The Functional Influence of PIPKIγi5 on Endosomal Trafficking

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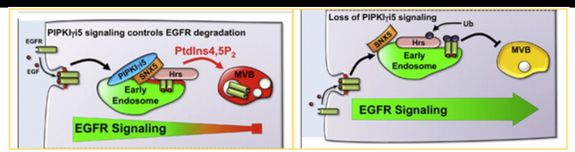
ABSTRACT

Type I gamma phosphatidylinositol phosphate kinase (PIPKIγ) is an enzyme that generates PtdIns4,5P2, 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 PIPKIγi1, i2, i3, i4, i5, and i6. Of these, PIPKIγi5 is found to distinctively localize to endosomes and the plasma membrane; however, its biological functions, including its effect on endosomal trafficking, are yet to be determined. PIPKIγi5 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, PIPKIγi5 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. PIPKIγi5 modulates Rab7 activation and subcellular location. While the underlying molecular mechanism is not known yet, the present study aims to understand whether PIPKIγi5 interacts with MON1, a Rab7 modulator protein and whether this interaction affects Rab7 function and endosomal trafficking. To assess the interaction of PIPKIγi5 with MON1, purified MON1-GST and HA-PIPKIγi5 were allowed to interact in vitro to determine the extent of interaction between MON1 and PIPKIγi5. Additionally, 293FT cells were transfected with GFP-Rab7 and HA-PIPKIγi5 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 PIPKIγi5 is significant in endosomal trafficking owing to its interaction with MON1. This study would also help in understanding if the interaction of PIPKIγi5 with MON1 affects the trafficking of other cellular proteins.

BACKGROUND



PIPKIγi5 has been determined to have a significant influence on EGFR signaling with studies reporting that in the absence of PIPKIγi5, the EGFR signaling is unregulated and abnormal (Sun et al., 2013). This dysregulated signaling can lead to many diseases like cancer and immunodeficiencies, thus suggesting that PIPKIγi5 is an essential component in ensuring that the signaling is normal. While PIPKIγi5 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 PIPKIγi5 directly interacts with Rab7a and is critical for the modulation of Rab7a 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.

RESULTS

1) MDA-MB-231 cells transfected with MON1 exhibited colocalization with endogenous Rab7. Rab7 (stained in green) and MON1 (stained in red) interacted to exhibit a yellow fluorescence (Figure 1.). Additionally, both proteins being markers of late endosomes, there is a possibility that this interaction occurred in the late endosome.

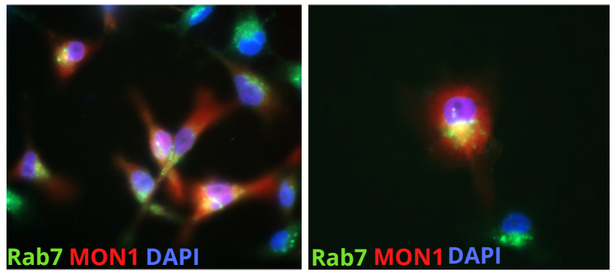
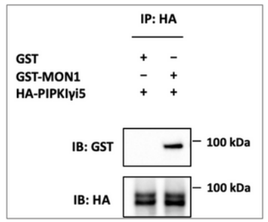


Figure 1.
Immunofluorescence staining of MDA-MB-231 cells transfected with MON1 (stained in red) and endogenous Rab7 (stained in green). The yellow fluorescence indicates the subcellular colocalization of MON1 and Rab7.

2) In-vitro binding assay using purified MON1-GST and HA-PIPKIγi5 (Figure 2.). Pulldown with anti-HA beads showed a band for MON1 and not GST, indicating that PIPKIγi5 specifically pulled down MON1, therefore confirming that PIPKIγi5 and MON1 interact

Figure 2.
In-vitro binding assay using purified MON1-GST and HA-PIPKIγi5 was used to assess the interaction of MON1 with PIPKIγi5.



3) Coimmunoprecipitation of GFP-Rab7 and HA-PIPKIγi5 carried out using anti-flag coated beads in HEK293FT cells (Figure 3A.) shows that in the presence of PIPKIγi5 the pulldown of Rab7a is reduced compared with the pulldown in the absence of HA-PIPKIγi5. Additionally, anti-Flag coated beads were used to assess the pulldown of HA-PIPKIγi5 (Figure 3B) in the presence of Rab7. In the presence of Rab7a and MON1, pulldown of PIPKIγi5 was observed, which is indicative of interaction between MON1 and PIPKIγi5

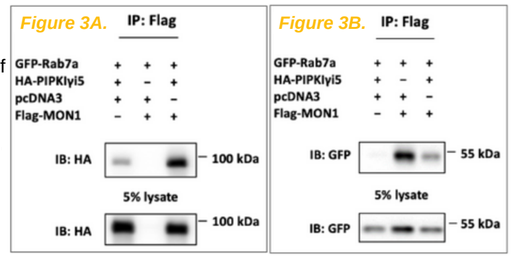


Figure 3A.
Coimmunoprecipitation of Rab7 and PIPKIγi5 in HEK293FT cells. The pulldown of PIPKIγi5 by MON1 in the presence of Rab7 was evaluated using anti-Flag coated beads

Figure 3B.
Coimmunoprecipitation of Rab7 and PIPKIγi5 in HEK293FT cells. The pulldown of Rab7 by MON1 in the presence of PIPKIγi5 was evaluated using beads coated with anti-Flag antibodies

CONCLUSION

The interactions of PIPKIγi5 with MON1 and Rab7 as shown through immunoprecipitation and in-vitro binding assays, confirm the role of PIPKIγi5 and its significance in endosomal trafficking. Because Rab7 and MON1 are markers of late endosomes, we can conclude that the findings of this study support the hypothesis that PIPKIγi5 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.

FUTURE DIRECTIONS

Further exploration of this study should be done to explore other possible trafficking components that might be affected by PIPKIγi5 and how those findings could aid in identifying critical functions of PIPKIγi5, which can be utilized in clinical research.

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