Original Communications

Neural stem cell delivery to the spinal cord in an ovine model of fetal surgery for spina bifida

Dario O. Fauza, MD,[a,b] **Russell W. Jennings, MD,**[a,b] **Yang D. Teng, MD, PhD,**[b,c,d] *and* **Evan Y. Snyder, MD, PhD,**[e] *Boston, Mass and San Diego, Calif*

Background. *We introduce the notion of prenatal neural stem cell (NSC) delivery to the spinal cord as an adjuvant to fetal repair of spina bifida.*
Methods. *Fetal lambs with experimental myelomeningocele (MMC;* n = *25) were divided in 3 groups: group I, no repair; group II, standard surgical MMC coverage; and group III, MMC coverage plus delivery of a murine NSCs clone into the spinal cord defect. Donor cells constitutively expressed lacZ encoding for* Escherichia coli *β-galactosidase, yet they were further labeled by exposure to either BrdU and/or to the fluorescent membrane dye PKH-26. Blinded initial clinical evaluations and multiple spinal cord analyses were undertaken soon after birth.*
Results. *Both survival and the incidence of major paraparesis were significantly worse in group I compared with groups II and III. In group III, NSC density was highest within the most damaged areas of the spinal cord, with selective engraftment within those regions. Donor NSCs retained an undifferentiated state in vivo, producing neurotrophic factors within the defect. No animals in group III had a worsened condition following this intervention.*
Conclusions. *Neural stem cells retain an undifferentiated state and produce neurotrophic factors in the short term after delivery to the fetal spinal cord, in the setting of experimental MMC. Further scrutiny of NSC delivery to the spinal cord as a therapeutic strategy against spina bifida is warranted. (Surgery 2008;144:367-73.)*

[a]*Department of Surgery and Advanced Fetal Care Center, Children's Hospital Boston, Mass;* [b]*Harvard Medical School, Boston, Mass;* [c]*Department of Neurosurgery, Children's Hospital Boston/Brigham & Women's Hospital, Boston, Mass;* [d]*Spinal Cord Injury Research, VA Boston Healthcare System, Boston, Mass*
[e]*Stem Cell Center, The Burnham Institute, San Diego, Calif*

Congenital neural tube anomalies occur before the end of the 4th week of gestation and are characterized by a midline vertebral defect (spina bifida), most often in the dorsal portion of the lumbosacral vertebrae. When only a meningeal sac protrudes through the defect, it is called a meningocele; if the spinal cord also protrudes, it is called a myelomeningocele (MMC). Isolated neural tube defects are multifactorial in inheritance in ≥90% of cases.[1] The most commonly held belief is that MMC is formed as a consequence of lack of fusion of the neural folds over the invaginating neural plate.[2] More recently, however, other theories have been advanced, including the possibility of a primary failure of caudal mesenchymal closure and/or the prospect of a reopening of the neural tube.[3-5] Folic acid deficiency has been implicated as a contributory, if not etiologic, factor in the development of neural tube defects, including MMC.[6,7]

MMC leads to injury/loss of spinal cord tissue at and below the lesion. Common manifestations include paraplegia, urinary and fecal incontinence, sexual dysfunction, and secondary musculoskeletal deformities. In addition, variable degrees of the Arnold-Chiari II hindbrain malformation and hydrocephalus are present in the majority of

Supported by a grant from the Harvard Center for Minimally Invasive Surgery and by the Kevin and Kate McCarey Fund for Surgical Research, at Children's Hospital Boston.

Accepted for publication May 5, 2008.

Reprint requests: Dario O. Fauza, MD, Children's Hospital Boston, Dept. of Surgery - Fegan 3, 300 Longwood Avenue, Boston, MA 02115 E-mail: dario.fauza@childrens.harvard.edu.

