

## Review Article

## Capturing the mammalian Hippo: Elucidating its role in cancer

Miki Nishio,<sup>1</sup> Kohei Otsubo,<sup>1</sup> Tomohiko Maehama,<sup>2</sup> Koshi Mimori<sup>3</sup> and Akira Suzuki<sup>1,4</sup><sup>1</sup>Division of Cancer Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka; <sup>2</sup>Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases, Tokyo; <sup>3</sup>Department of Surgery, Kyushu University Beppu Hospital, Beppu, Japan

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The Hippo pathway is an evolutionarily conserved kinase cascade involved in cell growth, apoptosis, development and migration. It is also crucial for stem cell self-renewal and the maintenance of genomic stability. In addition, this pathway has the unique capacities to sense aspects of tissue architecture, such as cell polarity and mechanical tensions imposed by the surrounding microenvironment, and to control organ size and shape. All of these properties are frequently altered in tumor cells. In this review, we summarize how dysregulation of mammalian Hippo signaling is implicated in cancer. (*Cancer Sci* 2013; 104: 1271–1277)

## Overview of the Hippo pathway

## Hippo pathway core components.

Most components of the Hippo signaling pathway were originally identified in *Drosophila* by screening for mutations resulting in organomegaly. The Hippo pathway is highly conserved in mammals (Fig. 1, Table 1), and serves to shut down cell proliferation under conditions of high cell density or other stress. Core components include the Mammalian sterile 20-like kinases (MSTs), Large tumor suppressor kinases (LATs), and the adaptor proteins Salvador homolog 1 (SAV1; also called WW45) and Mps One Binder kinase activator proteins (MOBs). Under conditions of low cell density, Hippo signaling is inactivated and the mammalian transcriptional activator Yes-associated protein (YAP) (or its paralog Transcriptional coactivator with PDZ-binding motif [TAZ]) is activated and free to translocate into the nucleus. Nuclear YAP activates or suppresses transcription factors that regulate target genes involved in cell proliferation, tissue growth, or control of organ size and shape.<sup>(1,2)</sup> These transcription factors include: TEAD1-4 (important for growth promotion and the epithelial–mesenchymal transition [EMT]); SMADs (TGF- $\beta$  signaling); RUNXs (blood and bone formation); p63/p73 (apoptosis); PAX3 (neural crest formation); PPAR $\gamma$  (adipogenesis); TTF1 (thyroid and lung morphogenesis); and TBX-5 (WNT/ $\beta$ -catenin signaling and cardiac and limb development).<sup>(3,4)</sup> Under conditions of high cell density (or stress stimuli or mechanical compression), MST and LATS are activated. Activated LATS phosphorylates YAP and TAZ, promoting their cytoplasmic retention through binding to 14-3-3 protein. This binding prevents activation of YAP/TAZ target transcription factors and facilitates YAP/TAZ proteasomal degradation by the E3 ligase complex SCF <sup>$\beta$ -TRCP</sup> (Fig. 2).<sup>(5)</sup>

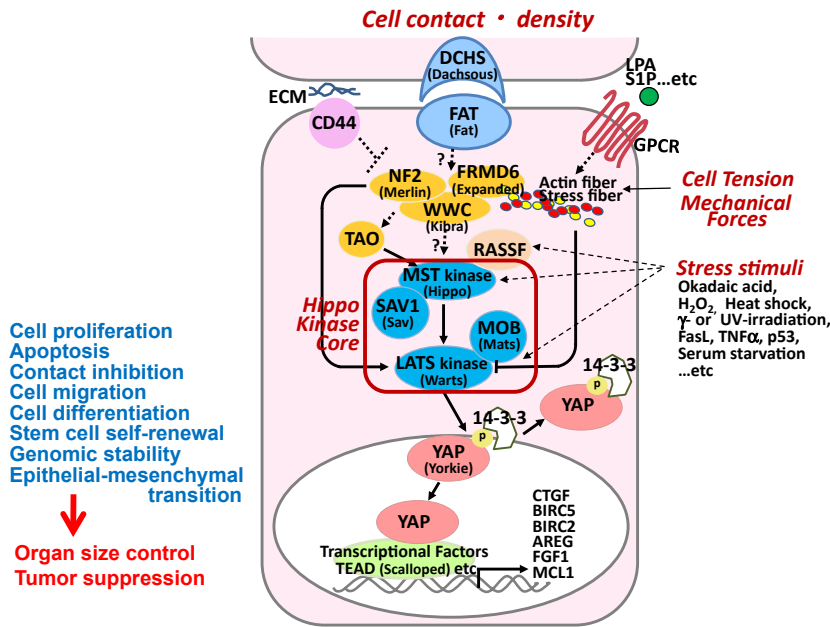
**Hippo pathway upstream components.** Candidate upstream sensors activating the mammalian Hippo pathway are FAT tumor suppressor homolog4 (FAT4), Dachous1/2 (DCHS1/2),

Neurofibromin2 (NF2), FERM domain-containing 6 (FRMD6), and WW and C2 domain-containing 1 (WWC1; also called KIBRA) (Fig. 1, Table 1). Mice mutated in *FAT4* and/or *DCHS1*, which regulate apical membrane organization, show unexpectedly small intestines, lungs and kidneys. However, the absence of organomegaly and the presence of normal LATS1 and YAP phosphorylation in these mutants suggest that mammalian Hippo signaling is not regulated by FAT4 or DCHS1, unlike in *Drosophila*.

