Ubiquitination-deubiquitination in the Hippo signaling pathway (Review)

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Abstract. The Hippo signaling pathway is considered to be a tissue growth regulator and tumor suppressor pathway that controls cell proliferation, differentiation, survival, regeneration and tissue homeostasis. Defects in Hippo kinases and hyperactivation of transcriptional co-activator with PDZ-binding motif and Yes-associated protein (YAP) may contribute to the development of different types of cancer. The Hippo pathway is regulated in a variety of way, of which ubiquitination is of considerable importance. Ubiquitination is a crucial post-translational protein modification in cancer cells and is an applicable target for pharmacological intervention. Ubiquitin modifications are involved in regulating various physiological processes and are counteracted by deubiquitination. Imbalanced ubiquitination-deubiquitination is closely associated with tumor initiation and progression. Therefore, the examination of the specific association between the Hippo pathway and ubiquitination is of interest. The present study reviews the modulatory mechanism of ubiquitination-deubiquitination in the Hippo signaling pathway, the recent progress in identifying therapeutic targets and strategies, and the future directions in the field that may contribute to better tumor diagnosis and treatment.

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Abbreviations: AIP4, atrophin-1 interacting protein 4; AMOT-p130, angiomotin-p130; AMOTL, angiomotin-like; β-Trcp, β-transducin repeat-containing E3 ubiquitin protein ligase; CHIP, C terminus of Hsp70 interacting protein; CRB, Crumbs homolog; CRL2, cullin 2-RING ubiquitin ligase; CRL4, cullin 4-RING ubiquitin ligase; DCAF1, DDB1 and CUL4 associated factor 1; DDB1, DNA damage-binding protein 1; DUB, deubiquitinase; KIBRA, kidney and brain protein; LATS, large tumor suppressor; LRR1, leucine-rich repeat protein 1; MARK, microtubule affinity-regulating kinase; MOB1, MOB kinase activator 1; MST, mammalian STE20-like protein kinase; NEDD4, neural-precursor-cell-expressed developmentally downregulated 4; NEDD4L, NEDD4-like; NEDL2, NEDD4-like ubiquitin protein ligase 2; NF2, neurofibromin 2; PTPN14, protein tyrosine phosphatase non-receptor type 14; RASSF, Ras association domain family; RUNX, RUNT-related transcription factor; SAV1, Salvador homolog 1; SCF, Skp1-Cullin1-F-box; SIAH2, seven in absentia homolog 2; TAO, thousand and one amino acid protein; TAZ, transcriptional co-activator with PDZ-binding motif; TEAD, TEA domain-containing sequence-specific transcription factor; USP, ubiquitin-specific protease; VGLL4, vestigial-like family member 4; WBP2, WW domain-binding protein 2; WWP1, WW domain containing E3 ubiquitin protein ligase 1; YAP, Yes-associated protein

Key words: Hippo pathway, ubiquitination, deubiquitination, cancer diagnosis, therapeutic strategies

Contents

1. Introduction

The Hippo signaling pathway was originally discovered using genetic screens of *Drosophila melanogaster* (1) and is highly conserved from *D. melanogaster* to mammals (2). It has been a focus of research in the past due to its fundamental role in modulating cell proliferation and differentiation, apoptosis, cell survival and migration (3,4). The Hippo pathway is considered to be a tissue growth regulator and tumor suppressor (5). A number of studies have demonstrated that dysregulation of the Hippo pathway closely correlates with tumor initiation, progression and the acquisition of drug resistance (6-12). Therefore, there is considerable interest in and speculation about targeting the Hippo pathway to treat human cancer. However, the activity and stability of the pathway components contribute to internal environmental homeostasis and are regulated in various ways, including through ubiquitination.

Ubiquitination-deubiquitination is a common and vital post-translational modification that serves critical roles in protein degradation and localization, autophagy, antigen presentation, signal transduction and other cellular processes (13,14). Ubiquitination-deubiquitination is primarily regulated by the

E3 ubiquitin ligase and deubiquitinases (DUBs), which target the pathway components, mediate their turnover and activity, and lead to different biological effects (15). The balance between ubiquitination and deubiquitination is essential for maintaining normal functional output of the Hippo pathway. Impairment of this balance may result in severe pathological states, including cancer. Here, the modulatory mechanism of ubiquitination-deubiquitination in the Hippo pathway, the recent progress in identifying therapeutic targets, and strategies and future directions in the field are reviewed.

2. Overview of the Hippo pathway

The Hippo signaling pathway, also termed the Salvador/Warts/Hippo (SWH) pathway, was initially identified through genetic mosaic screens of *D. melanogaster* by its suppression of tissue overgrowth and was named after one of its key signaling components, the protein kinase Hippo (Hpo) (1). The pathway comprises a large network of proteins that have coordinating functions. At the heart of the Hippo pathway in *D. melanogaster* is a kinase cassette consisting of two serine/threonine kinases known as the Hippo and Warts kinases, along with their regulatory proteins Salvador and Mats (3). In mammals, the Hippo kinases are known as mammalian STE20-like protein kinase 1 (MST1; also known as STK4) and MST2 (also known as STK3), whereas the Warts kinases are known as large tumor suppressor 1 (LATS1) and LATS2 (4). In addition, Salvador homolog 1 (SAV1) and MOB kinase activator 1A/B (MOB1A/B) are homologs of Salvador and Mats, respectively (6). Yes-associated protein (YAP) and the transcriptional co-activator with PDZ-binding motif (TAZ; also known as WWTR1) are two transcriptional co-activators (Yorkie homologs) that are the principal downstream effectors and are negatively regulated by the Hippo pathway (16).

A variety of upstream stimuli, including apicobasal polarity, mechanotransduction, cell-cell adhesion, cellular stress, contact inhibition and extracellular signaling, are able to activate the Hippo pathway (17). Canonically, in mammals, the Hippo pathway is switched on when activated MST1/2 phosphorylates SAV1 and they subsequently phosphorylate and activate MOB1A, MOB1B, LATS1 and LATS2, resulting in the phosphorylation of YAP and TAZ. Phosphorylated YAP and TAZ bind to the 14-3-3 protein, resulting in their cytoplasmic retention and proteasomal degradation in a β-transducin repeat-containing E3 ubiquitin protein ligase (β-Trcp)-dependent manner (4). YAP and TAZ are transcriptional co-activators that lack the ability to directly bind DNA, although they form complexes with TEA domain-containing sequence-specific transcription factors (TEADs) as their primary partners (16,18). YAP/TAZ also bind to other transcription factors, including tumor protein p73 (19), mothers against decapentaplegic homologs (SMADs) (20-22), forkhead box protein M1 (23), T-box transcription factor 5 (24,25), RUNT-related transcription factor 1 (RUNX1), and RUNX2 (26), along with other factors, to regulate target gene expression. The transcription cofactor vestigial-like family member 4 (VGLL4) competes with YAP and TAZ for binding with TEADs to suppress the co-activators of YAP and its target gene expression (27). By contrast, when the Hippo pathway is switched off, MST1, MST2, LATS1 and LATS2 are inactivated, resulting in hypophosphorylated YAP and TAZ and their accumulation in the nucleus, where they associate with TEADs instead of VGLL4 to promote the expression of target genes, including connective tissue growth factor (CTGF), amphiregulin, cysteine-rich angiogenic inducer 61, and surviving (28-30). A similar mechanism of action has been identified in *D. melanogaster* (31); however, for simplicity, the present review focused primarily on the mammalian Hippo pathway (Fig. 1).

In addition to the aforementioned key components, there are a number of other vital branches of the Hippo pathway involved in regulating functional outputs. For example, MST kinases may be phosphorylated and activated by thousand and one amino acid protein (TAO) kinase (32), microtubule affinity-regulating kinase (MARK) (33), and kidney and brain protein (KIBRA; also known as WWC1) (34). In addition to MST, the mammalian misshapen homolog mitogen-activated protein kinase kinase kinase kinase (35), the apical membrane-associated FERM-domain protein neurofibromatosis 2 (NF2) (36), angiomotin (AMOT) (37), and even heat-shock proteins including HSP90 (38), are all able to activate LATS kinases, whereas members of the junction-associated Ajuba protein family directly repress LATS activity (39). LATS, WW domain-binding protein 2 (WBP2) (40), and protein tyrosine phosphatase non-receptor type 14 (PTPN14) (41) each contribute to the regulation of YAP and TAZ in different ways. Notably, the regulatory mechanisms controlling the activity of the Hippo pathway appear to vary depending on the specific cells and tissues.

Further examination of the Hippo pathway in murine embryonic stem cells and induced pluripotent cells has demonstrated that YAP is capable of inhibiting cell differentiation, expanding stem cell populations, and reprogramming differentiated cells to more primitive states (42). In human embryonic stem cells, TAZ seems to act in a similar manner (21). Additional examples, including stem and progenitor cells of the liver, intestine, heart and skin, support the function of YAP and TAZ in cell stemness, pluripotency and differentiation (43-48), which implies their potential roles in repair and regeneration. Furthermore, a growing body of evidence reveals pivotal roles for the Hippo pathway in growth control in cells. In *D. melanogaster*, inhibition of the Hippo or Warts kinases, or overexpression of Yorkie, results in marked overgrowth phenotypes leading to oversized tissues (49,50). In mammals, deregulation of the Hippo pathway, including through the inactivation of LATS or the overexpression of YAP, induces liver and heart hypertrophy in mice (51,52). It is hypothesized that there are two mechanisms leading to the overgrowth phenotype. First, normal cell proliferation may be disrupted, resulting in rapid cell proliferation, even when the tissue reaches its proper size. Second, ectopic cells may become insensitive or resistant to apoptotic signals (53). Notably, deregulation of the Hippo pathway does not cause overgrowth of every tissue and organ, for example the skin (47), indicating other functions of the pathway.

