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REVIEW

DECODING ASTROCYTE HETEROGENEITY: NEW TOOLS FOR CLONAL ANALYSIS

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Abstract—The importance of astrocyte heterogeneity came out as a hot topic in neurosciences especially over the last decades, when the development of new methodologies allowed demonstrating the existence of big differences in morphological, neurochemical and physiological features between astrocytes. However, although the knowledge about the biology of astrocytes is increasing rapidly, an important characteristic that remained unexplored, until the last years, has been the relationship between astrocyte lineages and cell heterogeneity. To fill this gap, a new method called *StarTrack* was recently developed, a powerful genetic tool that allows tracking astrocyte lineages forming cell clones. Using *StarTrack*, a single astrocyte progenitor and its progeny can be specifically labeled from its generation, during embryonic development, to its final fate in the adult brain. Because of this specific labeling, astrocyte clones, exhibiting heterogeneous morphologies and features, can be easily analyzed in relation to their ontogenetic origin. This review summarizes how astrocyte heterogeneity can be decoded studying the embryonic development of astrocyte lineages and their clonal relationship. Finally, we discuss about some of the challenges and opportunities emerging in this exciting area of investigation.

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Key words: glial cells, clonal, progenitor, lineage, NG2-glia, cortical lesion.

Contents

INTRODUCTION

In recent years, the development of new and innovative genetic techniques opened up the possibility of studying the heterogeneity of brain cell populations in relation to their ontogenetic origin. In particular, *in vivo* single-cell analysis offers a direct correlation between the embryonic origin of brain cells and their adult fate and heterogeneity (García-Marqués and López-Mascaraque, 2013; García-Moreno et al., 2014; Loulier et al., 2014; Siddiqi et al., 2014; Figueres-Oñate et al., 2015).

Among all cells forming the Central Nervous System (CNS), astrocytes are one of the most abundant and heterogeneous cell types in the brain and spinal cord, representing nearly 20% of the total number of cells in humans (Pelvig et al., 2008). Classically astrocytes were classified into two general categories: protoplasmic and fibrous; protoplasmic astrocytes occupy the gray matter whereas fibrous astrocytes reside in the white matter (Fig. 1A, B; Kölliker, 1889; Andriezen, 1893; Lenhossek, 1893; Cajal, 1913; Rio-Hortega, 1928). Cajal already used this general distinction of astrocytes at the beginning of the last century (1913), describing the presence of morphologically distinct types of astrocytes in the human hippocampus (Fig. 1C). Nowadays, these differences are easily highlighted using genetic tools that allow visualizing the totality of cell bodies such as dye-filling (Bushong et al., 2002; Ogata and Kosaka, 2002; Wilhelmsson et al., 2004; Pekny and Nilsson, 2005), fluorescent protein labeling either by specific transgenic mice and viral injections (Buffo et al., 2008), or by the most recently developed, DNA fluorescent reporter vectors like the *StarTrack* method (García-Marqués and López-Mascaraque, 2013). Unlike the dye-filling or the viral or transgenic mediated fluorescent labeling of astrocytes, the *StarTrack* technique specifically labels astrocyte progeny distinguishing unequivocally each individual cell clone at the end of the

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Abbreviations: hGFAP, human glial fibrillary acidic protein promoter; NPC, neural progenitor cell; NSCs, neural stem cells; OPCs, oligodendrocyte precursor cells; RGCs, radial glial cells; SGZ, subgranular zone.

