Uncovering the Origins of Autophagosomes: How Cells Recycle Waste

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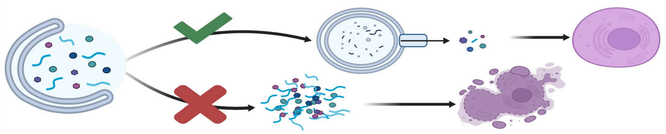
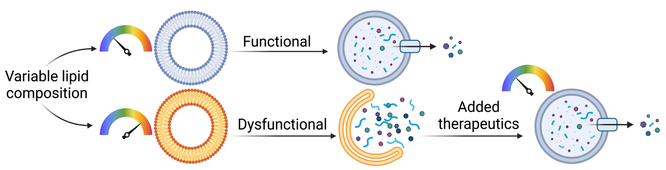
Introduction

Autophagy describes "self-digestion" in cells[1]. This self-digestion provides nutrients by recycling superfluous or damaged organelles, misfolded proteins, and invading microorganisms[2]. Diseases like Alzheimer's are suspected to arise from dysfunctional autophagosomes[3].

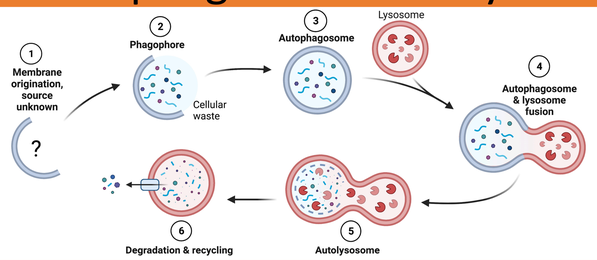


Autophagosomes are comprised of a lipid bilayer where in the ratio and composition of these lipids play a critical role in dictating proper functionality[4]. Sadly, the complete lipid profile of a mature autophagosome membrane is unknown[5]. This gap in knowledge hinders our study of therapeutics to help maintain proper autophagosome functionality.



Our objective is to provide a comprehensive lipid profile of mature autophagosome membranes through matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. This data may be used to better track how autophagosomes form and locate irregularities in lipid compositions as a cause of dysfunctional autophagosomes.

Autophagosome Life Cycle



Autophagy Visualized

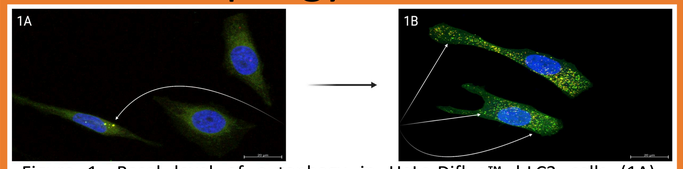
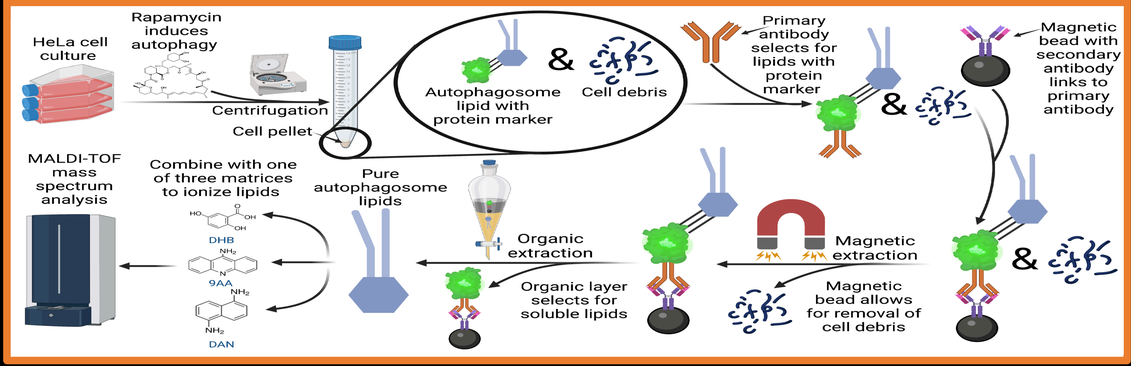


Figure 1: Basal level of autophagy in HeLa-Difluo™ hLC3 cells (1A). Enhanced level of autophagy in HeLa-Difluo™ hLC3 cells after induction with rapamycin (1B). Autophagosome membranes are spherical bilayers which fluoresce green due to the green fluorescent protein (GFP) marker.

