



Regulation of the Hippo pathway in cancer biology

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Abstract

The Hippo tumor suppressor pathway, which is well conserved from *Drosophila* to humans, has emerged as the master regulator of organ size, as well as major cellular properties, such as cell proliferation, survival, stemness, and tissue homeostasis. The biological significance and deregulation of the Hippo pathway in tumorigenesis have received a surge of interest in the past decade. In the current review, we present the major discoveries that made substantial contributions to our understanding of the Hippo pathway and discuss how Hippo pathway components contribute to cellular signaling, physiology, and their potential implications in anticancer therapeutics.

Keywords Hippo pathway · YAP/TAZ · TEAD · Cancer · Therapeutic target

Overview of the Hippo pathway

The core Hippo components in *Drosophila*

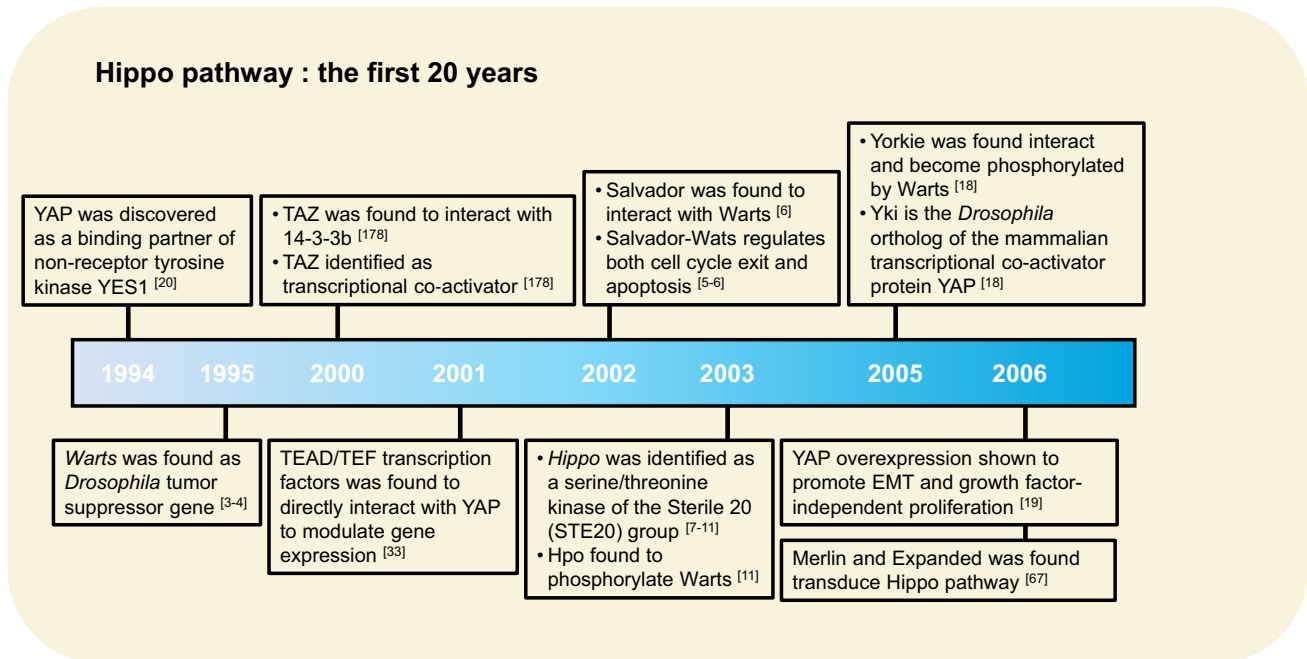
Since it was first established as a genetic model by Thomas Hunt Morgan, *Drosophila melanogaster* has made a large contribution to our understanding of metazoan development [1]. The genetic mosaic screening of *Drosophila* using tissue-specific loss-of-function assays by the flippase/FRT system is a powerful tool to investigate genetic pathways [2]. Surprisingly, this led to the discovery of almost all components of the Hippo tumor suppressor pathway in experiments designed to identify growth suppressors. Gene inactivation in this pathway resulted in massive tissue overgrowth in the developing eyes or wings. In 1995, two studies discovered that homozygous loss of Warts (Wts), which encodes a

nuclear Dbp-2-related (NDR) family Ser/Thr kinase, caused large outgrowth of multiple tissues in *Drosophila* [3, 4]. These initial findings indicated that Wts regulates tissue growth and functions as a tumor suppressor gene. Following the discovery of Warts, Hariharan, Halder, and collaborators reported that Salvador (Sav; also known as Shar-Pei), a WW domain-containing protein, genetically and physically interacts with Wts and that a Sav mutant clone showed excess interommatidial cells in *Drosophila* eye development via elevated cyclin E-induced cell cycle progression [5, 6]. Moreover, Sav-deficient cells induced DIAP1 (Drosophila inhibitor of apoptosis protein 1), which attenuates apoptosis by inhibiting caspase, resulting in cell proliferation and organ growth. These results suggested that Wts–Sav restrain massive cell growth during development and may have critical roles in tumor suppression.

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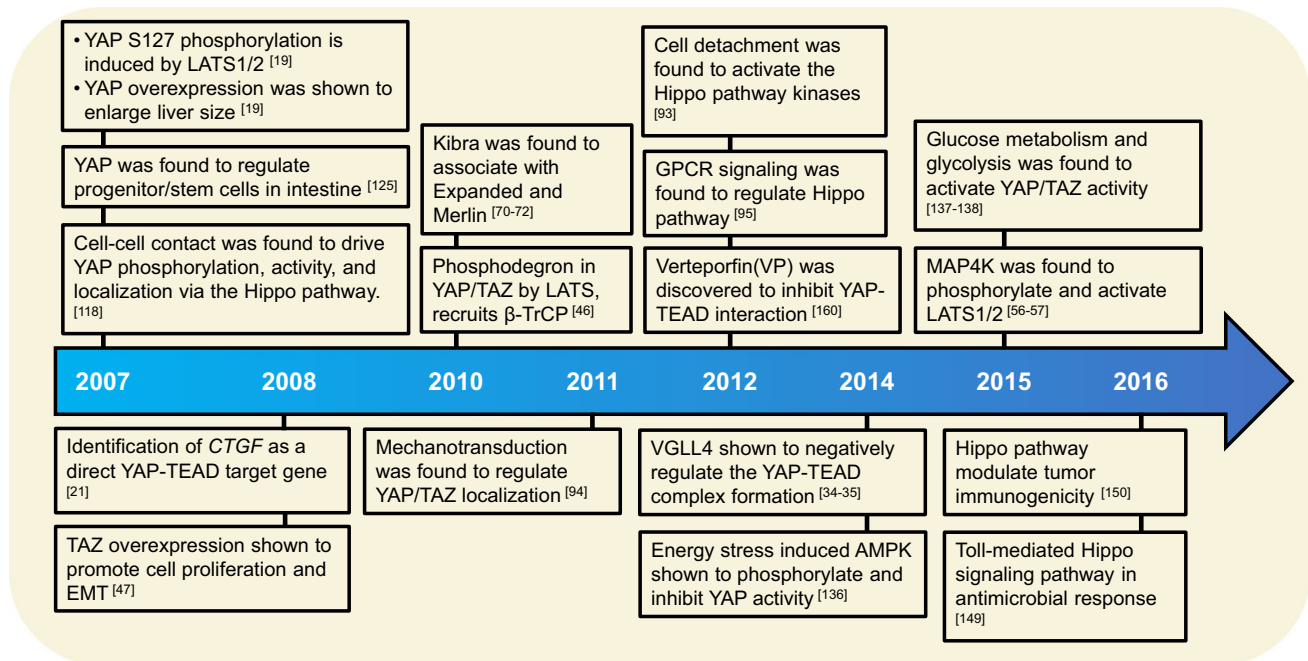
Timeline



