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PERSPECTIVE

Astroglial heterogeneity: merely a neurobiological question? Or an opportunity for neuroprotection and regeneration after brain injury?

Pioneer studies by Ramon y Cajal in the early nineteenth century evidenced that astrocytes are a heterogeneous cell population. The initial division of the glial family proposed by Rudolf Albert von Kölliker and William Lloyd Andriezen that separated glia into two groups, fibrous glia and protoplasmic glia, was further refined by Ramon y Cajal, who adopted the term *astrocyte* for both populations. The term astrocyte was originally coined by Michael von Lenhossek in 1893 to describe the many star-shaped cells observed in histological brain specimens (for an historical perspective see Kettenmann and Ransom, 2012). Cajal's work showed that processes of fibrous astrocytes are fewer and longer and branch less frequently, and at a more acute angle, than those of protoplasmic astrocytes. While protoplasmic astrocytes are those localized in the gray matter, fibrillar astrocytes are those restricted to the white matter. Early Cajal's studies also noticed that some astrocytes retain their ability to divide; he called them *twin astrocytes* (see excellent revisions of Cajal's work in neuroglia by Navarrete and Araque, 2014; Garcia-Marin and Garcia-Lopez, 2007; and for historical perspective Kettenmann and Ransom, 2012).

From the anatomical perspective, gray matter and white matter astrocytes differ, not only in morphology, but also in their role in central nervous system (CNS) physiology. While gray matter astrocytes participate in the neurovascular unit in close relationship with blood vessels, neuronal somata and synaptic cleft (del Zoppo, 2009), white matter astrocytes are related to axons and blood vessels. For almost a century astrocytes were disregarded when studying the CNS neuronal complexity. It was not until this last decade that the glia field, and specifically the study of astroglial heterogeneity, has been further explored using state of the art tools to identify astroglial subtypes. We now recognize that there are significant differences not only in morphology, but also in the neurochemical and physiological features among astrocytes, that define a yet unknown number of astroglial subfamilies. The aim of this short article is to share some facts and think beyond the neurobiological problem of studying the astroglial heterogeneity, which has been thoroughly revised in several full length recent reviews (Zhang and Barres, 2010; Götz et al., 2015; Bribian et al., 2016; Scheller and Kirchhoff, 2016), to discuss the opportunities that astroglial heterogeneity may offer to translational investigation in neuroprotection and neuroregeneration. While interesting differences among white matter astrocytes and gray matter astrocytes have been described (see Kettenmann and Ransom, 2012), this short article will refer mainly to the gray matter astrocytes heterogeneity and their potential role in translational medicine.

In the early days of neurogenesis, the concept of astroglial heterogeneity emerged back from the past and gained a lot of attention. It was then shown that specific astroglial populations essentially behave as stem cells in specific regions of the adult CNS. These astroglial GFAP-expressing cells actively divide and have the potential to give rise to different adult CNS cell populations (Doetsch et al., 1999). Following these seminal works, a large number of reports have shown that neurogenic niches in the subventricular zone (SVZ) and dentate gyrus (DG) retain astroglial cells with stem cell potential; however they are essentially indistinguishable from typical astrocytes in brain sections and also in electrophysiological recordings (Zhang and Barres, 2010). The question that remains open still today is whether this type of stem astrocytes that share the same morphology, undistinguishable immunohistochemical pattern and immunolabeling, could be intermingled in the rest of CNS parenchyma. Unfortunately, since these stem astrocytes are not located in a specific anatomical region, it is likely that they have not been individualized yet. A long standing hypothesis in the field is that, beneath a common immunohistochemical and morphological pattern, the intrinsically heterogeneous astroglial population might be masking astrocytic phenotypes with different potential and physiological roles (Zhang and Barres, 2010; Götz et al., 2015).

Subsequent studies based on the transcriptional profile of astroglial cells have shown extensive differences in the gene expression of astrocytes found in different brain regions (Doyle et al., 2008). Microarray studies also showed diverse patterns of gene expression in cultured astrocytes from different anatomical origins (Yeh et al., 2009). In addition to these reports, numerous studies have shown and identified a large number of genes that are differentially expressed by subsets of astrocytes *in vivo* and *in vitro* (reviewed in Zhang and Barres, 2010). Considering that many of these differentially expressed genes are related to surface receptors and channels sensitive to neurotransmitters, it is conceivable that astrocytes from different brain regions have the ability of interacting in a wide variety of ways with neurons.

