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Review

Review

Hippo signaling in epithelial stem cells

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Abstract

Over the past decade, discoveries on Hippo signaling have revealed a complex signaling network integrating various signaling pathways to modulate tissue homeostasis, organ size control, tissue repair, and regeneration. Malfunction of the Hippo pathway is associated with tumor and cancer development. Moreover, Hippo signaling has been proposed to act in numerous stem cells in a variety of organisms. Recently, more attention has been paid to define the functions of the Hippo pathway in tissue-specific stem cells, which have great potential to be used in cell-based therapies. Here we provide an overview of its roles in regulating stem cells in epithelial tissues and its potential implications in related cancers.

Key words: Hippo, YAP, epithelial, stem cells

The Hippo Signaling Network

The Hippo pathway was identified to orchestrate proliferation and apoptosis to control organ size, initially in *Drosophila melanogaster* from genetic screens for seeking tissue growth regulators [1–7]. It has also been shown to be involved in diverse cellular and tissue properties, including cell–cell adhesion, contact inhibition, apicobasal polarity, planar cell polarity, and mechanotransduction [8–10]. The central to Hippo signaling is a highly conserved kinase cascade that contains the STE20 family serine/threonine kinase Hippo (Hippo) or MST1/MST2 in mammals [1–5] and the NDR family kinases Warts (Wts) or LATS1/LATS2 in mammals [7,11,12]. Hippo/MST, activated by auto-phosphorylation and dimerization [7,13], phosphorylate and activate Wts/LATS with the aid of adaptor proteins Salvador (Sav) or SAV1 in mammals [6,14] and Mob as tumor suppressor (Mats) or MOBKL1A/MOBKL1B in mammals [15]. Subsequently, activated Wts/LATS phosphorylate the transcriptional coactivator Yorkie (Yki) or Yes associated-protein (YAP) and its paralog TAZ in mammals [16], resulting in a sequestration of Yki in the cytoplasm upon binding with 14-3-3 proteins at a key residue Ser168 of Yki or Ser127 of human YAP or Ser89 of human TAZ and at two additional serine residues [10,17–19]. Yki/YAP/TAZ are the final effectors of the Hippo pathway [8–10]. By promoting cytoplasmic retention of Yki/YAP/TAZ, their growth promoting function is inhibited [8–10]. Suppression of Wts/LATS activity promotes nuclear translocation of Yki/YAP/TAZ, enabling the association of Yki/YAP/TAZ with a range of DNA-binding transcription factors to execute their transcriptional functions (**Fig. 1**) [8–10]. The most well studied binding partner of Yki/YAP/TAZ in the nucleus is the TEAD family transcriptional factor Scalloped (Sd) or TEAD1–4 in mammals [20–23], which drive the oncogenic potential of Yki/YAP/TAZ by inducing the expression of a diverse array of genes involved in proliferation and anti-apoptosis.

Although the core kinase cascade is well defined, the regulation of the core of the Hippo pathway seems to be rather complicated. Unlike the highly conserved core kinase cascade, the regulations of the Hippo pathway are diverse. In the initial steps of Hippo signal transduction, contact inhibition and mechano-transduction seem to have significant impacts. The FERM domain protein Merlin (also known as NF2), encoded by the *Neurofibromatosis2* gene, is an important mediator of contact inhibition [24]. Conditional knockout of NF2 in the mouse liver results in massive organ enlargement and tumor development. Studies in both fly and mammals linked Merlin to the Hippo pathway, and uncovered the function of Merlin-Expanded (FRMD6 in mammals)-Kibra that acts as a negative regulator of growth upstream of Hippo and Wts [24,25]. A recent research suggested that Merlin promotes downstream Hippo signaling without activating the intrinsic kinase activity of Hippo but recruiting Wts/LATS to the plasma membrane to promote Wts phosphorylation induced by the Hippo–Sav kinase complex [26]. In addition, *Drosophila* protein

