The reality of stem cell grafting for CNS injury

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Statement of Purpose: We hypothesized that pluripotent stem cells would be useful in grafting experiments to repair the injured spinal cord. We examined the potential for neuronal and oligodendrocyte replacement in a variety of spinal cord injury models. We grafted cells into both normal and injured spinal cord and examined a number of parameters: constitutive differentiation of pluripotent stem cells, differentiation of partial lineage restricted precursor cells, the effects of additional growth factors that had been shown to influence lineage specific differentiation *in vitro*, and blocking endogenous signaling pathways in the engrafted cells in response to factors upregulated in the injured spinal cord which direct or restrict stem/precursor cell differentiation.

Methods: The following types of stem/precursor cells were grafted into either the normal or injured spinal cord: pluripotent stem cells isolated from the embryonic day 14 (E14) rat cerebral cortex[1], neuronal-restricted precursors (NRPs)[2] or glial-restricted precursors[3] (GRPs) isolated from the E14 rat or mouse spinal cord, and GRPs genetically modified to express the multineurotrophin D15A (with BDNF and NT3 activities), CNTF, neuregulin 1 type III, the BMP antagonist noggin, or a dominant-negative BMP receptor 1a construct, and human embryonic stem cells (hESCs) pre-differentiated towards a GRP phenotype.

The injury models that were used were either a moderate thoracic (T9) contusive injury or an ethidium bromide demyelinating lesion of the ventral lateral funiculus (VLF). We demonstrate that the mediolateral VLF is the anatomical region of the spinal cord in both the rat and the mouse in which electrical signals elicited by transcranial magnetic motor evoked potentials (tcMMEPs) are carried to the muscles of the hindlimb[4,5]. The contusive spinal cord injury that we use was titrated to just eliminate tcMMEPs while leaving intact many demyelinated axons in the VLF.

Animals were evaluated functionally by open field analysis of hindlimb locomotion, tcMMEP responses and other electrophysiological tests and histologically for spared white matter, lesion volume, and the extent of remyelination by engrafted cells.

Results: Our results can be summarized as follows:

1. Pluripotent stem cells which *in vitro* express only the stem cell marker nestin in their proliferative state, can be induced to differentiate *in vitro* into neurons, astrocytes, and oligodendrocytes. Grafting these cells into the uninjured adult spinal cord results in predominantly astrocyte differentiation, with a few oligodendrocytes, but no neurons. Many cells remain undifferentiated, nestin$^+$ stem cells. After grafting into the contused spinal cord, most cells remain nestin$^+$, some astrocytes are observed, but no oligodendrocytes or neurons are seen[1].

2. NRPs *in vitro* proliferate and express the neuronal markers β-III tubulin and Map2a,b and differentiate only into neurons many of which express the neurotransmitters GABA, glutamate, ChAT, and glycine. Similar differentiation was observed following grafting into the uninjured spinal cord. However, after grafting into the contused spinal cord, NRPs are restricted in their differentiation as most express nestin, but have lost β-III tubulin and Map2a,b expression, indicating active de-differentation[2].

3. GRPs *in vitro* differentiate only into astrocytes and oligodendrocytes. If naïve GRPs are grafted into the contused VLF, some differentiate into oligodendrocytes but most are astrocytes and tcMMEP responses are not observed. However, if GRPs are genetically modified *in vitro* prior to grafting to express either D15A or CNTF, significantly more oligodendrocytes are observed and tcMMEP responses return and hindlimb locomotor function improves[3].

Conclusions:

1. The injured spinal cord expresses factor(s) that restrict the differentiation of neurons and oligodendrocytes from pluripotent stem cells and lineage restricted precursors. Data are suggestive that both BMP and Notch signaling are involved[6].

2. Obtaining large numbers of oligodendrocytes or neurons from engrafted stem cells requires: a) partial pre-differentiation along the desired lineage, b) the presence of factor(s) that potentiate cell survival and differentiation, and c) inhibition of intracellular signaling pathways that respond to endogenous factors that inhibit cell-specific differentiation.

References:

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