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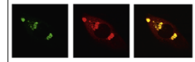

Research Report

Morphological changes of radial glial cells during mouse embryonic development

Xi Lu, Minghui Duan, Lingzhen Song, Wei Zhang, Xinde Hu, Shanting Zhao, Shulin Chen*

College of Veterinary Medicine, Northwest A&F University, No. 22 Xinong Road, Yangling, Shaanxi 712100, P.R. China

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ABSTRACT

During brain development, the radial glial cell acts as a scaffold to support radial migration of postmitotic neurons. However, the morphological changes of radial glial cells during embryo development are poorly understood. We used *in utero* electroporation and immunohistochemistry to study the dynamics of radial glial cells accompanied by cortical development in mice from embryonic day 14 to postnatal day 0. We found that different segments of radial glial cells changed by the growth of different layers of cortex, such as marginal zone, cortical plate, intermediate zone and ventricular zone. Moreover, the length, angle and number of branches of the radial glial cell changed significantly at the late stage of neurogenesis. All these changes were consistent with the distinct phases of locomotion. Thus, we speculated that morphological changes of the radial glial cell were associated with the neuronal migration and dendritic development.

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1. Introduction

The radial glial cell (RGC) is a widely distributed non-neuronal cell type in the developing central nervous system (CNS) of all vertebrates analyzed so far. Their first identified function during the initial phase of corticogenesis is to support radial migration of postmitotic neurons (Hatten, 2002; Zhao and Frotscher, 2010). The radial pathway of migration uses the processes of RGCs as a guide (Noctor et al., 2004). RGCs, therefore, play a pivotal role in establishing the laminar structure of the cerebral cortex. Recently, radial glial cells have acquired further interest due to their central role in a variety of developmental processes including their role as neuronal precursors (Malatesta and Gotz, 2013).

RGCs are well defined by their characteristic radial and bipolar morphology and their glial properties. The soma of a RGC lies in the ventricular zone (VZ) and gives rise to a long basal process to pial surface and a very short apical process to the ventricular surface (Pinto and Gotz, 2007). The basal process extends from its soma throughout the neural wall to the pial surface. At the end, the basal process often gives rise to several branches that terminate with multiple endfeet

Abbreviations: BLBP, brain lipid-binding protein; BM, basement membrane; BSA, bovine serum albumin; CNS, central nervous system; CP, cortical plate; DAPI, 4′,6-diamidino-2-phenylindole; GFAP, glial fibrillary acidic protein; IUE, *in utero* electroporation; IZ, intermediate zone; MZ, marginal zone; PBS, phosphate-buffered saline; PFA, paraformaldehyde; RC2, radial glial cell maker 2; RGC, radial glial cell; SVZ, subventricular zone; VZ, ventricular zone

*Corresponding author.
E-mail address: cslin9719@nwsuaf.edu.cn (S. Chen).

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that form the outer cerebral surface (glia limitans), that is coated with a basement membrane (BM) (Rakic, 2003).

The radial glial scaffold is altered in mice with defects in the reelin signaling pathway (Forster et al., 2002; Frotscher et al., 2003; Weiss et al., 2003). Forster et al. (2002) first showed that a regular radial glial scaffold failed to form in the dentate gyrus of *reeler* mutants and mice lacking the adapter protein Dab1. In addition, glial fibrillary acidic protein (GFAP)-positive glial cells preferred a reelin-containing substrate, and reelin promoted branching of GFAP-positive glial fibers. Members of the integrin family have been implicated in neuronal migration in the cerebral cortex (Galileo et al., 1992; Anton et al., 1999; Dulabon et al., 2000), likely by regulating the anchorage of glial endfeet and the formation of the glial scaffold (Graus-Porta et al., 2001). Recently, we found that few radial glia cells branched in the *reeler* mutant mouse, without endfeet at the end of the branches (Chai et al., 2014). These preliminary findings suggested that the neuronal migration defects observed in these mutants are caused by malformations of the radial glial scaffold, particularly the altered branching and disappeared endfeet of RGCs.

Proliferation, migration and differentiation are pivotal steps during the development of the CNS, and RGCs play a key role in CNS morphogenesis. However, the morphological dynamics of RGCs during the CNS development is poorly understood. The present study was to investigate the morphological changes of glial development in the mouse cerebrum and the relationship between morphological changes of radial glia and neuronal migration. Our results indicated that morphology of the radial glial cell changed during the embryonic days, which was related to the neuronal migration.

