Molecular Immunology 87 (2017) 122–131

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Quantitative proteomic profiling for clarification of the crucial roles of lysosomes in microbial infections

Benhong Xu[1], Yanpan Gao[1], Shaohua Zhan, Wei Ge*

Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, National Key Laboratory of Medical Molecular Biology & Department of Immunology, No 5 Dongdan Santiao, Dongcheng District, Beijing 100005, China

ARTICLE INFO

Keywords:
Lysosome
Proteomics
Hypoxia
Microbes

ABSTRACT

Lysosomes play vital roles in both innate and adaptive immunity. It is widely accepted that lysosomes do not function exclusively as a digestive organelle. It is also involved in the process of immune cells against pathogens. However, the changes in the lysosomal proteome caused by infection with various microbes are still largely unknown, and our understanding of the proteome of the purified lysosome is another obstacle that needs to be resolved. Here, we performed a proteomic study on lysosomes enriched from THP1 cells after infection with *Listeria monocytogenes* (*L.m*), Herpes Simplex Virus 1 (HSV-1) and Vesicular Stomatitis Virus (VSV). In combination with the gene ontology (GO) analysis, we identified 284 lysosomal-related proteins from a total of 4560 proteins. We also constructed the protein-protein interaction networks for the differentially expressed proteins and revealed the core lysosomal proteins, including SRC in the *L. m* treated group, SRC, GLB1, HEXA and HEXB in the HSV-1 treated group and GLB1, CTSA, CTSB, HEXA and HEXB in the VSV treated group, which are involved in responding to diverse microbial infections. This study not only reveals variable lysosome responses depending on the bacterial or virus infection, but also provides the evidence based on which we propose a novel approach to proteome research for investigation of the function of the enriched organelles.

1. Introduction

Lysosomes, which are organelles comprising a single-lipid bilayer with membrane proteins and an acidic lumen, exist widely in eukaryotic cells (Saftig and Klumperman, 2009; Luzio et al., 2007). It is generally accepted that lysosomes not only play roles in the degradation of various macromolecules that are transported to lysosomes by endocytosis or autophagy (Dunn, 1994), but are also involved in fundamental functions required for plasma membrane repair (Reddy et al., 2001), signaling transduction and energy metabolism (Settembre et al., 2013). Lysosomal functions are closely related to the proteins present in the both membrane and lumen (Schwake et al., 2013). Among these proteins, hydrolases and acidification proteins are crucial for the execution of lysosomal function under different physiological and pathological conditions, such as starvation and infection (Yu et al., 2010; Balaji et al., 2014). Thus, clarification of the changes in the lysosomal proteins in response to different pathological conditions is important in providing an improved understanding of lysosomal function.

One striking aspect of the functions of the autophagy-lysosome degradation pathway is its involvement in the defense of host cells against microbial infection (Castrejon-Jimenez et al., 2015). In addition, different pathogens employ various strategies to escape lysosomal degradation. For example, *Listeria monocytogenes* (*L.m*) bacteria secrete toxins that destabilize the lysosome membrane to evade phagocytosis. It is widely accepted that lysosomes fuse with late endosomes, autophagosomes and the plasma membrane to perform the function of degradation and signaling (Mullock et al., 1998). Mass spectra-based studies have also revealed the pivotal roles of lysosomal membrane and related proteins against virus infections (Jean Beltran et al., 2016; Chapel et al., 2013). However, the changes in lysosome-related proteins and signaling pathways involved in the degradative processes that occur in lysosomes in response to diverse microbial infections remain to be elucidated.

THP1 is a human monocytic leukemia cell line and widely used as an in vitro model of monocytes and macrophages after stimulation with phorbol 12-myristate 13-acetate (PMA) or 1α, 25-dihydroxy vitamin D3 (Auwerx, 1991; Chanput et al., 2014). THP1 cells also exhibit similarities with primary peripheral blood mononuclear cell (PBMC)-derived macrophages induced by lipopolysaccharide (LPS) alone (Sharif et al.,

* Corresponding author.
E-mail address: wei.ge@chem.ox.ac.uk (W. Ge).
[1] These two authors contributed equally to this article.

http://dx.doi.org/10.1016/j.molimm.2017.04.002
Received 24 January 2017; Received in revised form 22 March 2017; Accepted 4 April 2017
Available online 25 April 2017

2007). Here, we used a combination of differential and density ultracentrifugation processes to enrich the lysosomes from THP1 monocytes cells infected with *L. m* (bacterial), HSV-1 (an DNA virus) and VSV (a RNA virus). The aim of this study was to investigate the responses and proteome changes of intact and mature lysosomes to various types of microbial infection using a comprehensive proteomics strategy. The results revealed that the lysosomal enrichment strategies used in this study provided material suitable for use in the subsequent proteomics studies. The proteomic profiles combined with bioinformatics analysis revealed the diversity of lysosomal responses to different pathogenic infections.

2. Methods

2.1. Cell culture and infection

THP1 cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS). *Listeria monocytogenes* (*L.m*) was amplified in Brain Heart Infusion (BHI) medium. Herpes Simplex Virus 1 (HSV-1) and Vesicular Stomatitis Virus (VSV) were expanded in VERO cells as described previously (Yasuhara-Bell et al., 2010). THP1 cells were treated with *L. m*, HSV-1 or VSV at a multiplicity of infection (MOI) of 10. *L. m*- and VSV-infected cells were harvested 9 h post-infection and HSV-1-infected cells were harvested 20 h post-infection.

