Alzheimer’s Disease: From Genes to Therapies in the Whole Genome Era

Dr. Rudolph E. Tanzi, PhD
Professor of Neurology, Harvard Medical School
Director, Genetics and Aging Research Unit, Massachusetts General Hospital

Heibert Lounge (L-14)

12:00 pm  | Keynote Lecture
1:15 pm    | Lunch and Short Talks

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**LIST OF ABSTRACTS**
Exosomal tau transcytosis mechanism through neuron-microglia interaction

H Asai, S Ikezu, T Ikezu

Boston University School of Medicine, Massachusetts, USA

In the early Braak stage of AD neuropathology, neurofibrillary tangles (NFTs) is confined in the layer II trans-entorhinal cortex (EC) region, then stereotypically disseminate into the dentate gyrus (DG) of hippocampal fields. Recent studies suggest that the spread of aggregated tau is mediated by trans-synaptic dissemination from EC neurons to the granular neurons of DG, however, the mechanism is currently unknown.

Neuroinflammation is closely associated with tauopathy, and mononuclear phagocytes may play important roles in exosomal secretion of intracellular molecules. We hypothesized that microglia played a major role in the scavenging and secretion of tau species via exosomes in the central nervous system.

To test this hypothesis, we first investigated whether microglia secrete tau protein in exosomes in vitro. Recombinant human tau protein was phagocytosed by primary cultured mouse microglia and subsequently secreted via exosome after sequential stimulation with lipopolysaccharide and ATP. The tau proteins in the exosomal fraction were readily transferred into the primary cultured mouse cortical neurons.

Next, we tested the involvement of microglia and exosome in dissemination of tau aggregation in mouse models in vivo. We have successfully developed a rapid tau dissemination model from the EC to DG by stereotaxic injection of AAV6-synapsin-1-promoter-P301L tau and GFP into EC layer II/III. Selective depletion of microglia by intracerebroventricular injection of clodronate liposome reduced tau dissemination to DG region. Furthermore, pharmacologic inhibition of exosome secretion also reduced the dissemination of tau. Taken together, our results suggest that both microglia and exosome are significantly involved in dissemination of aggregated tau.
Cleavage sites determination of the anti-aging protein Klotho

**Ci-Di Chen**, Ella Zeldich and Carmela R. Abraham

Boston University School of Medicine, Department of Biochemistry

Shedding of membrane proteins is a critical regulatory step in many normal and pathological processes. The anti-aging protein Klotho (KL) is shed from the cell surface by ADAM10 and ADAM17. However, the cleavage site is unknown. There are two major cleavage sites in KL: one close to the juxtamembrane region and another one between the KL1 and KL2 domains in KL. We attempted to identify the cleavage motif of KL by mutating potential sheddase(s) recognition sequences and examined the secretion of KL extracellular fragments in COS-7 cells by transfection and western blot. We report that deletion of amino acids T958 and L959 results in 50-60% reduction in KL shedding, and an additional mutation of P954E results in further reduction of KL shedding by 70-80%. Deletion of amino acids 954-962 resulted in 94% reduction in KL shedding. The deletion mutant does not change the subcellular localization of KL as demonstrated by immunofluorescence, and it does not interfere with its function as a FGFR co-receptor as tested by FGF23 signaling assay. Mutation in the juxtamembrane region results in reduction in secretion of both the 130 kDa and 70 kDa fragments, suggesting that cleavage between the KL1 and KL2 domains is dependent on the C-terminal cleavage. Our results shed light into KL shedding and provide a target for potential pharmacologic intervention aiming on regulating KL secretion and alpha-secretase activities.
A Genome-wide Meta-analysis of Plasma Clusterin Concentration in the CHARGE Consortium

V Chouraki\textsuperscript{1,2}, J Jakobsdottir\textsuperscript{3}, H Adams\textsuperscript{4}, D Levy\textsuperscript{1,5}, K Mather\textsuperscript{6}, V Gudnason\textsuperscript{3}, M. A Ikram\textsuperscript{4}, S Seshadri\textsuperscript{1,2}

\textsuperscript{1} The Framingham Heart Study, MA; \textsuperscript{2} Boston University School of Medicine, MA; \textsuperscript{3} The Icelandic Heart Association, Iceland; \textsuperscript{4} Erasmus MC, the Netherlands; \textsuperscript{5} National Heart, Lung, and Blood Institute, MD; \textsuperscript{6} UNSW Medicine, Australia

