that match a GBM subtype. Under these conditions, astrocytes enter a reprogramming mode, dedifferentiate and generate tumors that present a progressive loss of *GFAP* expression and are positive for several progenitor/stem cell markers including nestin and SOX2 (Friedmann-Morvinski et al., 2012). This is reminiscent of the high plasticity of reactive astrocytes under CNS insults. Likewise, following particular cues from the local microenvironment or genetic alteration, mature astrocytes dedifferentiate and obtain characteristics of neural precursor cells which can maintain their pluripotency and initiate tumorigenesis. A second study reported that the transduction of primary human astrocytes with lentiviral vectors expressing four defined genetic factors (Myc, Oct-4, p53DD and Ras) induced efficient generation of malignant cells with powerful tumor-initiating capabilities (Li et al., 2016). Indeed, when transplanted into immunodeficient mice, these transduced cells were sufficient to induce tumor formation, showed unlimited self-renewal and expressed typical glioma stem-like cell markers. *In vitro* cultivation of the transformed astrocytes revealed their potential to form spheres and differentiate into neuron-, astrocyte- and oligodendrocyte-like cells. The observation that reactive astrocytes are able to share common progenitor markers might explain why, under specific conditions, astrocytes may encompass the starting point of brain tumor formation, further supporting the concept that a fine line separate the dedifferentiated astrocytic fate towards reprogramming or tumorigenesis.

In line with the loss of GFAP expression by dedifferentiated astrocytes, *GFAP* expression is usually silenced in a progressive manner with increasing grade of astrocytoma (Restrepo et al., 2011). The fact that no *GFAP* mutations or major DNA rearrangements or deletions are detected in human glioma, prompted investigation of the role of epigenetic mechanisms in the loss of *GFAP* expression. Aberrant DNA methylation was detected in the promoter region of *GFAP* leading to its downregulation (Restrepo et al., 2011). In contrast to *GFAP* silencing, *DNMT1* and *DNMT3B* were found to be overexpressed in GBM. Specifically, the *DNMT1* promoter was enriched with active chromatin marks, including acetylated histones H3, H4 and H3K4me2, in GBM patients and cell lines. Conversely, normal brain tissue exhibited enrichment of the repressive histone modifications H3K9me2 and H3K27me3. Furthermore, the *DNMT3B* promoter was hypomethylated in the same tissue and overexpression of these DNMTs led to inactivation of the tumor suppressor genes *p21* and *PTEN* (Rajendran et al., 2011). Promoters of additional tumor suppressor genes including *p16/CDKN2 A* (Costello et al., 1996), *RB1* (Nakamura et al., 2001), *p14(ARF)* (Yin et al., 2002), *PTEN* (Baeza et al., 2003), *MGMT* (Blanc et al., 2004), *RASSF1A* (Gao et al., 2004), *p73* (Yu et al., 2004) were all shown to be hypermethylated and silenced in gliomas, thus resulting in the deregulation of multiple signaling pathways responsible for cell growth and apoptosis.

6.1. The importance of IDH genes

Integrated genomic analysis of human GBM samples revealed recurrent mutations in the active site of isocitrate dehydrogenase 1 gene (*IDH1*) in a small number of GBM patients (Parsons et al., 2008), that later turned out to largely represent secondary GBM. Mutations in *IDH2* gene have also been identified in gliomas (Yan et al., 2009). Under normal conditions, these enzymes catalyze the oxidative carboxylation of isocitrate to α-ketoglutarate, resulting in the production of NADPH. Glioma bearing mutated *IDH1* or *IDH2* display a glioma-CpG island methylator phenotype (G-CIMP) and produce high levels of D-2-hydroxyglutarate, proposed to inhibit several histone and DNA (de)methylases, including the TET enzymes (Chowdhury et al., 2011; Xu et al., 2011). Almost all mutations in *IDH1* in glioma occur at the amino acid residue 132 or the analogous residue 172 in *IDH2*, with the vast majority presenting a substitution of arginine with histidine (R132 H) (Balss et al., 2008). Introduction of this single point mutation in immortalized human astrocytes was sufficient for the alteration of specific histone marks and for inducing DNA hypermethylation, influencing the expression of approximately 600 genes (Turcan et al., 2012), although hypermethylation alone was not sufficient to induce tumorigenesis. Recently, the same research group identified enrichment of the active

histone mark H3K4me3, the repressive histone marks H3K9me3, H4K20me3 and H3K36me3, which prevent intragenic cryptic transcript initiation in these cells. One gene that showed increased H3K4me3 was *PDGFRA*, already linked to gliomagenesis (Turcan et al., 2018). Taken together, these results show that *IDH1* R132H mutation is able to reshape the chromatin state and transcriptome, however how this contributes to gliomagenesis remains to be determined.

6.2. The methylation phenotype of brain tumors

As indicated above, the IDH mutation is associated with a collective hypermethylation at a large number of loci signifying a G-CIMP (Noushmehr et al., 2010).

Another critical epigenetic mark in glioma is the DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT), which repairs the naturally occurring mutagenic O^6-methylguanine to guanine, thereby protecting against mutagenesis and malignant transformation. The *MGMT* promoter is frequently methylated in GBM patients, leading to gene silencing, which has been related with increased sensitivity to alkylating agents used for cancer therapy in patients with GBM (Weller et al., 2010). A meta-analysis study conducted in GBM patients showed that MGMT promoter methylation was associated with better progression-free survival and overall survival in these patients regardless of the fact of having received any therapeutic treatment and linked to longer overall survival in GBM patients treated with alkylating agents (Zhang et al., 2013). Therefore, the prognostic and predictive value of MGMT promoter methylation in GBM patients is highly relevant.

Analysis of diffuse glioma samples using different omics technologies including DNA methylation profiling, identified six distinct methylation clusters allowing the most robust and relevant distinction (Ceccarelli et al., 2016). A recent study suggests a new DNA methylation-based classification of CNS tumors, offering a novel approach to tumor classification and diagnosis precision, further strengthening the importance of comprehending and assessing the cancer methylome and thus the epigenetic mechanisms that contribute to tumorigenesis (Capper et al., 2018). Generally, global DNA hypomethylation is common in cancer, including gliomas, and it is estimated to influence 10 million CpG dinucleotides per haploid tumor genome (Cadieux et al., 2006). Demethylation was described to mainly occur in satellite sequences and pericentromeric regions promoting genomic instability and reactivation of transposable elements, thereby leading to tumorigenesis through activation of oncogenes (Cadieux et al., 2006).

6.3. Histone changes linked to Glioblastoma

Pediatric GBM as well as a subtype of adult GBM are characterized by the presence of somatic histone mutations. Two single point mutations have been described in the genes encoding the H3.3 (*H3F3A*) and H3.1 (*HIST1H3B, HIST1H3C*) histone variants. These mutations result in the substitution of lysine-to-methionine at position 27 (K27 M) and glycine-to-arginine or -valine at position 34 (G34R/V) (Jones et al., 2017; Williams et al., 2017). It was reported that aberrant binding of mutant K27 M to EZH2 inhibits the enzymatic activity of Polycomb repressive complex 2 which is needed to maintain gene repression. In addition, K27 M mutation leads to a global reduction of H3K27me3 levels and to global DNA hypomethylation, events that drive gene expression, including those involved in neuronal differentiation and gliomagenesis (Bender et al., 2013; Lewis et al., 2013; Venneti et al., 2013). However, a substantial gain of H3K27me3 and EZH2 at specific gene loci has also been reported, which can be advantageous for tumor cells if the silenced gene is a tumor suppressor as has been described for *p16/CDKN2 A* (Chan et al., 2013).

Comparative analysis of the chromatin state in GBM stem-like cells (GSCs) *versus* more differentiated tumor cells and nonmalignant neural cells revealed a prevalence of gene activation. Repressed promoters in human astrocytes lose the H3K27me3 mark in GSCs and become activated. Among the upregulated genes, a large set of developmental transcription factors (TFs) was activated in GSCs. In particular, ASCL1, a member of the bHLH family and known to play a role in neuronal commitment and differentiation, was found to directly activate Wnt signaling and to be necessary for GSCs maintenance and their tumorigenicity *in vivo* (Rheinbay et al., 2013). The Wnt pathway has long been associated with oncogenesis (Polakis, 2007) and is also essential for maintaining NSCs in a self-renewing state (Kalani et al., 2008). Notably, ASCL1 is important for Müller glia activation, cells essential for retina regeneration in fish. LIN28, a downstream ASCL1 effector, decreases let-7 miRNA levels enabling expression of the pluripotency genes, *KLF-4*, *OCT4* and *MYC* (Alunni and Bally-Cuif, 2016). The same LIN28/let-7 regulatory loop seems to be active in cancer stem cells, regulating their properties and contributing to oncogenesis (Chien et al., 2015; Sun et al., 2016), revealing how some molecular mechanisms are shared during development and tumorigenesis.

6.4. MiRNAs associated with Glioblastoma

A systematic review reported that 253 miRNAs were found to be significantly upregulated and 95 miRNAs downregulated in GBM (Moller et al., 2013). Target genes of these miRNAs are implicated in many cancer-associated processes, such as cell proliferation, cell cycle regulation, apoptosis, autophagy, drug resistance, angiogenesis, invasion and metastasis (Banelli et al., 2017; Luo et al., 2015).

For example, a significant decrease in the levels of miR-145 is not only observed in reactive astrocytes (see above), but also in astrocytic tumors, and it is correlated with poor prognosis in GBM patients (Lee et al., 2013a) and also inhibits glioma cell migration and self-renewal (Lee et al., 2013a, b). The latter is consistent with experiments in hESCs where increased levels of miR-145 inhibit self-renewal (Xu et al., 2009). In addition, *OCT4,* a miR-145 target, is expressed in human gliomas and OCT4 protein levels correlate with increasing glioma grade (Du et al., 2009).

In addition to its role in regulating astrocytic responses following SCI (see above), elevated levels of miR-21 were found in various types of cancer, including GBM (Akers et al., 2013). MiR-21 promotion of cancer growth (Chung et al., 2013; Yang et al., 2014) depends on STAT3 (Loffler et al., 2007). Could miR-21 represent a link in the transition from reactive astrogliosis to brain tumorigenesis? Reactive astrocytes might upregulate miR-21, thereby acquiring persistent stem cell characteristics leading to uncontrolled cell proliferation, growth and ultimately resulting in malignancy. The identification of molecular pathways which contribute to the transition from reactive astrocytes to neoplastic cells may pave the way to the development of anti-tumorigenic therapies.