0039-6060/$ - see front matter

doi:10.1016/j.surg.2008.05.009

patients. Overall mortality of MMC at 5 years of age is approximately 14%, reaching 35% in children with hydrocephalus.[8] Morbidity rates are much higher, with the level of the lesion determining the type and severity of motor and sensory dysfunction.

Classical treatment of MMC consists of surgical closure of the spinal canal soon after birth. Lifelong support, rehabilitation, and institutionalization are usually necessary. However, given that the neural damage associated with MMC is thought to be, at least in part, secondary to the exposure of the spinal cord to the amniotic fluid and local trauma, prenatal surgical closure of the defect has been performed at a few centers in an attempt to improve outcomes. Although a large multicenter clinical trial of fetal repair (the Management of Myelomeningocele Study) is ongoing, clinical experience to date has unfortunately suggested that prenatal MMC closure as early as 21 weeks gestation has had a limited impact on spinal cord function.[9–11] For prenatal surgical repair of MMC to improve cord function, it is likely that the actual spinal cord tissue would have to be repaired or replaced—or further protected.

The neural stem cell (NSC)—the most primordial cell of the nervous system and a key participant in neural development—has been observed, after transplantation from an exogenous source, to mediate repair in a variety of central nervous system (CNS) impairments, including in the spinal cord, by invoking a number of developmental mechanisms.[12-20] In nearly all of these conditions, however, NSCs have been administered after birth or in adulthood. Here, we introduce the notion of prenatal delivery of NSCs as a potential means of enhancing prenatal surgical coverage of the defect by promoting spinal cord repair in fetuses with MMC, essentially a developmental problem. Our main goal in this first study was to simply validate this fetal model, specifically by determining the early engraftment and phenotypic patterns after prenatal NSC delivery to the spinal cord in surgically created MMC.

MATERIAL AND METHODS

Maternal and fetal surgical manipulation. This study was approved by the Standing Committee on Animals of Harvard Medical School and Children's Hospital Boston, under protocols #s 03113 and A04-10-131, respectively. Time-dated pregnant ewes at 97–112 days gestation (term, 145 days) were anesthetized with 2% halothane (Halocarbon Laboratories, River Edge, NJ). Animals received 1 g of cefazolin (G.C. Hanford, Syracuse, NY) intravenously before surgery. Through a median longitudinal laparotomy, we exposed the bicornuate uterus. Fetal lambs (n = 25) underwent open creation of a MMC defect through a posterior laminectomy encompassing lumbar vertebrae 1–4 (L1–4), as previously described.[21] We partially drained the amniotic fluid from the uterine cavity and kept it at 37°C before fetal manipulation, then reinfused it into the amniotic cavity at the end of the procedure, together with 500 mg of cefazolin. We closed the gestational membranes and uterine wall in a single layer with a reusable TA 90-mm Titanium surgical stapler (US Surgical, Norwalk, Conn) and the mother's abdomen in layers. On the first postoperative day, the ewes received 1.2 million units of benzatin penicillin (Wyeth Laboratories, Philadelphia, Penn) intramuscularly.

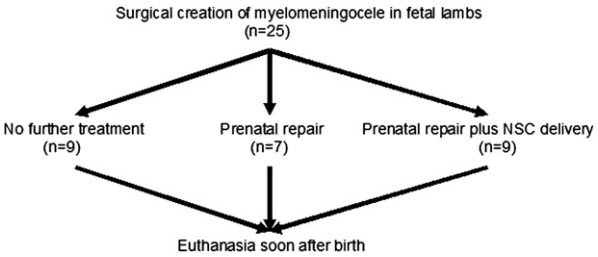

Fig 1. Overall experimental outline. Fetal lambs underwent operative creation of a myelomeningocele (MMC) defect and then were divided in 3 groups, depending on a subsequent procedure performed, still in utero: group I, no further manipulation; group II, simple surgical MMC coverage; and group III, MMC coverage plus NSC delivery into the spinal cord.

