Acta Biochim Biophys Sin, 2015, 47(1), 10–15
doi: 10.1093/abbs/gmu108
Advance Access Publication Date: 9 December 2014
Review

Review

G protein-coupled receptors: bridging the gap from the extracellular signals to the Hippo pathway

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Received 15 September 2014; Accepted 31 October 2014

Abstract

The Hippo pathway is crucial in organ size control, whereas its dysregulation contributes to organ degeneration or tumorigenesis. The kinase cascade of MST1/2 and LATS1/2 and the coupling transcription co-activators YAP/TAZ represent the core components of the Hippo pathway. Extensive studies have identified a number of upstream regulators of the Hippo pathway, including contact inhibition, mechanic stress, extracellular matrix stiffness, cytoskeletal rearrangement, and some molecules of cell polarity and cell junction. However, how the diffuse extracellular signals regulate the Hippo pathway puzzles the researchers for a long time. Unexpectedly, recent elegant studies demonstrated that stimulation of some G protein-coupled receptors (GPCRs), such as lysophosphatidic acid receptor, sphingosine-1-phosphate receptor, and the protease activated receptor PAR1, causes potent YAP/TAZ dephosphorylation and activation by promoting actin cytoskeleton assemble. In this review, we briefly describe the components of the Hippo pathway and focus on the recent progress with respect to the regulation of the Hippo pathway by GPCRs and G proteins in cancer cells. In addition, we also discuss the potential therapeutic roles targeting the Hippo pathway in human cancers.

Key words: Hippo, YAP, TAZ, GPCR, G proteins, cancer

Introduction

The Hippo pathway was initially identified by genetic mosaic screen using an Flp/Frt recombination system in *Drosophila*, and subsequent studies have identified an ever-increasing number of pathway components [1,2]. The Hippo pathway is evolutionarily conserved from *Drosophila* to mammals, and plays an important role in regulating cell proliferation, migration, differentiation, and apoptosis during development, regeneration, and the pathological status such as cancer [1,3]. To date, up to 30 members of the Hippo pathway have been identified in mammals [4]. The core of the Hippo pathway comprises a conserved kinase cascade (consisting of MST1/2 and LATS1/2) and the downstream transcription co-activator YAP/TAZ (**Fig. 1**). MST1/2 in the GCK family phosphorylate and complex with the regulatory subunit Sav1 to enhance their own kinase activities. The Ndr-family kinases LATS1/2 and the non-catalytic partner Mob1 are also the substrates of MST1/2. In complex with the phosphorylated Mob1, LATS1/2 phosphorylate the homologous transcription co-activator YAP and TAZ directly at multiple sites, and result in the inhibition of YAP/TAZ activities ultimately [5–7]. The phosphorylated YAP/TAZ are sequestrated by 14-3-3 in the cytoplasm and degraded via

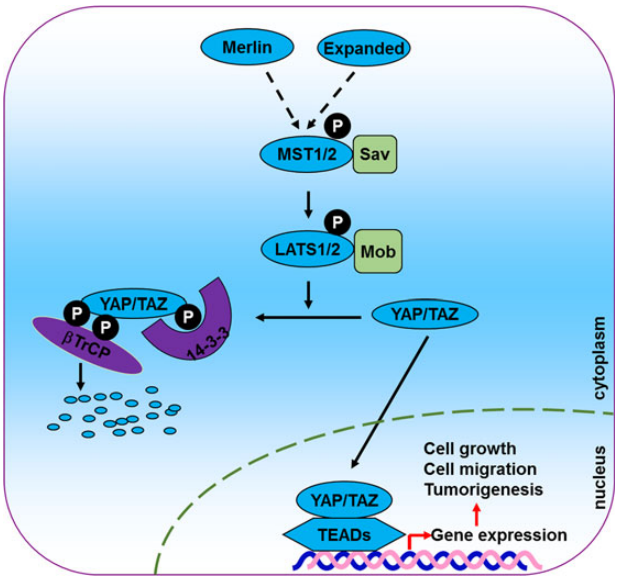

Figure 1. The core components of the Hippo pathway The kinase cassette consists of MST1/2 and LATS1/2. LATS1/2 directly phosphorylate two transcriptional co-activators YAP/TAZ, promoting cytoplasmic retention or proteasome degradation through enabling the binding of 14-3-3 or βTrCP; when the kinase cassette is inhibited, the dephosphorylated YAP/TAZ are translocated to nuclei and induce gene expression by interacting with transcription factors TEADs.

