Localization of Rab35 to Lipidosome During Crystalline Silica-Induced Inflammation

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Introduction

- Lung cancer is the most common cancer worldwide and the leading cause of cancer-related deaths in the US.
- Around two million US workers and several million worldwide are occupationally exposed to crystalline silica (CS) in industrial settings such as mining, construction, pottery, and glass production.
- Persistent CS exposure leads to chronic lung inflammation, causing silicosis, which can ultimately lead to lung cancer.
- Leukotriene B4 (LTB_4), a potent lipid chemoattractant that initiates inflammation, is an important mediator of CS-induced inflammation. Mast cells and macrophages are the main producers of LTB_4.
- LTB_4 synthesis occurs in the lipidosome, a cytosolic complex where all enzymatic machinery is localized for LTB_4 production. Currently, little is known about the mechanisms that lead to lipidosome activation for LTB_4 production in CS-induced inflammation.
- Rab GTPases, part of the Ras superfamily of small GTPases, are known as master regulators of vesicle budding, trafficking, motility of fusion through the recruitment of effector proteins.
- Rab35, a Rab GTPase, plays essential roles in endocytic recycling and cytokinesis, and has since been widely studied in other cellular functions such as phagocytosis, cell migration, and neurite outgrowth. LTB_4 production was reduced through siRNA knockdown of the Rab35 gene, suggesting a potential role for Rab35 in lipidosome activation.

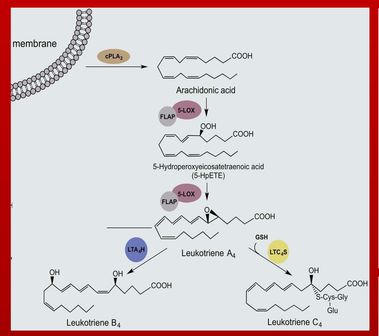


Figure 1. Biosynthesis pathway of LTB_4. cPLA2, 5-LOX, FLAP, and LTA_4H are essential for the formation of LTB_4.

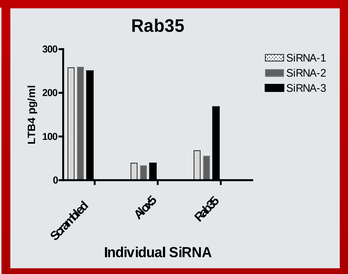


Figure 2. Rab35 has previously been shown to have an effect on LTB_4 production, prompting current studies on Rab35's function in lipidosome activation and LTB_4 production.

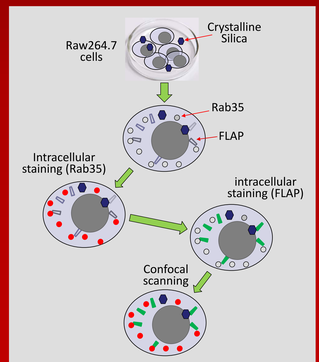
Hypothesis

- To further understand the mechanisms of LTB_4 production in CS-induced inflammation, we focused on Rab35 and its potential role in lipidosome formation.
- Based on the known functions of Rab35 in phagocytosis and endocytic recycling, we hypothesize that Rab35 is a key factor of lipidosome formation and LTB_4 production.

Methods

Cell Culture → LPS Priming (3 hours) → CS Induction (3 hours) → Antibody Staining → Confocal Microscopy

- Raw264.7 macrophage cells were plated on 35 mm dishes and primed with LPS and stimulated with CS to promote CS-induced phagocytosis and lipidosome activation.
- The cells were fixed in 4% paraformaldehyde solution for 15 minutes, permeabilized with 0.1% saponin for 10 minutes, and blocked with 5% BSA for 30 minutes.
- The cells were incubated with anti-Rab35 rabbit antibody at 4°C overnight and then incubated with Alexa Flour 594 goat anti-rabbit IgG.
- Cells were again blocked with 5% BSA then incubated with anti-FLAP (an essential enzyme for LTB_4 production) rabbit antibody and then with Alexa Flour 488 goat anti-rabbit IgG.
- DAPI was added.
- Cells were imaged using a Nikon AIR confocal microscope.