2.2. Lysosome purification

The Lysosome Isolation Kit (Cat No. LYSISO1, Sigma, MO, USA) was used for lysosome purification according to the manufacturer's instructions with slight modifications. Cells were harvested and washed twice with ice-cold PBS before centrifugation at 500 × *g* for 10 min. The pellet was then resuspended in extraction buffer and homogenized with a 15 ml Dounce homogenizer (30 cycles). Cell disruption was evaluated under an optical microscope (Olympus CKX31, Tokyo, Japan). After ultracentrifugation (150,000 × *g* for 4 h), samples were separated into nine fractions according to the bands. The location of the lysosomal fractions was verified using an acid phosphatase (AP) assay kit and the total protein concentration of each fraction was measured with a BCA protein assay kit according to the manufacturer's instructions.

2.3. Western blot analysis

Lysates from each fraction prepared by gradient centrifugation were subjected to Western blot analysis. Different antibodies for specific detection of organelle biomarkers were used for verification of the lysosome-enriched fraction: Lamp1 (ab25245), Ezrin (ab75840) and ERp72 (cst-5053), RCAS1 (cst-12290) and Cox7b (ab140629).

2.4. Tandem mass tag (TMT) labeling

Lysosomes were disrupted in 8 M urea lysis buffer (dissolved in 0.01 M PBS, pH 7.2) containing proteinase inhibitors (Cocktail, Roche, IN, USA) followed by protein concentration measurement using a Nano Drop 2000. Equal amounts of protein (100 μg) from each group were treated with 10 mM DTT at 37 °C for 40 min followed by 25 mM IAA at room temperature for 1 h in the dark. Subsequently, trypsin/Lys-C (50:1) was added to the reaction for protein digestion and incubated for 1 h before dilution with PBS to achieve a final urea concentration of 1 M. The reaction mix was then digested overnight at 37 °C prior to quenching by heating to 65 °C and acidification with 0.1% TFA. The samples were then desalted by reversed-phase column chromatography (Oasis HLB; Waters, MC, USA) and TMT isobaric labeling was performed according to the manufacturer's instruction with a slight modification. Equal amounts of peptides derived from each group were labeled with different TMT labels: control, TMT-126; *L. m*, TMT-127; HSV-1, TMT-128; and VSV, TMT-130. The labeled peptides were mixed, desalted and then separated into 50 fractions by HPLC as previously described (Xu et al., 2016). Briefly, the peptides were loaded onto the C18 peptide separation column (Xbridge BEH300, C18, 2.5 μm, 4.6 × 250 mm, Waters) and separated into 50 fractions by gradient elution in water/acetonitrile at pH 10.0.

2.5. LC–MS/MS and database searches

The fractions derived from HPLC were dried using a vacuum concentrator and then dissolved in 0.1% TFA before combining into 20 aliquots. A Thermo Orbitrap Fusion mass spectrometer was used for detection of each fraction after separation by an EASY-nLC 1000 system under gradient elution (120 min at a flow rate of 0.30 μl/min). A "homemade" fused silica capillary column (75-μm inner diameter, 150-mm length; Upchurch, Oak Harbor, WA, USA) packed with C-18 resin (300 Å, 5 μm; Varian, Lexington, MA, USA) was used as an analytical column. Mobile phase A and mobile phase B consisted of 0.1% formic acid/ddH_2O and 0.1% formic acid/acetonitrile, respectively. Xcalibur 3.0 software was used for the visualization of the mass spectrometer operation. The Orbitrap mass spectrometer (350–1550 *m/z*, 120,000 resolution) was used for a single full-scan mass spectrum and 3-s data-dependent MS/MS scans at 38% normalized collision energy (HCD). The MS/MS raw data were searched against the UniProt/SwissProt mouse database (released on 1st November, 2016) using Proteome Discovery (version 1.4). Mass spectra filters for the SEQUEST HT analysis were set at a minimum number of peaks of 200 and intensity of 20,000. Trypsin/Lys-C and a maximum of two missed cleavage sites for a range of 6–144 peptides were applied as tryptic parameters. The precursor tolerance and fragment tolerance were set at 20 ppm and 0.02 Da, respectively. Cysteine carbamidomethylation (+57.021) and lysine and N-terminal TMT 6-plex modification (+229.163) were set as static modifications. Proteins with at least one unique peptide (with a false discovery rate (FDR) less than 1%) were considered to be valid. All peptide ratios were normalized to the median protein ratio and protein ratio was determined for all the identified unique peptides. The mass spectrometry proteomics data have been deposited into the ProteomeXchange consortium via the PRIDE partner repository with the dataset identifier PXD005748.

2.6. Bioinformatic analysis

For functional pathway analysis, the different subsets of proteins identified in the dataset were subjected to functional analysis using DAVID bioinformatics resources. Gene ontology (GO) terms for biological processes (BP), molecular functions (MF) charts and cellular components (CC) were obtained using default statistical parameters. The STRING database 10.0 (http://string-db.org) was used to predict the protein-protein interaction networks. All STRING network analyses were performed using protein accessions as input and with medium (0.4) confidence levels. Cytoscape 3.4 was employed for the visualization of protein networks in each group.

3. Results

3.1. Lysosome enrichment and identification

To clarify the role of lysosomes in the responses to different microbial infections, we conducted a comprehensive proteomic study of lysosomes enriched from THP1 cells by density gradient fractionation following infection with *L. m*, HSV-1 and VSV; PBS was used as a control (Fig. 1A). Gradient fractions were separated into nine parts after the ultracentrifugation according to the interphases. The protein concentration and AP assays showed that lysosomes were enriched mainly within Fraction 3, which showed the highest AP activity and a relatively low total protein concentration (Fig. 1B). All the fractions