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DATA NOTE

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Proteomics of Ishikawa endometrial cancer cells: impact of liposomal backbone

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Abstract

Objectives The Ishikawa cell line is the most widely used model system for investigating implantation and endometrial cancer. Understanding the biology of this cell line is essential for developing effective interventional strategies. To gain a deeper understanding of its cellular protein profile, we extracted cellular proteins from Ishikawa cells and analyzed the peptides using mass spectrometry. Our goal was to create a proteomic resource specifically tailored for Ishikawa cells. This data set is of particular significance in the realm of targeted drug delivery. Liposomes are synthetic spherical vesicles composed of hydrophobic bilayer phospholipids and have received immense recognition as highly effective carriers for the delivery of pharmaceutical drugs and essential nutrients to the endometrium. Phosphatidylcholine and phosphatidylethanolamine are often combined to create functional liposomal systems. To discern any potential interfering effects originating from the liposome backbone, our investigation involved direct effects of phospholipid liposomes on endometrial epithelial cells.

Data description The data set includes peptide spectra derived from the intracellular proteomes of Ishikawa endometrial cancer cell isolates and their phospholipid-treated counterparts. Representing a proteome-wide profile, this dataset aims to contribute to a broader understanding of the physiology of endometrial epithelial cells. Proteomic analysis identified key proteins involved in the intricate regulation of cellular metabolism, cell cycle progression, and signaling. Between-group analysis revealed no differentially expressed proteins after adjusting for multiple testing using the applied thresholds (p-value < 0.05 and |logFC| > 1). Data are available via ProteomeXchange with identifier PXD050871.

Keywords Endometrial cells, LC-MS/MS, Liposomes, Phosphatidylcholine, Proteomics

Objective

The Ishikawa cells, a well-characterized epithelial-like model of the endometrium, is the most widely used model system for investigating implantation and endometrial cancer [1]. Liposomes, artificial spherical vesicles composed of a hydrophobic bilayer of phospholipids, have gained significant recognition as effective carriers for delivery of pharmaceutical drugs and essential nutrients to the endometrium [2, 3]. This study provides the first reported proteomic analysis of Ishikawa cells treated with liposomes composed of phosphatidylcholine and phosphatidylethanolamine, two phospholipids frequently combined to develop functional liposomal systems. The comprehensive impact of phospholipid liposomes is one of the least studied aspects of carrier-based drug delivery.

With limited studies available on the cellular dynamics of endometrial cancer cells, this research aims to fill a crucial gap by revealing any potential interfering effects

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originating from the liposome backbone in endometrial epithelial cells. These findings offer insight into the physiology of endometrial epithelial cells, providing a foundation for further investigations into the dynamics of cellular states in endometrial cells. Furthermore, this dataset will be instrumental in studying the response of Ishikawa endometrial cells to liposome-based delivery.

Data description

We used liquid chromatography–mass spectrometry (LC–MS/MS) analysis to generate a proteome-wide profile of cellular proteins extracted from Ishikawa endometrial cancer cells.

Raw data files (.raw), peptide spectrum match (.tsv) as well as a list of identified proteins (.fasta) were deposited in the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository [4] and are available with the identifier PXD050871 (Table 1).

Methodology

Liposomes were synthesized using 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (PC) alone or in combination with 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (PE) (Cayman Chemical, MI), two essential phospholipids in cell membranes [5]. PC is a naturally abundant phospholipid found in cell membranes and facilitate the formation of fluid bilayers at physiological temperatures. PE, which contains arachidonic acid—a long-chain polyunsaturated fatty acid—can modulate membrane fluidity and potentially affect interactions with cell membranes. Moreover, arachidonic acid may introduce biological activity, as it plays a crucial role in cell signaling pathways [5]. The synthesis protocol followed a previously developed protocol [2]. Stock solutions of PC and PE were prepared in methanol at a concentration of 20 mM. A lipid mixture containing a PC: PE molar ratio of 70:30 was then generated. To form small liposomes, aliquots of these solutions were dried under nitrogen, followed by hydration with 1 mL of sterile PBS. The solution was allowed to swell for 2 h at room temperature. After 30 min of bath sonication, the sample was centrifuged to separate the large liposomes [6]. Aliquots of liposomes were stored at -80 °C until treatment.