Uncontrolled overgrowth tends to be a primary step in neoplasia. Indeed, a large number of studies have reported that dysregulation of the Hippo pathway is observed at a relatively high frequency in various human carcinomas. For instance, overexpression of YAP1 in mice leads to the development

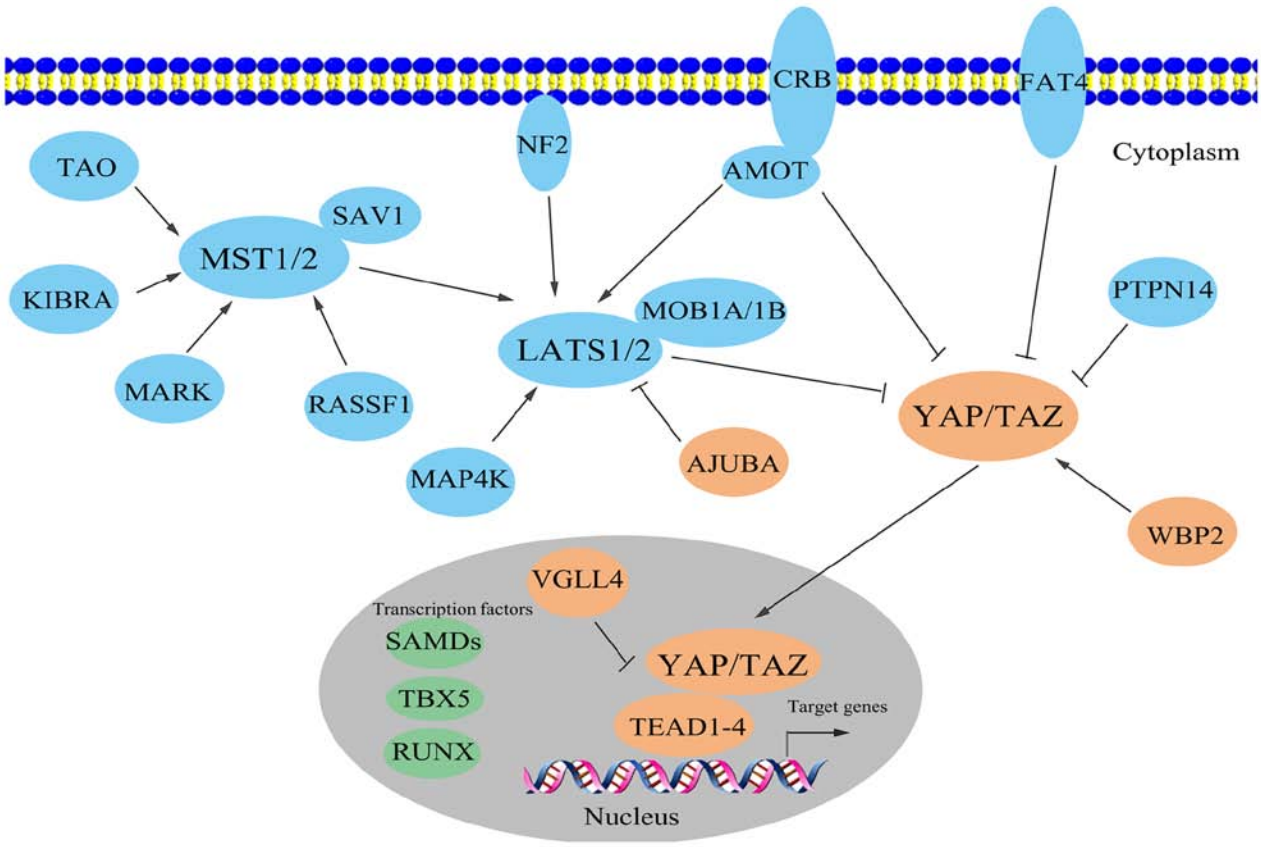

Figure 1. Brief graphical presentation of the mammalian Hippo pathway. The Hippo pathway is composed of a large network of proteins that function in a coordinated manner. Putative oncoproteins (blue) and putative tumor suppressors (orange) are illustrated, along with a number of other transcription factors (green) involved in the Hippo pathway, apart from TEAD1-TEAD4 and VGLL4. AMOT, angiomotin; CRB, Crumbs; KIBRA, kidney and brain protein; LATS, large tumor suppressor; MAP4K, mitogen-activated protein kinase kinase kinase kinase; MARK, microtubule affinity-regulating kinase; MOB1, MOB kinase activator 1; MST, mammalian STE20-like protein kinase; NF2, neurofibromin 2; PTPN14, protein tyrosine phosphatase non-receptor type 14; RASSF1, Ras association domain family 1; RUNX, RUNT-related transcription factor; SAV1, Salvador homologue 1; TAO, thousand and one amino acid protein; TAZ, transcriptional co-activator with PDZ-binding motif; TBX5, T-box transcription factor 5; TEAD, TEA domain-containing sequence-specific transcription factor; VGLL4, vestigial-like family member 4; WBP2, WW domain-binding protein 2; YAP, Yes-associated protein.

of hepatocellular carcinoma (HCC) (43) and a deficiency in MST1/2 kinases causes YAP hyperactivation, marked liver overgrowth and the development of HCC (54). TAZ is upregulated and stabilized by SKI like proto-oncogene, which enhances its oncogenic activity and epithelial-mesenchymal transition (EMT) (55). Elevated YAP expression resulting from the downregulation of LATS contributes to cell proliferation and invasion in gastric cancer (56). However, the exact mechanisms associated with tumorigenesis and progression are not completely understood.

The Hippo pathway is modulated in various ways, and diverse modulations exert different effects on physiological and pathological processes. First, in common human cancer types, somatic and germline mutations in Hippo pathway genes are exceptionally rare (6). However, evidence of mutations in Hippo pathway genes is beginning to emerge, owing to the rapid advancements in large genomic sequencing projects. Tumor-associated mutations have been identified in LATS2 in esophageal cancer and non-small-cell lung cancer (57,58), and mutations occur in LATS1 and LATS2 in basal cell carcinoma of the skin (59). NF2 (also known as Merlin) is considered to be a tumor suppressor, and mutations in the NF2 gene correlate with neurofibromatosis type II (53). In addition to mutations, loss of heterozygosity, genomic deletions and promoter hypermethylation all contribute to the ectopic activity of the Hippo pathway at the DNA level. For example, loss of LATS1 heterozygosity has been reported in ovarian and breast cancer (60-62). In renal cell carcinoma, downregulation of LATS1, at least in part by promoter hypermethylation, leads to YAP overexpression, resulting in tumor initiation and progression (63). FAT atypical cadherin 4 (FAT4), an important member of the FAT cadherin protein family, is a critical regulator of the Hippo pathway and probably acts as a tumor suppressor (64). Genomic deletion and promoter hypermethylation of FAT4 have been detected in gastric cancer and breast cancer (65,66), respectively.

Second, microRNAs (miRNAs), small noncoding RNAs of 21-25 nucleotides in length that negatively modulate gene expression at the post-transcriptional or translational level (67), are closely associated with the regulation of the Hippo pathway at the RNA level and with carcinogenesis. Mature forms of miRNAs bind to the 3'-untranslated region (UTR) of target mRNAs and cleave and/or hinder their translation (68). Studies have demonstrated that in endometrial cancer, miRNA 31 is able to bind the 3'-UTR of LATS2 mRNA, causing the downregulation of LATS2, and promoting YAP accumulation in the nucleus and the transcription of cyclin D1 (69). Another miRNA, miRNA (miR)-129-5p, is also able to directly inhibit

YAP and TAZ expression in a similar manner, leading to the inactivation of TEAD transcription and a reduction in CTGF and cyclin A, offering a possible explanation for its tumor suppressive ability in ovarian cancer (70). Additionally, it is noteworthy that there exists a YAP-miR-130a-VGLL4 positive feedback loop in the control of organ size and tumorigenesis (71). miR-130a is induced by YAP and represses VGLL4, promoting the formation of the YAP-TEAD complex and enhancing YAP activity, which has been confirmed in liver cancer and glioblastoma (71,72). In addition to miRNAs, long noncoding RNAs (lncRNAs) are also involved in the regulation of the Hippo pathway. These are noncoding transcripts of >200 nucleotides in length that interact with DNA, RNA and proteins, and are associated with numerous human diseases, including tumors (73,74). In gastric cancer, lncRNA p21 (lincRNA-p21) is capable of negatively regulating YAP expression. Knockdown of lincRNA-p21 results in elevated expression levels of YAP mRNA and protein in a Hippo-independent manner, with the opposite effects observed with increased expression of lincRNA-p21 (75). However, the precise underlying mechanism remains to be determined. Another type of noncoding RNA, circular RNA, usually has miRNA sponge functionality and interacts with RNA-binding proteins associated with tumorigenesis (76,77). A recent report revealed that by sponging miR-424-5p and modulating LATS1 expression, circular RNA_LARP4 suppresses cell proliferation and invasion in gastric cancer (78), indicating an indirect regulatory mechanism of the Hippo pathway.

Third, post-translational modifications (PTMs) have attracted considerable attention in recent years due to their crucial roles in mediating the activation and subcellular localization of signaling components, which results in the induction, strengthening or repression of the corresponding signaling pathways. An increasing number of reports have revealed that the Hippo pathway is regulated by a variety of post-translational modifications, including phosphorylation, acetylation, methylation and ubiquitination, whose imbalance and malfunction are involved in tumor formation and progression (79). Among the various types of post-translational modifications, phosphorylation may be regarded as the most common. In the canonical Hippo pathway, phosphorylation of LATS1/2 promotes the phosphorylation of YAP/TAZ, leading to YAP/TAZ cytoplasmic retention, degradation via ubiquitination, and non-expression of relevant target genes (4). There are multiple phosphorylation sites on proteins responsible for diverse biological effects, which are activated by various enzymes. For instance, YAP has five currently identified sites (T119, S138, T154, S317 and T362) that may be phosphorylated by JUN N-terminal kinases (JNK1 and JNK2), triggering YAP to serve a dual role under different circumstances (25,80). Furthermore, phosphorylation of MST1 at T120 and T387 by PI3K/Akt prevents caspase-mediated cleavage of MST1, thereby inhibiting its activation (81,82). JNK1 facilitates MST1-mediated pro-apoptotic signaling by phosphorylating MST1 at S82 (83).

Additionally, acetylation has been demonstrated to specifically modify YAP at highly conserved C-terminal lysine residues catalyzed by the nuclear acetyltransferases CREB binding protein and p300 in response to S(N)2 alkylating agents, which may be reversed by the nuclear deacetylase NAD-dependent protein deacetylase sirtuin-1 (SIRT1) (84,85). The regulation of YAP by SIRT1-mediated deacetylation may be associated with HCC tumorigenesis and drug resistance (85).

Methylation of non-histone proteins is another essential regulatory mechanism that controls the functions of proteins. It has been reported that Set7 forms a complex with YAP and directly monomethylates YAP at lysine 494, which is beneficial for its cytoplasmic sequestration (86). This indicates a methylation-dependent checkpoint in the Hippo pathway. O-GlcNAcylation, catalyzed by O-GlcNAc transferase (87), is a notable type of post-translational modification and has been associated with tumorigenesis by mediating signal transduction, transcription, metabolism and other cellular functions (88-90). In a recent study, researchers reported that O-GlcNAcylation of YAP at Thr241 within the WW domain is responsible for high-glucose-induced liver oncogenesis (91). This kind of modification antagonizes the phosphorylation of YAP at Ser127, partly by preventing the binding of LATS to YAP, and enhances its stability and pro-tumorigenic capacity (91).

Apart from the PTMs described above, ubiquitination-deubiquitination has emerged as an important type post-translational modification. Due to this, an introduction to ubiquitination-deubiquitination and its modulation of the Hippo pathway is provided below.