CD44 (absent in flies) inactivates NF2 upon cell–cell contact to control Hippo signaling (Fig. 1). NF2 acts as a potent tumor suppressor gene (TSG) by controlling LATS1/2 and YAP.<sup>(6)</sup> Several G protein-coupled receptor (GPCR) agonists, such as LPA, S1P, and thrombin (Gi-coupled GPCR agonists), as well as glucagon and epinephrine (Gs-coupled GPCR agonists), also negatively or positively regulate LATS1/2.<sup>(7)</sup> WWC1(KIBRA) binds to NF2 and FRMD6 to promote Hippo signaling independently of MSTs. While TAO-1 kinase promotes MST phosphorylation, PP2A dephosphorylates MST1/2 and YAP (Fig. 2). RASSF1A activates MST1 by preventing PP2A-mediated dephosphorylation.

Apical-basal cell polarity in mammalian epithelial cells is established by the Crumbs, Par, and Scribble complexes, which influence the Hippo pathway (Fig. 3). The Crumbs complex component AMOT co-localizes with MST1/2, LATS1/2 and YAP in a complex at the tight junction to control cell growth. Zona occludens-2 (ZO-2) in the tight junction, and  $\alpha$ -catenin,  $\beta$ -catenin, or PTPN14 in the adherence junction, also bind to YAP/TAZ<sup>(8)</sup> (Fig. 3). Inhibition of any of cell–cell adhesion; Crumbs, Scribble, or Lgl complex formation;  $\alpha$ -catenin, ZO-2, or PTPN14 expression; F-actin polymerization, stress fiber formation, or Rho activity induced by mechanical forces, may decrease YAP phosphorylation in a manner dependent<sup>(9)</sup> or independent<sup>(10)</sup> of Hippo core kinases. The result is increased cell growth.<sup>(8,11)</sup> Nuclear translocation of YAP is also promoted when the cells are “stretched” or growing on a stiff extracellular matrix (ECM), and repressed when cells are compressed or growing on a soft surface. Importantly, loss of  $\alpha$ -catenin, which occurs in many human cancers, is linked to a poor prognosis. Thus, the Hippo pathway is tightly linked to tissue architecture and regulated by upstream cytoskeletal or tension signaling. For a detailed review of Hippo upstream components, please refer to Yu *et al.*<sup>(12)</sup>

<sup>4</sup>To whom correspondence should be addressed.  
E-mail: suzuki@bioreg.kyushu-u.ac.jp



**Fig. 1.** Overview of the mammalian Hippo signaling pathway. Core components of the mammalian Hippo pathway are: *MST* and *LATS* kinases, and *SAV1* and *MOB* adaptor proteins. Orthologous *Drosophila* components are indicated in parentheses. The Hippo pathway is activated in response to increased cell density, tension or stress, and inhibits cell proliferation, and other processes potentially contributing to tumorigenesis. Candidate sensory components upstream of the Hippo kinase core include *FAT4*, *DCHS1/2*, *NF2*, *FRMD6* and *WWC1*. *TAO-1* kinase and *RASSF* regulate *MST* phosphorylation. When activation of the Hippo core is triggered, *SAV1* allows *MST* to phosphorylate *LATS*, and *MOB* binds to *LATS* to enhance its catalytic activity. *LATS* then phosphorylates and inactivates *YAP* (or its paralog *TAZ*), promoting their cytoplasmic retention through binding to 14-3-3 protein. *YAP* (or *TAZ*) is ultimately degraded so that transcription factors (such as *TEAD*) promoting cell survival are not activated. In contrast, under conditions of low cell density or minimal stress, *YAP* dissociates from 14-3-3, translocates into the nucleus, and activates transcription factors that induce the expression of pro-survival genes such as *CTGF*. Solid lines, known direct interactions; dashed lines, unknown mechanisms.

**Table 1. Names of Hippo component molecules in *Drosophila* and in Human**

| <i>Drosophila</i> | Human   |
|-------------------|---|
| Dachsous          | DCHS1,2                                       |
| Fat               | FAT1-4  |
| Expanded          | FRMD6 (WILLIN)                                |
| Kibra             | WWC1-3  |
| Merlin            | NF2   |
| Rassf             | RASSF1-10                                     |
| Hippo             | MST1 (STK4), MST2 (STK3), MST3, MST4          |
| Salvador          | SAV1 (WW45)                                   |
| Warts             | LATS1,2                                       |
| Tricornered       | NDR1 (STK38), NDR2 (STK38L)                   |
| Mats              | MOB1A (MOBK1B), MOB1B (MOBK1A), MOB2, 3A-C, 4 |
| Yorkie            | YAP1, TAZ (WWTR1)                             |
| Scalloped         | TEAD1-4                                       |

