Abstracts for Oral Presentations, Thursday, Sept 27th

Oral Session: Proteostasis, Seeding/Spreading of Protein Aggregates I

1:00-1:20 p.m. Wilfried Rossoll (Mayo)

Intracellular transport defects in TDP-43 proteinopathies

The cytoplasmic mislocalization and aggregation of TDP-43 is a common histopathological hallmark of the amyotrophic lateral sclerosis and frontotemporal dementia disease spectrum (ALS/FTD). However, the composition of aggregates and their contribution to the disease process has long remained unknown. We have used proximity-dependent biotin identification (BioID) to interrogate the interactome of detergent-insoluble TDP-43 aggregates and found them enriched for proteins associated with components of the ER-Golgi trafficking endomembrane system, as well as the nucleocytoplasmic transport machinery. Aggregated and disease-linked mutant TDP-43 triggered the sequestration and/or mislocalization of nucleoporins and transport factors, and interfered with nuclear protein import and RNA export in ALS models. These data strongly implicated TDP-43-mediated nucleocytoplasmic transport defects as a common disease mechanism in ALS/FTD. An unexpected additional finding was that while some of these nucleocytoplasmic transport factors co-aggregated with TDP-43, certain transport proteins act as modifiers of cytoplasmic TDP-43 aggregation and TDP-43 mediated toxicity, suggesting potential novel targets of therapeutic intervention for TDP-43 proteinopathies.

1:20-1:40 p.m. Cara Croft (UF)

Proteinopathies in a dish: a platform for therapeutic discovery and mechanistic insight

Intracellular inclusions are hallmark pathologies found across a spectrum of neurodegenerative proteinopathies including Alzheimer's and Parkinson's disease. Specifically, mature neurofibrillary tau inclusions like those in Alzheimer's disease have only been observed in transgenic rodent models. These models limit throughput, show heterogeneity and are relatively expensive to maintain and age. Similarly, α-synuclein pathology has predominantly been studied in vivo with similar drawbacks or in vitro in single cell types lacking the milieu of the diseased brain. This has somewhat slowed both therapeutic development and mechanistic insight into these neurodegenerative proteinopathies

We have developed an approach to recapitulate mature tau and α-synuclein inclusions like those seen in Alzheimer's and Parkinson's disease, respectively, in a three-dimensional, cytoarchitecturally and functionally intact system. We transduced organotypic brain slice cultures (BSCs) from post-natal mice using recombinant adeno-associated viruses (rAAVs) to express human wild-type (WT) or mutant MAPT or SNCA and maintained them ex vivo for several weeks. We found that BSCs transduced with mutant MAPT progressively develop a multitude of sarkosyl-insoluble, filamentous, Thioflavin S positive inclusions between 7 and 28 days ex vivo resembling mature tau pathology and in extended culture periods begin to show neuronal loss. In addition, BSCs expressing human SNCA develop Lewy body-like inclusions comprised of insoluble phosphorylated α-synuclein. These BSC models have been treated chronically with small molecule compounds or cotransduced with other genes of interest to identify appropriate therapeutic targets.

These rAAV-based BSC models of neurodegenerative proteinopathies can provide a cost-effective and facile alternative to in vivo studies. This system can be used to determine novel therapeutic strategies against tau and α-synuclein inclusions and provide a platform to understand mechanisms of neurodegeneration.

1:40-2:00 p.m. – Valerie Joers (Emory)

Cannabinoid type 2 receptors regulate peripheral and central inflammation following alpha-synuclein overexpression in the substantia nigra