3. Overview of ubiquitination and deubiquitination

Ubiquitin (Ub) is a highly conserved small protein consisting of 76 amino acids with a molecular mass of ~8.5 kDa that is widely expressed in eukaryotes (92). The process by which one or more molecules of ubiquitin bind target proteins via enzyme catalysis is called ubiquitination, with its fundamental function being protein degradation (93). The ubiquitin-proteasome system (UPS) modulates 80-85% of all protein degradation in eukaryotic cells. It comprises ubiquitin, ubiquitin activating enzymes (E1), ubiquitin conjugating enzymes (E2), ubiquitin protein ligases (E3), the proteasome and the substrate (target protein) (94). In general, ubiquitination involves two stages. The first stage includes ubiquitin activation by an E1 enzyme using ATP as an energy resource, followed by the transfer of the activated ubiquitin to an E2 enzyme via a trans(thio) esterification reaction. Finally, an isopeptide bond is created between a lysine of the target protein and the C-terminal glycine of ubiquitin. This step requires an E3 enzyme to transfer ubiquitin to the target protein. Additional ubiquitin molecules may be added to the substrate by binding to the initial ubiquitin, yielding a polyubiquitin chain. The second stage involves the recognition of the activated target protein by the 26S proteasome, which subsequently degrades the protein into small peptides, typically of 3-24 amino acids in length, and releases the ubiquitin for cyclic utilization (95).

The process in which numerous lysine residues of the target protein are ubiquitinated is termed multiubiquitination. Ubiquitin has seven lysine residues, K48, K63, K6, K11, K27, K29 and K33, which may serve as polyubiquitination points. Polyubiquitination brings about different functional consequences depending on whether the polyubiquitin chains are linked through K48, K63 or other K residues of ubiquitin (96).

For instance, K48-linked polyubiquitination targets substrates in the 26S proteasome for destruction, whereas K63-linked polyubiquitination is able to stabilize proteins, direct their translocalization and transmit signals associated with intracellular trafficking, cell signaling, ribosomal biogenesis and DNA repair (97-99).

Ubiquitination is an important post-translational modification. Besides regulating protein degradation (93), it also serves important roles in modulating the activity and localization of proteins, receptor-mediated endocytosis and the transportation of lysosomes (100), insulin levels (101) and the transforming growth factor (TGF)-β pathway (102). Thus, various cellular biological processes, including gene expression, cell proliferation and differentiation, cell senescence and apoptosis, autophagy, antigen presentation, signal transduction, DNA repair and immune responses are all associated with ubiquitination to varying degrees (103).

It has been demonstrated that ubiquitination is a reversible process that is counteracted by deubiquitinating enzymes (DUBs). DUBs recognize specific sequences of ubiquitin, its substrates or the proteasome, and primarily remove ubiquitin from the target proteins to recycle the ubiquitin, to inhibit and proofread ubiquitination, or to alter the activity state of proteins (104). Based on their Ub-protease domains, DUBs are divided into five subclasses consisting of ubiquitin C-terminal hydrolases, ubiquitin-specific proteases (USPs), ovarian tumor-related proteases, JAB1/PAB1/MPN-domain-containing metallo-enzymes, and Machado-Joseph disease protein domain proteases (105). Likewise, deubiquitination also functions in metabolism, stress responses, inflammation, immunity and other cellular activities.

An increasing number of studies have revealed that ubiquitination and deubiquitination are associated with the pathogenesis of numerous diseases, including cancer (106-113). These processes serve as promoters and/or suppressors of cancer initiation and progression by regulating cell proliferation and differentiation, cell migration, the cell cycle, signal transduction and DNA repair (114,115). It is generally considered that the ectopic expression of oncogenes and anti-cancer genes contributes to tumorigenesis. Specifically, if tumor suppressors and oncoproteins are not properly sequestered or eliminated, it may lead to neoplasia. For instance, p53 is an anticancer protein known as the 'guardian of the genome' (116). Murine double minute chromosome 2 (MDM2) acts as an E3 enzyme and marks p53 for proteasomal degradation (117). MDM2 expression is increased in numerous tumors and is a crucial reason for the decreased expression of wild-type p53 in malignancies (118). The ubiquitin-specific proteases USP7 (also known as HAUSP) and USP42 can also cleave ubiquitin from p53, thereby protecting it from proteasome-dependent degradation via the ubiquitin ligase pathway (119,120). Another classical example is the nuclear factor-κB (NF-κB) signaling pathway, which is ubiquitous in eukaryotic cells and is regarded as a facilitator of cancer initiation (121). NF-κB inhibitor (IκB) functions as a tumor suppressor by preventing the entry of NF-κB into the nucleus, and thus preventing the activation of the downstream signaling pathways (122). β-Trcp is a member of the E3 protein family that targets IκB for degradation, and activates NF-κB signaling to drive cell malignant transformation (123). Conversely, USP15 inhibits IκB turnover (124) and cylindromatosis downregulates TNF receptor associated factor 6 to repress this pathway (125). Additionally, USP10, USP11, USP22 and USP48 are overexpressed in malignant lymphoma, and USP17 expression is elevated in esophageal and cervical carcinoma (126).

Notably, enzymes involved in ubiquitination and deubiquitination may serve as either oncoproteins or tumor suppressors, depending on the functional output of their substrate. For example, USP2 stabilizes fatty acid synthase (FAS), an apoptotic protein, and MDM2 via deubiquitination, leading to p53 degradation, escape from apoptosis and the development of drug resistance in prostate cancer cells (127). On the other hand, USP2 has also been reported to be downregulated in breast cancer (128), implying that it may be a tumor suppressor. Taken together, it may be considered that ubiquitination and deubiquitination are closely associated with cancer and serve roles in oncogenesis and tumor progression via different mechanisms. Considering the importance of the Hippo pathway in cancer, it is of interest to investigate the precise association between ubiquitination-deubiquitination and the Hippo pathway.

4. Ubiquitination-deubiquitination in the Hippo pathway

There are multiple components of the Hippo pathway, including LATS1/2, YAP/TAZ, AMOT and VGLL4 which are crucial and have been extensively studied. Each of these is described below, in addition to a number of other components (Figs. 2 and 3; Tables I and II).

LATS1/2. LATS kinases have gained much research interest owing to their wide range of activities in numerous biological processes. During mammalian evolution, a genomic duplication event led to the emergence of two paralogs (LATS1 and LATS2) (129). These are serine/threonine kinases of the AGC subfamily sharing extensive sequence similarity within their kinase domain (85% similarity) situated at the C terminus of the proteins, where they each contain a hydrophobic motif (130). In addition, the N terminus contains two stretches of conserved amino-acid sequences (LCD1 and LCD2) and ubiquitin-associated (UBA) domains that bind to ubiquitinated proteins. LATS1 harbors a proline-rich domain, whereas a PAPA repeat exists in LATS2. In addition, LATS2 contains one PPxY (P, proline; X, any amino acid; Y, tyrosine) motif and LATS1 contains two (131,132). There also exist a number of phosphorylation, auto-phosphorylation and ubiquitination sites on the two proteins at different locations. It is hypothesized that the structural distinctions between LATS1 and LATS2 are conducive to their divergent regulation and functions (133).

LATS1 and LATS2 are notable regulators of cell fate and are associated to cancer initiation and progression by mediating the functions of oncogenic or anti-tumor effectors during the processes of the cell cycle, apoptosis, migration and EMT (134). The most well-known mechanism is that of LATS1/2 as tumor suppressors, which restrict YAP/TAZ activity in the classical Hippo pathway (2). However, beyond the Hippo pathway, studies have determined that LATS1 is able to interact with HSPA2 and FKBP5 to modulate the estrogen signaling pathway (135). In breast tissues, LATS2 controls estrogen receptor (ER) activity (136); whereas, in the