In 2003, five independent groups identified Hippo (Hpo) from genetic mosaic screens for mutant clones that show excess overgrowth phenotypes in *Drosophila* [7–11]. Interestingly, all five reports noticed that the phenotypic characteristics of Hpo mutants were identical to those of Wts or Sav loss-of-function mutants. Hpo deletion increased the number of interommatidial cells in *Drosophila* eye disc via robust cyclin E [12–14] and DIAP [15] expression. In contrast, overexpression of Hpo showed a loss of eye, head, and wing tissue phenotype during imaginal disc development

through cell cycle arrest and apoptosis. Collectively, these findings indicated that Hpo, Sav, and Wts control cell cycle exit and apoptosis in *Drosophila*, and thus function as negative growth regulators, identifying Salvador/Warts/Hippo (SWH) signaling as a tumor suppressor pathway [16]. Another core component, Mats (Mob as tumor suppressor), was later identified as a Wts-interacting partner that enhances Wts kinase activity [17]. Loss of Mats also results in an excessive overgrowth phenotype similar to Hpo or Wts mutation in *Drosophila*.

Timeline



Notably, in 2005, Pan's group discovered Yorkie (Yki), a transcriptional co-activator that acts as a Wts-binding partner [18]. Overexpression of Yki recapitulates the tissue overgrowth phenotype similar to Sav, Wts, or Hpo mutants, while Yki mutants suppress tissue proliferation. Moreover, Yki mutants combined with Hpo, Sav, or Wts mutants did not show any phenotypic difference when compared with the Yki mutation alone. These results suggested that Yki is genetically epistatic to Hpo, Wts, and Sav and that the Sav/Wts/Hpo complex possibly acts upstream of Yki. Two years later, Pan and colleagues reported that Wts phosphorylates Yki at serine 168 residue, which induces cytoplasmic retention of Yki via interaction with the 14-3-3 protein, thus providing the first mechanism of Yki regulation by Wts [19] (Fig. 1).

The Yki transcription co-activator lacks a DNA-binding domain, which prompted research to identify the transcription factors that mediate Yki target gene expression [18, 20]. In 2008, independent groups observed that non-phosphorylated Yki localizes in the nucleus and interacts with the Scalloped (Sd) transcription factor [21–24], which in turn induces Sd target genes. Yki–Sd-induced Bantam micro-RNA expression, which promotes the overgrowth phenotype, and overexpression of bantam partially rescued the growth defect phenotype, caused by the Yki loss-of-function mutation [25, 26]. Bantam also links the epidermal growth factor receptor (EGFR) and Hippo pathway-mediated tissue growth during development [27]. Yki also induces gene expression of the proto-oncoprotein and transcription factor dMyc [28, 29], as well as cell cycle regulator E2F [24]. In addition to

Sd, Homothorax (Hth), and Teashirt (Tsh) are reported to interact with Yki, and mediate the transcriptional output of Hippo growth-regulatory pathway [30].

Collectively, this kinase cascade has been grouped into a new signal transduction called the “Hippo pathway”, which was named after the Hpo mutant *Drosophila* overgrowth head phenotype that resembles the folded skin around the neck of a hippopotamus.

The Hippo signaling pathway in mammals

The Hippo signaling pathway is evolutionarily well conserved from flies to mammals. Hence, most of the core components of the *Drosophila* Hippo pathway have mammalian homologs. These include mammalian STE20-like protein kinase 1/2 (MST1/2) (Hippo homolog, also known as STK4 and STK3), Salvador family WW domain-containing protein 1 (SAV1) (Salvador homolog), large tumor suppressor 1/2 (LATS1/2) (Warts homolog), MOB kinase activator 1A/B (MOB1A/B) (Mats homolog), YAP [20] and TAZ (Yorkie homologs), and TEA domain family members 1–4 (TEAD1–4) (Scalloped homolog). Similar to the *Drosophila* Hippo pathway, the mammalian Hippo pathway is also considered a tumor suppressor pathway that is mainly regulated by a phosphorylation-dependent protein kinase cascade. For example, upon receiving various upstream signals, activated MST1/2 phosphorylates and activates the downstream kinases LATS1/2, MOB1, and SAV1. MST1/2 interacts with SAV1, which enhances MST1/2 kinase activity to interact and phosphorylate LATS1/2. Activation of

