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Data Article

Data for identification of GPI-anchored peptides and ω-sites in cancer cell lines

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ABSTRACT

We present data obtained using a focused proteomics approach to identify the glycosylphosphatidylinositol (GPI)-anchored peptides in 19 human cancer cell lines. GPI-anchored proteins (GPI-APs), which localize to the outer leaflet of the membrane microdomains commonly referred to as lipid rafts play important roles in diverse biological processes. Due to the complex structure of the GPI-anchor moiety, it has been difficult to identify GPI-anchored peptide sequences on the proteomic scale by database searches using tools such as MASCOT. Here we provide data from 73 ω-sites derived from 49 GPI-APs in 19 human cancer cell lines. This article contains data related to the research article entitled "Identification of glycosylphosphatidylinositol-anchored proteins and ω-sites using TiO_2-based affinity purification followed by hydrogen fluoride treatment" (Masuishi et al., 2016) [1].

Specifications Table

Subject area	Biology
More specific subject area	Proteomics, Post-translational modification

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Type of data	MS data, Table, Figure,
How data was acquired	MS data were acquired using data-dependent acquisition mode on a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific).
Data format	Raw, Analyzed
Experimental factors	Digested GPI-anchored peptides were treated with hydrogen fluoride.
Experimental features	GPI-anchored proteins were isolated from human cancer cell lines using Triton X-114 phase separation and PI-PLC treatment. The digested peptides were analyzed by nano-LC-MS/MS.
Data source location	*Fukuura 3-9, 8 Kanazawa, Yokohama 236-0004, Japan*
Data accessibility	Data is within this article and have been deposited in the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository with the dataset identifier PRIDE: PXD003105.

Value of the data

- Identification of novel GPI-APs and ω-sites.
- These data provide the first evidence that the GPI-anchor attaches to multiple amino acids in the C-terminal site, yielding a variety of protein species.
- These data may be helpful in understanding the mechanisms of GPI anchoring.

1. Data

We present 46 RAW mass spectrometry data files with negative controls that correspond to our LC-MS/MS analysis of 19 human cancer cell lines (ovarian cancer cell lines OVISE, A2780, OVCAR-3, OVMANA, OVSAHO, and OVSAYO, renal cancer cell lines 786-O, A498, ACHN, Caki-1, Caki-2 and UMRC3, bladder cancer cell lines 5637, T24 and UMUC3, prostate cancer cell lines DU145, LNCaP and PC3, and neuroblastoma cell line SH-SY5Y).

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository [2] with the dataset identifier PRIDE: PXD003105.

2. Experimental design, materials and methods

The detailed method is described elsewhere [1].

2.1. Isolation of GPI-Aps

This procedure was performed as previously described [3]. The GPI-AP–enriched fraction, termed the detergent-resistant membrane (DRM) fraction, was purified by sucrose gradient fractionation. The DRM fraction was resuspended in 50 mM HEPES [pH 7.5] and 1% (v/v) Triton X-114. This solution was subjected to Triton X-114 two-phase separation with or without Phosphatidylinositol-specific phospholipase C (PI-PLC) (from *Bacillus cereus*; Molecular-Probes USA) treatment. Supernatants were concentrated by trichloroacetic acid (TCA)/acetone precipitation and subjected to in-solution digestion (digestion with either trypsin, chymotrypsin).

2.2. GPI-APs digestion

For in-solution digestion, PI-PLC treated GPI-APs were resuspended in 20 μl of 8 M urea, and dithiothreitol (DTT) was added to a final concentration of 10 mM. This mixture was incubated for 30 min at 37 °C, chilled, brought to a final concentration of 10 mM iodoacetic acid for S-alkylation,