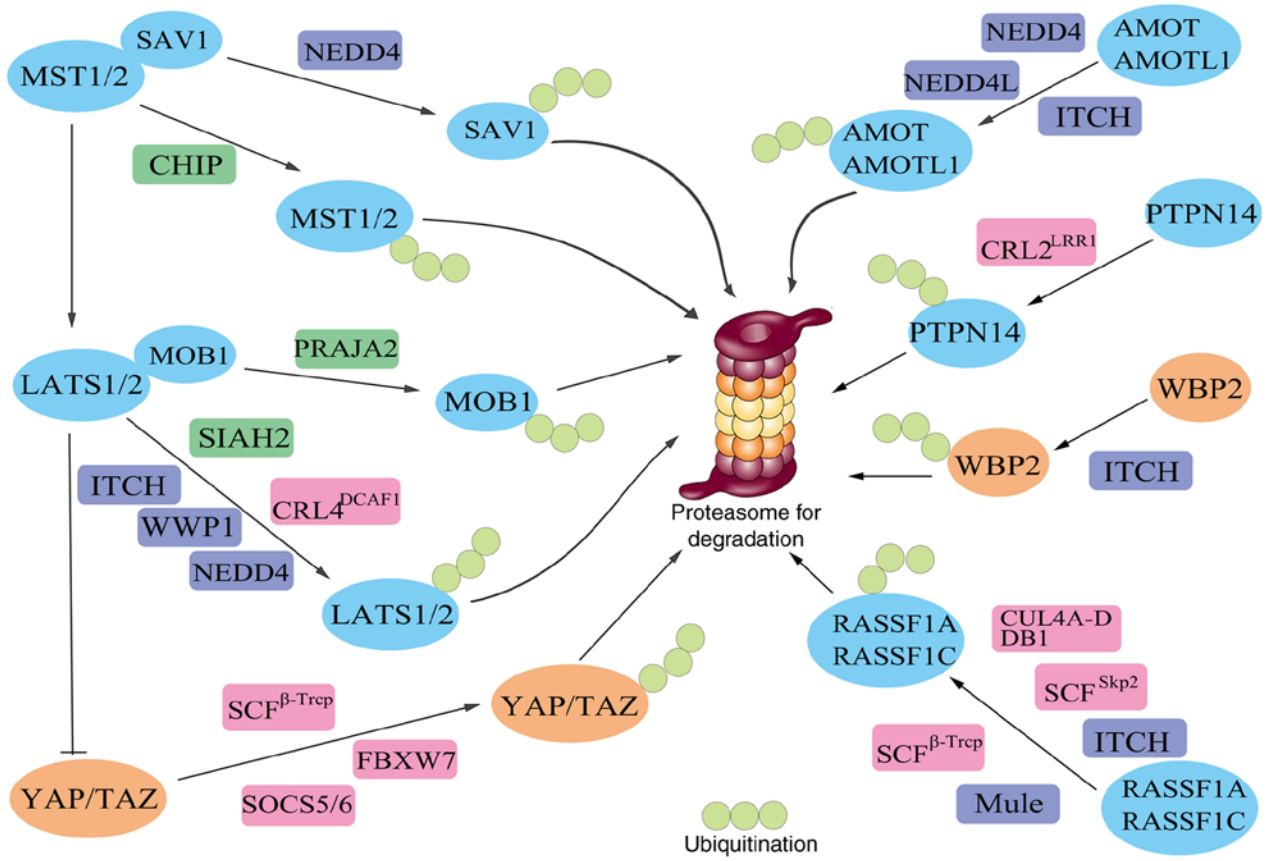

Figure 2. Ubiquitination and proteasomal degradation of components of the Hippo pathway. E3 ubiquitin ligases (dark blue, green and pink) transfer ubiquitin (light green) to the Hippo pathway components (blue and orange), which are subsequently recognized by the 26S proteasome and targeted for proteolysis. AIP4, atrophin-1 interacting protein 4; AMOT-p130, angiomotin-p130; AMOTL, angiomotin-like; β-Trcp, β-transducin repeat-containing E3 ubiquitin protein ligase; CHIP, C terminus of Hsp70 interacting protein; CRL2, cullin 2-RING ubiquitin ligase; CRL4, cullin 4-RING ubiquitin ligase; DCAF1, DDB1 and CUL4 associated factor 1; DDB1, DNA damage-binding protein 1; LATS, large tumor suppressor; LRR1, leucine-rich repeat protein 1; MOB1, MOB kinase activator 1; MST, mammalian STE20-like protein kinase; NEDD4, neural-precursor-cell-expressed developmentally downregulated 4; NEDD4L (NEDD4-2), (neural-precursor-cell-expressed developmentally downregulated 4)-like; NEDL2, NEDD4-like ubiquitin protein ligase 2; PC2, polycystin 2; PTPN14, protein tyrosine phosphatase non-receptor type 14; RASSF1, Ras association domain family 1; SAV1, Salvador homologue 1; SCF, Skp1-Cullin1-F-box; SIAH2, seven in absentia homolog 2; TAZ, transcriptional co-activator with PDZ-binding motif; WBP2, WW domain-binding protein 2; WWP1, WW domain containing E3 ubiquitin protein ligase 1; YAP, Yes-associated protein.

prostate, androgen receptor chromatin binding and transcriptional activities are restrained by LATS2 (137). Furthermore, LATS kinases have been demonstrated to suppress the activity of ER by promoting its degradation (138). All these findings support the inhibitory roles of LATS in breast and prostate cancers. In addition, LATS2 associates with p53, proliferating cell nuclear antigen, Aurora A and other proteins that are involved in cell cycle metabolism (135). Strikingly, loss of LATS1/2 kinases has been reported to result in the induction of anti-cancer immune responses that decrease tumor growth and improve vaccine efficacy (139), revealing a critical role of the Hippo pathway in modulating tumor immunogenicity. The wide range of regulatory functions of LATS1/2 are of great importance in biological homeostasis. More detailed studies of LATS1/2 may provide effective methods for the detection and treatment of cancer.

In recent years, ubiquitination has been identified as a mode of post-translational modification shared by LATS1 and LATS2 proteins. According to their structural characteristics, E3 ubiquitin ligases are divided into two main subfamilies, RING finger and HECT (140). The neural-precursor-cell-expressed developmentally downregulated 4 (NEDD4)-like ubiquitin ligase family belongs to the HECT subfamily, consisting of E3 ubiquitin-protein ligase Itchy homolog (ITCH), NEDD4, NEDD4-2, WW domain containing E3 ubiquitin protein ligase 1 (WWP1), WWP2, E3 ubiquitin-protein ligase SMURF1 (Smurf1), Smurf2, NEDD4-like ubiquitin protein ligase (NEDL)1 and NEDL2 in humans (141). Each member of this subfamily contains a Ca^{2+}/lipid-binding (C2) domain associated with membrane localization, 2-4 WW domains conferring substrate specificity, and a HECT-type ligase domain contributing to the catalytic E3 activity (142,143). An increasing number of studies have indicated that members of the NEDD4-like ubiquitin ligase family interact with LATS1/2, thereby affecting the activity and functional outcomes of the Hippo pathway.

The ITCH ligase contains four WW domains and physically interacts with LATS1, primarily through binding of its first WW domain (WW1) to the PPxY motifs of LATS1, leading to LATS1 ubiquitination and degradation by the 26S proteasome (144). ITCH-mediated turnover of LATS1 results in increased cell proliferation, decreased apoptosis, greater tumorigenesis and increased EMT, which may be associated in part with the accumulation of nuclear YAP and its enhanced

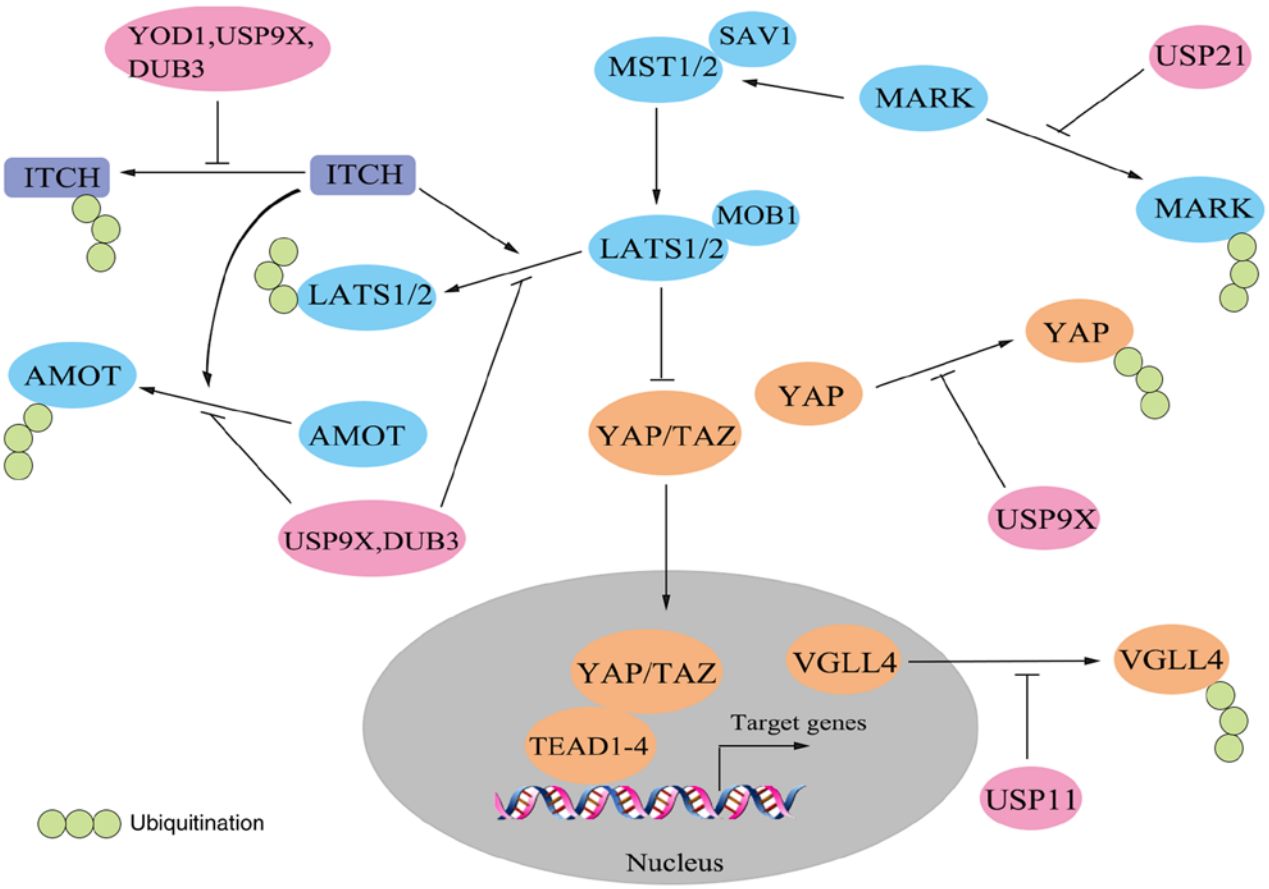

Figure 3. Deubiquitination in the Hippo pathway. Deubiquitinases (pink) counteract the ubiquitination of ITCH and Hippo pathway components (blue). AMOT, angiomotin; DUB, deubiquitinase; LATS, large tumor suppressor; MARK, microtubule affinity-regulating kinase; MOB1, MOB kinase activator 1; MST, mammalian STE20-like protein kinase SAV1, Salvador homologue 1; TAZ, transcriptional co-activator with PDZ-binding motif; TEAD, TEA domain-containing sequence-specific transcription factor; USP, ubiquitin-specific protease; VGLL4, vestigial-like family member 4; YAP, Yes-associated protein.

transcriptional coactivation function (144). Furthermore, activation of the Hippo pathway, for example by MST activation, upregulates ITCH and thereby promotes the ITCH-LATS1 interaction, which indicates the presence of a negative feedback loop (144). Other studies have demonstrated that overexpression of ITCH in breast cancer cells is associated with increased tumor formation and progression, and that in cases of invasive and metastatic breast cancer, ITCH expression is strikingly elevated (145). Thus, it may be inferred that ITCH exerts a tumor-boosting function by triggering LATS1 degradation and YAP activation, and may be considered a biological marker and therapeutic target in breast cancer. Likewise, WWP1, another member of the NEDD4-like ubiquitin ligase family, negatively regulates LATS1 turnover in a similar manner, which is also crucial for enhanced cell proliferation in breast cancer cells (146). In contrast to the ITCH-LATS1 interaction, LATS1 primarily binds to WW domains 1-3 of WWP1 through its two PPxY motifs (146). However, in another study, NEDD4 (also termed NEDD4-1) was reported to control intestinal stem cell homeostasis by regulating WW45 (SAV1) and LATS1/2, which are polyubiquitinated by NEDD4 and degraded by the 26S proteasome, resulting in the inactivation of the Hippo pathway (147). Additionally, these findings reveal that NEDD4 modulates LATS directly and indirectly. In general, WW45 associates with its binding partners through either the WW domains or the C-terminal Sav-Ras association domain family (RASSF)-Hpo (SARAH) domain (148). In one study, NEDD4 and WW45 were demonstrated to interact with each other directly through their N-terminal regions (147). In contrast to WW45, the C-terminal region of NEDD4 likely serves important roles in its interaction with LATS2 (147). Furthermore, NEDD4 is capable of destabilizing LATS1 (147), although the underlying interaction and mechanism remain unclear and merit additional investigation.

In addition to the distinct E3 ligase NEDD4-like ubiquitin ligase, seven in absentia homolog 2 (SIAH2), which is an important component of the hypoxia response pathway (149), promotes LATS1 and LATS2 degradation in a UPS-dependent manner to stimulate YAP activity (150). Hypoxia is known to be a common feature of solid tumors (151). Low SIAH2 levels with stabilized LATS2 and upregulated pYAP suppress tumorigenesis in a xenograft mouse model (150), indicating that the SIAH2-LATS2 axis likely serves a role in tumor formation. Amino acids 403-480 and amino acids 667-720 are two critical regions of LATS2 that are responsible for the SIAH2-LATS2 interaction and contain a relatively larger number of lysine residues in LATS2, among which Lys 670 and Lys 672 are two key sites that contribute to SIAH2-mediated ubiquitination and proteolysis (150). According to the structural similarity of LATS1 and LATS2, it may be hypothesized that an analogous binding mechanism is involved in the SIAH2-LATS1 interaction, although this requires further study.