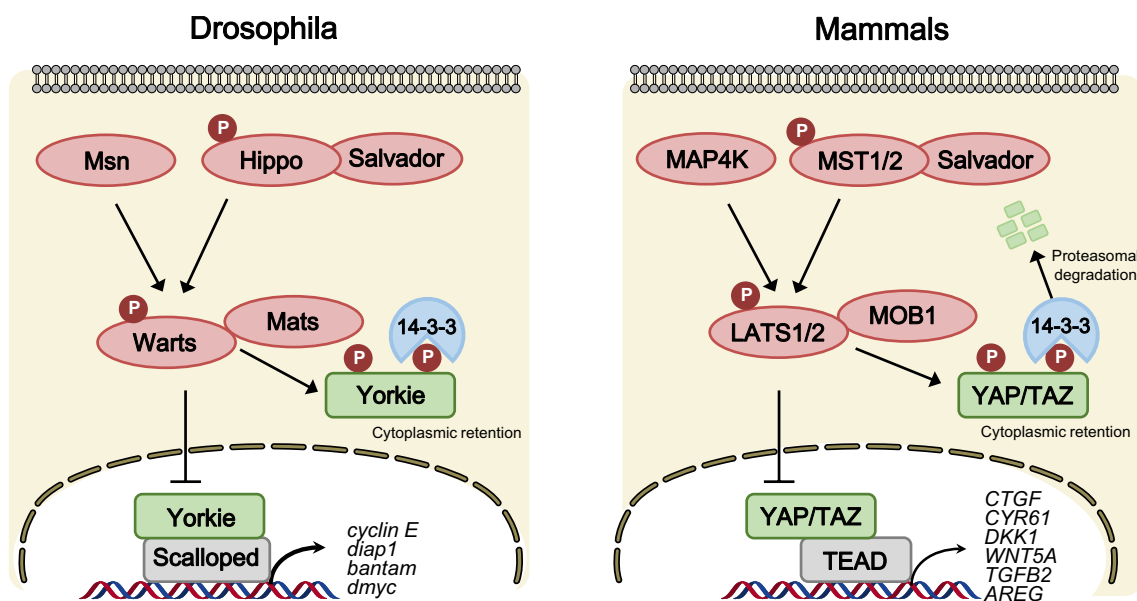


Fig. 1 Core Hippo pathway components in *Drosophila* and mammals. In *Drosophila* (left), Hippo (Hpo) and Misshapen (Msn) kinase phosphorylate and activate Warts (Wts) kinase, which in turn inactivates the transcriptional co-activator Yorkie (Yki). Phosphorylation of Yki leads to cytoplasmic retention via a 14-3-3 interaction. Upon Hippo pathway inactivation, dephosphorylated Yki translocates to the

nucleus and binds the transcription factor Scalloped (Sd) to induce gene expression involved in cell proliferation and anti-apoptosis. In mammals (right), the core Hippo pathway components are evolutionarily conserved. MST1/2 kinase phosphorylates LATS1/2, which in turn phosphorylates and inhibits YAP/TAZ. Phosphorylation of YAP/TAZ leads to cytoplasmic sequestration and proteasomal degradation

LATS1/2 subsequently phosphorylates and inhibits YAP, and its paralog TAZ. Therefore, the final physiological output of the Hippo kinase cascade is to restrict the transcriptional activity of YAP/TAZ and TEAD (Fig. 1).

Regulation of the Hippo pathway

YAP/TAZ regulation by the Hippo pathway

The core Hippo pathway consists of the cytoplasmic kinase modules MST1/2 and LATS1/2, which are serine–threonine kinases with tumor suppressive functions, and the nuclear transcriptional modules YAP/TAZ and TEAD, which harbor oncogenic functions. Upon Hippo pathway inactivation, YAP/TAZ translocate into the nucleus, which leads to enhanced gene transcription via interactions with TEAD1–4 transcription factors [21, 31–33]. In contrast, when YAP/TAZ is sequestered in the cytoplasm by LATS1/2-mediated phosphorylation, TEAD activity is suppressed by Vestigial-like 4 (VGLL4), which acts as a tumor suppressor, and the TEAD–VGLL4 interaction is dissociated by YAP/TAZ–TEAD activation [34–37]. In addition to TEAD, YAP/TAZ can interact with diverse transcription factors via their WW domain–PPXY motif, including FoxO1, ErbB4, TBX5, Pax, Smads, p73, and RUNX1/2 to regulate multiple aspects

of tissue growth and cell differentiation [38]. Through interacting with TEAD and other transcription factors, YAP/TAZ promote the expression of target genes including *CTGF*, *CYR61*, *WNT5A*, *TGFB2*, *NOTCH2*, *IL6*, and *AREG*, which are involved in cell proliferation, anti-apoptosis, and tumorigenesis [21, 39–44].

Regulation of protein stability by post-translational modifications is a common mechanism to alter transcriptional activity. In *Drosophila*, Leash E3 ubiquitin ligase induces Yki degradation via Hippo protein–protein interaction network [45]. In addition, Zhao and colleagues showed that Hippo signaling promotes YAP/TAZ cytoplasmic retention via YAP/TAZ phosphorylation and 14-3-3 interaction, followed by β -TrCP E3 ubiquitin ligase-mediated ubiquitination and proteasomal degradation [46–49]. YAP/TAZ degradation depends on LATS1/2-mediated direct phosphorylation of YAP/TAZ at multiple sites. YAP S127 phosphorylation by LATS1/2 is essential for YAP to associate with 14-3-3, while phosphorylation on YAP S381 triggers subsequent phosphorylation by Casein kinase 1 δ/ϵ , thus creating a phospho-degron motif for β -TrCP, which leads to YAP/TAZ ubiquitination and degradation. Conversely, upon Hippo pathway inactivation, dephosphorylated YAP/TAZ translocates into the nucleus and interacts with the TEAD family transcription factors, to activate the transcription of genes encoding proteins involved in cell proliferation and

tissue growth [46]. Consistently, mouse models with deletion of upstream Hippo components, MST1/2, NF2 (Merlin homolog), SAV1, MOB1A/B, or LATS1/2, all exhibit hyperactivated YAP-induced progenitor cell expansion and tumorigenesis [50]. Therefore, YAP/TAZ phosphorylation by the Hippo pathway is a critical process that restricts their transcriptional activity. In addition to phosphorylation and ubiquitination, multiple post-translational modifications such as acetylation, methylation, sumoylation, and glycosylation have been recently identified to regulate the Hippo pathway (for a review, see [51]).

Recent studies discovered that Tao Kinases 1–3 (TAOK1/2/3) and Misshapen (Msn) function similar to Hpo kinase and are responsible for Wts activation. The sterile 20 family kinase, TAO1/3, directly phosphorylates MST1/2 and LATS1/2 via NF2 and RHOA upstream signaling [52–54]. Msn is conserved in mammals as MAP4K4, and its activation induces phosphorylation of LATS1/2 [55], which in turn inhibits YAP/TAZ activity [56]. In addition, two recent studies revealed that multiple MAP4K family kinases, including MAP4K1/2/3/5 (Happyhour in *Drosophila*) and MAP4K4/6/7 (Msn in *Drosophila*), could directly phosphorylate and activate LATS1/2 [57, 58]. Thus, MST1/2 and MAP4Ks act in parallel to phosphorylate and activate LATS1/2, and loss of both MST1/2 and MAP4Ks can completely inhibit the Hippo pathway by turning off LATS1/2 hydrophobic motif phosphorylation and activation [55, 57, 58].

Regulation of the Hippo pathway by cell polarity and cell adhesion

Apical–basal polarity separates two complementary membrane domains, the adherens junctions, and tight junctions (adherens and septate junctions in *Drosophila*). Cell polarity is connected to diverse signaling pathways, and controls development and tissue growth [59]. Genetic studies showed that two protocadherins Fat (Ft) and Dachshous (Ds), both of which are highly involved in cell polarity and planar-cell polarity (PCP), regulate the Hippo–Yki pathway during *Drosophila* wing growth [60, 61]. The *Drosophila* Zyxin family gene, Zyx102 (Zyx), a downstream target of Ds, interacts with Wts and inhibits its kinase activity, linking Fat signaling to the Hippo pathway [62, 63]. Moreover, the *Drosophila* Crumbs (Crb) protein has been identified as a cell surface Hippo signaling regulator [64–67]. Crb interacts with FERM (4.1, Ezrin, Radixin, and Moesin) domain proteins, Merlin (Mer) and Expanded (Ex), which are homologs of NF2 and Willin/FRMD6 in mammals, respectively, and function as tumor suppressors by interacting with Tao-1 through Schip1, which activates the Hippo pathway [68, 69]. Crb also inhibits Yki by regulating the Ex level through ubiquitin-mediated degradation [70]. Kibra (WW and C2

domain-containing protein 1) interacts with Mer and Ex, which in turn activate Wts to suppress Yki activity [71–73]. Inactivation of Kibra by forming a complex with Par3/aPKC promotes cancer metastasis via Hippo inactivation [74]. Thus, the Mer/Ex/Kibra complex recruits the Hippo pathway kinases to the apical plasma membrane for activation.