The clusterin gene, CLU, is a genetic risk factor for late onset Alzheimer’s disease (AD). Clusterin, also called apolipoprotein J, may help clear A\textsubscript{\beta} from the brain, and plasma clusterin levels have been associated with greater cognitive impairment in AD. We performed a genome-wide meta-analysis of plasma clusterin concentration to understand the genetic determinants of plasma clusterin, and provide insight into pathophysiological mechanisms underlying AD. Using genome-wide data imputed to the 1000G Phase 1v3 reference panel, and linear regression models adjusted for age, sex, and population stratification in 11148 participants from the Framingham, Age, Gene/Environment Susceptibility (AGES), Rotterdam, and Older Australian Twins (OATS) studies, we found suggestive associations (p<1x10^{-6}) near the TOX3 gene on chromosome 16, previously associated with breast cancer and neuronal survival, and in the LDLR-AD4 gene on chromosome 18, associated with blood pressure, schizophrenia, bipolar disorder and pancreatic adenocarcinoma. These interesting results need to be confirmed, and we are expanding our sample to include the AddNeuroMed, Sydney Memory and Aging (MAS), Baltimore longitudinal study of aging (BLSA) and Hunter Valley studies.
Non-Gaussian water diffusion in the aging white matter

J.-P. Coutu\textsuperscript{1,2}, J.J. Chen\textsuperscript{1}, H.D. Rosas\textsuperscript{1}, D.H. Salat\textsuperscript{1,3}

1. MGH/MIT/HMS Athinoula A. Martinos Center for Biomedical Imaging, MA;  
2. Massachusetts Institute of Technology, MA;  
3. VA Boston Healthcare System, MA

Age-associated white matter degeneration has been well-documented and is likely an important mechanism contributing to cognitive decline in older adults. Recent work has explored a range of non-invasive neuroimaging procedures to differentially highlight alterations in the tissue microenvironment. Diffusion kurtosis imaging (DKI) is an extension of diffusion tensor imaging (DTI) that accounts for non-Gaussian water diffusion and can reflect alterations in the distribution and diffusion properties of tissue compartments. We used DKI to produce voxel-based maps of mean, axial and radial diffusional kurtoses, quantitative indices of the tissue microstructure’s diffusional heterogeneity, in 111 participants ranging from 33 to 91 years of age. Greater age was associated with regional reductions in all three DKI metrics with prominent effects in prefrontal and association white matter compared to relatively preserved primary motor and visual areas. Although DKI metrics co-varied with DTI metrics on a global level, DKI provided unique regional sensitivity to the effects of age and were useful in combination with DTI metrics for the multivariate classification of regions according to their pattern of relative age effect sizes, which may represent different types of diffusional processes with aging. These results suggest that DKI provides important complementary indices of brain microstructure for the study of brain aging and neurological disease such as Alzheimer’s disease.
Emotional perception in Alzheimer’s disease: Contributions to the caregiving experience

**R.T. Daley**$^{1,2}$ and M.K. O’Connor$^{1,2}$

$^1$Boston University Medical Center, MA; $^2$Bedford Veterans Administration Hospital, MA

While primarily considered a memory disorder, Alzheimer’s disease (AD) pathology also affects the amygdala, resulting in emotional perception changes, such as ability to recognize emotion through facial expression and tone of voice. This study investigates (1) whether AD patients perform worse on tasks of emotional perception compared to healthy older controls (OC) and (2) whether changes in emotional perception influence the experience of spousal caregivers (SC).

Emotional perception tasks from the Advanced Clinical Solutions (ACS) test were administered to both ADs and OCs: tasks of affect naming, prosody-face matching, prosody-pair matching, and emotion labeling. The Montreal Cognitive Assessment was administered, controlling for global cognitive deficits. SCs completed an interview and three caregiving questionnaires.