7. Conclusions and perspectives

Astrocytes are implicated in the orchestration of neural development and constitute essential regulators of brain functions both in health and disease. Coordinated astrocyte differentiation is critical during development, and dysfunction of astrocytes can lead to several brain disorders. In the presence of a CNS insult, astrocytes respond by altering their morphology and molecular profile. Notably, they are able to be reprogrammed to a stem cell-like state permitting their eventual transdifferentiation into neurons or oligodendrocytes, which might be harnessed to restore brain damage. These processes rely on widespread transcriptional and epigenetic modifications that act in a highly coordinated manner leading to a fine balance between a healthy and pathological state.

So far, the (de)differentiation potential of reactive astrocytes has been mainly characterized *in vitro*, based on experimental tractability and the ease of monitoring the role of specific growth factors favoring commitment to specific lineages, a task that is much more difficult within the complex cellular milieu of the brain. However, the capacity

of astrocytes *per se* to dedifferentiate holds significant promise in developing endogenous brain repair strategies. Studying astrocyte plasticity and exploring the means that will allow such a switch of cellular fate *in vivo*, will provide new opportunities to implement novel endogenous therapeutic approaches. The ease of manipulation of 2D astrocyte cultures has provided the first vital mechanistic insight into their plasticity, but more complex cell systems that better recapitulate the cellular complexity of the brain will be necessary to fully understand the intricate relationship between signaling, transcriptome and epigenome that ultimately drive their phenotypic response *in vivo*. For example, 3D cell culture using microfluidic devices or brain organoids may represent a valuable compromise between *in vitro* and *in vivo* approaches, which will allow us to study the effect of other CNS cell types on astrocyte programs. Using these systems, it will be possible to study transcriptional and epigenetic changes in astrocytes to analyze, for example, the effect of familial AD or PD mutations. Screening compounds in a 3D system that show a specificity for differentially expressed epigenetic modulators in astrocytes may allow us to identify promising therapeutic treatments.

Apart from the identification of epigenetic drugs which usually shows a lack of selectivity, another arising and promising approach is the targeted epigenome editing that enables a precise alteration in the epigenome. For example, designer methyltransferases can be constructed to achieve targeted methylation of a gene promoter (Siddique et al., 2013). Furthermore, by using the deactivated Cas9 (dCas9) nuclease in combination with the catalytic domain of a DNMT, it is possible to enable targeted CpG methylation of single or even multiple CpG sites, with the DNA methylation activity being heritable across mitotic divisions (Vojta et al., 2016). On the other hand, the dCas9 system can be used for targeted DNA demethylation of specific genomic loci (Xu et al., 2016). In this way, the induced methylation/demethylation silences or activates, respectively, the expression of the target gene and may represent a potential locus-specific therapeutic strategy of the epigenome. For example, dCas9:Tet1 was recently used to target demethylation of a specific STAT3-binding site upstream of the *Gfap* promoter resulting in decreased methylation of the promoter and flanking region accompanied by increased *Gfap* expression (Morita et al., 2016).

Another critical, and often underappreciated feature of astrocytes is their heterogeneity. Due to their multiple roles in the CNS it would not be surprising to uncover the existence of several astrocytic subpopulations, each displaying specific properties and functions. To fully understand the extent, studies including transcriptomics combined with chromatin accessibility and proteomics analyses at single cell resolution will be crucial. In fact, methods that utilize average responses of a cell population are not able to detect subtle differences across individual cells. Applying single-cell techniques, for example in homeostatic and reactive astrocytes, will provide pivotal information on their cellular state under specific conditions. In addition, single-cell analyses will highlight any heterogeneity within the different brain regions and reveal the existence of possible astrocytic subpopulations. Such data will allow to infer the functional states of the identified cellular diversity and to understand the fate of specific subpopulation of astrocytes under homeostatic and pathological conditions. Such analyses are beginning to emerge and, for example, single-cell transcriptomic analysis of the adult NSC lineage revealed their existence on a continuum through the processes of activation and differentiation (Dulken et al., 2017).

In the last few years, more consideration has been given to the roles and potential therapeutic contribution of non-coding RNAs. For example, the long non-coding RNA (lncRNA) MALAT1, secreted by transplanted adipose-derived stem cells, has been shown to exert neuroprotective effects and ameliorate motor and cognitive impairments in a TBI mouse model. In the absence of MALAT1, both motor and cognitive improvements were prevented and neuronal loss was not reduced (Tajiri et al., 2014). Transcriptional analyses of the brain tissue in a TBI rat model revealed that inflammatory, cell cycle, cell death, and regenerative molecular pathways are dependent on MALAT1 modulation, reinforcing the therapeutic potential of this lncRNA (Patel et al., 2018). Similar studies conducted in NSCs or astrocytes may reveal important aspects of the lncRNAs in these cells unveiling potential therapeutic applications of these molecules.

There are clearly many parallels between the signaling, transcriptional and epigenetic mechanisms guiding astrocyte development and those that regulate astrocyte pathology. It is this commonality of mechanism that underwrites the possibility of identifying therapeutic mechanisms in neurodegeneration and tumorigenesis. For the latter, this approach would result particularly relevant, where the identification of novel developmental factors influencing the molecular and cellular astrocytic identities would enable adopting new approaches in the context of the differentiation therapy (Laug et al., 2018), as attempts towards this direction have not been prosperous so far. Efforts to induce cell cycle arrest and differentiation of GSCs conducted *in vitro*, revealed that only a subset of GSCs responded the desired way. The cells still remained vulnerable to re-enter cell cycle and dedifferentiate as the differentiation-accompanied epigenetic modifications were not complete (Caren et al., 2015). Therefore, caution must be exerted since these overtly differentiated cells still retain the potential to re-acquire their old identity and become once more tumorigenic. Single cell genetic fate mapping of the differentiated GSCs will allow to identify which of these cells truly altered their epigenome and gene expression profile towards a non-proliferating and mature cell type. In this way, it may be possible to select specific subpopulations for further usage in therapeutic applications. From a different perspective, genetically engineered human NSCs have been successfully transplanted into mice bearing brain metastases from lung cancer where they were able to target and inhibit tumor growth. In this study, the NSCs were essentially acting as transient drug delivery vehicles, by converting a prodrug into its cytotoxic form that preferentially targeted dividing cells and then undergoing programmed cell death (Hong et al., 2013). Thus, NSC strategies employed to arrest aberrant cell proliferation could also be considered in brain tumor therapies.

Although an increasing number of studies implicate specific epigenetic events with gliomagenesis, further research is needed to fully comprehend their role in tumor initiation, progression and recurrence. In this way, biomarkers of diagnostic and prognostic value can be identified together with derivation of new tools to manipulate the epigenome of the patients potentially opening new avenues for GBM treatment. Pharmacological intervention on DNA methylation and histone acetylation has been broadly exploited in tumor biology with some compounds showing promise as tools to combat cancer. However, only few are approved by the US FDA and they have serious limitations. For example, HDAC inhibitors target only a small percentage of all acetylation sites, indicating that there is still room for improvement and large-scale screenings and trials will be needed to identify novel lead compounds. The construction of target specific drugs, *i.e.* intervening with a unique acetylation, methylation event or selective inhibition of specific HDACs and DNMTs is a highly demanding task but, if successful, it will reduce the side effects that occur with global inhibition of these epigenetic modulators. Identification of the genome-wide DNA methylation profile of patients with glioma tumors is becoming relatively straightforward (Capper et al., 2018). Characterizing the methylome of these patients may enable physicians to utilize personalized medicine approaches and make informed choices on the potential efficacy of therapeutic agents that target DNA methylation. Pharmacoepigenomics is an emerging field that makes use of epigenetic processes that underpin CNS diseases. The ability to use individuals' epigenetic profiles in order to identify sensitivity or resistance to existing drugs, represents a valuable tool in the emerging field of personalized medicine. In addition, these advances allow a theranostic strategy whereby changes in the epigenome can be monitored during the course of treatment to provide a dynamic biomarker of drug efficiency and disease progression.

Taken together, it is evident that, in astrocytes, epigenetic mechanisms, such as DNA methylation and histone modifications, are extremely complex and their causative role in pathological conditions is still a matter of investigation. However, it is clear that a deep molecular understanding of their mechanism of action will bridge the gap between extrinsic and intrinsic factors that drive change of astrocytes identity as well as to shed light on their involvement in brain disease. Understanding how these various mechanisms eventually alter gene expression and define or re-define astrocytic cell fate is critically important. Not only will it unveil neurodevelopmental mechanisms, but it will also provide the knowledge base to exploit these processes to manipulate astrocytes *in vivo*, thereby paving the way to novel therapies for multiple CNS disorders.

Acknowledgements

M.A.S.P. and L.G. were supported by the University of Luxembourg (IRP ASTROSYS). A.M. was supported by the Luxembourg Institute of Health and the Luxembourg Centre for Systems Biomedicine (MIGLISYS).

