Magnetic resonance imaging of murine EG-derived neural stem cells in mouse lower motoneuron paralysis

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Introduction

Pluripotent stem cells are currently viewed as an exiting strategy for neuronal repair. In animal studies, many beneficial effects ranging from cell replacement to growth factor excretion have so far been documented. Human embryonic germ (EG) cells, for instance have already been differentiated into neurally biased, pluripotent cell populations [1]. In this study, we adapted a virally induced lower motor neuron degeneration model to study the therapeutic effects of transplanted murine EG cell-derived neural stem cells (NSC) and to image their distribution in the spinal cord by magnetic resonance imaging (MRI). The neuroadapted *sindbis*-virus (NSV) used in this study causes self-limiting encephalomyelitis, and selective death of ventral motor neurons, yet upper motoneurons and other neuronal populations are mostly spared [2]. Infection results in total paralysis of hind limbs after 3-4 days.

Methods

Male mice (40-50 g) were infected with *sindbis*-virus intracranially. Animals were treated with murine embryonic germ cell-derived NSC 10-14 days or baby hamster kindney (BHK) cells after induction of viral infection (n=18). These cells maintain pluripotency even in prolonged culture conditions. The cells also stain positively to classical markers of neural progenitor state, such as neurofilament-L and beta-tubulin, and can be differentiated into excitatory and inhibitory neurons and glia [3]. For imaging studies, NSC were labeled in culture with MD-100 magnetodendrimers (corresponding to 20 µg/mL Fe) for 24 hours [4]. The labeled NSC were then washed twice and injected into the spinal cords of mice (30 x 10^4 cells/animal) intraparenchymally (n=4). Freeze-thaw inactivated cells (n=2) and saline (n=2) were injected into control animals. 10 days after NSC transplantation, animals were anesthetized, saline perfused and fixed with 4% paraformaldehyde (PFA). Spinal cords were removed & immersed in saline in a 5 mm high-resolution NMR tube. Imaging experiments were performed at 9.4 T *ex vivo* using a double spin echo sequence (NA=2, TR=2.0 s, TE=60 ms, 3D data matrix size 80 x 40 x 40 µm).

Results

Treatment results are shown in Figure 1. Sham injected animals showed no recovery after 8 weeks. In animals treated with NSC however, moderate recovery could be observed after the same time period. On MRI, dark regions corresponding to iron-laden cells could be observed (Figure 2). Migration distances were determined from maximal extent of negative contrast, yielding 6.8±2.8 mm in the cranial, and 4.8±1.9 mm in the caudal directions (n=4). Interestingly, migration could be detected into the lesioned ventral horns, verified by Hoechst 33342 staining (Fig. 3). No migration or spread of negative contrast could be detected in the controls.

Discussion and Conclusions

The results show that murine EG-derived NSC cells have restorative potential in a mouse model of lower motoneuron paralysis. Typically, some extension movement was gained, including moderate weight support in some animals. This may be due to functional engraftment, as well as trophic factors [5]. There were 11 nonresponders in the NSC treatment group, most of which could be counted as technical failures in cell delivery. MRI shows that this labeling technique can be utilized to detect stem cell transplants in the spinal cord, and to accurately define migration distances and sites. The results also show that in this model, extensive cell migration takes place during the first 10 days post-transplantation. The results are encouraging when considering future therapeutic and imaging trials in humans.

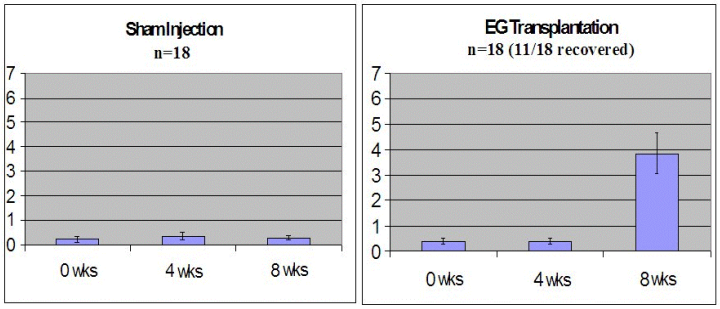

Figure 1. *Basso-Beattie-Bresnahan (BBB) motor recovery data from mice injected with baby hamster kidney cells (left) or EG-derived NSC (right). 0=paralysis, 3=extension movement, 9=weight support, 21=normal movement.*

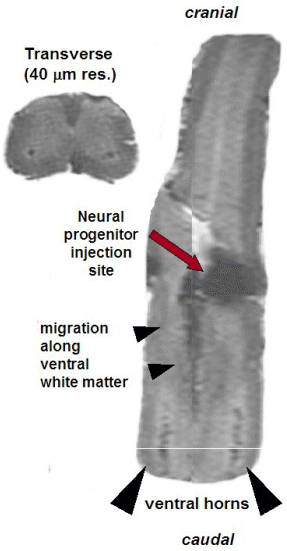

Figure 2. (left) *MRI sections from a high resolution 3D spin echo data set of mouse spinal cord. In this respresentative image, neural stem cell injection site is depicted by the red arrow. Black arrowheads point to migrating cells. The transverse section on the left is from the caudal end, depicted by the white line.The white area in the middle is a sample preparation artifact*

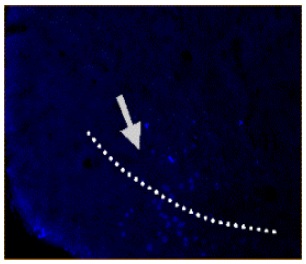

Figure 3. (above) *Hoechst 33342 positive NSC in the ventral horn of treated mouse (white arrow)..*

References

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