Table I. Summary of the ubiquitination in the Hippo pathway.

Author, year	Components of the hippo pathway	E3 ubiquitin ligases	Interaction	Effects	(Refs.)
Salah *et al*, 2011; Yeung *et al*, 2013	LATS1	ITCH, WWP1	WW-PPxY	LATS1 ubiquitination and degradation, YAP/TAZ activation	(144,146)
Bae *et al*, 2015		NEDD4	Uncertain	LATS1 ubiquitination and degradation, YAP/TAZ activation	(147)
Ma *et al*, 2015		SIAH2	Uncertain	LATS1 ubiquitination and degradation, YAP activation	(150)
Li *et al*, 2014		$CRL4^{DCAF1}$	Ubiquitination sites-DCAF1	LATS1 poly-ubiquitination and degradation in the nucleus, YAP/TAZ activation	(152)
Bae *et al*, 2015	LATS2	NEDD4	Uncertain	LATS2 ubiquitination and degradation, YAP/TAZ activation	(147)
Ma *et al*, 2015		SIAH2	Lysine residues of LATS2 are involved	LATS2 ubiquitination and degradation, YAP activation	(150)
Li *et al*, 2014		$CRL4^{DCAF1}$	Ubiquitination sites-DCAF1	LATS2 oligo-ubiquitination and inactivation in the nucleus, YAP/TAZ activation	(152)
Bae *et al*, 2015	WW45(SAV1)	NEDD4	N-terminal regions	WW45 ubiquitination and degradation, the Hippo pathway inactivation	(147)
Zhao *et al*, 2010	YAP	$SCF^{\beta\text{-Trcp}}$	Phosphorylated phosphodegron of YAP (S381 phosphorylation by LATS, S384/387phosphorylation by CK1)-β-Trcp	YAP ubiquitination and degradation	(174)
Tu *et al*, 2014		FBXW7	Uncertain	YAP ubiquitination and degradation	(185)
Hong *et al*, 2015		Elongin B/C-Cullin5- SOCS5/6	YAP-SOCS5/6 degradation	YAP ubiquitination and	(186)
Liu *et al*, 2010	TAZ	$SCF^{\beta\text{-Trcp}}$	Phosphorylated phosphodegron of TAZ (S311 phosphorylation by LATS,S314 phosphorylation by CK1)-β-Trcp	TAZ ubiquitination and degradation	(175)
Tian *et al*, 2007		$SCF^{\beta\text{-Trcp}}$	Phosphorylated S314 by NEK1-β-Trcp	TAZ serves as an adaptor for β-TrCP to promote ubiquitination of PC2	(180)
Huang *et al*, 2012		$SCF^{\beta\text{-Trcp}}$	Phosphorylated S58/62 by GSK3-β-Trcp	TAZ ubiquitination and degradation	(182)
Wang *et al*, 2012	AMOT-p130	NEDD4, NEDD4-2 (NEDD4L),ITCH	L/P-PxY-WW	AMOT-p130 ubiquitination and degradation	(201)
Adler *et al*, 2013		AIP4(ITCH)	L/P-PxY-WW	AMOT-p130 ubiquitination and stabilization, YAP degradation in the YAP-AMOT p130-AIP4 complex	(202)

Table I. Continued.

Author, year	Components of the hippo pathway	E3 ubiquitin ligases	Interaction	Effects	(Refs.)
Wang *et al*, 2012	AMOTL1	NEDD4, NEDD4-2 (NEDD4L),ITCH	L/P-PxY-WW	AMOTL1 ubiquitination and degradation	(201)
Choi *et al*, 2016		NEDL2(HECW2)	K63 ubiquitination site of AMOTL1 is	AMOTL1 increased stability involved	(204)
Kim *et al*, 2016	AMOTL2	Uncertain	K347, K408 ubiquitination sites of AMOTL2 are involved.	AMOTL2 mono-ubiquitination, LATS activation and YAP inhibition in response to cell confluence	(205)
Wang *et al*, 2012	PTPN14	$CRL2^{LRR1}$	PTPN14-LRR1	PTPN14 ubiquitination and degradation, YAP/TAZ activation	(218)
Lim *et al*, 2016	WBP2	ITCH	PPxY-WW	WBP2 ubiquitination and degradation	(222)
Pefani *et al*, 2016	RASSF1A	ITCH	Uncertain	RASSF1A ubiquitination and degradation, allowing YAP1 to interact with SMAD2 and drive target genes transcription	(235)
Song *et al*, 2008		SCF^{Skp2}	Ser203 of RASSF1A is involved	RASSF1A ubiquitination and degradation, promoting the transition from G_1 to S phase	(238)
Jiang *et al*, 2011		CUL4A-DDB1	A region containing amino acids 165–200-DDB1	RASSF1A ubiquitination and degradation during M phase, promoting cell cycle progression	(239)
Zhou *et al*, 2012	RASSF1C	Mule, $SCF^{\beta\text{-Trcp}}$	Uncertain	RASSF1C ubiquitination and degradation in response to stress signals	(240)
Lignitto *et al*, 2013	MOB1	PRAJA2	Uncertain	MOB1 ubiquitination and degradation, attenuation of the Hippo pathway	(243)
Ren *et al*, 2008	MST	CHIP	Uncertain	MST ubiquitination and degradation, attenuation of the Hippo pathway	(245)

AIP4, atrophin-1 Interacting Protein 4; AMOT-p130, Angiomotin-p130; AMOTL, angiomotin-like; β-Trcp, β-transducin repeat-containing E3 ubiquitin protein ligase; CHIP, C terminus of Hsp70 interacting protein; CRL2, cullin 2-RING ubiquitin ligase; CRL4, cullin 4-RING ubiquitin ligase; CUL4A, Cullin-4A; DCAF1, DDB1 and CUL4 associated factor 1; DDB1, DNA damage-binding protein 1; LATS, large tumor suppressor; LRR1, leucine-rich repeat protein 1; MOB1, MOB kinase activator 1; MST, mammalian STE20-like protein kinase; NEDD4, neural-precursor-cell-expressed developmentally downregulated 4; NEDD4L (NEDD4-2), (neural-precursor-cell-expressed developmentally downregulated 4)-like; NEDL2, NEDD4-like ubiquitin protein ligase 2; PC2, polycystin 2; PTPN14, protein tyrosine phosphatase non-receptor type 14; RASSF, Ras association domain family; SAV1, Salvador homologue 1; SCF, Skp1-Cullin1-F-box; SIAH2, seven in absentia homolog 2; TAZ, transcriptional co-activator with PDZ-binding motif; WBP2, WW domain-binding protein 2; WWP1, WW domain containing E3 ubiquitin protein ligase 1; YAP, Yes-associated protein.

In addition, the activity of the nuclear E3 ubiquitin ligase CRL4-DDB1 and CUL4 associated factor 1 ($CRL4^{DCAF1}$), which belongs to the RING-finger subfamily, is repressed by NF2, thereby inhibiting the proteasomal degradation of LATS1 and the ubiquitination-induced conformational alteration in LATS2 (152). $CRL4^{DCAF1}$ comprises the scaffold

Table II. Summary of the deubiquitination in the Hippo pathway.

Author, year	Deubiquitinases	Targets	Effects	(Refs.)
Kim *et al*, 2017	YOD1	ITCH	Stabilizes ITCH, thereby promoting LATS degradation and YAP activation	(154)
Toloczko *et al*, 2017	USP9X	LATS	Deubiquitinates LATS, thereby inhibiting YAP/TAZ activity	(156)
Li *et al*, 2018		YAP1	Deubiquitinates and stabilizes YAP1, thereby promoting YAP1 activity	(189)
Thanh Nguyen *et al*, 2016		AMOT	Deubiquitinates AMOT, thereby inhibiting YAP/TAZ activity	(157)
Mouchantaf *et al*, 2006		ITCH	Antagonizes ITCH auto-ubiquitination	(206)
Nguyen *et al*, 2017	DUB3	ITCH	Antagonizes ITCH auto-ubiquitination	(159)
Nguyen *et al*, 2017		LATS	Stabilizes LATS and increases LATS protein level, thereby inhibiting YAP/TAZ activity	(159)
Nguyen *et al*, 2017		AMOT	Stabilizes AMOT, thereby inhibiting YAP/TAZ activity	(159)
Zhang *et al*, 2016	USP11	VGLL4	Stabilizes VGLL4, thereby inhibiting YAP-TEAD interaction	(214)
Nguyen *et al*, 2017	USP21	MARK	Stabilizes MARK, thereby activating LATS and inhibiting YAP/TAZ activities	(242)

AMOT, Angiomotin; DUB, deubiquitinase; LATS, large tumor suppressor; MARK, microtubule affinity-regulating kinase; TAZ, transcriptional co-activator with PDZ-binding motif; TEAD, TEA domain-containing sequence-specific transcription factor; USP, ubiquitin-specific protease; VGLL4, vestigial-like family member 4; YAP, Yes-associated protein.

protein Cullin 4A (CUL4A), the catalytic subunit Roc1/Rbx1, the adaptor DNA damage-binding protein 1 (DDB1), and the substrate recognition module DCAF1 (DDB1 and CUL4 associated factor 1), which directly binds the ubiquitination sites of LATS via its WD40 domain (153). The number and location of ubiquitination sites differ between LATS1 and LATS2, which may be one of the reasons for the distinct regulatory mechanisms employed by $CRL4^{DCAF1}$ and the consequences of $CRL4^{DCAF1}$ mediation.

Deubiquitination, the inverse process of ubiquitination, is also involved in regulation of the Hippo pathway. Kim *et al* (154) demonstrated using unbiased small interfering RNA screening that the DUB ubiquitin thioesterase OTU1 (YOD1) controls the biological responses mediated by YAP/TAZ. YOD1 deubiquitinates and stabilizes ITCH, contributing to the degradation of LATS1 and the activation of YAP. Overexpression of YOD1 leads to enhanced hepatocyte proliferation and hepatomegaly in a YAP-dependent manner in a transgenic mouse model, and a strong connection exists between YOD1 and YAP expression in patients with liver cancer, which implies that YOD1 may promote tumor initiation (155). In comparison, USP9X (FAM) deubiquitinates LATS kinases to suppress tumor growth (156). The DUB3 family of deubiquitinating enzymes, which includes 25 USP17-like proteins, was identified, using a short hairpin RNA-based screening technique, to be a mediator of YAP/TAZ activity (157,158). Notably, DUB3 antagonizes ITCH auto-ubiquitination, protecting ITCH from degradation. Higher ITCH expression is believed to decrease the levels of LATS kinase; conversely LATS kinases are able to bind to DUB3, resulting in its increased stability and elevated protein expression (159).

