these experiments that this lysosomal enzyme can spread in the mouse brain by diffusion and axonal transport, leading to complete correction of storage in the injected structure but also in physically or synaptically-connected areas of the brain.

962. Treatment of Feline G_{M1} Gangliosidosis with Mesenchymal Stem Cells and Lentiviral Gene Therapy

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Deficiency of lysosomal β-galactosidase causes G_{M1} gangliosidosis, an inherited, progressive neurological disorder in which G_{M1} ganglioside accumulates in all tissues, including thymus, liver and brain. In addition to abnormal neuronal histology and function, gangliosidosis pathology involves a significant inflammatory component and therefore is similar to other neurodegenerative disorders such as Alzheimer and Parkinson disease. G_{M1} gangliosidosis occurs in humans, mice and domesticated animals, including cats, and the feline gangliosidosis model has been used previously by our laboratory to test several therapeutic strategies. In this study, stem cell transplantation and gene therapy have been evaluated separately and in combination as therapeutic approaches for feline G_{M1} gangliosidosis.

A lentiviral vector (TK291) was constructed in which a human β-galactosidase cDNA is driven by a modified long terminal repeat promoter. Pseudotyped with the vesicular stomatitis virus G protein, TK291 transduced at least 75% of fibroblasts and mesenchymal stem cells (MSC) isolated from normal and G_{M1} gangliosidosis-affected cats after one round of infection. Both wild-type and diseased cells expressed above normal levels of β-galactosidase activity after transduction and were evaluated for therapeutic potential in cross-correction assays *in vitro*. Also, TK291 was injected directly into the cerebral cortex of diseased cats, which subsequently expressed elevated levels of β-galactosidase.

In addition, bone marrow MSC were evaluated for therapeutic potential from at least 2 perspectives. First, mesenchymal stem cells were tested as β-galactosidase delivery vehicles by assessing enzyme transfer to recipient cells *in vitro*. MSC were evaluated both before and after transduction with lentiviral vector TK291 and found to deliver β-galactosidase to recipient cells through conditioned media. Additionally, mesenchymal stem cells were tested by *in vitro* differentiation assays for their potential to replace diseased neurons in G_{M1} gangliosidosis cats. After exposure to a neuronal induction cocktail for 24 hours, feline mesenchymal stem cells increased by up to 20-fold the expression of several neuronal markers, including neurofilament M, TrkA, tau and GAP43. Expression of the stem cell marker nestin decreased substantially after induction, as did non-neuronal markers such as osteopontin and glial fibrillary acidic protein. When transplanted into organotypic brain slice cultures from fetal cats, MSC migrated broadly throughout the slices and assumed morphologies typical of neuronal or glial cells.

Finally, a combination of stem cell and gene therapy was tested for therapeutic potential by intracortical injection of mesenchymal stem cells transduced by TK291. Transplanted cells survived for at least 6 weeks in immunosuppressed cats, where they migrated from the injection site and expressed β-galactosidase activity for the duration of the experiment. These encouraging results provide the impetus for continued studies of stem cell and gene therapy for G_{M1} gangliosidosis.

963. Efficient Suppression of GluR1 Receptor Expression *In Vitro* and *In Vivo* by Infection with HIV1 Vectors Expressing siRNA

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We have previously demonstrated efficient and stable transduction of neural cells and cell-type specific transgene expression with HIV1-based lentivirus vectors. In the present study, we evaluated efficacy of spinal parenchymal delivery of HIV1 vectors expressing siRNAs against an AMPA receptor GluR1.

To construct HIV1 vectors expressing shRNAs for GluR1, we chose three different regions in rat GluR1 cDNA as target for siRNA. Synthesized oligonucleotides correspond to each shRNA were linked to U6 promoter and inserted into the HIV1 vector backbone plasmid. Similarly, as control, HIV1 vector expressing either EGFP gene or shRNA for luciferase were also constructed. HIV1 vectors were produced by co-transfection of HEK293 cells and concentrated by ultracentrifugation.

To assess the efficacy of HIV1-GluR1-shRNA vector delivery in suppressing GluR1 receptor expression, virus vectors were i) applied directly into a mixed primary cortical culture isolated from E14 cortices, or, ii) directly pressure-injected into lumbar ventral spinal cord using a glass microcapillary in spinally mounted, halothane anesthetized rats. Four days after infection, cells were fixed with 4% paraformaldehyde and stained for GluR1 receptor and combined with GFAP or NeuN staining. Similarly, animals injected spinally with HIV1 vector were perfusion fixed with 4% paraformaldehyde 4 days after injections. Frozen transverse spinal cord sections (10-15mm) were stained with a specific GluR1 antibody and glial (GFAP) and neuronal (NeuN) markers.

Staining with anti-GluR1 antibody of control cultures infected with HIV1-shLuc vector revealed highly specific expression of GluR1 in a subpopulation of neurons and astrocytes. This expression pattern was similar as seen in control non-infected cultures. In contrast, in cultures infected with HIV1-GluR1-shRNA, near complete block of GluR1 expression was seen in both neurons and astrocytes. Western blot analysis of cell membrane protein with anti-GluR1 antibody revealed significant decrease of GluR1 protein in HIV1-GluR1-shRNA infected cells.

Staining with NeuN and GluR1 antibody of spinal cord sections taken from animals injected with HIV1-GluR1-shRNA showed a potent downregulation of GluR1 receptor. In contrast no change in GluR1 staining pattern in HIV1-shLuc injected animals was detected.

These data demonstrate that by using HIV1-GluR1-shRNA lentivirus, it is possible to achieve a potent downregulation of GluR1 receptor in both neurons and astrocytes. Such approach provides implications in treatment of neuropathological disorders associated with increased a-motoneuronal activity as seen in patients with spinal injury-induced spasticity.

964. Adenoviral-Mediated Expression of Brain-Derived Neuroptrophic Factor or Neurotrophin-3 Promotes Sprouting of Lesioned Serotonergic Axons in the Central Nervous System

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We tested whether adenoviral-mediated expression of the neurotrophins Brain-Derived Neurotrophic Factor (BDNF),

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