But astroglial heterogeinity is not just a matter of anatomical localization. Modern cell fate tracking techniques, such as dye-filling, fluorescent protein labeling either by specific transgenic mice or viral-delivered genes encoding the markers, as well as specific labeling techniques based on modifications of the *brainbow* approach have allowed to differentiate astroglial cell populations even in the same brain region (revised in Bribian et al., 2016). These techniques have shown that astroglial heterogeneity is determined early in the CNS development and that astrocytes have clonal identity. However, astrocytes coming from the same clones do not necessarily end up in the same brain subregions and having the same functions or physiological roles. Bribian and colleagues (2016) observed that clones of protoplasmic astrocytes form domains of spatially restricted cells showing diverse arrangements throughout the cortical layers: some clones are located throughout several cortical layers while others occupy restricted layers. The dispersion of astrocytes suggests that the heterogeneity is not only related to their clonal origin but also influenced by local environment and

their function (Martin-López et al., 2013) .

Although not formally considered as astrocytes, NG2 glial cells or polydendrocytes are other intriguing members of the glial cell family in the adult brain. During embryonic development, NG2 glia from gray matter can give rise to astrocytes and oligodendrocytes while NG2 glia from white matter only generates oligodendrocytes (Zhu et al., 2011; Kettenmann and Ransom, 2012). In the normal adult brain NG2 cells are distributed through the CNS and they are supposed to give rise to oligodendrocytes as shown by lineage tracing through *in vivo* imaging (Hughes et al., 2013). Thus, they still remained classified as oligodendrocyte precursor cells (OPC). NG2 glial cells actively divide in the adult CNS and they undergo increased proliferation after CNS injury. After several years of controversy as to whether NG2 cells can derive into astrocytes after CNS injury, recent evidence has shown that NG2 cells *in vivo* can give rise to a lineage of reactive astrocytes by a mechanism controlled by the Sonic hedgehog (Shh) signaling pathway (Honsa et al., 2016). Whether these NG2-derived reactive astrocytes represent a specific subfamily in astroglial population is still unknown.

The evidence of astroglial heterogeneity is overwhelming, even when considering the same anatomical region. Furthermore, brain injury certainly exposes another, maybe even more complex, layer of astroglial heterogeneity. Animal models of traumatic or ischemic brain injury and transgenic animals showing features of human neurodegenerative pathologies such as Alzheimer's disease have been repeatedly used for studying CNS pathological response. At the same time these models clearly exposed and highlighted the astroglial heterogeneity. Ben Barres laboratory proposed, in an elegant transcriptome study of reactive astrocytes obtained from animals subjected to brain ischemia by middle cerebral artery occlusion (MCAO) or from animals exposed to bacterial lipopolysaccharide (LPS), that these cells polarize into different profiles depending on the stimulus that induces reactive gliosis (Zamanian et al., 2012). In this way, LPS induces a pro-inflammatory pro-neurodegenerative profile while MCAO experimental model of ischemia induces the expression of anti-inflammatory-neuroprotective genes (Zamanian et al., 2012). An interesting question that these results raise is whether these polarized, extreme phenotypes, are part of the same process of reactive gliosis on *naïve* astrocytes, or if they are the result of the selective expansion of specific astroglial clones already present in the adult brain? Some evidence support the idea of a clonal expansion induced by CNS damage. For example, Wanner and colleagues (2013) have shown that glial scar borders are formed by newly proliferated astrocytes with elongated processes that surround the ischemic core. In addition, atypical astrocytes named aberrant astrocytes (AbA) have been purified from primary spinal cord cultures of symptomatic transgenic rats expressing the $SOD1^{G93A}$ mutation that leads to ALS-like pathology in rodents (Diaz-Amarilla et al., 2011). These AbA cells have a marked proliferative capacity, lack of replicative senescence and secrete soluble factors that induce motor neuron death (Diaz-Amarilla et al., 2011). We have also recently reported the *ex vivo* isolation and amplification of IDA (ischemia-derived astrocytes) from ischemic tissue containing ischemic core and penumbral regions (Villarreal et al., 2016). IDA cultures can be started from very few dissociated cells obtained from the ischemic region or directly from ischemic tissue explants, thus supporting the idea that initially, only very few cells have the IDA phenotype, that later become expanded *in vitro*. The most striking characteristics of the IDA astroglial cell type include the facilitation of neuronal death of oxygen-glucose deprived neurons and the IDA ability to induce reactive gliosis on quiescent astrocytes. Furthermore, transplantation of *in vitro* amplified IDA into normal non-ischemic brains led to focal reactive gliosis that propagated into the vicinity of the injection site, thus showing the IDA potential to induce reactive gliosis *in vivo* (Villarreal et al., 2016). Going beyond these findings, we wonder if these atypical astrocytes (AbA, IDA or even the scar-forming astrocytes) are specific types of hidden astrocytes already present in the normal brain that become expanded or activated by the environmental clues generated by the injury? Again, this is a very interesting question in terms of the basic neurobiology of glial cells, but may be the most important question in translational medicine is whether we are able to prevent the expansion of these pro-neurodegenerative or scar-forming astrocytes.

