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Invited Mini Review

The role of extracellular biophysical cues in modulating the Hippo-YAP pathway

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The Hippo signaling pathway plays an essential role in adult-tissue homeostasis and organ-size control. In *Drosophila* and vertebrates, it consists of a highly conserved kinase cascade, which involves MST and Lats that negatively regulate the activity of the downstream transcription coactivators, YAP and TAZ. By interacting with TEADs and other transcription factors, they mediate both proliferative and antiapoptotic gene expression and thus regulate tissue repair and regeneration. Dysregulation or mutation of the Hippo pathway is linked to tumorigenesis and cancer development. Recent studies have uncovered multiple upstream inputs, including cell density, mechanical stress, G-protein-coupled receptor (GPCR) signaling, and nutrients, that modulate Hippo pathway activity. This review focuses on the role of the Hippo pathway as effector of these biophysical cues and its potential implications in tissue homeostasis and cancer. [BMB Reports 2017; 50(2): 71-78]

INTRODUCTION

The size of each organ is determined by cell number and cell size. This process involves many signaling pathways during development, and regeneration controls cell number in tissue and organs. In recent years, the Hippo tumor-suppressor pathway has emerged as a key regulator of organ size and tumorigenesis by inhibiting cell proliferation, promoting apoptosis, and limiting stem/progenitor-cell expansion (1). This pathway was initially identified by means of genetic mosaic screens for growth-control genes. In *Drosophila*, the core components of the Hippo pathway include a kinase cascade of Ste20-like kinase Hippo (Hpo), with the scaffolding protein Salvador (Sav), and NDR family kinase Warts (Wts), with its regulatory protein Mob as Tumor Suppressor (Mats) (2-8). Hpo forms a complex with Sav to phosphorylate and activate Wts, which then interacts with Mats (9-11). Wts directly phosphorylates the transcriptional co-activator Yki (Yorkie), promoting its interaction with 14-3-3 and leading to YAP cytoplasmic retention (12-16). Inactivation of the Hippo pathway reduces its downstream kinase-mediated YAP phosphorylation. The unphosphorylated YAP translocates to the nucleus, where it binds with the TEAD/TEF- family transcription factor Sd (Scalloped) to activate transcription of target genes, promoting cell survival and proliferation (17, 18). Then the pathway and its cellular functions, including cell survival, proliferation, and organ-size control is evolutionally conserved in mammals (13, 19). Core components of the mammalian Hippo pathway include a kinase cascade of mammalian STE20-like protein kinase 1/2 (MST1/2) and the large tumor suppressor 1/2 (Lats1/2). MST1/2 in complex with its regulatory protein Sav1 phosphorylates and activates Lats1/2 kinases, which also form a complex with its regulatory protein, Mob1. The Yes-associated protein (YAP) is a transcription co-activator and an important downstream effector of the Hippo pathway. YAP was first identified as a non-receptor tyrosine kinase YES1 binding partner (20). The physiological importance of YAP/TAZ was uncovered after the identification of *Drosophila* Yki as a key effector of the Hippo pathway (12). In a detailed study of Hippo kinase cascade, the Hippo pathway kinase Lats1/2 inhibits YAP by direct phosphorylation of five consensus HXRXXS motifs (13, 19, 21-23). Phosphorylation of S127 in YAP results in cytoplasmic sequestration via 14-3-3 binding and therefore inactivates YAP. Thus YAP is degraded by the proteasome in a ubiquitin-dependent manner following phosphorylation of Ser 397. A transcriptional co-activator with PDZ-binding motif (TAZ, also called WWTR1), a paralog of YAP in mammals, was initially identified as a 14-3-3 binding protein in a phosphorylation-dependent manner (24). TAZ contains four consensus Lats1/2 target motifs and is similarly regulated by Lats1/2 (23, 25). Conversely, unphosphorylated YAP localizes in the nucleus and acts mainly through the TEAD family transcription factors to stimulate expression of genes that promote proliferation and inhibit apoptosis (26, 27). Besides TEADs, YAP/TAZ can also interact with several different transcription factors, including Smad, Runx1/2, p73, ErbB4, Pax3, and T-box transcription factor 5 (TBX5) to mediate transcription and a diverse array of cellular functions (28).

