Using genetically modified stem cells to halt the progression of ALS

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Abstract—The following was completed as part of the 2011 Route 28 Summit at the International Symposium on Neural Regeneration. The topic of the Route 28 Summit was, "Novel Ways to Exploit Stem Cells for Recovery of Central Human Nervous System Function." Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the loss of motor neurons leading to paralysis and death. The vast majority of ALS cases are idiopathic; however, at least 2% are caused by mutation of the copper-zinc superoxide dismutase 1 gene on chromosome 21. Here, we propose a three-pronged approach: (1) identify the molecular trigger for the onset of symptomatic ALS using a microray approach, (2) develop a genetically modified cell-based treatment, and (3) restore lost respiratory function once disease progression has been halted by an implanted stem cell treatment.

BACKGROUND AND SIGNIFICANCE

ALS is a neurodegenerative disease affecting about 30,000 Americans [1]. The typical timecourse of the disease from onset to death is two to five years. Most cases of ALS are idiopathic and the precipitating factor in genetic cases is yet unknown. Currently, the only approved clinical treatment is Riluzole, which blocks glutamatergic transmission in the CNS [2]. Clinical trials have been conducted with varied success using modified stem cells [3–4], anti-glutamatergic factors [5–7], and neurotrophic factors [8]. Further investigation is needed to determine the cause and molecular triggers of the ALS, and the development of an effective treatment. First, we propose an extensive microarray study using induced pluripotent stem cells (iPSCs) derived from patients with ALS-SOD1 to determine what molecular change occurs at the onset of symptomatic ALS. Second, we propose a novel intervention/therapy using genetically modified autologous hematopoietic stem cells. Finally, we present a simple method for restoring respiratory function in patients using stem cells to form interneuronal relays.

PROPOSED STUDY AND METHODS

Hypothesis Statement

We hypothesize that stem cells can be modified to deliver protective factors to the CNS in order to halt the progression of ALS.

Aim 1: To Determine the Molecular Trigger For Motor Neuron Death And Symptom Presentation in ALS-SOD1 Patients

We will use gene microarray technology to investigate the gene expression profiles of cells from ALS-SOD1 patients before and after onset of symptoms, and cells from healthy subjects (controls). Fibroblasts will be harvested from ALS-SOD1 patients (n = 20) and age matched controls (n = 5) every four months over the five year period during which symptomatic onset typically occurs. The fibroblasts will be transformed into iPSCs which will be induced to become motor neurons, oligodendrocytes, astrocytes, microglia and macrophages [9–12]. Since the initiating trigger for ALS is not known, all of these cell types need to be investigated. Mixed cell cultures of all possible combinations of ALS-SOD1 and control cells will be grown. mRNA will be isolated from each culture condition, hybridized to the Affymetrix GeneChip Human Genome U133 Plus 2.0 array for microarray analysis, and fold changes will be calculated. The resulting gene data sets will be further analyzed with Ingenuity Pathway Analysis (IPA, Ingenuity Systems, Inc.) for comparison analysis. Gene expression patterns that correlate with disease progression and cell type will be identified. For this exercise, we hypothesize that we will find transcription regulators that correlate with symptomatic progression. Based on current literature, we propose that these transcription factors will include c-Fos and JunD because the expression levels increase dramatically at the time symptoms are observed [13]. Identifying these molecular triggers will allow for therapeutic interventions that target these molecules and their related signaling pathways.

Aim 2: To Develop Hematopoietic Derived Monocytes Modified to be Protective Against ALS While Retaining the Innate Ability to Home to Lesioned Areas

In order to deliver therapeutic factors to the sites of neuronal loss in ALS, macrophages derived from autologous bone marrow derived hematopoietic cells by standard protocols [14] will be used to

home to areas of inflammation by endogenous mechanisms. Similar cell types have been shown to home to areas of inflammation in myocardial infarction and glomerular nephritis [15–16]. These cells will then be infected with multiple replication incompetent lentiviral expression vectors to drive the cells toward a wound healing macrophage phenotype, alleviate the damage caused by ALS, and allow for elimination of these cells at later times. Cell lines will then be generated that stably express these factors. Gene expression in all viral vectors will be driven by the MMP-9 promoter.

As presented in the **Figure**, IL4 and IL13 will be used to drive monocytes into an M2 type macrophage phenotype with wound healing properties [17]. To alleviate damage caused by ALS, insulin-like growth factor-1 (IGF-1), somatostatin, c-Jun N-terminal kinase inhibitor (D-JNK-1) and excitatory amino-acid transporter 2 (EAAT2) will be expressed. IGF-1 promotes cellular proliferation, cellular differentiation and inhibition of apoptosis when activated. Although unsuccessful in clinical trials when delivered by subcutaneous injection [18], IGF-1 was shown to exert neuroprotective effects in a mouse model of ALS when delivered by lenti-viral vector [19], and has also shown increases in mesenchymal stem cell engraftment when expressed by transplanted cells [15]. Somatostatin and D-JNKI-1 inhibit c-Fos and JunD, respectively, and, turn off the trigger of ALS that we (hypothetically) derived from our microarray studies [13,20–22]. EAAT2, which increases glutamate re-uptake at the synaptic cleft, will reduce the excitotoxic effect of glutamate in ALS [5,23–24]. Herpes simplex virus-thymidine kinase (HSV-TK) generates monocyte susceptibility to Ganciclovir [25], allowing removal of any excess cells.

