P2561

Board Number: B714

Modeling Protein Aggregation and the Heat Shock Response in ALS iPSC-derived Motor Neurons.

E.R. Seminary[1], S.L. Sison[1], A.D. Ebert[1];
[1]Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder caused by the selective loss of the upper and lower motor neurons. Only 10% of all cases are caused by a mutation in one of the two dozen different identified genes, the most common of which are C9orf72, SOD1, and TDP-43, together responsible for at least 60% of familial ALS. The remaining 90% of cases are likely caused by a combination of as yet unidentified genetic and environmental factors. Remarkably, despite the large degree of heterogeneity, all cases of ALS have protein aggregates in the brain and spinal cord that are immunopositive for SOD1, TDP-43, OPTN, and/or p62. Protein inclusions are normally prevented and cleared by heat shock proteins (Hsps), suggesting that ALS motor neurons have an impaired ability to induce the heat shock response (HSR). Accordingly, there is evidence of decreased induction of Hsps in ALS mouse models and in human post-mortem samples compared to controls. However, the role of Hsps in protein accumulation in human motor neurons has not been fully elucidated. Here we generated motor neuron cultures from human induced pluripotent stem cell (iPSC) lines carrying mutations in SOD1, TDP-43, or C9orf72. We show that despite a lack of overt motor neuron loss, there is an accumulation of insoluble aggregation prone proteins in iPSC-derived motor neuron cultures but that content and levels vary with genetic background. Additionally, protein aggregation corresponds to an incomplete induction of the HSR and minimal stress granule formation. We therefore conclude that ALS iPSC-derived motor neurons recapitulate key early pathological features of the disease and fail to endogenously upregulate the HSR in response to increased protein burden. As such, we believe that iPSCs represent a valuable model to further study the role of the HSR in ALS.

P2562

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The activated endoplasmic reticulum stress sensor IRE1 oligomerizes into filaments contained in 30 nm membrane tubes of complex topology.

N.T. Tran[1,2], S.D. Carter[2,3], V. Belyy[1,2], D. Acosta-Alvear[4], G.J. Jensen[2,3], P. Walter[1,2];
[1]Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA, [2]Howard Hughes Medical Institute, Chevy Chase, MD, [3]Biophysics and Biology, California Institute of Technology, Pasadena, CA, [4]Molecular, Cellular, and Developmental Biology, University of California Santa Barbara, Santa Barbara, CA

The unfolded protein response (UPR) is an intracellular signaling network that adjusts the abundance and protein folding capacity of the endoplasmic reticulum (ER) according to need. The most conversed branch of the UPR is mediated by the ER-resident transmembrane kinase/endoribonuclease IRE1. It senses unfolded protein accumulation within the ER and transduces the signal via a non-conventional mRNA splicing mechanism. In response to direct binding of unfolded proteins in the ER lumen, IRE1 activates by oligomerization and accumulates in dynamic foci. IRE1 foci are not autophagosomes as they did not colocalize with the autophagosomal marker LC3. Fluorescence recovery after photobleaching (FRAP) experiments indicate that IRE1 molecules in the foci remain in equilibrium with IRE1 molecules in the surrounding ER network. We determined the structure of IRE1 foci in cells by whole cell correlative light – electron tomography. Our results show that IRE1 oligomers induce

membrane deformations, leading to the protrusion of narrow 30 nm ribosome-free tubes that remain connected to the ER and are twisted into glomeruli of complex topology. The tubes contain two parallel filaments in their lumen, likely representing oligomerized IRE1 ER-lumenal domains. Taken together, our findings define a previously unrecognized subdomain of the ER membrane and shed new light on the structure and organization of active mammalian IRE1 inside the cell.

P2563

Board Number: B716

Autoregulatory transcriptional control of prions by G-Quadruplex motifs in prion promoter.

P. Pradhan[1], V. Perumal[1], B. Kundu[1];
[1]Kusuma School of Biological Sciences, Indian Institute of Technology Delhi, New Delhi, India

Cellular prion protein (PrP^C) misfolds into an aggregated and infectious conformer, PrP^{Sc} (scrapie) that forms the hallmark of pernicious diseases commonly known as transmissible spongiform encephalopathies (TSEs). The association of prions with random quadruplex forming nucleic acid sequences has been recently identified but the exact physiological role of this interaction still remains obscure. Herein, we show that the promoter region of the human prion gene (*PRNP*) incorporates two G-rich sequences (Q1 and Q2) that could assume stable hybrid intra-molecular G-quadruplex structures (T_m >70 ^{o} C). We found that both Q1 and Q2 specifically bind to native, α-helical PrP^C with high affinity (K_D ~70-100 nM) but showed very weak or no association with β sheet rich-PrP oligomers (PrP^{Sc} like). To further probe the relevance of these interactions, a battery of kinetic and structural studies have been utilized that involved surface plasmon resonance (SPR), fluorescence, NMR, circular dichroism and molecular modeling. We demonstrated that the N-terminal unstructured tail of PrP (residues 23-88) is crucial for binding and unwinding of both the quadruplex sequences. Interestingly, cell-based luciferase assays showed that PrP^C auto-regulated its expression by binding to G-quadruplexes present in its own promoter sequence. Evidently, the autoregulatory function was lost on mutating one of the quadruplex sequences. Overall our data indicate the presence of feedback transcriptional regulation of *PRNP* gene by native PrP^C through the dynamic unwinding of quadruplex structures by native PrP^C that apparently gets affected by pathogenic PrP^{Sc} formation. The identification of feedback transcriptional regulation could be a crucial event in the pathogenic cycle of prions which may be targeted for developing newer therapeutic interventions.

Cell Death

P2564

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Potential mechanisms of platelet-activating factor induced neutrophil NETosis.

Y. LI[1,2], V.P. Werth[1,2], M. Liu[1,2];
[1]Department of Dermatology, Perelman School of Medicine at University of Pennsylvania , Philadelphia, PA, [2]Department of Dermatology, Michael J. Crescenz V.A. Medical Center, Philadelphia, PA

Background/Purpose: Platelet-activating factor (PAF) is a proinflammatory lipid mediator, and plays a critical role in autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, psoriasis, and lupus, by affecting different immune cells. Increasing evidence indicates the importance of neutrophils in development of autoimmune diseases. Neutrophil NETosis is a newly described form of cell death, and NETotic neutrophils release neutrophil extracellular traps (NETs) that are involved in various human diseases. Although the PAF signaling cascades have been widely investigated in other human