The role of fat cells in treatment of ALS

Amyotrophic lateral sclerosis is a (heterogeneous) neurodegenerative disorder with a variable site of disease initiation and rate of progression. There are no established blood or CSF-based markers that outperform clinical observation to provide an early diagnosis, accurate prediction of disease progression, or clinical stratification of ALS phenotypic variants. Proinflammatory cytokines, reactive oxygen species, and pro-inflammatory lipid-derived compounds result in neuronal damage and supply a positive feedback loop of neuroinflammation. Pathways of inflammation have multiple and redundant initiation sites and that means that many proinflammatory cytokines can compensate in the absence of any single factor. Thus, it is unlikely that efforts to target a single factor in humans will provide significant therapeutic benefit in patients with ALS. It may be necessary to develop pluripotent treatments that can regulate multiple steps in inflammatory pathways.

Since many factors affect the onset and progression of ALS, it is important to identify biomarkers that aid the identification of drugs that influence and mitigate the damage. Targets are prevention of motor neuron loss and neuromuscular denervation. One approach to identifying biomarkers, as well as potential treatment targets, is by analyzing signals from adipose tissue cells (ATC's). The population of endothelial cells (supporting cells in fat tissue that contain stem cells) change over time and understanding how disease changes cell phenotype may be useful. If detrimental changes can be identified, patients can receive patient-specific treatments. An *in vitro* analysis of an individual's current disease state may direct treatment and is a worthy goal. Patient derived stem cells are active areas of investigation in ALS therapies and could provide useful treatment information.

Stem cells are used as a restorative therapy in ALS. The risk of damaging the nervous system and exacerbating disease with stem cells is apparent. In addition to physical damage, the transplanted cells are not homogenous across patients or appropriate at all stages of disease. Autologous (donated by the same person that is receiving them) stem cells are sensitive to their microenvironment. Consideration of the microenvironment is appropriate to both the source and destination of the transplanted cells.

Locally, motor neuron oxidative stress, as it occurs in ALS, is made worse by induction of uncoupling proteins. Uncoupling proteins disassociate specific energy (metabolic) pathways from mitochondria, often with harmful outcomes. Interestingly, in disease there is increased aerobic glycolysis, mitochondrial cell stress, low ATP and uncoupling protein upregulation that results in intracellular toxicity. In diabetes and mild obesity, there is an association with increased blood glucose that may be protective against ALS.

The pathogenesis of ALS may be due to a "crosstalk" between vascular endothelium in the central nervous system and cells in adipose (fat) tissue. Theoretically, as subclinical disease progresses to a clinical state, the crosstalk could signal and alter the adipose tissue. One possible effect is called "browning". Browning increases the beige/brown fat population, this fat produces heat at the expense of energy to the mitochondria. A local outcome of browning is an "uncoupling of ATP production from oxygen consumption in the electron transport chain. Mitochondrial uncoupling leads to futile cycling of ATP synthase, consuming oxygen, expending calories, and producing heat" (Yang 2017). We propose to evaluate ATC's from ALS patients for markers that would indicate browning.

When cells are cultured *in vitro* they secrete cytokines and growth factors, the media is called secretome. Paracrine secretions from stem cells found in some secretome benefit survival when tested in animal models of ALS. It is useful to study cell secretions provided in secretome/conditioned media

from *in vitro* culture of stem cells and their ability to modulate the immune and inflammatory pathways associated with ALS. It is possible that the secretome could effect a neuroprotective function for motor neurons. In this way, beneficial components associated with stem cells are administered to the patient once the correct consortium of agents are recognized. Stem cells are sourced from multiple tissues, ATC may prove uniquely beneficial.

Critical information to understand the application of stem cell secretome/conditioned media is adequately characterizing the effective components mediating therapeutic benefits and optimizing the medium for clinical application. A proposed source of conditioned media is from adipose stem cells, ASC. We anticipate that the diseased environment may influence the phenotype of ASC's and propose to evaluate the phenotype difference between normal and diseased donor derived ASC's.

The progress toward a stem cell based, cell-free therapy in humans has a precedent. The dysfunction and function of endothelial cells can be determined. Analysis of ASC's could provide a snapshot of an individuals disease and indicate an appropriate therapy.