References

Ahmed, A.U., Auffinger, B., Lesniak, M.S., 2013. Understanding glioma stem cells: rationale, clinical relevance and therapeutic strategies. Expert Rev. Neurother. 13, 545–555.
Akers, J.C., Ramakrishnan, V., Kim, R., Skog, J., Nakano, I., Pingle, S., Kalinina, J., Hua, W., Kesari, S., Mao, Y., Breakefield, X.O., Hochberg, F.H., Van Meir, E.G., Carter, B.S., Chen, C.C., 2013. MiR-21 in the extracellular vesicles (EVs) of cerebrospinal fluid (CSF): a platform for glioblastoma biomarker development. PLoS One 8, e78115.
Alunni, A., Bally-Cuif, L., 2016. A comparative view of regenerative neurogenesis in vertebrates. Development 143, 741–753.
Anderson, M.A., Ao, Y., Sofroniew, M.V., 2014. Heterogeneity of reactive astrocytes. Neurosci. Lett. 565, 23–29.
Andersson, E.R., Sandberg, R., Lendahl, U., 2011. Notch signaling: simplicity in design, versatility in function. Development 138, 3593–3612.
Aravantinou-Fatorou, K., Ortega, F., Chroni-Tzartou, D., Antoniou, N., Poulopoulou, C., Politis, P.K., Berninger, B., Matsas, R., Thomaidou, D., 2015. CEND1 and NEUROGENIN2 reprogram mouse astrocytes and embryonic fibroblasts to induced neural precursors and differentiated neurons. Stem Cell Rep. 5, 405–418.
Arenas, E., 2014. Wnt signaling in midbrain dopaminergic neuron development and regenerative medicine for Parkinson's disease. J. Mol. Cell Biol. 6, 42–53.
Asano, H., Aonuma, M., Sanosaka, T., Kohyama, J., Namihira, M., Nakashima, K., 2009. Astrocyte differentiation of neural precursor cells is enhanced by retinoic acid through a change in epigenetic modification. Stem Cells 27, 2744–2752.
Baeza, N., Weller, M., Yonekawa, Y., Kleihues, P., Ohgaki, H., 2003. PTEN methylation and expression in glioblastomas. Acta Neuropathol. 106, 479–485.
Balss, J., Meyer, J., Mueller, W., Korshunov, A., Hartmann, C., von Deimling, A., 2008. Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol. 116, 597–602.
Banelli, B., Forlani, A., Allemanni, G., Morabito, A., Pistillo, M.P., Romani, M., 2017. MicroRNA in glioblastoma: an overview. Int. J. Genom. 2017, 7639084.
Barcia, C., Guillemin, G.J., Curtin, J.F., Zirger, J.M., 2016. Editorial: glial cells: managers of neuro-immunity. Front. Cell. Neurosci. 10, 60.
Bardehle, S., Kruger, M., Buggenthin, F., Schwausch, J., Ninkovic, J., Clevers, H., Snippert, H.J., Theis, F.J., Meyer-Luehmann, M., Bechmann, I., Dimou, L., Gotz, M., 2013. Live imaging of astrocyte responses to acute injury reveals selective juxtavascular proliferation. Nat. Neurosci. 16, 580–586.
Barres, B.A., 2008. The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 60, 430–440.
Beebe, K., Lee, W.C., Micchelli, C.A., 2010. JAK/STAT signaling coordinates stem cell proliferation and multilineage differentiation in the Drosophila intestinal stem cell lineage. Dev. Biol. 338, 28–37.
Ben Haim, L., Carrillo-de Sauvage, M.A., Ceyzeriat, K., Escartin, C., 2015. Elusive roles for reactive astrocytes in neurodegenerative diseases. Front. Cell. Neurosci. 9, 278.
Bender, S., Tang, Y., Lindroth, A.M., Hovestadt, V., Jones, D.T., Kool, M., Zapatka, M., Northcott, P.A., Sturm, D., Wang, W., Radlwimmer, B., Hojfeldt, J.W., Truffaux, N., Castel, D., Schubert, S., Ryzhova, M., Seker-Cin, H., Gronych, J., Johann, P.D., Stark, S., Meyer, J., Milde, T., Schuhmann, M., Ebinger, M., Monoranu, C.M., Ponnuswami, A., Chen, S., Jones, C., Witt, O., Collins, V.P., von Deimling, A., Jabado, N., Puget, S., Grill, J., Helin, K., Korshunov, A., Lichter, P., Monje, M., Plass, C., Cho, Y.J., Pfister, S.M., 2013. Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. Cancer Cell 24, 660–672.
Berninger, B., Costa, M.R., Koch, U., Schroeder, T., Sutor, B., Grothe, B., Gotz, M., 2007. Functional properties of neurons derived from in vitro reprogrammed postnatal astroglia. J. Neurosci. 27, 8654–8664.
Berwick, D.C., Harvey, K., 2014. The regulation and deregulation of Wnt signaling by PARK genes in health and disease. J. Mol. Cell Biol. 6, 3–12.
Bhalala, O.G., Pan, L., Sahni, V., McGuire, T.L., Gruner, K., Tourtellotte, W.G., Kessler, J.A., 2012. microRNA-21 regulates astrocytic response following spinal cord injury. J. Neurosci. 32, 17935–17947.
Bi, F., Huang, C., Tong, J., Qiu, G., Huang, B., Wu, Q., Li, F., Xu, Z., Bowser, R., Xia, X.G., Zhou, H., 2013. Reactive astrocytes secrete lcn2 to promote neuron death. Proc. Natl. Acad. Sci. U. S. A. 110, 4069–4074.
Bignami, A., Dahl, D., 1976. The astroglial response to stabbing. Immunofluorescence studies with antibodies to astrocyte-specific protein (GFA) in mammalian and submammalian vertebrates. Neuropathol. Appl. Neurobiol. 2, 99–110.
Birck, C., Koncina, E., Heurtaux, T., Glaab, E., Michelucci, A., Heuschling, P., Grandbarbe, L., 2016. Transcriptomic analyses of primary astrocytes under TNFalpha treatment. Genom. Data 7, 7–11.
Bird, A., 2002. DNA methylation patterns and epigenetic memory. Genes Dev. 16, 6–21.
Blanc, J.L., Wager, M., Guilhot, J., Kusy, S., Bataille, B., Chantereau, T., Lapierre, F., Larsen, C.J., Karayan-Tapon, L., 2004. Correlation of clinical features and methylation status of MGMT gene promoter in glioblastomas. J. Neurooncol. 68, 275–283.
Bonni, A., Sun, Y., Nadal-Vicens, M., Bhatt, A., Frank, D.A., Rozovsky, I., Stahl, N., Yancopoulos, G.D., Greenberg, M.E., 1997. Regulation of gliogenesis in the central nervous system by the JAK-STAT signaling pathway. Science 278, 477–483.
Borggrefe, T., Oswald, F., 2009. The Notch signaling pathway: transcriptional regulation at Notch target genes. Cell. Mol. Life Sci.: CMLS 66, 1631–1646.
Bouvier, D.S., Jones, E.V., Quesseveur, G., Davoli, M.A., A Ferreira, T., Quirion, R., Mechawar, N., Murai, K.K., 2016. High resolution dissection of reactive glial nets in Alzheimer's disease. Sci. Rep. 6, 24544.
Brambilla, R., Bracchi-Ricard, V., Hu, W.H., Frydel, B., Bramwell, A., Karmally, S., Green, E.J., Bethea, J.R., 2005. Inhibition of astroglial nuclear factor kappaB reduces inflammation and improves functional recovery after spinal cord injury. J. Exp. Med. 202, 145–156.
Brown, D.R., 1999. Neurons depend on astrocytes in a coculture system for protection from glutamate toxicity. Mol. Cell. Neurosci. 13, 379–389.
Buffo, A., Rite, I., Tripathi, P., Lepier, A., Colak, D., Horn, A.P., Mori, T., Gotz, M., 2008. Origin and progeny of reactive gliosis: a source of multipotent cells in the injured brain. Proc. Natl. Acad. Sci. U. S. A. 105, 3581–3586.
Cadieux, B., Ching, T.T., VandenBerg, S.R., Costello, J.F., 2006. Genome-wide hypomethylation in human glioblastomas associated with specific copy number alteration, methylenetetrahydrofolate reductase allele status, and increased proliferation. Cancer Res. 66, 8469–8476.
Capper, D., Jones, D.T.W., Sill, M., Hovestadt, V., Schrimpf, D., Sturm, D., Koelsche, C., Sahm, F., Chavez, L., Reuss, D.E., Kratz, A., Wefers, A.K., Huang, K., Pajtler, K.W., Schweizer, L., Stichel, D., Olar, A., Engel, N.W., Lindenberg, K., Harter, P.N., Braczynski, A.K., Plate, K.H., Dohmen, H., Garvalov, B.K., Coras, R., Holsken, A., Hewer, E., Bewerunge-Hudler, M., Schick, M., Fischer, R., Beschorner, R., Schittenhelm, J., Staszewski, O., Wani, K., Varlet, P., Pages, M., Temming, P., Lohmann, D., Selt, F., Witt, H., Milde, T., Witt, O., Aronica, E., Giangaspero, F., Rushing, E., Scheurlen, W., Geisenberger, C., Rodriguez, F.J., Becker, A., Preusser, M., Haberler, C., Bjerkvig, R., Cryan, J., Farrell, M., Deckert, M., Hench, J., Frank, S., Serrano, J., Kannan, K., Tsirigos, A., Bruck, W., Hofer, S., Brehmer, S., Seiz-Rosenhagen, M., Hanggi, D., Hans, V., Rozsnoki, S., Hansford, J.R., Kohlhof, P., Kristensen, B.W., Lechner, M., Lopes, B., Mawrin, C., Ketter, R., Kulozik, A., Khatib, Z., Heppner, F., Koch, A., Jouvet, A., Keohane, C., Muhleisen, H., Mueller, W., Pohl, U., Prinz, M., Benner, A., Zapatka, M., Gottardo, N.G., Driever, P.H., Kramm, C.M., Muller, H.L., Rutkowski, S., von Hoff, K., Fruhwald, M.C., Gnekow, A., Fleischhack, G., Tippelt, S., Calaminus, G., Monoranu, C.M., Perry, A., Jones, C., Jacques, T.S., Radlwimmer, B., Gessi, M., Pietsch, T., Schramm, J., Schackert, G., Westphal, M., Reifenberger, G., Wesseling, P., Weller, M., Collins, V.P., Blumcke, I., Bendszus, M., Debus, J., Huang, A., Jabado, N., Northcott, P.A., Paulus, W., Gajjar, A., Robinson, G.W., Taylor, M.D., Jaunmuktane, Z., Ryzhova, M., Platten, M., Unterberg, A., Wick, W., Karajannis, M.A., Mittelbronn, M., Acker, T., Hartmann, C., Aldape, K., Schuller, U., Buslei, R., Lichter, P., Kool, M., Herold-Mende, C., Ellison, D.W., Hasselblatt, M., Snuderl, M., Brandner, S., Korshunov, A., von Deimling, A., Pfister, S.M., 2018. DNA methylation-based classification of central nervous system tumours. Nature 555, 469–474.
Caren, H., Stricker, S.H., Bulstrode, H., Gagrica, S., Johnstone, E., Bartlett, T.E., Feber, A., Wilson, G., Teschendorff, A.E., Bertone, P., Beck, S., Pollard, S.M., 2015. Glioblastoma stem cells respond to differentiation cues but fail to undergo commitment and terminal cell-cycle arrest. Stem Cell Rep. 5, 829–842.
Carlen, M., Meletis, K., Goritz, C., Darsalia, V., Evergren, E., Tanigaki, K., Amendola, M., Barnabe-Heider, F., Yeung, M.S., Naldini, L., Honjo, T., Kokaia, Z., Shupliakov, O., Cassidy, R.M., Lindvall, O., Frisen, J., 2009. Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. Nat. Neurosci. 12, 259–267.
Cate, H.S., Sabo, J.K., Merlo, D., Kemper, D., Aumann, T.D., Robinson, J., Merson, T.D., Emery, B., Perreau, V.M., Kilpatrick, T.J., 2010. Modulation of bone morphogenic protein signalling alters numbers of astrocytes and oligodendroglia in the subventricular zone during cuprizone-induced demyelination. J. Neurochem. 115, 11–22.
Ceccarelli, M., Barthel, F.P., Malta, T.M., Sabedot, T.S., Salama, S.R., Murray, B.A., Morozova, O., Newton, Y., Radenbaugh, A., Pagnotta, S.M., Anjum, S., Wang, J., Manyam, G., Zoppoli, P., Ling, S., Rao, A.A., Grifford, M., Cherniack, A.D., Zhang, H., Poisson, L., Carlotti Jr., C.G., Tirapelli, D.P., Rao, A., Mikkelsen, T., Lau, C.C., Yung, W.K., Rabadan, R., Huse, J., Brat, D.J., Lehman, N.L., Barnholtz-Sloan, J.S., Zheng, S., Hess, K., Rao, G., Meyerson, M., Beroukhim, R., Cooper, L., Akbani, R., Wrensch, M., Haussler, D., Aldape, K.D., Laird, P.W., Gutmann, D.H., Network, T.R., Noushmehr, H., Iavarone, A., Verhaak, R.G., 2016. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. Cell 164, 550–563.
Chan, K.M., Fang, D., Gan, H., Hashizume, R., Yu, C., Schroeder, M., Gupta, N., Mueller, S., James, C.D., Jenkins, R., Sarkaria, J., Zhang, Z., 2013. The histone H3.3K27M