In mammals, tight junction (TJ)-associated scaffold protein angiomin (Amot) has emerged as a critical regulator of the Hippo pathway. Amot has both PY- and PDZ-binding motifs, which interact with diverse proteins involved in cell polarity and junction formation [75–77]. Zhao et al. reported that Amot family proteins directly interact with YAP/TAZ through PPXY motif and WW domain interactions [78]. Amot inhibits YAP/TAZ activity by promoting their cytoplasmic translocation [78–80]. In addition, Amot can bind both LATS1/2 and MST1/2 to promote LATS kinase activity [81], which in turn suppresses YAP/TAZ activity. Scribble, a polarity protein localized to the epithelial junctions, has been shown to physically interact with and inhibit TAZ. Upon EMT epithelial-to-mesenchymal transition (EMT), Scribble delocalization activates TAZ, which induces cancer stem cell traits [82]. Another tight junction cytoplasmic scaffolding protein, Zonula occludens-2 (ZO-2) interacts with the C-terminal PDZ domain of YAP/TAZ, and promotes YAP/TAZ nuclear translocation [83, 84] (Fig. 2).

α -Catenin is a major component of adherens junctions (AJs), and acts as a linker for the cadherin complex and actin cytoskeleton [85]. α -Catenin functions as a tumor suppressor by negatively regulating YAP activity during epidermal stem cell proliferation and tissue expansion [86]. Consistently, Silvis et al. reported that deletion of α -catenin in the hair follicle stem cells led to skin cancer because of the constitutive nuclear localization of YAP [87]. In addition, the Hippo pathway is required for E-cadherin/catenin-dependent contact inhibition of proliferation via YAP inhibition [88]. Another AJ component, protein tyrosine phosphatase 14 (PTPN14), also directly interacts with YAP and contributes to YAP cytoplasmic retention [89–92] (Fig. 2). Therefore, the apical cell polarity complex, cadherin/catenin, and adherens junction proteins are important regulators of Hippo signaling to suppress cell proliferation and tumorigenesis.

Regulation of the Hippo pathway by extracellular ligands and cellular stress

The Rho family of GTPase are molecular switches that play an essential role in converting external cues into signal transduction pathways that regulate actin cytoskeleton polymerization, membrane transport, and cell migration [93]. Importantly, Rho GTPases, including RhoA, Rac1, and Cdc42, play pivotal roles in Hippo pathway regulation linking extracellular stimuli, such as membrane receptor signaling and mechanotransduction, to YAP/

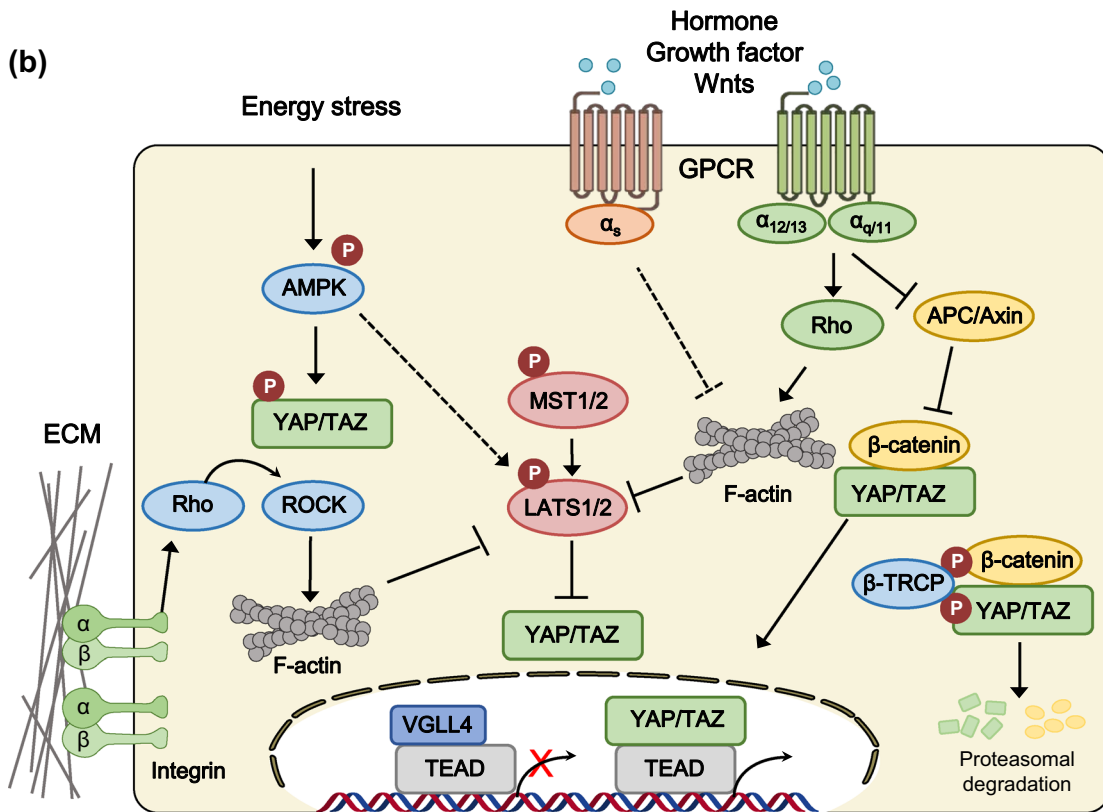
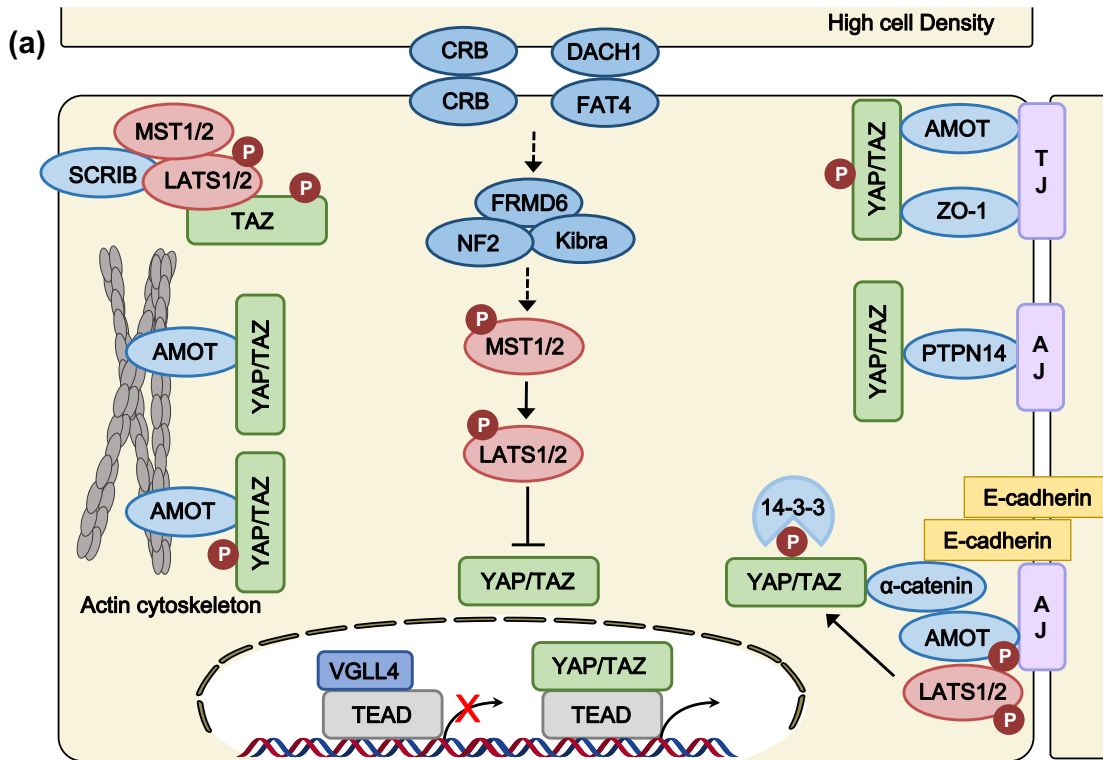


