IPD Project Details

Project ID: IPD8258

Project Title: Cell membrane proteome analysis in HEK293T cells challenged with ?-Synuclein amyloids

Description: Amyloids interact with plasma membranes. Extracellular amyloids cross the plasma membrane barrier. Internalized extracellular amyloids are reported to trigger amyloidogenesis of endogenous proteins in recipient cells. To what extent these extracellular and intracellular amyloids perturb the plasma membrane proteome is not investigated. Using ?-Synuclein as a model amyloid protein, we performed membrane shaving followed by mass spectrometry experiment to identify the conformational changes in the cell surface proteins after extracellular amyloid challenge. We also performed membrane proteomics after the biogenesis of intracellular ?-Synuclein amyloids. Our results suggest that promiscuous interaction with extracellular amyloids stochastically alter the conformation of plasma membrane proteins. This affects the biological process through the plasma membrane and result in loss in cell viability. Cells that survive the extracellular amyloid shock can grow normally and gradually develop intracellular amyloids which do not directly impact the plasma membrane proteome and associated biological processes. Thus, ?-Synuclein amyloids can damage the plasma membrane and related processes only during cell to cell transfer and not during their intracellular biogenesis.

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Sample Preparation: Human recombinant ?-synuclein was overexpressed in E. coli BL21(DE3) using the pET 28a+ expression vector.

Peptide Separation: In solution trypsin digestion 100 mM ammonium bicarbonate (AmBic) was prepared in 0.22 µm membrane filtered deionized water. 200 mM dithiothreitol (DTT) and 1 M iodoacetamide (IAA) were prepared separately in 100 mM AmBic. 150 ng/µl MS grade trypsin (sigma) was prepared in 25 mM AmBic, 1 mM CaCl2. 10 mM DTT was added to the membrane-shaved fraction and incubated at 37°C for 1 hr followed by incubation in 10 mM IAA at 37 °C for 1 hr in dark. Then DTT was added to the tubes to 20 mM final concentration and incubated at 37°C for 30 min followed by addition of trypsin to 2 µg/ml and incubated at 37°C for 16 hrs. In gel trypsin

digestion 60 µg of protein was loaded on NuPAGE 4%–12% Bis–Tris Protein Gel (Invitrogen) and ran in MES buffer (100 mM MES, 100 mM Tris–HCl, 2 mM EDTA, 7 mM SDS) at 200 V for 40 min. The gel was stained with Coomassie brilliant blue R250 and destained. Each lane was cut into 10 bands. Each band was chopped into smaller pieces and destained using 35 mM AmBic, 5.74 M acetonitrile (ACN). Gel pieces were shrunken using ACN, swelled using 50 mM AmBic and re-shrunken using ACN. Gel pieces were vacuum dried and treated with 10 mM DTT in 50 mM AmBic for 45 min at 56 °C. Equal volume of 55 mM IAA in 50 mM AmBic was added and incubated for 30 min in dark. Gel pieces were washed thoroughly through two cycles of AmBic and ACN. Gel pieces were vacuum dried. Trypsin buffer (MS grade trypsin 643.8 nM, AmBic 25 mM, calcium chloride 1 mM) was added in 1:20 molar ratio, incubated for 16 hr at 37°C at 300 rpm. Peptides were extracted using extraction buffer (ACN 30%, formic acid 5%), sonicated for 5 min and supernatant containing peptides was collected. Peptides were kept in vacuum concentrator till dry. Desalting of peptides 100 µl and 10 µl volume C18 tips (thermo) were used for membrane shaving and membrane fraction experiments, respectively. All solutions were acidified using 0.1% trifluoroacetic acid. Tips were activated by 100% ACN, then sequentially washed with 50% and 5% ACN. Peptides dissolved in 5% ACN were slowly passed through column 30 times. Column was then sequentially washed with deionized water and 5% ACN before elution of peptides in 50% ACN. Peptides were vacuum dried, dissolved in 5% ACN, 0.1% formic acid and sonicated for 5 min before running for mass spectrometry. Mass spectrometry Peptides were run on Thermo Scietific EasySpray Nano Column PepMapTM RSLC C18 (75 ?m× 15 cm; 3 ?m; 100 ?) attached to Thermo Scientific EASY-nLC 1200 nano-flow LC system. A mobile phase gradient from 5% to 95% ACN (both containing 0.1% formic acid) was run for 60 min at 300 nl/min flow rate. Mass spectra was captured on Thermo Scientific Q Exactive mass spectrometer from m/z 400 to 1,750. MS/MS spectra for top 10 peptides with z ?2 was recorded.

Protein Characterization: All mass spectrometry data was analyzed using MaxQuant 2.0.3.0 against human Uniprot reference proteome dated 27/04/2021 using Andromeda search engine. Cysteine carbamidomethylation was kept as the fixed modification; amino acid oxidation and methylation were set as variable modification. Maximum number of modifications per peptide was set to 5. Min. peptide length was set to 7 and maximum peptide mass was set to 4600 kDa. Time of flight MS/MS match tolerance was kept at 40 ppm and deisotoping tolerance was kept at 0.01 ppm. Peptides were quantified in unique+razor mode. False detection rate was kept at 1%. Match between runs option was used for membrane shavings experiment. MaxLFQ was calculated for membrane fraction dataset. Reverse, contaminants and only identified by site rows were removed before initiating calculations. A list of human plasma membrane, nuclear, mitochondrial and all membrane proteins were downloaded from The Human Protein Atlas, Mitocarta 3.0 and Uniprot KB, respectively

Experiment Type: Shotgun proteomics, Gel-based experiment

Species: Homo sapiens-9606

Tissue: Cell culture (bto:0000214)

Cell Type: Cell line cell

Disease: Unknown

Instrument Details: Other Q Exactive (MS:1001911), Q Exactive HF (MS:1002523)

Protein Modifications: iodoacetamide derivatized residue

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