mutation in pediatric glioma reprograms H3K27 methylation and gene expression. Genes Dev. 27, 985–990.
Chen, Y., Vartiainen, N.E., Ying, W., Chan, P.H., Koistinaho, J., Swanson, R.A., 2001. Astrocytes protect neurons from nitric oxide toxicity by a glutathione-dependent mechanism. J. Neurochem. 77, 1601–1610.
Cheng, P.Y., Lin, Y.P., Chen, Y.L., Lee, Y.C., Tai, C.C., Wang, Y.T., Chen, Y.J., Kao, C.F., Yu, J., 2011. Interplay between SIN3A and STAT3 mediates chromatin conformational changes and GFAP expression during cellular differentiation. PLoS One 6, e22018.
Chien, C.S., Wang, M.L., Chu, P.Y., Chang, Y.L., Liu, W.H., Yu, C.C., Lan, Y.T., Huang, P.I., Lee, Y.Y., Chen, Y.W., Lo, W.L., Chiou, S.H., 2015. Lin28B/Let-7 regulates expression of Oct4 and Sox2 and reprograms oral squamous cell carcinoma cells to a stem-like state. Cancer Res. 75, 2553–2565.
Chowdhury, R., Yeoh, K.K., Tian, Y.M., Hillringhaus, L., Bagg, E.A., Rose, N.R., Leung, I.K., Li, X.S., Woon, E.C., Yang, M., McDonough, M.A., King, O.N., Clifton, I.J., Klose, R.J., Claridge, T.D., Ratcliffe, P.J., Schofield, C.J., Kawamura, A., 2011. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. EMBO Rep. 12, 463–469.
Chung, W.M., Chang, W.C., Chen, L., Chang, Y.Y., Shyr, C.R., Hung, Y.C., Ma, W.L., 2013. MicroRNA-21 promotes the ovarian teratocarcinoma PA1 cell line by sustaining cancer stem/progenitor populations in vitro. Stem Cell Res. Ther. 4, 88.
Clarke, L.E., Liddelow, S.A., Chakraborty, C., Munch, A.E., Heiman, M., Barres, B.A., 2018. Normal aging induces A1-like astrocyte reactivity. Proc. Natl. Acad. Sci. U. S. A. 115, E1896–E1905.
Colodner, K.J., Montana, R.A., Anthony, D.C., Folkerth, R.D., De Girolami, U., Feany, M.B., 2005. Proliferative potential of human astrocytes. J. Neuropathol. Exp. Neurol. 64, 163–169.
Costello, J.F., Berger, M.S., Huang, H.S., Cavenee, W.K., 1996. Silencing of p16/CDKN2 expression in human gliomas by methylation and chromatin condensation. Cancer Res. 56, 2405–2410.
Del Sol, A., Buckley, N.J., 2014. Concise review: a population shift view of cellular reprogramming. Stem Cells 32, 1367–1372.
Domingues, H.S., Portugal, C.C., Socodato, R., Relvas, J.B., 2016. Oligodendrocyte, astrocyte, and microglia crosstalk in myelin development, damage, and repair. Front. Cell Dev. Biol. 4, 71.
Du, Z., Jia, D., Liu, S., Wang, F., Li, G., Zhang, Y., Cao, X., Ling, E.A., Hao, A., 2009. Oct4 is expressed in human gliomas and promotes colony formation in glioma cells. Glia 57, 724–733.
Dulken, B.W., Leeman, D.S., Boutet, S.C., Hebestreit, K., Brunet, A., 2017. Single-cell transcriptomic analysis defines heterogeneity and transcriptional dynamics in the adult neural stem cell lineage. Cell Rep. 18, 777–790.
Elneihoum, A.M., Falke, P., Axelsson, L., Lundberg, E., Lindgarde, F., Ohlsson, K., 1996. Leukocyte activation detected by increased plasma levels of inflammatory mediators in patients with ischemic cerebrovascular diseases. Stroke 27, 1734–1738.
Eng, L.F., Vanderhaeghen, J.J., Bignami, A., Gerstl, B., 1971. An acidic protein isolated from fibrous astrocytes. Brain Res. 28, 351–354.
Escartin, C., Pierre, K., Colin, A., Brouillet, E., Delzescaux, T., Guillermier, M., Dhenain, M., Deglon, N., Hantraye, P., Pellerin, L., Bonvento, G., 2007. Activation of astrocytes by CNTF induces metabolic plasticity and increases resistance to metabolic insults. J. Neurosci. 27, 7094–7104.
Faiz, M., Sachewsky, N., Gascon, S., Bang, K.W., Morshead, C.M., Nagy, A., 2015. Adult neural stem cells from the subventricular zone give rise to reactive astrocytes in the cortex after stroke. Cell Stem Cell 17, 624–634.
Fan, G., Martinowich, K., Chin, M.H., He, F., Fouse, S.D., Hutnick, L., Hattori, D., Ge, W., Shen, Y., Wu, H., ten Hoeve, J., Shuai, K., Sun, Y.E., 2005. DNA methylation controls the timing of astrogliogenesis through regulation of JAK-STAT signaling. Development 132, 3345–3356.
Faulkner, J.R., Herrmann, J.E., Woo, M.J., Tansey, K.E., Doan, N.B., Sofroniew, M.V., 2004. Reactive astrocytes protect tissue and preserve function after spinal cord injury. J. Neurosci. 24, 2143–2155.
Fiore, R., Khudayberdiev, S., Saba, R., Schratt, G., 2011. MicroRNA function in the nervous system. Prog. Mol. Biol. Transl. Sci. 102, 47–100.
Follert, P., Cremer, H., Beclin, C., 2014. MicroRNAs in brain development and function: a matter of flexibility and stability. Front. Mol. Neurosci. 7, 5.
Friedmann-Morvinski, D., Verma, I.M., 2014. Dedifferentiation and reprogramming: origins of cancer stem cells. EMBO Rep. 15, 244–253.
Friedmann-Morvinski, D., Bushong, E.A., Ke, E., Soda, Y., Marumoto, T., Singer, O., Ellisman, M.H., Verma, I.M., 2012. Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. Science 338, 1080–1084.
Gabel, S., Koncina, E., Dorban, G., Heurtaux, T., Birck, C., Glaab, E., Michelucci, A., Heuschling, P., Grandbarbe, L., 2016. Inflammation promotes a conversion of astrocytes into neural progenitor cells via NF-kappaB activation. Mol. Neurobiol. 53, 5041–5055.
Gadea, A., Schinelli, S., Gallo, V., 2008. Endothelin-1 regulates astrocyte proliferation and reactive gliosis via a JNK/c-Jun signaling pathway. J. Neurosci. 28, 2394–2408.
Gaiano, N., Nye, J.S., Fishell, G., 2000. Radial glial identity is promoted by Notch1 signaling in the murine forebrain. Neuron 26, 395–404.
Gao, Y., Guan, M., Su, B., Liu, W., Xu, M., Lu, Y., 2004. Hypermethylation of the RASSF1A gene in gliomas. Clin. Chim. Acta 349, 173–179.
Gao, K., Wang, C.R., Jiang, F., Wong, A.Y., Su, N., Jiang, J.H., Chai, R.C., Vatcher, G., Teng, J., Chen, J., Jiang, Y.W., Yu, A.C., 2013. Traumatic scratch injury in astrocytes triggers calcium influx to activate the JNK/c-Jun/AP-1 pathway and switch on GFAP expression. Glia 61, 2063–2077.
Gascon, S., Masserdotti, G., Russo, G.L., Gotz, M., 2017. Direct neuronal reprogramming: achievements, hurdles, and new roads to success. Cell Stem Cell 21, 18–34.
Glushakova, O.Y., Glushakov, A.O., Borlongan, C.V., Valadka, A.B., Hayes, R.L., Glushakov, A.V., 2018. Role of caspase-3-mediated apoptosis in chronic caspase-3-cleaved tau accumulation and blood-brain barrier damage in the Corpus callosum after traumatic brain injury in rats. J. Neurotrauma 35, 157–173.
