Type I γ Phosphatidylinositol Phosphate 5-Kinase Regulates Hippo Signaling Pathway

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Abstract

Hippo pathway plays an essential role in organ-size control, which exhibits its potential to contribute to cancer progression. Hippo signaling impacts cellular composition by causing the phosphorylation of YAP1 (Yes-associated Protein 1), a transcriptional coactivator. The phosphorylated YAP1 binds to 14-3-3, inhibiting the YAP1 pathway and directing YAP1 towards degradation. When the Hippo pathway is deactivated, YAP1 translocates to the nucleus and enhances transcription of the oncogene TEA domain family members (TEAD). Moreover, YAP1 overexpression causes resistance to chemotherapeutic agents by promoting insensitivity to apoptosis and allows cells to overcome contact inhibition to prolong tissue proliferation. Our recent research suggests that type I-gamma phosphatidylinositol phosphate 5-kinase (PIPKIγ), an enzyme that synthesizes phosphatidylinositol-4,5-bisphosphate (PI4,5P2), can interact with and potentially regulate YAP1. Thus, it is necessary to determine whether PIPKI modulates YAP1 expression, phosphorylation, degradation, and downstream YAP1 signaling. For this study, UM-SCC-1 and Cal27 cells were transfected with siRNA to knock down PIPKIγ, PIPKIγi5, and PIPKIγi2 expression. Western blot was used to measure protein levels, while real-time PCR was used to measure mRNA expression. Co-immunoprecipitation (co-IP) assay was conducted to determine interactions between PIPKIγ subunits and YAP1. We show that PIPKIγ and its sub-families PIPKIγi5 and PIPKIγi2 may play an unanticipated role in cancer development by regulating YAP1 signaling, according to mRNA expression. In PIPKIγ, PIPKIγi5, and PIPKIγi2 knockdown UM-SCC-1 and Cal27 cells, the transcription levels of YAP1 target genes are markedly elevated. This shows that PIPKIγ, PIPKIγi5, and PIPKIγi2 have a universal role in modifying YAP1 signaling in various species. PIPKIγi5 and PIPKIγi2 are directly linked to YAP1 according to a co-IP result. These results demonstrate a new role for PIPKIγi5 and PI4,5P2 in the immune system. In future studies, the function of PIPKIγi5 in YAP1 modulation will be determined through sub-cellular localization and YAP1 nuclear translocation.

Background

Phosphoinositides are membrane-containing phospholipids that play a vital role in membrane trafficking, which is essential in maintaining immune response and cellular development. PIPKIγ, a specific isoform of the PIPKIs subfamily, has been found to contain six splice variants in humans, named as PIPKIγi1-i6 (Han, 2019). PIPKIγi2 is found at cell adhesions and promotes cancer cell directional migration and invasion by controlling endosomal trafficking to the cell membrane. PIPKIγi5 is found at endosomes and is required for Epidermal Growth Factor Receptor (EGFR) sorting from the endosome to lysosome for degradation. Upstream signals within the Hippo signaling pathway cause the phosphorylation of YAP1 (Han, 2019). Once phosphorylated, YAP1 binds to 14-3-3, inhibiting the YAP1 pathway and directing YAP1 towards degradation. Without phosphorylation, YAP1 translocates to the nucleus and enhances transcription of TEAD, an oncogene. YAP1 overexpression may also lead to prolonged tissue proliferation, insensitivity to chemotherapy induced apoptosis, and ability to counteract tumor suppression.

Aim

Our recent research suggests that PIPKIγ can interact with and potentially regulates YAP1. Thus, we will determine whether PIPKIγ can regulate YAP1 expression, phosphorylation, degradation, and the downstream signaling.

Methods

Expression profile of YAP1 related genes

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Knock down with control and PIPKIγi2, PIPKIγi5 and PIPKIγ siRNA (Cell line:UM-SCC-1 and Cal27)

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Estimation of protein levels by western blot and mRNA expression levels by qPCR

Identification of molecular mechanisms

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Transient transfection with respective plasmids (Cell line: HEK293FT)

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Co-immunoprecipitation to assess the interaction between PIPKIγi5 and YAP1