Fig. 2 Regulators and regulations of the Hippo pathway. Hippo pathway components in mammals are shown in various colors. Pointed arrows indicate activation, and blunt-ended lines indicate inhibition. Hippo cascade kinases are shown in red and inhibitory regulators of YAP/TAZ activity are shown in blue. **a** Hippo pathway is regulated by cell polarity (Crumbs, DACH1–FAT4) and cell–cell junctions (adherens junction, tight junction). *AMOT* angiomin, *AJ* adherens junction, *CRB* Crumbs homolog, *DACH1* Dachous-1, *FRMD6* FERM domain-containing protein 6, *LATS* large tumor suppressor homolog, *MST* mammalian STE20-like protein kinase, *NF2* neurofibromin 2 (also known as Merlin), *PTPN14* protein tyrosine phosphatase, non-receptor type 14, *SCRIB* scribbled planar-cell polarity protein, *TAZ* transcriptional co-activator with PDZ-binding motif, *TJ* tight junction, *VGLL4* vestigial-like protein 4, *YAP* Yes-associated protein, *ZO* zona occludens protein. **b** Hippo pathway is regulated by extracellular ligands, stress responses, and mechanotransduction. *AMPK* 5' AMP-activated protein kinase, *APC* adenomatous polyposis coli, *β -TRCP* β -transducin repeat-containing E3 ubiquitin protein ligase, *ECM* extracellular matrix, *GPCR* G protein-coupled receptor, *Rho* Ras homolog gene family, *ROCK* Rho-associated protein kinase

TAZ activity [94–96]. First, various G protein-coupled receptors (GPCRs) and their associated heterotrimeric G protein signaling regulate Hippo pathway activity via Rho GTPases. Serum-borne lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) act as ligands for the $G\alpha_{12/13}$ -coupled receptor, and its downstream RhoA signaling to inhibit LATS1/2 [96]. Thrombin, thromboxane A₂, and Kaposi sarcoma-associated herpesvirus also activate YAP via Rho GTPases. Similarly, $G\alpha_{q/11}$ -coupled GPCR ligands such as endothelin-1 and estrogen can activate YAP/TAZ via Rho GTPases [97, 98]. Moreover, constitutively active YAP/TAZ resulting from $G\alpha_{q/11}$ mutations is oncogenic drivers in patients with uveal melanoma [99, 100]. In contrast, upon stimulation of the $G\alpha_s$ -coupled receptor, activation of PKA by cAMP inhibits Rho GTPases, which in turn activates LATS kinase activity [99, 101, 102]. Rho GTPases link ligand stimuli to the Hippo pathway through F-actin formation. Both in *Drosophila* and mammalian cells, modulating actin polymerization by Rho GTPases and actin-capping proteins affect YAP/TAZ and Yorkie activities [103, 104]. However, how the actin cytoskeleton controls the core Hippo kinase activity requires further investigation.

Wnt signaling has been intensively studied as a YAP/TAZ upstream regulator. YAP/TAZ interacts with β -catenin, which is a key transcriptional co-activator in canonical Wnt signaling. Imajo et al. found that YAP inhibits Wnt signaling by sequestering β -catenin in the cytoplasm [105]. Piccolo and colleagues identified that YAP and TAZ are essential components of the β -catenin destruction complex [106, 107]. In addition, alternative Wnt signaling by Wnt ligands, including Wnt3 and Wnt5a/b, activates YAP/TAZ through the $G\alpha_{12/13}$ -Rho–LATS signaling pathway by interacting with the Frizzled/ROR receptors [40]. Amino acids regulate YAP/TAZ activity through the TSC–mTORC1 pathway.

TSC1/2 deficiency potentiates YAP through mTORC1-mediated inhibition of autophagosome degradation [108]. YAP in turn activates mTORC1. YAP-induced miR-29 downregulates PTEN, which in turn activates the PI3K–mTOR pathway [109]. YAP/TAZ also promotes mTORC1 activation via a TEAD-induced LAT1 amino acid transporter under low nutrient conditions [110]. In *Drosophila*, positive cross-talk between Yki and insulin/Tor pathway also suggested coordinated regulation of these two oncogenic pathways in development and cancer [111].

MAPK, including JNK and p38, link stress responses to the Hippo pathway. During wound healing and tumor growth, JNK increases Yki and YAP activity by promoting the binding of Ajuba family proteins to Warts and LATS [112]. When activated by UV irradiation, JNK and p38 MAPK phosphorylate and potentiate YAP [113, 114]. Moreover, p38 promotes TEAD cytoplasmic translocation and suppresses YAP-driven cancer cell growth upon various cellular stress signals [115]. NLK also phosphorylates YAP on Ser128 during osmotic stress and disrupts 14-3-3 interactions, thereby promoting nuclear accumulation of YAP [116, 117]. Stress caused by cytokinesis failure has been shown to activate LATS2, which in turn inactivates YAP/TAZ and stabilizes p53 [118]. Hippo pathway activation by cellular stresses is considered an important tumor suppression mechanism.