### Tumor-Related Roles of Downstream Hippo Components

The loss of Hippo signaling is a key prognostic factor in predicting cancer patient survival and sensitivity to chemotherapeutic drugs. Cancer onset and/or development is generally associated with Hippo pathway inactivation or *YAP* activation, which is caused mainly by epigenetic changes (e.g. methylation of *MST* or *LATS*). In addition, genetic mutations (e.g. germline or somatic *NF2* mutations) and *YAP* amplification (somatic) have also been reported. However, mutations in the Hippo pathway genes themselves are uncommon. For a detailed review of Hippo pathway alterations in various human cancers, please refer to Harvey *et al.*<sup>(13)</sup>

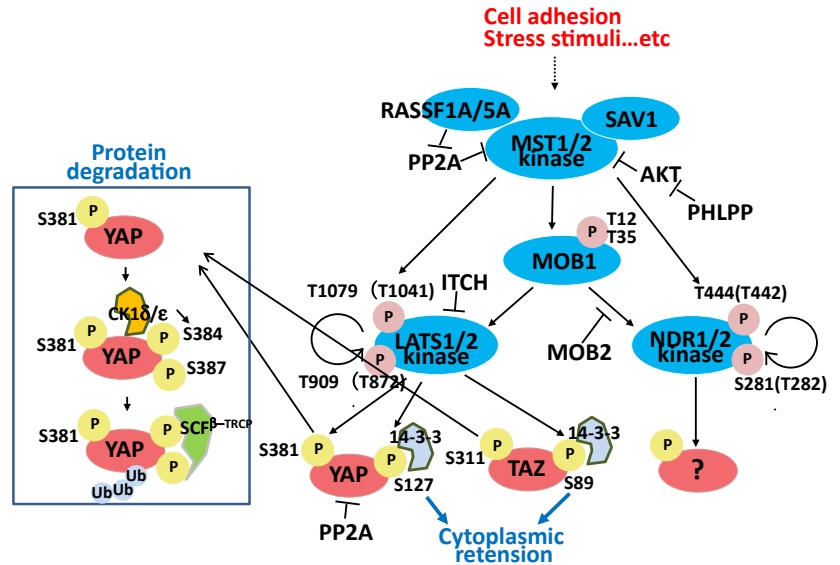
**Tumor-related roles of *YAP*.** *YAP* can act as an oncogene or a TSG, depending on the cellular context and *YAP*'s interacting partners. Conditional *YAP* activation in the liver reversibly increases liver size, and *YAP* dysregulation leads to hepatocellular carcinomas (HCCs). Conversely, *YAP*

inactivation leads to loss of hepatocytes and bile duct cells.<sup>(14,15)</sup>

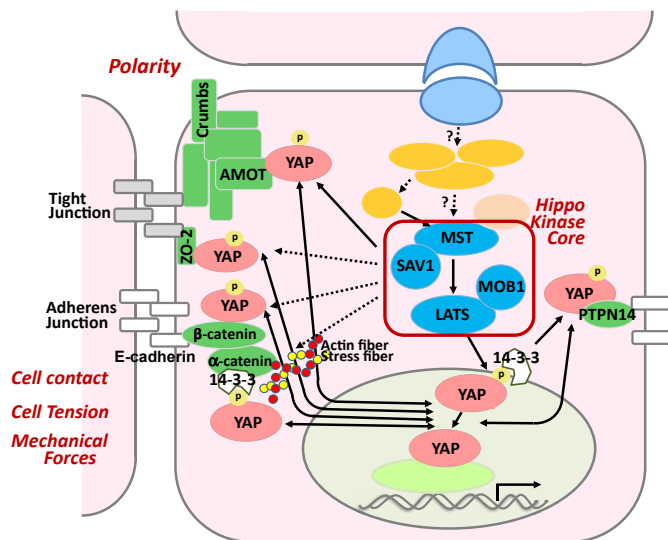
In mice, Camargo *et al.*<sup>(14)</sup> reported that conditional *YAP* activation in the intestine expanded multipotent progenitor cells and resulted in early onset of polyps following DSS treatment. However, Barry *et al.* found that transgenic *YAP* expression reduced WNT target gene expression and induced rapid destruction of intestinal crypts,<sup>(16)</sup> while loss of *YAP* led to excessive WNT signaling causing hyperplasia, intestinal stem cell expansion, and formation of ectopic crypts and microadenomas. Barry *et al.* therefore speculated that cytoplasmic *YAP* may normally bind to *DVL* proteins and dampen WNT signaling. The discrepancy between these two reports requires resolution, since either increased nuclear *YAP* or decreased cytoplasmic *YAP* heightens the risk of colon cancer. In a third report, Rosenbluh *et al.*<sup>(17)</sup> showed that *YAP* and the transcription factor *TBX5* form a complex with  $\beta$ -catenin. Phosphorylation of *YAP*(Thr357) by the tyrosine kinase *YES1* induces localization of this complex to the promoters of the anti-apoptotic genes *BCL2L1* and *BIRC5*, inducing the transformation and survival of  $\beta$ -catenin-driven tumor cells. *YAP* is thus a key driver of  $\beta$ -catenin-related intestinal cancers.