Neural stem cells. We plated a suspension of primarily dissociated murine NSCs (5×10^5 cells/mL), prepared from the cerebellum, as detailed elsewhere.[12,22] We diluted dissociated cells to 1 cell/15 μL and plated them at 15 μL/well of a Terasaki or 96-well dish. Wells with single cells were noted immediately. We expanded and maintained single-cell clones and confirmed monoclonality by identifying in all progeny a single and identical genomic insertion site on Southern analysis for either the *lacZ*- or the v*myc*-encoding provirus, as previously detailed.[18] Although this clone of murine NSCs (clone C17.2[12]) constitutively expresses *lacZ* [encoding for *E coli* β–galactosidase (β-gal)] to control for and circumvent the risk of transgene downregulation; we also prelabeled cells either by ex vivo exposure to BrdU (20 μmol/L; 48 hours before transplantation) and/or to the nondiffusible vital fluorescent membrane dye PKH-26 (Sigma, St. Louis, Mo), immediately before transplantation, as per the manufacturer's protocol. The nondiffusibility of PKH-26, even from killed

Table. Basic qualitative functional evaluation of surviving lambs

	Presence of hindlimb withdrawal	*Presence of tail withdrawal*	*Hindlimb power (examiner 1)*	*Hindlimb power (examiner 2)*
Group I	2/4	1/4	1A/1L	1A/1L
Group II	5/6	5/6	3A/2L	2A/3L
Group III	8/8	7/8	5A/3L	4A/4L

The presence of hindlimb and tail withdrawals was tested via application of a moderate noxious stimulus (needle piercing) to the skin of the interdigital clef of both hooves and to the distal fourth of the tail, respectively. A paraparetic lamb could have hindlimb and/or tail withdrawal. A, adequate; L, low.

cells, had previously been verified for NSCs.[23] Donor cells could also be recognized by a 4th independent marker—species difference.

Operative repair of MMC. We reoperated on the fetuses 14–25 days after creation of the MMC defect, when they were divided in three groups, depending on the procedure performed (Fig 1): group I, no further manipulation (n = 9); group II, simple MMC coverage with acellullar human dermis (AlloDerm; LifeCell, Branchburg, NJ; n = 7); and group III, MMC coverage with Alloderm plus NSC delivery into the spinal cord (n = 9). There was no significant difference in the interval between the 2 operations among the groups. The surgical technique for closure of the fetal MMC defect was as previously described.[24,25] Delivery of the NSC suspension (2 mL, at a density of 1×10^8 cells/mL) to the spinal cord was by direct injection into the grey matter of the spinal cord at 4–6 equally spaced points (depending on the actual size of the L1–4 span) through the MMC defect. Despite the xenologous origin of the donor NSCs, immunosuppression was not employed, taking advantage of the unique immune milieu within the fetal CNS.

Assessment of newborn lambs and analysis of donor NSCs in vivo. All ewes had vaginal delivery. Two examiners blinded for groups II and III performed basic clinical evaluations for motor and sensory status on all newborn lambs within 24 hours of birth using a basic qualitative scale (Table). Immediately after the clinical examination we performed euthanasia and removed the spinal cords en bloc, after fixing it through whole body perfusion with 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.2), as previously detailed.[12] We postfixed the specimens overnight at 4°C, and then transferred them to 30% sucrose for 72 hours. We searched for donor-derived cells on 35-μm sagittal sections of the spinal cords by Xgal histochemistry[12]; immunocytochemistry with antibodies against β-gal[22]; BrdU immunostaining, and/or by PKH-26 fluorescence (through a Texas Red filter[26]). We employed at least 2 of these cell identification techniques in each animal to confirm their donor origin. To visualize cellular nuclei, sections were incubated in the blue fluorescent nuclear label DAPI (10 minutes at 20°C).