Methods



Results

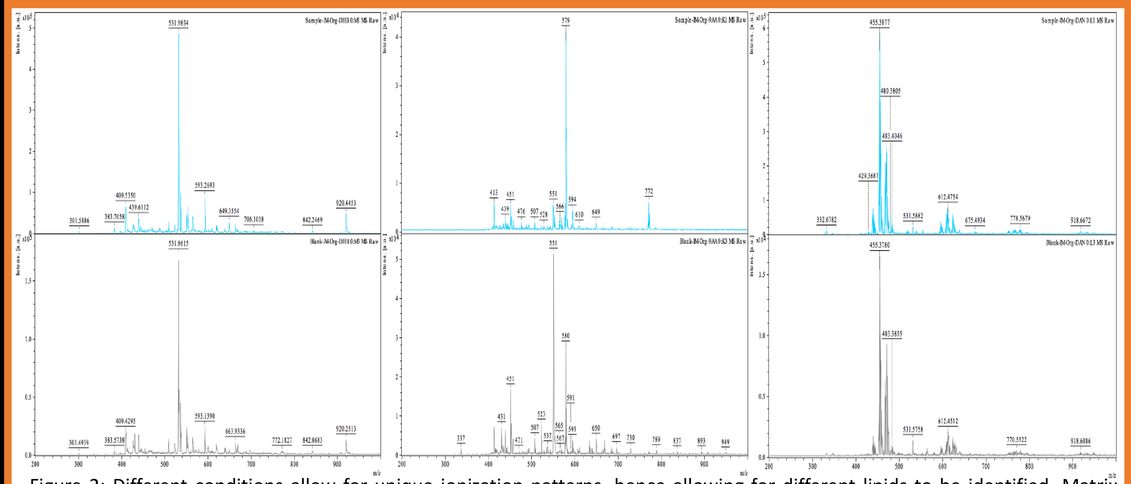


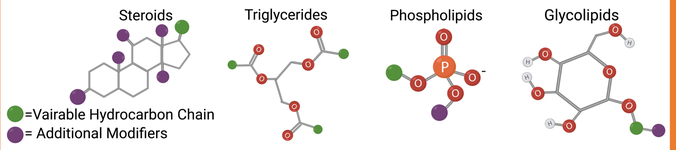
Figure 2: Different conditions allow for unique ionization patterns, hence allowing for different lipids to be identified. Matrix effects are a major cause of unique ionization patterns as various lipid categories ionize better in different matrices. Method blanks were used to account for any matrix peaks which match lipid masses by coincidence. Said peaks were removed as lipid candidates.

Lipid IUPAC Name	Lipid Reference ID	Mass Difference in ppm	Sample m/z	Reference Lipid m/z	S/N value	Matrix	Lipid Class
N-Octadecanoyl-4-sphingosine 2-aminoethylphosphonate	PSP8005	0.698964925	629.5027	629.50226	20	DAN	Sphingophospholipid
rac-1,2-Dilauroyl-glycero-3-phosphoserine	PGP3089	6.549560967	592.41	592.40612	7	9AA	Glycerophospholipid
1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamines	850804	7.42625533	767.5527	767.547	13	DAN	Phosphatidylethanolamine
1-pentadecanoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (sodium salt)	330719	-9.356846743	769.4828	769.49	13	9AA	Phosphatidylserine
1,2-dimyristoleoyl-sn-glycero-3-phosphocholine	850346	9.503049885	673.4744	673.468	9	DAN	Phosphatidylcholines
1,2-dimyristoyl-sn-glycero-3-phosphocholine	850345	15.94095941	677.5108	677.5	8	DAN	Dimyristoylphosphatidylcholine
1-lignoceroyl-2-hydroxy-sn-glycero-3-phosphocholine	855800	-17.12052521	607.4476	607.458	19	DAN	Phosphatidylcholines
1,2-dilinoleoyl-sn-glycero-3-phosphocholine	850385	-18.42464194	781.5476	781.562	12	DAN	phosphatidylcholine
N-(1-Adamantaneacetyl)-galactosyl ceramide	860479	26.51167533	637.4719	637.455	10	DAN	Ceramide
1,2-dilinolenoyl-sn-glycero-3-phosphocholine	850395	27.39440614	777.5523	777.531	13	DAN	Phosphatidylcholines
1,2-diundecanoyl-sn-glycero-3-phosphocholine	850330	29.82780761	593.4237	593.406	10	DAN	Phosphatidylcholines
1,2-dilauroyl-sn-glycero-3-phosphocholine	850335	37.17190962	621.4601	621.437	17	DAN	phosphatidylcholine
1-(1Z-octadecenyl)-2-arachidonoyl-sn-glycero-3-phosphocholine	852469	-46.11900973	793.5624	793.599	11	DAN	Phosphatidylcholines
1-O-octadecyl-2-hydroxy-sn-glycero-3-phosphate (ammonium salt)	857228	89.73040093	441.3616	441.322	7	9AA	lysophosphatidic acid

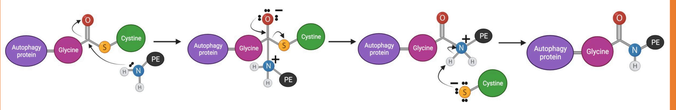
Table 1: Lipid candidates ordered by increasing Absolute Mass Difference (AMD) in ppm. AMDs of less than 100 ppm are shown as they represent lipids who identities have the highest confidence. Lipids were first screened by matched rounded integer values of m/z peaks in the sample spectrum to the reference list, then reanalyzed to obtain the mass difference between the reference and sample m/z in ppm .