Ishikawa cells were seeded in culture plates 24 hours before treatment with either vehicle or liposomes at a concentration of 100 µM concentration in MEM medium supplemented with Glutamax, sodium pyruvate and 2% exosome-depleted fetal bovine serum. Following a 72-hour incubation, whole-cell protein extraction was performed. This incubation period was selected based on previous studies demonstrating significant proteomic changes after in vitro treatment [7, 8] and effective liposomal drug delivery within this timeframe [9]. The cells were lysed in RIPA buffer for 10 minutes to extract the proteins. The lysate was then centrifuged to separate the supernatant containing the extracted proteins. LC–MS/MS and data analysis are detailed in Data file 1 (Table 1) [4]. Briefly, proteins were digested with trypsin, and subjected to LC-MS/MS analysis on an Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer (Thermo) equipped with a Nanospray Flex™ ion source operating in positive ion mode. Data-dependent acquisition with dynamic exclusion was employed for MS/MS analysis to prevent re-acquisition of previously analyzed peptides. Each treatment condition was assessed in three independent experiments. Raw data analysis was performed using FragPipe (version 20.0), a proteomics pipeline incorporating MSFragger (version 3.8) for peptide identification, IonQuant (version 1.9.8) for label-free quantification, and Philosopher (version 5.1.0) for statistical validation. Searches were conducted against a Homo sapiens UniProt database using specified parameters. Subsequent data analysis, including differential expression analysis and normalization, was performed in R programming language using the 'limma' and 'vsn' packages, respectively.

Proteomic analysis identified a total of 6750 proteins, including key regulators of cellular metabolism, cell cycle progression, and signaling. ACAD10, essential for fatty acid oxidation and energy generation, was prominently represented. CAMK4 and CHP, critical in calcium signaling and cell cycle control, were also identified, highlighting their potential roles in processes such as proliferation, differentiation, and apoptosis. Essential components of the protein synthesis machinery, RPS18 and RPL23A,

Table 1 Overview of data files/data sets

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data set 1	Ishikawa cell line LC-MS/MS analysis	RAW data file (.raw), peptide spectrum match (.tsv), metadata (.csv), peptide sequence assignment files (.fasta), feature quantification data (.fp-manifest)	PRIDE (http://identifiers.org/pride.project:PXD050871) [4]
Data set 2	Results table	Plain text file of results	FigShare (https://doi.org/10.6084/m9.figshare.25466794) [10]
Data set 3	Enrichment_molecular functions	Plain text file of comparisons (.csv)	
Data file 1	Description of the methodology	LC–MS/MS (.pdf)	
Data file 2	Mapping of the molecular functions	Network plots (.pdf)	

