Data Article

Dataset of growth cone-enriched lipidome and proteome of embryonic to early postnatal mouse brain

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A B S T R A C T

A growth cone is a part of a neuron considered as a hub for axon growth, motility and guidance functions. Growth cones are thought to play a critical role during development of neurons. Growth cones also play a significant role in adult regeneration. Here, we present a dataset on the lipid and protein profiling of the growth cone-enriched fractions derived from C57BL/6J mice forebrains of developmental stage: E18, P0, P3, P6, and P9. For comparison, we analyzed non-growth cone membranes from the same samples. Lipid data is available at the Metabolomics Workbench [http://www.metabolomicsworkbench.org (Project ID: PR000746)]. Protein data is available at Proteomics Identifications (PRIDE) partner repository (PRIDE identifier PXD012134).

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1. Data

Here, we carried out lipid and protein profiling of the growth cone (GC)-enriched fractions derived from forebrains of E18 – P9 C57BL6/J mice. Lipids were extracted using chloroform, methanol and water mixture to obtain phase separation. We then used untargeted liquid chromatography Q-Exactive Orbitrap tandem mass spectrometry (LC-MS/MS) for lipid profiling. Peak extraction, identification, relative quantification, and alignment were performed using Lipid Search 4.1 software. The list of

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identified lipids is provided in Supplementary Table S1. In parallel, protein samples were reduced, alkylated and digested using trypsin and Lys-C proteases, followed by LC-MS/MS. Proteome Discoverer 2.2 was used for bioinformatics analysis. The list of identified proteins is provided in Supplementary Table S2. Samples clustering is presented in Fig. 1 and Fig. 2 for lipid and protein profiling data, respectively.

2. Experimental design, materials, and methods

2.1. Brain tissue collection and subcellular fractionation

All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and policies of the UM Institutional Animal Care & Use Committee (IACUC). Forebrains of E18, P0, P3, P6, and P9 C57BL/6J mice were fractionated by sucrose gradient ultracentrifugation into growth cone-enriched (GC) and non-growth cone membrane (M) fractions as described previously [1–5]. Protein concentrations were determined using BCA assay (Thermo Fisher Scientific, Waltham, MA), according to manufacturer's instruction. For samples list, see Table 1.

2.2. Lipid profiling

2.2.1. Sample preparation

6 mL of methanol (LC-MS grade) and 3 mL of chloroform (LC-MS grade) were added to each sample (100 μg of total protein). After 2 min vigorous vortexing and 2 min sonication in an ultrasonic bath, the
samples were incubated at 48 °C overnight (in borosilicate glass vials, PTFE-lined caps). The following day, 3 mL of water (LC-MS grade) and 1.5 mL of chloroform were added, samples vigorously vortexed for 2 min and centrifuged at 3000 RCF, 4 °C for 15 min to obtain phase separation. Lower phases were collected. Remaining samples (upper and interphases) were re-extracted by addition of 4.5 mL of chloroform and centrifugation at 3000 RCF, 4 °C for 15 min to obtain phase separation. Lower phases from both extractions were combined and dried in a centrifugal vacuum concentrator. Samples were stored at −20 °C until reconstituted in 150 μL of chloroform:methanol (1:1) before mass spectrometric analysis.

2.2.1. High-performance liquid chromatography (HPLC). Reversed-phase chromatographic separation utilized Accela Autosampler (Thermo), Accela 600 pump (Thermo) and Acclaim C30 column: 3 μm, 2.1 × 150 mm (Thermo). The column temperature was maintained at 30 °C and tray temperature at 20 °C. Solvent A was composed of 10 mM ammonium acetate (LC-MS grade) in 60:40 methanol:water (LC-MS grade) with 0.2% formic acid (FA; LC-MS grade). Solvent B was composed of 10 mM ammonium acetate with 60:40 methanol:chloroform with 0.2% FA. The flow rate was 260 μL/min, and the injection volume was 10 μL. The gradient was 35—100% solvent B over 13.0 min, 100% solvent B over 13.0—13.8 min, 100—35% solvent B over 13.8—14.5 min, 35% solvent B over 14.5—18.0 min, 0% solvent B over 18.0—20.0 min.