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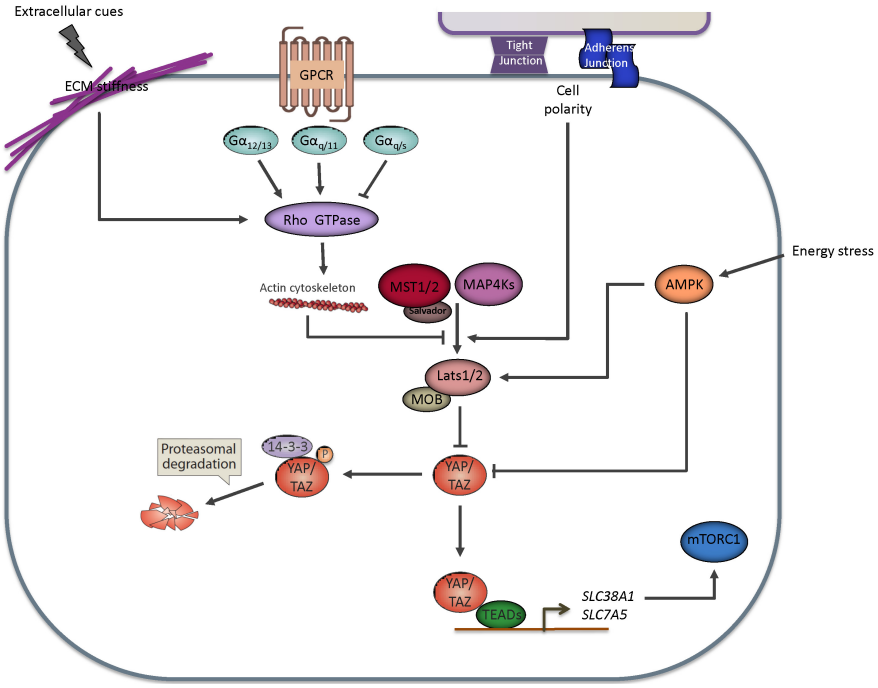

Fig. 1. Regulation of the Hippo-YAP pathway by extracellular biophysical cues. Mechanical stress inhibits Lats1/2 kinase activity via Rho GTPase and the actin cytoskeleton. GPCR signaling can either activate or inhibit YAP/TAZ activity through the coupled Gα protein. Cellular junction and cell polarity modulate the Hippo pathway. Nutrient signaling modulates the core Hippo kinase and YAP activity through AMPK. YAP/TAZ activity is involved in amino-acid induced mTORC1 activation.

In recent years, beyond the main components of the Hippo pathway defined above, many other additional regulators have been found to regulate the Hippo pathway. Accumulating evidence suggests that the core Hippo kinase cascade and YAP/TAZ incorporate various upstream responses, enabling dynamic regulation of tissue homeostasis and cancer (29). In this review we will focus on the expanding roles of YAP/TAZ as mediators of responses to biophysical cues, especially mechanical stress, GPCR signaling, and nutrient signaling (Fig. 1).

REGULATION OF HIPPO-YAP PATHWAY BY EXTRACELLULAR MECHANICAL CUES

Growth and development is the net result of various harmonized events of cells to adjust to physical restraints and extracellular mechanical signals. For instance, the cell-density-mediated cell-cell contact causes a growth-inhibitory signaling pathway that in large part is mediated by the Hippo pathway (19, 30, 31). Abundant cell-cell contact activates Lats and inactivates YAP which is critically important for contact inhibition. The regulation of YAP/TAZ-TEAD mediated transcription in response to contact inhibition is also essential for embryo development (32). In addition, the apical-basal cell polarity protein, adherens junctions, and tight junctions provide the intrinsic cues to regulate Lats1/2 and restrict YAP activity (33). Interestingly, it was found that YAP /TAZ activity and subcellular localization are regulated by extracellular matrix (ECM) stiffness. When cells are cultured on stiff ECM, YAP/TAZ predominantly localizes to nuclei and promotes YAP/TAZ transcriptional activity. However, when cells are cultured on soft ECM, cells are round and adhesion with ECM is limited. Likewise, YAP/TAZ activity and subcellular localization depend on the adhesive area. Furthermore, YAP/TAZ activity is modulated by cell stretching, spreading, and cell size through changes in the cytoskeleton (34-36). More importantly, activation of YAP/TAZ by rigidity of the extracellular matrix greatly improves differentiation of human pluripotent stem cells in motor neurons (37).

