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Review

Hippo signaling in stress response and homeostasis maintenance

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Abstract

Co-ordination of cell proliferation, differentiation, and apoptosis maintains tissue development and homeostasis under normal or stress conditions. Recently, the highly conserved Hippo signaling pathway, discovered in *Drosophila melanogaster* and mammalian system, has been implicated as a key regulator of organ size control. Importantly, emerging evidence suggests that Hippo pathway is involved in the responses to cellular stresses, including mechanic stress, DNA damage, and oxidative stress, to maintain homeostasis at the cellular and organic levels. The mutation or deregulation of the key components in the pathway will result in degenerative disorder, developmental defects, or tumorigenesis. The purpose of this review is to summarize the recent findings and discuss how Hippo pathway responds to cellular stress and regulates early development events, tissue homeostasis as well as tumorigenesis.

Key words: Hippo, stress response, homeostasis

Introduction

Cell proliferation, differentiation, and death have been extensively studied. However, how these processes cooperate together to maintain homeostasis under physiological and pathological conditions is poorly understood. The discovery of Hippo pathway may provide an important entry point to address this question.

Hippo pathway is a highly conserved signaling network that controls cell proliferation, differentiation, and cell death. It has been first defined in *Drosophila* by genetic mosaic screening that loss-of-function mutation of Hippo leads to a strong overgrowth phenotype [1]. Consistently, genetic inactivation of main components including Warts [2], Hippo [3], Salvador, and Mats [4,5] resulted in robust tissue overgrowth. As the major downstream effector of the Hippo pathway [6], Yorkie (Yki) functions as an oncogene and regulates gene transcription by interacting with the transcription factor Scalloped (Sd) [7]. Interestingly, the components of Hippo pathway are highly conserved in mammals. MST1/2 (Hpo orthologs), Sav1, LATS1/2 (Wts orthologs), and Mob1 (MOBKL1A and MOBKL1B, Mats orthologs) constitute a kinase cascade that phosphorylates YAP/TAZ (Yki orthologs) and promotes its binding with 14-3-3 and cytoplasmic retention. YAP/TAZ, in conjunction with TEAD1–4 (Sd orthologs), mediate major physiological functions of the Hippo pathway [8,9].

The Hippo pathway can be stimulated by multiple types of cellular stress, including mechanical stress, DNA damage, and reactive oxygen species (ROS) (**Fig. 1**). In this review, we will summarize recent advances in understanding how different cellular stress signals or stress stimuli regulate Hippo pathway, and in turn, how the Hippo pathway regulates tissue homeostasis under the cellular stresses.

Hippo Pathway and Stress Response

Mechanical stress

Biomechanic is recognized as an important regulator of development and pathological abnormalities. For instance, tumor growth and progression are slowed down in the soft microenvironment [10]. It is also known that mesenchymal stem cells differentiate into adipocytes on soft matrix whereas osteoblasts on stiff matrix [11]. Organs and cells are perpetually subjected to mechanical stresses, including stretching, strain, compression, and pressure arising from different stiffness of extracellular matrices. YAP/TAZ are identified as sensors and mediators of mechanical cues represented by the rigidity of the
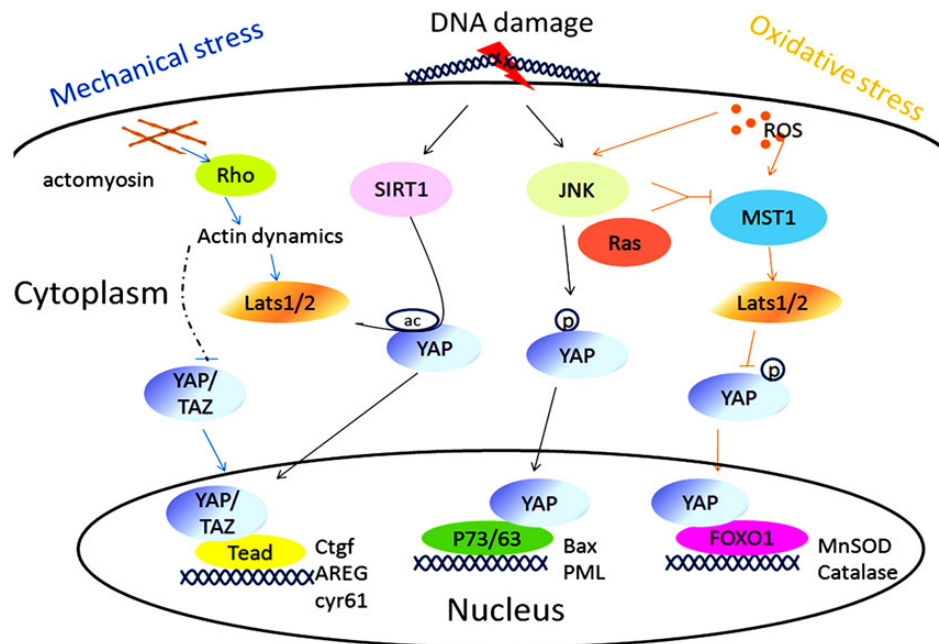
Figure 1. Stress response of Hippo pathway Mechanisms of Hippo pathway regulation by mechanical stress, DNA damage, and oxidative stress. Arrows or blunted ends indicate activation or inhibition, respectively. Dashed lines indicate unknown mechanisms.