2.2.2. Mass spectrometry
The Q Exactive (Thermo) mass spectrometer was operated under heated electrospray ionization (HESI) in positive and negative mode separately. The spray voltage was 4.4 kV, the heated capillary was held at 310 °C (negative mode) or 350 °C (positive mode) and heated at 275 °C (positive mode). The S-lens radio frequency (RF) level was 70. The sheath gas flow rate was 30 (negative mode) or 45 units
(positive mode), and auxiliary gas was 14 (negative mode) or 15 units (positive mode). Full scan used resolution 70,000 with automatic gain control (AGC) target of $1 \times 10^6$ ions and maximum ion injection time (IT) of 100 ms. Data-dependent MS/MS (top10) were acquired using the following parameters: resolution 17,500; AGC $1 \times 10^5$; maximum IT 75 ms; 1.3 m/z isolation window; underfill ratio 0.1%; intensity threshold $1 \times 10^3$; dynamic exclusion time 3 s. Normalized collision energy (NCE) settings were: 15, 30, 45, 60, 75, 90 (in positive and negative mode separately; total 12 runs per sample).

2.2.2.1. Lipid identification and quantification. Lipid identification and relative quantification were performed using LipidSearch 4.1 software (Thermo). The search criteria were as follows: product search; parent m/z tolerance 5 ppm; product m/z tolerance 5 ppm; quantification: m/z tolerance 5 ppm,

Table 1

| Fraction                        | Age | Biological replicate |
|---------------------------------|-----|----------------------|
| growth cone-enriched membranes (GC) |     |                      |
| E18                             | 1–6 |                      |
| P0                              | 1–6 |                      |
| P3                              | 1–6 |                      |
| P6                              | 1–6 |                      |
| P9                              | 1–6 |                      |
| non-growth cone membranes (M)   |     |                      |
| E18                             | 1–6 |                      |
| P0                              | 1–6 |                      |
| P3                              | 1–6 |                      |
| P6                              | 1–6 |                      |
| P9                              | 1–6 |                      |

Fig. 2. Principal component analysis (PCA) of protein profiling data. Data from Supplementary Table S2 has been filtered to protein accessions detected in at least 4 biological replicates. Missing data was replaced by column min, followed by quantile normalization and log transformation (glog). Samples are plotted in 2 dimensions using their projection onto the first 2 principal components (in brackets % of total variance explained).
retention time tolerance 1 min. The following adducts were allowed in positive mode: +H, +NH4, +H–H2O, +H–2H2O, +2H, +Na, +K and negative mode: H, +HCOO, +CH3COO, -2H, –Cl. All classes were selected for search. LipidSearch nomenclature is used (Table 2).

2.2.3. Data processing

Positive and negative mode identifications at different NCE were aligned in LipidSearch, allowing calculation of unassigned peaks. The following settings were applied: product search, alignment method max; retention time tolerance 0.1 min; filters: top rank, main isomer peak; M-score 5; molecular lipid identification grade: A–B (A: lipid class and fatty acid completely identified or B: lipid class and some fatty acid identified).

2.3. Protein profiling

2.3.1. Sample preparation

100 µg of proteins were precipitated with 4 vol of ice-cold acetone overnight at −20 °C. The following day, proteins were pelleted by centrifugation (15 min, 4 °C, 18,000 RCF). Pellets were