Gotz, M., Sirko, S., Beckers, J., Irmler, M., 2015. Reactive astrocytes as neural stem or progenitor cells: in vivo lineage, in vitro potential, and genome-wide expression analysis. Glia 63, 1452–1468.
Graff, J., Mansuy, I.M., 2008. Epigenetic codes in cognition and behaviour. Behav. Brain Res. 192, 70–87.
Grandbarbe, L., Bouissac, J., Rand, M., Hrabe de Angelis, M., Artavanis-Tsakonas, S., Mohier, E., 2003. Delta-Notch signaling controls the generation of neurons/glia from neural stem cells in a stepwise process. Development 130, 1391–1402.
Guo, Z., Zhang, L., Wu, Z., Chen, Y., Wang, F., Chen, G., 2014. In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. Cell Stem Cell 14, 188–202.
Hara, M., Kobayakawa, K., Ohkawa, Y., Kumamaru, H., Yokota, K., Saito, T., Kijima, K., Yoshizaki, S., Harimaya, K., Nakashima, Y., Okada, S., 2017. Interaction of reactive astrocytes with type I collagen induces astrocytic scar formation through the integrin-N-cadherin pathway after spinal cord injury. Nat. Med. 23, 818–828.
Hatada, I., Namihira, M., Morita, S., Kimura, M., Horii, T., Nakashima, K., 2008. Astrocyte-specific genes are generally demethylated in neural precursor cells prior to astrocytic differentiation. PLoS One 3, e3189.
Heinrich, C., Blum, R., Gascon, S., Masserdotti, G., Tripathi, P., Sanchez, R., Tiedt, S., Schroeder, T., Gotz, M., Berninger, B., 2010. Directing astroglia from the cerebral cortex into subtype specific functional neurons. PLoS Biol. 8, e1000373.
Herrmann, J.E., Imura, T., Song, B., Qi, J., Ao, Y., Nguyen, T.K., Korsak, R.A., Takeda, K., Akira, S., Sofroniew, M.V., 2008. STAT3 is a critical regulator of astrogliosis and scar formation after spinal cord injury. J. Neurosci. 28, 7231–7243.
Hirabayashi, Y., Gotoh, Y., 2005. Stage-dependent fate determination of neural precursor cells in mouse forebrain. Neurosci. Res. 51, 331–336.
Hong, S.H., Lee, H.J., An, J., Lim, I., Borlongan, C., Aboody, K.S., Kim, S.U., 2013. Human neural stem cells expressing carboxyl esterase target and inhibit tumor growth of lung cancer brain metastases. Cancer Gene Ther. 20, 678–682.
Howng, S.Y., Huang, Y., Ptacek, L., Fu, Y.H., 2015. Understanding the role of dicer in astrocyte development. PLoS One 10, e0126667.
Hutchison, E.R., Kawamoto, E.M., Taub, D.D., Lal, A., Abdelmohsen, K., Zhang, Y., Wood 3rd, W.H., Lehrmann, E., Camandola, S., Becker, K.G., Gorospe, M., Mattson, M.P., 2013. Evidence for miR-181 involvement in neuroinflammatory responses of astrocytes. Glia 61, 1018–1028.
Imayoshi, I., Sakamoto, M., Yamaguchi, M., Mori, K., Kageyama, R., 2010. Essential roles of Notch signaling in maintenance of neural stem cells in developing and adult brains. J. Neurosci. 30, 3489–3498.
Inestrosa, N.C., Arenas, E., 2010. Emerging roles of Wnts in the adult nervous system. Nat. Rev. Neurosci. 11, 77–86.
Iwata-Ichikawa, E., Kondo, Y., Miyazaki, I., Asanuma, M., Ogawa, N., 1999. Glial cells protect neurons against oxidative stress via transcriptional up-regulation of the glutathione synthesis. J. Neurochem. 72, 2334–2344.
Jin, M., Jang, E., Suk, K., 2014. Lipocalin-2 acts as a neuroinflammatogen in lipopolysaccharide-injected mice. Exp. Neurobiol. 23, 155–162.
John Lin, C.C., Deneen, B., 2013. Astrocytes: the missing link in neurologic disease? Semin. Pediatr. Neurol. 20, 236–241.
Jones, C., Karajannis, M.A., Jones, D.T.W., Kieran, M.W., Monje, M., Baker, S.J., Becher, O.J., Cho, Y.J., Gupta, N., Hawkins, C., Hargrave, D., Haas-Kogan, D.A., Jabado, N., Li, X.N., Mueller, S., Nicolaides, T., Packer, R.J., Persson, A.I., Phillips, J.J., Simonds, E.F., Stafford, J.M., Tang, Y., Pfister, S.M., Weiss, W.A., 2017. Pediatric high-grade glioma: biologically and clinically in need of new thinking. Neurooncology 19, 153–161.
Kalani, M.Y., Cheshier, S.H., Cord, B.J., Bababeygy, S.R., Vogel, H., Weissman, I.L., Palmer, T.D., Nusse, R., 2008. Wnt-mediated self-renewal of neural stem/progenitor cells. Proc. Natl. Acad. Sci. U. S. A. 105, 16970–16975.
Kanski, R., Sneeboer, M.A., van Bodegraven, E.J., Sluijs, J.A., Kropff, W., Vermunt, M.W., Creyghton, M.P., De Filippis, L., Vescovi, A., Aronica, E., van Tijn, P., van Strien, M.E., Hol, E.M., 2014. Histone acetylation in astrocytes suppresses GFAP and stimulates a reorganization of the intermediate filament network. J. Cell Sci. 127, 4368–4380.
Karimi-Abdolrezaee, S., Billakanti, R., 2012. Reactive astrogliosis after spinal cord injury-beneficial and detrimental effects. Mol. Neurobiol. 46, 251–264.
Kim, J.H., Ko, P.W., Lee, H.W., Jeong, J.Y., Lee, M.G., Kim, J.H., Lee, W.H., Yu, R., Oh, W.J., Suk, K., 2017. Astrocyte-derived lipocalin-2 mediates hippocampal damage and cognitive deficits in experimental models of vascular dementia. Glia 65, 1471–1490.
Klose, R.J., Bird, A.P., 2006. Genomic DNA methylation: the mark and its mediators. Trends Biochem. Sci. 31, 89–97.
Kong, S.Y., Kim, W., Lee, H.R., Kim, H.J., 2018. The histone demethylase KDM5A is required for the repression of astrocytogenesis and regulated by the translational machinery in neural progenitor cells. FASEB J. 32, 1108–1119.
Kouzarides, T., 2007. Chromatin modifications and their function. Cell 128, 693–705.
Kriaucionis, S., Heintz, N., 2009. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science 324, 929–930.
Kroehne, V., Freudenreich, D., Hans, S., Kaslin, J., Brand, M., 2011. Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. Development 138, 4831–4841.
L'Episcopo, F., Tirolo, C., Testa, N., Caniglia, S., Morale, M.C., Cossetti, C., D'Adamo, P., Zardini, E., Andreoni, L., Ihekwaba, A.E., Serra, P.A., Franciotta, D., Martino, G., Pluchino, S., Marchetti, B., 2011. Reactive astrocytes and Wnt/beta-catenin signaling link nigrostriatal injury to repair in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. Neurobiol. Dis. 41, 508–527.
Lathia, J.D., Mack, S.C., Mulkearns-Hubert, E.E., Valentim, C.L., Rich, J.N., 2015. Cancer