Two transgenic mouse strains overexpressing *YAP* in the skin have been described.<sup>(8,18)</sup> Both strains initially show expanded basal epidermal progenitors that fail to undergo normal terminal differentiation, and later develop squamous cell carcinomas (SCCs). These mutants have a severe defect in hair follicle (HF) formation that may be due to impaired planar cell polarity caused by strong expression of *YAP* before HF morphogenesis.

**Crosstalk between Hippo and other tumor-related pathways.** *YAP/TAZ* binds to phosphorylated R-SMADs,<sup>(19,20)</sup> connecting the Hippo pathway to TGF- $\beta$ - and BMPR-related signaling (Fig. 4). In general, nuclear *YAP/TAZ* enhances SMAD activity in the nucleus, while cytoplasmic *YAP/TAZ* restricts SMAD nuclear accumulation. TGF- $\beta$ -SMAD activation induced by low cell density depends on nuclear *YAP/TAZ*, while *YAP/TAZ*-induced stem cell self-renewal



**Fig. 2.** Mechanisms of YAP inactivation. Activation of the Hippo kinase core as described in Figure 1 leads to the indicated phosphorylation events on YAP/TAZ, and their cytoplasmic retention via 14-3-3 binding. Alternatively, phosphorylated YAP/TAZ can bind to the E3 ligase complex SCF $\beta$ -TRCP, which facilitates YAP/TAZ ubiquitination and proteasomal degradation. RASSF1A activates MST1 by preventing PP2A-mediated dephosphorylation of MST1/2 and YAP. NDR1/2 are alternative MST substrates.



**Fig. 3.** Regulation of YAP by upstream polarization or adhesion protein complexes. Polarization proteins sense cell contact and mechanical forces and transmit signals to the Hippo pathway to inhibit cell proliferation and control organ size and shape. The Crumbs complex component AMOT can co-localize with MST1/2-LATS1/2-YAP complexes at the tight junction to promote cytoplasmic retention of YAP. ZO-2 in the tight junction, and  $\alpha$ -catenin,  $\beta$ -catenin, or PTPN14 in the adherence junction, can also bind to YAP and prevent nuclear translocation. YAP nuclear localization can also be reduced by F-actin polymerization, stress fiber formation or Rho activity induced by mechanical forces.

depends on nuclear SMADs.<sup>(19,20)</sup> Thus, Hippo signaling may underlie the opposing roles of the TGF- $\beta$  pathway in early versus late stages of cancer. TGF- $\beta$  inhibits cell-cycle progression during tumor initiation but subsequently promotes cancer cell proliferation, EMT, and metastasis. Normally-polarized epithelial cells possess cytoplasmic YAP/TAZ, which restricts TGF- $\beta$ -induced SMAD activity.<sup>(19)</sup> In contrast, cells that have lost Crumbs complex function exhibit impaired cell polarity, elevated nuclear YAP/TAZ, increased nuclear SMADs, and enhanced sensitivity to TGF- $\beta$  ligand.<sup>(19)</sup> EMT and the transformed phenotype are then promoted. Crosstalk between Hippo

signaling and the TGF- $\beta$ -SMADs pathway may thus explain the differential functions of TGF- $\beta$  as a tumor progresses.

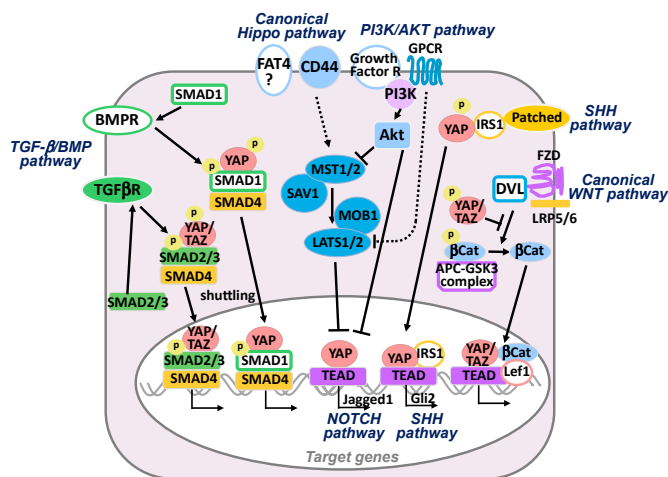
The Hippo pathway also regulates WNT-induced signaling.<sup>(21,22)</sup> YAP/TAZ is recruited to TCF/LEF binding sites together with  $\beta$ -catenin. Heart-specific conditional deletion of SAV1, MST1/2 or LATS2 in mice increases nuclear YAP activity, which promotes WNT/ $\beta$ -catenin signaling and triggers cardiomyocyte proliferation.<sup>(22)</sup> Conversely, WNT signaling enhances YAP mRNA expression. Nuclear accumulation of  $\beta$ -catenin is augmented by nuclear TAZ via LATS inhibition. Phosphorylated YAP/TAZ also inhibits Disheveled (DVL), promoting  $\beta$ -catenin degradation and dampening WNT signaling.<sup>(16,21)</sup> Thus, nuclear YAP/TAZ enhances WNT activity, whereas cytoplasmic YAP/TAZ restricts it.