Trafficking of immune cells into the brain is a normal response to injury, yet there are key regulatory mechanisms that are impaired during disease that alter the homeostatic central-peripheral crosstalk of the immune system and increase immune cell infiltration into the brain further promoting central inflammation, microglia activation and disease progression. The cannabionoid type 2 (CB2) receptor is highly expressed on activated microglia and circulating monocytes and is upregulated in the substantia nigra of Parkinson's disease (PD) patients. Research from others suggest CB2 receptors on immune cells exert an immunosuppressive effect and may critically control progression of neurodegeneration, yet its precise role in regulating peripheral and central immunity following alpha-synuclein overexpression is not known. Here we report the peripheral and central immunomodulatory effects following treatment with a novel CB2 inverse agonist, SMM-189, in an AAV2/5-hAsyn rat model of PD. We hypothesize that targeting CB2 will benefit the central inflammatory environment by inhibiting activation of central and peripheral immune cells and promoting phagocytosis and clearance of Asyn to protect against PD-like pathology. Spraque-Dawley rats were unilaterally injected in the nigra with low (3E11) titers of AAV2/5-hAsyn and followed for 8 weeks. One week later, animals were randomly selected to receive daily systemic SMM-189 (6 mg/kg) or vehicle. Our preliminary results indicate SMM-189 decreased PBMC gene expression of pro-inflammatory marker TNF ($p=0.0084$) while promoting anti-inflammatory behavior with increased TGFβ ($p=0.0204$). Analyses by flow cytometry demonstrated reduced circulating monocyte subsets and their MHCII intensity after 7 weeks of SMM-189 treatment. Additionally, nigral IBA1+ cell size was significantly decreased in SMM-189-treated ($p<0.0001$) compared to vehicle-treated, suggesting that targeting CB2 ameliorates persistent microgliosis from Asyn overexpression. We are currently completing analyses of circulating and central immune populations following SMM-189 treatment in a cohort of animals with moderate nigral degeneration.

2:00-2:20 p.m. – Na Zhao (Mayo)

ApoE4 promotes α-synuclein aggregation and pathology-induced behavioral deficits in a mouse model of synucleinopathy

Apolipoprotein E (APOE) ε4 allele is the strongest genetic risk factor for late-onset Alzheimer's disease mainly by driving amyloid-β pathology. Most recently, APOE4 has also been discovered as a strong risk gene for dementia with Lewy bodies (DLB). However, how apoE4 drives DLB risk and whether it involves effects directly on α-synuclein pathology are not clear. Here we generated a mouse model of synucleinopathy using AAV gene delivery system in human apoE isoform-targeted replacement (TR) mice. We found that apoE4 significantly increases α-synuclein aggregation and pathology compared with apoE2 and apoE3. Consistent with a functional effect of α-synuclein aggregates, we also found that apoE4 accelerated behavioral abnormalities including motor and memory deficits. Our data demonstrate a pathogenic role of apoE4 in driving α-synuclein pathology and related behaviors and provide pathogenic insights on how APOE4 increases the risk of DLB.

Oral Session: Proteostasis, Seeding/Spreading of Protein Aggregates II

2:35-2:40 p.m.– Rita Cowell (Southern Research)

Defining cellular identity and cell-specific expression changes in the pre-formed fibril model of Parkinson Disease

Parkinson Disease (PD) is a neurodegenerative movement disorder that is characterized by post-mortem observation of two pathological hallmarks including loss of dopaminergic (DAergic) neurons of the substantia nigra (SN) and alpha-synuclein aggregates (Lewy bodies and Lewy neurites). Current therapies for PD focus on managing symptoms after substantial loss of DAergic neurons with no current method of prevention of neurodegeneration. Elucidation of the underlying causes of neuronal cell dysfunction has the potential to provide new therapeutic targets to prevent loss of DAergic neurons. Post-translationally modified alpha-synuclein is the major component of filamentous inclusions in neurons throughout the brain in PD. Addition of alpha-synuclein preformed fibrils in vivo or in culture leads to corruption of endogenous alpha-synuclein over time, resulting in inclusions, defects in neuronal function, and cell death. It is not known how DAergic neurons respond to inclusion formation; defining their transcriptional profile during the cell death process could identify pathways for preventing cell death. However, it is very difficult to identify and sort inclusion-containing neurons for traditional transcriptomic analyses. To enable quantification of transcripts in cells containing alpha-synuclein aggregates, we optimized a new technique combining RNAscope fluorescent in situ hybridization with immunofluorescence for phosphorylated alpha-synuclein. With this protocol, we demonstrate that neurons with inclusions are glutamatergic and always express mRNA for alpha-synuclein, consistent with the model that inclusion formation requires cell-autonomous endogenous alpha-synuclein expression. Interestingly, inclusions were not observed in GABAergic neurons, which express very low levels of alpha-synuclein mRNA. In the future, we will use this method to track transcriptional changes in a cell-specific manner during the process of inclusion formation and cell death.