Mechanotransduction and the Hippo pathway

Organ and cell growth are constantly subjected to mechanical stresses, extra cellular matrix (ECM) stiffness, cell adhesion, and cell–matrix interaction. For instance, cell–cell contact at high density results in a growth inhibitory signal, which is an important cue for Hippo pathway activation [31, 32, 119]. Recently, YAP/TAZ has emerged as critical sensors of mechanotransduction, demonstrating that ECM stiffness, cell shape, or tension of cells controls YAP/TAZ activity and localization in the context of stem cell differentiation and tumorigenesis [95, 120, 121] (Fig. 2). Small adhesive areas or soft ECM conditions retain YAP/TAZ in the cytoplasm. Conversely, large adhesive areas or a stiffer ECM induces nuclear localization of YAP/TAZ and enhances TEAD transcriptional activity [95]. Wada et al. showed that stress fiber-dependent F-actin signaling suppresses YAP phosphorylation and induces YAP nuclear translocation [121]. Consistent findings showed that F-actin formation induced a strong overgrowth phenotype in *Drosophila* imaginal discs by inhibiting the Hippo pathway [104]. Aragona et al. showed that F-actin-capping/severing proteins Cofilin, CapZ, and Gelsolin inhibit YAP/TAZ transcriptional activity during cell contact inhibition by sensing mechanical forces [122]. Mechanical regulation promotes LIMD1–LATS1 binding through activation of c-Jun N-terminal kinase

(JNK), which in turn suppresses LATS activity, leading to YAP transcriptional activity [123].

The Hippo pathway in cancer biology

The dramatic overgrowth observed upon Hippo pathway dysregulation has led to the investigation of its role in cancer development. Numerous reports indicate that hyperactivation of YAP/TAZ or TEAD confers proliferative advantage, promotes cell invasion and migration, enhances cancer stem cell traits, and elicits metastasis and drug resistance [124, 125]. One of the early studies was performed in mouse liver. Liver-specific induction of YAP, or depletion of upstream Hippo components, caused aberrant tissue expansion and a dramatic increase in liver size, which subsequently developed into liver tumors [19, 126]. Deletion of Mst1/2 or SAV1 in the mouse intestinal epithelium also resulted in crypt hyperplasia and tumorigenesis through hyperactivation of YAP [127–129]. Moreover, loss of the APC tumor suppressor in colon cancer led to YAP/TAZ activation [106, 130]. YAP also promotes resistance to anticancer drugs targeting RAF and MEK in tumor cells harboring BRAF, KRAS, and NRAS mutations, which is a major clinical challenge [131]. Indeed, the protein levels and nuclear localization of YAP/TAZ and TEAD are elevated in many human cancers, such as lung carcinoma, thyroid, ovarian, colorectal, prostate, pancreatic, esophageal, liver, and breast cancer [125, 132]. Higher YAP/TAZ protein levels positively correlate with poorly differentiated tumors and are associated with shorter patient overall survival [125]. In addition to mammals, the Hippo pathway in *Drosophila* also plays an important role in tumorigenesis. Nutritional cues activate salt-inducible kinase (SIK), which inhibits the Hippo pathway and activates Yorkie in Ras/Src-induced tumorigenesis [133]. In addition, Hippo pathway inhibition by mitochondrial dysfunction has been shown to drive non-autonomous tumor progression via JNK/Ras signaling [134]. Therefore, the conserved components and tumorigenic phenotypes in various genetic models, as well as in humans, underscore the role of the Hippo pathway in cancer biology.

The Hippo pathway in cancer metabolism

Cancer cells utilize different metabolism from that of normal tissues, because they require ample amount of cellular building blocks, such as glucose, amino acids, and fatty acids for their rapid and unrestricted proliferation. Importantly, Warburg showed that under aerobic conditions, cancer cells metabolize a significantly higher amount of glucose into lactate than normal tissues do a phenomenon called the Warburg effect [135]. In addition to altered glucose metabolism or glycolysis, cancer-associated metabolic changes in amino acid uptake, increased demand for nitrogen via glutamine,

and the use of intermediate metabolites for biosynthesis are reported [136]. Recent studies highlighted the role of the Hippo pathway in several aspects of these altered metabolisms in cancer cells.

Glucose metabolism and the cellular energy level have emerged as critical upstream regulators of the Hippo pathway. The AMP-activated protein kinase (AMPK), a major energy sensor, directly phosphorylates angiomin-like protein 1 (AMOTL1) at Ser 793, which in turn stabilizes AMOTL1 to inhibit YAP activity via the Hippo pathway [137]. Two independent groups explored whether AMPK directly phosphorylates YAP on multiple residues, including S94, and should that these additional phosphorylations diminished the YAP–TEAD interaction and YAP-driven cancer cell growth [138, 139]. Moreover, YAP increases glucose uptake in cancer cells by increasing the transcription of mRNA encoding the GLUT3 transporter [139]. Phosphofructokinase (PFK1), which catalyzes the first rate-limiting step of glycolysis, interacts with TEAD in the nucleus to promote the YAP–TEAD interaction and transcription activity [140]. In *Drosophila*, liver kinase B1 (LKB1) directly activates AMPK, which further attenuates Yki activity [141]. In addition, SIKs sense nutrients such as glucose to activate Yki during tumorigenesis [133, 142]. Two studies also indicate that glucose stimulates O-GlcNAcylation of YAP via O-GlcNAc transferase (OGT) at residues S109 and T241, which is critical for high glucose-induced tumorigenesis [143, 144]. Collectively, these studies suggest that glucose metabolism is a critical regulator of YAP transcriptional activity in cancer cells.

The mevalonate pathway or the cholesterol synthesis pathway activates YAP/TAZ via Rho GTPases. Inhibition of this pathway by statins and bisphosphonates suppresses YAP nuclear localization and YAP-driven tumor growth [145, 146]. Reliance on Glutamine is considered as another hallmark of cancer cell metabolism. YAP can directly enhance glutamine synthase (GLUL) expression to elevate intracellular glutamine levels and stimulate nucleotide biosynthesis, which is required for liver growth and tumorigenesis [147]. Thus, further understanding of the role of the Hippo pathway in both normal and cancer cell metabolism will lead to novel therapeutic strategies to treat cancer.

