P3-136  A GENETICALLY IMMORTALIZED HUMAN STEM CELL LINE: A PROMISING NEW TOOL FOR ALZHEIMER'S DISEASE THERAPY

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Background: Amyloid-β peptide and hyperphosphorylated tau are the main pathological hallmarks of Alzheimer’s disease (AD) (Reitz et al., 2014). Given the recent failure of several large-scale clinical trials and the lack of disease-modifying pharmacological treatments, there is an urgent need to develop alternative therapies. CTX0E03 is a clinical-grade human neural stem cell line which has recently passed Phase I trials in people with stroke (Hick et al., 2013). However, this line has not been investigated in other neurodegenerative disorders. Methods: CTX0E03 cells were seeded into laminin-precoated 96-well plates at cell density 5x10^4 cells/ml. Cells were treated with amyloid peptides (Aβ1-40 and Aβ1-42) at concentrations of 0.5, 1, 5, 10, and 15 μM and oka-daic acid (OA) at 0.5, 1, 5, 10, and 15 nM. Vehicle control cultures were treated with 0.1% DMSO or PBS (control for OA and Aβ respectively). After 24 h incubation, the cells were examined for cell viability using PrestoBlue reagent and lactate dehydrogenase-cytotoxicity assay. Results: Cell viability assays showed a concentration dependence of this cell line to the toxic effects of Aβ1-42, but not Aβ1-40, and OA. Notably, CTX0E03 cell line displayed toxicity at concentrations significantly higher than both rat neural stem cells and those previously reported for primary cultures. Conclusions: Our study indicates the ability of clinical grade CTX0E03 stem cell line to resist the inhospitable milieu associated with AD. Thus, CTX0E03 stem cells provide a potential candidate for cell therapy in AD patients.

P3-137  AFIQ INTERACTIONS WITH TCF7 TO FACILITATE NEURAL STEM CELL PROLIFERATION

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Background: ALL1-fused from chromosome 1q (AF1q), originally considered as an oncogenic factor, has been implicated in the pathogenesis of eurodegeneration. AF1q is highly expressed during neurodevelopment, but its specific functions and molecular mechanisms in neural system remained elusive. Methods: Luciferase reporter assay, Co-IP, Edu cell proliferation assay, Western Blot, Site-directed mutagenesis. Results: Our study here demonstrated that AF1q facilitated neural stem cell proliferation. Since previous studies have shown WNT signaling being involved in neural stem cell proliferation, we examined whether AF1q could induce cell proliferation by activating Wnt signaling pathway. Reporter assay showed AF1q could activate WNT signaling. And communoprecipitation (Co-IP) analysis demonstrated that AF1q bound specifically to T-cell factor/lymphoid enhancer binding factor-7 (TCF7/LEF7), which stabilized TCF7 and facilitated TCF7 translocation into nucleus. Additionally, we identified the amino acid 11-20 on AF1q is sufficient for the binding of TCF7. Furthermore, the phosphorylation of Serine 11 on AF1q is required for the binding of TCF7. The AF1Q-S11F mutant decreased the activation of WNT signaling and was unable to induce cell proliferation. Conclusions: Our study here identified AF1Q as an important factor in neurodevelopment by interacting with TCF7 and regulating WNT signaling pathway.

P3-138  DEVELOPMENT OF IPS-C-BASED BIOMARKERS TO IDENTIFY THE PATIENT POPULATION RESPONSIVE TO ALLOPREGNANOLONE

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Background: Alzheimer’s disease (AD) is a national and global epidemic with complex pathoetiology including compromised brain metabolic activity and decreased regenerative capacity. Allopregnanolone (Allo) is an investigational neuroregenerative therapeutic, currently in Phase 1b clinical trial for AD (NCT02221622, https://clinicaltrials.gov/ct2/show/NCT02221622?term=NCT02221622&rank=1). In rodent preclinical models, Allo promotes neural stem cell (NSC) proliferation and neural differentiation and improves mitochondrial function. To develop biomarkers to predict regenerative response to Allo, we have initiated proof of concept analyses to determine the impact of Allo on human induced pluripotent stem cells (iPSCs) and iPSC-derived neural cells. Methods: T-cells from a patient with familial AD due to the A431E presenilin-1 point mutation were reprogrammed via a non-integrating, non-viral method, to iPSCs. Additional iPSCs were provided by the University of California Irvine Alzheimer’s Disease Research Center (UCI-ADRC) and the Institute for Memory Impairments and Neurological Disorders. Isogenic iPSCs were generated using CRISPR-Cas9. Using dual inhibition of SMAD signaling, iPSCs were differentiated to NSCs. Mitochondrial respiration and regenerative capacity were determined using metabolic analyzer and FACS. Results: Mitochondrial respiration and proliferation analyses were conducted in AD-derived and healthy control iPSCs and NSCs. Initial data indicates that AD iPSCs have similar proliferation rates, but increased ATP production compared to healthy controls. Analyses were conducted to determine the regenerative and bioenergetic effect of Allo. In iPSC-derived NSCs, Allo increased basal mitochondrial respiration by 78% and maximal mitochondrial respiratory capacity by 35%. Conclusions: Initial data indicate that iPSCs from AD patients demonstrate a metabolic phenotype distinct from healthy controls and that Allo improves mitochondrial function of iPSC-derived NSCs. Going forward the effect of Allo on the regenerative capacity and metabolic phenotype of iPSC-derived NSCs will be evaluated. These data will form the foundation for developing the first regenerative biomarker to determine and monitor response to therapeutics. Research supported by NIH National Institute on Aging U01AG031115 and U51AG046148 to RDB; NIH/NINDS R00-NS07743 and the Donald E. and Delia B. Baxter Foundation to JKI; NIH National Institute on Aging AG005142 to HCC; UCI-ADRC funded by NIH/NIA Grant P50 AG16573; USC Provost Fellowship, CIRM Predoctoral Research Traineeship, and American Foundation for Pharmaceutical Education Fellowship to CMS.

P3-139  INDUCTION OF NEURONAL DIFFERENTIATION, TAU EXPRESSION, AGGREGATION AND PHOSPHORYLATION BY GROWTH FACTOR REMOVAL AND ACIDIC PH CORRELATES WITH AN ENHANCED PROTEIN CLEARANCE AND RECYCLING IN HUMAN HIPPOCAMPAL NEURAL PRECURSOR CELLS (HIPPNPCS)

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