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MRI Detection of Progenitor Cell Migrations during Postnatal Rat Brain Development by in situ MPIO Labeling

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INTRODUCTION

Recently Shapiro et al demonstrated that the neural progenitor cells (NPCs) can be labeled in situ by intraventricular injection of micron-sized iron oxide particles (MPIOs) [1]. MRI can detect the migrating MPIO-labeled NPCs along the rostral migratory stream (RMS) to the olfactory bulb (OB) in normal adult rats. The NPC migration patterns are known to differ between in adult and developing brains. The objective of this study was to determine whether this MR cellular imaging approach can reliably track the migration of endogenous NPCs from subventricular zone (SVZ) during normal postnatal brain development.

MATERIALS and METHOD

Animal preparation*:* 10-day-old normal SD neonatal rats (n=6) were stereotactically injected with 10ul (1.25×10^7) of 0.9 um MPIOs (Bangs Laboratories) in anterior left lateral ventricle (LV). The injection was localized at 1 mm caudal from bregma, left 1.5mm and down 2mm. **MRI scanning:** All animals were scanned using 7T rodent MRI scanner (70/16 Bruker BioSpin MRI PharmaScan, Germany) at day 1, 3, 7 and 14 after MPIO injection. 3D-GE images were acquired with TR/TE = 50/8 ms, flip angle = 10°, FOV = 2.5×2.5×1.2 cm^3, spatial resolution= 106×106×100 um^3 and NEX=1. 2D-GE T2*-weighted images (T2*WI) were acquired in transverse and sagittal direction with TR/TE1/TE2/TE3 = 550/8/14/20ms, NEX=6, FOV=2.5 cm, slice thickness =0.3 mm, spatial resolution = 98×98 um^2 and 20 slices. T2-weighted images (T2WI) were acquired using a RARE sequence in coronal direction with TR/TE1/TE2=6000/60/200ms, NEX=2, spatial resolution = 98×98 um^2 and 20 slices. **Histology**: 3 animals were sacrificed at 7 days post injection (dpi) and 14dpi, respectively. Brains were perfused transcardially with 10% formaldehyde and embedded in paraffin. The sagittal and transverse sections (8μm) were prepared and processed for Prussian blue (PB) iron staining only and PB plus immunohistochemical double staining for MPIO in different migrating cells: NPCs (anti-Nestin) and astrocytes-like progenitor cells (anti- GFAP).

RESULTS and DISCUSSION

All animals were successfully injected with MPIOs in left LV. By 1 dpi, the dark contrast permeated into both sides of LV (see top row in Fig. 1) in 3 out of 6 rats, with remaining 3 showing dark contrast only in the ispilateral LV side (to MPIO injection site). Between 3 and 14 dpi, the dark contrast in LV pervaded into the 3rd ventricle (3V) and the arachnoid space, such as the hippocampal fissure (HF) (see black arrows in 3rd to 4th rows in Fig.1).

MRI of migrating NPCs in normal neonatal rats: We mainly observed a bidirectional, tangential pattern at the rostral and caudal orientation in the serial MRI scans. ① At the rostral side, the dark migration contrast extended from the anterior part of SVZ (SVZa) into the RMS by 3 dpi, and clearly entered into the RMS pathway in 7 dpi. By 14 dpi, the dark contrast reached the end of RMS and extended into OB in 4 out of 6 rats. In the 2 remaining animals, the dark contrast only reached the middle of RMS (see the yellow arrows in the 2nd, 3rd and 4th rows in Fig.1). This migration pattern in neonatal rats is similar to that previously reported in adult rats [1]. ② At the caudal side, we observed the dark contrast from anterior part of left LV into the central part corpus callosum (CC) in all rats by 3 dpi, and further into the contralateral side of CC in 3 out of 6 rats. By 14 dpi, the caudal migration could be detected along CC backwards into the one-side or two-side external capsule (EC). Moreover, the dark migration paths could also be observed from posterior LV (PLV) into the EC at the hippocampal outer border in all rats (see white arrows in the 2nd , 3rd and 4th rows in Fig.1).

