Type I gamma phosphatidylinositol phosphate kinase (PIPKIγ) is an enzyme that generates PtdIns5P2, a secondary messenger. The PIPKIγ gene is alternatively spliced, resulting in protein variants with conserved N termini and kinase domains, but unique sequences at the C termini. In humans, these variants include PIPKIγ1, 2, 3, 4, 5, and 6. Of these, PIPKIγ5 is found to distinctly localize to endosomes and the plasma membrane; however, its biological functions, including its effect on endosomal trafficking, are yet to be determined. PIPKIγ5 controls the trafficking of epidermal growth factor receptor (EGFR), an oncogene that dictates cell differentiation and survival, from the endosomes to the lysosomes. In this way, PIPKIγ5 is required for the lysosomal degradation of EGFR, which then downregulates EGFR signaling. Rab7 is a marker of late endosomes and a key regulator of the endosome maturation to the lysosome. PIPKIγ5 modulates Rab7β activation and subcellular localization. While the underlying molecular mechanism is not known yet, the present study aims to understand whether PIPKIγ5 interacts with MON1, a Rab7 modulator protein and whether this interaction affects Rab7 function and endosomal trafficking. To assess the interaction of PIPKIγ5 with MON1, purified MON1-GST and HA-PIPKIγ5 were allowed to interact in vitro to determine the extent of interaction between MON1 and PIPKIγ5. Additionally, 293FT cells were transfected with GFP-Rab7 and HA-PIPKIγ5 and their interaction was studied using coimmunoprecipitation. Immunofluorescence was used to study the subcellular colocalization of MON1 and Rab7. The findings support the hypothesis that PIPKIγ5 is significant in endosomal trafficking owing to its interaction with MON1. This study would also help in understanding if the interaction of PIPKIγ5 with MON1 affects the trafficking of other cellular proteins.

ABSTRACT

PIPKIγ5 has been determined to have a significant influence on EGFR signaling with studies reporting that the absence of PIPKIγ5, the EGFR signaling is unregulated and abnormal (Sun et al., 2013). This dysregulated signaling can lead to many diseases like cancer and inflammation, suggesting that PIPKIγ5 is an essential component in ensuring that the signaling is normal. While PIPKIγ5 has shown to be a critical component in EGFR signaling its underlying molecular mechanisms in endosomal trafficking have not been determined. It has been previously reported that PIPKIγ5 directly interacts with Rab7β and is critical for the modulation of Rab7β activity and localization, thus being an active component of the endosomal trafficking pathway (Sun et al., 2019). Additionally, the endosomal trafficking system is responsible for maintaining the spatial organization of cell membrane proteins along with regulation of cellular functions like cell signaling, nutrient uptake, membrane turnover, cell movement, development, and also in metastasis. Thus, elucidating how endosomal trafficking is regulated will help in developing therapeutic approaches to combat cancer and other disorders.

BACKGROUND

VI. CONCLUSION

The interactions of PIPKIγ5 with MON1 and Rab7β as shown through immunoprecipitation and in-vitro binding assays, confirm the role of PIPKIγ5 and its significance in endosomal trafficking. Because Rab7β and MON1 are markers of late endosomes, we can conclude that the findings of this study support the hypothesis that PIPKIγ5 is involved and has a significant role in endosomal trafficking. Using different approaches across varied cell types also indicates that this phenomena is conserved across cell types.

REFERENCES


BACKGROUND

VI. CONCLUSION

The interactions of PIPKIγ5 with MON1 and Rab7β as shown through immunoprecipitation and in-vitro binding assays, confirm the role of PIPKIγ5 and its significance in endosomal trafficking. Because Rab7β and MON1 are markers of late endosomes, we can conclude that the findings of this study support the hypothesis that PIPKIγ5 is involved and has a significant role in endosomal trafficking. Using different approaches across varied cell types also indicates that this phenomena is conserved across cell types.

REFERENCE

ACKNOWLEDGEMENTS

Further exploration of this study should be done to explore other possible trafficking components that might be affected by PIPKIγ5 and how those findings could aid in identifying critical functions of PIPKIγ5, which can be utilized in clinical research.

I would like to thank my mentor, Ruchi Kakar, for her exemplary guidance throughout this process and my PI, Dr. Yue Sun, for the valuable information I’ve learned through my time in his lab.

Furthermore, I’d like to thank the VCU Medical Science Internship Program co-directors Aranya Surve, Aditya Koha, and Siddharth Venigalla for being such helpful guides and giving great suggestions. Finally, I’d like to thank Dr. Whitehurst Cook for sponsoring this program.

I would like to thank my mentor, Ruchi Kakar, for her exemplary guidance throughout this process and my PI, Dr. Yue Sun, for the valuable information I’ve learned through my time in his lab.

Furthermore, I’d like to thank the VCU Medical Science Internship Program co-directors Aranya Surve, Aditya Koha, and Siddharth Venigalla for being such helpful guides and giving great suggestions. Finally, I’d like to thank Dr. Whitehurst Cook for sponsoring this program.

I would like to thank my mentor, Ruchi Kakar, for her exemplary guidance throughout this process and my PI, Dr. Yue Sun, for the valuable information I’ve learned through my time in his lab.

Furthermore, I’d like to thank the VCU Medical Science Internship Program co-directors Aranya Surve, Aditya Koha, and Siddharth Venigalla for being such helpful guides and giving great suggestions. Finally, I’d like to thank Dr. Whitehurst Cook for sponsoring this program.

I would like to thank my mentor, Ruchi Kakar, for her exemplary guidance throughout this process and my PI, Dr. Yue Sun, for the valuable information I’ve learned through my time in his lab.

Furthermore, I’d like to thank the VCU Medical Science Internship Program co-directors Aranya Surve, Aditya Koha, and Siddharth Venigalla for being such helpful guides and giving great suggestions. Finally, I’d like to thank Dr. Whitehurst Cook for sponsoring this program.

I would like to thank my mentor, Ruchi Kakar, for her exemplary guidance throughout this process and my PI, Dr. Yue Sun, for the valuable information I’ve learned through my time in his lab.

Furthermore, I’d like to thank the VCU Medical Science Internship Program co-directors Aranya Surve, Aditya Koha, and Siddharth Venigalla for being such helpful guides and giving great suggestions. Finally, I’d like to thank Dr. Whitehurst Cook for sponsoring this program.

I would like to thank my mentor, Ruchi Kakar, for her exemplary guidance throughout this process and my PI, Dr. Yue Sun, for the valuable information I’ve learned through my time in his lab.

Furthermore, I’d like to thank the VCU Medical Science Internship Program co-directors Aranya Surve, Aditya Koha, and Siddharth Venigalla for being such helpful guides and giving great suggestions. Finally, I’d like to thank Dr. Whitehurst Cook for sponsoring this program.

I would like to thank my mentor, Ruchi Kakar, for her exemplary guidance throughout this process and my PI, Dr. Yue Sun, for the valuable information I’ve learned through my time in his lab.

Furthermore, I’d like to thank the VCU Medical Science Internship Program co-directors Aranya Surve, Aditya Koha, and Siddharth Venigalla for being such helpful guides and giving great suggestions. Finally, I’d like to thank Dr. Whitehurst Cook for sponsoring this program.