extracellular matrix (ECM), cell geometry, cell density, and the status of the actin cytoskeleton [12,13].

Matrix stiffness not only controls the subcellular localization of YAP/TAZ but also modulates their expression. YAP/TAZ localize to the cytoplasm when cells are grown on a soft matrix, whereas when cells are grown on a stiff matrix, YAP/TAZ translocate to the nucleus and activate the transcription of proliferation-related genes. This regulation requires Rho GTPase activity and tension of the actomyosin cytoskeleton, but is independent of the Hippo/LATS cascade [12,14]. Hippo pathway is also regulated by G-protein-coupled receptor (GPCR) signaling. For example, lysophosphatidic acid stimulates Gα12/13-coupled receptor to induce YAP/TAZ activity by inhibiting LATS. In contrast, stimulation of Gs-coupled receptors by glucagon or epinephrine activates Lats1/2 kinase activity, thereby inhibiting YAP function. The Gα12/13-induced YAP/TAZ activation can be blocked by the F-actin disrupting agent, Latrunculin A, suggesting that GPCRs and RhoA act upstream of LATS to regulate YAP/TAZ [15]. Additionally, different substrate stiffness also alters the expression of YAP/TAZ in human trabecular meshwork cells [16,17]. Interestingly, remodeling of the ECM is partially dependent on the YAP, as the activation of YAP in cancer-associated fibroblasts enhances matrix stiffening through promoting an extensive deposition of collagen [18].

Changes in ECM stiffness also affect cell spreading [11,19]. Cell morphology is another important factor in the regulation of the Hippo pathway. It is known that YAP/TAZ sense the changes of cell geometry during cell proliferation regulation [20,21]. YAP/TAZ are localized in the cytoplasm when a cell is plated on the small adhesive micro-patterned surface, while the cell on a large adhesive micro-patterned surface will have epithelial cell-like geometry with active YAP/TAZ localized in the nucleus [12,22]. Cell morphology and F-actin regulated phosphorylation of YAP, and the effects of F-actin were suppressed by modulation of LATS [23].

Tensile forces are also well known to be involved in the regulation of YAP/TAZ activity. Myosin motor proteins together with actin filaments generate contractile forces and tension inside of cells, which regulates the activity of YAP/TAZ to modulate the proliferation status of cells [12,14]. Taken together, Hippo signaling plays a vital role in the cellular adaption to the extracellular environments. However, there is still lack of *in vivo* evidence that mechanical stress affects Hippo signaling.

Oxidative stress

Recently, multiple lines of evidence link Hippo pathway with oxidative stress or ROS-initiated signaling pathway and various pathological processes. MST1 is the first studied Hippo pathway component that exerts vital effect on ROS-induced cell death and ROS defense [24]. We first reported that MST1 was activated upon oxidative stress and the activated MST1 kinase phosphorylated FOXO3 and enhanced FOXO3-mediated Bim expression, leading to neuronal cell death. Conservatively, Cst-1 (MST1 ortholog in worm) also regulates lifespan through daf-16 (FOXO ortholog)-mediated gene transcription in *C. elegans* [24]. Our group also reported that MST1 is phosphorylated by protein kinase c-Abl at Tyr433 and increases its interaction with FOXO3 and subsequently phosphorylates FOXO3 to initiate oxidative stress-induced neuronal cell death [25,26]. Accordingly, in cancer cells, MST1 was reported to mediate cisplatin-induced cell death. Upon cisplatin treatment, peroxiredoxin-I, a ROS responsive protein, is specifically associated with MST1 and subsequently induces apoptosis in U2OS cells [27].