2:40-3:00 p.m. – Talene Yacoubian (UAB)

14-3-3θ regulates cell-to-cell spread of alpha-synuclein

Research points to a prion-like mode for alpha-synuclein (αsyn) toxicity: αsyn is released as aggregated species that cause further aggregation and toxicity in neighboring cells. 14-3-3s are chaperone-like proteins that reduce protein aggregation and regulate protein secretion. We tested the effect of 14-3-3s on αsyn propagation in an αsyn fibril model. The formation of insoluble S129-phosphorylated (pS129) αsyn inclusions was reduced in 14-3-3θ transgenic neurons at 10 and 14 days after fibril treatment. We also observed a dramatic reduction in neuron loss at 14 days after fibril treatment. In contrast, we observed that 14-3-3 inhibition by the pan 14-3-3 peptide inhibitor difopein potentiated αsyn aggregation and toxicity. pS129-αsyn-positive aggregation was increased in difopein neurons after fibril treatment compared to control. Neuronal loss induced by fibrils was observed earlier in difopein cultures at ten days after treatment, at a time point when neuronal loss is normally not observed in wildtype cultures. At 14 days after treatment, difopein cultures showed a 31% increase in neuronal death compared to wildtype. To test whether 14-3-3θ blocks cell-to-cell spread of pathogenic αsyn, we used microfluidic culture devices with three compartments connected by microgrooves. We plated wildtype or 14-3-3θ neurons in the first compartment and wildtype neurons into the second and third compartments. Fibrils were added to the first compartment. At 14 days, pS129-αsyn was detectable in all chambers when wildtype neurons were plated in all compartments, with a gradient of highest pS129-αsyn in chamber one to lowest pS129-αsyn in the most distal chamber. When 14-3-3θ neurons were plated in the first compartment, pS129-αsyn staining was dramatically reduced in all compartments, with almost no detectable pS129-αsyn in chambers 2 and 3. When difopein neurons were plated in the first compartment, we saw a significant increase in pS129-αsyn positive aggregates in chambers 2 and 3. We conclude that 14-3-3θ regulates αsyn propagation and may serve as a target for therapeutic intervention in Parkinson's disease.

3:00-3:20 p.m. – Xu Hou (Mayo)

Selective autophagy in Alzheimer's disease and related dementias

Alzheimer's disease (AD) is characterized by neuronal loss and the deposition of intra- and extracellular aggregates consisting of tau and amyloid beta (Aβ). Age-related decline of autophagy/lysosomal degradation is accumulatively suggested to contribute to neurofibrillary tangle and senile plaque accumulation. Dysfunctional mitochondria are common and among the earliest events observed in the progression of AD. Mitophagy, a cargo-specific autophagy- lysosomal pathway for removal of damaged mitochondria, constitutes a key cellular pathway in mitochondrial quality control. Failure of mitophagy is a feature of both familial and sporadic AD and appears to play an early key role in AD pathophysiology. Both Aβ/APP and tau have been shown to induce mitochondrial stress directly or indirectly through alterations of the flux through the autophagy-lysosome system. Upon mitochondrial stress, PINK1 and Parkin cooperatively label damaged mitochondria with phosphorylated ubiquitin (pS65-Ub) to tag them for destruction. The selective clearance of damaged organelles ensures the integrity and function of the mitochondrial network thereby preventing cell death. We have generated antibodies against pS65-Ub as specific and sensitive tools to monitor and quantify mitophagy in neurons and in brain tissue. Here, we set out to quantify pS65-Ub in human post-mortem brain sections from age-matched neurologically normal and pathological aging controls as well as from early and late stage AD patients. On the whole tissue level, we observed significant increases of pS65-Ub levels in both early and late stage AD across selectively vulnerable regions. The increase of the mitophagy tag was closely associated with tau pathology and granulovacuolar degeneration bodies (GVBs) that are thought to be autophagic remnants of incomplete degradation. On the single cell level, and similar to GVBs, pS65-Ub levels appeared to increase early on with tangle development, but disappeared in cells with fully mature tau pathology. Altogether, our study provides novel hints to potential mechanisms underlying the mitophagy deficits in AD and suggests that pS65-Ub may serve as an early marker and quantitative trait to identify modifiers of disease pathogenesis.

