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Data Article

Myristoylation profiling in human cells and zebrafish

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ABSTRACT

Human cells (HEK 293, HeLa, MCF-7) and zebrafish embryos were metabolically tagged with an alkynyl myristic acid probe, lysed with an SDS buffer and tagged proteomes ligated to multifunctional capture reagents via copper-catalyzed alkyne azide cycloaddition (CuAAC). This allowed for affinity enrichment and high-confidence identification, by delivering direct MS/MS evidence for the modification site, of 87 and 61 co-translationally myristoylated proteins in human cells and zebrafish, respectively. The data have been deposited to ProteomeXchange Consortium (Vizcaíno et al., 2014 *Nat. Biotechnol.*, 32, 223–6) (PXD001863 and PXD001876) and are described in detail in Multifunctional reagents for quantitative proteome-wide analysis of protein modification in human cells and dynamic protein lipidation during vertebrate development' by Broncel et al., *Angew. Chem. Int. Ed.*

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Specifications table

Subject area	*Biology, chemical proteomics, post-translational modification*
More specific subject area	*Protein myristoylation*
Type of data	*Proteomics, tandem mass spectrometry*
How data was acquired	*Mass spectrometry using a Q Exactive mass spectrometer (Thermo Fisher Scientific)*
Data format	*Raw LC–MS/MS, searched with PEAKS7 and MaxQuant 1.5*
Experimental factors	*Samples were processed using tagging by substrate methodology*
Experimental features	*Tryptic peptides were detected by LC–MS/MS and identified by database searches with two independent proteomics platforms*
Data source location	*http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD001863* *http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD001876*
Data accessibility	*The data have been uploaded to the ProteomeXchange Consortium* [1] *(PXD001863 and PXD001876) and are described in detail by Broncel* et al. [2]

Value of the data

- The largest repository for myristoylated proteins in human cells.
- First database for myristoylated proteins in zebrafish.
- First quantitative description of myristoylation dynamics in a developing vertebrate.

1. Experimental design

Cells and zebrafish were metabolically tagged with an alkynyl myristic acid analogue or myristic acid control. Following lysis the tagged proteins were ligated via CuAAC to multifunctional capture reagents, [2] affinity enriched and digested to peptides before LC–MS/MS analysis (Fig. 1). Generated spectra were processed with MaxQuant [3] and PEAKS [4] software for protein identifications and lipid modified peptide discovery, respectively. Myristoylated proteins and peptides detected in this study have been deposited as PXD00186 (human) and PXD001876 (zebrafish). Quantitative data (triplex dimethyl labelling) [5] revealing myristoylation dynamics during zebrafish development was included as a part of repository submission PXD001876.

2. Materials and methods

2.1. Cell and zebrafish culture

Cells (HEK 293, HeLa, MCF-7) were maintained in DMEM + 10% FBS, at 10% CO_2, and at 37 °C. Zebrafish were maintained according to standard practices. All procedures were conformed to U.K. Home Office regulations (ASPA 1986) under Animal Project Licence no. PPL 70/7472. Adult zebrafish strains AB were kept at 28 °C on 14 h light and 10 h dark cycle. Embryos were obtained from natural spawnings and were maintained in system water (combination of tap and reverse osmosis water).

2.2. Metabolic tagging and lysis

Cells: the medium was supplemented with 20 μM YnMyr or Myr for 24 h, then washed twice with Phosphate 10 mM Buffered pH 7.4 Saline 0.154 M (PBS) and lysed on ice using the following lysis buffer: PBS, 0.1% SDS, 1% Triton X-100, 1 × EDTA-free complete protease inhibitor. Lysates were kept on ice for 20 min, and then centrifuged at 17,000*g* for 20 min and supernatants collected and stored at −80 °C. The Bio-Rad DC Protein Assay was applied to determine protein concentration.

Zebrafish: embryos were placed in system water containing 0.0001% methylene blue (zebrafish water). The embryos were treated as described in Laughlin et al. [6] with minor adjustments. At 4–5 h post-fertilization (hpf), 48 hpf and 96 hpf the embryos were dechorionated with pronase (1 mg/mL,