Experimental Gene Therapy with Serine Histogranin and Endomorphin-1 for Spinal Cord Injury-Induced Pain in Rats

Stanislava Jergova, Nirmal Pathak, Shivani Jani, Shyam Gajavelli, Jacqueline Sagen

TO CURE PARALYSIS
The Miami Project to Cure Paralysis, University of Miami, Miller School of Medicine, Miami, Florida, sjergova@med.miami.edu

INTRODUCTION
The insufficient pain relief of chronic pain following spinal cord injury (SCI) provided by the current pharmacological treatment represents a serious medical problem. Hypothesized mechanisms underlining chronic neuropathic pain include increased hyperexcitability of spinal dorsal horn neurons due to loss or dysfunction of inhibitory γ-aminobutyric acid (GABA)-ergic interneurons, and enhanced excitatory glutamate signaling through NMDA receptors. Therefore, restoration of inhibitory tone and/or reduction of hyperexcitability via transplantation of selected cell types is a potential long-term intervention.

Genetically engineering is a suitable tool to enhance antinociception by combining small peptides with synergistic action into a single cDNA construct with potentially stronger analgesic effect. The aims of this study were to i) engineer GABAergic progenitor cells producing NMDA receptor antagonist serine-histogranin (SHG) and evaluate analgesic action of the graft in SCI rats and ii) to evaluate synergistic action of SHG and endomorphin-1 after intraspinal injection of SHG/EM genes.

MATERIAL AND METHODS
Surgery: All surgeries were done under 2% isoflurane/O2. The spinal cord was transected at L4/L5 level for SCI and kept moist with saline for the procedures through the SCI model. For L4/L5 spinal injury rat embryos (E18.5) were transplanted into SCID mice, (as previously described in [18, 19]), and 10 days post-surgery almost 100% of transplanted NPCs were detected by TH immunostaining in the spinal cord of SCID mice. All surgical and therapeutic procedures were performed under the guidelines approved by the Animal Care Committee of the University of Miami. Data from five experiments were analyzed to get a representative result.

RESULTS
Intraspinal injection of recombinant GABA/SHG neuroprogenitors

Rat E14 GABAergic progenitors

In vitro micrographs of rat E14 neuronal progenitor cells before transplantation. Immunostaining using GABA (red) and MAP2 (green) antibodies revealed that the majority of P0 E14 NPCs are in immature stages of neuronal differentiation.

Lenti-SHG-mRFP transfection of E14 NPCs and transplantation

Schematic of lentiviral construct. SHG cDNA was cloned between flgM and XmaI sites. pPGL-β signal peptide enables production of secretory peptides. Red fluorescent signal of mRFP helped to identify transplanted cells (Gajavelli, 2008).

Successful transduction of NPC was confirmed by immunofluorescence. A) lenti-SHG-mRFP transfection of NPCs. The red signal suggests the presence of construct within the neurosphere. B) Combined immunofluorescence of SHG (red) and GABA (green) shows transduction of GABAergic neurons by construct. C) No transduction was observed in GAP-43 cells.

Identification of graftshel SHG NPCs: A) Dorsal horn immunostained with NeuN (green), red fluorescence identifies SHG construct labeled by Red Fluorescence Protein. B) Higher magnification of the outlined area in A. Colocalization of green and red color suggests transduction of neuronal cells with lenti-SHG construct.

CONCLUSIONS
• Recombinant GABAergic NPCs transduced by 1x or 6xSHG construct improved pain-like behavior of animals after SCI.
• SHG treatment enhanced analgesic action of intraspinal injected endomorphin-1 in SCI rats.
• Combined treatment with intraspinal 1xSHG and EM1 constructs was more effective than EM1 construct alone, although less or equally effective as SHG NPCs treatment.
• Compound construct with multiple SHG and EM1 cDNAs is developed and will be used to produce recombinant cells.
• Recombinant neuronal replacement therapy utilizing gene constructs of synergistic analgesic peptides could be an alternative strategy in chronic neuropathic pain management.

Supported by NNS1667 and CNF 190926