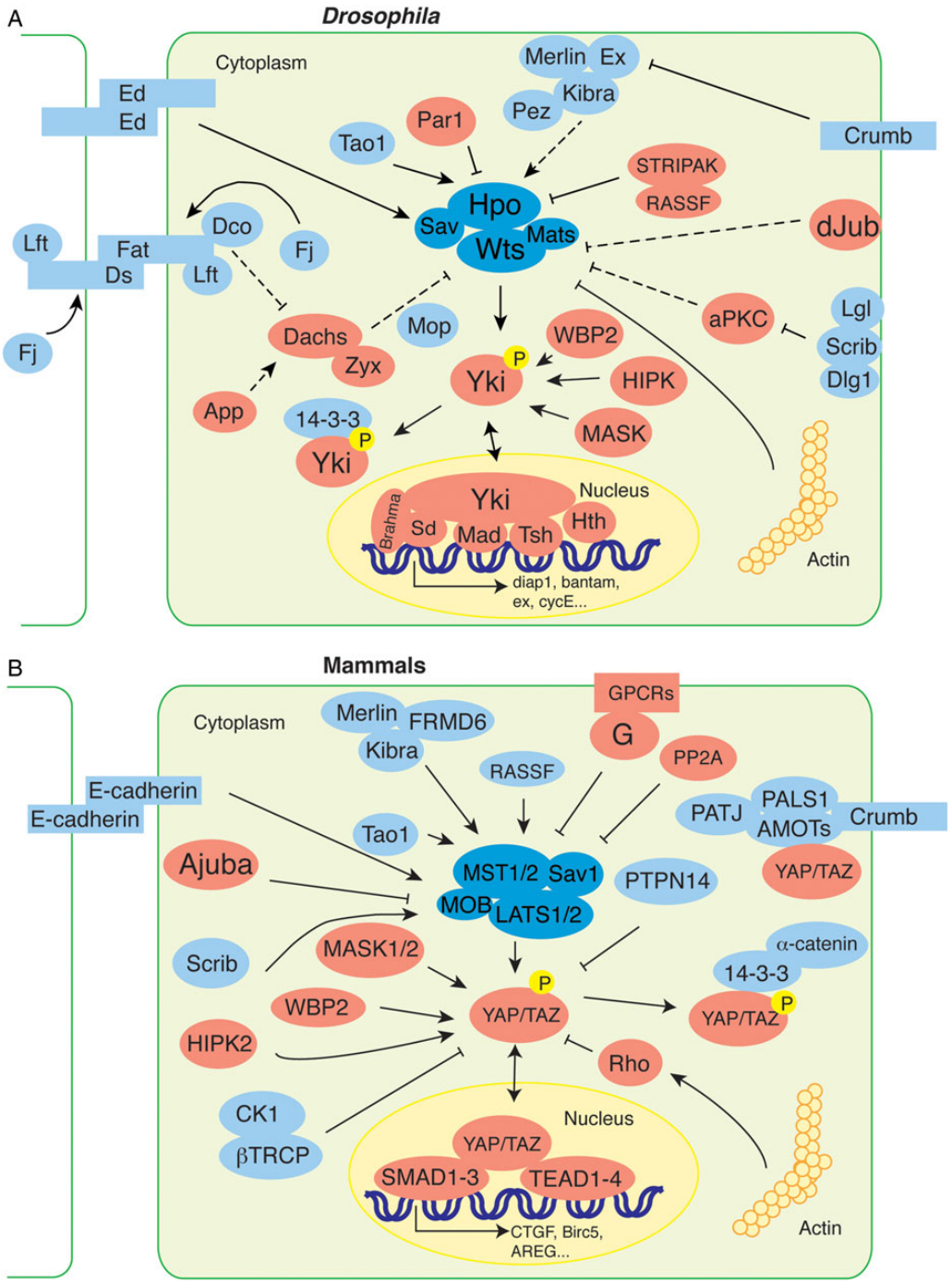

Figure 1. A schematic diagram of the Hippo pathway in *Drosophila* and mammals Growth promoting components are in red, and inhibitory components are in blue. Arrowed or blunted ends indicate activation or inhibition, respectively. Dashed lines indicate unknown mechanisms.