stem cells in glioblastoma. Genes Dev. 29, 1203–1217.
Laug, D., Glasgow, S.M., Deneen, B., 2018. A glial blueprint for gliomagenesis. Nat. Rev. Neurosci. 19, 393–403.
Lee, S., Kim, J.H., Kim, J.H., Seo, J.W., Han, H.S., Lee, W.H., Mori, K., Nakao, K., Barasch, J., Suk, K., 2011. Lipocalin-2 is a chemokine inducer in the central nervous system: role of chemokine ligand 10 (CXCL10) in lipocalin-2-induced cell migration. J. Biol. Chem. 286, 43855–43870.
Lee, H.K., Bier, A., Cazacu, S., Finniss, S., Xiang, C., Twito, H., Poisson, L.M., Mikkelsen, T., Slavin, S., Jacoby, E., Yalon, M., Toren, A., Rempel, S.A., Brodie, C., 2013a. MicroRNA-145 is downregulated in glial tumors and regulates glioma cell migration by targeting connective tissue growth factor. PLoS One 8, e54652.
Lee, H.K., Finniss, S., Cazacu, S., Bucris, E., Ziv-Av, A., Xiang, C., Bobbitt, K., Rempel, S.A., Hasselbach, L., Mikkelsen, T., Slavin, S., Brodie, C., 2013b. Mesenchymal stem cells deliver synthetic microRNA mimics to glioma cells and glioma stem cells and inhibit their cell migration and self-renewal. Oncotarget 4, 346–361.
Lewis, P.W., Muller, M.M., Koletsky, M.S., Cordero, F., Lin, S., Banaszynski, L.A., Garcia, B.A., Muir, T.W., Becher, O.J., Allis, C.D., 2013. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. Science 340, 857–861.
Li, X., Zhang, J., Gao, L., McClellan, S., Finan, M.A., Butler, T.W., Owen, L.B., Piazza, G.A., Xi, Y., 2012. MiR-181 mediates cell differentiation by interrupting the Lin28 and let-7 feedback circuit. Cell Death Differ. 19, 378–386.
Li, F., Liu, X., Sampson, J.H., Bigner, D.D., Li, C.Y., 2016. Rapid reprogramming of primary human astrocytes into potent tumor-initiating cells with defined genetic factors. Cancer Res. 76, 5143–5150.
Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., Bennett, M.L., Munch, A.E., Chung, W.S., Peterson, T.C., Wilton, D.K., Frouin, A., Napier, B.A., Panicker, N., Kumar, M., Buckwalter, M.S., Rowitch, D.H., Dawson, V.L., Dawson, T.M., Stevens, B., Barres, B.A., 2017. Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541, 481–487.
Liu, Y., Miao, Q., Yuan, J., Han, Se., Zhang, P., Li, S., Rao, Z., Zhao, W., Ye, Q., Geng, J., Zhang, X., Cheng, L., 2015. *Ascl1* converts dorsal midbrain astrocytes into functional neurons *in vivo*. J. Neurosci. 35, 9336–9355.
Loffler, D., Brocke-Heidrich, K., Pfeifer, G., Stocsits, C., Hackermuller, J., Kretzschmar, A.K., Burger, R., Gramatzki, M., Blumert, C., Bauer, K., Cvijic, H., Ullmann, A.K., Stadler, P.F., Horn, F., 2007. Interleukin-6 dependent survival of multiple myeloma cells involves the Stat3-mediated induction of microRNA-21 through a highly conserved enhancer. Blood 110, 1330–1333.
Louis, D.N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W.K., Ohgaki, H., Wiestler, O.D., Kleihues, P., Ellison, D.W., 2016. The 2016 world health organization classification of tumors of the central nervous system: a summary. Acta Neuropathol. 131, 803–820.
Louvi, A., Artavanis-Tsakonas, S., 2006. Notch signalling in vertebrate neural development. Nat. Rev. Neurosci. 7, 93–102.
Lozano, D., Gonzales-Portillo, G.S., Acosta, S., de la Pena, I., Tajiri, N., Kaneko, Y., Borlongan, C.V., 2015. Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatr. Dis. Treat. 11, 97–106.
Luo, J.W., Wang, X., Yang, Y., Mao, Q., 2015. Role of micro-RNA (miRNA) in pathogenesis of glioblastoma. Eur. Rev. Med. Pharmacol. Sci. 19, 1630–1639.
Mabie, P.C., Mehler, M.F., Kessler, J.A., 1999. Multiple roles of bone morphogenetic protein signaling in the regulation of cortical cell number and phenotype. J. Neurosci. 19, 7077–7088.
Magnusson, J.P., Goritz, C., Tatarishvili, J., Dias, D.O., Smith, E.M., Lindvall, O., Kokaia, Z., Frisen, J., 2014. A latent neurogenic program in astrocytes regulated by Notch signaling in the mouse. Science 346, 237–241.
Maragakis, N.J., Rothstein, J.D., 2006. Mechanisms of disease: astrocytes in neurodegenerative disease. Nat. Clin. Pract. Neurol. 2, 679–689.
McIver, S.R., Faideau, M., Haydon, P.G., 2013. Astrocyte–neuron communications. In: Noronha, C.C.G. (Ed.), Neural-Immune Interactions in Brain Function and Alcohol Related Disorders. Springer, Boston, MA, pp. 31–64.
Meares, G.P., Rajbhandari, R., Gerigk, M., Tien, C.L., Chang, C., Fehling, S.C., Rowse, A., Mulhern, K.C., Nair, S., Gray, G.K., Berbari, N.F., Bredel, M., Benveniste, E.N., Nozell, S.E., 2018. MicroRNA-31 is required for astrocyte specification. Glia 66, 987–998.
Mehler, M.F., 2008. Epigenetic principles and mechanisms underlying nervous system functions in health and disease. Prog. Neurobiol. 86, 305–341.
Michelucci, A., Bithell, A., Burney, M.J., Johnston, C.E., Wong, K.Y., Teng, S.W., Desai, J., Gumbleton, N., Anderson, G., Stanton, L.W., Williams, B.P., Buckley, N.J., 2016. The neurogenic potential of astrocytes is regulated by inflammatory signals. Mol. Neurobiol. 53, 3724–3739.
Miska, E.A., Alvarez-Saavedra, E., Townsend, M., Yoshii, A., Sestan, N., Rakic, P., Constantine-Paton, M., Horvitz, H.R., 2004. Microarray analysis of microRNA expression in the developing mammalian brain. Genome Biol. 5, R68.
Moller, H.G., Rasmussen, A.P., Andersen, H.H., Johnsen, K.B., Henriksen, M., Duroux, M., 2013. A systematic review of microRNA in glioblastoma multiforme: micro-modulators in the mesenchymal mode of migration and invasion. Mol. Neurobiol. 47, 131–144.
Molne, M., Studer, L., Tabar, V., Ting, Y.T., Eiden, M.V., McKay, R.D., 2000. Early cortical precursors do not undergo LIF-mediated astrocytic differentiation. J. Neurosci. Res. 59, 301–311.
Molofsky, A.V., Krencik, R., Ullian, E.M., Tsai, H.H., Deneen, B., Richardson, W.D., Barres, B.A., Rowitch, D.H., 2012. Astrocytes and disease: a neurodevelopmental perspective. Genes Dev. 26, 891–907.
Morita, S., Noguchi, H., Horii, T., Nakabayashi, K., Kimura, M., Okamura, K., Sakai, A., Nakashima, H., Hata, K., Nakashima, K., Hatada, I., 2016. Targeted DNA demethylation in vivo using dCas9-peptide repeat and scFv-TET1 catalytic domain fusions. Nat. Biotechnol. 34, 1060–1065.
Murao, N., Noguchi, H., Nakashima, K., 2016. Epigenetic regulation of neural stem cell property from embryo to adult. Neuroepigenetics 5, 1–10.
Myer, D.J., Gurkoff, G.G., Lee, S.M., Hovda, D.A., Sofroniew, M.V., 2006. Essential protective roles of reactive astrocytes in traumatic brain injury. Brain: A J. Neurol. 129, 2761–2772.
Naka, H., Nakamura, S., Shimazaki, T., Okano, H., 2008. Requirement for COUP-TFI and II in the temporal specification of neural stem cells in CNS development. Nat. Neurosci. 11, 1014–1023.
Naka-Kaneda, H., Nakamura, S., Igarashi, M., Aoi, H., Kanki, H., Tsuyama, J., Tsutsumi, S., Aburatani, H., Shimazaki, T., Okano, H., 2014. The miR-17/106-p38 axis is a key regulator of the neurogenic-to-gliogenic transition in developing neural stem/progenitor cells. Proc. Natl. Acad. Sci. U. S. A. 111, 1604–1609.
Nakamura, M., Yonekawa, Y., Kleihues, P., Ohgaki, H., 2001. Promoter hypermethylation of the RB1 gene in glioblastomas. Lab. Invest. 81, 77–82.
Nakashima, K., Yanagisawa, M., Arakawa, H., Kimura, N., Hisatsune, T., Kawabata, M., Miyazono, K., Taga, T., 1999. Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. Science 284, 479–482.
Namihira, M., Nakashima, K., Taga, T., 2004. Developmental stage dependent regulation of DNA methylation and chromatin modification in a immature astrocyte specific gene promoter. FEBS Lett. 572, 184–188.
Namihira, M., Kohyama, J., Semi, K., Sanosaka, T., Deneen, B., Taga, T., Nakashima, K., 2009. Committed neuronal precursors confer astrocytic potential on residual neural precursor cells. Dev. Cell 16, 245–255.
Nato, G., Caramello, A., Trova, S., Avataneo, V., Rolando, C., Taylor, V., Buffo, A., Peretto, P., Luzzati, F., 2015. Striatal astrocytes produce neuroblasts in an excitotoxic model of Huntington's disease. Development 142, 840–845.
Nedergaard, M., 1994. Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. Science 263, 1768–1771.
Nieto, M., Schuurmans, C., Britz, O., Guillemot, F., 2001. Neural bHLH genes control the neuronal versus glial fate decision in cortical progenitors. Neuron 29, 401–413.
Niranjan, R., 2014. The role of inflammatory and oxidative stress mechanisms in the pathogenesis of Parkinson's disease: focus on astrocytes. Mol. Neurobiol. 49, 28–38.
Niu, W., Zang, T., Zou, Y., Fang, S., Smith, D.K., Bachoo, R., Zhang, C.L., 2013. In vivo reprogramming of astrocytes to neuroblasts in the adult brain. Nat. Cell Biol. 15, 1164–1175.
Niwa, H., Burdon, T., Chambers, I., Smith, A., 1998. Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. Genes Dev. 12, 2048–2060.
Noguchi, H., Kimura, A., Murao, N., Namihira, M., Nakashima, K., 2016a. Prenatal deletion of DNA methyltransferase 1 in neural stem cells impairs neurogenesis and causes anxiety-like behavior in adulthood. Neurogenesis 3, e1232679.
Noguchi, H., Murao, N., Kimura, A., Matsuda, T., Namihira, M., Nakashima, K., 2016b. DNA methyltransferase 1 is indispensable for development of the hippocampal dentate gyrus. J. Neurosci. 36, 6050–6068.
Noushmehr, H., Weisenberger, D.J., Diefes, K., Phillips, H.S., Pujara, K., Berman, B.P., Pan, F., Pelloski, C.E., Sulman, E.P., Bhat, K.P., Verhaak, R.G., Hoadley, K.A., Hayes, D.N., Perou, C.M., Schmidt, H.K., Ding, L., Wilson, R.K., Van Den Berg, D., Shen, H., Bengtsson, H., Neuvial, P., Cope, L.M., Buckley, J., Herman, J.G., Baylin, S.B., Laird, P.W., Aldape, K., Cancer Genome Atlas Research, N, 2010. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 17, 510–522.
O'Callaghan, J.P., Kelly, K.A., VanGilder, R.L., Sofroniew, M.V., Miller, D.B., 2014. Early activation of STAT3 regulates reactive astrogliosis induced by diverse forms of neurotoxicity. PLoS One 9, e102003.
Oberheim, N.A., Wang, X., Goldman, S., Nedergaard, M., 2006. Astrocytic complexity distinguishes the human brain. Trends Neurosci. 29, 547–553.
Okada, S., Nakamura, M., Katoh, H., Miyao, T., Shimazaki, T., Ishii, K., Yamane, J., Yoshimura, A., Iwamoto, Y., Toyama, Y., Okano, H., 2006. Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. Nat. Med. 12, 829–834.
Okano, M., Bell, D.W., Haber, D.A., Li, E., 1999. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99, 247–257.
Ostrom, Q.T., Gittleman, H., Xu, J., Kromer, C., Wolinsky, Y., Kruchko, C., Barnholtz-Sloan, J.S., 2016. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2009–2013. Neurooncology 18, v1–v75.
Palma, V., Lim, D.A., Dahmane, N., Sanchez, P., Brionne, T.C., Herzberg, C.D., Gitton, Y., Carleton, A., Alvarez-Buylla, A., Ruiz i Altaba, A., 2005. Sonic hedgehog controls stem cell behavior in the postnatal and adult brain. Development 132, 335–344.
Parpura, V., Basarsky, T.A., Liu, F., Jeftinija, K., Jeftinija, S., Haydon, P.G., 1994. Glutamate-mediated astrocyte-neuron signalling. Nature 369, 744–747.
Parsons, D.W., Jones, S., Zhang, X., Lin, J.C., Leary, R.J., Angenendt, P., Mankoo, P., Carter, H., Siu, I.M., Gallia, G.L., Olivi, A., McLendon, R., Rasheed, B.A., Keir, S., Nikolskaya, T., Nikolsky, Y., Busam, D.A., Tekleab, H., Diaz Jr., L.A., Hartigan, J., Smith, D.R., Strausberg, R.L., Marie, S.K., Shinjo, S.M., Yan, H., Riggins, G.J., Bigner, D.D., Karchin, R., Papadopoulos, N., Parmigiani, G., Vogelstein, B., Velculescu, V.E., Kinzler, K.W., 2008. An integrated genomic analysis of human glioblastoma multiforme. Science 321, 1807–1812.
Patel, N.A., Moss, L.D., Lee, J.Y., Tajiri, N., Acosta, S., Hudson, C., Parag, S., Cooper, D.R., Borlongan, C.V., Bickford, P.C., 2018. Long noncoding RNA MALAT1 in exosomes drives regenerative function and modulates inflammation-linked networks following traumatic brain injury. J. Neuroinflammation 15, 204.
Pavlou, M.A.S., Pinho, R., Paiva, I., Outeiro, T.F., 2017. The yin and yang of alpha-synuclein-associated epigenetics in Parkinson's disease. Brain: A J. Neurol. 140, 878–886.
Pekny, M., Nilsson, M., 2005. Astrocyte activation and reactive gliosis. Glia 50, 427–434.