We performed immunostaining of donor NSC for the immature neural stem/progenitor marker Nestin, as well as markers for neural cell-type differentiation, namely β-III tubulin, microtubule-associated protein-2 (MAP-2), glial fibrillary acidic protein (GFAP), choline acetyl transferase (ChAT), pan-neuronal neurofilament (NF), CNPase, and myelin basic protein (MBP), as previously described.[18] We also analyzed local production of neurotrophic and neuroprotective factors, such as glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF), both by donor NSCs and other cells. Briefly, chicken polyclonal antibody to either GDNF or BDNF (Promega, Madison, Wisc) was incubated with tissue sections that had been blocked with 3% Normal Goat Serum (NGS). Fluorescent (Texas red) conjugated (for GDNF) or biotinylated goat–anti-chicken immunoglobulin (for BDNF) was used in combination with a Vectastain ABC kit (Vector, Burlingame, Calif) to demonstrate antigen presence.

Statistical analysis. For statistical comparisons we used the Fisher's exact test, with significance set at $P < .05$.

RESULTS

The survival of lambs was significantly lower in group I (4/9; 44%), than in groups II (6/7; 86%) or III (8/9; 89%). The incidence of major paraparesis (defined as inability to walk) was significantly higher in group I (3/4; 75%) than in groups II (2/6; 33%) and III (2/8; 25%). Group III trended toward a lower incidence of major paraparesis than group II, yet this did not reach statistical significance in this large animal model, which typically precludes having a sufficiently large number of lambs per group to power statistical significance. No engrafted animal (group III) worsened clinically or was more impaired than animals in groups I and II. In fact, both the power of muscular contractions and tactile function in the affected areas seemed somewhat

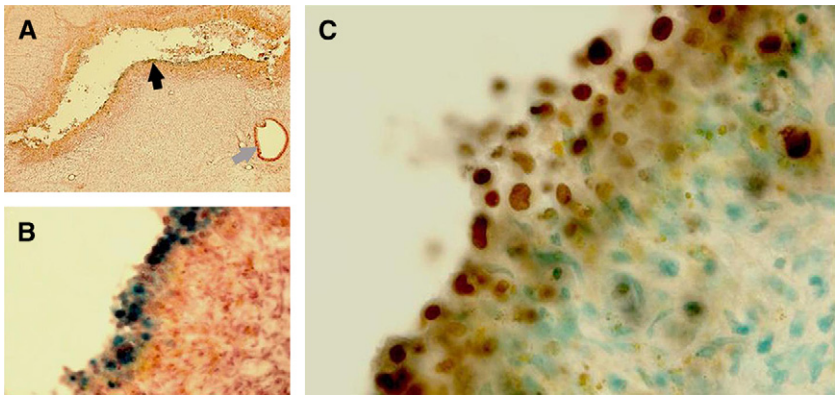

Fig 2. Engraftment and phenotypical patterns of donor neural stem cells within the fetal spinal cord. Donor NSCs engrafted robustly within the MMC-afflicted fetal lamb spinal cord, with greatest concentration in the damaged regions. **A**, **B**, Labeled donor *lacZ*-expressing NSCs identified by Xgal histochemistry (*dark blue*) within the lamb spinal cord. NSC density was highest within the most damaged portions of the spinal cords. The *black arrow* points to a site of hydro/syringomyelia-like injury, next to the central canal (*grey arrow*). This injured area is lined by NSCs, although NSCs could not be found within the normal portions of the cord. **C**, Donor-derived cells were also identified by an independent marker, their immunoreactivity to BrdU, having been preincubated ex vivo in the thymidine analogue BrdU before transplantation. In this panel, dually immunostained BrdU (*black*) and Nestin-expressing (*brown*) donor NSCs line a peripheral, damaged portion of the host's spinal cord. Original magnifications: **A**, ×50; **B**, ×200; **C**, ×400.

enhanced in group III compared with group II, based on a simple qualitative clinical scale (Table). Variable degrees of spinal cord tethering to the Alloderm patch were noted in all animals in groups II and III, in accordance with recent clinical experience with this method of coverage.[27]