YAP, the major Hippo downstream target, also mediated ROS-triggered signaling. In murine hearts, YAP over-expression was reported to protect cardiomyocytes against H_2O_2-induced cell death [28]. In cardiomyocytes, YAP functions as a transcriptional co-activator of FOXO1 that leads to the up-regulation of antioxidant genes, such as catalase and MnSOD [29]. Recently, Zhou's group reported that the transcriptional level of YAP is regulated by GABP (an Ets family member) in liver and YAP expression is reduced when

GABP is inactivated by oxidative stress, indicating that the reduced expression of YAP accounts for the weak oxidative stress defense [30]. Controversially, the protein levels of YAP in glioma cells are elevated whereas phosphorylation levels are reduced when treated with Chaetocin, a histone methyltransferase inhibitor that is known to induce ROS generation. In addition, the activated YAP coordinates with p73 and p300/CBP to induce apoptosis in glioma cells [31].

Mitochondria are the major source and target of ROS among the subcellular organelles. In *Drosophila*, over-expression of Yki up-regulates the transcription of genes such as opa1-like (opa1) and mitochondria assembly regulatory factor (Marf), leading to mitochondrial fusion and reduced ROS levels [32]. However, other than apoptosis induction, moderate ROS levels can promote tumor progression *in vivo*. In *Drosophila* imaginal epithelium, the combination of Ras activation and mitochondrial dysfunction stimulates ROS production and subsequently inactivates Hippo pathway in JNK-dependent manner, as well as drives non-autonomous tumor progression [33]. Taken together, key components of the Hippo pathway participate in ROS-mediated cell death or ROS scavenge in a variety of species and organs as well as the subcellular organelles.

DNA damage

The genomic DNA is constantly exposed to various genotoxic insults, including UV radiation and oxidative stress. Prevention and repair of DNA damage are critical for the maintenance of genomic integrity and cell survival. The latest work in hematological cancer reveals the role of Hippo pathway in DNA damage-induced apoptosis. Unlike epithelial cancer in which YAP is amplified and functions as an oncogene, the multiple myeloma has been found to contain a specific deletion in YAP gene locus with DNA damage. Re-expression of YAP induces apoptosis and reduces proliferation, which is mediated by stabilization of p73 and increased expression of its pro-apoptotic downstream targets. In addition, MST1 inactivation increased YAP protein levels and induced a robust apoptotic response [34]. Consistently, p73 is stabilized by YAP protein in HCT116 and H1299 cells upon cisplatin-induced DNA damage [35]. DNA damage also stabilizes YAP protein through PML-mediated sumoylation and ubiquitination, which reinforces YAP's transcriptional co-activation and induces p73-dependent apoptosis [36].

In contrast, cisplatin induces SIRT1-mediated deacetylation of YAP, which promotes the nuclear localization and transcriptional activation as well as drug resistance in hepatocarcinoma [37]. Accordingly, down-regulation of MST1 by Hsp70 mediates cisplatin resistance in prostate cancer cells [38]. In addition to chemotherapeutic drug resistance, YAP activation also renders cancer cells resistant to UV or gamma-radiation-induced apoptosis. It has been reported that YAP enables cells to enter mitosis with un-repaired DNA through driving insulin-like growth factor-2 (IGF-2) expression and Akt activation in response to radiation [39]. It has also been reported that YAP protects keratinocytes from UV irradiation by binding and stabilizing the pro-proliferative DNp63a isoform in a JNK-dependent manner [40]. Therefore, the biological outcomes of YAP activation in response to DNA damage are dependent on the downstream effectors, such as p73, p63, and other transcription factors. These conflict results suggest that Hippo pathway is a double-edged sword in response to DNA damage and might exert a tight control on DNA damage response in different cell contexts.

Hippo Signaling and Homeostasis

Cellular homeostasis is a process that cell adapts itself to a number of environmental factors, including pH, membrane potential, nutrients, oxygen, and ROS. In terms of tissue or whole organ, the coordination among cell proliferation, differentiation, and death is essential for homeostasis under physiological and pathological conditions. During the process of development, cell proliferation is required for growing organ and body size; meanwhile, proper cell fate determination will ensure the appropriate function of tissue and organs. When the balance is disrupted (as a result of external perturbations and insults), the body engages in a stress response that aims to restore homeostasis at different levels (systemic, tissue, and cellular) [41]. Recently, emerging evidence showed that Hippo pathway plays an important role in the homeostasis maintenance through regulating the cell proliferation, progenitor renewal and differentiation, and stress-induced cell apoptosis. The current knowledge of the *in vivo* roles of Hippo pathway is briefly discussed below.

