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Origin of the Neocortical Subependymal Cells Speculated by Emx1 and GAD67 Expression

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Introduction

More than a decade has passed since adult neurogenesis in the murine telencephalon was described and accepted widely. The adult neurogenesis has been attributed to the progenitors in the subgranular layer in the dentate gyrus and the subependymal layer of the telencephalon. However, the developmental origin of the progenitors in the dentate gyrus and the subependymal layer is poorly understood. It is well known that the neurogenesis in the embryo brains occurs in the ventricular zone (VZ). Recently it has been proposed that the progenitors in the VZ of the telencephalon turn into the subventricular zone (SVZ) astrocytes (Tramontin *et al.*, 2003).

The subependymal layer of the neocortex has been regarded as a source of the granule cells and periglomerular cells in the adult olfactory bulb. The subependymal layer produces neurons that migrate to the olfactory bulb as the rostral migratory stream, and the newly produced neurons are going to be GABAergic inhibitory neurons in the olfactory bulb. However, we felt discontinuity in the idea that the neocortical VZ cells turn into SVZ astrocytes and then produce GABAergic neurons. By now, it is well known that most of the neocortical GABAergic neurons were supplied by tangential cell migration originating in the subpallial structures (Anderson *et al.*, 1997; Tamamaki *et al.*, 1997). It is also speculated that the neocortical VZ almost lack the ability to produce GABAergic neurons. Under these circumstances, we speculated that the GABAergic neurons for the olfactory bulb may be produced by progenitors derived from other than the neocortex. In this paper we will show that the subependymal cells in the adult neocortex are heterogeneous and may have different origin.

Materials and methods

Animals, histology and histochemistry

Mice used in this study were obtained by mating homozygous Emx1-Cre knock-in mice (Iwasato *et al.*, 2000) and Cre-mediated lacZ/AP (alkaline phosphatase) double reporter mouse (Lobe *et al.*, 1999) or by mating heterozygous GAD67-GFP knock-in neo+ mice (Tamamaki *et al.*, 2003) with wild-type mice. These mice were anesthetized with pentobarbital (50 mg/kg) and perfused at 3 months old with saline and a fixative containing 4% formaldehyde in phosphate buffer (pH 7.4). After post fixation with the same fixative and cryoprotection, cryostat sections (12–50 μm) were made and processed for histochemistry or observation. For histochemistry of lacZ and AP, we followed the methods described previously (Lobe *et al.*, 1999).

Results

In this study we started to investigate how the neocortical subependymal cells obtained the ability to produce GABAergic neurons in the neocortex. First, we examined whether the neocortical subependymal cells were on the Emx1-negative cell lineage or Emx1-positive cell lineage.

We mated homozygous Emx1-Cre knock-in mice and Cre-mediated lacZ/AP double reporter mouse (Lobe *et al.*, 1999). In these mice, cells in Emx1-negative cell lineage appear as positive for lacZ and cells in Emx1-positive cell lineage appear as positive for AP. We investigated the subependymal layer in this mouse after X-gal reaction for lacZ and NBT reaction for AP (Figure 1A). Vast majority of the neocortical neurons were positive for AP and revealed as Emx1-positive cell lineage. The majority of the subependymal cells were also positive for AP as a continuation of the neocortex. However, the AP-positive cell layer was interrupted by lacZ-positive cell clusters in the subependyma (Figure 1A). Several lacZ-positive cell clusters were found in every frontal section at 50 μm thickness. The size of the cell cluster varied much and we counted the lacZ-positive cells in a cluster from a few to close to a hundred.

We also investigated the characteristics of cells in the subependymal layer of the GAD67-GFP knock-in mouse (Tamamaki *et al.*, 2003). We found several GFP-positive cell clusters in every frontal section at 50 μm thickness (Figure 1B). The number of GFP-positive cells in a cluster ranged from a few to nearly a hundred.

We also found the lack of Emx1 immunoreactivity in the GAD67-GFP-positive cells in the knock-in mouse neocortex and reported it previously (Nakamura *et al.*, 2003). Thus the lacZ-positive cell clusters seemed to correspond to the GAD67-GFP-positive cell clusters in the subependymal layer.

