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Cortical lesion stimulates adult subventricular zone neural progenitor cell proliferation and migration to the site of injury

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Abstract The subventricular zone (SVZ) is the principal neurogenic niche present in the adult non-human mammalian brain. Neurons generated in the SVZ migrate along the rostral migratory stream to reach the olfactory bulb. Brain injuries stimulate SVZ neurogenesis and direct migration of new progenitors to the sites of injury. To date, cortical injury-induced adult SVZ neurogenesis in mice remains ambiguous and migration of neural progenitors to the site of injury has not been studied in detail. Here we report that aspiration lesion in the motor cortex induces a transient, but significant increase in the proliferation as well as neurogenesis in the SVZ. New neural progenitors migrate ectopically to the injured area with the assistance of blood vessels and reactive astrocytes. The SVZ origin of these progenitors was further confirmed using lentiviral transduction. In addition, we show that astrocyte-assisted ectopic migration is regulated by CXCR4/SDF-1 signaling pathway. Finally, upon reaching the lesion area, these progenitors differentiate mainly into glial cells and, to a lesser extent, mature neurons. These data provide a detailed account of the changes occurring in the SVZ and the cortex following lesion, and indicate the potential of the endogenous neural progenitors in cortical repair.

Introduction

Loss of cortical neurons is characteristic of many pathological conditions such as stroke, trauma and neurodegeneration. Two main approaches have been proposed for neural repair following cortical damage: cell transplantation (Gaillard et al., 1998, 2007; Gaillard and Jaber, 2007, 2008) and recruitment of endogenous neural precursors (reviewed in Saha et al., 2012).

In the adult rodent brain, neurogenesis continues in two regions, the subventricular zone (SVZ) lining the lateral ventricle and the subgranular zone of the hippocampus (Altman and Das, 1965; Doetsch et al., 1997; Gage et al., 1998). New neurons generated in the SVZ migrate along a well-defined pathway, the rostral migratory stream (RMS), to reach the olfactory bulb (OB) and differentiate into

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granule and periglomerular cells and integrate into the existing circuitry (Luskin, 1993; Lois and Alvarez-Buylla, 1994).

The discovery of adult neurogenesis and neuronal migration has generated great interest in the field of cell replacement therapy following neuronal loss. In several pathophysiological conditions, the proliferative capacity of the SVZ is altered (reviewed in Saha et al., 2012). For instance, in animal models of cerebral ischemia and Huntington's disease, the SVZ proliferation is increased significantly (Arvidsson et al., 2002; Decressac et al., 2010), whereas it is reduced in Parkinson's disease and Alzheimer's disease (Hoglinger et al., 2004; Rodriguez et al., 2009). In different cortical injury models, however, conflicting reports on SVZ proliferation have created confusion in the understanding of lesion-induced proliferation. While in controlled cortical impact (Ramaswamy et al., 2005), fluid-percussion injury (Chirumamilla et al., 2002; Chen et al., 2003) and focal acute injury (Blizzard et al., 2011), a significant increase in the SVZ progenitor proliferation has been observed, no significant change was reported with the aspiration lesion model (Dizon et al., 2006).

Besides inducing proliferation, brain diseases and injuries also induce migration of neural progenitors to the sites of injury (Arvidsson et al., 2002; Ramaswamy et al., 2005; Sundholm-Peters et al., 2005; Faiz et al., 2008). In cerebral ischemia, blood vessels were reported to guide ectopic neural progenitor migration (Thored et al., 2007; Kojima et al., 2010). However, the mechanisms underlying the ectopic migration of the neural progenitors in response to cortical lesion are still largely unknown.

Here, we studied changes taking place in the forebrain following motor cortical lesion in adult mice. We showed that aspiration lesion in the motor cortex induces a transient, but significant increase in the proliferation as well as neurogenesis in the SVZ. We analyzed lesion-induced changes in the cortical microenvironment and their role in the ectopic migration of new neural progenitors and demonstrated, for the first time, that both re-organized blood vessels and reactive astrocytes form scaffolds to assist migration. GFP-expressing lentiviral transduction in the SVZ confirmed that these neurons are indeed originating in the SVZ. Finally, we report that neural progenitors migrating to the lesion area differentiate into glial cells and also into mature neurons, although to a much lesser extent.

Material and methods

Cortical lesion

For all experiments, adult (4–6 month-old, females and males) C57BL/6 mice were used. Housing of the animals and all animal experimental procedures were carried out according to the guidelines of the French Agriculture and Forestry Ministry (decree 87849) and of the European Communities Council Directive (86/609/EEC). All efforts were made to reduce the number of animals used and their suffering.

Mice were anesthetized with avertin (250 mg/kg of body weight), placed in a stereotaxic apparatus and the left motor cortex was aspirated from approximately 0.5 to 2.5 mm rostral to the Bregma and from 0.5 to 2.5 mm lateral to the midline using a fire-polished pipette, with the corpus callosum left intact. After lesion, the skin was stapled and the mice were kept for recovery.

Lentivirus injection

A custom-made GFP-expressing lentivirus [under phosphoglycerate kinase (PGK) promoter] was prepared as described before (Consiglio et al., 2004; Grubb et al., 2008) to transduce SVZ/RMS progenitors. For stereotactic injections, mice were anesthetized three days after lesion and approximately 1.5 μl of solution containing the virus (2×10^{10} U/ml) was injected near the head of SVZ (anteroposterior +0.4 mm from Bregma, mediolateral 1.08 mm and dorsoventral 1.8 mm) using a picoinjector (Picospritzer III, Parker) using the following parameters: pressure 8 psi, time: 4.0 s. Mice were sacrificed either 8 days or 21 days following injection.

BrdU injections

For proliferation studies, mice were injected with a single BrdU dose (50 mg/kg body weight, in 0.9% NaCl solution) 4 h before perfusion. For differentiation studies, long-term BrdU assay was performed at a similar dose twice daily for 3 days (at day 6, day 7 and day 8 after lesion) and mice were sacrificed 30 days after last injection.

AMD3100 injection

SDF1/CXCR4 signaling was blocked in lesioned mice using AMD3100, a specific CXCR4 blocker. Following cortical lesion, animals were injected with 1.25 mg/kg AMD3100 (Sigma) subcutaneously twice per day (with an interval of 6 h between two injections) for seven days as described in Rubin et al. (2003). After the final injection, mice were perfused and brains were harvested and processed for immunostaining.

Brain harvesting

Mice were injected with a lethal dose of avertin and transcardially perfused with 50 ml of saline (0.9%), followed by 200 ml ice-cold paraformaldehyde (PFA, 4%) in 0.1 M phosphate buffer (PB). Brains were removed and kept overnight in 30% sucrose solution for cryoprotection. 40 μm tissue sections (coronal and parasagittal) were cut with a freezing microtome (RM2145, Leica) for antibody staining. For proliferation studies, lesioned mice were perfused 1, 2, 3, 5, 7, 9, 12, 15, 30 and 60 days after lesion.

Immunohistochemistry

Free-floating sections were washed in Tris-buffered saline (TBS, 0.05 M, pH 7.6) and blocking was done at room temperature (RT) for 1 h in TBS containing 3% bovine serum albumin (Sigma) and 0.3%Triton X-100. Primary antibodies, diluted in blocking solution, were applied overnight at 4 °C. Secondary antibodies were applied for 1 h at RT. The following antibodies were used in this study: goat anti-Doublecortin (1:300, Santacruz Biotech.), mouse anti-PSA-NCAM (IgM, 1:500, AbCys), rat anti-CD31 (1:250, BD