Investigating the role of PIKfyve in modulating membrane contact site dynamics between the lysosome and endoplasmic reticulum

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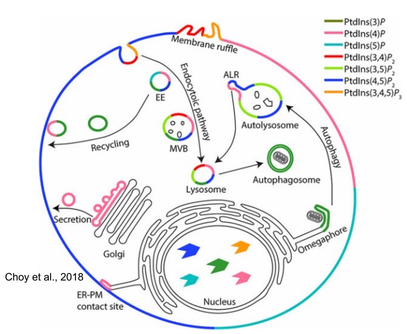
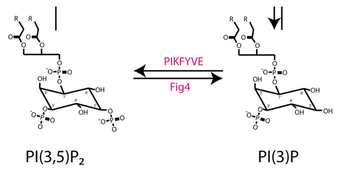
Introduction

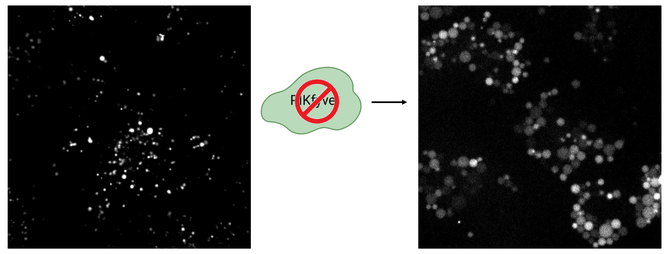
PIKfyve regulates production of PtdIns(3,5)P_2



- Phosphoinositides (PI) are phospholipids derived from phosphatidylinositol
- There are 7 possible PI species and these can be interconverted via specific PI kinases and phosphatases
- PI(3)P and PI(3,5)P_2 are considered lysosome and late endosome specific PI species. This interconversion is catalyzed by the lipid kinase PIKfyve.

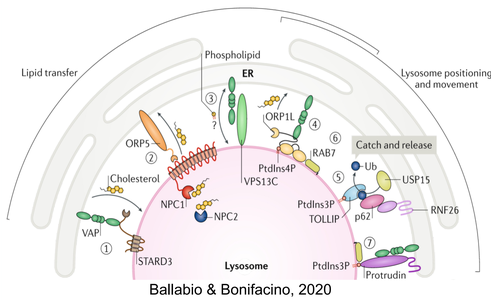


Lysosome size and dynamics is controlled by PIKfyve



- Lysosomes are the catabolic center of the cell and are responsible for removing cellular waste but also act as a signaling hub.
- PIKfyve plays a significant role in regulating the morphology and trafficking of endo-lysosomes(Ho, Alghamdi, & Botelho, 2012; Wallroth & Haucke, 2018)
- Loss of PIKfyve results in fewer but severely enlarged lysosomes causing lysosome coalescence (Choy et al., 2018).

Lysosomes form many membrane contact sites with other organelles



Ballabio & Bonifacino, 2020

- Organelles communicate and integrate their biochemical processes via close contacts known as “membrane contact sites” (Lackner & Voeltz, 2017; Prinz et al., 2020; Silva et al., 2020)
- The ER is a large network and can form contact sites with various organelles such as the mitochondria and late endosomes/lysosomes via anchoring proteins.
- ER-lysosome contacts may be important for endosomal and lysosomal fission and are considered signaling hubs.

Objective & Hypothesis

- **Understand how alterations to lysosome morphology and dynamics in PIKfyve-inhibited cells impact ER morphology and dynamics and ER-lysosome interactions.**
- **We hypothesize that the changes in lysosome morphology and dynamics due to PIKfyve inhibition disturbs ER structure and dynamics**

