LYVAC mediates Lysosomal Vacuolation Associated with Skeletal Dysplasia

Jinrui Xun[1,2], Haoxiang Yang[2], Jay Xiaojun Tan[2,3]
[1]Department of Sports Medicine & Research Center of Sports Medicine, Xiangya Hospital, Central South University, Changsha, Hunan, China, [2]Aging Institute, University of Pittsburgh School of Medicine and University of Pittsburgh Medical Center; Pittsburgh, PA, USA, [3]Department of Cell Biology, University of Pittsburgh School of Medicine; Pittsburgh, PA, USA.
Email of Presenting Author: Jinrui_xun@foxmail.com

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INTRODUCTION: Lysosomes are central organelles for cellular degradation, nutrient sensing, and signaling transduction. Increasing evidence indicates that lysosomal dysfunction contributes to skeletal diseases, including osteopetrosis, osteoarthritis, and congenital skeletal dysphasia. A characteristic manifestation of impaired lysosomal homeostasis is lysosomal vacuolation, which disrupts intracellular trafficking, signaling, and bone cell function. Recent genetic studies of Yunis-Varón syndrome (YVS) have revealed that mutations in the PIKfyve–VAC14–FIG4 complex lead to severe skeletal developmental abnormalities accompanied by prominent vacuolation in multiple cell types. However, the molecular mediators and mechanisms that drive vacuole formation remain poorly defined. Therefore, the objective of this study was to identify the specific mediator of lysosomal vacuolation during skeletal dysplasia.

METHODS: To identify the molecules responsible for lysosomal vacuolation associated with skeletal dysplasia, we used apilimod, which induces lysosomal vacuolation by inhibiting PIKfyve, mimicking the vacuolation in YVS syndrome. Then, we applied proximity-dependent biotinylation (TurboID) proteomics and identified LYVAC as a central regulator of lysosomal vacuolation. By knocking out different target genes from the proteomics, we identified the signaling pathway mediating LYVAC recruitment and activation. Domain mapping was performed using truncation and mutagenesis to define the sequence regions critical for LYVAC recruitment and vacuole development. Lysosomal vacuolization was assessed in mammalian cells under stress conditions using confocal microscopy and quantitative image analysis. The lipid transfer activity was further validated by in vitro reconstitution assays and molecular dynamics simulations.

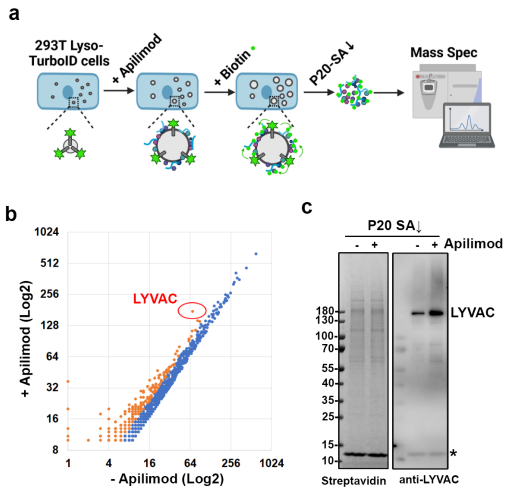

Fig 1. Unbiased proteomics reveal LYVAC as an essential mediator of lysosomal vacuolation.

RESULTS SECTION: Our proteomics analysis identified PDZD8 (PDZ domain–containing 8), which we propose to be renamed as LYVAC (lysosomal vacuolator), as the top hit selectively enriched on vacuolating lysosomes after PIKfyve inhibition. LYVAC is an endoplasmic reticulum (ER)–anchored lipid transfer protein, suggesting its potential role in mediating vacuole formation by transferring lipids from the ER to lysosomes.
Genetic deletion of LYVAC strongly suppressed lysosomal vacuolation caused by either inhibition or genetic deletion of PIKfyve. LYVAC was also essential for lysosomal vacuolation triggered by a variety of unrelated stressors, including weak base compounds, sucrose storage, and hypotonic stress. These findings establish LYVAC as a general mediator of lysosomal vacuole formation.
Domain analysis revealed that LYVAC was recruited through multivalent weak interactions mediated by two distinct domains: one that binds to RAB7, a marker of late endosomes and lysosomes, and another that binds to negatively charged lipids, particularly phosphatidylserine (PS). Lysosomal osmotic stress triggered phosphoinositide signaling that drove the ER-to-lysosome transfer of PS and cholesterol, both of which were required for lysosomal vacuolation. PS and cholesterol not only promoted LYVAC recruitment but also activated its lipid transfer function. The lipid transfer domain of LYVAC directly binds to PS- and cholesterol-enriched membranes through conserved motifs recognizing the presence of both PS and cholesterol. In cells, label-free chemical imaging revealed LYVAC-dependent large-scale, ER-to-lysosome lipid movement after vacuole induction. In vitro reconstitution assays and molecular dynamics simulations supported the idea that this directional lipid movement was driven by lysosomal osmotic membrane tension and lipid composition differences between the ER and lysosomes.

DISCUSSION: Our findings identify LYVAC as a critical mediator of lysosomal vacuolation, bridging lipid signaling with ER–lysosome interactions. By coupling lysosomal stress to vacuolar remodeling, LYVAC contributes to the disruption of bone cell homeostasis. This pathway provides new insights into how lysosomal dysfunction underlies skeletal developmental abnormalities.

SIGNIFICANCE/CLINICAL RELEVANCE: Defective lysosomal function is increasingly recognized in musculoskeletal pathology, ranging from genetic skeletal syndromes to degenerative bone and joint disorders. Our findings uncover LYVAC as a key mediator of lysosomal vacuolation, providing mechanistic insights into how lysosomal stress translates into skeletal abnormalities. Understanding this pathway may open new therapeutic avenues for skeletal diseases characterized by impaired lysosomal homeostasis and suggest lysosomal lipid signaling as a potential target for modulating bone development and repair.

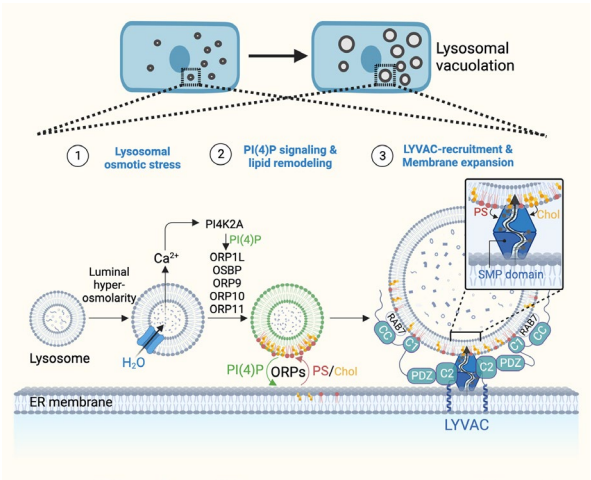

Fig 2. Schematic model of LYVAC-mediated lysosomal vacuolation through ER-to-lysosome lipid transfer.