Our preliminary results (data collected from 5 OCs and 7 ADs and SCs) revealed significant difference in performance on the ACS for OCs ($M = 35.20$, $SD = 7.259$) compared to ADs ($M = 24.86$, $SD = 4.67$), because ADs performed worse on the task, $F(1,10) = 9.13$, $p = .01$. Correlations were run and as emotional perception decreased so did relationship satisfaction, $r = .23$, $p = .61$. Emotional perception decreased as caregiver burden increased, $r = -.70$, $p = .08$. And emotional perception decreased as neuropsychiatric symptoms severity, as rated by the caregiver, increased, $r = -.42$, $p = .35$. Preliminary results trended toward study hypotheses despite small sample size.
A Potential molecular mechanism of tau accumulation and dissemination via collapsin response mediator protein-2 and tau-tubulin kinase 1

S Ikezu, H Asai, T Ikezu

Boston University School of Medicine, Massachusetts, USA

Tau-tubulin kinase 1 (TTBK1) is specifically expressed in neurons, activates Cdk5, and enhance tau phosphorylation both in vitro and in vivo. Genetic variations of the TTBK1 gene are associated with late-onset Alzheimer’s disease (AD) in two large cohorts of Chinese and Spanish populations. We have created transgenic mice model of TTBK1 and reported that cognitive impairment was a major phenotype of this mice model. However, its precise mechanism is uncovered. We hypothesize that TTBK1 regulates axonal growth via phosphorylation of microtubule-associated molecules, such as tau and collapsin response mediator protein-2 (CRMP2).

Transient expression of TTBK1 in SH-SY5Y cells showed significant phosphorylation of CRMP2 at Thr-514. This phosphorylation was accelerated by fresh amyloid beta treatment and was blocked by overexpression of dominant negative-Rho. Furthermore, transient expression of TTBK1 in primary cultured mouse hippocampal neurons induces reduction of axonal length and branching.

Next, we crossed TTBK1 transgenic mice with Tg2576 expressing Swedish familial AD mutant of amyloid precursor protein (APP). APP/TTBK1 double transgenic mice showed severe spatial learning impairment, and somatic accumulation of phosphorylated CRMP2 in layer II/III of entorhinal cortex and dentate gyrus of the hippocampus, suggesting the axonal collapse in the affected regions.

These results suggest that up-regulation of TTBK1 accelerates axonal degeneration in entorhinal cortex and hippocampus, which is physiologically involved in learning and memory and affected in AD brain. The study also supports TTBK1 as a potential therapeutic target of AD.
Distinguishing dementia with Lewy bodies from Alzheimer’s disease using EEG

H. Lee, G.J.F. Brekelmans, G. Roks
St. Elisabeth Ziekenhuis, the Netherlands

Present diagnostic criteria for dementia with Lewy bodies (DLB) regard EEG abnormalities as a supportive feature. Still the use of the EEG as a diagnostic tool to differentiate between DLB and Alzheimer’s disease (AD) remains rare in daily practice in part because of the absence of a reliable scoring method. Roks et al. (2007) used the Grand Total EEG (GTE) score to differentiate between DLB and AD with good sensitivity and specificity.

We reproduced the former study and visually rated EEGs using the GTE score in 29 patients with DLB and 54 with AD recruited from a memory clinic in a general hospital in the Netherlands.

Patients with DLB had significantly higher median scores than patients with AD: 9 vs. 4. ROC analyses revealed that patients with DLB could be distinguished from those with AD at a GTE cut-off score of 6.5 with a sensitivity of 79% and a specificity of 76%. The association between GTE and DLB was independent of age, gender, Mini Mental State Examination and medication. Frontal intermittent rhythmic delta activity (FIRDA) was found in 17.2% of patients with DLB compared to 2% with AD.

The GTE score has proven to be a reliable and easy to apply scoring method. The EEG can help to differentiate between DLB and AD with good sensitivity and specificity. Future revisions of the diagnostic criteria for DLB should consider FIRDA as a suggestive feature.
Effects of Space Radiation on Hippocampal-Dependent Learning and Neuropathology in Wild-Type and Alzheimer’s Disease Transgenic Mice

J. A. Moncaster$^{1,2}$, M. Wojnarowicz$^1$, A. Fisher$^{1,2}$, C. Tagge$^{1,2}$, S. Sarangi$^{1,2}$, O. Minaeva$^{1,2}$, K. Gopaul$^3$, S. A. Miry$^3$, P. K. Stanton$^3$, K.A. Bjornstad$^4$, P. Chang$^4$, E. A. Blakely$^4$, L. E. Goldstein$^{1,2}$

$^1$Molecular Aging and Development Laboratory, Boston University, School of Medicine, Boston, MA; $^2$Photonics Center, Boston University, Boston, MA; $^3$Massachusetts General Hospital, Harvard Medical School, Boston, MA; $^4$Children’s Hospital Boston, Boston, MA. $^5$University of Washington School of Medicine, Seattle, WA.