tyrosine phosphatase Pez may precipitate in Hippo activation by binding to Kibra [27]. Another well-known upstream regulatory branch of the Hippo pathway is Fat (Fat1–4 in mammals)–Dachsous (Dachs, in *D. melanogaster*, Dchs1–2 in mammals). Fat is the giant atypical cadherin, whose activity is regulated by its ligand Ds in a ligand-concentration independent manner. Fbxl7, an F box protein, functions in a subset of pathways downstream of Ft and links Ft to Ds localization [28]. The activity of Fat–Ds is also regulated by Golgi kinase Four-jointed (Fj, in *D. melanogaster*)-induced phosphorylation and by casein kinase Discs overgrown (Dco) and Lowfat (Lft). The sharpness of Ds–Fj gradients in neighboring cells suppresses the activity of the Hippo pathway (reviewed in [29]). However, this branch is only evidenced to operate in *D. melanogaster*. In spite of Merlin–Kirbra–Expended complex and Fat branch of upstream regulations, several other membrane associated proteins are involved in transducing cell contact-mediated signals to the core component of the Hippo pathway, including apicobasal polarity proteins Crumbs complex and Scrib complex in mammals, the Crumbs complex-associated protein AMOT, Lethal giant larvae and Discs large 1 in *D. melanogaster*, the adherens junction-associated proteins Echinoid,

E-cadherin, and α-catenin, and the atypical PKC kinases (reviewed in [29,30]).

Regardless of the regulation of Hippo signaling by cell contact inhibition, mechano-transduction also plays important role in the modulation of Hippo pathway activity. Modulation of the apical level of filament actin (F-actin) by actin-capping proteins in both flies and mammals influences the Hippo pathway activity. F-actin stabilization leads to YAP/TAZ activation, while its disruption results in YAP/TAZ inactivation [31,32]. Furthermore, mammalian YAP/TAZ are identified as sensors and mediators of mechanical signals exerted by extracellular matrix rigidity and cell shape, which requires Rho GTPase activity and tension of the actomyosin cytoskeleton, but independent of the core Hippo signaling kinase cascade [33]. In addition to that, the Hippo pathway responds to extracellular diffusible signals (reviewed in [34]). It has been discovered that the Hippo pathway is linked to diverse G-protein-coupled receptor (GPCR) ligands and receptor signaling [35]. GPCR signaling can either stimulate or inhibit the activity of YAP/TAZ. Activation of G12/13-coupled receptors results in LATS kinase inactivation and YAP/TAZ activation in an MST1/2-independent manner, while stimulation of Gs-coupled receptors acts through cAMP via protein kinase A and Rho GTPases to stimulate LATS kinase activity and YAP phosphorylation [35].

Several other Hippo signaling modulators have also been discovered, such as Hippo/MST activity regulators Tao-1 [36,37], Par-1 [38], RASSF [39–41], Wts/LATS activity regulators *D. melanogaster* Jub and mammalian Ajuba LIM proteins [42–44], Sav regulator salt-inducible kinase (SIK1–3) [45], and Yki/YAP regulators MASK (multiple ankyrin repeats single KH domain-containing protein) [46,47], homeodomain-interacting protein kinase 2 (HIPK2) [48,49], WBP2 [50], and PTPN14 [51] (for details, see **Fig. 1**). Of note, the Hippo pathway has been shown not to act in isolation but to crosstalk with diverse growth signaling pathways, indicating it is regulated at a network level. To date, the epidermal growth factor receptor (EGFR)–MAPK signaling pathway, the TGFβ–SMAD pathway, the Wnt pathway, the Jak/Stat and the Notch pathway have been reported to integrate with Hippo signaling for growth control and other cellular behaviors (reviewed in [52]).