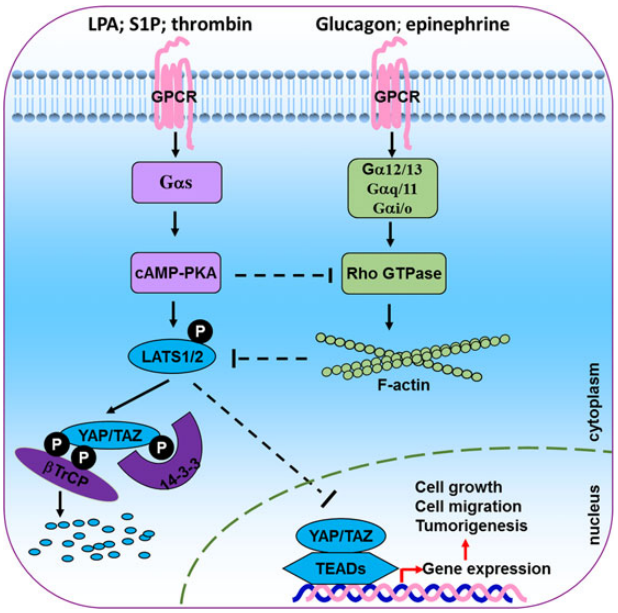

Figure 2. Regulation of the Hippo-YAP/TAZ pathway by GPCR Ligands/agonists of Gα12/13-, Gαq/11- and Gαi/o-coupled GPCRs activate Rho GTPases and cytoskeleton assembly, leading to the inhibition of LATS1/2, which suppresses YAP/TAZ activities. In contrast, Gαs-coupled receptors and ligands induce LATS1/2 via cAMP-PKA, resulting in the inhibition of YAP/TAZ active.

proteasome [6–11]. Under proliferating conditions, the module (MST1/2–LATS1/2) is repressed, resulting in nucleus localization of the dephosphorylated YAP/TAZ and the expression of a subset of genes relating to cell proliferation, migration, and survival via co-activating the TEAD family transcription factors (**Fig. 1**) [12,13].

Consistent with the striking overgrowth phenotypes caused by mutations in different Hippo pathway genes in *Drosophila melanogaster* tissues [1], increasing studies in mammalian have proved that the Hippo pathway plays a crucial role in cancer development [2]. Genetic manipulation of the Hippo pathway genes in mice leads to tumorigenic phenotypes. Mutation, amplification, and epigenetic silence of a subset of the Hippo pathway genes have also been observed in various human cancers [2]. For instance, elevated expression of YAP was observed in multiple human cancers [6,7,14–16]; TAZ protein expression level and activity were up-regulated in high-grade metastatic breast cancers [17,18].

Compared with the well-established core components of the Hippo pathway, the upstream signals, which are crucial to understanding how the MST–LATS–YAP/TAZ core is regulated in response to growth signals of the intracellular or intercellular environments, have not been well defined. Several different modulators have been identified via extensive genetic and biochemical analysis, including contact inhibition, mechanic stress, extracellular matrix stiffness, cytoskeletal rearrangement, and some molecules involved in cell polarity and cell junction [3,4,19,20]. Previous work has shown that cell–cell contact could inactivate YAP oncoprotein via inhibiting MST1/2 and LATS1/2 kinases [6]. Dupont *et al.* [21] showed that YAP/TAZ could be tightly regulated by extracellular matrix (ECM) stiffness. Zhao *et al.* [22] reported that cell detachment could repress YAP/TAZ activities, leading to cell detachment-induced anoikis. Over-expression of NF2 in mammalian cells leads to YAP inhibition by activating LATS [6,23]. More importantly, deletion of YAP could largely rescue the phenotypes of *NF2* knockout in the liver [23]. AMOT proteins, a family of cell junction proteins have been reported to inhibit YAP/TAZ activities by both phosphorylation-dependent and phosphorylation-independent mechanisms [24–26]. Interestingly, AMOT has also been shown to interact with both NF2 and YAP/TAZ and may serve as a connection between NF2 and YAP/TAZ [25,27,28]. Several other proteins, such as KIBRA, PTPN14, NPHP4, LKB1, Ajuba, and ZO-2, were also reported to be involved in the regulation of the Hippo-YAP/TAZ pathway [29–35]. However, the extracellular signals/ligands and cell surface receptors regulating the mammalian Hippo pathway remain elusive until the recent studies revealed that G protein-coupled receptors (GPCRs) serve as a link between extracellular signals and the Hippo pathway (**Fig. 2**) [36,37].

