

Title
(Conclusion)

Aim

Method

Results

Conclusion/
interpretation

Foci of exogenous SHIP164 reflect accumulations of small vesicles

To gain further insight into the precise nature of foci of exogenous tagged SHIP164, we performed correlative light-electron microscopy (CLEM) of COS-7 cells co-expressing the early endosome marker GFP-WDFY2 and SHIP164^{mScarlet} (Fig. 3 A). Both conventional transmission electron microscopy (TEM) and focused ion beam scanning electron microscopy (FIB-SEM) were performed. We employed Ras^{G12V} to induce the formation of macropinosomes (Porat-Shliom et al., 2008) to facilitate the alignment of fluorescence and EM images. This analysis revealed that spots of SHIP164 fluorescence reflected accumulations of hundreds of tightly packed ~50-60-nm vesicles juxtaposed to endosomal membranes positive for GFP-WDFY2 (Fig. 3 A), thus providing an explanation for the dynamic nature of the fluorescence spots. An ~70-nm-thick matrix appeared to anchor these clusters to the WDFY2-positive vacuoles, and the space occupied by this matrix was nearly devoid of vesicles (Fig. 3, A-C). ER tubules were present in proximity of these clusters and often penetrated them, but there did not seem to be sites of preferential accumulation of vesicles (Fig. 3 B). The mechanism responsible for the clustering of SHIP164 positive vesicles remains unclear. Also unclear is how such clusters are anchored to the large vacuoles.

Collectively, the findings described above, based on exogenous tagged SHIP164, strongly supported a role of SHIP164 in the endocytic pathway. However, large accumulation of small vesicles, such as those described here, were never observed to our knowledge in WT cells, making it critical to assess the localization of SHIP164 when expressed at the endogenous level.

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