The function of Hippo signaling exhibits through the downstream effectors, Yki/YAP/TAZ. Therefore, the regulation of the Hippo pathway eventually leads to fine-tuning of the activities of Yki/YAP/TAZ. It has been proposed that upstream components modulate Yki/YAP/TAZ activities through at least two mechanisms, regulating their phosphorylation status and governing their activities through physical interactions. The best example of phosphorylation-dependent regulatory mechanisms is the Wts/LATS-mediated Yki/YAP/TAZ phosphorylation. Wts/LATS phosphorylate Yki/YAP/TAZ on consensus motifs and subsequently promotes 14-3-3 binding, resulting in cytoplasmic retention of Yki/YAP/TAZ and inhibition of their activities [11,12,53,54]. It has also been reported that HIPK may promote Yki/YAP activity through phosphorylation-dependent regulation [48,49]. Furthermore, by modulating the YAP phosphorylation status on Ser381 site, CK1δ/ε induces the binding of YAP and β-TRCP E3 ubiquitin ligase, leading to polyubiquitination and degradation of YAP/TAZ [55]. WW domains and PPxY or PY motifs interactions participate in the regulation of the Hippo pathway [56]. Yki/YAP/TAZ contain WW domains [57], where several regulators physically associate. For instance, PTPN14, the negative regulator of YAP/TAZ, and WBP2, the promoter of Yki/YAP, contain PY motifs, by which they interact with Yki/YAP/TAZ during the regulation [50,51]. In addition to the regulation by phosphorylation and physical interaction, Yki/YAP/TAZ activities are also regulated through other mechanisms. For example, the Ets family member GABP has been reported to be an activator of *YAP* gene expression and hence affects YAP activity [58]. Furthermore, two independent studies showed that Sd-binding-protein (SdBP/Tgi) directly competes with Yki in binding to Sd and inhibits the transcriptional activity of Sd–Yki complex [59,60]. The mammalian ortholog of SdBP/Tgi potently suppresses the YAP oncoprotein in transgenic mice [60].

Hippo Signaling in Epithelial Stem Cells

During the past several years, accumulating evidence implied that Hippo signaling shows stem cell like properties and its effectors, Yki/YAP/TAZ, function as regulators of stem cell homeostasis. Hence, the Hippo pathway is thought to not only control tissue growth but also regulate cell fate decision, stem cell proliferation, and tissue regeneration. Much progress has been made in exploring the cellular and molecular functions of Hippo signaling in various types of stem cells [61,62]. Stem cells have the remarkable potential to develop into different cell types in the body during early life and growth. In many tissues, such as epithelial tissues, they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells. Epithelial stem cells undergo constant renewal and line the cavities and surfaces of structures throughout the body, making it a great model to study stem cell self-renewal and regeneration. In the next section, we will outline the recent discoveries of Hippo signaling in epithelial stem cells.

Hippo signaling in skin

The skin interfaces with the environment and undergoes constant replenishing to defense external factors. The association between Hippo signaling and the development and homeostasis of skin has been previously shown. Sav is the first component of the Hippo pathway to be discovered functioning in skin development. Inactivation of *Sav1* not only results in early embryonic lethality but also displays a thickening of the epidermal skin layer in the embryos [63]. Studies in mice have implicated that YAP is required for normal skin development and influences epidermal stem cell fate [64,65]. YAP is concentrated in the nuclei of epidermal basal layer, where the most proliferative progenitors are. Skin-inducible activation of YAP promotes the proliferation and self-renewal of epidermal stem cells and maintains their undifferentiated state, leading to squamous cell carcinoma-like tumors. After initiating epidermal progenitor cell differentiation, YAP shuttles into cytoplasm with increased phosphorylation level [64,65]. In contrast, skin-specific loss of YAP reduces proliferative potential of the epidermis and even loss of skin [64]. The function of YAP in maintaining skin hemostasis relies on its interaction with TEAD in the nucleus, but not MST1/2, since skin-specific depletion of MST1 and MST2 causes no phenotypes, such as cell fate abnormalities and alterations in YAP phosphorylation [64]. In addition, its function is independent on its C-terminal domain *in vivo* [66]. Yet, upon phosphorylation, YAP is retarded in cytoplasm by 14-3-3 proteins and an adherens junction component α-catenin [64,67,68]. In addition, Yap activity in promoting epidermal differentiation is affected by actin-related protein2/3-mediated F-actin assembly [69]. These findings indicated that cell density regulates epidermal expansion by inactivating YAP.