The hippocampus and dentate gyrus are critically important brain regions required for long-term memory formation. Damage to these critical brain regions contributes to memory deficits in patients with Alzheimer’s disease. Exposure to space radiation can result in impairments in learning and long-term reduction in hippocampal neurogenesis. It is unknown how radiation causes these impairments and whether and by what mechanism(s) radiation exposure might predispose individuals to develop Alzheimer’s disease.

Mice are irradiated with $^{56}$Fe and $^{28}$Si ions at Brookhaven National Laboratory, NY. Alzheimer's disease pathology will be assessed in the brain post-mortem by immunohistochemical and biochemical methods.

Our studies directly address key objectives of the NASA Human Space Flight Program, including determination of potential space-related SR dependencies related to late CNS risks such as early-onset dementia or Alzheimer’s disease, assessment of SR effects on molecular, cellular and tissue environment changes in hippocampus indicative of increased risk of dementia or Alzheimer's disease, and evaluation of biological models of Alzheimer's disease or other forms of dementia that occur in humans. The existing knowledge gap is immense and presents a major obstacle to rational assessment of short- and long-term risk to the central nervous system posed by SR exposure expected during extended human space travel.

Our experiments will examine the mechanisms by which space radiation impairs synaptic function in normal brain and assess whether space radiation enhances long-term risk of Alzheimer's disease.
Cyclin G-Associated Kinase Expression is Increased in Parkinson’s Disease Brain and Affects A-Synuclein Expression in Vitro

M. Nagle, J. Latourelle, A. Dumitriu, RH. Myers

Boston University School of Medicine

Parkinson’s Disease (PD) is pathologically characterized by the formation of cellular aggregates primarily composed of the protein a-synuclein. The genetic contribution to the risk of acquiring PD is not well understood; however, our group previously identified a single nucleotide polymorphism (SNP) near the Cyclin G-associated Kinase (GAK) gene, whose minor allele is associated with significantly increased PD risk. The GAK protein is important in the dissociation of clathrin from coated vesicles; however, its relationship to PD and a-synuclein is unclear. We assayed the prefrontal cortex of 49 control and 29 PD human brains for transcriptomic expression using RNA-seq analysis. We observed several exons with significantly increased expression in the clathrin binding and J domains of GAK in PD versus controls. Conversely, carriers of the GAK risk SNP displayed reduced expression of these exons relative to non-carriers in an additive model. Expression of a-synuclein (SNCA) exons were found to be reduced in PD versus controls; however, the reverse was observed in carriers of the GAK risk SNP. We next sought to understand the effect of GAK expression on a-synuclein levels in HEK293 cells. When GAK is over-expressed, we observed a decrease in expression of a-synuclein. These results suggest a relationship between a-synuclein levels and GAK expression in PD and indicate that transcripts containing these specific exons may be involved in PD pathogenesis.
Falling on deaf ears: gamma generation impaired following systemic administration of the cholinergic antagonist scopolamine in rats

Newman, E.L.¹, Gillet, S.N.², Climer, J.R.¹, & Hasselmo, M.E.¹

¹Boston University, MA; ²University of California, San Diego, CA

Acetylcholine release is greatly reduced in Alzheimer’s patients. We explored the influences of systemic blockade of muscarinic acetylcholine receptors on the activation dynamics of the superficial layers of the medial entorhinal cortex (sMEC) in rats. These layers are among the first anatomical areas to show cell loss during the progression of Alzheimer’s disease. We focused our analyses on the fast rhythmic activity observed in the local field potential (LFP) known as gamma. Gamma is believed to reflect inter-regional communication and to mediate the induction of neural plasticity. In healthy individuals, gamma power is coupled to the phase of a 6 – 9 Hz theta rhythm and this coupling is predictive of successful encoding of new memories. In our study, rats ran laps on a circle track for food rewards as we collected LFP recordings in the sMEC before, during and after the influence of systemically administered scopolamine, a muscarinic antagonist. We found that systemic blockade of muscarinic receptors selectively decreased the power of 60 – 120 Hz gamma rhythm and altered the pattern of coupling between gamma and theta. These shifts in power and coupling would be expected to result in deficits in inter-regional communication and memory encoding and may account for some of the observed behavioral symptoms in Alzheimer’s patients.
Non-invasive detection of Alzheimer’s disease Aβ pathology in the lenses of people with Down syndrome