Morphological manipulation and stress-fiber quantity changes in response to physical forces inhibit the Hippo pathway and promote nuclear YAP localization in a way similar to matrix stiffness (38). Also, induction of F-actin polymerization by loss of capping proteins, Cpa and Cpb, or overexpressing an activated actin nucleation factor Diaphanous, leads to cell proliferation and overgrowth in imaginal discs. Studies on *Drosophila* have demonstrated that changing F-actin levels correlates with activation of Yki and causes overgrowth (39). In contrast, reduction of actin-capping protein or inhibition of Capulet, which all induce abnormal F-actin polymerization, sustains Hippo pathway activity, thereby inducing expression of Yki target genes near the apical surface in *Drosophila* (40). The outcome of F-actin in regulation of YAP is also likely evolutionarily conserved in mammals, since deletion of the destrin gene, an actin-depolymerizing factor, increases the aberrant actin cytoskeleton and leads to epithelial hyperproliferation (41). This was further established by the observation that CapZ or Cofilin restricts YAP nuclear localization and YAP transcriptional activity (35). The structure of actin

cytoskeleton is responsible for the transduction of mechanical stress in cells. The Rho GTPases, which have great effects on actin cytoskeleton organization, is a crucial regulator of YAP/TAZ activity. For example, disruption of F-actin or inhibition of Rho by specific inhibitors considerably reduces YAP nuclear translocation and activity (36, 38, 42). The molecular mechanism of YAP/TAZ regulation by actin cytoskeleton and mechanical stress has not yet been fully understood. Previous studies ignore MST1/2 and Lats1/2 in the regulation of YAP/TAZ nuclear translocation and transcriptional activation, because knockdown of Lats1/2 is not enough to recover YAP/TAZ activity by ECM stiffness (36). However, under detached conditions, the Lats1/2 leads to YAP inhibition in a cytoskeleton-dependent manner (42). Similar to that observed in cell detachment, mechanical strain lead to Lats1/2 inhibition to activate YAP in a JNK-dependent manner (43). Accordingly, it is possible that both Lats1/2-dependent and -independent mechanisms are included in the YAP/TAZ regulation by mechanical stress. Recent findings have implied that YAP/TAZ plays a role in breast-cancer development in response to mechanical stress. For instance, many cancers such as breast cancer have elevated extracellular stiffness accompanied by a changed ECM composition compared with that of normal mammary tissue. Remarkably, it was shown that YAP is activated in cancer-associated fibroblasts (CAFs), and that its function is required for matrix stiffing (44). Higher extracellular stiffness affects YAP activity and hence contributes to the tumor microenvironment. It was proposed that YAP conditioned the tumor microenvironment by modulating matrix stiffening and production of YAP/TAZ target genes, such as AREG, CYR61, and CTGF, to promote tumorigenesis. TAZ is shown to be upregulated in high-grade and metastatic breast tumors (45). In addition, TAZ confers cancer stem-cell traits on breast cancer cells, and cancer stem cells showing high levels of TAZ are observed in high-grade tumors (46). The YAP/TAZ activity and the extracellular matrix provide a positive feedback mechanism, in which cancer cells promote matrix stiffening that further activates YAP/TAZ as transcriptional co-activators. Recent studies also show that disturbed flow activates YAP/TAZ target-gene expression through the modulation of Rho-GTPase activities, demonstrating a significant role for YAP/TAZ in mediating mechanical cues and vascular homeostasis (47-49).

Overall, many studies have suggested that actin Rho-GTPases serves as a sensor to connect mechanical cues to YAP/TAZ activity. However, the involvement of the Hippo pathway kinases MST1/2 and Lats1/2 are not completely understood. Future studies are required to define the mechano-transducers as YAP/TAZ effectors as well as the role of the core Hippo kinase cascade in regulation of YAP/TAZ by mechanical cues.