Nanotechnology has provided a large number of nanocompounds that can be used as carriers for the cell-specific delivery of therapeutic drugs. These compounds include several different chemical families, but the dendrimer-based platforms emerged as promising carriers for different types of drugs due to their capacity to carry different loads, the possibility of chemically modifying their structure and the feasibility of chemically engineering the structure of the carrier (see revision in Kannan et al., 2014). Specifically, polyamidoamine dendrimers hydroxyl-modified generation 4 (G4-OH) have been successfully used to deliver N-acetyl cysteine to astrocytes and microglia (Kannan et al., 2012). The systemic treatment with the loaded dendrimer improved recovery and reduced neuroinflammation in different models of CNS injury, including maternal inflammation-induced cerebral palsy, neonatal ischemic stroke and circulatory arrest (Nance et al., 2016). Indubitably, the engineering of dendrimer-based carriers to specifically deliver active compounds to astroglial clones polarized to the proinflammatory-neurodegenerative phenotype is a concrete possibility. Several laboratories, including ours, are working on these strategies and we envision, in the near future, an explosive growth of this incipient field that will take advantage of basic findings on astroglial heterogeneity to reduce neuroinflammation and secondary neuronal death.

A number of reports have shown that undifferentiated and/or multipotent local astroglial cell precursors emerge or are expanded in CNS lesions; however until now their amplification has required extensive genetic or chemical manipulation. For example, several groups have reported the formation of self-renewing multipotential neurospheres from injured rodent brains; however there is still an intense debate on the astroglial or NG2 nature of these neurosphere-forming cells (reviewed in Götz et al., 2015). While NG2 cells are the unique cell type showing cell division capability in the adult CNS, genetic fate mapping experiments have shown

that, after cortical stab injury, a limited subset of reactive astrocytes seem to resume clonal cell division, but evidencing an astroglial lineage restriction (Bardehle et al., 2013). However, this reactive astrocyte subset is likely considered as the neurospheres-forming cells when relieved of the *in vivo* gliogenic non-neurogenic environment by *in vitro* culture (Götz et al., 2015). The identification of the non-permissive environmental clues that restrict neurogenic expansion would lead to new opportunities for neurorepair in the injured CNS. Taking advantage of the neurosphere-forming astroglial subfamily and facilitating its expansion is also another interesting possibility to design potential neuroreparative strategies.

In summary, astroglial heterogeneity has been passively observed by neuroscientists during the last century, but it was not until the last decade that it was seriously accepted that there are a –yet undefined- number of astroglial subfamilies beyond the classical protoplasmic and fibrous phenotypes, even in the same anatomical CNS regions. We are currently facing a new challenge that is to define whether these different subfamilies come from different precursors, or if they are determined by environmental clues that lead to the preferential clonal expansion of specific subfamilies. This astroglial heterogeneity has raised an interesting problem in basic neurobiology but, at the same time, is opening a whole new era in the development of therapeutic options. Taking advantage of new nanocompounds and other specific carriers that would target specific beneficial or detrimental astroglial cell subpopulations, could set the basis for new treatment strategies in neuroprotection and neuroregeneration.