S. Sarangi1,2, O. Minaeva1,2, J.A. Moncaster1, N. Casey1, R. H. Webb1,2,3, D. Ledoux4, J.I. Clark5, D.G. Hunter4, L.E. Goldstein1,2

1Molecular Aging and Development Laboratory, Boston University, School of Medicine, Boston, MA; 2Photonics Center, Boston University, Boston, MA; 3Massachusetts General Hospital, Harvard Medical School, Boston, MA; 4Children’s Hospital Boston, Boston, MA. 5University of Washington School of Medicine, Seattle, WA.

The hallmark pathology in Alzheimer’s disease (AD) is characterized by age-related deposition in the brain of amyloid-β peptides (Aβ) which eventually results in plaque formation. Aβ is a cleavage product derived from the amyloid precursor protein (APP) encoded on chromosome 21. DS is the most common chromosomal disorder in humans and carries 100% risk of early-onset AD due to chromosome 21 triplication. Triplication of the APP gene (21q21) results in increased expression of APP and Aβ overproduction. We discovered that Aβ accumulates in the supranuclear region of the lens in patients with AD (Goldstein et al., Lancet, 2003) and DS (Moncaster et al., PloS One, 2010). These Aβ lens aggregates qualify as Raleigh scattering centers that clinically manifest as distinctive specific supranuclear lens opacities that are phenotypically, anatomically, and biochemically distinguishable from common age-related nuclear cataracts. We use quasi-elastic light scattering (QLS) to detect and monitor these aggregates in vivo and in vitro. QLS measurements were performed on DS subject and controls. We demonstrate early age-related increases in light scattering from the lens of subjects with DS compared to age-matched controls. In vitro and AD transgenic mouse studies confirm Aβ dose- and time-dependent increases in QLS light scattering.

Conclusions: QLS can be used to detect early AD-linked Aβ pathology in the lenses of human subjects with Down Syndrome.
Correlational Analysis of Five Commonly Used Measures of Cognitive Functioning and Mental Status:

**Solomon, T.M.**¹,² Debrois, G², Budson A.E¹, Mirkovic, N², Murphy, C², & Solomon, P.R.²,³

¹Boston University Alzheimer’s disease Center, Boston, MA; ²The Memory Clinic, Bennington, VT; ³Williams College, Williamstown, MA

Alzheimer’s disease (AD) continues to be the most prevalent form of dementia. Currently, there are numerous measures for detecting the presence of dementia and quantifying its severity and progression. We analyzed the relations between scores on 5 commonly used measures (Mini Mental State Exam, Montreal Cognitive Assessment, Alzheimer’s Disease Assessment Scale-cognitive subscale, Activities of Daily Living Scale and Global Deterioration Scale) of 101 successive admissions to a memory clinic. Patients were only included in the analysis if they received a diagnosis of Mild Cognitive Impairment due to Alzheimer’s disease pathophysiological process or probable Alzheimer’s disease and if all five measures were administered during the same evaluation. Regression analysis yielded 20 linear equations that allow for conversion between test scores on any two measures. Results indicated that all five measures analyzed were highly correlated ($r = .67$-$86$). ($p < .001$). Further, participants were grouped by MMSE scores with regard to level of disease severity, allowing for the creation of a quick reference table for estimating an approximate score range between measures. Results from this study provide a useful tool for clinicians when comparing between multiple different instruments that measure the mental status and functional ability of individuals with Alzheimer’s disease and MCI due to AD pathology.
The effect of repetitive mild traumatic brain injury on the pathologies of Alzheimer disease

T.D. Stein\textsuperscript{1,2,3}, V.E. Alvarez\textsuperscript{3}, A.C. McKee\textsuperscript{1,3}

VA Boston Healthcare System\textsuperscript{1}; Bedford VA Medical Center\textsuperscript{2}; Boston University School of Medicine\textsuperscript{3}, MA

Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease induced by repeated mild traumatic brain injuries (TBIs). It is a tauopathy characterized by neurofibrillary tangles and tau-positive processes that preferentially involve the cortical sulci, medial temporal lobe, diencephalon, and brainstem. In CTE the tau pathology is predominantly subpial, perivascular, and within the sulcal depths. This tau pathology may be due to vascular damage, disruptions of the blood brain barrier, and axonal injury-alterations that also have been linked to Alzheimer disease (AD). In fact, trauma is a known risk factor for AD, and multiple mild TBIs may play a causative role in the development of AD as well as CTE. Thus, we hypothesize that the distribution of AD pathologies is altered by the mechanical injury that occurs in those subjects with both CTE and AD (CTE-AD). Immunohistochemistry, confocal microscopy, and quantitative ELISA were performed using antibodies against beta-amyloid, tau, GFAP, and MAP-2 on fixed or frozen post-mortem brain tissue from subjects with CTE-AD and compared to subjects with CTE or AD alone. Protein levels at the depths of sulci were compared to gyral crests in brain regions both affected and not affected by CTE. In CTE-AD subjects, \( \alpha \)-\text{beta} is significantly greater within the sulcal depths as compared to the gyral crests. The increased sulcal beta-amyloid is associated with marked tau pathology within neuronal processes. In conclusion, \( \alpha \)-\text{beta} and tau deposition are altered in AD subjects with CTE and a history of repetitive mild TBIs, suggesting that trauma may initiate or accelerate these pathologies.
AAV2/1-mediated gene delivery of CD200 into the hippocampus enhances neurogenesis and amyloid clearance in the APP mouse

MM Varnum¹, T Kiyota², K Ingraham¹, S Ikezu¹, T Ikezu¹

¹Boston University School of Medicine, Boston, US; ²University of Nebraska Medical Center, Omaha, US

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive decline of cognitive function and memory formation. Up-regulation of inflammatory cytokines, microglial activation, and toxic Aβ in the AD brain negatively affect the ability of neural stem cells to mature and survive. CD200 is an anti-inflammatory glycoprotein expressed in many cell types, including neurons, and its receptor is expressed in myeloid cells, like microglia. It has been shown in AD patients and mouse models that an age-related increase in microglial activation is accompanied by an age-related or Aβ-induced decrease in CD200. We hypothesize that chronic neuroinflammation in AD contributes to reduced neurogenesis in the hippocampus and that this suppression can be restored by gene delivery of CD200. In this study the AD transgenic mouse model expressing an APP mutant was intrahippocampally injected with adeno-associated virus expressing CD200 at the pre-symptomatic disease stage. Following treatment with CD200, mice were assessed for Aβ load and neurogenesis. CD200 expression enhanced proliferation and differentiation of neural stem cells in the dentate gyrus and reduced Aβ load. These data indicate that CD200 may be able to delay the onset of AD-like pathology and restore neurogenesis, thus giving it potential as a therapeutic against neurodegeneration.
Accelerated neurodegeneration and neuroinflammation in transgenic mice expressing P301L tau mutant and tau-tubulin kinase 1

**Woodbury ME, Asai H, Ikezu S, Yonemoto GMS, Cui L, Ikezu T**

Boston University School of Medicine, MA, USA

Tau-tubulin kinase-1 (TTBK1) is a central nervous system (CNS)-specific protein kinase implicated in the pathological phosphorylation of tau. TTBK1 transgenic mice show enhanced neuroinflammation in the CNS. Double transgenic mice expressing TTBK1 and FTDP-17-linked P301L (JNPL3) tau mutant (TTBK1/JNPL3) show increased accumulation of oligomeric tau protein in the CNS, and enhanced loss of motor neurons in the ventral horn of the lumbar spinal cord. To determine the role of TTBK1-induced neuroinflammation in this tauopathy-related neuropathogenesis, age-matched TTBK1/JNPL3, JNPL3, TTBK1, and non-transgenic littermates were systematically characterized. There is a striking switch in activation phenotype and population of mononuclear phagocytes (resident microglia, infiltrating macrophages) in the affected spinal cord region: JNPL3 mice show accumulation of alternatively activated microglia, whereas TTBK1 and TTBK1/JNPL3 mice show accumulation of classically activated infiltrating peripheral monocytes. Further, primary cultured mouse motor neurons show axonal degeneration by transient expression of TTBK1 gene or after treatment with conditioned media derived from lipopolysaccharide-stimulated microglia, which is partially blocked by silencing of endogenous TTBK1 gene in neurons. These data suggest that TTBK1 accelerates neurodegeneration of motor neurons by recruiting pro-inflammatory infiltrating monocytes and by enhancing sensitivity to neurotoxicity in inflammatory conditions.
G3BP1 Protects Against Pathological Tau-Induced Neurotoxicity