| Group          | ClassKey | Lipid name                                      |
|----------------|----------|-------------------------------------------------|
| phospholipid   | BisMePA  | bismethyl phosphatidic acid                     |
|                | dMePE    | dimethylphosphatidylethanolamine                 |
|                | LdMePE   | lysodimethylphosphatidylethanolamine            |
|                | LPC      | lysophosphatidylcholine                         |
|                | LPE      | lysophosphatidylethanolamine                    |
|                | LPEt     | lysophosphatidylethanol                         |
|                | LPS      | lysophosphatidylserine                         |
|                | PA       | phosphatic acid                                |
|                | PAF      | platelet-activating factor                      |
|                | PC       | phosphatidylcholine                             |
|                | PE       | phosphatidylethanolamine                        |
|                | PEt      | phosphatidylethanol                             |
|                | PG       | phosphatidylglycerol                           |
|                | PI       | phosphatidylinositol                           |
|                | PIP      | phosphatidylinositol                           |
|                | PMe      | phosphatidylmethanol                           |
|                | PS       | phosphatidylerine                              |
| sphingolipid   | Cer      | ceramide                                        |
|                | CerP     | ceramide phosphate                              |
|                | SM       | sphingomyelin                                   |
|                | So       | sphingosine                                     |
| glycosphingolipid | CerG1   | hexosyl ceramide                                |
|                 | CerG2   | dihexosyl ceramide                              |
|                 | GD1a    | ganglioside                                     |
|                 | ST       | sulfatide                                       |
| cardiolipin    | CL       | cardiolipin                                     |
| neutral glycerolipid | MG | monoglyceride                                    |
|                 | DG       | diglyceride                                     |
|                 | TG       | triglyceride                                    |
| steroid        | ChE      | cholesterol ester                              |
|                 | ZyE      | zymosterol                                      |
| coenzyme       | Co       | coenzyme                                        |
| fatty esters   | AcCa     | acyl carnitine                                  |
| glycocepheroacid | MGMG | monogalactosyldiacylglycerol                     |
|                 | MDG     | monogalactosyldiacylglycerol                    |
|                 | DGMG    | digalactosyldiacylglycerol                      |
|                 | SQMG    | sulfoquinovosyldiacylglycerol                   |
|                 | SQDG    | sulfoquinovosyldiacylglycerol                   |
resuspended in 20 μL of 0.2% ProteaseMAX (Promega)/50 mM ammonium bicarbonate (ABC). 50 mM ABC was added to a final volume of 93.5 μL. Proteins were reduced with dithiothreitol (DTT): 1 μL of 50 mM (final concentration ~5 mM) for 20 min at 56 °C. Next, proteins were alkylated using iodoacetamide (IAA): 2.7 μL of 0.55 M (final concentration ~15 mM) for 15 min at room temperature in the dark. 1 μL of 1% ProteaseMAX/50 mM ABC with 4 μg of Trypsin/Lys-C mix (Promega; 1:25 trypsin-to-protein ratio) was added. Digestion was carried out in a total volume of 100 μL (1 μg of total protein/μL) for 4 h at 37 °C. Samples were adjusted to 300 μL of 0.1% trifluoroacetic acid/50 mM ABC. Pierce High pH Reversed-Phase Fractionation Kit (Thermo) was used to separate peptides into eight fractions (according to manufacturer’s instruction). We also collected flow-through, wash (water) and an additional 100% acetonitrile (ACN) elution fraction (total: 11 fractions). Fractions were dried in a centrifugal vacuum concentrator at 45 °C and then stored at −20 °C. Before LC-MS analysis, peptides were re-suspended in 30 μL of 0.1% FA in water.

2.3.2. Ultra-high performance chromatography (UHPLC)

The chromatographic separation utilized EASY-nLC 1000 system (Thermo) and Acclaim PepMap RSLC 75 μm × 15 cm, nanoViper column (Thermo). Solvent A was composed of 0.1% FA in water (LC-MS grade). Solvent B was composed of 0.1% FA in ACN (LC-MS grade). The flow rate was 260 μL/min and injection volume was 10 μL. The gradient was 2–5% solvent B over 5.0 min, 5–60% solvent B over 5–70 min, 60–98% solvent B over 70–98 min, 98% solvent B over 98–118 min, 98–2% solvent B over 118–120 min.

2.3.3. Mass spectrometry

The Q Exactive (Thermo) mass spectrometer was operated under heated electrospray ionization (HESI) in positive mode. The spray voltage was 1.8 kV, the heated capillary was held at 250 °C, and the S-lens radio frequency (RF) level was 70. Full scan (m/z 150–2000) used resolution 70,000 with automatic gain control (AGC) target of 2 × 10^6 ions and maximum ion injection time (IT) of 100 ms. Data-dependent MS/MS (top15) were acquired using the following parameters: resolution 17,500; AGC 1 × 10^5; maximum IT 200 ms; 1.3 m/z isolation window; NCE 28. Underfill ratio was set to 0.1%; intensity threshold to 5 × 10^2; dynamic exclusion time to 20 s; peptide match to preferred; charge exclusion to unassigned and 1.

2.3.4. Protein identification and quantification

The acquired raw files were analyzed with Proteome Discoverer 2.2 (Thermo) using the SEQUEST HT engine. The data was searched against 83,916 Mus musculus entries (Swiss-Prot + TrEMBL, UniProt 8/14/2018). Search parameters included: precursor mass tolerance 10 ppm and 0.02 Da for fragments, 2 missed trypsin cleavages, oxidation (Met) and acetylation (protein N-term) as variable modifications, carbamidomethylation (Cys) as a static modification. Percolator PSM validation used the following parameters: strict FDR of 0.01, relaxed FDR of 0.1, maximum ΔCn of 0.05, validation based on q-value. For label-free quantification (LFQ), the Minora Feature Detector was used along with the Feature Mapper and Precursor Ions Quantifier. Data were filtered by peptide filter: medium (FDR 0.1) and high confidence (FDR 0.01) and protein filter: medium (FDR 0.1) and high confidence (FDR 0.01).

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Transparency document

Transparency document associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2019.103865.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.103865.

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