Lysophosphatidic Acid (LPA) and Sphingosine-1-phosphate (S1P) Are Potent Extracellular Signals Regulating the Hippo-YAP/TAZ Pathway

Cell proliferation is tightly regulated by a large number of growth factors via activating membrane receptors and intracellular signaling pathways [38]. As an important pathway regulating cell growth, the Hippo pathway is expected to be modulated by some of these growth factors. However, the phosphorylation of YAP/TAZ is not significantly affected by several well-known growth factors, such as insulin and EGF [6,37]. Unexpectedly, recent studies from two independent groups revealed that LPA and S1P could activate YAP/TAZ through their corresponding GPCRs [36,37].

These discoveries arose from the observation that YAP/TAZ were dephosphorylated and restricted in the cytoplasm under serum starvation conditions in cultured cells [36,37]. It was also noticed that this process is rapid and reversible, indicating that YAP/TAZ activities could be directly stimulated by serum in a dynamic manner.

By extensive elegant biochemical analysis of YAP activating fractions of serum, two most abundant phospholipids present in serum, LPA and S1P were identified as the major components responsible for YAP/TAZ activations [36,37]. Both reports showed that these phospholipids act through their corresponding membrane GPCRs, specific G proteins Gα12 and Gα13, Rho GTPases, and actin cytoskeleton assemble to activate YAP/TAZ (**Fig. 2**) [36,37]. Yu *et al.* [37] reported that LATS1/2, but not MST1/2, serve as key mediators in the regulation of YAP/TAZ from upstream GPCRs. Interestingly, Miller *et al.* [36] proposed that neither LATS1/2 nor MST1/2 are involved in the regulation from GPCRs to YAP/TAZ. Although controversial at this point, they suggested the same important signaling cascade from the extracellular diffuse signals to YAP/TAZ via GPCRs.

As transcription co-activators, the major function of YAP/TAZ is to stimulate gene expressions via binding to TEAD family transcription factors [12]. Yu *et al.* [37] showed that LPA could up-regulate the expression of *CTGF*, *CYR61*, *ANKRD1*, *TAGLN*, and *EDN1* in a YAP/TAZ-dependent manner. They also observed that YAP/TAZ are required by LPA-induced cell proliferation and migration, indicating that YAP/TAZ play important roles in mediating the physiological functions of LPA [37]. Consistent with previous observation that TAZ is over-expressed in breast cancer [17,18], they found that TAZ was dephosphorylated and localized in the nucleus of the mammary tissues and tumors of LPA receptor (LPA2) transgenic mice. In addition, the protein levels of TAZ and CTGF (the most important target gene of TAZ) were largely elevated in LPA2 tumors [37]. These observations support that LPA–TAZ axis is crucial in mammary tumor development *in vivo*. Collectively, these findings not only provided the first example that YAP/TAZ can be truly regulated by diffusible extracellular signals and cell surface receptors, but also unveiled the ability of GPCRs to activate the YAP/TAZ oncogenes.

Two subsequent studies further confirmed the stimulation of YAP/TAZ by LPA in ovarian cancer cells [39,40]. Cai and Xu [39] showed that LPA induced YAP dephosphorylation and nuclear localization in dose and time-dependent manner in several ovarian cancer cell lines. LPA3 and the cognate Gα13 transmitted the signal of LPA to RhoA–ROCK, and subsequently induced YAP/TAZ activation. They also noticed that the kinase activities of LAST1/2 and MST1/2 were not affected by LPA in ovarian cancer cells. Instead, protein phosphatase 1A (PP1A) serves as the downstream of RhoA in mediating LPA-induced YAP dephosphorylation. Finally, they observed that the phosphorylation level of YAP was reduced in epithelia ovarian cancer (EOC) specimens compared with normal or benign ovarian tissues, supporting that the phosphorylated YAP could serve as a biomarker for EOC [39]. Jeong *et al.* [40] also found that the activation of TAZ by LPA treatment could be blocked by specific LPA receptor inhibitor Ki16425 or siRNA target LPA1, and TAZ is required for LPA-induced ovarian cancer cell migration. These two studies strengthened the potential role of LPA in regulating YAP/TAZ in cancer cells and indicated that this axis may play crucial role in numerous cancer types.