Results

1. ER morphology is altered upon PIKfyve inhibition

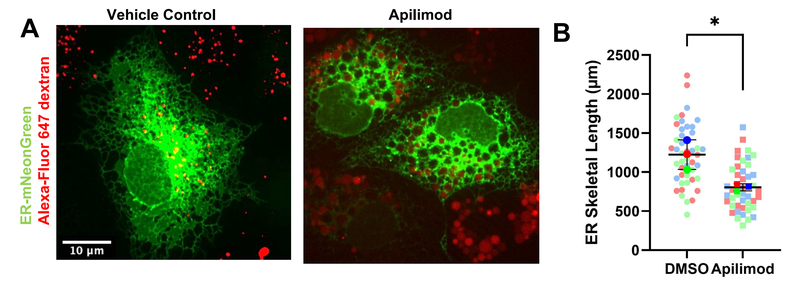


Figure 1: ER and lysosome morphology appears to be altered as a result of PIKfyve inhibition. A) Wild-type COS-7 cells were transfected with ER-mNeonGreen (a general ER marker) for 24h and pre-labelled with Alexa-647 dextran at 200 μg/mL for 1 hour washed 1X with PBS and treated with either vehicle or 80 nM Apilimod for 2 hours. In the apilimod treated cells, as expected we see enlarged lysosomes which is altering the ER morphology by creating large spaces within the ER network. However, it is interesting to note that there are large empty spaces that are not dextran labelled in addition to dextran labelled lysosomes within the ER. This begs the question as to whether these spaces are filled by enlarged lysosomes, or perhaps late endosomes. B) Overall, we observed a significant decrease in ER skeletal length with apilimod treatment compared to vehicle (Student’s t-test, $p<0.05$, $n=4$)

2. ER vacuoles are not cytosolic in nature and may consist of endo-membrane compartments

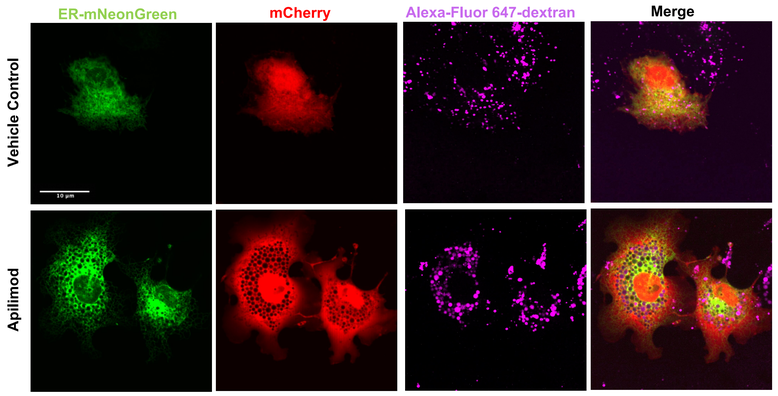


Figure 2: ER vacuoles are not filled with cytosolic mCherry upon lysosome enlargement by PIKfyve inhibition. Wild-type COS-7 cells were transfected with ER-mNeonGreen (a general ER marker) and mCherry (a general cytosolic marker) for 24h and pre-labelled with Alexa-647 dextran at 200 μg/mL for 1 hour washed 1X with PBS and treated with either vehicle or 80 nM Apilimod for 2 hours. In the apilimod treated cells, as expected we see enlarged lysosomes which is altering the ER morphology by creating large spaces within the ER network. We were curious to investigate whether these large empty spaces are filled with cytosol and interestingly found that they are not. This could suggest that there is a population of enlarged organelles in addition to lysosomes and these could perhaps be early or late endosomes.

3A. Development of a method to study ER membrane dynamics

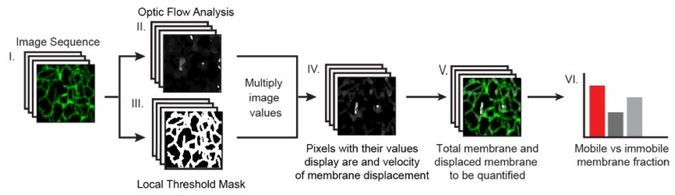


Figure 3: Schematic flow of membrane displacement analysis adapted from Spits et al.,2021 Membrane displacement analysis was used to quantify ER motility over a 60 s period as identifying such heterogeneous networks is challenging using particle tracking methods. This automated analysis method uses the number of pixels that have moved in a specific region of interest where the extent of membrane movement is reflected by colour intensity. The higher the colour intensity the further the pixel moved in a particular frame in relation to the previous frame.

