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Human spinal autografts of olfactory epithelial stem cells recapitulate donor site histology, maintaining proliferative and differentiation capacity many years after transplantation

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Many early trials are investigating neural stem cells (NSCs) and glia in spinal cord injury (SCI). One paradigm involves autologous transplant of olfactory mucosa (OM), containing NSCs and olfactory ensheathing cells (OECs). In preclinical SCI models, OM cells provide a scaffold for regenerating axons, facilitate remyelination, and even form new neurons [1, 4, 7]. Still, long-term persistence and differentiation of OM autografts in humans is undefined.

We evaluated two patients who underwent autologous OM transplant [5] (data collection performed in accordance with local Institutional Review Board policies). The first patient was seen 7 years after transplantation (Supplemental Table 1). Magnetic resonance imaging (MRI) demonstrated a mass at the transplant site (Supplemental Figure 1 A-B). A second patient was evaluated 5 years after OM transplantation with similar spinal mass (Supplemental Figure 1 C-D), and demonstrated progressive arm pain and weakness. Given progressive symptomatology, the patient underwent surgical decompression. Intra-operative findings revealed a multiloculated mass with mucinous, septated compartments (Supplemental Figure 1 E-G).

Histologic investigation demonstrated structures reminiscent of the source olfactory epithelium (OE-like) and respiratory epithelium (RE-like). Hematoxylin and eosin (H&E) showed a ciliated columnar epithelium overlying spindled cells and blood vessels (Supplemental Figure 2A). Ciliated cells stained for AE1/AE3 cytokeratins (Supplemental Figure 2D). Underlying tissue stained for nestin and neurofilament (Supplemental Figure 2E-F), revealing continued presence of immature neuroglial cells (although contribution from spinal cord cannot be excluded). By contrast, minimal astrocyte formation was seen by

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glial fibrillary acidic protein (GFAP) staining (Supplemental Figure 2B), and only occasional cells were positive for Ki67 (Supplemental Figure 2C).

Immunofluorescence revealed further evidence of OE-like and RE-like structures. Staining for acetylated α -tubulin revealed cilia at the edges of the excised tissue, covering both the thinner RE-like and thicker OE-like regions (Figure 1A). RE-like cells were positive for both keratin 18 and CD133 (Figure 1B, Supplemental Figure 3A). By contrast, OE-like areas showed keratin 18-positive and CD133-negative cells, consistent with sustentacular and microvillar cells (Figure 1B).

Self-renewing stem cells were demonstrated by co-expression of the stem cell markers p63 and keratin 5 (Supplemental Figure 3B). Expression of p63 and keratin 18 were mutually exclusive, thus defining a distinct population of stem cells (Figure 1C) amid differentiated tissue. These stem cell markers may represent the source of OE-like and RE-like tissue; alternatively, metaplasia of olfactory epithelium may have given rise to RE-like tissue.

A number of cells were reactive for the olfactory G-protein ($G_{\alpha/olf}$) in OE-like areas but not RE-like regions (Figure 1D). Additionally, olfactory marker protein (OMP) and Tuj1 positive processes were seen in OE-like regions (Supplemental Figure 3C, D). These data demonstrate that cells resembling immature and mature olfactory sensory neurons could be identified many years after OM autograft.

Trials using olfactory cells for SCI have been reported by 3 groups [3, 5, 6]. One strategy expanded OECs *ex vivo* prior to transplantation, with no reports of mass formation [6]. By contrast, the 2 patients described here as well as another spinal mass were reported after direct OM transplant [2]. We have demonstrated that direct OM autografts continue to recapitulate donor site RE-like and OE-like structures. Cells expressing stem-cell markers also persist within this RE-like and OE-like epithelium. The preservation of these structures for many years after transplantation suggests ongoing proliferation, turnover and differentiation. To our knowledge, this is the first evidence that transplanted OM and olfactory stem cells not only survive, but proliferate and form tissue reminiscent of the donor site. As technology in stem cells progresses, our understanding of stem cell transplants must be expanded as stem cell therapy enters this nascent era.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig 1.

Tissue recapitulating respiratory epithelium (RE-like) and olfactory epithelium (OE-like) is demonstrated many years after olfactory mucosal autograft. (a) A ciliated epithelium is revealed, staining for acetylated α -tubulin (4',6-diamidino-2-phenylindole, DAPI, nuclear stain). (b) CD133 co-staining with keratin 18 is present in RE-like regions, but absent in OE-like regions. (c) Keratin 18 and p63 do not co-localize, demonstrating a distinct stem-cell population. P63-positive cells have a dispersed pattern in RE-like regions, but form a continuous line in OE-like regions. (d) Olfactory G-protein alpha subunit ($G_{\alpha/olf}$) labels cells in the OE-like epithelium, but not in the RE-like epithelium. Scale bars; A = 250 μ m, B-D = 50 μ m