All animals in group III showed NSC engraftment and survival in the spinal cord, despite their xenologous origin and lack of host immunosuppression. NSC density was noticeably higher within the most damaged portions of the spinal cord, suggesting selective NSC homing to abnormal areas (Fig 2, *A*, *B*]. This was particularly evident in the periphery of the cord at the level of the defect, which had been previously exposed to chemical insult from the amniotic fluid and possibly mechanical damage as well. Donor cells were also found in hydro/syringomyelia-like lesions present within the cord of some of the animals in this group, which, although known to occur in MMC, may simply have been direct consequences of the needle-based cell injections, in this model. Nonperipheral areas with no morphologic evidence of injury had exceedingly few, if any, engrafted cells. We did not find any clear relationship between degree of NSC engraftment and clinical outcome in this initial series. There was no evidence of tumor formation at the engrafted sites in any animal.

Interestingly, although numerous, the NSCs (recognized by their *lacZ* reporter cell expression or the BrdU immunoreactivity) remained undifferentiated in vivo, retaining a small, round morphology and immunoreactivity for the immature neuroepithelial stem cell marker nestin (Fig 2, *C*). Nestin-positive cells were not found in groups I and II. No donor cells expressed the neuronal markers β-III tubulin, NF, MAP-2, and ChAT, or the astroglial marker GFAP, or the oligodendroglial markers CNPase and MBP. Donor NSCs did, however, produce GDNF and BDNF within the defects. It is known that these neurotrophic factors perform both supportive and protective functions for spinal motor neurons and can promote axonal growth.[28-30] Local production of GDNF and BDNF was detectable within all group III spinal cords, but not in group II cords. GDNF and BDNF immunoreactivity was detected predominantly within the zone of donor nestin-positive NSCs (Fig 3). Encouragingly, immunoreactivity for these neurotrophic factors was present not only within the cytoplasm of the donor-derived cells, but also in the vicinity of extracellular and intercellular matrix regions, suggesting that the GDNF and BDNF was secreted and made available to surrounding host cells (Fig 3). The use of a species-specific marker such as murine nestin further confirmed that donor-derived cells were associated with expression of these neurotrophic factors.

DISCUSSION

The spinal cord damage associated with MMC is thought to be both primary, as a result of abnormal spinal cord development, and secondary, as a

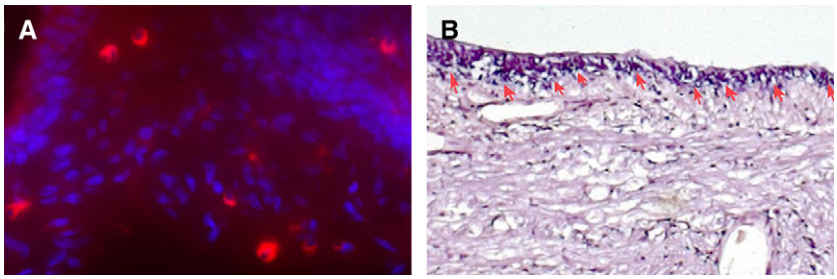

Fig 3. Functional pattern of donor neural stem cells within the fetal spinal cord. Undifferentiated NSCs within the damaged spinal cord constitutively produced neurotrophic/neuroprotective factors. **A**, Immunofluorescence for GDNF (*pink*) within a cluster of donor NSCs, suggesting that they produce this molecule (all cell nuclei are identified by a blue DAPI counterstain). **B**, NSCs lining a damaged, peripheral portion of the spinal cord are immunoreactive for BDNF (*red arrows*), with hematoxylin and eosin counterstain. Original magnifications: **A**, ×400; **B**, ×100.

