Electronic Supplementary Material (ESI) for Food & Function.
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Details on proteomics

1. Experimental Instruments and Softwares

Experimental Instruments: Q Exactive Mass Spectrometer（Thermo Scientific）、Easy nLC Liquid Chromatograph (Thermo Scientific)

Softwares: MaxQuant 1.3.0.5 (Max Planck Institute of Biochemistry in Martinsried, Germany)、Perseus 1.3 (Max Planck Institute of Biochemistry in Martinsried, Germany)

2. Methods

2.1 SDT Lysis：

SDT buffer was added to the sample. The lysate was sonicated (this step can be skipped for protein solution) and then boiled for 15 min. After centrifuged at 14000g for 40 min, the supernatant was quantified with the BCA Protein Assay Kit (Bio-Rad, USA). The sample was stored at -80℃

2.2 SDS-PAGE Separation

20 μg of proteins for each sample were mixed with 5X loading buffer respectively and boiled for 5 min. The proteins were separated on 12.5% SDS-PAGE gel (constant current 14 mA, 90 min). Protein bands were visualized by Coomassie Blue R-250 staining.

2.3 Filter-aided sample preparation (FASP Digestion)

200 μg of proteins for each sample were incorporated into 30 μl SDT buffer (4% SDS, 100 mM DTT, 150 mM Tris-HCl pH 8.0). The detergent, DTT and other low-molecular-weight components were removed using UA buffer (8 M Urea, 150 mM Tris-HCl pH 8.0) by repeated ultrafiltration (Microcon units, 10 kD). Then 100 μl iodoacetamide (100 mM IAA in UA buffer) was added to block reduced cysteine residues and the samples were incubated for 30 min in darkness. The filters were washed with 100 μl UA buffer three times and then 100 μl 25mM NH4HCO3 buffer twice.

Finally, the protein suspensions were digested with 4 μg trypsin (Promega) in 40 μl 25 mM NH_4HCO_3 buffer overnight at 37°C, and the resulting peptides were collected as a filtrate. The peptides of each sample were desalted on C18 Cartridges (Empore™ SPE Cartridges C18 (standard density), bed I.D. 7 mm, volume 3 ml, Sigma), concentrated by vacuum centrifugation and reconstituted in 40 µl of 0.1% (v/v) formic acid. The peptide content was estimated by UV light spectral density at 280 nm using an extinctions coefficient of 1.1 of 0.1% (g/l) solution that was calculated on the basis of the frequency of tryptophan and tyrosine in vertebrate proteins.

3. Mass Spectrometry

3.1 HPLC

Each fraction was injected for nanoLC-MS/MS analysis. The peptide mixture was loaded onto a reverse phase trap column（Thermo Scientific Acclaim PepMap100, 100μm*2cm, nanoViper C18）connected to the C18-reversed phase analytical column (Thermo Scientific Easy Column, 10 cm long, 75 μm inner diameter, 3μm resin) in buffer A (0.1% Formic acid) and separated with a linear gradient of buffer B (84% acetonitrile and 0.1% Formic acid) at a flow rate of 300 nl/min controlled by IntelliFlow technology. The linear gradient was determined by the project proposal:

i. 1 hour gradient：0-35% buffer B for 50 min, 35-100% buffer B for 5 min, hold in 100% buffer B for 5 min.

ii. 2 hours gradient：0-55% buffer B for 110 min, 55-100% buffer B for 5 min, hold in 100% buffer B for 5 min.

iii. 4 hours gradient：0-55% buffer B for 220 min, 55-100% buffer B for 8 min, hold in 100% buffer B for 12 min.

3.2 LC-MS/MS Analysis

LC-MS/MS analysis was performed on a Q Exactive mass spectrometer (Thermo Scientific) that was coupled to Easy nLC (Proxeon Biosystems, now Thermo Fisher Scientific) for 60/120/240 min (determined by project proposal). The mass spectrometer was operated in positive ion mode. MS data was acquired using a data-dependent top10 method dynamically choosing the most abundant precursor ions from the survey scan (300–1800 m/z) for HCD fragmentation. Automatic gain control (AGC) target was set to 3e6, and maximum inject time to 10 ms. Dynamic exclusion duration was 40.0 s. Survey scans were acquired at a resolution of 70,000 at m/z 200 and resolution for HCD spectra was set to 17,500 at m/z 200, and isolation width was 2 m/z. Normalized collision energy was 30 eV and the underfill ratio, which specifies the minimum percentage of the target value likely to be reached at maximum fill time, was defined as 0.1%. The instrument was run with peptide recognition mode enabled.