REGULATION OF THE HIPPO-YAP PATHWAY BY CELL-SURFACE RECEPTORS AND SOLUBLE MOLECULES

Under normal physiological conditions, hormones are chemical messengers that stimulate cell growth and proliferation. Such molecules are released from the cell sending the signal, cross over the gap between cells by diffusion, and interact with specific receptors in another cell, triggering a response in that cell by activating intracellular signaling which leads to physiological changes inside the cell. Physiological changes that result from soluble molecules tightly regulate cell growth, proliferation, and differentiation. It has been hypothesized that the extracellular environment, such as hormones, might regulate tissue growth and homeostasis through cell-surface receptors and Hippo pathway components. An important discovery came with the demonstration that diffusive lipid molecules, such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), could trigger intracellular signaling cascades and activate YAP/TAZ through their cognate G protein-coupled receptors (GPCRs) (50, 51). Additional study confirmed that both LPA1 and LPA3 are involved in LPA-induced YAP/TAZ activation, which is likely to be relate to long-term cell migration; PP1A is required for the LPA-YAP effects in epithelial ovarian cancer cells (52). Consistent with the roles of LPA and S1P in regulating YAP/TAZ, thrombin, the ligand of protease-activated receptors (PARs), stimulated YAP/TAZ activities by inducing its dephosphorylation and target-genes expression (53). GPCRs recognize numerous extracellular signals and transduce them to heterotrimeric G proteins, which further transduce these intracellular signals to appropriate downstream effectors and thereby play a main role in various signaling pathways (54). Mechanistically, LPA, S1P, and thrombin counteract $G\alpha_{12/13}$- and $G\alpha_{q/11}$-coupled GPCRs to activate Rho-GTPases. Activation of Rho-GTPase serves as a key mediator in the activation of YAP/TAZ from upstream GPCRs. YAP/TAZ activity could be either activated or inhibited, depending on the G protein coupled to the GPCRs. Activation of $G\alpha_S$-coupled GPCRs by epinephrine and glucagon increases Lats1/2 kinase activities and inactivates YAP/TAZ in a manner dependent on protein kinase A (PKA) (55). Hence, depending on the kind of G proteins, GPCRs can differentially regulate Lats1/2 to stimulate or suppress YAP activity. Other studies further demonstrate that the core Hippo kinase cascade and YAP/TAZ activity are regulated by GPCRs in response to various hormonal cues. For instance, GPR68, a proton-sensing GPCR, is activated in response to a decrease in extracellular pH and is required for the pH-dependent regulation of the proliferation and apoptosis. Under a decrease in extracellular pH, GPR68 leads to an increase in the proliferation and a decrease in apoptosis of cells with abundant proton-sensing GPCR expression. In addition, it was found that YAP functions as a potent downstream effector of GPR68 through $G\alpha_{12/13}$ and Rho GTPase (56, 57). Besides, YAP is required for the pH-

dependent regulation of the differentiation of mesenchymal stem cells (MSCs) into cancer-associated fibroblasts, CAFs. Furthermore, stimulation of the G-protein-coupled estrogen receptor (GPER) by estrogen activates YAP/TAZ and regulates the expression of numerous genes, including well-characterized target genes via the $G\alpha_{q/11}$, PLCβ/PKC, and Rho/Rock signaling pathways. It was proposed that TAZ was required for breast cancer cell proliferation, migration, and tumor growth. As expected, TAZ expression positively correlated with GPER expression in human invasive ductal carcinoma (IDC) specimens, indicating that YAP/TAZ may be activated by estrogen in breast cancer (58). TxA2 exerts its biological activity through its cognate thromboxane A2 receptor (TP) receptor that couples with $G\alpha_{q/11}$, $G\alpha_{12/13}$, and other trimeric G proteins to regulate downstream effectors. TP has been implicated in promoting cell migration and proliferation of vascular smooth muscle cells (VSMCs). Treatment of the cells with thromboxane A2 (TP) activation promotes DNA synthesis and induces VSMC proliferation and migration in a manner dependent on YAP/TAZ (59). Thromboxane A2 signaling increases YAP/TAZ activity in VSMCs and other cell types via $G\alpha_{12/13}$, providing YAP/TAZ as potential therapeutic target for VSMC-mediated vascular disease. This study shows for the first time that AngII binding to the angiotensin II type 1 receptor (AT1R) can inhibit the Hippo pathway and activate YAP (60). As GPCR's coupling to the G protein subclass $G\alpha_{q/11}$, in general, are able to activate YAP, we therefore expected the same influence from the AT1R, which mainly couples to $G\alpha_{q/11}$. Stimulation of the AT1R with AngII showed decreased Lats1/2 activation, which was accompanied by decreased phosphorylation of its target YAP in HEK293T cells. Despite the initial observation of AngII as a stimulant of YAP dephosphorylation and nuclear localization, the Hippo pathway is not activated by stimulation with AngII in podocytes, which show a deactivated pathway. However, the actin cytoskeleton disruption with Latrunculin B reactivates Lats1/2 kinase activity, resulting in increased cytoplasmic YAP localization accompanied by a strong induction of apoptosis. Angiotensin II receptor serves as an upstream regulator of the Hippo pathway. The control of Lats1/2 activation and subsequent YAP localization is important for podocyte homeostasis and survival.