3:20-3:25 – Rachael Earls (UGA)

Intracerebral injection of pre-formed alpha-synuclein fibrils into mice alters immune cell profiles

Parkinson's disease (PD) is pathologically characterized by the accumulation of alpha-synuclein (α-syn), a highly soluble presynaptic protein that is the major component of Lewy bodies (LBs). Within the central nervous system (CNS), extracellular α-syn aggregates act as a damage-associated molecular pattern (DAMP) for microglia and promote a pro-inflammatory response. Various autoantibodies against α-syn species (monomer, oligomer and fibrils) were detected in the cerebrospinal fluid and blood in PD patients, implicating its effect on peripheral immune cells. The purpose of this study is to evaluate whether pre-formed fibrils (PFF) α-syn-induced synucleinopathies alter immune cell profiles in the periphery and the CNS. Recombinant human α-syn fibril seeds or monomers were intrastriatally injected into mice and then the systemic and central immune responses were evaluated at 5 months post injection. Immune cell profiling was performed by flow cytometry analyses on blood, lymph node, spleen and the brain. We confirmed synucleinopathies in the cortex, striatum and midbrain in PFF α-syn mice but not in monomer α-syn mice. In blood, we observed a significant increase in frequency of the natural killer (NK) cell population in PFF α-syn mice compared to monomer α-syn mice. We observed significant increases in the frequencies of B cells, monocytes, and neutrophils but decreased T and NK cells in the lymph node while there were no significant changes in the spleen. In the brain, we observed increased percentages of B, T, activated myeloid cells (CD11b+CD45hi) and NK cells in PFF α-syn mice implicating substantial peripheral immune cell infiltration into the brain. Collectively, our study provides novel information regarding intracerebral-initiated synucleinopathies altering immune cell profiles both in the periphery and the CNS.

3:25-3:45 – Marion Delenclos (Mayo)

Mitochondrial SIRT3 in Parkinson's disease

The sirtuins are highly conserved nicotinamide adenine dinucleotide (NAD+)-dependent enzymes that have long been known for cell survival, metabolism, lifespan determinants and recently have shown beneficial effects in the context of neurodegeneration. Mitochondrial sirtuins, especially sirtuin3 (SIRT3), play a major role in maintaining mitochondrial integrity, regulation of mitochondrial function and in the prevention of oxidative stress. Mitochondrial dysfunction is central to the progression of Parkinson's disease (PD) and mutations in mitochondrial-associated proteins are found in familial cases of PD. Here, we demonstrate that the presence of alpha-synuclein (αsyn) oligomers induce a corresponding decrease in mitochondrial SIRT3 activity and decreased mitochondrial biogenesis in experimental PD-like models as well as post mortem brain of Lewy Body Disease patients. Decreased SIRT3 levels are accompanied by changes in mitochondria dynamics, and decreased mitochondrial respiration that can be rescued with the AMP-activated protein kinase (AMPK) agonist AICAR (5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside). Our data suggest that pharmacologically increasing SIRT3 levels can counteract αsyn-induced mitochondrial dysfunction by normalizing mitochondrial bioenergetics and confirm SIRT3 as a target for therapeutic intervention for Lewy body diseases.