Results (cont’d)

3B. ER dynamics are altered upon PIKfyve inhibition

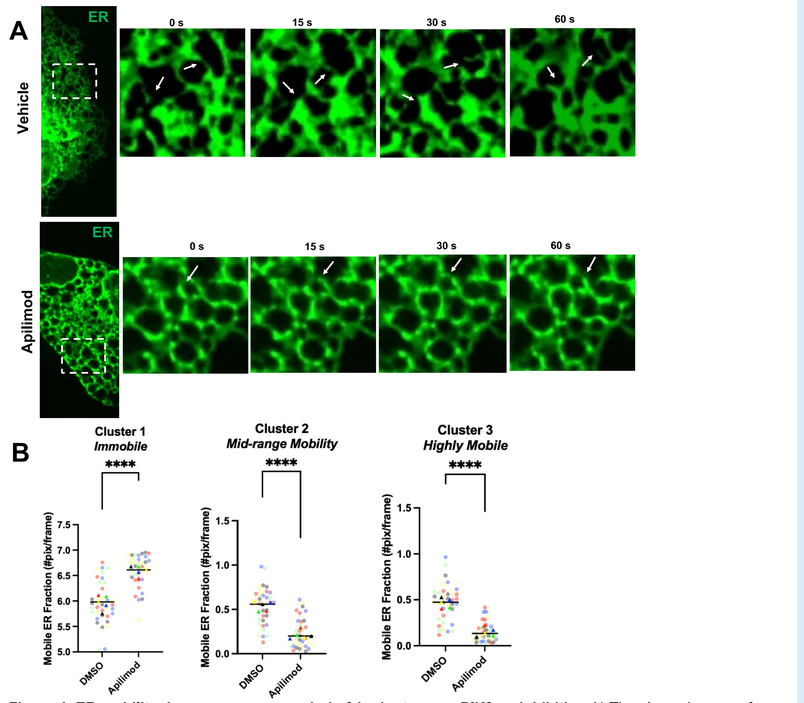


Figure 4: ER mobility decreases over a period of 1 minute upon PIKfyve inhibition A) Time-lapse images of wild-type COS-7 cells labelled with ER-mNeonGreen (a general ER marker) showing dynamic ER movement and remodeling over 60 s period, compared to static and less dynamic ER movement with Apilimod treatment. White arrows indicate areas of mobility (vehicle) and no mobility (apilimod) B) Mobile ER fractions were clustered into 3 categories – immobile, mid-range mobility and highly mobile for statistical analysis where mobile ER fractions decreased with Apilimod compared to highly mobile ER fractions in the control (Student’s t-test, $p<0.0001$, $n=5$).

4. Lysosome – ER contact sites may be altered with PIKfyve inhibition

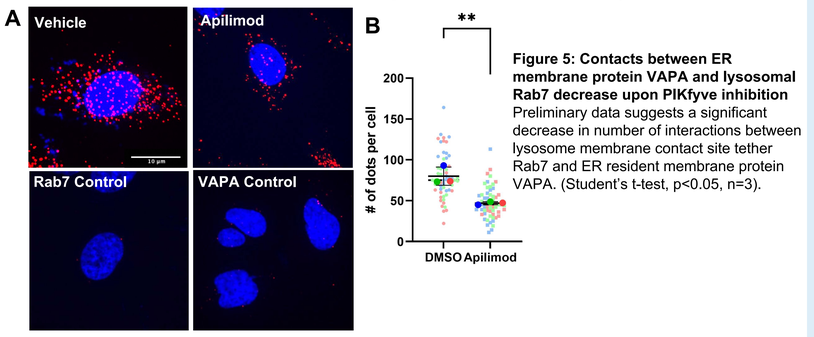


Figure 5: Contacts between ER membrane protein VAPA and lysosomal Rab7 decrease upon PIKfyve inhibition Preliminary data suggests a significant decrease in number of interactions between lysosome membrane contact site tether Rab7 and ER resident membrane protein VAPA. (Student’s t-test, $p<0.05$, $n=3$).

Future Directions

We hypothesize that lysosome fission and morphology may be governed by ER-lysosome contact sites and that changes in lysosome morphology as a result of PIKfyve inhibition may be altering this process.

Conclusions

Changes in lysosome morphology and dynamics as a result of PIKfyve inhibition are altering ER morphology and dynamics. This in turn may impact ER-lysosome membrane contact sites that demarcate lysosome fission sites.