Hippo signaling in intestine

Stem cells of the adult midguts are essential for maintaining tissue homeostasis and replenishing lost cells. The structural simplicity and the multi-potency of *D. melanogaster* posterior midgut make it an

excellent model for studying adult epithelial tissue homeostasis and regeneration. Intestinal stem cells (ISCs) in *D. melanogaster* undergo symmetrical or asymmetrical division to self-renew or give rise to enteroblasts, which subsequently differentiate into enterocytes or enteroendocrine cells [70–72]. The Hippo pathway has been reported to exhibit restrictive role in *D. melanogaster* ISC proliferation, as inactivation of Hippo signaling in either midgut precursor cells or differentiated enterocytes induced ISC proliferation and expression of Jak/Stat pathway ligands and EGFR pathway ligands [73–76]. Yki is dispensable for normal midgut function but is required for the damage-induced ISC proliferation in both ISCs and enterocytes [73–76]. Furthermore, Pez functions as an adaptor protein and negative upstream regulator of Yki, independent of its potential phosphatase activity, to restrict ISC proliferation in adult midgut enterocytes [27]. The most recent studies have demonstrated that Yki recruits the Brahma chromatin-remodeling complex, which is essential for ISC proliferation and regeneration, to perform its function in mediating ISCs [77,78]. The protein level of the ATPase subunit of Brahma complex is tightly controlled by Hippo activity induced caspase-dependent cleavage [78].

In conditional YAP knockout mouse intestines, YAP also had little effect on normal homeostasis of the gut, and similar growth phenotypes were observed as it has been shown in *D. melanogaster*, implying a conserved regenerative role for the Hippo pathway [79,80]. As in the skin, YAP is highly expressed in the nucleus of the cells near the Basel layer. However, the precise role of YAP is not well defined in mammalian intestines. Although loss of Yki/YAP does not show impacts on the intestine, loss of upstream Hippo components, Sav1 or MST1/2, leads to YAP-dependent formation of sessile serrated polyps, which is molecularly and morphologically distinct from typical Wnt-driven adenomas [80,81], showing that they are not dispensable in normal intestine growth. It suggested that, under normal homeostasis, the Hippo pathway keeps the YAP oncoprotein in a relatively inactive state by suppressing its abundance [80,81]. In addition, overexpressed YAP synergizes with β-catenin to drive intestinal stem cell proliferation for epithelial repair as well as proliferation of colon cancer cells, making the expression of YAP attractive target for cancer therapy.

Hippo signaling in liver

Compared with other organs, the liver has a very unique regenerative way with a remarkable regenerative capacity. It can replenish its mass following a two-third hepatectomy or injury by differentiated hepatocytes rather than by multi-potent stem cells [82]. The first evidence showing that the Hippo pathway is involved in regulating liver enlargement is the conditional over-expression of YAP S127A in the mouse liver, which resulted in increased hepatocyte proliferation with hepatocellular carcinoma characteristics [79,83,84]. This function of YAP in liver requires the TEAD activity, as a dominant-negative TEAD molecule suppresses YAP over-expression-induced or Merlin inactivation-mediated hepatomegaly [85].

