● PERSPECTIVE

Astrogenesis *versus* astrogliosis

In the mammalian cortex, certain astrocyte subtypes can serve as local adult progenitors. This process of local astrogenesis has been linked to astrogliosis, a nebulous term to describe the astrocytic response to injury and disease. The current evidence indicates that astrogliosis occurs in a context-specific manner along a graded continuum in response to injury: from subtle perturbations to severe insult to the parenchyma (Sofroniew, 2015). Since astrocytes can proliferate locally during astrogliosis in the cortex, this has important implications on the effect of nervous system communication due to astrocytic communicative contacts with other cell types and the vasculature. Also, while a subset of astrocytes undergoing astrogliotic processes have been shown to proliferate, some evidence demonstrates localized astrogenesis can occur in the cortex independent of a response to injury and disease.

During development, fate-mapping studies have shown that local astrocyte expansion in the cortex occurs 3 weeks postnatally in the rodent brain (~6–8 fold). After cell migration from the germinal layers *via* radial glia precursors perinatally, astrocytes differentiate in the cortex with substantial heterogeneity, and divide symmetrically, becoming integrated within their communicative microenvironment and establishing astrocyte tiling domains, with all cells surviving up to 20 days postnatally in mice (Ge et al., 2012). It is then thought that mature cortical astrocytes remain quiescent until undergoing astrogliosis in response to injury and disease states in the mature brain. Studies on clonal lineage of astrocytes formed in the cortex during development has demonstrated that certain subtypes retain proliferative ability into adulthood in response to injury, and that this is more robust closer to the injury site. Additionally, clones exhibited a varied response to injury, with some becoming hypertrophic, while other members of the same clonal lineage did not, which also did not necessarily correlate with distance from the injury site (Martin-Lopez et al., 2013).

In the adult, the proliferative ability of astrocytes undergoing astrogliosis was initially based on immunohistochemical biomarker studies of astrocyte intermediate filament glial fibrillary acidic protein (GFAP), which is upregulated in injury and disease as part of the graded continuum of astrogliosis, with more GFAP expression correlating with the severity of injury or disease and scar formation. However, since most astrocytes in healthy cortical tissue do not label for GFAP, an upregulation of GFAP in response to injury had traditionally been confused with cell proliferation (Mohn and Koob, 2015). Therefore, it is also possible that local cortical astrocyte proliferation does not contribute to the scar. In order to gain a better understanding of local astrogenesis and astrogliosis, fate-mapping studies were conducted in the brain of mice after stab would injury. Most astrocytes upregulated GFAP proximal to the injury, with some cells exhibiting hypertrophic increase in size to the soma without significant change to the bushy morphology of most cells (Bardehle et al., 2013). With two different severities of stab wound to the cortex, a comparable increased upregulation of GFAP was seen in correlation with injury size. 45% of astrocytes also became polarized 3–5 days post severe injury, extending a process at least 3 times longer than the average radius of the astrocyte domain. Interestingly, no astrocytes migrated towards the injury site even in severe injury. Likewise, only 14% of astrocytes in the stab wound region proliferated, with a portion becoming polarized before division, and 71% of proliferating astrocytes had somata juxtaposed with blood vessels. 45% of astrocytes juxtaposed to blood vessels proliferated overall, but none of them migrated towards the injury site. Therefore, a subset of astrocytes undergoing astrogenesis first upregulated GFAP, then divided symmetrically ~5–7 days post injury, producing two daughter cells in similar fashion to local postnatal astrogenesis during establishment of the cortical environment. Astrocytes only divided once and remained in this state up to 30 days post injury (Bardehle et al., 2013). Studies on ischemia in mice confirmed the heterogeneous nature of astrogenesis in response to injury, observing an ischemia-induced subset of astrocytes proliferating locally. When the proliferative astrocyte cell type was isolated in tissue culture, they influenced quiescent astrocytes to proliferate as well (Villarreal et al., 2016).