Katherine L. Youmans¹, M. Medalla², T. Vanderweyde¹, J. L. Luebke², B. L. Wolozin¹,³

Departments of Pharmacology and Experimental Therapeutics¹, Anatomy and Neurobiology², and Neurology³, Boston University School of Medicine

Alzheimer's disease (AD) is a devastating neurodegenerative disease that was originally diagnosed over 100 years ago, yet no disease modifying treatment strategies currently exist. Tau is a microtubule binding protein normally expressed in all neurons throughout the brain, but during AD hyperphosphorylated tau forms pathological aggregates to generate the neurofibrillary tangles that are a hallmark of AD. P301L mutant tau has been shown to exacerbate this pathological aggregation in human brain, leading to tauopathies. A hypothesis in the field is that these aggregated species of tau are neurotoxic, and that their removal may reverse or lessen the cognitive deficits associated with disease. The Wolozin lab has identified stress granules (SG) as a major component of AD pathology. SGs accumulate in the brains of subjects with mild cognitive impairment (MCI) and AD, and in many cases are physically distinct from classic markers of AD pathology. This finding provides insights into putative biological pathways mediating neurodegeneration in AD, as well as novel opportunities for early disease diagnosis. RNA binding proteins are a large group of proteins that regulate RNA translation through formation of RNA/protein complexes. Stress, such as that occurring during neurotoxicity or inflammation, induces alterations in the pattern of protein synthesis, and these stress-induced regulatory events are mediated in part by SGs. SGs are formed via reversible aggregation of RNA and protein, and mediate critical modifications in RNA processing, including sequestration, splicing, degradation and translation. This project focuses on understanding the role of RNA binding proteins, particularly Ras GTPase Activating Protein Binding Protein-1 (G3BP1), in the progression of tau pathology at the neuronal cell body and the synapse. G3BP1 stabilizes tau mRNA and is selectively absent from neurons containing pathological tau filaments, while the RNA binding protein TIA-1 promotes apoptosis and may exacerbate tau aggregation. Our data indicates that G3BP1 reduces levels of pathological tau in primary cortical neurons and immortalized cells, and this reduction is mediated in part by interactions between G3BP1 and tau mRNA. In addition, G3BP1 rescues the effects of P301L tau on spontaneous excitatory postsynaptic currents (EPSCs) in pyramidal neurons in vitro and increases action potential firing rates, suggesting that this RNA binding protein may induce functional changes at the synapse. With these findings, we hope to reveal a potential new therapeutic target by which to modulate pathological tau in vivo, which could translate to the human condition for the prevention or treatment of AD and age-related neurodegenerative tauopathies.
Klotho protects hippocampal neurons from oxidative stress via regulation of the redox system

Zeldich E, Abraham CR
Boston University School of Medicine, Boston, MA

Generation of reactive oxygen species (ROS), leading to oxidative damage and neuronal cell death plays an important role in the pathogenesis of neurodegenerative disorders including Alzheimer’s disease. The present study aimed to examine the mechanism by which the anti-aging protein Klotho exerts neuroprotective effects against oxidative stress in neurons. Pretreatment of hippocampal neurons with recombinant Klotho protected them from glutamate-induced cytotoxicity, which was reflected by decreased cell death, reduced accumulation of ROS and reduced release of LDH to the medium. In addition, primary hippocampal neurons obtained from Klotho overexpressing mouse embryos were more resistant to glutamate-induced oxidative stress, as compared to neurons from WT littermates. An anti-oxidative stress array revealed Klotho-induced upregulation of members of the thioredoxin/peroxiredoxin (Trx/Prx) system and the induction of Prx-2 in particular, which was confirmed at the mRNA and protein levels. Klotho-induced phosphorylation of PI3K/Akt pathway was associated with sustained inhibitory phosphorylation of forkhead box O3a (FoxO3a) and was found as essential for the protective effect of Klotho and the induction of Prx-2. Downregulation of Prx-2 expression using a lentivirus harboring shRNA almost completely abolished Klotho’s ability to rescue the neurons, suggesting that the induction of Prx-2 is a key modulator of neuroprotection. The induction of Prx-2 was associated with an activation of NRF-2. Our results demonstrate, for the first time, the neuroprotective role of Klotho and reveal a novel mechanism underlying this effect.