Conclusions

Unlike proteins which are defined by their amino acid backbones[7], or DNA and RNA defined by their nucleic acids[8] lipids represent such a diverse category with varying hydrocarbon chains, core motifs, and numerous additional lipid specific modifications[9]. As a result, only select phospholipids and glycolipids were included as possible lipid candidates because these lipid classes are the primary components of lipid bilayers[10].



In spite of this diversity, we've identified several lipid candidates which may comprise autophagosome membranes based on their average mass numbers. This includes phosphatidylserines (PS) and phosphoethanolamines (PE), which previous research may indicate play a role in autophagy through covalent linking receptor proteins to the autophagosome membrane[11].



Further lipid verification will be done through tandem mass spectroscopy (MS) with MALDI or Electrospray Ionization TOF. MS research along with experiments such as like genetic knockouts, isotopic labeling, and live cell imaging to further validate lipid identities must be done before any definitive list of autophagosome lipid composition can be used for medical research.

Acknowledgements

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References

[1] Yin, Z., Pascual, C., & Klionsky, D. J. (2016). Autophagy: machinery and regulation. Microbial cell (Graz, Austria), 3(12), 588–596. https://doi.org/10.15698/mic2016.12.546

[2] Levine, B., & Kroemer, G. (2008). Autophagy in the pathogenesis of disease. Cell, 132(1), 27–42. https://doi.org/10.1016/j.cell.2007.12.018

[3] Farine, L., Niemann, M., Schneider, A., & Bütikofer, P. (2015). Phosphatidylethanolamine and phosphatidylcholine biosynthesis by the Kennedy pathway occurs at different sites in Trypanosoma brucei. Scientific Reports, 5(1). https://doi.org/10.1038/srep16787

[4] Li, L., Tong, M., Fu, Y., Chen, F., Zhang, S., Chen, H., Ma, X., Li, D., Liu, X., & Zhong, Q. (2021). Lipids and membrane-associated proteins in autophagy. Protein & cell, 12(7), 520–544. https://doi.org/10.1007/s13238-020-00793-9

[5] Lamb, C. A., Yoshimori, T., & Tooze, S. A. (2013). The autophagosome: origins unknown, biogenesis complex. Nature reviews. Molecular cell biology, 14(12), 759–774. https://doi.org/10.1038/nrm3696

[6] Nakamura, S., & Yoshimori, T. (2018). Autophagy and Longevity. Molecules and cells, 41(1), 65–72. https://doi.org/10.14348/molcells.2018.2333

[7] NCI Dictionary of Cancer terms. National Cancer Institute. (n.d.). Retrieved July 12, 2022, from https://www.cancer.gov/publications/dictionaries/cancer-terms/def/protein

[8] Bates, S. A. (2022, July 8). Nucleic acids. Genome.gov. Retrieved July 12, 2022, from https://www.genome.gov/genetics-glossary/Nucleic-Acids#:~:text=Nucleic%20acids%20are%20large%20biomolecules,cells%20need%20to%20make%20proteins

[9] Ahmed, S., Shah, P., & Ahmed, O. (2022, May 8). Biochemistry, lipids - statpearls - NCBI bookshelf. Biochemistry, Lipids. Retrieved July 12, 2022, from https://www.ncbi.nlm.nih.gov/books/NBK525952/

[10] Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. The Lipid Bilayer. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26871/

[11] Funderburk, S. F., Marcellino, B. K., & Yue, Z. (2010). Cell "self-eating" (autophagy) mechanism in Alzheimer's disease. The Mount Sinai journal of medicine, New York, 77(1), 59–68. https://doi.org/10.1002/msj.20161

Cartoon graphics created in Biorender.

Lipid atomic mass categories. In addition to avantilipids.com's PA, LPA, Cyclic LPA, PC, LPC, PE, LPE, PG, references from lipidbank.jp's Glycosphingolipid, Glycerophospholipid, and Sphingophospholipid LPG PI, PIP, LPI, PS, and LPS product categories.

Background image of HeLa cell

Complete lipid list and reference links