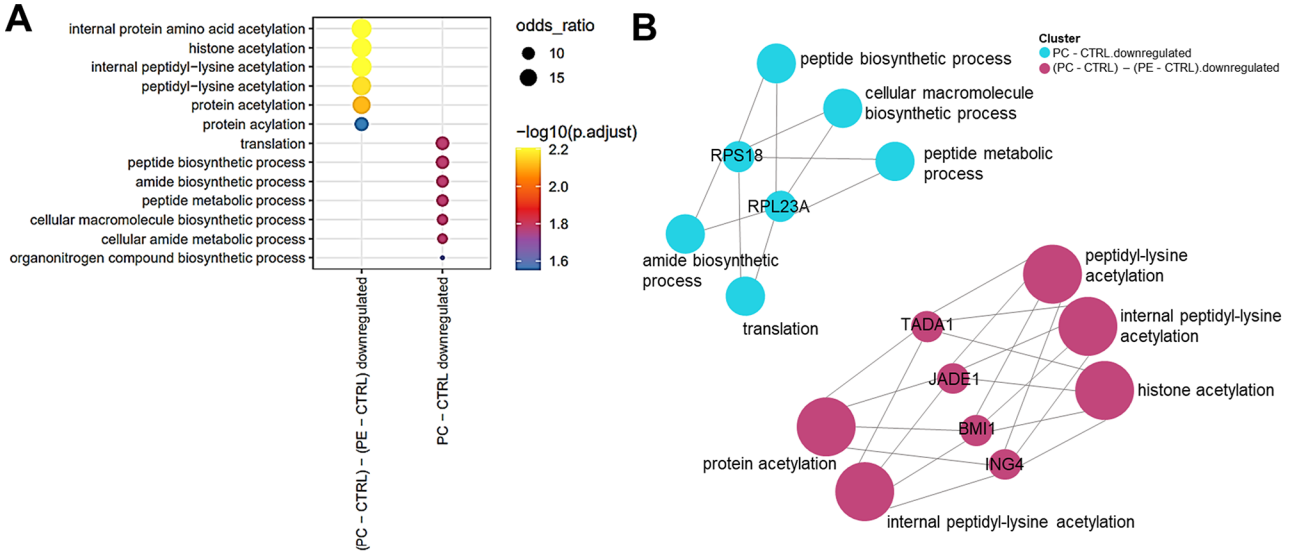

Fig. 1 Dotplot (**A**) and circular network plot (**B**) of gene ontology enrichment analysis for biological processes. Proteomic analysis of Ishikawa cells treated with control (CTRL), phosphatidylcholine liposomes (PC), and phosphatidylethanolamine liposomes (PE) ($n = 3$). The size of the circle represents the fold change compared to the control group. Limma statistics were used to assess the significance of enrichment

were detected, along with heat shock proteins involved in protein homeostasis and stress responses. To demonstrate the utility of the data, we applied spectral information to map the biological processes in the two liposome-treated cell isolates compared to the untreated control group (Fig. 1). Several key proteins associated with cytoskeleton regulation, vesicle trafficking, and cell division, including SYAP1, ARFGAP1, and ACAD10, exhibited a trend of increased expression levels in the PE condition compared to the PC condition (Table 1, Data file 2). However, between-group analysis revealed no differentially expressed proteins after adjusting for multiple testing using the applied thresholds (p-value < 0.05 and $|\text{logFC}| > 1$). This integration of LC–MS/MS analysis not only provides a detailed protein profile, but also highlights the potential to understand complex cellular dynamics underlying endometrial function, including embryo receptivity and cancer development.

Limitations

The present data are based on in vitro studies of growth and treatment of the Ishikawa endometrial cancer cell line, wherein peptide detection may be subject to the specific cell culture condition and the selected harvest time point. Subsequent investigations would be strengthened by incorporating primary patient samples and employing time-course analyses to elucidate the dynamic fluctuations within the proteome.

Abbreviations

ACAD10	Acyl-CoA Dehydrogenase Family Member 10
ARFGAP1	ADP-Ribosylation Factor GTPase Activating Protein 1
CAMK4	Calcium/Calmodulin-Dependent Protein Kinase IV
CHP	Calcineurin B Homologous Protein
LC	MS/MS-Liquid Chromatography Tandem-Mass Spectrometry
logFC	Log Fold Change
MS	Mass Spectrometry
PC − 1	Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine
PE − 1	Stearoyl-2-Arachidonoyl-sn-Glycero-3-Phosphoethanolamine
RPL23A	Ribosomal Protein L23a
RPS18	Ribosomal Protein S18
SYAP1	Synapse Associated Protein 1

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Author contributions

SF acquired funding, led the project conceptualization, and drafted the original manuscript. SF and AG supervised the study. AG and TS provided resources. AJ extracted the proteins. All authors reviewed the manuscript and approved the final manuscript.

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Data availability

The data described in this Data Note can be freely and openly accessed in the PRIDE partner repository of the ProteomeXchange Consortium and the Figshare repository under accession number PXD050871 and https://doi.org/10.6084/m9.figshare.25466794. Please see Table 1 for details and links to the data.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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