Nervous system

In nervous system, YAP and TEAD play critical roles in regulating neural progenitor cell number by affecting proliferation, fate choice, and cell survival. In *Drosophila melanogaster*, Hippo and Warts are required for the maintenance of *Drosophila* sensory neuron dendrites incooperation with polycomb proteins [42]. During chick neural tube development, gain of function of YAP and TEAD results in a marked expansion of the neural progenitor through inducing cyclin D1 transcription and a decreased differentiation by suppressing NeuroM expression. Consistently, loss of YAP and TEAD leads to the increased apoptosis and premature neuronal differentiation [43]. In *Xenopus*, YAP is required for the expansion of Sox2-positive neural plate progenitors and Pax3-positive neural crest progenitors at the neural plate border and for maintaining them in an undifferentiated state [44].

Consistent with the result in chick, YAP modulates the proliferation of mouse neural progenitor cell through transcriptional regulation of cyclin D1. Our group showed that BMP2 induces Smad1/4 activation that competes with YAP for the interaction with TAED1 and inhibits YAP's cotranscriptional activity during neural progenitor division [45]. Cappello *et al.* [46] reported that knockdown of FAT4 or DCHS1 promoted neural progenitor cell proliferation and malpositioning of cells in the development of cerebral cortex by decreasing the phosphorylation of YAP, which revealed a novel upstream signaling of YAP/TAZ during brain development. Moreover, mechanistic studies revealed that motor neuron differentiation of human pluripotent stem cells (hPSCs) was regulated by stiffness-dependent Hippo/YAP activities [47]. In sum, YAP/TEAD are critical for neuronal progenitor cell proliferation and development [48].

Heart

Hippo/YAP pathway was found to play essential roles in the regulation of heart development and postnatal cardiomyocyte regeneration and apoptosis. In accordance with that Hippo pathway suppresses proliferation in cancer cells, Heallen *et al.* [49] found that inactivation of Mst1/2, Sav1, and Lats2 resulted in enlarged embryo hearts due to increased cardiomyocyte proliferation. Consistently, cardiac-specific over-expression of MST1 in transgenic mice increased caridomyocytic apoptosis and led to dilated cardiomyopathy [50], whereas over-expression of a dominant-negative MST1 prevented myocardial infarction (MI)-induced myocyte apoptosis, fibrosis, and preserved systolic contraction [50,51]. Similar to MST1, LATS2 over-expression led to heart dysfunction and reduced heart size in mice at 5 months of age. Inhibition of endogenous LATS2 by over-expression of its

dominant negative form resulted in heart hypertrophy both at baseline and under pressure overload [52].

As the major target of Hippo pathway, the role of YAP in the development of cardiomyocyte diseases has recently been extensively studied. Fetal YAP inactivation caused marked, lethal myocardial hypoplasia and decreased cardiomyocyte proliferation, whereas fetal activation of YAP stimulated cardiomyocyte proliferation [53]. Three independent studies showed that cardiomyocyte-specific inactivation of YAP caused myocyte apoptosis, dilated cardiomyopathy and premature death. Conversely, forced expression of a constitutively active form of YAP in the adult heart stimulates cardiac regeneration and improves contractility after MI [28,54,55]. In addition, Wang *et al.* [56] found that cardiomyocyte-specific transgene of human YAP induced cardiac hypertrophy and increased fetal gene expression in the heart of 3 months old mice. In contrast, von Gise *et al.* [53] reported that YAP1 stimulates heart growth through promoting cardiomyocyte proliferation but not hypertrophy in postnatal cardiomyocytes. The mechanistic controversy might be resulted from different techniques. Wang *et al.* used YAP transgenic mice, whereas von Gise *et al.* used retro-orbital delivery of Ad:Tnnt2-Cre to achieve YAP conditional knockout. These findings suggest that YAP is critical for heart homeostasis.

Ongoing studies will explain how YAP regulates heart growth and cardiomyocyte proliferation. Hippo/YAP directly interact with β-catenin on Sox2 and Snai2 genes. YAP could also increase the abundance of β-catenin by activating IGF signaling which led to the inactivation of GSK3β during embryonic cardiomyocyte proliferation [57]. Additionally, microarray data indicated that YAP transcriptionally regulates the expression of numerous cell cycle-related genes, which contributes to the function of YAP in cardiomyocyte regeneration [54,55]. Modulating YAP activity might therefore provide a novel therapeutic strategy for heart diseases.