SHH pathway activation leads to YAP mRNA expression, followed by YAP protein stabilization and nuclear accumulation.<sup>(23)</sup> SHH also increases TEAD1 and IRS1, and induces IRS1 nuclear translocation. YAP, TEAD1 and IRS1 form a nuclear complex that regulates GLI2 and GLI1 expression, enhancing cell-cycle progression. In mice, YAP overexpression results in hyperactivation of SHH signaling, causing a neuronal differentiation defect in primary cortical progenitors. In humans, increased YAP occurs in medulloblastomas with aberrant SHH signaling, suggesting that therapeutic targeting of YAP might eliminate medulloblastoma recurrence.<sup>(23)</sup>

AKT inhibits apoptosis by inactivating MST1/2 via phosphorylation at Thr120/117. In addition, AKT-mediated MST2 (Thr117) phosphorylation reduces MST2 binding to RASSF1A while increasing its binding to RAF1, again inhibiting MST2 activity. AKT also phosphorylates MST1 (Thr387), which reduces MST1 activity and decreases its cleavage in response to H<sub>2</sub>O<sub>2</sub>.<sup>(24)</sup> Reciprocal regulation exists, since MST1/2 overexpression in a prostate cancer cell line inhibits AKT activity. However, AKT-mediated YAP (Ser127) phosphorylation promotes YAP localization in the cytoplasm and attenuation of p73-mediated apoptosis.<sup>(25)</sup> Thus, AKT's effects on YAP localization are cell context-dependent.

MST1/2 deficiency in murine intestinal epithelium decreases YAP phosphorylation and activates NOTCH and  $\beta$ -catenin signaling.<sup>(26)</sup> Jagged-1 (NOTCH ligand) is a YAP target in hepatocytes, and Hippo pathway activation in these cells inhibits their proliferation and survival. Conversely, overexpression of constitutively active YAP upregulates Jagged-1 and increases hepatocyte proliferation. Jagged-1 induction is required for





**Fig. 4.** Crosstalk between the Hippo pathway and tumor-related signaling pathways. The Hippo pathway is influenced by and interacts with components of several tumor-related pathways, including the transforming growth factor- $\beta$  (TGF- $\beta$ )/BMP, WNT/ $\beta$ -catenin, PI3K/Akt, SHH, and NOTCH pathways, as indicated. Please see main text for details.

YAP binding to TEAD4 after MST stimulation. Accordingly,  $\gamma$ -secretase inhibitors suppress intestinal dysplasia caused by YAP overexpression in mice.<sup>(14)</sup> Thus, YAP and NOTCH may be therapeutic targets for gastrointestinal cancer.

### Regulation and Tumor-Related Roles of Hippo Core Components

**MST1/2 kinases. MST activation.** Unlike *Drosophila* Hippo kinase, mammalian MST1/2 contain cleavage sites for caspase-3 and caspase-6/7. Cleavage at these sites induced by stresses such as heat shock, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) or UV generate cleaved MST1/2 proteins (~36 kDa) that have lost the autoinhibitory domain and gained catalytic activity. These shorter MST proteins also localize in the nucleus due to the removal of nuclear export signals. In mouse splenocytes or embryonic fibroblasts, MST1/2 are usually present as 55–60 kDa proteins that are rarely phosphorylated at the autophosphorylation site (Thr183/Thr180) required for full activation. Accordingly, these full-length MST kinases show little activity and are localized in the cytoplasm. In contrast, in mouse liver, most MST1/2 molecules are 36 kDa and abundantly phosphorylated.<sup>(26)</sup> The mechanism by which MST1/2 are phosphorylated and cleaved in the liver is unclear.

MST1/2 are also controlled by RASSF proteins (Fig. 2). *Drosophila* RASSF has 10 orthologs in humans (RASSF1–10) but only RASSF1–6 bind to MST1/2. RASSF1 and RASSF5 each have two splice variants. The longer splice variants, RASSF1A and RASSF5A, activate MST1/2 and sustain Hippo signaling. The RASSF1A-MST1 complex normally localizes at microtubules and regulates the cell-cycle and microtubule dynamics. The RASSF1A-MST2 complex promotes MST2 activity by enhancing its autophosphorylation and preventing its degradation. RASSF1A also protects MST1/2 against dephosphorylation and inactivation by PP2A. RASSF6 binds to MST2 but antagonizes Hippo signaling. Interestingly, the RASSF1 gene is localized at 3p21, a chromosomal region frequently deleted in human lung tumors. Notably, RASSF1A is one of the most frequently epigenetically silenced TSGs in human cancers.<sup>(27)</sup>

MST1 activity can be enhanced by PH-domain and leucine-rich-repeat protein phosphatase (PHLPP) via a RASSF1A-independent mechanism (Fig. 2). MST1 cleavage is normally

prevented by AKT-mediated phosphorylation of Thr387,<sup>(24)</sup> but PHLPP reverses this phosphorylation by binding to MST1 in its PP2C domain. Thus, PHLPP functions as a TSG through its effects on AKT. In mice, PHLPP1 loss cooperates with PTEN loss and p53 mutation to promote prostate carcinogenesis.

