IPD Project Details

Project ID: IPD2655

Project Title: Aggregation of respiratory complex subunits marks the onset of proteotoxicity in proteasome inhibited cells

Description: Proteostasis is maintained by optimum expression, folding, transport, and clearance of proteins. Deregulation of any of these processes triggers protein aggregation and is implicated in many age-related pathologies. Here, using quantitative proteomics and microscopy we show that aggregation of many nuclear-encoded mitochondrial proteins is an early protein-destabilization event during short-term proteasome inhibition. Among these, Respiratory Chain Complex (RCC) subunits represent a group of functionally related proteins consistently forming aggregates under multiple proteostasis-stresses with varying aggregation-propensities. Sequence analysis reveals that several RCC subunits, irrespective of cleavable mitochondrial targeting sequence (MTS), contain low complexity regions (LCR) at N-terminus. Using different chimeric and mutant constructs we show that these low complexity regions partially contribute to intrinsic instability of multiple RCC subunits. Taken together, we propose that physicochemically driven aggregation of unassembled RCC subunits destabilizes their functional assembly inside mitochondria. This deregulates the biogenesis of respiratory complexes and marks the onset of mitochondrial dysfunction.

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Sample Preparation: Sample preparation for mass spectrometry 1.5 million cells were plated in 100 mm culture dishes. The light-labeled cells (L) served as solvent control whereas medium-labeled (M) and heavy-labeled (h) cells were treated with 2.5 µM and 5 µM of MG132 respectively. After 8 hr of incubation, equal number of L-, M- and H-labeled cells were pooled together and total, soluble and insoluble fractions were prepared as described earlier. The fractions were separated on NuPAGE 4-12% Bis-Tris Protein Gels (Invitrogen). The gel was run in MES buffer (100 mM MES, 100 mM Tris-HCl, 2 mM EDTA, 7 mM SDS) at 200V 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. Finally, peptides were desalted. LC-MS/MS Peptides eluted from desalting tips were dissolved in 2% formic acid and sonicated for 5 min. Soluble fraction was analyzed on Linear Trap Quadrupole (LTQ)-OrbitrapVelos interfaced with nanoflow LC system (Easy nLC II, Thermo Scientific). Peptide fractions were separated on a Bio Basic C18 pico-Frit nanocapillary column (75 ?m × 10 cm; 3 ?m) using a 120 min linear gradient of the mobile phase [5% ACN containing 0.2% formic acid (buffer-A) and 95% ACN containing 0.2% formic acid (buffer-B)] at a flow rate of 300 nL/min. Full scan MS spectra (from m/z 400–2000) were acquired followed by MS/MS scans of Top 20 peptides with charge states 2 or higher. Total and insoluble fractions were analyzed on Q Exactive (Thermo Scientific) interfaced with nanoflow LC system (Easy nLC II, Thermo Scientific). Peptide fractions were separated on a Bio Basic C18 pico-Frit nanocapillary column (75 ?m × 10 cm; 3 ?m) using a 60 min linear gradient of the mobile phase [5% ACN containing 0.2% formic acid (buffer-A) and 95% ACN containing 0.2% formic acid (buffer-B)] at a flow rate of 400 nL/min. Full scan MS spectra (from m/z 400–2000) were acquired followed by MS/MS scans of Top 10 peptides with charge states 2 or higher.

Protein Characterization: Peptide identification and statistical analysis For peptide identification, raw MS data files were loaded onto MaxQuant proteomics computational platform (Ver. 1.3.0.5) and searched against Swissprot database of Mus musculus (release 2016.03 with 16790 entries) and a database of known contaminants. MaxQuant used a decoy version of the specified database 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, 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. iBAQ option was selected to compute abundance of the proteins. Bioinformatics and statistical analysis was performed in Perseus environment (Ver. 1.5.2.4)

Experiment Type: Shotgun proteomics, Gel-based experiment

Species: Mus musculus-10090

Tissue: Unknown

Cell Type: Unknown

Disease: Unknown

Instrument Details: Other LTQ Orbitrap Velos (MS:1001742), Q Exactive (MS:1001911)

Protein Modifications: No PTMs

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