Oral Session: Disease Mechanism

5:00-5:05 – Danielle Gulick (USF)

Inhibition of casein kinase 1ε and δ reduces circadian disruption and improves behavior in APP-PS1 mice

There is no effective treatment to restore circadian rhythmicity in AD, largely because it remains unclear how the molecular circadian clock interacts with the mechanisms of AD pathology. Casein kinase 1 (CK1) isoforms ε and δ, key circadian regulators, are significantly upregulated in AD. Both CK1ε and δ phosphorylate tau and contribute to its pathologic aggregation, whereas only CK1ε increases β-amyloid (Aβ) production. However, this work was done in cell lines, which limits examination of regional changes in pathology. In the current studies, we have examined how inhibition of CK1δ alone, or CK1 ε and δ, impact regional Aβ and circadian gene expression in APP-PS1 mice, and assessed circadian, cognitive, and affective behavioral correlates of these neural changes. Here, the circadian rhythms of 10-13 month old APP-PS1 mice were observed under normal lighting and constant darkness (DD) in wheel-running chambers. Mice were then pseudo-randomized and treated with either vehicle or the CK1 inhibitor PF-670462 (CK1i; at 10mg/kg, δ isoform selective, or 30 mg/kg, □ and □ selective) for 7 days in DD. Finally, brain tissue was harvested for immunofluorescent examination of A□ and circadian gene expression in the hippocampus, suprachiasmatic nucleus, and prefrontal cortex. In Experiment 2, mice were similarly pseudo-randomized, and run through a behavioral battery. At baseline, mice showed a short period, fragmented activity patterns, and hyperactivity in the sleep phase, as well as impaired cognitive performance in both prefrontal- and hippocampus-dependent tasks. Both doses of CK1i reduced overall activity and fragmentation, whereas only the δ-isoform selective dose improved cognition and only the dose targeting both isoforms lengthened the period. Further, CK1i appeared to dose-dependently break up plaques – overall Aβ signal decreased in the hippocampus and suprachiasmatic nucleus, but in the hippocampus, plaque quantification revealed increasing numbers of plaques with CK1i dose, but a decrease in average plaque size.

5:05-5:15 – Shon Koren (UF)

Tau impairs translational selectivity via interacting with ribosomal proteins

Pathological tau species disrupt many neuronal and cognitive processes, but identifying the molecular mechanisms behind that dysfunction remains a major challenge. We recently determined that tau levels correlate with differential translation of RNA subgroups in Alzheimer's disease (AD) brain, and tau expression in vitro reversibly reduces protein synthesis. Therefore, we hypothesized that tau directly alters selective transcript affinity of the ribosome, impairing translation. We measured protein synthesis by coupling RNA microarrays and a novel puromycin-based method to detect nascent translation in vivo in the TET/OFF rTg4510 mouse model of tauopathy. As expected, tau overexpression substantially modifies the transcriptome, with a subset of affected pathways rescued by tau suppression. Our nascent proteome method involves intraperitoneal injections of puromycin, a tRNA analog that stably incorporates into growing polypeptide chains. Puromycin immunostaining reveals a 60% decrease in cortical protein synthesis in rTg4510. Immunoprecipitating for puromycin-tagged proteins followed by LC-MS/MS analysis shows distinct shifts in the nascent proteome. Strikingly, comparing the nascent proteome to the transcriptome reveals tau reversibly impairs the synthesis of ribosomal proteins. We validated these results in human AD brains by focusing on rpS6, a ribosomal protein which regulates the selectivity of translational machinery depending on cellular stress signals. We show that the filtering activity of rpS6, as measured by its phosphorylation, inversely correlates with tau pathology. Tau and rpS6 form a stable complex in human brain with an increased association in AD brain. This association correlates with impaired protein synthesis of transcripts under translational control by rpS6 in AD brain. Furthermore, this rps6 dysfunction is reversibly dependent on tau expression in vitro. Taken together, we report strong evidence that tau pathology derails the selective translational capability of the ribosome via direct association, potentially impairing ribosome function overall, and thereby progressing neurodegenerative disease.