Stimulation of PAR1 Activates YAP/TAZ

Consistent with the roles of LPA and S1P in regulating YAP/TAZ, another study showed that thrombin, the ligand of protease-activated receptors (PARs), also stimulated YAP/TAZ activities (**Fig. 2**) [41]. In this study, Mo *et al.* [41] demonstrated that PAR1-induced dephosphorylation and activation of YAP as well as its target gene expression are blunted by depletion of Gα12/13 but not Gαq/11. They also provided evidence that RhoA acts as an important mediator in PAR1-induced YAP activation by transfecting the dominant inhibitory mutant of RhoA (RhoAN19) or using the RhoA-inactivating toxin C3. In line with the role of RhoA in cytoskeleton regulation, PAR1 stimulation induced actin rearrangement and disruption of cytoskeleton dynamic resulted in complete inhibition of PAR1-induced YAP dephosphorylation and nuclear localization [41]. However, ROCK (Rho kinase) is not required in Rho mediating YAP activation, which contrasts with the fact that ROCK is involved in S1P-induced YAP nuclear localization and also in YAP regulation under contact inhibition and mechanical forces [18,36]. They also observed that YAP/TAZ are required for PAR1-induced gene transcription and cell migration. Due to the vital role of PAR1 in the metastasis of tumor, such as hepatocellular carcinoma [42], the potential involvement of YAP/TAZ in human cancers which have elevated thrombin or PAR1 abundance deserves more attention.

The Hippo Pathway Is Potentially Regulated by a Variety of GPCRs

GPCRs, comprising the largest cell surface receptor family, involve in signal transmission in a wide range of physiological regulation as well as pathological conditions [43]. More than 50% of all prescription drugs are targeting at GPCRs [43]. As the center of signal transmission, GPCRs could regulate several different pathways, such as PLC-PKC, AKT/mTOR, and MAPKs, in promoting cancer cell proliferation and migration [43]. Notably, GPCRs play critical roles in cancer development by stimulating Rho GTPases and actin cytoskeletal dynamic [44,45]. As it is already known that LPA, S1P, and thrombin transmit the signals to the Hippo-YAP/TAZ pathway via the corresponding GPCRs, another very critical question raised is whether other GPCRs (and their ligands) could regulate the Hippo-YAP/TAZ pathway.

To answer this question, Yu *et al.* [37] transiently co-transfected 34 individual GPCRs, most of which were related to human cancers, with FLAG-YAP in HEK293A cells, and the phosphorylation state of FLAG-YAP was assessed on phos-tag gels. They found that the large majority of GPCRs used in their study, such as purinergic receptor 1 (P2YR1) and the platelet-activating factor receptor (PTAFR), promoted YAP/TAZ dephosphorylation. Interestingly, they also found that endothelin receptor type A, dopamine receptor D1, and glucagon receptor could induce YAP phosphorylation. This observation was further confirmed by cell treatments with glucagon or epinephrine, and consistent result was obtained in mice injected with epinephrine in the heart as well [37].

Given that the enormous number of GPCRs were used in the study, Yu *et al.* [37] established a possible role that the Hippo-YAP/TAZ pathway is robustly regulated by a wide range of diffused signals and their corresponding GPCRs (**Fig. 2**) [37]. Indeed, depending on the hormones, cell types, and GPCR expression level, YAP/TAZ might be differently regulated. It would be important to characterize whether YAP/TAZ are activated in cancers in which GPCRs are over-expressed or activated. Whether YAP/TAZ made a major contribution to the cancer development under control of specific GPCR(s) is an open question.

YAP/TAZ Are Differently Regulated by Different Gα proteins

Upon agonist binding, the conformation of GPCRs is rapidly changed, leading to the dissociation and activation of heterotrimeric G proteins

that are comprised of Gα, β and γ subunits [46]. The 15 mammalian Gα subunits could be divided into four classes, i.e. Gα12/13, Gαq/11, Gαi/o, and Gαs [46]. By transfecting both the wild-type and the constitutive activation form of Gα, Yu *et al.* [37] observed that Gα12/13, Gαq/11, Gαi/o repressed LATS1/2 activities, leading to YAP dephosphorylation and activation, whereas only Gαs elevated YAP phosphorylation by inducing LATS1/2 activities. Taken together with the effect of GPCRs on YAP phosphorylation, GPCRs cognate to Gα12/13, Gαq/11, or Gαi/o, such as LPA, S1P, and thrombin, could activate YAP/TAZ. In contrast, glucagon and epinephrine which transmit signal from Gαs, inhibit YAP/TAZ (**Fig. 2**) [37]. Actually, previous study has identified dobutamine, an agonist for the Gαs-coupled adrenergic receptor, as a YAP inhibitor [47], supporting that YAP/TAZ could be either up-regulated or down-regulated by different GPCRs through different Gα proteins.