2. Results

2.1. Immunostaining could not distinguish individual intact RGC

Immunostaining is a common method to visualize the localization of proteins or the morphology of the positive cell. RC2, Nestin and BLBP are the specific markers for RGCs. Immunostaining for RC2, Nestin and BLBP was conducted on mouse brain slices at E15.5. All three antibodies could label the radial processes of RGC (Fig. 1 A–C). The tip of process generates branches after entering the marginal zone (MZ) (Rakic, 2003). However, RC2 and BLBP antibodies labeled branches partially, while Nestin-positive branches were mixed up with each other (Fig. 1 D–F). In the intermediate zone (IZ), a large number of glial fibers intermingled with each other (Fig. 1 G–I). In the VZ, BLBP antibodies, rather than RC2 and Nestin, labeled the cell body of RGC (Fig. 1 J–L). Therefore, immunostaining did not distinguish the morphological appearance of individual intact RGC.

2.2. GFP plasmid with BLBP promoter was specifically expressed in the RGCs

Plasmids with pCAG-EGFP and pBLBP-EGFP were transfected into cells in the VZ at E15.5, and both plasmids expressed GFP in the RGC (Fig. 2 A, A′). After transfection with pCAG-EGFP plasmid, branches of GFP-labeled glial cells overlapped in the MZ (Fig. 2B). The pCAG-EGFP expressed GFP not only in RGCs, but also in newly generated neurons. 24 h after transfection, GFP-labeled neurons had entered the IZ, and contacted with RGCs (Fig. 2C). Meanwhile, GFP-labeled neuronal precursors in the VZ mixed together with the somata and apical processes of RGCs (Fig. 2D). The typical morphology of the RGC was observed in the section of brain transfected with pBLBP-EGFP plasmid: The soma and apical process of a radial glial cell lay in the VZ and a long radial process extended from its cell-body throughout the neural wall to the pial surface with branches at the end (Fig. 2 A′–D′).

Immunostaining with BLBP antibody was conducted on transfected slices. The ratio between BLBP-positive cells and GFP-labeled cells in the VZ was calculated, which represented the specificity of promoter in the RGC. The ratio of pBLBP-EGFP was significantly higher than pCAG-EGFP ($F_{1,16}=1.136$, $P<0.0001$) (Fig. 2E). Therefore, BLBP promoter was more specifically expressed than CAG promoter in the RGC, suggesting that pBLBP-EGFP is more suitable for the morphological observation of RGCs.

2.3. Different segments of radial glial cells changed by the development of different layers of cortex

With the development of the different layers of the cortex, the length of different segments of RGCs changed at different time points. ANOVA indicated significant differences in the length of different segments of radial glial cells and the thickness of different layers of cortex among the ages.

In the cortical plate (CP), the length of basal process increased gradually from E14 (60.1±1.0 μm) to E16.5 (113.1±5.6 μm) and subsequently increased significantly from E16.5 to P0 (468.7±33.0 μm) ($P<0.0001$). The thickness of CP also had a significant increase from E16.5 (101.9±12.6 μm) to P0 (456.5±27.9 μm) ($P<0.0001$) (Fig. 3A).

The changes of thickness of VZ and IZ and the length of glial fiber in both layers followed the similar variation tendency: increased at the beginning, reached the maximum, and then decreased. However, they reached their climax at different time points. The length of the apical processes in VZ slightly increased from E14 (62.6±5.2 μm), reached the peak at E14.5 (67.1±4.4 μm), and then decreased until P0 (44.6±2.3 μm) ($P=0.0015$). Likewise, the thickness of the VZ increased significantly from E14 (87.7±3.3 μm) to E14.5 (111.3±2.6 μm) ($P<0.0001$), and then decreased significantly at E15.5 (72.4±3.6 μm) ($P<0.0001$) and maintained around 75–85 μm subsequently. (Fig. 3B). In the IZ, after a gradual increase at mid-neurogenesis, the basal process increased significantly from E15.5 (150.0±22.4 μm) to E17.5 (252.3±6.3 μm) ($P<0.0001$). Subsequently, the fiber length decreased gradually. The thickness of the IZ reached the peak at E17.5 (215.9±8.6 μm). And then the thickness of IZ decreased dramatically from E17.5 (215.9±8.6 μm) to P0 (134.3±8.1 μm) ($P<0.0001$) (Fig. 3C). It is notable that the basal processes in the IZ were straight up to E15.5, but became bent from E16.5 (Fig. 3 D–E′). That could explain why the length of basal process decreased slower than the thickness of IZ.

Based on the above results, the length of apical process in VZ reached the peak at E14.5, and the basal process in IZ increased from E15.5 to E17.5. Whereafter, the length of basal process in CP started a significant increase until birth. Thus, the changes of glial fiber length and the thickness of cortex

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