Discussion

We used the Emx1-Cre knock-in mouse and the GAD67-GFP knock-in mouse to reveal heterogeneity in the subependymal cells in the adult mouse neocortex. It has been well documented that Cre expression in the former mouse and GFP expression in the latter mouse were regulated by Emx1 promoter and GAD67 promoter properly (Iwasato *et al.*, 2000; Tamamaki *et al.*, 2003). Thus, we interpreted the AP reporter expression as Emx1 expression and GFP expression as GAD67 expression in the neocortex.

According to the distribution, size and number of the lacZ-positive cell clusters and the GAD67-GFP-positive cell clusters, they seemed like a structure almost completely overlapping each other. In good agreement, the GAD67-GFP-positive cells lacked Emx1-immunoreactivity in the knock-in mouse (Nakamura *et al.*, 2003). Since Emx1 has been regarded as primarily a pallial marker, it was discussed repeatedly that neocortical cells which lack the Emx1 expression might be derived from the subpallial structures (Chan *et al.*, 2001; Gorski *et al.*, 2002). Thus we speculate that the Emx1-negative cells in the subependymal layer are derived from the subpallial structures and the Emx1-positive cells are derived from the pallium. Although our speculation depends exclusively on whether the Emx1 is really a reliable pallial marker, we believe that Emx1 expression indicates that the subependymal cells in the neocortex are heterogeneous. In the case of Emx1-negative subependymal cells proliferating and functioning in adult neurogenesis, the major products of them will be inhibitory GABAergic neurons.

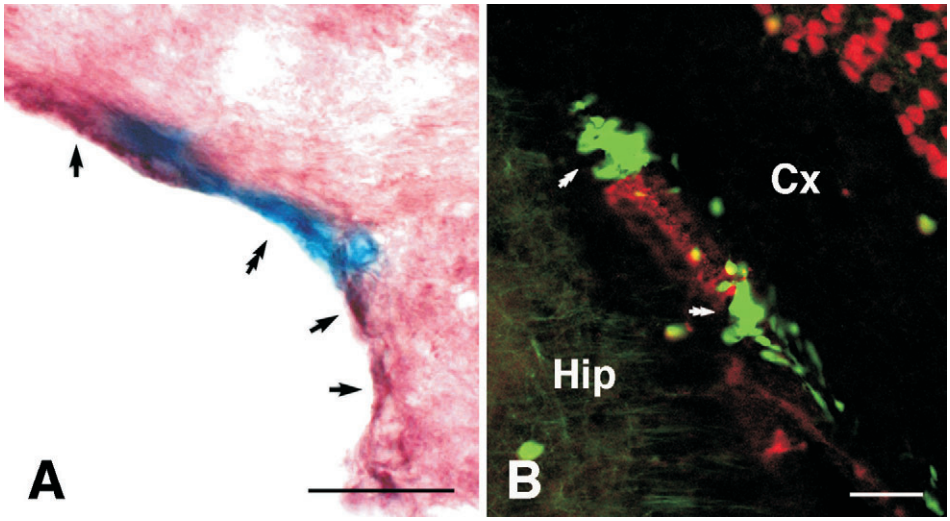

Figure 1 **(A)** A lacZ-positive-cell cluster (double arrowhead) found in the subependymal layer of a 3-month-old mouse obtained by mating homozygous Emx1-Cre knock-in mice with Cre-mediated lacZ/AP double reporter mouse. Surroundings were AP-positive cells (arrows). **(B)** GAD67-GFP-positive cell clusters (arrows) in the subependymal layer of the GAD67-GFP knock-in mouse. Cx, neocortex; Hip, hippocampus. Calibration bars: 50 μm.

Summary

The subependymal layer of the telencephalon has been regarded as a source of the granule cells and periglomerular cells in the adult olfactory bulb. However, the developmental origin of the neocortical subependymal layer is poorly understood. Here we examined the expression of a neocortical marker, Emx1, in the adult neocortical subependymal cells in mice. The subependymal cells were heterogeneous, some of them being of an Emx1-negative cell lineage and others being Emx1-positive. The Emx1-negative cell clusters seemed to overlap with GAD67-postive cell clusters in the subependymal layer. These observations imply that some of the neocortical subependymal cells were derived from the subpallial structures. When these two types of subependymal cells proliferate and function for adult neurogenesis, their product might be different.

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