In addition to GPCRs, several other morphogenic factors elicit diverse receptor-mediated signaling pathways to control development and tissue homeostasis. The cytokine receptor leukemia inhibitory factor receptor (LIFR) activates the Hippo kinase cascade (61). The PI3K-PDK1 pathway disrupts the core Hippo complex in response to EGF, leading to inactivation of Lats1/2 and activation of YAP (62). Furthermore, YAP/TAZ is a critical mediator of the canonical Wnt/b-catenin and noncanonical alternative Wnt signaling. Two independent groups revealed that Wnt ligands could activate YAP/TAZ through their corresponding GPCRs, frizzled (FZD) receptors, although distinct signaling mechanisms are utilized (63-69). In the present studies, TGFβ and bone morphogenetic protein (BMP) sustain YAP/TAZ activity. Interaction between TAZ and TGFβ-regulated SMAD2 and SMAD3 governs their nuclear localization and target-genes expression. YAP can also be involved with SMAD1 and synergize transcriptional activation of BMP signaling (70-72).

GPCRs are the superfamily of the cell-surface receptors mediating the actions of hundreds of extracellular molecules that have a pivotal role in many physiological functions and in multiple diseases, including the development of cancer and cancer metastasis (54). Elevated expression of GPCRs or activating mutation of Gα leads to aberrant YAP activation and has been found in several types of cancers (58, 73-75). The regulation of YAP/TAZ by GPCRs implies that the Hippo pathway not only is modulated by many extracellular signals and cell-surface receptors, but also contributes to a wide range of physiological regulation and may function as the key mediator of GPCR agonists or antagonists for disease progression.

REGULATION OF THE HIPPO-YAP PATHWAY BY NUTRIENT SIGNALING

Nutrients and energy metabolism such as glucose, amino acids, and fatty acids are building blocks of the cells that promote cell growth. Glucose is an abundant fuel and the most widely used as an energy source in living organisms. Therefore, it is anticipated that nutrient signals can modulate YAP and TAZ activities. As expected, deprived of glucose, AMPK directly phosphorylates S793 of AMOLT1 and increases AMOTL1 protein levels, resulting in YAP inhibition in a Lats1/2 dependent manner (76). Furthermore, energy stress-activated AMPK directly phosphorylates YAP at multiple sites, and this phosphorylation interferes with the interaction between YAP and TEAD, thus contributing to its inactivation and inhibition of TEAD-mediated transcription (77, 78). LKB/STK11 is a known tumor suppressor and a major upstream regulator of AMPK. LKB1 represses YAP activity via either the core Hippo kinase cascade dependent or independent pathway (79, 80). On the other hand, loss of LKB1 and AMPK contributes to Yki activation and accelerated proliferation in the *Drosophila* (81). LKB1-mediated inhibition of Yki activity is mediated by AMPK and is independent of the Hpo/Wts kinase cascade, suggesting a potential energy-dependent pathway controlling proliferation in the central brain (CB) and ventral nerve-cord developmental neural systems (VNC).

