Human neural stem cells and cell transplantation are being investigated as potential therapies for neurodegenerative diseases, congenital disorders, stroke, spinal cord injury (SCI), traumatic brain injury, and brain tumors. However, concerns have been raised over the safety of these potential therapies for treatment of a variety of neurological disorders. However, significant safety concerns have surrounded these trials due to the possibility of ectopic, uncontrolled cellular growth and tumor formation.

The authors present the case of an 18-year-old woman who sustained a complete spinal cord injury at T10–11. Three years after injury, she remained paraplegic and underwent olfactory mucosal cell implantation at the site of injury. She developed back pain 8 years later, and imaging revealed an intramedullary spinal cord mass at the site of cell implantation, which required resection. Intraoperative findings revealed an expanded spinal cord with a multicystic mass containing large amounts of thick mucus-like material. Histological examination and immunohistochemical staining revealed that the mass was composed mostly of cysts lined by respiratory epithelium, submucosal glands with goblet cells, and intervening nerve twigs.

This is the first report of a human spinal cord mass complicating spinal cord cell transplantation and neural stem cell therapy. Given the prolonged time to presentation, safety monitoring of all patients with cell transplantation and neural stem cell implantation should be maintained for many years.

Key Words • stem cells • olfactory ensheathing cells • spinal cord injury • tumor • olfactory mucosa • human • cell transplantation • oncology

Case report

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Over the last decade, human cell transplantation and neural stem cell trials have examined the feasibility and safety of these potential therapies for treatment of a variety of neurological disorders. However, significant safety concerns have surrounded these trials due to the possibility of ectopic, uncontrolled cellular growth and tumor formation.

The authors present the case of an 18-year-old woman who sustained a complete spinal cord injury at T10–11. Three years after injury, she remained paraplegic and underwent olfactory mucosal cell implantation at the site of injury. She developed back pain 8 years later, and imaging revealed an intramedullary spinal cord mass at the site of cell implantation, which required resection. Intraoperative findings revealed an expanded spinal cord with a multicystic mass containing large amounts of thick mucus-like material. Histological examination and immunohistochemical staining revealed that the mass was composed mostly of cysts lined by respiratory epithelium, submucosal glands with goblet cells, and intervening nerve twigs.

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H uman neural stem cells and cell transplantation are being investigated as potential therapies for neurodegenerative diseases, congenital disorders, stroke, spinal cord injury (SCI), traumatic brain injury, and brain tumors. However, concerns have been raised over the safety of this experimental therapeutic approach. Most concerning is whether de novo tumors can develop from transplanted stem cells or supporting cells. Utilizing the neural stem cell potential and neuronal supporting cells of olfactory mucosa, intraspinal olfactory mucosal cell transplantation has been used as an experimental treatment strategy for human spinal cord injury. However, the efficacy and the long-term safety of this treatment strategy are uncertain. Two cell types within olfactory mucosa purported to be useful in repair of the nervous system are stem-like progenitor cells and olfactory ensheathing cells (OECs). The ability of these cell types to differentiate into organized neural tissue in humans or support new neural growth in humans in the setting of spinal cord injury is unclear. Here we present a case of a spinal cord mass that developed after olfactory mucosal cell transplantation in a patient with a spinal cord injury. We demonstrate the development of this spinal cord mass on radiological imaging with corresponding intraoperative photographs obtained during resection and correlative histopathology and immunohistochemical staining.

This article contains some figures that are displayed in color online but in black-and-white in the print edition.
Ectopic spinal cord mass following cell transplant with sensory level at T-11 and no motor strength in the lower extremities. She underwent reduction, realignment, and internal instrumentation of the spinal fracture for stabilization at our institution. She was discharged to a rehabilitation facility but did not regain sensation or motor strength below T-11. Given the lack of neurological improvement, she sought experimental stem cell treatment.

Three years after the injury, the patient underwent intraspinal olfactory mucosal cell transplantation at the site of the spinal cord injury at an outside institution. The treatment team’s method for isolation, preparation, and implantation of olfactory mucosal autografts in spinal cord injury patients was patented and published. Here it will be described in brief. Endonasal endoscopic olfactory mucosa was obtained from the olfactory groove. Laminectomy over the site of injury was performed and the dura opened. Scar tissue was removed and the mucosal grafts were implanted at the site of injury.