4. Data Analysis

The MS data were analyzed using MaxQuant software version1.3.0.5 (Max Planck Institute of Biochemistry in Martinsried, Germany). The protein database was uniprot_mouse_83374_20170814. fasta. (83,374 total entries, downloaded 2017-08-14, http://www.uniprot.org). The following parameters were set.

Item	Value
Enzyme	Trypsin
Max Missed Cleavages	2
Main search	6 ppm
First search	20 ppm
MS/MS Tolerance	20 ppm

Fixed modifications	Carbamidomethyl (C)
Variable modifications	Oxidation (M), Acetyl (Protein N-term)
Database	uniprot_mouse_83374_20170814.fasta
Database pattern	Reverse
Peptide FDR	≤0.01
Protein FDR	≤0.01
Time window (match between runs)	2min
Protein Quantification	Razor and unique peptides were used for protein quantification.
LFQ	True
LFQ min. ratio count	1

5. Bioinformatic Analysis

5.1 Gene Ontology (GO) Annotation

The protein sequences of differentially expressed proteins were in batches retrieved from UniProtKB database (Release 2016_10) in FASTA format. The retrieved sequences were locally searched against SwissProt database (mouse) using the NCBI **BLAST+** client software (ncbi-blast-2.2.28+-win32.exe) to find homologue sequences from which the functional annotation can be transferred to the studied sequences. In this work, the top 10 blast hits with E-value less than 1*e*-3 for each query sequence were retrieved and loaded into **Blast2GO9** (Version 3.3.5) for GO mapping and annotation. In this work, an annotation configuration with an E-value filter of 1e-6, default gradual EC weights, a GO weight of 5, and an annotation cutoff of 75 were chosen. Un-annotated sequences were then re-annotated with more permissive parameters. The sequences without BLAST

hits and un-annotated sequences were then selected to go through an InterProScan10 against EBI databases to retrieve functional annotations of protein motifs and merge the InterProScan GO terms to the annotation set. The GO annotation results were plotted by R scripts.

5.2 KEGG Pathway Annotation

The FASTA protein sequences of differentially changed proteins were blasted against the online Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://geneontology.org/) to retrieve their KOs and were subsequently mapped to pathways in KEGG11. The corresponding KEGG pathways were extracted.

5.3 Functional Enrichment analysis

To further explore the impact of differentially expressed protein in cell physiological process and discover internal relations between differentially expressed proteins, enrichment analysis was performed. GO enrichment on three ontologies (biological process, molecular function, and cellular component) and KEGG pathway enrichment analyses were applied based on the Fisher' exact test, considering the whole quantified protein annotations as background dataset. Benjamini-Hochberg correction for multiple testing was further applied to adjust derived p-values. And only functional categories and pathways with p-values under a threshold of 0.05 were considered as significant.

5.4 Hierarchical Clustering

The studied protein relative expression data was used to performing hierarchical clustering analysis. For this purpose, Cluster3.0(http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm) and the Java Treeview software (http://jtreeview.sourceforge.net) were used. Euclidean distance algorithm for similarity measure and average linkage clustering algorithm (clustering uses the centroids of the observations) for clustering were selected when performing hierarchical clustering. Heatmap is

often presented as a visual aid in addition to the dendrogram.

5.5 Protein-Protein Interact Network（PPI）

The protein–protein interaction information of the studied proteins was retrieved from IntAct molecular interaction database (http://www.ebi.ac.uk/intact/) by their gene symbols or STRING software (http://string-db.org/). The results were downloaded in the XGMML format and imported into Cytoscape5 software (http://www.cytoscape.org/,version 3.2.1) to visualize and further analyze functional protein-protein interaction networks. Furthermore, the degree of each protein was calculated to evaluate the importance of the protein in the PPI network.