CELL BIOLOGY

A connection between phosphatidylinositol 5-phosphate and the Hippo pathway to prevent epithelial-mesenchymal transition in cancer

Emilio Hirsch*, Emanuele Fantastico, Lorenzo Prever, Federico Gulluni

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The Hippo pathway blocks epithelial-mesenchymal transition and metastasis in cancer mediated by the transcriptional coactivator YAP. In this issue of *Science Signaling*, Palamiuc *et al.* demonstrate that phosphatidylinositol 5-phosphate (PI5P) enhances Hippo pathway activation and that simultaneously the Hippo pathway initiates a positive feedback loop by inhibiting the conversion of PI5P into PIP_2.

Lipids such as phosphoinositides regulate diverse cellular processes through complex kinase and phosphatase networks (*1*). Among these, phosphatidylinositol 5-monophosphate (PI5P) serves as a substrate for type II phosphatidylinositol 5-phosphate 4 kinases (PI5P4Kα and PI5P4Kβ), converting it into phosphatidylinositol 4,5-bisphosphate (PIP_2), a crucial component of cellular membranes (*1*). Although PIP_2 is involved in cytoskeletal organization and in various signaling pathways (*1*, *2*), the specific signaling function of PI5P still remains obscure. The relative scarcity of PI5P in cells suggests that its conversion into the more abundant PIP_2 may not contribute to the PIP_2 pool but rather inhibit a potential autonomous role yet to be discovered.

Palamiuc *et al.* (*3*) now unveil the conversion of PI5P into PIP_2 as a determinant of the activity of the Hippo pathway, a signaling route that controls cell growth regulation and tissue homeostasis but that is also implicated in diseases such as cancer (*4*). Within the Hippo signaling cascade, the kinases MST1 and MST2 phosphorylate and activate the downstream effectors LATS1/2 and MOB1/2, leading to the inhibition of the transcriptional coactivator YAP and ultimately allowing proper cell proliferation and organ size maintenance. Palamiuc *et al.* (*3*) demonstrated that kinases such as MST1, MST2, and their functional analog HGK directly phosphorylated and inhibited PI5P4Kα and PI5P4Kβ (Fig. 1A). They identified specific phosphorylation sites in these two enzymes that exhibit high homology and appear to be conserved across different species. These phosphorylation sites are absent in type I PI5P4Ks, suggesting their unique regulatory role in the turnover of PI5P downstream of the Hippo pathway.

Unexpectedly, inhibition of PI5P4Ks by MST1/2 triggered feedback regulation, sustaining Hippo pathway activation (Fig. 1A). Biochemical studies showed that MOB1 preferentially bound to PI5P over PIP_2 and that this binding stabilized an active complex, with LATS promoting YAP phosphorylation, thus demonstrating an unforeseen direct signaling function for PI5P in regulating the Hippo pathway. Intriguingly, computational modeling of MOB1/PI5P association suggested that the fatty acid chains of PI5P are retained inside a pocket in MOB1, potentially implying that membrane interaction might not be needed. Although this prediction is fascinating, a more detailed description of the structural dynamics of PI5P binding by MOB1 is still needed, and future work will likely more clearly define this interaction. Nonetheless, the overall effect of the blockade of PI5P conversion into PIP_2 was to enhance YAP phosphorylation and cytoplasmic retention, thereby inhibiting YAP-dependent transcriptional activity (Fig. 1A). In agreement, RNA sequencing analysis in both normal and cancer cells revealed down-regulation of YAP target genes upon PI5P4K inhibition, supporting the role of PI5P4Ks in regulating YAP transcriptional activity.

In cancer, inhibition of the Hippo pathway can trigger a transcriptional program leading to epithelial-mesenchymal transition (EMT) and consequent cancer progression (*5*), and accordingly, down-regulation of PI5P4Ks decreased YAP-dependent expression of EMT genes such as *TWIST1*, *Vimentin*, *FN1*, and *CDH2*. Consistent with inhibition of a YAP-driven EMT phenotype, breast and ovarian cancer cells lacking PI5P4K activity better preserved their epithelial features by maintaining E-cadherin expression as well as reduced migration and invasion. Given these findings indicating that down-regulation of PI5P4Ks could activate the functional output of the Hippo pathway, the authors next tested whether expression of the genes encoding these lipid kinases was up-regulated in aggressive forms of cancer. Additional correlative analysis of expression datasets from patients with invasive breast carcinoma supported the association between increased *PI5P4K* expression, YAP transcriptional activity, and EMT gene signatures, suggesting the potential relevance of PI5P4K-Hippo pathway interaction in cancer progression and metastasis (Fig. 1B).