The patient returned to our institution 8 years after cell transplantation complaining of progressively worsening mid/low-back pain (pain at the level of the thoracolumbar junction) of 1 year’s duration. Imaging revealed a 3.9 × 1.2 cm expansile cystic and heterogeneously enhancing intramedullary mass at the level of the spinal cord injury (T10–11), which raised concern for tumor (Fig. 2). On examination, there was no identifiable clinical improvement subsequent to the olfactory mucosal graft transplant.

**Operation and Postoperative Course.** Surgery was undertaken for diagnosis and resection of the mass. Intraoperatively, an expanded spinal cord was observed with a heterogeneous multicystic mass with fibrous walls containing thick, white mucus-like material (Fig. 3). The mass appeared separate from the cord, but defining a distinct border was a challenge. The patient did well postoperatively and her mid/low-back pain subsided.

**Histology and Immunohistochemical Staining.** The specimen consisted of 2 red, tan, and brown soft tissue fragments measuring 1.4 × 0.8 × 0.7 cm and 1.6 × 1.3 × 0.7 cm. Histological examination (Fig. 4) with H & E revealed multiple cysts lined by respiratory mucosa with underlying submucosal glands (Fig. 4A and B), some containing goblet cells (Fig. 4D). In addition, there were small fragments of bone. Of particular interest were numerous nerve twigs coursing through the submucosa (Fig. 4C and D). The majority of the nerve twigs were small and therefore had the appearance of sprouting or regenerating nerve fibers. The tissue was further characterized by immunohistochemical staining (Fig. 4E and F). The nerves stained positive for neurofilament and S100 protein, and staining for epithelial membrane antigen (EMA) outlined a thin nerve sheath around nearly all the small nerves (Fig. 4E). Gliotic neural tissue staining positive for glial fibrillary acidic protein (GFAP) was observed adherent to scar-like fibrosis and adjacent to the respiratory mucosal tissue and was focally lined by respiratory epithelium (Fig. 4F).

**Discussion**

In this paper we describe the occurrence of a spinal cord mass after olfactory mucosal cell transplantation in a patient with a spinal cord injury. Olfactory mucosa contains stem-like progenitor cells and olfactory ensheathing cells (OECs) thought to mediate repair of the central nervous system. Olfactory mucosal stem cells have been shown to be effective in regenerating neuronal cell populations in vitro, OECs, which function normally to surround the olfactory axons and support axonal regeneration, have shown promise in preclinical animal models as a cell transplantation therapy for repair of the injured spinal cord. Although the goal of the olfactory mucosal cell transplantation was to capitalize on the regenerating properties of the stem-like progenitor cells and OECs, histological examination revealed that the mass was composed mostly of cysts lined by respiratory epithelium, submucosal glands with goblet cells, and intervening nerve twigs. This histology resembled olfactory mucosa, indicating cell transplant survival and autograft

![Fig. 1. Sagittal CT reconstruction (A) and T1-weighted (B) and T2-weighted (C) MR images obtained after spinal cord injury showing fracture dislocation at T10–11 (white arrows) causing spinal cord compression and spinal cord edema (black arrows, B and C).](image-url)
derivation of the mass. The intraoperative finding of thick copious mucus-like material within the cysts suggests that these glands maintained secretory function after transplantation. Progressive expansion of the cyst spaces by accumulating mucus produced symptoms, prompting treatment 8 years after cell transplantation. Although human clinical trials have reported safety of olfactory mucosal cell transplantation and OEC transplantation in human spinal cord injury patients, the duration of follow-up in those trials was less than 4 years.\textsuperscript{5,11}

It is unclear whether the intervening nerve twigs represented functioning corticospinal tract regeneration or development of newly sprouting nerve fibers from transplanted stem-like progenitor cells and support from OECs. Intraoperatively, the mass appeared circumscribed and distinct from the surrounding spinal cord, suggesting that the nerve twigs were newly developing nerve fibers from the transplanted tissue rather than regeneration of lesioned axons in the spinal cord. In either case, the presence of these nerves within the mass indicates the capacity of olfactory mucosa to support nerve fiber regeneration or new nerve formation. However, given the lack of clinical improvement in the patient, the functional capacity of these nerve twigs was clinically insignificant.