In conclusion, ITCH and WWP1 specifically destabilize LATS1. NEDD4 and SIAH2 may target LATS1 and LATS2 for destruction, leading in certain contexts to YAP/TAZ-driven overgrowth and neoplasia. Given that YAP and TAZ are capable of binding LATS1/2 through their WW domains (160), the WW-PPxY interaction between these NEDD4-like E3 ligases and LATS1/2 may reduce LATS1/2 expression levels and displace YAP/TAZ from its PPxY-binding site, possibly causing YAP/TAZ dephosphorylation and increased oncogenic activity. The E3 ubiquitin ligases and DUBs involved in modifying LATS act as oncogenes and/or tumor suppressors, suggesting a new and attractive strategy for the prognosis and treatment of tumors.

YAP/TAZ. YAP and TAZ are regarded as the prime mediators of the major functional outputs of the Hippo pathway, and act as a signaling nexus and integrators of numerous other signaling pathways, including the Wnt, G protein-coupled receptor (GPCR), epidermal growth factor and Notch pathways (17). TAZ shares ~50% sequence identity with YAP (161) and they structurally have much in common. The most prominent feature of YAP or TAZ is the WW domain, which contains two conserved and consistently-positioned tryptophan residues. These domains are responsible for conferring signaling specificity and thereby controlling YAP/TAZ localization and activity (160). In the C-terminal region, YAP and TAZ each contain a PDZ-binding motif that interacts with the PDZ domain of other proteins, including tight junction protein ZO-2 and Na(+)/H(+) exchange regulatory cofactor NHE-RF2 to direct the localization of YAP and TAZ (162,163). An unstructured transcriptional activation domain exists in the extended C terminus of YAP and TAZ, which is likely to control their transcriptional roles (164). They also possess a domain that modulates TEAD transcription factor binding. However, in YAP, this region possesses a PxxΦP motif (x, any

amino acid; Φ, a hydrophobic residue) that is absent in TAZ, resulting in differences between YAP and TAZ interactions with TEAD transcription factors (165). Though YAP and TAZ share numerous structural features, certain distinctions are apparent. For example, YAP possesses two WW domains, whereas TAZ has only one. YAP contains a proline-rich region in the N terminal region, whose interplay with heterogeneous nuclear ribonuclear protein U is involved in mRNA processing, although TAZ lacks this proline-rich region (166). In addition, an SH3-binding motif is present on YAP that does not exist in TAZ (167).

Growing evidence supports the idea that YAP and TAZ are oncogenes in mammalian cells. Overexpression of YAP/TAZ in normal epithelial cells promotes cell transformation and confers a cancer stem cell phenotype (168,169). In mice, upregulated YAP levels enhance tissue hyperproliferation and lead to cancer development in various epithelial tissues (43). Furthermore, as a signaling nexus, YAP and TAZ may be influenced by the aberrant activity of other pathways, including the GTPase KRAS signaling pathway (170), which is critical for tumor development. It is generally considered that YAP and TAZ exert their oncogenic functions by regulating the expression of target genes. Further investigation has suggested that YAP in certain context acts as a tumor suppressor, in part by dampening Wnt signaling (171) and inducing DNA damage-induced apoptosis (172). Considering the fact that YAP and TAZ are able to interact with various transcription factors that have dissimilar functions, it may be inferred that their function as oncogenes may depend on the cellular and signaling context and varies in different types of cancer.

Like LATS, YAP and TAZ are also directly modulated by ubiquitination and deubiquitination. YAP has five consensus HXRXXS motifs in which Ser 127 is phosphorylated following the activation of LATS, leading to 14-3-3 binding and the cytoplasmic retention of YAP (173). This results in YAP being sequestered from the nucleus and being unable to drive target gene expression. In TAZ, the well-studied Ser 89 is required for 14-3-3 binding and cytoplasmic retention (161). Apart from this spatial separation, YAP and TAZ are similarly degraded in a β-Trcp-dependent manner (174,175).

A member of the RING-finger E3 family, Skp1-Cullin1-F-box (SCF), consists of two scaffold proteins (Skp1 and Cullin1), the RING-finger domain protein Rbx1 and an F-box (176). There are a variety of F-box proteins, which may be further subclassified into three families, FBXL, FBXO and FBXW. The FBXW family comprises ten proteins that contain the F-box motif in their N-terminal for interacting with Skp1 (177), and seven WD40 repeats in the C-terminal for substrate specificity and promoting ubiquitination (178). β-Trcp belongs to the FBXW family and is highly conserved with two paralogs, β-Trcp1 (also termed FBXW-7) and β-Trcp2 (also termed FBXW-11) in mammals (179). $SCF^{β\text{-}Trcp}$ functions by recognizing a DSGXXS motif in the substrate. Only when the serine residues are phosphorylated in this motif, which is termed phosphodegron, may $SCF^{β\text{-}Trcp}$ bind to the substrate and thus lead to ubiquitination and degradation of the target protein (179). A recent study has reported that YAP has a DSGXS motif, analogous to the classical DSGXXS motif (174). When the Hippo pathway is switched on, activated LATS kinases phosphorylate YAP on Ser 381 in one of the HXRXXS motifs, priming YAP for subsequent phosphorylation at Ser384 and Ser387 in the C-terminal phosphodegron by casein kinase I (CK1). Phosphorylated phosphodegron is recognized by $SCF^{β\text{-}Trcp}$, resulting in the ubiquitination and proteasome-mediated degradation of YAP, thereby inhibiting the oncogenic activity of YAP (174). In addition, Ser387 phosphorylation is known to be crucial for YAP ubiquitination and is indispensable (174). Likewise, TAZ is recognized by $SCF^{β\text{-}Trcp}$ and degraded via a similar mechanism. LATS and CK1 phosphorylate TAZ at Ser 311 and Ser 314, respectively (175). Furthermore phosphorylation of TAZ at Ser314 by another kinase, serine/threonine-protein kinase NEK1, also recruits β-TrCP; however, under these circumstances, TAZ serves as an adaptor for β-TrCP to promote the ubiquitination of the calcium-permeable cation channel protein polycystin 2 (180), thereby mediating cilia-directed signaling (181). Notably, the N-terminal phosphodegron of TAZ, which is not shared by YAP, also affects the regulation of TAZ protein abundance. It is phosphorylated at Ser58/62 by GSK3, recruiting $SCF^{β\text{-}Trcp}$ and thus triggering TAZ ubiquitination and degradation, which notably does not appear to require prior phosphorylation by LATS (182). Elevated levels of TAZ with increased activation of PI3K signaling has been observed in cancer (183), and activated PI3K may inhibit GSK3. It is therefore possible that TAZ works as a downstream effector of the PI3K pathway to regulate tissue growth and tumor development. Therefore, ubiquitination induced by the either the C-terminal or N-terminal phosphodegron may serve roles in modulating the activity and biological functions of TAZ.

Another FBXW family protein, FBXW7, is regarded as a tumor suppressor in human cancer (184). A recent study demonstrated that decreased expression of FBXW7 is associated with poor clinicopathological features. It induces apoptosis and growth arrest, at least in part, by targeting YAP for ubiquitination and degradation in HCC (185). However, further investigation is required to confirm whether the interaction between FBXW7 and YAP is similar to that between $SCF^{β\text{-}Trcp}$ and YAP, and whether FBXW7 is able to target TAZ for degradation. In addition, YAP protein turnover may be mediated by RAS signaling through regulation of suppressor of cytokine signaling (SOCS)5 and SOCS6 expression (186). SOCS5 and SOCS6 serve as substrate recognition modules of the Elongin B/C-Cullin 5 ubiquitin ligase complex (187) and are able to recruit YAP for ubiquitination. Activated RAS signaling may downregulate SOCS5 and SOCS6, promoting the stability of YAP. In addition, the RAS/mitogen-activated protein kinase pathway acts via phosphorylation of Ajuba to inactivate LATS kinases (188), which causes reduced YAP phosphorylation, $SCF^{β\text{-}Trcp}$-dependent YAP proteolysis and increased YAP activity. RAS exerts its oncogenic functions, at least in part, by these mechanisms.

As for deubiquitination, a recent study determined that USP9X targets YAP1 for deubiquitination and stabilization, thereby promoting breast cancer cell survival and progression (189). Elimination of USP9X upregulates YAP1 degradation and renders cells more sensitive to chemotherapy (189), suggesting an oncogenic role for USP9X and identifying it as a potential therapeutic target in breast cancer treatment.

Collectively, the turnover of YAP and TAZ is primarily controlled by $SCF^{\beta\text{-}Trcp}$, which is of central importance regarding the abundance of YAP/TAZ and the functional outcomes of the Hippo pathway. In addition to $SCF^{\beta\text{-}Trcp}$, FBXW7 and Elongin B/C-Cullin5-SOCS5/6, along with certain other E3 ubiquitin ligase enzymes, have been demonstrated to directly modulate YAP/TAZ degradation and their activity. However, there is still a need to identify whether there are other DUBs involved in counteracting these E3 ligases and rescuing YAP and TAZ from degradation.

Angiomotin (AMOT). AMOT was originally identified as a protein that interacted with angiostatin, an inhibitor of angiogenesis (190). The AMOT family is composed of AMOT, which exists as AMOT-p130 or AMOT-p80, angiomotin-like 1 (AMOTL1), and angiomotin-like 2 (AMOTL2), which are characterized by coiled-coil domains in the N terminus and a consensus PDZ-binding domain in the C terminus (191). AMOT-p130 differs from AMOT-p80 in its N-terminal cytoplasmic extension of 409 amino acids, which is rich in glutamine and mediates the binding of AMOT-p130 to filamentous (F)-actin and cell-cell tight junctions (192). The AMOT family members usually serve as tight junction proteins that control endothelial cell (EC) junction stability and permeability (190) and are expressed predominantly in the endothelial cells of capillaries, in addition to angiogenic tissues such as solid tumors (193). Furthermore, an increasing number of studies have demonstrated that AMOT is able to interact with LATS and YAP/TAZ to exert their regulatory roles in the Hippo pathway.

