Characterization of metastasis related lysosomal subpopulations by correlative live cell- 3D electron microscopy

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Lysosomes are degradative compartments, and major signaling hubs that sense the nutrient status of the cells. They are predominantly located in the central part of the cell, however there is a dramatic increase in the peripherally located population of lysosomes in malignant cells. These peripherally located lysosomes are gaining increasing attention as they may have dedicated functions in the regulation of cancer cell invasion[1,2]. Even though they constitute a very promising target for cancer cell invasion, the exact functions of this peripheral pool of lysosomes and how they differ from the perinuclear pool regarding their ultrastructure, membrane composition, hydrolytic capacity, and dynamic behavior remain as standing questions.

Correlative live-cell imaging - electron microscopy (live cell-CLEM) approaches provide elegant means to directly link molecular composition and dynamic behavior of organelles to their morphology at nanometer resolution[3]. A major challenge however, is to retrace single, especially small, organelles in 3 dimensions (3D). Mastering this challenge, we have recently presented an approach where we have introduced focused ion beam scanning electron microscopy (FIB-SEM) to live-cell CLEM pipeline, and for the first time used live-cell volume CLEM to link the dynamics and ultrastructural context of individual, highly dynamic organelles[4]. We presented that live-cell imaged organelles can be reliably retraced in FIB-SEM data, and fusion, fission and trafficking dynamics of single organelles can be linked to their ultrastructure. Strikingly, live cell-CLEM show that lysosomal marker LAMP-1 positive organelles greatly vary in their morphology, highlighting how ultrastructural information on the membrane organization is crucial to identify compartments. Here, we utilize and extend this live cell-CLEM approach to address and characterize the metastasis related peripheral subpopulation of lysosomes.

We have generated and imaged highly metastatic HT1080 fibrosarcoma cells stably expressing lysosomal marker LAMP-1-GFP with live-cell fluorescence microscopy. Single LAMP-1 positive organelles located either at the periphery or the perinuclear areas of the cells are identified, and their dynamic behaviors are characterized. After collection of the live-cell data, the cells are prepared for electron microscopy, the exact organelles are retraced, and imaged. This way, we assess and correlate distinct movement dynamics and morphological characteristics of peripheral and perinuclear subpopulations of lysosomes. Additionally, as a unique possibility brought by live-cell CLEM, we perform functional assays to assess the enzyme activity, degradative capacity, and pH gradient of these lysosomal subpopulations; and are currently in the process of linking this functional information directly to the ultrastructure.

In conclusion, using live-cell CLEM, we have assessed the distinct dynamics and composition of peripheral vs. perinuclear lysosomes by characterization/comparison of their luminal properties (pH, presence and activity of hydrolases). The (co)localization of lysosomal markers in peripheral and perinuclear populations, their motility (speed, displacement), and luminal characteristics were assessed, and directly linked to their morphology by correlative (live-cell) fluorescence and 3D electron microscopy. The novel way presented here to integrate subcellular dynamics, functional character, and ultrastructure onto a single organelle is relevant to address also many other biological questions related to membrane trafficking, organelle biogenesis and positioning.

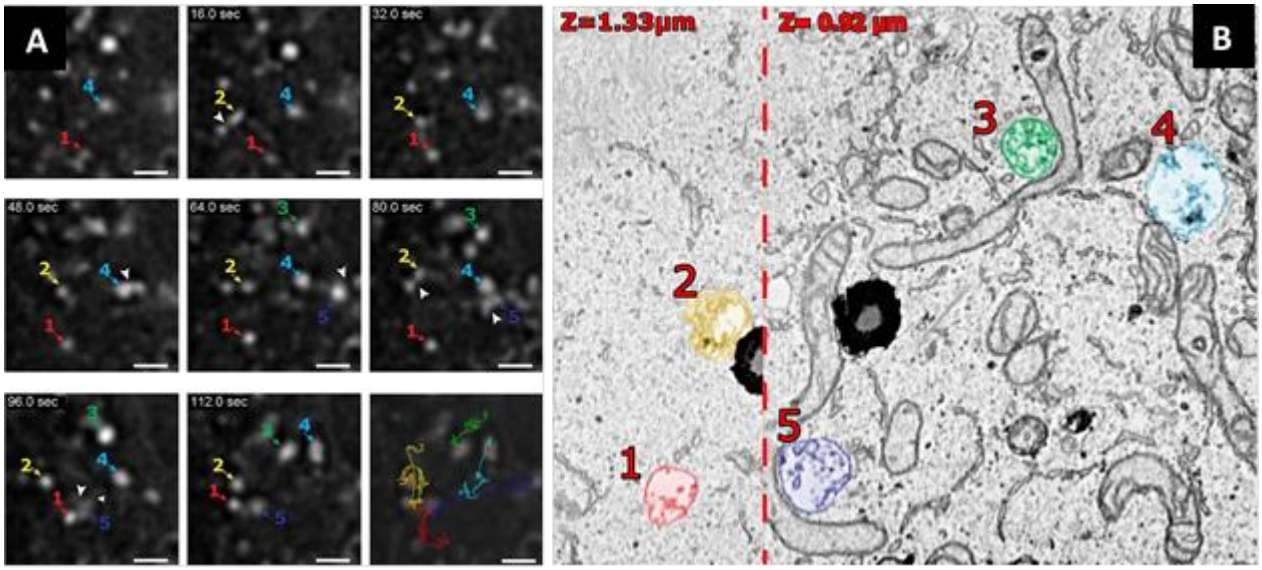

Figure: Correlative live cell - FIB-SEM links live-cell dynamics of compartments to their ultrastructural context

A. Stills from a ROI from a live-cell movie of LAMP-1 positive compartments. Tracked compartments are numbered and color-coded. The bottom right panel shows the tracks of the compartments over the duration of imaging.

B. FIB-SEM reconstruction of the same ROI as A. Organelles 1 through 5 were all retraced from live cell to FIB-SEM data. Compartments are numbered and color coded as in A**.**

References

1. Hämälistö, S. & Jäättelä, M. Lysosomes in cancer - living on the edge (of the cell). *Curr. Opin. Cell Biol.* **39,** 69 - 76 (2016).
2. Olson, O. C. & Joyce, J. A. Cysteine cathepsin proteases": regulators of cancer progression and therapeutic response. *Nat. Publ. Gr.* **15,** 712 - 729 (2015).
3. van Rijnsoever, C., Oorschot, V. & Klumperman, J. Correlative light-electron microscopy (CLEM) combining live-cell imaging and immunolabeling of ultrathin cryosections. *Nat. Methods* **5,** 973 - 80 (2008).
4. Fermie, J. *et al.* Single organelle dynamics linked to 3D structure by correlative live-cell and 3D electron microscopy. *Traffic* (2018).