Cell

Long-Distance Migration and Colonization of Transplanted Neural Stem Cells

A recent study reported remarkable survival and exuberant axon outgrowth from transplants of neural stem cells (NSCs) in a fibrin matrix with growth factors that were grafted into a complete spinal cord transection site in rats (Lu et al., 2012). As part of the NIH-supported replication project (Facilities of Research Excellence-Spinal Cord Injury), we repeated key parts of that study. Because this is a surgical intervention that may depend on skills that require extensive experience, we felt that the goals of the replication would be best served if the same surgeon performed the lesion surgeries and transplants. Accordingly, Dr. P. Lu traveled to UCI to perform the surgical procedures with aid of our staff. Also, because the cells could be considered to be a "product" that was obtained for use in much the same way as stem cells from a company, the cells were prepared at UCSD and were delivered to UCI on the day of the grafting procedure.

Rats (n = 20) received complete spinal cord transections at thoracic level 3 (T3) and 2 weeks later received transplants of neural stem cells (NSCs) isolated from E14 rat embryos that express GFP. Spinal cord injury and transplant surgeries were done on 4 separate days over a 2 week period.

By 9–10 weeks, NSC grafts had grown and differentiated to completely fill in the space left at the site of the spinal cord injury (Figure 1A). There was exuberant axon outgrowth from the grafts into the host spinal cord as reported by Lu et al. (2012) (Figure 1B). We were surprised, however, to find ectopic colonies of graft-derived cells at long distances from the transplant site, including in the central canal of the spinal cord, adhered to the surface of the spinal cord, and in the fourth ventricle of the brainstem.

An example of ectopic colonies in the central canal is illustrated in Figure 1A in a horizontal section through the center of the spinal cord that had been immunostained for GFP to reveal graft-derived cells. Ectopic colonies of graft-derived cells were evident in the central canal approximately 4–7 mm rostral to the transplant. The central canal was expanded around the ectopic cell masses.

Having discovered ectopic colonies of NSC-derived cells at long distances from grafts of NSC's, we screened for ectopic colonies in other locations. Examination of cross-sections taken from cervical through high lumbar levels of the spinal cord revealed ectopic colonies of graft-derived cells in the central canal at cervical levels in four rats (Figures 1C and 1J), and on the pial surface of the spinal cord in nine rats. Figure 1D illustrates a colony capping the dorsal horn near the dorsal root entry zone. Examination of sections through the brain revealed ectopic colonies adhered to the brainstem and in the fourth ventricle in four rats (Figure 1F). Overall, ectopic cell masses were seen in rats that received transplants on all four of the separate days.

The ectopic cell masses extended GFP-positive axons into the surrounding host parenchyma. The masses in the central canal extended axons into the spinal cord parenchyma forming a halo of labeled axons around the central canal (Figure 1E). Ectopic colonies capping the dorsal horn extended GFP-labeled axons into the dorsal horn and dorsal root (Figure 1D). The ectopic colony in the floor of the fourth ventricle extended axons into the dorsal part of the tegmentum near the nucleus of the solitary tract (Figures 1G and 1H).

Immunostaining of colonies with cell-type-specific markers revealed GFAP-positive cells with astrocyte morphology (Figure 1I) and MAP2-positive processes resembling dendrites (Figure 1J). Immunostaining with Ki67, a marker for proliferating cells, revealed abundant Ki67-positive cells in the ectopic mass in the brainstem (Figure 1K), indicating ongoing proliferation.

The fact that ectopic masses are found in the central canal, on the surface of the spinal cord and in the fourth ventricle suggests that cells may be carried by the CSF. The size of the cell masses makes it unlikely that the entire mass moved because the masses were considerably larger than the diameter of the central canal. Thus it is likely that the cell masses derived from a few cells that pioneered the outpost and proliferated in place.

The very surprising discovery of the presence of ectopic cell clusters at long distances from the transplant site raises concerns in terms of therapies involving stem cell transplants. It is especially concerning that NSCs continue to proliferate at ectopic locations, which could enlarge the cell masses.

Whether these ectopic colonies are harmful or not remains to be determined, but their presence highlights an important potential safety monitoring issue for therapies involving stem cell transplantation into the injured spinal cord. Cell masses in the central canal at cervical levels are a concern because the ectopic cell masses fill and expand the central canal. Patients with spinal cord injuries are already at risk for developing a syrinx, a condition in which the central canal expands leading to damage to surrounding neural tissue (el Masry and Biyani, 1996). Expansion of the central canal at the cervical level can lead to loss of pain sensitivity in the upper extremities due to interruption of axons of second-order sensory neurons that cross the midline beneath the central canal. Expansion of the central canal in the brainstem can be life threatening.

Connections formed by ectopic cell masses with host neurons could also be harmful. The cell masses that colonize near the dorsal root entry zone extend axons into the dorsal horn, which contains second-order sensory neurons that convey pain and temperature sensation. Activation of these second-order neurons by axons from ectopic masses could conceivably produce neuropathic pain.