On one hand, AMOT primarily utilizes its first and second L/P-PxY motifs in the N terminus to directly associate with the WW domains of YAP/TAZ, recruiting them to tight junctions and causing the cytoplasmic retention and decreased activity of YAP/TAZ (194,195). Since AMOT is an F-actin-binding protein, YAP/TAZ and F-actin compete for binding to AMOT (196). On the other hand, the Crumbs homolog (CRB) complex, localized to apical junctions, recruits AMOT, which may directly bind and activate LATS, thereby leading to the downregulation of YAP/TAZ activity (197,198). Additionally, activated LATS kinases phosphorylate AMOT through a conserved HXRXXS consensus site situated in the N-terminal regions of AMOT members, which results in the separation of AMOT from F-actin at the junctions of epithelial cells. This may enhance the interaction between AMOT and YAP/TAZ in the cytoplasm, resulting in the suppression of cell proliferation and tissue growth by AMOT (199). Taken together, AMOT downregulates YAP/TAZ activity in LATS-independent and LATS-dependent manners, and thereby functions as a tumor suppressor. However, a contradictory report has demonstrated that AMOT-p130 enhances YAP-mediated hepatic epithelial cell proliferation and tumorigenesis by promoting the nuclear localization of YAP, and by forming a functional complex with YAP and TEADs on target genes, indicating an oncogenic role of AMOT (200). Different cellular and molecular conditions may provide a possible explanation for this discrepancy, although further analysis is necessary.

Due to the significant roles of AMOT in the Hippo pathway, it is important to determine the modulatory mechanism of AMOT by ubiquitination and deubiquitination. A study demonstrated that three NEDD4-like ubiquitin ligases, NEDD4, NEDD4-2 (also known as NEDD4L) and ITCH, mediate the polyubiquitination of AMOT-p130 *in vivo* (201). Overexpression of NEDD4, NEDD4-2 or ITCH results in the efficient ubiquitination and proteasomal degradation of AMOT-p130, which depend on the interaction between the WW domains of NEDD4-like ubiquitin ligase and the L/P-PxY motifs of AMOT-p130. The short isoform AMOT-p80 cannot be ubiquitinated and degraded by NEDD4-like ubiquitin ligase due to a lack of L/P-PxY motifs (201). According to the pattern similarity between YAP/TAZ-AMOT and AMOT-NEDD4-like ubiquitin ligase, YAP/TAZ may compete with NEDD4 for binding to AMOT-p130. Another study demonstrated that atrophin-1 interacting protein 4 (AIP4; also termed ITCH) ubiquitinates AMOT-p130 in an analogous manner, although it reciprocally stabilizes AMOT-p130, which acts as a scaffold to form a complex in combination with ITCH and YAP. Consequently, ITCH degrades YAP, which is subsequently prevented from binding to LATS1, leading to the inhibition of cell proliferation and tissue growth (202). Collectively, ITCH may promote the degradation and stabilization of AMOT-p130 through ubiquitination, for which the precise mechanism requires investigation.

In addition, NEDD4-like ubiquitin ligases (NEDD4, NEDD4-2, and ITCH) also promote the degradation of AMOTL1 through the interaction with WW-L/P-PxY (201). Notably, cytoplasmic YAP1 is able to recruit the tyrosine kinase c-Abl to phosphorylate NEDD4-2 to maintain the cell tight junctions, thus hampering the NEDD4-2-mediated degradation of AMOTL1, which suggests that a feedback loop may exist between NEDD4-2 and YAP (203). On the other hand, NEDL2 (also termed HECW2) increases the protein stability of AMOTL1 via K63-linked polyubiquitination and enhances endothelial cell junctions through the AMOTL1-YAP pathway (204). With respect to AMOTL2, the ligases NEDD4, NEDD4-2 and ITCH are unable to influence AMOTL2 activity or promote its degradation since a phenylalanine replaces the tyrosine in the third L/P-P-X-Y motif of AMOTL2 compared with that of AMOT-p130 and AMOTL1. This results in the loss of WW domain-binding capacity (201); however, a recent study indicated that AMOTL2 is mono-ubiquitinated at K347 and K408 by a certain, currently unidentified, E3 ubiquitin ligase (205). This ubiquitinated AMOTL2, which may be counteracted by USP9X (157), binds to the LATS UBA domain, activating LATS and leading to YAP inhibition in response to cell confluency.

Regarding deubiquitination, using a cell-based RNA interference screen for YAP/TAZ activity, Thanh Nguyen *et al* (157) identified the DUB USP9X as a negative regulator of YAP/TAZ activity. USP9X deubiquitinates AMOT (AMOT-p130) at lysine 496, and thus protects AMOT from degradation and decreases the activity of YAP and TAZ. With reduced levels of USP9X, AMOT is unable to limit the activity of YAP and TAZ, which may be one of the reasons why low USP9X expression is associated with a number of cancer types; for instance, it is associated with poor outcomes in renal clear cell carcinoma (157). Another deubiquitinating enzyme, DUB3, is a potent tumor suppressor that acts by antagonizing ITCH auto-ubiquitination to prevent its degradation, simultaneously stabilizing LAST and AMOT to inhibit the activity of YAP

and TAZ (159). In fact, USP9X has also been reported to cleave ubiquitin from ITCH, acting as a protective factor (206). Therefore, from two reports (157,159), it appears that the protection of AMOT mediated by DUB3 and USP9X offsets the ubiquitination of AMOT on account of the stabilization and elevated levels of ITCH, although it is unclear whether the consequences are similar in other contexts.

Thus, AMOT regulates the functional outputs of the Hippo pathway primarily by controlling YAP/TAZ activity. The biological effects resulting from the ubiquitination and deubiquitination of different members of the AMOT family vary in diverse contexts, which possibly depends on the specific types of cells and tissues, upstream stimuli, signal transduction, and other factors. It is apparent that this is a complex network.

VGLL4 and other components of the Hippo pathway. VGLL proteins are transcriptional cofactors in the nucleus that are named after the *Drosophila* transcriptional co-activator Vestigial (207). VGLL1-VGLL4 proteins in mammals are able to interact with TEADs via their similar sequences in the TEAD-interacting domain (TDU domain) (207,208). Studies have demonstrated that VGLL proteins are associated with tumorigenesis. For example, the VGLL1-TEAD complex, like the YAP/TAZ-TEAD complex, promotes anchorage-independent cell proliferation by increasing the expression of proliferation-promoting genes, including such as IGFBP-5 (209). Downregulation of VGLL3 leads to a decrease in the proliferation and migration of soft tissue sarcoma (210). Furthermore, VGLL4 is considered to be a growth inhibitor and a common tumor suppressor in various human cancer types, including lung cancer, breast cancer and esophageal squamous cell carcinoma (27,211,212). Mechanistically, VGLL4 contains an extra TDU domain compared with that of VGLL1, VGLL2 and VGLL3, and is able to compete with YAP and TAZ for binding to TEADs through its two TDU domains (27). The extra TDU domain particularly hinders the formation of YAP-TEAD complexes and downregulates the expression of its target genes (27). Furthermore, VGLL4 may promote apoptosis by negatively regulating inhibitor of apoptosis proteins (213).

With respect to the PTMs of VGLL4, deubiquitinating enzyme USP11 is known to stabilize VGLL4 through binding of its USP domain to the N-terminal region of VGLL4 and, in the absence of USP11, cell proliferation and invasion is enhanced in a YAP-dependent manner (214). This suggests that USP11 may also function as a tumor suppressor. However, no studies have currently identified the E3 ubiquitin ligase of VGLL4. This E3 ubiquitin ligase may have an oncogenic function by targeting VGLL4 for proteolysis.

Another Hippo pathway component, tyrosine-protein phosphatase non-receptor type 14 (PTPN14; also known as Pez, PTPD2 and PTP36) is a classical non-transmembrane protein tyrosine phosphatase, and was initially identified as a cytoskeleton-associated protein that serves important roles in cell adhesion and proliferation (215,216). PTPN14 has an N-terminal FERM domain that mediates interactions with proteins at the plasma membrane, a C-terminal phosphatase domain, and central PPxY motifs (217). It has been reported that PTPN14 utilizes its PPxY motifs to bind to the WW domains of YAP, thereby negatively regulating the carcinogenic activity of YAP (218). In addition, PTPN14 may be ubiquitinated. The E3 ubiquitin ligase associated with PTPN14 is termed cullin 2-RING ubiquitin ligase-leucine-rich repeat protein 1 ($CRL2^{LRR1}$) and consists of the scaffold protein Cullin 2, the RING protein Roc1, the adaptor protein complex of Elongin B and Elongin C, and the substrate-recognizing adaptor protein peptidylprolyl isomerase-like 5 (also termed LRR1) (219,220). In response to low cell density, $CRL2^{LRR1}$ targets PTPN14 for degradation, thus promoting YAP nuclear localization and its transactivation activity (218). Additionally, WBP2 acts as an important co-factor of YAP and TAZ and enhances YAP/TEAD-mediated and TAZ/TEAD-mediated gene transcription (40,221). The E3 ligase ITCH mediates the proteasomal degradation of WBP2 to serve as a tumor suppressor, which relies on the interaction between its WW domains and the PPxY motifs of WBP2 (222). Noteworthy, it has been reported that this mode of degradation may be inhibited by WNT3A, contributing to the development of breast cancer (222). However, currently there are no reports regarding the role of DUBs in reversing the ubiquitination of PTPN14 and WBP2.

RASSF consists of two subclasses, C-RASSF and N-RASSF (223,224). Accumulating evidence indicates that C-RASSF proteins RASSF1-RASSF6 regulate the Hippo pathway through interaction with MST via the SARAH domain, which N-RASSF proteins RASSF7-RASSF10 lack (225). RASSF1A and RASSF1C, which are ubiquitously expressed, are the principal transcripts of the seven alternatively spliced variants of the RASSF1 gene, including isoforms A-G (226). Structurally, RASSF1A and RASSF1C contain Ras association and SARAH domains, while RASSF1A also contains a cysteine-rich diacylglycerol-binding C1 domain that is absent in RASSF1C (227). These differences between RASSF1A and RASSF1C may result in their distinctive functions. Ectopic expression of RASSF1A, by either deletions or promoter hypermethylation of the RASSF1 gene, is associated with various cancer types (226). RASSF1A is considered to be a tumor suppressor due to its critical roles in modulating apoptosis, microtubule stability and cell cycle arrest (225). One of the notable and well-known functions of RASSF1A is that it serves as an upstream regulator of the Hippo pathway. By stabilizing MST (228), preventing the dephosphorylation of MST (229), or releasing MST from inhibition by RAF1 and promoting the interaction between MST and LATS (230), RASSF1A activates MST, with SARAH-SARAH interactions between RASSF1A and MST serving a pivotal role (231). In FAS-induced apoptosis, RASSF1A-activated MST2 phosphorylates LATS1, leading to YAP1 phosphorylation and its release from LATS1 in the cytoplasm (230). Consequently, free YAP1 is able to translocate to the nucleus and form a complex with p73, which drives the transcription of pro-apoptotic target genes including BCL2 binding component 3 and BCL2-assocated X, apoptosis regulator (230). It may be assumed that this process is a possible explanation for the tumor-suppressive function of YAP. However, it is notable that RASSF1A is able to utilize its SARAH domain to associate directly with the SARAH domain of SAV, stimulating p73 independently of the canonical Hippo pathway (232). This adds another layer of complexity to the interplay between RASSF1A and p73. Additionally, studies have demonstrated that in cells with methylated RASSF1A, RASSF1C expression

is upregulated and enhances the SRC/YES-mediated phosphorylation of E-cadherin, and the tyrosine phosphorylation of β-catenin and YAP1, causing instability in cell junctions and the transcriptional activation of β-catenin/TBX-YAP/TEAD target genes in the nucleus (233). Also, increased RASSF1C expression with lower expression of RASSF1A may be observed in breast tumors (234). These findings, taken together, indicate that RASSF1C functions as an oncogene.