**Downstream of MST.** The MST/SAV1 complex activates LATS, which phosphorylates YAP/TAZ. MST1 also phosphorylates FOXOs, promoting their nuclear retention and transcriptional activity. Naive T cells from *MST1* knockout (KO) mice exhibit increased reactive oxygen species (ROS) and decreased expression of the FOXO targets catalase and SOD2. However, no reduction in either FOXO protein or phosphorylation is seen in *MST1* KO T cells or in *MST1/2* double KO (DKO) thymocytes.<sup>(28,29)</sup> Further examination is thus required to determine whether FOXOs are truly regulated by MSTs *in vivo*. Lastly, MST1/2 overexpression drives JNK/SAPK, p38, and histone H2B phosphorylation *in vitro*, but *in vivo* confirmation is lacking.<sup>(30)</sup>

**Tumor-related phenotypes in MST mutant mice.** Germline inactivating mutations of *MST1* result in an immunodeficiency syndrome both in mice and humans.<sup>(29)</sup> However, tumorigenesis has been reported only in mice bearing conditional tissue-specific *MST* mutations. For example, liver-specific *MST1/2* DKO mice show decreased YAP phosphorylation and hepatomegaly, and develop liver tumors by age 5–6 months.<sup>(26,31)</sup> These mutants accumulate oval cells (adult liver stem cells) in their hepatic periportal regions, resulting in enlarged livers and hepatomas. *NF2* and *SAV1* mutants have a similar phenotype. It is still unclear whether LATS1/2 are essential for tumor suppression by the Hippo pathway, at least in liver. One study showed loss of MST1/2 decreased MOB1 and YAP phosphorylation but had no effect on LATS1/2 phosphorylation,<sup>(26)</sup> while another study found that levels of phosphorylated YAP and LATS1/2 were reduced in *MST1/2* DKO liver.<sup>(31)</sup>

Mice lacking MST1/2 specifically in the intestinal epithelium show expansion of an undifferentiated cell population, enlarged crypts, and loss of secretory cells. These mutants die about 13 weeks after birth due to colonic adenomas.<sup>(26)</sup> *MST1/2* DKO intestinal epithelial cells exhibit reduced YAP phosphorylation but enhanced YAP protein and nuclear localization.

Keratinocyte-specific *MST1/2* DKO mice have no abnormalities and show normal YAP phosphorylation and activation.<sup>(8)</sup> This result was unexpected because *MOB1* KO (see below) and YAP transgenic mice both develop skin carcinomas. Thus, YAP may be phosphorylated by an MST-independent mechanism in keratinocytes.

**SAV1.** The binding of MST2 to the adaptor protein SAV1 allows MST2 to phosphorylate both SAV1 and LATS1 and enhance LATS1 activity. *SAV1* KO mutants develop hyperplasia of epithelial progenitors and are embryonic lethal.<sup>(32)</sup> Skin cells of keratinocyte-specific *SAV1* KO mice show impaired Ca<sup>2+</sup>-stimulated MST1/2 phosphorylation and nuclear translocation.<sup>(32)</sup> Liver-specific *SAV1* KO mice exhibit hepatomegaly and expanded oval cells, and eventually develop HCCs and cholangiocellular carcinomas.<sup>(31,33)</sup> YAP nuclear translocation occurs in *SAV1* KO oval cell-like cholangiocytes but not in hepatocytes, suggesting that SAV1-regulated MST1/2 activity may be required for YAP nuclear translocation in specific cell types.<sup>(33)</sup>

**LATS1/2 kinases. LATS1/2 activation.** MST1/2-mediated phosphorylation of LATS1/2 (Thr1079/Thr1041) is necessary but not sufficient to activate these kinases.<sup>(34)</sup> LATS1/2 must also bind to MOB1 to induce autophosphorylation of the serine residues (Ser909/Ser872) critical for complete LATS1/2 activation. LATS2 (Ser83/Ser380) are also phosphorylated by Aurora-A, leading to LATS2 localization at the mitotic

spindle. Similarly, LATS1 (Ser613) is phosphorylated by Cdc2/cyclinB during mitosis. However, whether YAP is involved in LATS1/2-mediated control of mitotic progression is unknown. PP2A may modulate LATS activity in this context since okadaic acid activates LATS1/2.

At the transcriptional level, p53 induces LATS2 mRNA in response to nocodazole or constitutive H-Ras expression. LATS2 transcripts are also regulated by microRNAs (e.g. miR-31, miR-372, miR-373) that are oncogenic when they inhibit LATS2 expression in lung, esophageal, gastric, or testicular tumors. At the protein level, WWC1(KIBRA) and/or NF2 stabilizes LATS2 by inhibiting its ubiquitination. Mitotic stress or constitutive H-Ras activation can also lead to LATS stabilization and nuclear accumulation, whereas LATS1 protein is destabilized by sustained ROS-PKC $\delta$  signaling or activation of the E3 ubiquitin ligase ITCH.