Pekny, M., Pekna, M., 2014. Astrocyte reactivity and reactive astrogliosis: costs and benefits. Physiol. Rev. 94, 1077–1098.
Pellerin, L., Bouzier-Sore, A.K., Aubert, A., Serres, S., Merle, M., Costalat, R., Magistretti, P.J., 2007. Activity-dependent regulation of energy metabolism by astrocytes: an update. Glia 55, 1251–1262.
Pereira, J.D., Sansom, S.N., Smith, J., Dobenecker, M.W., Tarakhovsky, A., Livesey, F.J., 2010. Ezh2, the histone methyltransferase of PRC2, regulates the balance between self-renewal and differentiation in the cerebral cortex. Proc. Natl. Acad. Sci. U. S. A. 107, 15957–15962.
Polakis, P., 2007. The many ways of Wnt in cancer. Curr. Opin. Genet. Dev. 17, 45–51.
Rajendran, G., Shanmuganandam, K., Bendre, A., Muzumdar, D., Goel, A., Shiras, A., 2011. Epigenetic regulation of DNA methyltransferases: DNMT1 and DNMT3B in gliomas. J. Neurooncol. 104, 483–494.
Rao, K.V., Panickar, K.S., Jayakumar, A.R., Norenberg, M.D., 2005. Astrocytes protect neurons from ammonia toxicity. Neurochem. Res. 30, 1311–1318.
Raz, R., Lee, C.K., Cannizzaro, L.A., d'Eustachio, P., Levy, D.E., 1999. Essential role of STAT3 for embryonic stem cell pluripotency. Proc. Natl. Acad. Sci. U. S. A. 96, 2846–2851.
Restrepo, A., Smith, C.A., Agnihotri, S., Shekarforoush, M., Kongkham, P.N., Seol, H.J., Northcott, P., Rutka, J.T., 2011. Epigenetic regulation of glial fibrillary acidic protein by DNA methylation in human malignant gliomas. Neurooncology 13, 42–50.
Rheinbay, E., Suva, M.L., Gillespie, S.M., Wakimoto, H., Patel, A.P., Shahid, M., Oksuz, O., Rabkin, S.D., Martuza, R.L., Rivera, M.N., Louis, D.N., Kasif, S., Chi, A.S., Bernstein, B.E., 2013. An aberrant transcription factor network essential for Wnt signaling and stem cell maintenance in glioblastoma. Cell Rep. 3, 1567–1579.
Rivetti di Val Cervo, P., Romanov, R.A., Spigolon, G., Masini, D., Martin-Montanez, E., Toledo, E.M., La Manno, G., Feyder, M., Pifl, C., Ng, Y.H., Sanchez, S.P., Linnarsson, S., Wernig, M., Harkany, T., Fisone, G., Arenas, E., 2017. Induction of functional dopamine neurons from human astrocytes in vitro and mouse astrocytes in a Parkinson's disease model. Nat. Biotechnol. 35, 444–452.
Rothstein, J.D., Dykes-Hoberg, M., Pardo, C.A., Bristol, L.A., Jin, L., Kuncl, R.W., Kanai, Y., Hediger, M.A., Wang, Y., Schielke, J.P., Welty, D.F., 1996. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16, 675–686.
Sahni, V., Mukhopadhyay, A., Tysseling, V., Hebert, A., Birch, D., McGuire, T.L., Stupp, S.I., Kessler, J.A., 2010. BMPR1a and BMPR1b signaling exert opposing effects on gliosis after spinal cord injury. J. Neurosci. 30, 1839–1855.
Sanosaka, T., Imamura, T., Hamazaki, N., Chai, M., Igarashi, K., Ideta-Otsuka, M., Miura, F., Ito, T., Fujii, N., Ikeo, K., Nakashima, K., 2017. DNA methylome analysis identifies transcription factor-based epigenomic signatures of multilineage competence in neural stem/progenitor cells. Cell Rep. 20, 2992–3003.
Schachtrup, C., Ryu, J.K., Helmrick, M.J., Vagena, E., Galanakis, D.K., Degen, J.L., Margolis, R.U., Akassoglou, K., 2010. Fibrinogen triggers astrocyte scar formation by promoting the availability of active TGF-beta after vascular damage. J. Neurosci. 30, 5843–5854.
Scholze, A.R., Foo, L.C., Mulinyawe, S., Barres, B.A., 2014. BMP signaling in astrocytes downregulates EGFR to modulate survival and maturation. PLoS One 9, e110668.
Schreiner, B., Romanelli, E., Liberski, P., Ingold-Heppner, B., Sobottka-Brillout, B., Hartwig, T., Chandrasekar, V., Johannssen, H., Zeilhofer, H.U., Aguzzi, A., Heppner, F., Kerschensteiner, M., Becher, B., 2015. Astrocyte depletion impairs redox homeostasis and triggers neuronal loss in the adult CNS. Cell Rep. 12, 1377–1384.
See, J., Mamontov, P., Ahn, K., Wine-Lee, L., Crenshaw 3rd, E.B., Grinspan, J.B., 2007. BMP signaling mutant mice exhibit glial cell maturation defects. Mol. Cell. Neurosci. 35, 171–182.
Sempere, L.F., Freemantle, S., Pitha-Rowe, I., Moss, E., Dmitrovsky, E., Ambros, V., 2004. Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol. 5, R13.
Setoguchi, T., Nakashima, K., Takizawa, T., Yanagisawa, M., Ochiai, W., Okabe, M., Yone, K., Komiya, S., Taga, T., 2004. Treatment of spinal cord injury by transplantation of fetal neural precursor cells engineered to express BMP inhibitor. Exp. Neurol. 189, 33–44.
Sharif, A., Legendre, P., Prevot, V., Allet, C., Romao, L., Studler, J.M., Chneiweiss, H., Junier, M.P., 2007. Transforming growth factor alpha promotes sequential conversion of mature astrocytes into neural progenitors and stem cells. Oncogene 26, 2695–2706.
Sher, F., Boddeke, E., Copray, S., 2011. Ezh2 expression in astrocytes induces their dedifferentiation toward neural stem cells. Cell. Reprogram. 13, 1–6.
Shimada, I.S., LeComte, M.D., Granger, J.C., Quinlan, N.J., Spees, J.L., 2012. Self-renewal and differentiation of reactive astrocyte-derived neural stem/progenitor cells isolated from the cortical peri-infarct area after stroke. J. Neurosci. 32, 7926–7940.
Siddique, A.N., Nunna, S., Rajavelu, A., Zhang, Y., Jurkowska, R.Z., Reinhardt, R., Rots, M.G., Ragozin, S., Jurkowski, T.P., Jeltsch, A., 2013. Targeted methylation and gene silencing of VEGF-A in human cells by using a designed Dnmt3a-Dnmt3L single-chain fusion protein with increased DNA methylation activity. J. Mol. Biol. 425, 479–491.
Sirko, S., Behrendt, G., Johansson, P.A., Tripathi, P., Costa, M., Bek, S., Heinrich, C., Tiedt, S., Colak, D., Dichgans, M., Fischer, I.R., Plesnila, N., Staufenbiel, M., Haass, C., Snapyan, M., Saghatelyan, A., Tsai, L.H., Fischer, A., Grobe, K., Dimou, L., Gotz, M., 2013. Reactive glia in the injured brain acquire stem cell properties in response to sonic hedgehog. [corrected]. Cell Stem Cell 12, 426–439.
Sloan, S.A., Barres, B.A., 2014. Mechanisms of astrocyte development and their contributions to neurodevelopmental disorders. Curr. Opin. Neurobiol. 27, 75–81.
Smirnova, L., Grafe, A., Seiler, A., Schumacher, S., Nitsch, R., Wulczyn, F.G., 2005. Regulation of miRNA expression during neural cell specification. Eur. J. Neurosci. 21, 1469–1477.
Sofroniew, M.V., 2009. Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci. 32, 638–647.
Sofroniew, M.V., Vinters, H.V., 2010. Astrocytes: biology and pathology. Acta Neuropathol. 119, 7–35.
Song, M.R., Ghosh, A., 2004. FGF2-induced chromatin remodeling regulates CNTF-mediated gene expression and astrocyte differentiation. Nat. Neurosci. 7, 229–235.
Song, S., Kong, X., Acosta, S., Sava, V., Borlongan, C., Sanchez-Ramos, J., 2016. Granulocyte colony-stimulating factor promotes behavioral recovery in a mouse model of traumatic brain injury. J. Neurosci. Res. 94, 409–423.
Sriram, K., Benkovic, S.A., Hebert, M.A., Miller, D.B., O'Callaghan, J.P., 2004. Induction of gp130-related cytokines and activation of JAK2/STAT3 pathway in astrocytes precedes up-regulation of glial fibrillary acidic protein in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of neurodegeneration: key signaling pathway for astrogliosis in vivo? J. Biol. Chem. 279, 19936–19947.
Sudo, G., Kagawa, T., Kokubu, Y., Inazawa, J., Taga, T., 2016. Increase in GFAP-positive astrocytes in histone demethylase GASC1/KDM4C/JMJD2C hypomorphic mutant mice. Genes Cells 21, 218–225.
Sun, Y., Nadal-Vicens, M., Misono, S., Lin, M.Z., Zubiaga, A., Hua, X., Fan, G., Greenberg, M.E., 2001. Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms. Cell 104, 365–376.
Sun, X., Liu, J., Xu, C., Tang, S.C., Ren, H., 2016. The insights of Let-7 miRNAs in oncogenesis and stem cell potency. J. Cell. Mol. Med. 20, 1779–1788.
Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L., Rao, A., 2009. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 324, 930–935.
Tajiri, N., Acosta, S.A., Shahaduzzaman, M., Ishikawa, H., Shinozuka, K., Pabon, M., Hernandez-Ontiveros, D., Kim, D.W., Metcalf, C., Staples, M., Dailey, T., Vasconcellos, J., Franyuti, G., Gould, L., Patel, N., Cooper, D., Kaneko, Y., Borlongan, C.V., Bickford, P.C., 2014. Intravenous transplants of human adipose-derived stem cell protect the brain from traumatic brain injury-induced neurodegeneration and motor and cognitive impairments: cell graft biodistribution and soluble factors in young and aged rats. J. Neurosci. 34, 313–326.
Takizawa, T., Nakashima, K., Namihira, M., Ochiai, W., Uemura, A., Yanagisawa, M., Fujita, N., Nakao, M., Taga, T., 2001. DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. Dev. Cell 1, 749–758.
Takouda, J., Katada, S., Nakashima, K., 2017. Emerging mechanisms underlying astrogenesis in the developing mammalian brain. Proc. Jpn. Acad. Ser. B: Phys. Biol. Sci. 93, 386–398.
Tan, S.L., Nishi, M., Ohtsuka, T., Matsui, T., Takemoto, K., Kamio-Miura, A., Aburatani, H., Shinkai, Y., Kageyama, R., 2012. Essential roles of the histone methyltransferase ESET in the epigenetic control of neural progenitor cells during development. Development 139, 3806–3816.
Tao, J., Wu, H., Lin, Q., Wei, W., Lu, X.H., Cantle, J.P., Ao, Y., Olsen, R.W., Yang, X.W., Mody, I., Sofroniew, M.V., Sun, Y.E., 2011. Deletion of astroglial Dicer causes non-cell-autonomous neuronal dysfunction and degeneration. J. Neurosci. 31, 8306–8319.
Taylor, M.K., Yeager, K., Morrison, S.J., 2007. Physiological Notch signaling promotes gliogenesis in the developing peripheral and central nervous systems. Development 134, 2435–2447.
Tessarz, P., Kouzarides, T., 2014. Histone core modifications regulating nucleosome structure and dynamics. Nat. Rev. Mol. Cell Biol. 15, 703–708.
Tian, W., Sawyer, A., Kocaoglu, F.B., Kyriakides, T.R., 2011. Astrocyte-derived thrombospondin-2 is critical for the repair of the blood-brain barrier. Am. J. Pathol. 179, 860–868.
Torper, O., Pfisterer, U., Wolf, D.A., Pereira, M., Lau, S., Jakobsson, J., Bjorklund, A., Grealish, S., Parmar, M., 2013. Generation of induced neurons via direct conversion in vivo. Proc. Natl. Acad. Sci. U. S. A. 110, 7038–7043.
Tsuyama, J., Bunt, J., Richards, L.J., Iwanari, H., Mochizuki, Y., Hamakubo, T., Shimazaki, T., Okano, H., 2015. MicroRNA-153 regulates the acquisition of gliogenic competence by neural stem cells. Stem Cell Rep. 5, 365–377.
Tu, Z., Li, Y., Dai, Y., Li, L., Lv, G., Chen, I., Wang, B., 2017. MiR-140/BDNF axis regulates normal human astrocyte proliferation and LPS-induced IL-6 and TNF-alpha secretion. Biomed. Pharmacother. 91, 899–905.
Tulina, N., Matunis, E., 2001. Control of stem cell self-renewal in Drosophila spermatogenesis by JAK-STAT signaling. Science 294, 2546–2549.
Turcan, S., Rohle, D., Goenka, A., Walsh, L.A., Fang, F., Yilmaz, E., Campos, C., Fabius, A.W., Lu, C., Ward, P.S., Thompson, C.B., Kaufman, A., Guryanova, O., Levine, R., Heguy, A., Viale, A., Morris, L.G., Huse, J.T., Mellinghoff, I.K., Chan, T.A., 2012. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature 483, 479–483.
Turcan, S., Makarov, V., Taranda, J., Wang, Y., Fabius, A.W.M., Wu, W., Zheng, Y., El-Amine, N., Haddock, S., Nanjangud, G., LeKaye, H.C., Brennan, C., Cross, J., Huse, J.T., Kelleher, N.L., Osten, P., Thompson, C.B., Chan, T.A., 2018. Mutant-IDH1-dependent chromatin state reprogramming, reversibility, and persistence. Nat. Genet. 50, 62–72.
Venneti, S., Garimella, M.T., Sullivan, L.M., Martinez, D., Huse, J.T., Heguy, A., Santi, M., Thompson, C.B., Judkins, A.R., 2013. Evaluation of histone 3 lysine 27 trimethylation (H3K27me3) and enhancer of Zest 2 (EZH2) in pediatric glial and glioneuronal tumors shows decreased H3K27me3 in H3F3A K27M mutant glioblastomas. Brain Pathol. 23, 558–564.
Vojta, A., Dobrinic, P., Tadic, V., Bockor, L., Korac, P., Julg, B., Klasic, M., Zoldos, V., 2016. Repurposing the CRISPR-Cas9 system for targeted DNA methylation. Nucleic Acids Res. 44, 5615–5628.
Voskuhl, R.R., Peterson, R.S., Song, B., Ao, Y., Morales, L.B., Tiwari-Woodruff, S., Sofroniew, M.V., 2009. Reactive astrocytes form scar-like perivascular barriers to leukocytes during adaptive immune inflammation of the CNS. J. Neurosci. 29,