Two recent studies further strengthened the model that YAP/TAZ could be tightly regulated by Gαs–PKA cascade [48,49]. Activation of Gαs-coupled receptors, such as glucagon, usually leads to the production of cyclic adenosine monophosphate (cAMP) which serves as a vital second messenger in signal transduction [50]. Yu *et al.* [48] showed that cAMP acts through PKA and Rho GTPases to stimulate LATS kinase, leading to YAP phosphorylation and inactivation. Moreover, they proved that PKA could also inhibit Yki in *Drosophila*, a homolog of YAP/TAZ in fruit fly. They provided evidence that YAP/TAZ play a crucial role in PKA-induced adipogenesis [48]. Another research group also demonstrated that cAMP/PKA signaling reinforces the LATS–YAP pathway to fully suppress YAP, in response to actin cytoskeletal changes [49]. They showed that cAMP-dependent PKA phosphorylates LATS directly at its (R/K)(R/K)xS/T motifs under cytoskeletal damage, serum starvation, or high cell density conditions, thus promoting YAP phosphorylation at Ser381, which is crucial for its proteasome degradation. This study also implicated that PKA is required for the canonical Hippo pathway (NF2, Angiomotin L1, and Angiomotin L2)-induced YAP phosphorylation [49]. Although these two studies proposed a different model in cAMP–PKA regulation on YAP/TAZ, they all pointed out that cAMP–PKA strongly inhibits YAP/TAZ and YAP/TAZ play crucial roles in the physiological functions of cAMP–PKA [48,49].

Although GPCR signaling plays crucial roles in various cancers, rare mutations of GPCR are found in human cancers. However, mutations frequently happen in G proteins, such as mutually exclusive activating mutations of GNAQ or GNA11 (encoding Gαq or Gα11, respectively) are observed in ~80% of uveal melanomas [51,52]. As discussed above, opposite to Gαs, Gαq/11 could stimulate YAP/TAZ activation. Over-expression of $G\alpha q/11^{Q209L}$ which is a constitutively active form by reducing their guanosine triphosphatase activity, but not the wild-type Gαq/11, is able to stimulate YAP/TAZ dephosphorylation in 293 cells [37]. More importantly, studies from two independent groups found that YAP/TAZ play crucial roles in uveal melanoma with GNAQ or GNA11 mutation [53,54]. Both groups showed that cancer-associated Gαq/11 mutants activate YAP by modulating actin polymerization and the Hippo pathway activity, and YAP is responsible for the oncogenic activity of mutant Gαq/11 in uveal melanomas development *in vitro* and *in vivo* [53,54]. In addition, they also showed that verteporfin (VP), an inhibitor of YAP, by disrupting YAP–TEAD interaction, blocked tumor growth of Gαq/11-mutated uveal melanomas cells, suggesting that YAP is a promising therapeutic target for uveal melanomas carrying mutations in GNAQ or GNA11 [6,55]. As an FDA-approved drug, VP is widely used in eye diseases. Hence, it would be relatively accessible to transfer and adapt it for uveal melanomas treatment, and other drugs which could inhibit YAP should also be considered for therapeutic use in uveal melanomas treatment.

Targeting YAP/TAZ for Cancer Therapy

The major readout of Hippo-YAP/TAZ pathway is the regulation of target gene expression by binding directly to TEAD transcription factors [12]. The most effective strategy to abolish YAP/TAZ-induced tumorigenic effects is to design compounds which could disrupt the interaction between YAP/TAZ and TEADs [56]. For this reason, Liu-Chittenden *et al.* [55] screened up to 3000 compounds based on Gal4-TEAD4 luciferase activity and identified the porphyrin family, such as VP, hematoporphyrin (HP), and protoporphyrin IX (PPIX), as a strong candidate of YAP inhibitors. Porphyrin exhibits anti-cancer potential in liver overgrowth induced by YAP overexpression or by inactivation of NF2 *in vivo* through dissociating the interaction between YAP and TEAD [55]. As discussed above, VP also blocked tumor growth of uveal melanoma cells containing GNAQ mutations [53]. Similarly, Jiao *et al.* [57] found that a peptide mimicking the function of VGLL4, which directly competed with YAP for binding to TEADs, potently suppressed gastric tumor growth *in vitro* and *in vivo*. These studies gave very good examples that disruption of YAP–TEADs interaction may be an attractive therapeutic approach against YAP-driven human cancers, and these compounds or peptides could also be used in other human cancers with abnormal GPCR signaling, in addition to uveal melanoma with GNAQ mutations.