Human clinical trials of treatment for spinal cord injury have used different techniques for preparation of

Fig. 2. Sagittal MR images showing spinal cord mass after olfactory mucosa transplant. The intramedullary spinal cord mass demonstrates hypointensity on T1-weighted imaging (A), heterogeneous enhancement with contrast (B), and heterogeneous hyperintensity on T2-weighted imaging (C), revealing a multicystic component.

Fig. 3. Intraoperative photographs of spinal cord mass.  A: Opened dura covering the spinal cord mass (large arrow) with small opening in a cyst revealing a white thick mucus-like material (small arrow).  B: Suction revealing the thickness of the white mucus-like material (small arrow) within the mass (large arrow).  C: A large ball of mucus-like material resected from a cyst cavity.  D: Empty resection cavity after complete resection of the multicystic mass and fibrous wall. Bar = 2 mm.
Ectopic spinal cord mass following cell transplant

Fig. 4. Photomicrographs of sections of the spinal cord mass showing results of histological examination and immunohistochemical analysis.  

A: Respiratory epithelium-lined connective tissue with small fragments of bone. H & E, original magnification ×40.  

B: Respiratory epithelium with underlying submucosal glands identical to that seen in normal nasal mucosa. H & E, original magnification ×100.  

C: Small nerve twigs (circled) with perineurium within the connective tissue deep to the respiratory epithelium. H & E, original magnification ×100.  

D: Respiratory epithelium with goblet cells and underlying thin nerve twigs lacking perineurium. H & E, original magnification ×400.  

E: Staining for EMA demonstrating the surrounding perineurium of the subepithelial nerve twigs. Original magnification ×400.  

F: GFAP-positive gliotic spinal cord adjacent to respiratory mucosal tissue. Original magnification ×400.
neural stem cells and OECs for transplantation. In the report described here, olfactory mucosa was transplanted to the site of the damaged cord.\textsuperscript{5,6} In other trials, OECs were grown and purified in vitro from nasal biopsies and injected into the region of the damaged spinal cord.\textsuperscript{11} There has been limited follow-up of these patients, and there have been few publications following the initial feasibility and safety trials. It is unclear if the difference in isolation and preparation of olfactory stem cells results in different outcomes. It is also unknown if spinal cord tumors or ectopic tissue masses have developed in other spinal cord injury patients after cell transplantation. Most importantly, it appears that the use of olfactory mucosa rather than purified OECs or stem cells may prove pathological and allow respiratory epithelial function to continue after transplantation, thus resulting in an ectopic mass.

Other studies have revealed the malignant potential of stem cell therapy.\textsuperscript{1,3} The only other case in which a tumor resulted from human neural stem cell therapy occurred in a boy with ataxia telangiectasia who was treated with intracerebellar and intrathecal injection of human fetal neural stem cells.\textsuperscript{1} The only other type of malignancy that has clearly been shown to develop as a result of stem cell therapy in humans is donor type leukemia following hematopoietic stem cell transplantation.\textsuperscript{13}

Our case and those described above confirm the concerns over human cell and stem cell transplantation. These cases should not deter the advancement of stem cell research and bench-to-bedside clinical trials. However, they do stand as a warning to the scientific and medical communities. Although the results of implantation of stem cells in animal studies are encouraging and have demonstrated improved function in many animal models of neurological conditions, there is still a need for better understanding of how to control cell proliferation, survival, migration, and differentiation in the pathological environment to foresee or prevent uncontrolled or abnormal cell growth in human patients.

Conclusions
This is the first report of a spinal cord mass complicating spinal cord cell transplantation and neural stem cell therapy in a human patient. Given the prolonged time to presentation, safety monitoring of all patients treated with cell transplantation and neural stem cell implantation should be maintained for many years.

Disclosure
The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Dlouhy, Hitchon. Acquisition of data: Dlouhy, Awe, Kirby, Hitchon. Analysis and interpretation of data: Dlouhy, Rao, Kirby, Hitchon. Drafting the article: Dlouhy. Critically revising the article: all authors.

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Disclosure
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