**LATS1/2 functions.** YAP and TAZ are the only substrates of LATS kinases identified to date. However, LATS1/2 directly or indirectly affect many other molecules involved in cell proliferation, apoptosis, senescence, migration, and/or mitosis, and do so in a YAP/TAZ-dependent or -independent manner. For example: (i) LATS1/2 arrest cells in G1/S or G2/M by inhibiting the kinase activities of the cyclinE/CDK2 and cyclinA/B-CDC2 complexes in a mechanism that may involve YAP.<sup>(35)</sup> (ii) LATS1 induces apoptosis by upregulating p53 and Bax while downregulating Bcl-XL and Bcl-2. (iii) LATS2 is involved in cellular senescence induced by oncogenic H-Ras-V12 or p16-RB-ROS activation. (iv) LATS1 interacts with zyxin, which is important for actin polymerization, and co-localizes with F-actin and LIMK1 at the contractile ring.<sup>(36)</sup> By binding to LIMK1, LATS1 inhibits its kinase activity and thus its effects on actin dynamics, cell migration and mitosis. (v) LATS2 associates with the microtubule-associated protein Ajuba, which then recruits  $\gamma$ -tubulin to centrosomes during normal mitosis.

LATS1/2 are key regulators of checkpoints required to maintain genomic stability. For example: (i) loss of *LATS1/2* results in centrosomal amplification and impaired spindle assembly. *LATS1/2* DKO cells therefore show loss of diploidy, chromosome misalignment, enlarged nuclei, presence of micronuclei, and defects in cytokinesis.<sup>(37,38)</sup> (ii) *LATS2* KO cells exhibit decreased expression of Aurora-B and PLK1,<sup>(37)</sup> while loss of LATS1 increases the production of LIMK1-induced polynucleated cells, leading to cytokinesis failure.<sup>(36)</sup> (iii) In response to DNA damage, LATS1/2 induce p53 by inhibiting MDM2, ensuring proper function of the G1 tetraploidy checkpoint.

In addition to these functions, LATS2 binds to the androgen receptor and inhibits its regulation of target genes, and microarray studies suggest that LATS1/2 participate in the RAS, p53 and WNT pathways.

**Tumor-related phenotypes in *LATS1/2* mutant mice.** The majority of *LATS1* KO mice die during embryogenesis. Survivors develop soft tissue sarcomas and ovarian stromal cell tumors.<sup>(39)</sup> All *LATS2* KO mutants die as embryos exhibiting failed cytokinesis and fragmented centrosomes.<sup>(37,38)</sup>

**NDR1/2 kinases.** NDR1/2 are mammalian orthologs of Tricornered (Trc), which regulates *Drosophila* morphogenesis. Full activation of mammalian NDR1/2 requires phosphorylation by MST1-3, which is facilitated by phospho-MOB1 or phospho-MOB2 with the assistance of the scaffold protein Furry<sup>(40)</sup> (Fig. 2). NDR1/2 mediate apoptosis downstream of RASSF1A and MST1 by an unknown mechanism. NDR1 also supports centrosome duplication early in S phase, and promotes chromosome attachment to the spindle in M phase.<sup>(41)</sup>

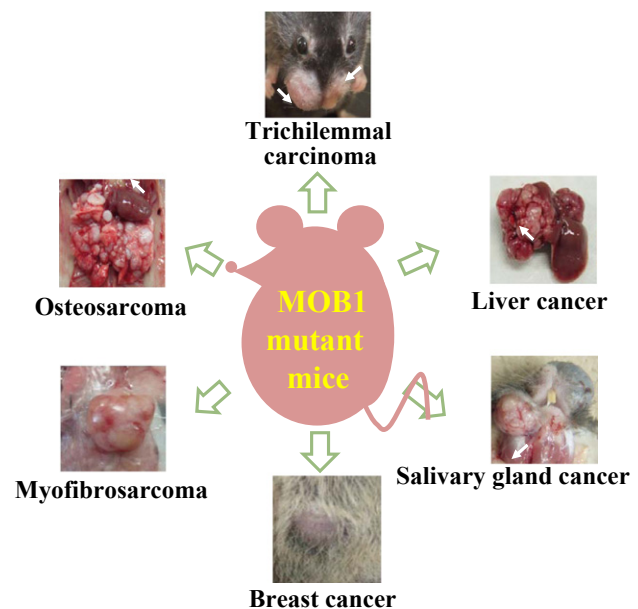
*NDR1* KO T cells show normal apoptosis in response to proapoptotic stimuli, possibly due to compensation by

increased NDR2 protein. However, mice deficient for NDR1 are prone to developing T cell lymphomas.<sup>(42)</sup>

**MOB1. MOB1 functions.** The MOB genes were originally discovered in yeast by searching for mutations interfering with the mitotic exit network and the separation initiation network pathways.<sup>(43)</sup> In mammals, there are four MOB families; MOB1A/1B (orthologs of *Drosophila* Mats), MOB2, MOB3A/3B/3C, and MOB4. MOB1A/1B are scaffold adaptor proteins that coactivate LATS1/2 and NDR1/2 kinase activities. Interaction with MOB1A/1B also enhances LATS1/2 and NDR1/2 phosphorylation by MSTs, downregulating proliferation.<sup>(44)</sup> MOB2 competes with MOB1 for binding to NDR1/2 and inhibits NDR1/2, but does not bind to LATS1/2. MOB3A/3B/3C and MOB4 proteins do not appear to regulate LATS or NDR kinases.

