IPD Project Details

Project ID: IPD6047

Project Title: Nuclear-injuries by aberrant dynein-forces defeat proteostatic purposes of Lewy Body-like InclusionsNuclear-injuries by aberrant dynein-forces defeat proteostatic purposes of Lewy Body-like Inclusions

Description: Biogenesis of inclusion bodies (IBs) facilitates protein quality control (PQC). Canonical aggresomes execute degradation of misfolded proteins while non-degradable amyloids quarantine into Insoluble Protein Deposits. Lewy Bodies (LBs) are well-known neurodegenerative IBs of Î±-Synuclein but PQC-benefits and drawbacks associated with LBs remain underexplored. Here, we report that a crosstalk between LBs and aggresome-like IBs of Î±-Synuclein (Syn-aggresomes) buffer amyloidogenic Î±-Synuclein load. LBs possess unorthodox PQC-capacities of self-quarantining Syn-amyloids and being degradable upon receding fresh amyloidogenesis. Syn-aggresomes equilibrate biogenesis of LBs by facilitating spontaneous degradation of soluble Î±-Synuclein and opportunistic turnover of Syn-amyloids. LBs overgrow at the perinucleus once amyloidogenesis sets in and are misidentified by cytosolic BICD2 as cargos for motor-protein dynein. Simultaneously, microtubules surrounding the perinuclear LBs are distorted misbalancing the dynein motor-force on nucleoskeleton leading to widespread lamina injuries. Like typical Laminopathies, nucleocytoplasmic mixing, DNA-damage, and deregulated transcription of stress chaperones defeat the proteostatic purposes of LBs.

Principal Investigator: Dr Swasti Raychaudhuri

PI Affiliation: Principal Scientist, CSIR- Centre for Cellular and Molecular Biology, Hyderabad, India

Sample Preparation: Hek293T - For total, soluble and insoluble proteome, cell-extracts were prepared as per the experimental scheme in Fig. S3G. Cells were pelleted down at day 3 and 6. Equal number (~1 million) of L-, M-, and H-labeled cells were pooled and lysed together. Proteome fractions were separated on NuPAGE 4%–12% Bis–Tris Protein Gels (Invitrogen) in MES buffer (100 mM MES, 100 mM Tris–HCl, 2 mM EDTA, 7 mM SDS) at 200 V for 40 min, fixed and stained with Coomassie brilliant blue.

Peptide Separation: Preparation of gel slices, reduction, alkylation, and in-gel protein digestion was carried out as described by Shevchenko et al. [86]. Finally, peptides were

desalted and enriched according to Rappsilber et al. [87] and stored in -20°C until mass spectrometry analysis. Similarly, equal number (~ 1.5 million) of L- and H-labeled cells were pooled together to prepare non-nuclear, nuclear and nuclear insoluble fraction as explained in experimental scheme in Fig. S5E. Primary Neurons – Primary neurons were directly dissolved in 4% SDS lysis buffer, Neurons were heat stressed as explained in experimental scheme (Fig. 4J). The lysate were precipitated by overnight incubating with chilled acetone at -20°C. The protein precipitate was then resuspended in 8M Urea with 7.5 mM DTT in 50 mM ammonium bicarbonate by vortexing for 10 min at RT. Resuspended proteins were reduced incubating with 45 mM DTT for 30 min at RT, followed by alkylation with 10 mM Iodoacetamide for 30 min in dark at RT. For trypsin digestion, Urea in the sample was diluted 10-fold and trypsin was added 1:20 ratio (15ng/µl in 25mM ammonium bi-carbonate/1mM calcium chloride) for 16 hours at 37°C. After 16 hours, Trifluoroacetic acid (10%) was added to stop the trypsin activity and samples were vacuum dried. Dried peptides were dissolved in 5% Acetonitrile/0.1% Trifluoroacetic acid. The peptides were desalted and fractionated using 200 µl Pierce C18 column tips (Merck Millipore). Peptides eluted from desalting tips were dissolved in 2% formic acid and sonicated for 5 min. Peptides were then analyzed on Q Exactive HF (Thermo Scientific) mass spectrometer interfaced with nano-flow LC system (EASY-nLC 1200, Thermo Scientific). EasySpray Nano Column PepMapTM RSLC C18 (Thermo Fisher) (75 ?m× 15 cm; 3 ?m; 100 ?) using 60 min gradient of mobile phase [5% ACN containing 0.1% formic acid (buffer A) and 90% ACN (acetonitrile) containing 0.1% formic acid (buffer B)] at flow rate 300 nL/min was used for separation of peptides. Full scan of MS spectra (from m/z 400 to 1750) were acquired followed by MS/MS scans of top 10 peptide with charge state 2 of higher.

Protein Characterization: For peptide identification, raw MS data files were loaded onto MaxQuant proteomics computational platform (Ver. 1.6.10.43) [89] and searched against Swissprot database of Homo sapiens (release 2019 with 20,371 entries) and a database of known contaminants. A decoy version of the specified database was used to adjust the false discovery rates for proteins and peptides below 1%. The search parameters included constant modification of cysteine by carbamidomethylation, enzyme specificity trypsin, and multiplicity set to 3 with Lys4 and Arg6 as medium label and Lys8 and Arg10 as heavy label. Other parameters included minimum peptide for identification 2, minimum ratio count 2, re-quantify option selected, and match between runs with 2-min time window. SILAC labelled cells downstream analysis and statistics was done by normalizing ratios (H/L and M/L) from two biological repeat experiments was done using Perseus (Ver. 1.6.0.4, 1.6.10.43) [90]. Individual experiment H/L ratios for each fraction were converted into log2 space and one-sample t-test was performed to calculate the statistical significance with Perseus. For Z-score calculation, average ratio H/L of 2 repeats was converted into log2 space and normalized using mean and standard deviations for each data set in Perseus. For Label free quantification, raw spectra files were loaded onto MaxQuant proteomics computational platform (Ver. 1.6.10.43) and searched against SwissProt Mus Musculus Fasta database (version November 2022). The search parameters included static and dynamic modification of cysteine by

carbamidomethylation and dynamic modification of methionine by oxidation, respectively, enzyme specificity of trypsin allowing for up to 2 missed cleavages. Precursor mass tolerance was set to 5 ppm and fragment mass tolerance 0.02 Da was used. Other parameters included minimum 1 peptide for identification with 6 amino acid length. Percolator (q-value) was used for validating peptide spectrum matches and peptides, accepting only the top-scoring hit for each spectrum, satisfying the cut off values for FDR 1% and Label Free Quantification (LFQ) option was selected. Log2 fold change was calculated from the ratios of LFQ intensities of SNCA(DM)-EGFP-NLS, 6day, +PFF (Heat Shock/ Non-Heat Shock) and SNCA(DM)-EGFP-NLS, 6day, -PFF (Heat Shock/ Non-Heat Shock) from average LFQ intensities of 3 independent experiments. Heatmaps were prepared using Morpheus (https://software.broadinstitute.org/morpheus) and other graphs were prepared in Perseus, GraphPad prism ver. 9.4.1 or OriginPro 2021b (OriginLab).

Experiment Type: Gel-based experiment

Species: Homo sapiens - 9606, Mus musculus-10090

Tissue:

Cell Type: Permanent cell line cell

Disease: Unknown

Instrument Details: Q Exactive HF (MS:1002523)

Protein Modifications: iodoacetamide derivatized residue

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