Liver

Liver is the extensively studied organ in mammals to investigate the role of Hippo pathway. In YAP transgenic mice, the liver size is nearly three times bigger than normal [58]. When mice lack the Hippo pathway upstream regulators (Mst1/2 [59], Nf2 [60], WW45 [61], and Sav1 [59]), YAP showed reduced phosphorylation level and increased nuclear localization. Histological and biochemical analysis showed that the expanded liver size is mainly due to increased cell number rather than cell size [58]. Mechanistically, YAP binds to TEAD family members to initiate the transcription of many target genes such as mitotic kinases, cell-division-associated proteins, and DNA replication proteins [62]. In mice, activated YAP promotes the proliferation of oval cells, a progenitor population of livers, which could differentiate into hepatocytes and biliary cells.

During liver regeneration, Hippo pathway also functions as a downstream target of integrin-linked kinase (ILK), which mediates the transmission of ECM signaling to cells and maintains the normal liver size. When ILK is mutated during partial hepatectomy, YAP is activated and promotes hepatocyte proliferation [63].

Recently, a research by Camargo's group showed that YAP's activation in hepatocytes leads to a progenitor-phenotype clonal outgrowth. Under normal conditions, YAP is inhibited by Hippo pathway to maintain the hepatocyte status [61]. However, when Hippo/MST are inactivated or YAP is over-expressed under pathological conditions, Notch signaling associated genes are transcriptionally upregulated, which promotes the transition of normal hepatocytes to hepatic progenitors [64]. The research linked Hippo pathway to the phenotypic plasticity in mature hepatocytes, which might be implicated in the treatment of liver diseases by targeting Hippo pathway.

Skin

The balance between proliferation and differentiation of progenitors controls the development and homeostasis of the epidermis. Zhang *et al.* [65] demonstrated that YAP over-expression causes hair placodes to evaginate into epidermis rather than invaginate into dermis. YAP also expands basal epidermal progenitors, promotes proliferation, and inhibits terminal differentiation. The phenomenon is supported by the observation that over-expression of a C-terminally truncated YAP mutant in the basal epidermis of transgenic mice caused marked expansion of epidermal stem/progenitor cell populations [66]. Similarly, the long-term effect of YAP activation leads to extensive proliferation of basal progenitors and results in squamous cell carcinoma-like tumors [67]. As an upstream regulator of Hippo pathway, WW45 deletion caused defects in terminal differentiation of epithelial progenitor cells [68]. Surprisingly, mice with skin-specific deletion of Mst1/Mst2 or Lats1/2 displayed no abnormalities in mice up to 5 months of age, suggesting that YAP might be regulated by alternative signals in keratinocytes that might be independent of the canonical Hippo pathway kinases [67].

Lung

The impact of Hippo pathway on lung development was first studied in TAZ-deficient mice. TAZ-deficient mice showed abnormal alveolarization during lung development and airspace enlargement mimicking emphysema in adult mice [69]. Yet early in 2004, TAZ was found to interact with thyroid transcription factor-1 to activate the expression of surfactant protein C [70]. Mice lacking Mst1/2 in the respiratory epithelium exhibited perinatal mortality with respiratory failure [71].

Kidney

In addition to lung developmental defect, TAZ knockout mice show renal cysts that are similar to the human polycystic kidney disease [72–74]. Consistently, mice with conditional knockout of YAP in kidney have reduced nephrogenesis and defective morphogenesis, in which YAP activity is needed for proper expression of a group of genes that control cell signaling and cell structure [75]. Ablation of Cdc42, a Rho GTPase, led to a reduced YAP-dependent gene expression and a defective nephrogenesis through decreasing nuclear localization of YAP. Thus, YAP responds to Cdc42-dependent signals in nephron progenitor cells to activate a genetic program required to shape the functional nephron [75].