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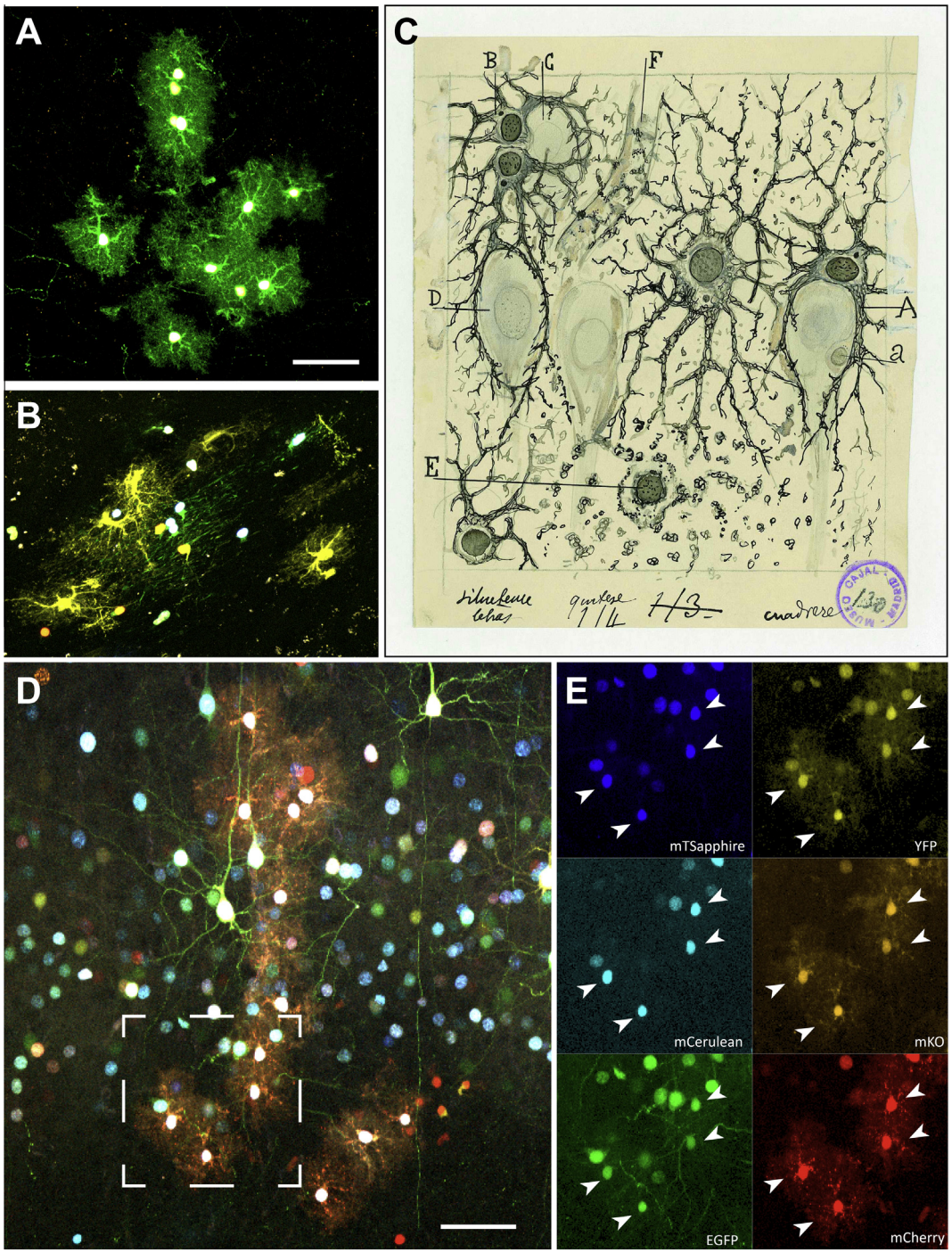

Fig. 1. Astrocyte classification. (A) Characteristic morphology of protoplasmic astrocytes after embryonic electroporation using the *StarTrack* method. (B) Typical morphologies of fibrous astrocytes within the white matter after embryonic electroporation using the *StarTrack* method. (C) Original Cajal drawing illustrating the human neuroglia in the pyramidal and radiated layers of Ammon's horn with the gold chloride method: *A* represents thick astrocyte wrapping a pyramidal cell; *B*, sibling astrocytes forming a nest around a cell (*C*), one of them extend two processes to another nest (*D*); *E*: cell ''with autolysis signs''. *F*: Astrocyte close to blood vessels. Cajal Legacy, Instituto Cajal, CSIC. (D) General view of the ubiquitous modification of the *StarTrack* method. The *UbC-StarTrack* allows the tracking of the whole neural progeny from single cells. This modification is based on the combinatorial expression of six fluorescent reporter proteins expressed both, in the cell nucleus and the cytoplasm under a ubiquitous promoter (Ubiquitin C). Decomposition of each fluorescent protein expression in the cells included in the box is represented in E. (E) Details of each confocal channel of labeled cells in D dotted box. Each clone is defined by analyzing the fluorescent expressing vectors incorporated in each cell. Separate confocal channels isolated each single emission of the six different reporter proteins. Fluorescent proteins are, organized by the spectral emission–excitation: mTsapphire, mCerulean, EGFP, Yellow fluorescent protein (YFP), monomeric Kusabira Orange (mKO) and mCherry. The *UbC-StarTrack* is driven by a ubiquitous promoter, which allows the tracing of the whole-cell progeny. Arrowheads point to the different fluorescent combination in astrocytes, although some neurons were also labeled, Fluorescent proteins mTsapphire, mCerulean, EGFP are expressed in those astrocytes just in the nuclear form while YFP, mKO and mCherry are expressed both in the nucleus and the cell cytoplasm. Scale bars: A, B 50 μm; D, E 50 μm.