Results

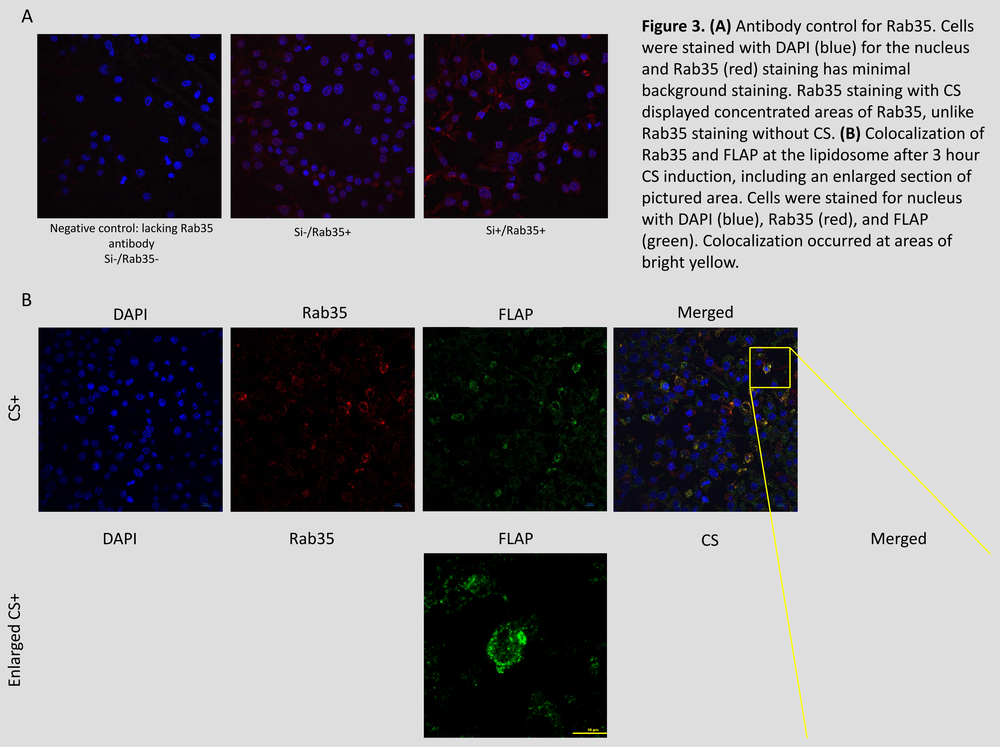


Figure 3. (A) Antibody control for Rab35. Cells were stained with DAPI (blue) for the nucleus and Rab35 (red) staining has minimal background staining. Rab35 staining with CS displayed concentrated areas of Rab35, unlike Rab35 staining without CS. **(B)** Colocalization of Rab35 and FLAP at the lipidosome after 3 hour CS induction, including an enlarged section of pictured area. Cells were stained for nucleus with DAPI (blue), Rab35 (red), and FLAP (green). Colocalization occurred at areas of bright yellow.

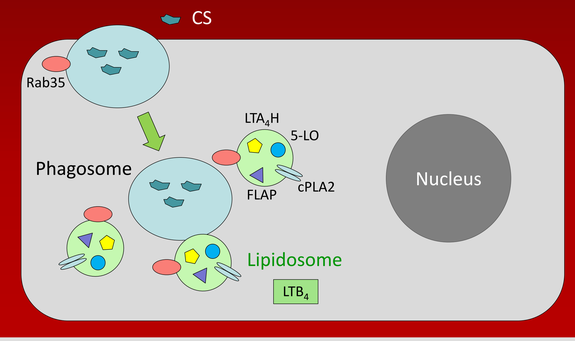


Figure 4. Lipidosome activation through CS uptake by macrophage. After phagocytosis of CS, the lipidosome is formed, containing enzymes needed for LTB_4 production. Rab35 was found to be colocalized with the lipidosome.

Conclusions

- In the absence of CS stimulation Rab35 was localized in the cytosol and plasma membrane. Upon CS stimulation an increase in vesicle localization of Rab35 was observed.
- Anti-FLAP antibody staining along with anti-Rab35 antibody staining showed colocalization of Rab35 and FLAP.
- Our results suggest Rab35 is likely an essential component of the LTB_4 producing CS-induced lipidosome.

Future Directions

- Future studies will be needed to better determine and confirm the role of Rab35 in lipidosome formation.
- Further studies of siRNA and CRISPR/Cas9 in combination with confocal microscopy may be used to confirm the Rab35 affects on lipidosome formation.
- Live imaging of cells throughout CS induction and staining of organelles and actin will aid in better understanding of Rab35 function in CS-induced phagocytosis.
- Determination of the effectors and activators of Rab35 are essential in understanding its role in lipidosome activation.

Acknowledgements

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