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References

Bardehle S, Krüger M, Buggenthin F, Schwausch J, Ninkovic J, Clevers H, Snippert HJ, Theis FJ, Meyer-Luehmann M, Bechmann I, Dimou L, Götz M (2013) Live imaging of astrocyte responses to acute injury reveals selective juxtavascular proliferation. Nat Neurosci 16:580-586.

Bribián A, Figueres-Oñate M, Martín-López E, López-Mascaraque L (2016) Decoding astrocyte heterogeneity: New tools for clonal analysis. Neuroscience 323:10-19.

del Zoppo GJ (2009) Inflammation and the neurovascular unit in the setting of focal cerebral ischemia. Neuroscience 158:972-982.

Díaz-Amarilla P, Olivera-Bravo S, Trias E, Cragnolini A, Martínez-Palma L,Cassina P, Beckman J, Barbeito L (2011) Phenotypically aberrant astrocytes that promote motoneuron damage in a model of inherited amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 108:18126-18131.

Doetsch F, Caillé I, Lim DA, García-Verdugo JM, Alvarez-Buylla A (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97:703-716.

Doyle JP, Dougherty JD, Heiman M, Schmidt EF, Stevens TR, Ma G, Bupp S, Shrestha P, Shah RD, Doughty ML, Gong S, Greengard P, Heintz N (2008) Application of a translational profiling approach for the comparative analysis of CNS cell types. Cell 135:749-762.

García-Marín V, García-López P, Freire M (2007) Cajal's contributions to glia research. Trends Neurosci 30:479-487.

Götz 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.

Honsa P, Valny M, Kriska J, Matuskova H, Harantova L, Kirdajova D, Valihrach L, Androvic P, Kubista M, Anderova M (2016) Generation of reactive astrocytes from NG2 cells is regulated by sonic hedgehog. Glia 64:1518-1531.

Hughes EG, Kang SH, Fukaya M, Bergles DE (2013) Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. Nat Neurosci 16:668-676.

Kannan RM, Nance E, Kannan S, Tomalia DA (2014) Emerging concepts in dendrimer-based nanomedicine: from design principles to clinical applications. J Intern Med 276:579-617.

Kannan S, Dai H, Navath RS, Balakrishnan B, Jyoti A, Janisse J, Romero R, Kannan RM (2012) Dendrimer-based postnatal therapy for neuroinflammation and cerebral palsy in a rabbit model. Sci Transl Med 4:130ra46.

Kettenmann and Ransom, 2012. Neuroglia. Oxford University Press. 3rd Ed. Edited by Kettenmann and Ransom, Oxford University Press, 2012

Martín-López E, García-Marques J, Núñez-Llaves R, López-Mascaraque L (2013) Clonal astrocytic response to cortical injury. PLoS One 8:e74039.

Nance E, Zhang F, Mishra MK, Zhang Z, Kambhampati SP, Kannan RM, Kannan S (2016) Nanoscale effects in dendrimer-mediated targeting of neuroinflammation. Biomaterials 101:96-107.

Navarrete M, Araque A (2014) The Cajal school and the physiological role of astrocytes: a way of thinking. Front Neuroanat 8:33.

Scheller A, Kirchhoff F (2016) Endocannabinoids and heterogeneity of glial cells in brain function. Front Integr Neurosci 10:24.

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

Wanner IB, Anderson MA, Song B, Levine J, Fernandez A, Gray-Thompson Z, Ao Y, Sofroniew MV (2013) Glial scar borders are formed by newly proliferated, elongated astrocytes that interact to corral inflammatory and fibrotic cells via STAT3-dependent mechanisms after spinal cord injury. J Neurosci 33:12870-12886.

Yeh TH, Lee DY, Gianino SM, Gutmann DH (2009) Microarray analyses reveal regional astrocyte heterogeneity with implications for neurofibromatosis type 1 (NF1)-regulated glial proliferation. Glia 57:1239-1249

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic analysis of reactive astrogliosis. J Neurosci 32:6391-6410.

Zhang Y, Barres BA (2010) Astrocyte heterogeneity: an underappreciated topic in neurobiology. Curr Opin Neurobiol 20:588-594.

Zhu X, Hill RA, Dietrich D, Komitova M, Suzuki R, Nishiyama A (2011) Age-dependent fate and lineage restriction of single NG2 cells. Development 138:745-753.