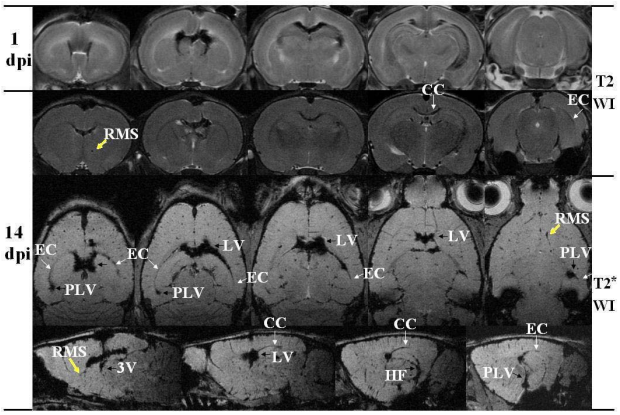

Fig.1 2D T2WIs and T2*WIs on coronal, axial and sagittal orientations in a normal neonatal rat showed the different migrating routes of endogenous NPCs carrying MPIOs by 1 and 14 dpi. The black, yellow and white arrows respectively denoted the MPIOs in cerebral ventricular system, the rostral and caudal migrating routes.

Colocalization of histological findings and MRI: ① PB iron staining: The MPIO-induced dark contrast in MRI spatially correlated with the iron positive staining in all rats. In Fig.2, the iron positive cells were found in the ependymal wall (Fig.2D), SVZa (Fig.2A), RMS (Fig.2B), OB (Fig.2C) and EC (Fig.2E), which corresponded to different MPIOs migration routes observed in MRI. In addition, certain dark spots could be seen in MRI in 2 out of 6 rats in the cortex close to the MPIO injected site, which spatially correlated with the iron positive staining in cortex (Fig.2F). This suggested the MPIO emigration from the anterior part of LV through white matter toward cortex via the local radial glial fibers as migratory scaffolds, thus presenting a radial migrating pattern [2]. ② PB plus immunohistochemical double staining: In Fig 3, the 3D-GE images by multiplanar reconstruction showed the two-side backwards migrations along the central bypass of CC at the inner side of LV. The long-distance migration stopped at the most caudal tip of CC close to the hippocampal inner border by 14 dpi, which spatially corresponded to iron+/Nestin+ and iron+/GFAP+ staining lines, respectively. Nestin, an intermediate filament, was first characterized in neuroepithelial stem cells during embryogenesis in rats [3]. In this study, nestin+/iron+, used as a marker for neural progenitor cells carrying MPIO, was expressed in the SVZ and the migrating neuroblasts mainly in the ependymal wall, OB, RMS, CC, EC, and rarely in cortex of normal neonatal rats. Moreover, the glial marker GFAP is not only a marker for astrocytes but also a marker of stem/progenitor cells [1, 2]. In present study, the GFAP+/ iron+ cells were found mainly in the ependymal wall, CC, EC and RMS, rarely in the OB and cortex. This demonstrated that the NPCs can differentiate into the astrocytes or/and astrocytes-like progenitor cells with MPIOs in developing brains.

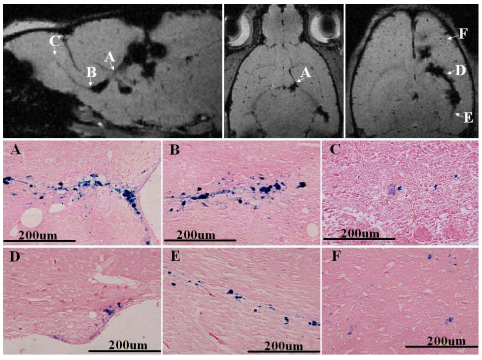

Fig.2 The migration of endogenous NPCs carrying MPIOs in MRI spatially correlated with the PB iron staining slices.

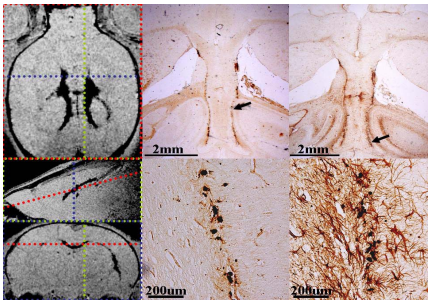

Fig.3 The migration of endogenous NPCs carrying MPIOs in MRI spatially correlated with the PB plus immunohistochemical double staining with nestin (the middle column) and GFAP (the right column). The magnified images of iron plus immunohistochemical staining in the 2nd row respectively corresponded to the black arrows in 1st row.

CONCLUSION

By in situ MPIO labeling of endogenous NPCs in 10-day-old normal rat brain, the migrating pathways were detected by MRI to mainly exhibit a bidirectional, rostrocaudal pattern in tangential orientation. The radially migrating pattern of NPCs for generating astrocytes in the cortex was rarely observed in this study. Such in situ MPIO labeling approach opens the possibility of using MRI to study the mechanism of cell migration in developing brain.

REFREENCES [1] Shapiro EM et al, Neuroimage 2006;32:1150. [2] Suzuki SO et al, J Neurosci 2003;23:4240. [3] Ernst C et al, Eur J Neurosci,2005;22 :3059.