5:15-5:20 – Zainuddin Quadri (USF)

The role of eIF5A in TDP-43 pathology in FTD; the tip of the iceberg?

TAR DNA-binding protein 43 (TDP-43) is a nuclear RNA/DNA binding protein that associates with Frontotemporal disorders. The clinical manifestations include motor neuron degeneration (ALS) and cognitive decline (FTD-TDP-43). FTD remains the second most common form of early-onset dementia after Alzheimer Disease. The hallmark of TDP-43 proteinopathy is nuclear loss-of-function and accumulation of nuclear and cytoplasmic TDP-43 inclusions, which acquire toxic gain-of-function. The unique post-translational modification of eIF5A; hypusination (eIF5AhypK50), within the hypusination loop denotes its activation and cytoplasmic localization where it further interacts with specific RNA binding proteins. eIF5A is implicated in translational elongation and translation silencing of certain mRNA in stress granules (SG). Together with our findings we posit that active eIF5A is position as a stress-response protein. Our data shows aberrant increase in enzymes responsible for hypusination in brain tissue from AD patient, TDP-43 animal models and arsenite-induced stress cellular models, suggesting that aberrant hypusination underlies the progression of disease. Further, we show that arsenite-induced stress induces interactions between eIF5AhypK50 and cytoplasmic TDP-43. We also find that eIF5AhypK50 binds TDP-43 and stress granule protein TIA-1 during pathology and arsenite-induced stress. Importantly, we find that pharmacological inhibitor of hypusination and sited-directed mutagenesis induces acetylation of eIF5A at lysine 47 (eIF5AacK47) resulting in significant reduction of phosphorylated and total TDP-43 in the cytoplasm and SG. We further confirm that potentiation of spermidine/spermine N1-acetyltransferase 1 (SSAT1) acetylates eIF5A and reduces the TDP-43 phenotype in cellular models. Hence, we argue that post-translational modifications specifically, hypusination vs. acetylation increases or subverts TDP-43 pathology, respectively. We predict that eIF5AhypK50 regulates TDP-43 fate via several potential mechanisms, including protein-protein binding properties, increasing TDP-43 cytoplasmic retention or perturbing the nucleocytoplasmic shuttling of TDP-43 via affecting the nuclear transport machinery. Here, we will discuses the strategies and approaches that we have employed to dissect the mechanism of action through which eIF5A affects TDP-43 pathology in FTD disorders and related dementia.

5:20-5:25 – Rachna Manek (UF)

The 5'UTR of ATXN1 is alternatively spliced and post-transcriptionally regulates Ataxin-1 expression

Molecular strategies aimed at suppressing Ataxin-1 protein levels in mouse models of Spinocerebellar Ataxia Type-1 (SCA1) have shown therapeutic promise in preclinical studies. Thus, understanding the endogenous cellular mechanisms that regulate Ataxin-1 expression, and how this regulation may be disrupted in SCA1, is key to the development of novel, clinically relevant therapeutics. Here we seek to better define how Ataxin-1 levels are regulated post-transcriptionally by evaluating the role that the 5'-untranslated region (5'UTR) of ATXN1 plays on the expression of Ataxin-1, in the context of normal and CAG-expanded transcripts. The 5'UTR of Ataxin-1 is unusually long (> 900bp), extending throughout at least 7 of the 9 exons that normally comprise the ATXN1 transcript. RT-PCR, quantitative PCR (qPCR), and single-allele analyses by PacBio SMRT sequencing of the 5'UTR region in control and SCA1 human brain tissue revealed some novel findings. First, a previously unidentified 5'UTR alternatively spliced variant lacking exons -2 and -3 appears to be the major ATXN1 transcript expressed in the brain. Second, there are alterations to 5'UTR ATXN1 splicing patterns in SCA1 human cerebellum when compared to 5'UTR ATXN1 splicing in control brains. Finally, an EGFP-based reporter assay showed that the 5'UTR of ATXN1 acts to repress Ataxin-1 expression, post-transcriptionally. Importantly, alternative splicing of the 5'UTR impacts the extent of this repression, suggesting a role for altered 5'UTR ATXN1 splicing in disease pathogenesis. Studies aimed at identifying the mechanism(s) by which the 5'UTR sequence and its alternatively spliced variants regulate Ataxin-1 expression revealed a potential role for uORFs and uAUGs, as deletion or mutation of all ATG codons in the 5'UTR ATXN1 sequence leads to greater than 3-fold rescue of EGFP expression in our reporter assay. Our data reveals a previously unknown role for the 5'UTR of ATXN1 in the regulation of Ataxin-1 levels and implicates mis-regulation of ATXN1 5'UTR alternative splicing in the pathogenesis of SCA1.