The Hippo pathway in cancer immunity

Immunotherapy provides novel therapeutic approaches for clinical oncology, and recently, several studies have demonstrated the importance of Hippo signaling in host immune responses and the tumor microenvironment. YAP activation in tumor-initiating cells (TICs) recruits M2 macrophages via TEAD-dependent Ccl2 and Csf1 expression, which protects the TIC from immune clearance, and thus enhances tumor growth [148]. By contrast, YAP activation

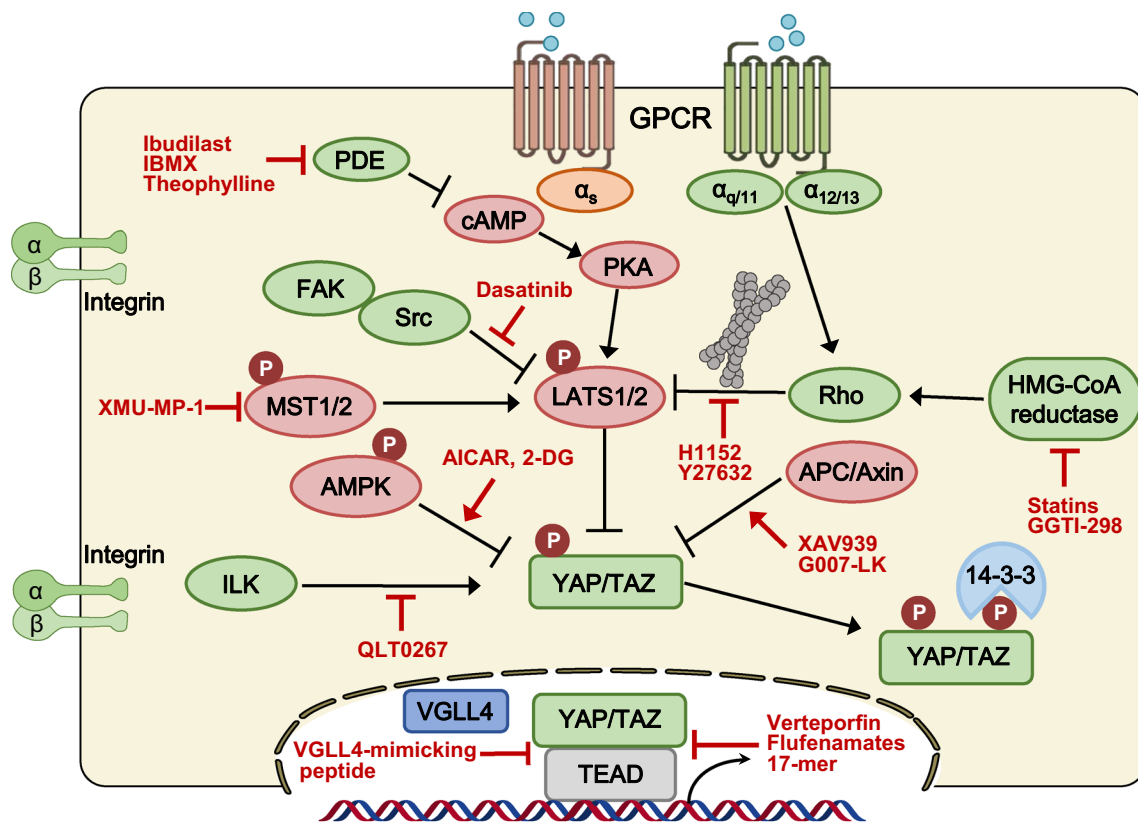


Fig. 3 Putative targets for therapeutic intervention in the Hippo pathway. Summary of the positive and negative regulators of Hippo kinases and YAP/TAZ, respectively. Activating components' upstream of YAP/TAZ is in green, and inhibitory components are in red

in cancer cells promotes myeloid-derived suppressor cell (MDSC) recruitment by inducing TEAD-dependent CXCL5 expression, thus suppressing tumor progression [149]. Further investigation may reveal how YAP-induced secreted ligands trigger context-dependent effects in the tumor microenvironment and immune cell infiltration. Similarly, YAP activation by LATS1/2 deletion in cancer cells triggers an anti-tumor immune response via activation of the TLR–MYD88/TRIF pathway by secreting nucleic-acid-rich extracellular vesicles (EVs) [150, 151]. Recent studies indicate that these nucleic-acid-rich EVs might also trigger an antiviral immune response in neighboring cells via the Hippo pathway. Independent studies demonstrate that MST1 and YAP/TAZ are critical regulators of the cytosolic RNA/DNA, sensing immune response by inhibiting TBK1 activity [152, 153]. Notably, YAP/TAZ has recently been shown to directly regulate PD-L1 expression in lung cancer cells. In EGFR–TKI-resistant lung cancer cells, YAP induces the transcription of PD-L1 [154]. In addition, TAZ activation is responsible for lactate-rich tumor microenvironment-induced PD-L1 expression in lung cancer cells, which led to apoptosis of T cells in vitro [155]. Therefore, the Hippo

pathway may provide new avenues for the development of immunotherapy targeting the PD1/PD-L1 pathway.

Next, in immune cells, the Hippo pathway plays a role in tumor immunity. Liu et al. reported that Gram-positive bacteria activate Hippo–Yki signaling through a Toll–Myd88–Pelle/Cha cascade in the *Drosophila* immune organ [150]. Several reports demonstrate that the Hippo pathway plays pivotal roles in T-cell differentiation and inflammation. Activation of CD8⁺ T cells induces the expression of core Hippo pathway components, including YAP and TEAD, through CTLA-4, which regulates T-cell terminal differentiation [156]. The Hippo pathway is also involved in T_H17 cell differentiation, which plays an important role in cancer immunity. Geng et al. showed that the TAZ/ROR γ t transcription complex is required for T_H17 differentiation and T_H17 cell-mediated inflammatory diseases, whereas the TAZ/TEAD complex promotes T_{reg}-cell differentiation [157]. The core Hippo kinase Mst1-null mice exhibit a low number of mature T cells by inhibiting naïve T-cell proliferation [158]. MST1 in dendritic cells inhibits T_H17-cell differentiation and regulates autoimmune diseases via p38 MAPK signaling [159]. Therefore, the status of the

Hippo pathway may be a critical factor in both innate and adaptive immune responses in the tumor microenvironment. Interestingly, unique to the hematopoietic system, YAP/TAZ expression is restricted to the hematopoietic stem cell (HSC) fraction, whereas upstream Hippo components are expressed at high levels across all fractions, including mature and lineage positive cells [160]. Further investigation may delineate the critical functions of YAP/TAZ and the Hippo pathway in immune cells and their role in cancer immunity.

Therapeutics targeting the Hippo pathway

The core Hippo pathway functions as a tumor suppressor pathway by inhibiting YAP/TAZ-dependent tumor growth. Therefore, current therapeutic approaches are mostly focused on inactivating YAP/TAZ oncogenic activity, but also negative upstream regulators of the pathway. Here, we focus on small molecules that are designed to target Hippo pathway components themselves, as well as various upstream regulators.