result of spinal cord exposure to both the amniotic fluid (chemical insult) and local trauma (mechanical insult). Several studies suggest that the secondary component is the most relevant. Pathologic analyses of human stillborns have shown that embryos or very young fetuses with MMC have little damage to the neural tube or spinal cord.[3,31-33] Fetal imaging studies have demonstrated that leg movement patterns tend to be normal in fetuses with MMC early on in gestation, only to deteriorate as pregnancy progresses.[34,35] Further evidence of the impact of secondary damage to the spinal cord can be found in reports suggesting that delivery of MMC fetuses by cesarean section is associated with improved neurologic outcome when compared with vaginal birth.[36,37] Animal models have also shown the importance of the secondary insult to the spinal cord in MMC.[21,24,25] These data, along with evidence of neurologic improvement after experimental prenatal closure of MMC, have served as the basis for fetal MMC repair in humans.

Although it is still too early for one to draw any definitive conclusions, clinical experience to date with fetal MMC repair by simple coverage of the defect suggests that this procedure plays a role more in preventing or minimizing the Arnold-Chiari malformation than in meaningfully improving spinal cord function after birth.[9-11] This is possibly due to the protracted interval between the occurrence of the spinal defect and its surgical repair (on average at least 17 weeks). Merely preventing further damage to the spinal cord by simple prenatal coverage may be too little too late. In this study, we have but started to analyze the short-term impact of NSC delivery to the fetal spinal cord in the setting of experimental MMC.

NSCs are primordial, uncommitted cells postulated to give rise to the array of more specialized cells of the CNS. They are operationally defined by their ability to self-renew, to differentiate into cells of all neural lineages (neurons, ideally of multiple subtypes, as well as oligodendroglia and astroglia) in multiple regional and developmental contexts and to populate developing and/or degenerating CNS sites. An unambiguous demonstration of monoclonal derivation of progeny is obligatory to the definition of NSC, namely, a single cell must possess all these attributes. In this first study, we used cells from a stable clone of murine NSCs (clone C17.2), with a well-documented tendency to integrate with host progenitors and their progeny and to possess in vivo repair potential.[12,16,18]

Our fetal approach was predicated on the hypothesis that the regenerative/reparative impact of NSCs might be maximized if delivered to the developing, rather than the more mature, spinal cord. The donor NSCs did selectively populate the damaged areas. In addition, they continued to express their foreign transgene robustly in vivo—in this case, *lacZ*—suggesting that a bioactive gene could be similarly delivered seamlessly to the cord parenchyma using NSCs as a vehicle.

We have previously learned that NSCs in their undifferentiated but quiescent neural progenitor state can exert a powerfully supportive impact on the abnormal CNS.[16,38,39] Undifferentiated NSCs inherently produced molecules known to be trophic for more differentiated cells, as if spontaneously assuming a "chaperone" role. Indeed, in this model, such support for endangered host neurons and fibers was likely more valuable—and tractable—than attempting to reconstitute the complexity of spinal cord neurogenesis and connectivity. Such an action would be particularly useful if the leading etiology for neurologic deficits in MMC is, in fact, postdevelopmental injury to the exposed cord. NSCs in their undifferentiated state

are known to constitutively secrete a number of neurotrophic and neuroprotective factors[18] (of which GDNF and BDNF are only 2 identified examples), which may be ideal for promoting survival, potential regrowth, and axonal regeneration of host neural elements during the initial phase of cell transplantation.

In this introductory study, we sought principally to validate this model and therapeutic concept by documenting NSC survival, engraftment, and early phenotypical patterns. Thus, we examined tissue samples collected from lambs only 24 hours after birth. Meaningful clinical benefit of prenatal NSCs delivery can only be ascertained in further studies in which preferably autologous NSCs are used, different cell delivery methods other than injections are examined, and animals are allowed to survive longer. We must also underline the fact that this is but a surgically created model of MMC, which may not necessarily mimic all aspects of the actual disease.