To interact with LATS1/2 and NDR1/2, MOB1 (Thr12/Thr35) must be phosphorylated by MST1/2. In *MST1/2* DKO mutants, MOB1 phosphorylation is abolished in all tissues.<sup>(26,29)</sup> *In vitro*, MOB1A overexpression inhibits mitosis, whereas suppression of MOB1A or MOB1B increases proliferation or impairs mitotic exit. *MOB1A/1B* DKO mice are early embryonic lethal (~E6.5) due to a defect in primitive endoderm formation.<sup>(45)</sup>

**Tumor-related phenotypes in *MOB1* mutant mice.** Mice with a partial loss of MOB1A/1B functions (i.e. homozygous for a MOB1A mutation and heterozygous for a MOB1B mutation, or the reverse), develop a variety of tumors, but trichilemmal carcinomas are most prominent (Fig. 5). All tumors in MOB1-deficient mutants show loss of the WT MOB1 allele, suggesting increased genomic instability.<sup>(45)</sup> *MOB1A/1B* DKO keratinocytes exhibit hyperproliferation, impaired contact inhibition, enhanced progenitor self-renewal, and increased centrosomes. Total LATS1/2, phospho-LATS1/2 and phospho-YAP proteins are reduced due to a post-transcriptional mechanism. Interestingly, MOB1-deficient mice show the most severe phenotype and develop the broadest range of tumors among strains lacking a Hippo signaling component, suggesting that MOB1s are key molecules in the Hippo pathway that may act on additional targets. Indeed, MOB1



**Fig. 5.** Tumors observed in MOB1 mutant mice. MOB1A/1B DKO mice show one or more of the indicated tumor types.

reportedly binds to TSSC1, NUP98, HDAC3, TRAF6, DIPA, and DOCK8.

Like keratinocyte-specific *YAP* transgenic mice,<sup>(8)</sup> keratinocyte-specific *MOB1*-deficient mutants show expanded basal epidermal progenitors. However, while *YAP* transgenic mice develop SCCs, *MOB1*-deficient mice developed trichilemmal carcinomas.<sup>(8)</sup> This difference may be explained by the fact that transgenic *YAP* expression occurred before HF morphogenesis, whereas the *MOB1A/1B* genes were deleted after birth (after HF formation). Examination of human tumors has shown that *YAP* is activated in many human trichilemmal carcinomas, some of which also exhibit *MOB1A/1B* inactivation.<sup>(45)</sup>

## Conclusion

Because the Hippo pathway controls cell proliferation, apoptosis, contact inhibition, cell migration, cell differentiation, stem cell self-renewal, genetic stability, and EMT, impaired Hippo signaling leads to altered organ size and the onset, development, and metastasis of cancers. The Hippo pathway is frequently inactivated in a range of tumor types, emphasizing its non-redundant and powerful functions in opposing oncogenesis and increasing its attractiveness as a target for new anti-cancer therapies. However, the kinases of the Hippo pathway are important tumor suppressors, precluding their selection as targets for the development of small-molecule inhibitor drugs. An alternative therapeutic target may be *YAP*. *YAP* overexpression is associated with tumorigenesis, and cancers arising due to inactivation of Hippo signaling can be largely suppressed by heterozygous

*YAP* deletion.<sup>(6)</sup> Certain porphyrin derivatives, such as verteporfin (VP) and protoporphyrin IX (PPIX) reportedly inhibit *YAP*-TEAD association and *YAP*-induced liver overgrowth.<sup>(46)</sup> Verteporfin is now in clinical use for photocoagulation therapy of age-related macular degeneration. Further screening to identify compounds blocking the interaction between *YAP* and its target transcription factors may lead to more effective anti-tumor drugs targeting the Hippo pathway. In addition, because GPCR signaling affects the Hippo pathway (Fig. 1), antagonists or inhibitors of *S1P*, *LPA* or  $\beta$ -adrenergic receptor signaling, or agonists of glucagon, epinephrine, or the dopamine receptor, may accomplish the same goal. For a recent review on Hippo pathway targeting as a therapeutic strategy, please refer to Harvey *et al.*<sup>(13)</sup>

In conclusion, continued molecular exploration of the Hippo pathway is likely to expose additional players or interactions that regulate its functions. We are optimistic that fresh insights will emerge which will both increase our understanding of various human cancers and open new avenues of exploitation for cancer treatment.

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## Disclosure Statement

The authors have no conflict of interest.

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