Cell masses in the fourth ventricle are concerning because they could grow to compress brainstem structures. Colonies that send axons into the brainstem could cause abnormal activation

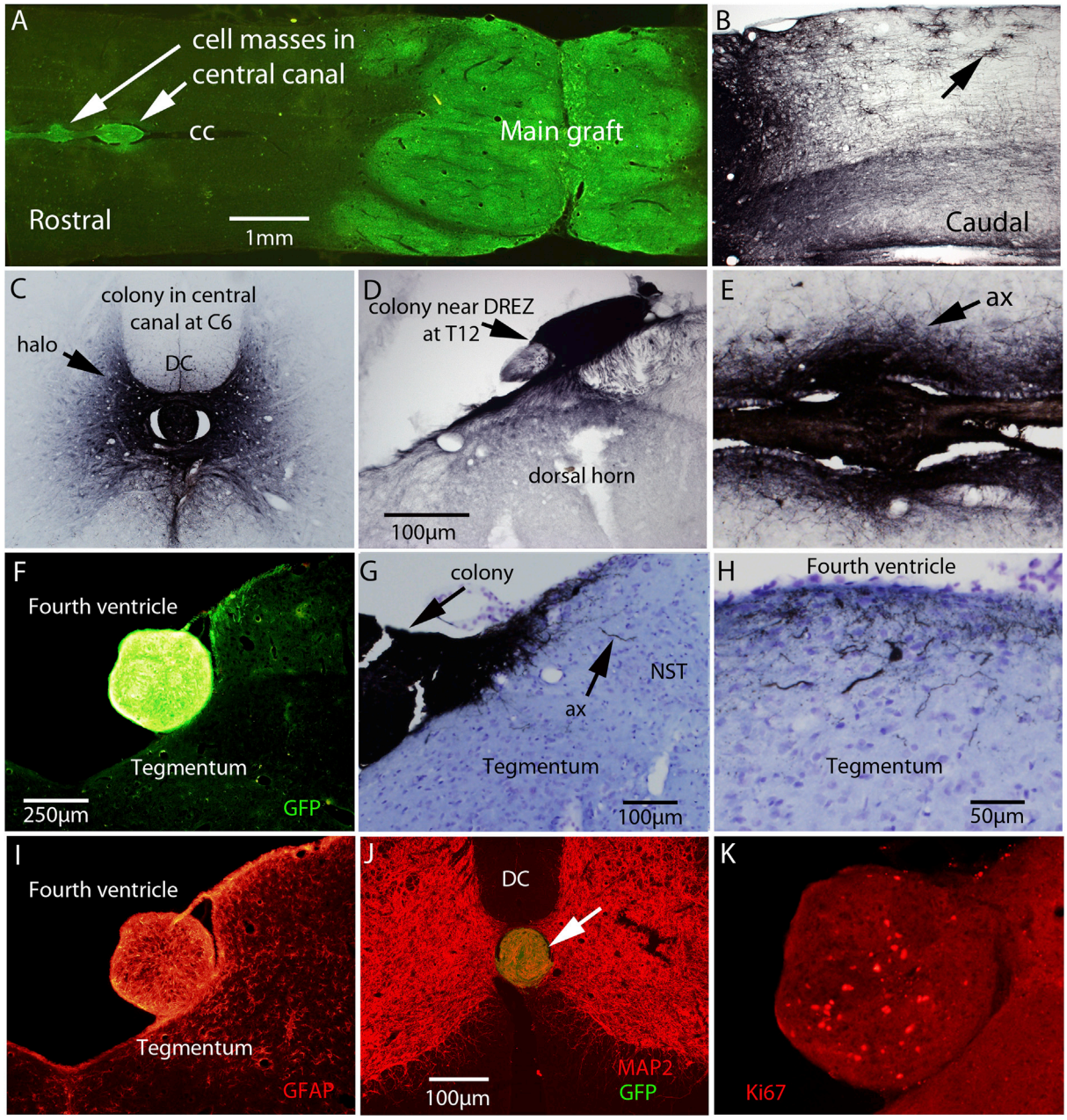

Figure 1. Migration and Colonization of NSCs from Grafts Placed at the Site of a Complete Spinal Cord Transection at T3

(A) Horizontal section through the lesion site immunostained for green immunofluorescence (GFP). The main body of the graft at T3 is evident on the right side of the image. Ectopic cell masses are evident in the central canal rostral to the injury.

(B) Image of GFP-labeled axons streaming from the graft in (A). Section was stained using DAB as the chromogen. Arrow indicates GFP-positive cells.

(C) Section through the cervical spinal cord at about C8 immunostained for GFP reveals a mass of graft-derived cells in the central canal (section stained using DAB as the chromogen).

(D) Ectopic colony adhered to the dorsal part of the spinal cord at T12 overlying the dorsal horn and dorsal root entry zone (DREZ) (section stained using DAB as the chromogen).

(E) High-magnification view of the rostral-most mass in the central canal in (A) stained using DAB as the chromogen. Note axons (ax) in a halo around the mass.

(F) Ectopic colony in the floor of the fourth ventricle adhered to the dorsal brainstem tegmentum.

(G) The same mass shown in (F) immunostained using DAB as the chromogen. Note axons (ax) extending toward the nucleus of the solitary tract (NST).

(H) higher-magnification view of axons in a section near the one shown in (G) extending along the dorsal part of the tegmentum.

(I) Same section illustrated in (F) immunostained for GFAP.

(J) Cross-section through the cervical region coimmunostained for GFP to reveal graft-derived cells and MAP2 to reveal dendrites. Brightness of the green channel is attenuated to allow visualization of MAP2 immunofluorescence.

(K) Ectopic mass in the fourth ventricle immunostained for Ki67.

of brainstem nuclei. The ectopic colony in the fourth ventricle sends axons near the nucleus of the solitary tract (NST), which controls critical functions such as blood pressure and heart rate. It remains to be seen whether other types of stem cells show similar potential to form ectopic colonies.

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