The core components of Hippo signaling inhibit YAP activity in liver [86–89]. Deletion of MST1/2 or Sav1 results in phenotypes those are similar to YAP S127A-induced liver overgrowth and hepatocellular carcinomas, suggesting that YAP activity is induced in those mutants. In addition, liver-specific ablation of mammalian Sav shows similar phenotypes and induces expansion of hepatic progenitor cells (oval cells), leading to the development of hepatomas [86–89], indicating a dual regulatory role of Hippo signaling in liver tumor suppression and transition of oval cells to fully differentiated hepatocytes. A strong link between NF2/Merlin and YAP activity in the liver has been reported [90,91], while one of the study claimed that overproliferation of Nf2(-/-) liver progenitors is driven by aberrant EGFR activity [91]. Furthermore, mice with a liver-specific angiomotin knockout suppress progenitor proliferation in response to toxin-induced injury or when crossed with mice lacking Nf2. The p130 isoform of angiomotin is identified to be a co-factor of YAP for hepatic progenitor cell proliferation [92]. Progressive expansion of progenitor cells throughout the liver without affecting differentiated hepatocytes has been observed in *Mst1/2*, *Sav1*, and *Nf2* mutant liver, suggesting a curial role of Hippo signaling in oval cell proliferation [89–91], which is thought to be able to give rise to hepatocellular carcinomas. Further studies that aim to define whether there is a direct function of YAP on oval cell proliferation may help us to better understand hepatocarcinogenesis and find rational medical therapy for hepatocellular carcinomas.

Hippo signaling in lung

Mammalian lungs separate the conducting airways and alveoli by distinct epithelial linings, yet how these two zones are specified and maintained are less known. Most recently, critical roles for the Hippo pathway not only in lung cancers but also in the regulation of lung progenitor cell differentiation are beginning to be delineated. It has been reported that knockdown of the Hippo mediators Yap1 or Taz decreased *in vitro* cellular migration and transplantation of metastatic disease and constitutively active Yap was sufficient to drive lung tumor progression *in vivo* [93]. During lung development, when epithelial tubules are forming and branching, a nucleocytoplasmic shift in Yap localization marks the boundary between the airway and the distal lung compartments [94]. At the boundary, Yap controls Sox2 expression and ultimately generates the airway epithelium. YAP loss-of-function and subcellular localization influence the proper response of epithelial progenitors to local TGF-β-induced cues and Sox2 expression, and subsequently the differentiation of adult airway progenitors [94]. Moreover, YAP displays a distinct activation pattern in lung adenocarcinoma (ADC) and squamous cell carcinoma [95]. It is initially activated by LKB1 loss in lung ADC, and then is inactivated during transdifferentiation and triggers squamous differentiation reprogramming. Functional studies showed that YAP acts as an essential barrier for lung cancer cell fate conversion, since disruption of the YAP barrier for phenotypic transition significantly accelerates squamous transdifferentiation, whereas constitutive YAP activation conversely inhibits this transition [95]. In addition, VGLL4 negatively regulates the formation of YAP–TEAD complex by directly competing with YAP in binding to TEADs and executing its growth-inhibitory function through two TDU domains to significantly inhibit lung cancer progression in *de novo* mouse model [96]. A full understanding of the role of the Hippo pathway in the lung may require future studies to examine the crosstalk between Hippo and other signaling pathways which are also thought to be involved in lung development.

Concluding Remarks

In this paper, we summarized the regulation and function of Hippo signaling and highlighted the rapid progress in the field. Even though influences of Hippo signaling in epithelial tissue *in vivo* have been observed, the challenge is now to uncover the regulatory mechanisms and to understand the cellular and physiological framework. Further researches on how Hippo signaling conducts and integrates cell-autonomous and non-cell-autonomous signaling, cell–cell contact, and mechanical cues together to regulate development, stem cell maintenance and tumorigenesis may provide new insights into stem cell

biology and organ growth, which could lead us to new, regenerative approaches to human medicine and disease treatment.

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