Targeting protein–protein interactions (PPIs)

Recent studies demonstrated that disrupting the formation of the YAP/TAZ–TEAD complex might be a potential strategy to develop anticancer therapeutics. In 2002, a screening of FDA-approved drugs by Pan and colleagues led to the identification of verteporfin (VP) and related porphyrin compounds as inhibitors of YAP–TEAD interactions [161] (Fig. 3). Verteporfin is a small molecule that inhibits YAP-induced tumor progression and suppresses liver overgrowth in mice, thereby demonstrating the therapeutic significance of interrupting YAP–TEAD interactions in cancer cells [99, 100, 161]. However, verteporfin has been reported to be toxic in non-malignant cells and has poor solubility, which may limit its specificity and bioavailability for cancer patients [162].

Vestigial-like family member 4 (VGLL4) is a transcriptional regulator that has two Tondu (TDU) domains. VGLL4 was initially reported to have interacted with TEAD1 and suppresses TEAD1-dependent α 1 adrenergic activation in cardiac myocytes [163]. Recent reports identified VGLL4 as an antagonist of YAP/TAZ–TEAD transcriptional activity by directly interacting with TEADs via its TDU domains. VGLL4 is downregulated in gastric, lung, and colorectal cancer, and is considered as a potential tumor suppressor and prognostic marker [34, 35, 164]. In addition, based on the structure of the VGLL4–TEAD interacting domain, Jiao and colleagues developed a new therapeutic strategy against YAP-driven human cancers: a VGLL4-mimicking inhibitor peptide termed “Super-TDU,” which disrupts

the YAP–TEAD interaction [35]. In addition, Jiao et al. reported that VGLL4 targets the TEAD4–TCF4 complex to interfere with the functional interplay between TEAD4 and TCF4 [155]. Similarly, cyclic YAP-like peptide (17mer) was designed to disrupt the YAP–TEAD interaction [165]. Flufenamic acid, a non-steroidal anti-inflammatory drug (NSAID), binds to the YAP-binding domain of TEADs and inhibits YAP–TEAD-dependent transcription and cell proliferation [166]. Interestingly, the structure of the TEAD–flufenamic complex showed that the flufenamic acid-contacting residue (C380 of TEAD2) is palmitoylated. Two studies indicated that palmitoylation of TEAD at evolutionarily conserved cysteine residues is required for TEAD stability and the YAP/TAZ interaction [167, 168]. Whether flufenamic acid affects TEAD palmitoylation requires further investigation. Mo and colleagues reported that AMP-activated protein kinase (AMPK) directly phosphorylates YAP at residue S94 and disrupts the YAP–TEAD interaction. 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) is a cell-permeable activator of AMPK that inhibits YAP activity [138]. Src family kinase inhibitor, Dasatinib, inhibits the kinase activity of YES, which disrupts the YAP– β -catenin–TBX5 complex, and thus attenuates cancer progression in both cell lines and animal models [169].

Targeting upstream components of YAP/TAZ

The Hippo pathway receives a wide range of upstream signals that lead to the regulation of YAP/TAZ. Thus, the primary targets for small-molecule therapeutics are modulators of YAP/TAZ cellular localization and activity.

Recent reports underscore the metabolic regulation of YAP/TAZ activity by the mevalonate pathway and energy stress. Inhibitors of the mevalonate pathway, such as statins, HMG–CoA reductase inhibitors, as well as the geranylgeranyl transferase inhibitor, GGTI-298, suppress YAP/TAZ activity via Rho GTPase inhibition [145, 146, 170] (Fig. 3). In addition, energy stress conditions inhibit YAP activity through AMPK and LATS kinase activity. AMPK directly suppresses the YAP–TEAD interaction, as mentioned above; however, it also promotes YAP cytoplasmic retention by activating Hippo kinase LATS1/2 [138, 139]. In addition, AMPK phosphorylates AMOTL1, which then facilitates YAP phosphorylation by LATS [137]. Thus, targeting the components of metabolic signaling might efficiently regulate YAP/TAZ activity.

Next, targeting GPCRs and their downstream effectors are promising approaches to modulate YAP/TAZ activity. Recent studies indicated that YAP/TAZ is activated through $G\alpha_{12/13}$ or $G\alpha_{q/11}$ -coupled GPCR ligands by Rho GTPases [40, 96, 171]. Hippo pathway activity is strongly affected by

actin dynamics through Rho GTPases and their downstream effector ROCK. Thus, ROCK inhibitors, such as fasudil and Y27632, may be potent inhibitors of YAP/TAZ. In contrast, activation of $G\alpha_s$ -coupled GPCR signaling by glucagon and epinephrine inhibits YAP/TAZ activity [96, 101, 102]. Similarly, phosphodiesterase (PDE) inhibitors elevate the cAMP level, which lead to LATS1/2 activation and suppression of YAP/TAZ [101]. Membrane proximal components are also targets for therapeutic approaches. Dasatinib, a Src family inhibitor, inhibits focal adhesion kinase (FAK)–Src–PI3K signaling, which in turn suppress YAP by promoting cytoplasmic retention [172].

Tankyrase inhibitors target YAP/TAZ by Hippo-independent mechanisms. XAV939, which is a small-molecule inhibitor of Wnt/ β -catenin signaling that acts through axin stabilization [173], not only decreases β -catenin stability, but also the TAZ protein level via a destruction complex [107]. In addition, a tankyrase inhibitor attenuated TEAD transcriptional activity by stabilizing angiomin and sequestering YAP in the cytoplasm [174, 175]. QLT0267, a small-molecule inhibitor targeting integrin-linked kinase (ILK), reduces tumor growth by inhibiting YAP/TAZ [176]. In addition, dobutamine, a sympathomimetic drug that antagonizes the β -adrenergic receptor, prevents YAP nuclear accumulation and YAP-dependent gene transcription [177] (Fig. 3). The core Hippo kinases are poorly druggable anticancer targets, because very few small-molecule kinase activators are available. By contrast, inhibition of the Hippo pathway may be valuable for wound healing and tissue regeneration. XMU-MP-1 has been identified as an MST1/2 inhibitor, which encourages multiple tissue repair and regeneration through YAP activation [178].

Conclusion/perspective

Hippo signaling has emerged as a pivotal regulatory pathway in mammalian development and tissue growth. Recent advances in research on the deregulation of Hippo pathway have attracted significant attention from researchers interested in all the stages of tumorigenesis from EMT, metastasis, and drug resistance to those involved in cancer metabolism and immunity. Accumulating evidence suggests that perturbation of YAP/TAZ activity has profound effects on various cancer types. Notably, since driver mutations of the core components of the Hippo pathway are relatively rare, and development of small-molecule activators of the Hippo kinases is difficult, it is important to identify a wide range of upstream regulators, as well as downstream targets, of Hippo–YAP signaling. In addition, identifying crosstalk with other oncogenic signal transduction pathways might provide valuable targets to modulate the Hippo pathway activity. Therefore, understanding the cellular determinants of the

Hippo pathway and its role in tumorigenesis and regeneration will be critical to classify cancers susceptible to YAP/TAZ modulation and to develop anticancer therapeutics.

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