Hippo Signaling in Human Cancers

Given the essential roles of Hippo pathway in controlling cell proliferation, stress response, and organ size, deregulated Hippo signaling including inactivation of the upstream kinase or hyperactivation of YAP/TAZ causes the destruction of tissue homeostasis and eventually tumorigenesis. Hereditary or sporadic inactivating mutations in neurofibromatosis tumor suppressor NF2 (Merlin) promotes brain tumor development [76]. Other than NF2, DNA mutations in the components of the Hippo pathway are rare in human cancers. However, deletions of WW45 were identified in two renal cancer cell lines [77]. Loss-of-function mutation of Mats1 was found in human skin melanoma and mouse mammary gland carcinoma [5]. Besides the mutation of coding sequence, nonmutational epigenetic modification is another prevalent mechanism silencing Hippo pathway. Promoter methylation of Mst1 was detected in soft tissue sarcomas [78], and hypermethylation of Lats1/2 in astrocytoma and breast cancers has also

Table 1. Studies of Hippo pathway in tumorigenesis in the mouse model

Mouse model	Tumor type	Time of tumor initiation	Reference
Nf2 heterozygous null	Osteosarcoma	10–30 months	[87]
	Hepatoceluar carcinoma	10–30 months	[87]
	Fibrosarcoma	10–30 months	[87]
	Malignant mesotheliomas	20 months	[88]
Conditional Nf2 null	Schwannomas	10 months	[89]
	Meningioma	14 months	[90]
	Renal cell carcinoma	3 months	[91]
	Hepatocellularcarcinoma	7.5 months	[60,92]
	Cholangiocarcinoma	7.5 months	[60]
	Bile duct hamartoma	1 month	[92]
Mst1 null	Lethal histiocytic sarcomas	18–24 months	[93]
Mst2 null	Mammary tumor	18–24 months	[93]
Mst1/2 double null	Hepatocellular carcinomas	15 months	[59,93,94]
	Cholangiocarcinoma	6 months	[93,94]
	Colonic adenoma	3 months	[95]
	T-cell acute lymphoblastic leukaemia	N.D.	[96]
Conditional Sav1 null	Hepatocellular carcinoma	13–14 months	[59,61]
	Cholangiocarcinoma	13–14 months	[59,61]
Mob1a/1b heterozygous null	Skin tumors	17 months	[97]
	Osteosarcomas	17 months	
	Fibrosarcoma	17 months	
	Breast cancers	17 months	
	Lung cancers	17 months	
	Salivary gland cancers	17 months	
	Liver cancers	17 months	
Lats1 null	Soft-tissue sarcoma	N.D.	[98]
	Ovarian stromal cell tumors	N.D.	
Yap over-expression	Hepatocellular carcinoma	3 months	[85,99]
	Intestinal dysplasia	N.D.	[58]
	Penetrant ductal metaplasia	N.D.	
	Squamous cell carcinoma-like tumors	20 days after grafting	[67]

been reported [79,80]. Different with the upstream kinases, gene amplification is common for YAP and TAZ. Amplification of the YAP gene locus has been reported in a wide range of human cancers, including oral squamous-cell carcinomas, medulloblastomas carcinomas of lung, pancreas, esophagus, liver, and mammary gland [81–85]. TEAD4 has also been found to be amplified in various cancers [86]. In line with the mutation or amplification of Hippo pathway components in human cancers, the mouse models with inactivation of the upstream kinase or over-expression of YAP/TAZ display spontaneous tumors (**Table 1**).

Conclusions and Perspectives

Hippo pathway plays key roles in organ size control, regeneration, and cancer development. The mutation or deregulation of key components in the pathway will result in developmental defects, degenerative disorder, or tumorigenesis. The Hippo pathway has gained significant attention in the past few years owing to its broad importance in animal development.

Although a large amount of studies have uncovered the signaling transduction and function of Hippo pathway, many questions still remain to be answered. For example, whether Hippo pathway responds to other stresses such as ER stress and, metabolic stress. Why Hippo pathway shows a different response to DNA damage in different cell context? Does Hippo pathway participate in glucose homeostasis, such as diabetes and hypoglycemia? Since Hippo pathway is critical

for tumorigenesis, what is the role of Hippo pathway in angiogenesis within the solid tumors? In the network of proliferation, differentiation, and cell death, how Hippo pathway coordinates with the other signals? How Hippo pathway is regulated by tissue-specific upstream regulators? Tissue-specific drugs for disease therapy need to be explored to stimulate or inactivate Hippo pathway in different tissues or organs. This might be achieved by the identification of beneficial downstream genes regulated by Hippo/YAP, which may yield more tissue-selective drug targets. Alternatively, localized or cell-type specific gene delivery technologies may also be used to achieve tissue-specific Hippo activation or inhibition. Thus, further research is necessary to address these issues, which will be significant for the understanding the occurrence of degenerative disorder and tumorigenesis.

Funding

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