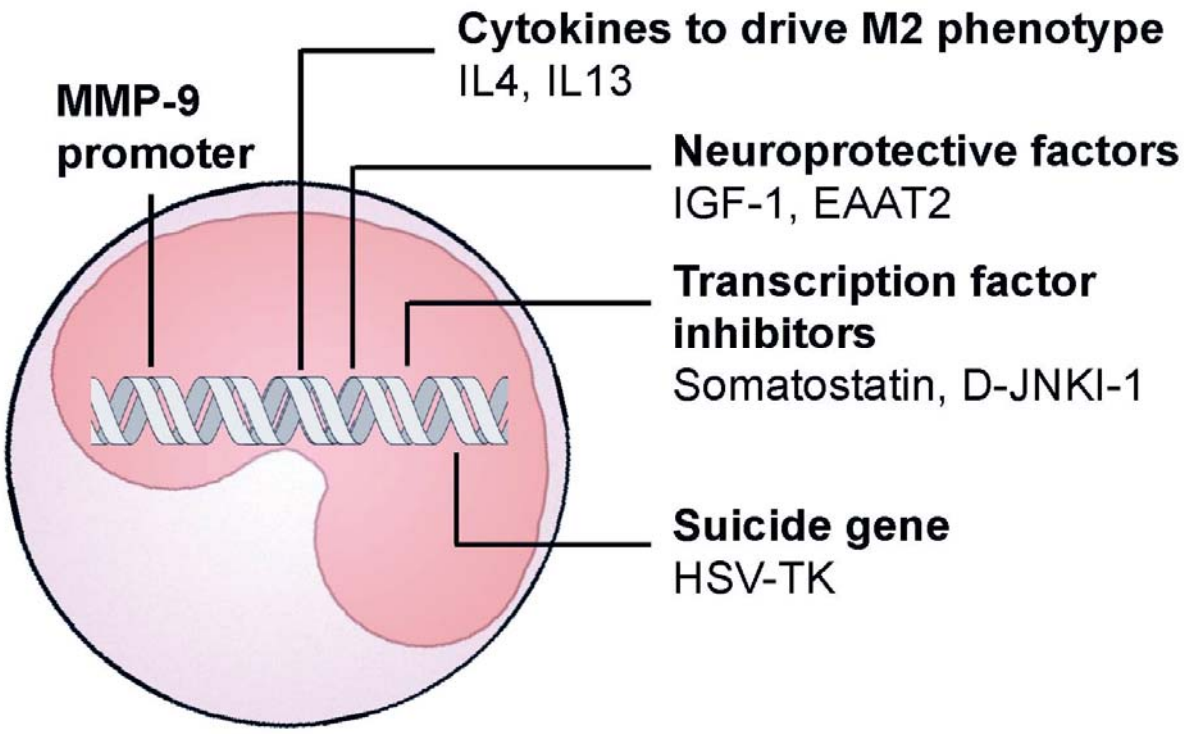

Figure. Proposed genetic modifications of hematopoietic derived monocytes.

We will transfer these modified monocytes into a SOD1-G93A mouse model of ALS using an established femoral vein systemic delivery technique [26]. After transplantation, animals will be monitored. Once symptoms have diminished or stabilized, animals will undergo a blood-brain barrier (BBB) integrity test using IV injection of biotin conjugated dextran [27]. At the point where the dextran is no longer found outside of the blood vessels in sectioned tissue, we will administer Ganciclovir conjugated to a high molecular weight dextran to prevent travel across the BBB and restrict HSV-TK mediated cell death to areas outside of the CNS. In order to prevent excess cell death due to the bystander effect, we will administer dexamethasone concomitantly [28].

Aim 3: To Augment Respiratory Function in a Rodent Model of ALS Once Disease Progression is Halted by Our Treatment Protocol

We will use the SOD1-G93A rat model to test whether autologous bone marrow derived

hematopoietic cells driven to become neural precursor cells (NPCs) [29–30] can promote improved respiratory behavior. NPCs will be stereotactically transplanted in the cervical spinal cord at the level of the phrenic motor nucleus of the transgenic rats. Several segmental injections will be used to deliver cells and populate the area around the phrenic motor neuron pool. NPCs transplanted in similar fashion have been shown to develop into interneuronal phenotypes that become integrated into the phrenic motor pathway and alter respiratory patterns [31–32]. Baseline plethysmographic and electrophysiological parameters will be evaluated and compared to post-transplant time points.

DISCUSSION AND CONCLUSIONS

This proposal describes an innovative approach to understanding and treating ALS. Three challenges are addressed: identification of a precipitating factor in development of ALS symptoms, application of a systemic treatment that will be able to reach the entire CNS in a biologically relevant way, and treatment of the devastating loss of respiratory function seen in late stages of the disease. However, this approach is currently technically unfeasible. First, discovery of the molecular trigger for ALS would require approximately 285,000 microarray chips to analyze all the mixed cell cultures proposed. Such an undertaking would be very expensive and require an enormous amount of labor for tissue processing and data analysis. Allowed unlimited resources, as we were in this exercise, we were freed from this limitation. Second, it is doubtful that a single cell could be stably transfected with as many genes as we have proposed and secrete all these factors at clinically relevant levels. However, this could be approximated with several genetically modified cells being co-transplanted. Transplantation of NPCs to augment respiratory function is feasible but would be insufficient for treating ALS without a treatment to halt or slow the progression of the disease. The idea that transcription factors are the key molecules for the progression of neurodegenerative diseases is being pursued [33] and, therefore, may yet prove to be part of the molecular trigger for symptomatic ALS. Focusing on the factors that lead to progression of the disease instead of the causative factors has the potential to extend the application of these results beyond the SOD1 form of ALS to the idiopathic cases as well.

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