Results

1. PIPKIγ regulates YAP1 targeted genes expression

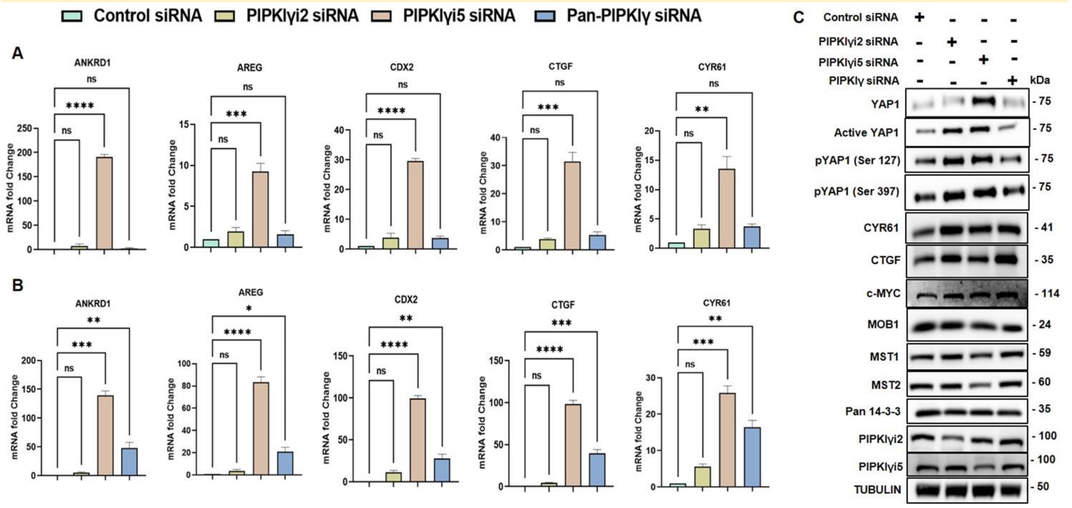

Figure 1. (A) UM-SCC-1 and (B) Cal27 cells were transfected with control siRNA or siRNAs targeting PIPKIγi2, PIPKIγi5, or PIPKIγ, and then the mRNA levels of YAP1 related genes were quantified by Real-Time PCR. Data analysis was conducted using Prism 8 (GraphPad). Error bars indicate mean ± SEM. Statistical significance was determined based using one-way ANOVA. *: p<0.1, **: p<0.01, ***: p<0.001, ****: p<0.0001, ns: not significant. (C) UM-SCC-1 cells were transfected with control, PIPKIγi2, PIPKIγi5, or PIPKIγ siRNA and protein levels of YAP1 related genes were detected by Western Blot.

2. PIPKIγi5 interacts with YAP1

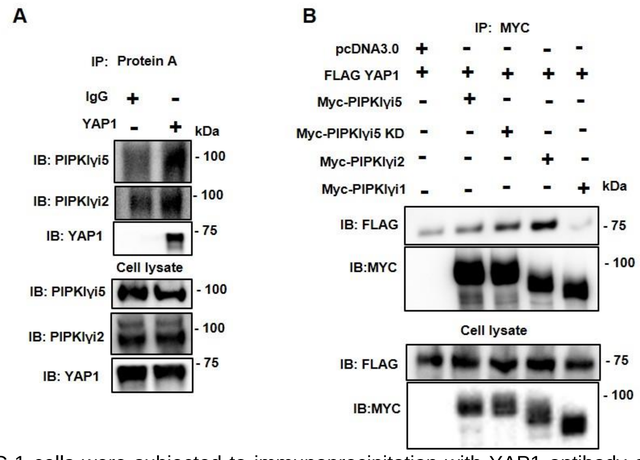

Figure 2. (A) UM-SCC-1 cells were subjected to immunoprecipitation with YAP1 antibody and then immunoblotted (IB) with PIPKIγi5, PIPKIγi2 or YAP1 antibodies. IgG serves as the negative control. (B) HEK293FT cells transfected with respective plasmids were subjected to immunoprecipitation with anti-myc antibody and immunoblot analysis is shown with indicated antibodies.

Conclusion

The results show that the hypothesis was supported because PIPKIγ and their sub-family PIPKIγi5 and PIPKIγi2 regulate YAP1 signaling, with PIPKIγi5 having the most significant effect. In PIPKIγi5 knockdown UM-SCC-1 and Cal27 cells, the transcription levels of YAP1 target genes are markedly elevated, as shown through mRNA expression using qPCR. Western blot results of the knockdown cells show stronger antibody binding to downstream signals in the YAP1 pathway, while co-IP results display that PIPKIγi5 and PIPKIγi2 are directly linked to YAP1. These results imply that PIPKIγ, PIPKIγi5, and PIPKIγi2 have a universal role in modifying YAP1 signaling among species. Thus, the presence of PIPKIγ may play a role in immune response as well as cancer progression.

Future Directions

The role of PIPKIγ in YAP1 modulation can be further investigated through sub-cellular localization of PIPKIγ and their sub-family PIPKIγi5 and PIPKIγi2. Through these techniques, the ability for 14-3-3 to bind to YAP1 in the absence of PIPKIγ can be determined.

Significance of our Study

Hippo pathway repression leads to insensitivity in apoptosis and promotes tumorigenesis. Furthermore, YAP1 is enriched in stem cells, and YAP1-mediated signaling is required for cancer stem cell self renewal. In this study, PIPKIγi5 was found to be a novel regulator of YAP1. As a kinase, PIPKIγ5 has the potential to be a target in drugs that inhibit cancer stem cell renewal through controlling YAP1 expression.

References

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