Nevertheless, these initial findings, taken together with the large body of data on the use of NSCs in other forms of spinal cord injury, support further investigation into this multifaceted, prenatal therapeutic approach, combining local NSC delivery to the cord with mechanical/surgical strategies to catalyze protective and/or regenerative processes within the abnormal host.

The authors are indebted to Mr. Jeffrey Pettit for his excellence in laboratory assistance.

REFERENCES

1. Main DM, Mennuti MT. Neural tube defects: issues in prenatal diagnosis and counselling. Obstet Gynecol 1986;67:1-16.
2. French BN. The embryology of spinal dysraphism. Clin Neurosurg 1983;30:295-340.
3. Hutchins GM, Meuli M, Meuli-Simmen C, Jordan MA, Heffez DS, Blakemore KJ. Acquired spinal cord injury in human fetuses with myelomeningocele. Pediatr Pathol Lab Med 1996;16:701-12.
4. Seller MJ. Recent developments in the understanding of the aetiology of neural tube defects. Clin Dysmorphol 1995;4:93-104.
5. Campbell LR, Sohal GS. The pattern of neural tube defects created by secondary reopening of the neural tube. J Child Neurol 1990;5:336-40.
6. Botto LD, Moore CA, Khoury MJ, Erickson JD. Neural-tube defects. N Engl J Med 1999;341:1509-19.
7. Dias MS, McLone DG. Hydrocephalus in the child with dysraphism. Neurosurg Clin North Am 1993;4:715-26.
8. Worley G, Schuster JM, Oakes WJ. Survival at 5 years of a cohort of newborn infants with myelomeningocele. Dev Med Child Neurol 1996;38:816-22.
9. Bruner JP, Tulipan N, Paschall RL, Boehm FH, Walsh WF, Silva SR, et al. Fetal surgery for myelomeningocele and the incidence of shunt-dependent hydrocephalus. JAMA 1999;282:1819-25.
10. Sutton LN, Adzick NS, Bilaniuk LT, Johnson MP, Crombleholme TM, Flake AW. Improvement in hindbrain herniation demonstrated by serial fetal magnetic resonance imaging following fetal surgery for myelomeningocele. JAMA 1999;282:1826-31.
11. Sutton LN, Adzick NS. Fetal surgery for myelomeningocele. Clin Neurosurg 2004;51:155-62.
12. Snyder EY, Deitcher DL, Walsh C, Arnold-Aldea S, Hartwieg EA, Cepko CL. Multipotent neural cell lines can engraft and participate in development of mouse cerebellum. Cell 1992;68:33-51.
13. Snyder EY, Wolfe JH. Central nervous system cell transplantation: a novel therapy for storage diseases? Curr Opin Neurol 1996;9:126-36.
14. Whittemore SR, Snyder EY. Physiological relevance and functional potential of central nervous system-derived cell lines. Mol Neurobiol 1996;12:13-38.
15. Snyder EY. Neural stem-like cells: developmental lessons with therapeutic potential. Neuroscientist 1998;4:408-25.
16. Teng YD, Lavik EB, Qu X, Park KI, Ourednik J, Zurakowski D, et al. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. Proc Natl Acad Sci U S A 2002;99:3024-9.
17. Yip S, Aboody KS, Burns M, Imitola J, Boockvar JA, Allport J, et al. Neural stem cell biology may be well suited for improving brain tumor therapies. Cancer J 2003;9:189-204.
18. Lu P, Jones LL, Snyder EY, Tuszynski MH. Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. Exp Neurol 2003;181:115-29.
19. Teng YD, Choi H, Onario RC, Zhu S, Desilets FC, Lan S, et al. Minocycline inhibits contusion-triggered mitochondrial cytochrome c release and mitigates functional deficits after spinal cord injury. Proc Natl Acad Sci U S A 2004;101:3071-6.
20. Imitola J, Raddassi K, Park KI, Mueller FJ, Nieto M, Teng YD, et al. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci U S A 2004;101:18117-22.
21. Meuli M, Meuli-Simmen C, Yingling CD, Hutchins GM, Hoffman KM, Harrison MR, et al. Creation of myelomeningocele in utero: a model of functional damage from spinal cord exposure in fetal sheep. J Pediatr Surg 1995;30:1028-32.
22. Snyder EY, Yoon C, Flax JD, Macklis JD. Multipotent neural precursors can differentiate toward replacement of neurons undergoing targeted apoptotic degeneration in adult mouse neocortex. Proc Natl Acad Sci U S A 1997;94:11663-8.
23. Flax JD, Aurora S, Yang C, Simonin C, Wills AM, Billinghurst LL, et al. Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes. Nat Biotechnol 1998;16:1033-9.
24. Meuli M, Meuli-Simmen C, Hutchins GM, Yingling CD, Hoffman KM, Harrison MR, et al. In utero surgery rescues neurological function at birth in sheep with spina bifida. Nat Med 1995;1:342-7.
25. Meuli M, Meuli-Simmen C, Yingling CD, Hutchins GM, Timmel GB, Harrison MR, et al. In utero repair of experimental myelomeningocele saves neurological function at birth. J Pediatr Surg 1996;31:397-402.

26. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group[see comments]. Lancet 1991;338:131-7.
27. Mazzola CA, Albright AL, Sutton LN, Tuite GF, Hamilton RL, Pollack IF. Dermoid inclusion cysts and early spinal cord tethering after fetal surgery for myelomeningocele. N Engl J Med 2002;347:256-9.
28. Yan Q, Elliott J, Snider WD. Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. Nature 1992;360:753-5.
29. Tuszynski MH, Mafong E, Meyer S. Central infusions of brain-derived neurotrophic factor and neurotrophin-4/5, but not nerve growth factor and neurotrophin-3, prevent loss of the cholinergic phenotype in injured adult motor neurons. Neuroscience 1996;71:761-71.
30. Vejsada R, Tseng JL, Lindsay RM, Acheson A, Aebischer P, Kato AC. Synergistic but transient rescue effects of BDNF and GDNF on axotomized neonatal motoneurons. Neuroscience 1998;84:129-39.
31. Patten BM. Embryological stages in the establishing of myeloschisis with spina bifida. Am J Anat 1953;93:365-95.
32. Osaka K, Tanimura T, Hirayama A, Matsumoto S. Myelomeningocele before birth. J Neurosurg 1978;49:711-24.
33. Meuli M, Meuli-Simmen C, Hutchins GM, Seller MJ, Harrison MR, Adzick NS. The spinal cord lesion in human fetuses with myelomeningocele: implications for fetal surgery. J Pediatr Surg 1997;32:448-52.
34. Korenromp MJ, van Gool JD, Bruinese HW, Kriek R. Early fetal leg movements in myelomeningocele. Lancet 1986;1: 917-8.
35. Sival DA, Begeer JH, Staal-Schreinemachers AL, Vos-Niel JM, Beekhuis JR, Prechtl HF. Perinatal motor behaviour and neurological outcome in spina bifida aperta. Early Hum Dev 1997;50:27-37.
36. Luthy DA, Wardinsky T, Shurtleff DB, Hollenbach KA, Hickok DE, Nyberg DA, et al. Cesarean section before the onset of labor and subsequent motor function in infants with meningomyelocele diagnosed antenatally. N Engl J Med 1991;324:662-6.
37. Shurtleff DB, Luthy DA, Nyberg DA, Benedetti TJ, Mack LA. Meningomyelocele: management in utero and post natum. Ciba Found Symp 1994;181:270-80.
38. Ourednik J, Ourednik V, Lynch WP, Schachner M, Snyder EY. Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons. Nat Biotechnol 2002;20: 1103-10.
39. Park KI, Teng YD, Snyder EY. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. Nat Biotechnol 2002; 20:1111-7.