Additionally, the Hippo pathway also responds to nutrients other than glucose. YAP/TAZ potentiates mTORC activity by increasing expression of the high-affinity L-type amino-acid transporter (LAT1), which is a heterodimer of SLC7A5 and SLC3A2. YAP/TAZ and TEAD directly induce transcription of LSC7A5, which rescues SLC3A2 protein expression by dimer formation, to increase LAT1 expression and amino-acid uptake (82, 83). In parallel, mTOR also is a master regulator of cellular growth and survival and stimulates cellular metabolic processes, such as protein synthesis. An mTORC signaling

pathway is reported to drive YAP activation and its target-genes expression in perivascular epithelioid cell tumors and glioblastomas (84-86). Both outputs of TOR are required for wing cells to divide and gain mass under Yki-Sd control in *Drosophila* (87). Previous evidence indicated that YAP, a main target of inhibition by the Hippo pathway, can activate AKT through miR-20-mediated inhibition of PTEN (88). These data, combined with a recent study, indicated that mTORC2 can regulate AKT activity, both directly and indirectly through inhibition of the Hippo pathway and activation of YAP (85). In addition, AKT and MST1 were previously shown to mutually inhibit each other (89, 90). Thus, mTOR2 and the Hippo pathway can engage in crosstalk at multiple levels. Of note, mTORC2 was also shown to activate SGK1 and PRKCA/PKCα (91-93). Besides lowering the cellular cholesterol levels, inhibition of the mevalonate pathway inhibits YAP/TAZ nuclear localization and transcriptional response, possibly because of inhibition of the Rho GTPases, which require a complex network by which cytoskeleton impinges on YAP/TAZ activation (94). In addition, other nutrients have been shown to be important in regulation of the Hippo pathway. For instance, the salt-induced kinases have been implicated in nutrient sensing that promotes Yki target-gene expression and tissue over-growth through phosphorylation of Sav at Ser413 (95).

The most recognized functional output of YAP and TAZ is to promote cell survival and proliferation by cellular nutrient status. Therefore, given the central role of the Hippo signaling pathway in nutrient sensing, understanding how nutrients contribute to cancer development remains an area of intense investigation.

CONCLUSIONS

Extensive research within recent decades has identified more components and other signaling pathways linked with the Hippo pathway and YAP/TAZ regulation, since many core Hippo-pathway components have been discovered in *Drosophila* and mammals. In recent years, the Hippo pathway has been influentially and intensely regulated by a wide array of extracellular biophysical cues, including mechanical cues, cell-surface receptors, and nutrient signaling from neighboring cells and the extracellular matrix. The core Hippo kinase cascade integrates multiple upstream inputs to control YAP/TAZ activity, allowing vigorous regulation of cellular processes, such as proliferation, differentiation, and apoptosis in intricate physiological contexts and in cancer.

However, it is important to realize that gaps still remain in understanding the key molecular mechanisms in extracellular biophysical cues. For example, it is unclear whether Lats1/2 kinase is involved in YAP/TAZ regulation by actin cytoskeleton under mechanical cues. Current evidence showed that Lats1/2 kinase activity is important for GPCR-mediated YAP/TAZ regulation, but Mst1/2 is not required for YAP/TAZ regulation by both mechanical cues and GPCR signaling. This suggests that other mechanisms or other unknown molecules may be involved in the process in response to the physiological environment. Furthermore, the detailed mechanism by which the actin cytoskeleton transmits upstream cues to modulate Lats1/2 kinase activity has yet to be uncovered. The possibly existing Lats1/2-independent mechanism of YAP/TAZ regulation by the actin cytoskeleton also has yet to be uncovered. It will also be interesting to define how YAP/TAZ may converge on these mechanical and hormonal cues to respond to the environment in an appropriate manner. For example, both mechanical cues and cell-surface receptors, especially GPCRs, signal input into regulation of Rho GTPase activity and thus affect YAP/TAZ activity.

Taken together, the YAP/TAZ are unquestionably important mediators of extracellular biophysical cues in regulation of organ size control, regeneration, and tumorigenesis, and thus would be legitimate attractive potential therapeutic targets for cancer therapy.

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CONFLICTS OF INTEREST

The authors have no conflicting financial interests.

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