Another approach to inhibit YAP/TAZ in cancer is to disrupt the upstream regulators. It is well known that Rho and actin cytoskeleton play a central role in the regulation of YAP/TAZ in response to multiple upstream signals such as ligands through GPCRs, cell detachment, extracellular stiffness, and mechanical stress. [4]. Hence, compounds targeting at Rho should have potential inhibitory effect on YAP/TAZ. Coincidently, Sorrentino *et al.* [58] screened a library containing 650 clinically used compounds for YAP inhibitors and found that statins, inhibitor of HMG-CoA reductase (HMGCR) which is the rate-limiting enzyme of mevalonate pathway, could strongly reduce YAP/TAZ nuclear localization [58]. Actually, it has been well known that HMGCR inhibitor is also a striking inhibitor for Rho GTPases, and could reduce the expression of CTGF which is a major target gene of YAP/TAZ [59]. More importantly, they also provided evidence that mevalonate–YAP/TAZ axis is required for proliferation and cell renew of breast cancer, and HMGCR inhibitors seem to be promising inhibitors of YAP/TAZ.

It is worth to note that the finding of GPCRs–YAP/TAZ axis not only provides potential therapeutic targets for GPCR signaling dysregulated cancers, but also elicits the approaches to target YAP/TAZ by small molecules which modulate the GPCR signaling for cancers carrying YAP/TAZ activation. To this end, the Hippo-YAP/TAZ network appears to be a very promising target for drug development in human cancers. Further in-depth characterization of inhibitors targeting at either YAP/TAZ–TEAD interaction or upstream GPCR signaling will provide valuable opportunities for cancer therapy.

Perspective

GPCRs represent the largest family of membrane receptors, and play crucial roles in various physiological function as well as pathological status, such as human cancer [43]. Elevated expression of GPCRs and activating mutations of GPCRs and G-proteins are sporadically present in human cancers. A recent study also revealed that nearly 20% of human cancers harbor mutations in GPCRs (TSHR, SMO, GRMs)

and G proteins by deep sequencing of the cancer genome [60]. Meanwhile, the elevated expression and nuclear localization of YAP/TAZ were observed in a wide range of human cancers, promoting cancer cells proliferation, migration, and metastasis [17,18,61–63]. These observations raise the possible role of GPCRs–YAP/TAZ axis in human cancer. Indeed, Yu *et al*. [37] have provided evidence that TAZ was predominantly localized in the nucleus in mammary and tumor tissue of LPA receptor transgenic mice. In the future, more investigation should be implemented in the potential role of YAP/TAZ in the initiation and development of cancer driven by abnormal or dysregulated GPCR signaling.

The influence of extracellular ligands on YAP/TAZ is transient and dynamic. Increasing evidence indicated that the dynamics of actin cytoskeleton appears to be crucial to integrate multiple signals, including GPCR signaling, to modulate YAP/TAZ. However, several critical questions regarding the regulation of the YAP/TAZ by these upstream regulators still need to be answered. How could YAP/TAZ sense the actin cytoskeleton structure either in LATS-dependent or LATS-independent way? Which pathway or protein complex is employed to transduct the signal from actin to YPP/TAZ? In which condition the Hippo pathway is or not required for GPCR signaling to modulate YAP/TAZ? Are there any key intracellular ligands of GPCRs crucial for the organ size control activity of the Hippo pathway? Further in-depth characterization of these challenging questions will advance our understanding of the regulation and function of the Hippo pathway.

Funding

This work was supported by the grants from the National Key Basic Research Program of China (Nos. 2015CB910401 and 2011CB910600), the National Natural Science Foundation of China (Nos. 31271454, 81225016, and 8143000167), Shanghai Key Basic Research Program (No. 12JC1401100), '100 Talents' Program of Shanghai Health (XBR2011041), Scholar of 'Dawn' Program of Shanghai Education Commission, and Shanghai Outstanding Academic Leader (No. 13XD1400600) to Q.Y.L.

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