Overall, dysregulation of lipid metabolism in cancer is a critical factor contributing to tumorigenesis and metastasis, and the connection between PI5P4Ks, PI5P signaling, and the Hippo pathway adds another layer of complexity to our understanding of how phosphoinositides may influence cancer progression. Given that dysregulation of the Hippo pathway is implicated in various cancers, in which its aberrant signaling contributes to tumorigenesis, metastasis, and drug resistance, this study opens the way to a previously unforeseen therapeutic option. The finding of PI4P5Ks as negative regulators of the MOB/LATS-mediated YAP cytoplasmic retention defines these lipid kinases as promising drug targets to antagonize the onset of EMT and the subsequent stimulation of cell motility and metastatic spreading.

Molecular Biotechnology Center, Department of Molecular Biotechnology and Health Sciences, University of Torino, Via Nizza 52, 10125 Torino Italy.
*Corresponding author. Email: emilio.hirsch@unito.it

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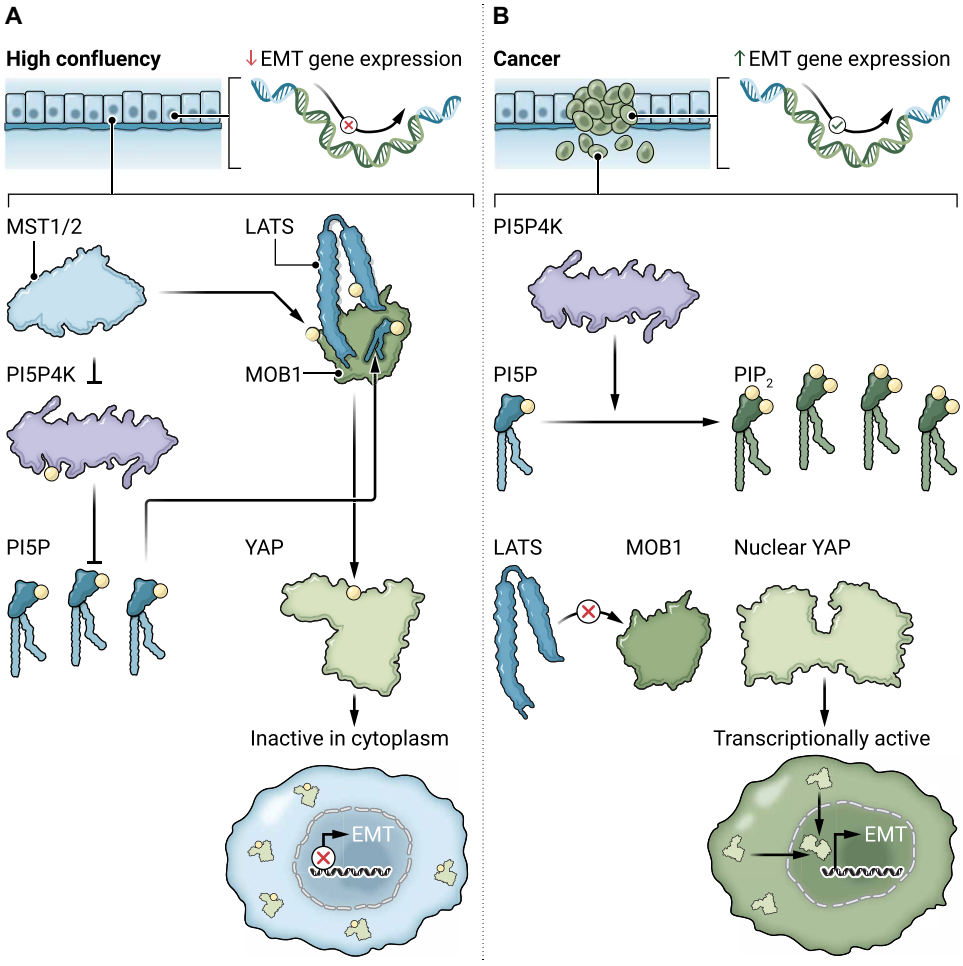

Fig. 1. PI5P4K negatively regulates the Hippo pathway, leading to EMT and cancer progression. (**A**) In healthy epithelia and in confluent cultures, the kinases MST1 and MST2 phosphorylate (yellow circle) and inhibit PI5P4Kα and PI5P4Kβ (PI5P4K) so that the lipid PI5P accumulates. Abundance of this lipid stabilizes the complex of LATS and MOB1, thereby increasing their activation by MST1/2-mediated phosphorylation. Active MOB1 leads to the phosphorylation of the cotranscriptional factor YAP, which is then retained in an inactive state within the cytoplasm. (**B**) In cancer cells, overexpressed PI5P4K switches off the PI5P signal by converting it into PIP_2, a lipid that poorly interacts with MOB1, leading to decreased stability of the MOB1/LATS complex. Therefore, YAP is not phosphorylated and can enter the nucleus, triggering the transcription of genes involved in EMT and driving cancer progression.

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