5:25-5:35 – April Darling (USF)

Repeat Problems: Combinatorial Effect of C9orf72-Derived Dipeptide Repeat Proteins

A microsatellite expansion mutation in C9orf72 is the most common genetic cause of sporadic and familial Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). The expansion mutation leads to C9orf72 loss of function, RNA foci, and five species of non-AUG RAN translated dipeptide repeat proteins (GA, GP, GR, PA, and PR). More than one dipeptide repeat protein species can be present in the same cell in a patient with the expansion mutation, however the interplay between the species has not been well established. We determined that PR localizes to the nucleus, causes cytotoxicity, triggers the phosphorylation of stress granule initiating proteins PERK and eif2α, induces the spontaneous formation of poorly dynamic stress granules, and undergoes liquid-liquid phase transitions in-vitro. However, co-transfection of PR with GA altered PR's localization and cytotoxic outputs. Additionally, we showed that the interaction between GA and PR leads to structural and morphological changes not seen when GA is with other highly charged polypeptides. Thus, the combined expression of distinct C9orf72-derived dipeptide repeat species produces cellular outcomes and structural differences that are unique compared to the expression of a single species.

5:35-5:40 – Aseel Eid (FIU)

DDT Exposure Increases Amyloid Precursor Protein Levels and Amyloid-Beta Pathology: Mechanistic Links to Alzheimer's Disease Risk.

The interaction of senescent-related, genetic, and environmental factors significantly contributes to the etiology of late-onset, sporadic Alzheimer's disease (AD). We previously reported that serum levels of p,p'DDE, a metabolite of the pesticide DDT, was significantly higher in patients with AD and associated with risk of AD diagnosis. Further, brain levels of DDE were similar to levels found in in the blood of 10 matched samples (Richardson et al., 2014). Here we report that in 20 post-mortem AD brains, detectable levels of DDE were present in every brain (14.5 – 43.7 ng/g), and that levels in the temporal lobe were similar to those in the cerebellum, indicating that DDE levels are uniform throughout the brain. However, the mechanisms by which DDT may contribute to AD pathogenesis is unknown. Here, we demonstrate that DDT exposure significantly increased the mRNA levels of APP, PSEN1 and APOE in differentiated SHSY5Y cells, as well as App mRNA and protein levels in mouse primary hippocampal neurons. This was accompanied by increased Aβ secretion in SHSY5Y cells exposed to DDT, which was abolished by the sodium channel antagonist tetrodotoxin. Treatment with 3 mg/kg DDT every 3 days for 30 days in male wild-type mice significantly increased APP mRNA and protein levels (~25%), along with increased mRNA expression of Nep (77%) and ApoE (150%) in the hippocampus. Similarly, the cortex of female 3xTG mice at 12 months of age exhibited significantly increased Aβ42 levels in the hippocampus, and Aβ40 and Aβ42 in the cortex following exposure to 3 mg/kg DDT for 3 months, along with enhanced plaque pathology in the female 3xTG mice exposed to DDT. These data, combined with our previous epidemiological findings, identify a potential mechanism by which DDT exposure may contribute to increased risk of AD. Supported in part by NIH R01ES026057, R01ES026067-S2 and P30ES005022.