However, proliferating astrocytes do not appear to migrate to form the glial scar, and evidence indicates the origin of the scar in the cortex derives from precursors undergoing astrogenesis from the subventricular zone (Benner et al., 2013). Astrocyte cell types in the cortex normally express low amounts of thrombospondin 4, whereas astrocytes derived from precursor cells in the SVZ are thrombospondin 4 high producing cells. Fate-mapping studies demonstrated that the thrombospondin 4 high producing cells from the SVZ differentiate into astrocytes which migrate to the injury site (Benner et al., 2013). Also, clonal studies also reveal that astrocytes from the SVZ migrate to contribute to injury site, a process that does not appear to occur in uninjured conditions (Martin-Lopez et al., 2013). Additionally, NG2 cells proliferate and migrate before astrocyte proliferation and form part of the glial scar, further indicating that local astrogenesis from mature cortical astrocytes was not the major constitute of the scar (Dimou and Götz, 2014). Therefore, as related to astrogliosis, subsequent astrogenesis likely occurs in response to injury and disease along a graded continuum in the cortex in a subset of cells, and does not seem to be the substantive contributor to scar formation up to a month after injury.

In the normal healthy cortex, astrogenesis has been observed as well. In the prefrontal cortex of rats performing learned operant conditioning tasks, 5-bromodeoxyuridine (BrdU) was used as a marker for cell division, and BrdU/GFAP$^+$ co-labeling was observed to increase significantly, indicating that local astrogenesis occurs in response to learning tasks in this region of the brain. Furthermore, if learning was not retained in rat populations, the new cells were also not retained. If rats did retain memory of the learned task new astrocytes remained in their niche (Rapanelli et al., 2011).

Additionally, it appears that astrogenesis occurs locally in the uninjured adult human cortex. In a study of the uninjured human brain, analysis of the cortex of individuals administered with BrdU that had been diagnosed with squamous cell carcinoma in non-nervous tissues, and who lived between 4.2 months–4.3 years after injection, demonstrated that 515 new nonneuronal cells were born in 200 mm^3 of tissue, with an average of 37% of those cells born in the last five years, and 54% of dividing cells in the grey matter of the cortex. No cells colabeled with neuronal marker NeuN. Furthermore, a subset of the cells colabeled with BrdU/GFAP$^+$, indicating in non-injured and non-diseased cortical tissue, a subset of astrocytes are capable of proliferation into adulthood. Additionally, use of flow cytometry to sort neuronal
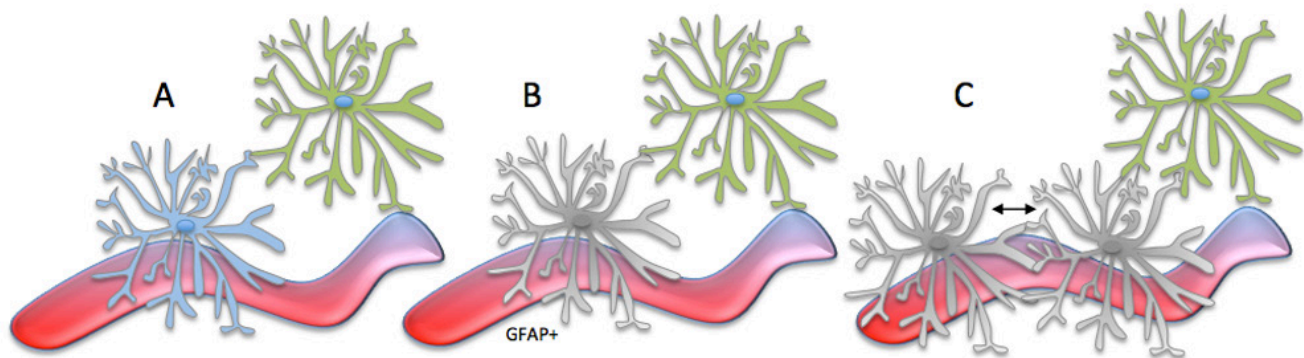
Figure 1 Adult cortical astrogenesis in response to changes in the microenvironment.
(A) Mature quiescent astrocytes in the cortex with soma juxtaposed to the vasculature (blue) or not intimately associated with a blood vessel (green). An astrocyte juxtaposed to a blood vessel upregulates glial fibrillary acidic protein (GFAP) in response to changes to the microenvironment (B; grey). (C) A subtype of astrocytes upregulating GFAP in response to injured states and with soma juxtaposed to the vasculature have been shown to divide symmetrically (grey), with daughter cells still active at 30 days post injury (Bardehle et al., 2013). It is unknown whether this can occur to other astrocytes subtypes with soma not intimately associated with the vasculature *in situ* (green). Additionally, it is unclear whether upregulation of intermediate filament is necessary before division in B.

and non-neuronal cells, and extrapolating when cell division occurred based on incorporation of C14 levels which were double at the time of above ground nuclear testing, they discovered that neuronal cells in the cortex were born around the time of birth of the individual, whereas non-neuronal cells were born later in the lifespan of the organisms, indicating there is some proliferation and renewal among these cell types (Bhardwaj et al., 2006).