As for the ubiquitination of RASSF1, it has been demonstrated that in response to TGF-β, RASSF1A is recruited to TGF-β receptor I at the cell membrane where it is ubiquitinated and degraded by E3 ligase ITCH. As a result, YAP1 is able to interact with mothers against decapentaplegic homolog 2, translocating to the nucleus and driving the transcription of TGF-β target genes (235). Furthermore, RASSF1A represses TGF-β-induced cell invasion (235), serving as a tumor suppressor, which is attenuated by ITCH-mediated proteolysis. ITCH targets RASSF5 for degradation through WW-PPxY interactions (236), although the PPxY motif is not present in RASSF1 (237). Thus, it may be hypothesized that there is another mechanism of interaction between RASSF1A and ITCH that requires investigation. Additionally, RASSF1A may be either a positive or a negative modulator of cell cycle progression by mediating the expression of cyclin, cyclin-dependent kinase, cyclin-dependent kinase inhibitors and other relevant cell-cycle components (225). A previous study determined that Skp2, the F-box protein and substrate-recognition component of SCF^{Skp2} ligase, targets RASSF1A for ubiquitination and proteolysis at the G_1/S transition, which requires prior phosphorylation of RASSF1A at Ser203 by cyclin D-Cdk4 (238). With the reduction of RASSF1A, cell cycle progression is accelerated from the G_1 to the S phase, which may contribute to tumorigenesis. Of note, during the M-phase of the cell cycle, RASSF1A associates with the substrate adaptor DDB1 via a region containing amino acids 165-200 and is targeted for degradation by CUL4A-DDB1 E3 ligase, leading to the promotion of cell cycle progression (239). Therefore, it appears that SCF^{Skp2} and CUL4A-DDB1 are able to modulate the expression of RASSF1A during the cell cycle; however, the factors that determine the interaction of RASSF1A with either of the two E3 ligases remain unknown. Furthermore, how decreases in the levels of RASSF1A may affect the Hippo pathway, including the impact on MST, merits further study.

Another isoform, RASSF1C, is a highly unstable protein and is primarily degraded in the nucleus. Ubiquitination has been identified as an important post-translational modification of RASSF1C. Exposure to stress signals, including those induced by ultraviolet irradiation, may activate Mule, a HECT family E3 ligase, and $SCF^{β\text{-}Trcp}$ to target RASSF1C for proteasomal destruction (240). Since the roles of RASSF1C in the Hippo pathway have been rarely reported, it is difficult to assess whether RASSF1C ubiquitination affects the pathway.

While the ubiquitination of RASSF1 has begun to be defined, currently there are no reports regarding the deubiquitination of RASSF1 or the DUBs that may be involved. With deubiquitination being such an important counterbalance to ubiquitination, more research is required to define this process.

MARK family proteins are serine/threonine kinases that have been reported to positively regulate MST and LATS (241). USP21 is able to deubiquitinate MARK proteins to control their stability. The stabilized MARK proteins in turn activate LATS and thereby promote the phosphorylation of YAP and TAZ (242). Furthermore, evidence has demonstrated that USP21 restricts the anchorage-independent growth of transformed primary cells and cancer cell lines, and that its expression is lower in renal clear cell carcinoma samples compared with normal renal cells (242), suggesting that USP21 may be useful as an anti-cancer molecule and a biomarker.

MOB1 is a regulator of LATS in the Hippo pathway that contributes to the complete activation of LATS, and is targeted and degraded by the RING ligase PRAJA2, which attenuates Hippo signaling, enhances YAP-dependent gene transcription and bolsters glioblastoma growth (243). In the canonical Hippo pathway, MST, another core component, is an important upstream activator of LATS. It is hypothesized that MST may also be regulated by ubiquitination. Consistent with this, it has been reported that the C terminus of an Hsp70 interacting protein [E3 ubiquitin-protein ligase CHIP (CHIP)], which is an E3 ubiquitin ligase of the U-box protein family (244), is able to target MST for proteasomal degradation (245). CHIP and its targeting of MST may be repressed during oxidative stress responses by the protein kinase c-Abl (246). The turnover and stability of the upstream regulators of ubiquitination and deubiquitination of MST, including KIBRA and serine/threonine-protein kinase TAO, likely also affects MST and leads to various biological outputs. In general, the ubiquitination and deubiquitination of MST require further study.

5. Conclusions and future perspectives

Ubiquitination and deubiquitination are widespread and important post-translational modifications associated with multiple biological processes. Numerous components of the Hippo pathway are modulated by these two PTMs, whose imbalance is conducive to tumor formation and metastasis. Therefore, it is necessary for cells to strike a balance between ubiquitination and deubiquitination for maintaining homeostasis. While much has been determined about ubiquitination and deubiquitination, a number of issues remain to be resolved.

For instance, LATS1 may be degraded by all NEDD4-like family member ligases, depending on the dosage; however, only the loss of endogenous ITCH and WWP1 increases the protein stability of LATS1, indicating that only ITCH and WWP1 are essential to the maintenance of LATS1 stability (146). However, the underlying mechanism and the effect on LATS2 are currently unknown. Furthermore, it is unclear whether there are additional types of E3 ligases and DUBs that modify Hippo pathway components (including TEAD1-4, NF2, FAT4 and CRB). Certain questions remain, including whether the E3 ligases and DUBs regulate the temporal and spatial organization of the Hippo pathway, and how the Hippo pathway may be used therapeutically in cancer.

Deregulation of the Hippo pathway is associated with cancer, allowing its targeting to be a promising therapeutic strategy. The pivotal roles of YAP and TAZ make them the most attractive targets, and studies have demonstrated that inhibiting YAP and TAZ may be effective in treating a variety of cancer types that are predisposed to YAP/TAZ activation. However, the long-term consequences of YAP/TAZ inhibition on normal and cancerous tissues require further investigation.

Therefore, a better method may be to selectively target the YAP/TAZ-TEAD complex in order to decrease side effects. For example, dobutamine, a β-adrenergic receptor antagonist, is able to recruit YAP from the nucleus to the cytosol, thereby repressing YAP-induced gene transcription. However, this drug has not yet been placed into clinical trials (247).

Verteporfin, a clinical photosensitizer used in photocoagulation therapy for macular degeneration (248), has been reported to interfere in the interaction between YAP and TEAD, and thus to inhibit YAP-induced transcription. Notably, verteporfin has been approved by the US Food and Drug Administration and is capable of blocking mouse hepatic tumorigenesis driven by either YAP1 overexpression or loss of NF2 (249), making it a promising drug in cancer therapy. To date, there has been a phase I/II study of verteporfin photodynamic therapy in locally advanced pancreatic cancer and it has exhibited a partial response (250).

miR-375 is an anti-oncogenic molecule in gastric cancer (GC) that acts, at least in part, by directly targeting YAP1, TEAD4 and CTGF, which may be exploited for treating GC (251). In addition, disruption of the YAP/TAZ-TEAD interaction by stimulating and enhancing VGLL4 expression may be a useful strategy against YAP/TAZ-driven tumors. In line with this concept, a peptide mimicking the function of VGLL4, which acts as a YAP antagonist, has recently been reported to inhibit tumor development in a *Helicobacter pylori* mouse model of GC (252). Such peptides may also be applicable to the treatment of other cancer types, including lung, breast and esophageal cancer. Additionally, based on the immune suppressing effect of LATS, targeting LATS1/2 in cancer immunotherapy may be considered. Furthermore, GPCR signaling regulates the Hippo pathway in multiple ways. Sphingosine 1-phosphate (S1P), serum-borne lysophosphatidic acid and thrombin each work through G12/G13-coupled receptors to inhibit LATS1/2 and activate YAP and TAZ. This has led to attempts to antagonize this signaling in an effort to repress the carcinogenic activities of YAP/TAZ (253). For instance, the S1P-blocking antibody sphinaomab has been reported to decrease lung tumor metastasis (254). Sphingosine kinase 1 (SPHK1) generates S1P. Phenoxodiol is an isoflavone-derived SPHK1 inhibitor that is currently in clinical trial in patients with platinum/taxane-refractory/resistant ovarian cancer, fallopian tube cancer or primary peritoneal cancers (255). MST and other components of the Hippo pathway may also be targeted; however, the therapeutic approach must be designed in light of their specific roles in different contexts.

Research into ubiquitination-deubiquitination in the Hippo pathway has provided additional effective methods of early detection, prognosis and treatment of cancer. Proteasome inhibitors, including bortezomib, have been used in the treatment of relapsed multiple myeloma and mantle cell lymphoma (256); however, they are associated with substantial toxicity due to the inhibition of overall protein degradation. Therefore, in comparison, the targeting of a specific E3 ligase may be a more ideal approach, with a higher level of specificity and potentially less associated toxicity. For example, NEDD4-like ubiquitin ligases are regarded as oncogenes due to their triggering of LATS degradation and promotion of YAP/TAZ-driven gene expression. Thus, drugs may be developed to interfere with the formation of NEDD4-like ubiquitin ligases or disrupt their interaction with LATS. Numerous types of miRNAs have been discovered to target NEDD4-like ubiquitin ligases. For instance, miR-497 exerts an anti-metastatic effect by targeting Smurf1 (257) and miR-1 directly regulates NEDD4/NEDD4L (258). These data imply that miRNAs may be applicable to mediating the function of NEDD4-like ubiquitin ligases and be used in drug development. However, currently there are very few studies on drug development targeting E3 ligases or DUBs. Thus, additional investigation is warranted.

Each type of E3 ligase or DUB usually has multiple substrates, and thus their specificity must be taken into consideration during drug development and targeted therapy for tumors. Beyond therapy, E3 ligases and DUBs may also be useful biomarkers allowing for improved diagnosis and detection of certain cancers. The Hippo pathway has a complex network of crosstalk with other signaling pathways that may also be regulated by ubiquitination-deubiquitination, and that may indirectly affect the Hippo pathway. Furthermore, cancer initiation and progression involve numerous interacting biological processes, of which a number remain unclear. All these facts add to the complexity of tumorigenesis. The field of ubiquitination-deubiquitination as it relates to the Hippo pathway remains in its infancy and further research in this area will undoubtedly contribute to the improvement of cancer diagnosis and therapy.

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Authors' contributions

YL and JD were both involved in the conception of the study. YL was a major contributor in writing the manuscript and JD revised the manuscript. Both authors read and approved the final manuscript, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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