11511–11522.
Wang, T., Pan, Q., Lin, L., Szulwach, K.E., Song, C.X., He, C., Wu, H., Warren, S.T., Jin, P., Duan, R., Li, X., 2012. Genome-wide DNA hydroxymethylation changes are associated with neurodevelopmental genes in the developing human cerebellum. Hum. Mol. Genet. 21, 5500–5510.
Wang, C.Y., Yang, S.H., Tzeng, S.F., 2015. MicroRNA-145 as one negative regulator of astrogliosis. Glia 63, 194–205.
Weller, M., Stupp, R., Reifenberger, G., Brandes, A.A., van den Bent, M.J., Wick, W., Hegi, M.E., 2010. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? Nat. Rev. Neurol. 6, 39–51.
Wilhelmsson, U., Bushong, E.A., Price, D.L., Smarr, B.L., Phung, V., Terada, M., Ellisman, M.H., Pekny, M., 2006. Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury. Proc. Natl. Acad. Sci. U. S. A. 103, 17513–17518.
Williams, M.J., Singleton, W.G., Lowis, S.P., Malik, K., Kurian, K.M., 2017. Therapeutic targeting of histone modifications in adult and pediatric high-grade glioma. Front. Oncol. 7, 45.
Wu, H., Coskun, V., Tao, J., Xie, W., Ge, W., Yoshikawa, K., Li, E., Zhang, Y., Sun, Y.E., 2010. Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes. Science 329, 444–448.
Xiao, Q., Du, Y., Wu, W., Yip, H.K., 2010. Bone morphogenetic proteins mediate cellular response and, together with Noggin, regulate astrocyte differentiation after spinal cord injury. Exp. Neurol. 221, 353–366.
Xu, N., Papagiannakopoulos, T., Pan, G., Thomson, J.A., Kosik, K.S., 2009. MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. Cell 137, 647–658.
Xu, W., Yang, H., Liu, Y., Yang, Y., Wang, P., Kim, S.H., Ito, S., Yang, C., Wang, P., Xiao, M.T., Liu, L.X., Jiang, W.Q., Liu, J., Zhang, J.Y., Wang, B., Frye, S., Zhang, Y., Xu, Y.H., Lei, Q.Y., Guan, K.L., Zhao, S.M., Xiong, Y., 2011. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Cancer Cell 19, 17–30.
Xu, X., Tao, Y., Gao, X., Zhang, L., Li, X., Zou, W., Ruan, K., Wang, F., Xu, G.L., Hu, R., 2016. A CRISPR-based approach for targeted DNA demethylation. Cell Discov. 2, 16009.
Yan, H., Parsons, D.W., Jin, G., McLendon, R., Rasheed, B.A., Yuan, W., Kos, I., Batinic-Haberle, I., Jones, S., Riggins, G.J., Friedman, H., Friedman, A., Reardon, D., Herndon, J., Kinzler, K.W., Velculescu, V.E., Vogelstein, B., Bigner, D.D., 2009. IDH1 and IDH2 mutations in gliomas. N. Engl. J. Med. 360, 765–773.
Yang, H., Feng, G.D., Olivera, C., Jiao, X.Y., Vitale, A., Gong, J., You, S.W., 2012. Sonic hedgehog released from scratch-injured astrocytes is a key signal necessary but not sufficient for the astrocyte de-differentiation. Stem Cell Res. 9, 156–166.
Yang, C.H., Yue, J., Pfeffer, S.R., Fan, M., Paulus, E., Hosni-Ahmed, A., Sims, M., Qayyum, S., Davidoff, A.M., Handorf, C.R., Pfeffer, L.M., 2014. MicroRNA-21 promotes glioblastoma tumorigenesis by down-regulating insulin-like growth factor-binding protein-3 (IGFBP3). J. Biol. Chem. 289, 25079–25087.
Yin, D., Xie, D., Hofmann, W.K., Miller, C.W., Black, K.L., Koeffler, H.P., 2002. Methylation, expression, and mutation analysis of the cell cycle control genes in human brain tumors. Oncogene 21, 8372–8378.
Yu, J., Zhang, H., Gu, J., Lin, S., Li, J., Lu, W., Wang, Y., Zhu, J., 2004. Methylation profiles of thirty four promoter-CpG islands and concordant methylation behaviours of sixteen genes that may contribute to carcinogenesis of astrocytoma. BMC Cancer 4, 65.
Zador, Z., Stiver, S., Wang, V., Manley, G.T., 2009. Role of aquaporin-4 in cerebral edema and stroke. Handb. Exp. Pharmacol. 159–170.
Zamanian, J.L., Xu, L., Foo, L.C., Nouri, N., Zhou, L., Giffard, R.G., Barres, B.A., 2012. Genomic analysis of reactive astrogliosis. J. Neurosci. 32, 6391–6410.
Zhang, Y., Barres, B.A., 2010. Astrocyte heterogeneity: an underappreciated topic in neurobiology. Curr. Opin. Neurobiol. 20, 588–594.
Zhang, K., Wang, X.Q., Zhou, B., Zhang, L., 2013. The prognostic value of MGMT promoter methylation in Glioblastoma multiforme: a meta-analysis. Fam. Cancer 12, 449–458.
Zhang, L., He, X., Liu, L., Jiang, M., Zhao, C., Wang, H., He, D., Zheng, T., Zhou, X., Hassan, A., Ma, Z., Xin, M., Sun, Z., Lazar, M.A., Goldman, S.A., Olson, E.N., Lu, Q.R., 2016. Hdac3 interaction with p300 histone acetyltransferase regulates the oligodendrocyte and astrocyte lineage fate switch. Dev. Cell 36, 316–330.