A subset of mature cortical astrocytes can divide symmetrically through adulthood after astrogliosis in response to injury and disease, but this process also appears to occur in the absence of injury or disease (Mohn and Koob, 2015). Therefore, astrogliosis as defined is not a necessary predeterminer for local cortical astrogenesis. In operant learning conditions, as well as in uninjured human cortical tissue, it should be noted that the astrocyte marker that was used to co-label cells undergoing astrogenesis with BrdU was GFAP. However, it is not certain whether upregulation of intermediate filaments GFAP and vimentin is a necessary process before symmetrical cell division, or whether soma juxtaposition to the vasculature as seen in proliferative astrocytes in injured states is required (**Figure 1**).

Since astrocytes can proliferate through normal processes in the brain, the perturbations that stimulate cell division of astrocytes in a brain that is not injured or diseased could be similar to the mechanisms which cause proliferation in injured and diseased states. Astrogenesis could occur if a single neuron is languishing in its microenvironment, or in response to slight changes to the vasculature in normal states that are enhanced in injury and disease. However, it is possible astrogenesis may also occur in conditions not traditionally associated with astrogliosis, such as during regulation of neurocommuncation in learning, maintenance of ionic homeostasis, or for constitutive cell replacement. Therefore it is uncertain whether the term astrogliosis should encompass the process of symmetrical division of astrocytes in healthy cortex even if traditional biomarkers for astrogliosis are present. As more is learned about the mechanisms by which astrogenesis can occur in the healthy adult brain, more information will be elucidated on the nature of nervous system communication, astrocyte domain organization, and neuronal protection.

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References

Bardehle S, Kruger M, Buggenthin F, Schwausch J, Ninkovic J, Clevers H, Snippert HJ, Theis FJ, 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.

Benner EJ, Luciano D, Jo R, Abdi K, Paez-Gonzalez P, Sheng HX, Warner DS, Liu CL, Eroglu C, Kuo CT (2013) Protective astrogenesis from the SVZ niche after injury is controlled by Notch modulator Thbs4. Nature 497:369-473.

Bhardwaj RD, Curtis MA, Spalding KL, Buchholz BA, Fink D, Bjork-Eriksson T, Nordborg C, Gage FH, Druid H, Eriksson PS, Frisen J (2006) Neocortical neurogenesis in humans is restricted to development. Proc Natl Acad Sci U S A 103:12564-12568.

Dimou L, Gotz M (2014) Glial cells as progenitors and stem cells: new roles in the healthy and diseased brain. Physiol Rev 94:709-737.

Ge WP, Miyawaki A, Gage FH, Jan YN, Jan LY (2012) Local generation of glia is a major astrocyte source in postnatal cortex. Nature 484:376-380.

Martin-Lopez E, Garcia-Marques J, Nunez-Llaves R, Lopez-Mascaraque L (2013) Clonal astrocytic response to cortical injury. PLoS One 8:e74039.

Mohn TC, Koob AO (2015) Adult astrogenesis and the etiology of cortical neurodegeneration. J Exp Neurosci 9:25-34.

Rapanelli M, Frick LR, Zanutto BS (2011) Learning an operant conditioning task differentially induces gliogenesis in the medial prefrontal cortex and neurogenesis in the hippocampus. PLoS One 6:e14713.

Sofroniew MV (2015) Astrogliosis. In: Glia, 1st ed. (Barres Freeman MR, Stevens BBA, eds), pp107-122. New York, NY: Cold Spring Harbor Laboratory Press.

Villarreal A, Rosciszewski G, Murta V, Cadena V, Usach V, Dodes-Traian MM, Setton-Avruj P, Barbeito LH, Ramos AJ (2016) Isolation and characterization of ischemia-derived astrocytes (IDAs) with ability to transactivate quiescent astrocytes. Front Cell Neurosci 10:139.