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Review

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Review

The Hippo signaling pathway in liver regeneration and tumorigenesis

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Abstract

The Hippo signaling pathway is an evolutionarily conserved signaling module that plays critical roles in liver size control and tumorigenesis. The Hippo pathway consists of a core kinase cascade in which the mammalian Ste20-like kinases (Mst1/2, orthologs of *Drosophila* Hippo) and their cofactor Salvador (Sav1) form a complex to phosphorylate and activate the large tumor suppressor (Lats1/2). Lats1/2 kinases in turn phosphorylate and inhibit the transcription co-activators, the Yes-associated protein (YAP) and the transcriptional co-activator with PDZ-binding motif (TAZ), two major downstream effectors of the Hippo pathway. Losses of the Hippo pathway components induce aberrant hepatomegaly and tumorigenesis, in which YAP coordinates regulation of cell proliferation and apoptosis and plays an essential role. This review summarizes the current findings of the regulation of Hippo signaling in liver regeneration and tumorigenesis, focusing on how the loss of tumor suppressor components of the Hippo pathway results in liver cancers and discussing the molecular mechanisms that regulate the expression and activation of its downstream effector YAP in liver tumorigenesis.

Key words: Hippo signaling, YAP, liver regeneration, liver cancer

Introduction

The Hippo pathway was originally identified in *Drosophila* through a genetic approach and found to be a conserved regulator of cell proliferation, apoptosis, and organ size in metazoans [1–3]. In *Drosophila*, the core of this signaling pathway is the Hippo kinase. Hippo binds and phosphorylates the scaffold protein Salvador (Sav), to facilitate Hippo-mediated phosphorylation of Warts kinase, as well as the adaptor protein Mats. Yorkie, a downstream transcriptional co-activator, binds to the transcriptional factor Scalloped and enhances the expression of proliferative and pro-survival genes. Activated Warts phosphorylates Yorkie, resulting in its binding to 14-3-3 protein, cytoplasmic retention, and inactivation. Overall, the main function of the Hippo signaling pathway is to inhibit the activation of Yokie. All of the core components of the Hippo signaling pathway are conserved in mammalians. Mst1 and Mst2, the Hippo orthologs in mammalians, utilize the WW45 (Salvador ortholog) to phosphorylate and activate the large tumour suppressor 1 (Lats1)/Lats2 (Warts orthologs) and the co-activator Mps one binder 1 A/B (Mob1A/B, Mats orthologs). Activated Lats1/2 kinases phosphorylate Yorkie orthologs, the transcriptional regulators Yes-associated protein (YAP) or transcriptional co-activator with PDZ-binding motif (TAZ), resulting in their binding to 14-3-3, promoting their nuclear exit and inhibiting the function (**Fig. 1**). Generally, intranuclear YAP and TAZ, and their *Drosophila* counterpart Yorkie, promote cell proliferation, cell viability, and tissue growth by regulating the activity of different transcription factors, including the TEA-domain-containing proteins (TEADs)/Scalloped and Sma- and Mad-related proteins SMADs [4,5]. Compared with the core kinase cascade from Hippo/Mst to YAP/Yki phosphorylation, proteins acting upstream of the Hippo kinase cascade are less well defined. Earlier studies in *Drosophila* have implicated that the apical membrane-associated FERM-domain proteins Merlin and Expanded might act as pathway components upstream of Hippo. Merlin's mammalian orthlog neurofibromatosis 2 (Nf2) is known as a tumor suppressor in mammals [6]. In recent years, numerous literature has

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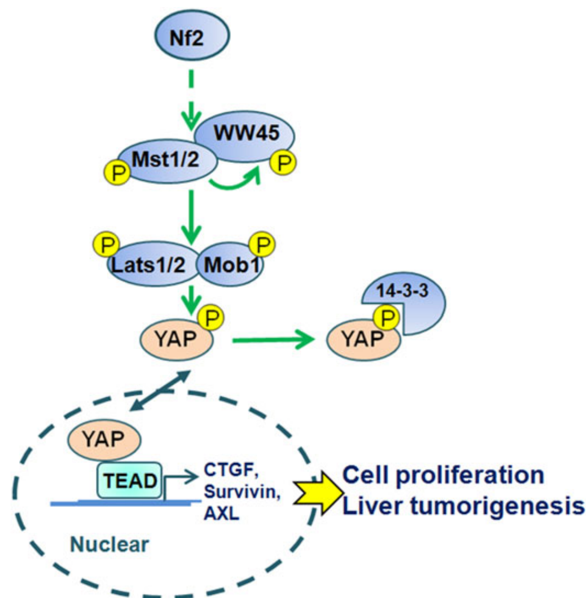

Figure 1. The Hippo signaling pathway in the liver Nf2 is an upstream factor for the activation of the Mst1/2–WW45 complex, which phosphorylates and activates Lats1/2 and the co-activator Mob1A/1B. Activated Lats1/2 kinases that mediated YAP phosphorylation results in YAP binding to 14-3-3, leading to cytoplasmic retention and inactivation of YAP. As a transcriptional co-activator, intranuclear YAP binds to the transcriptional factor TEAD and enhances the expression of down-stream target genes, including CTGF, Survivin, and AXL.

expanded the large protein network of the Hippo signaling, focusing on the regulation of YAP expression and activity that controls tissues' growth during development and regeneration, as well as tumorigenesis.

Liver is a vital internal organ of the digestive system and has a wide range of functions, including detoxification, protein synthesis, glycogen storage, decomposition of red blood cells, and bile acid production. There are two major types of cells in the liver lobes: parenchymal and non-parenchymal cells. The parenchymal cells, also called hepatocytes, make up ~80% of the liver, and carry out the major functions of the liver. Non-parenchymal cells, including bile ductal cells (cholangiocytes), sinusoidal hepatic endothelial cells, Kupffer cells, and hepatic stellate cells, constitute 40% of the total number of liver cells, but with only 6.5% of its volume [7]. Manipulation of Hippo signaling leads to profound changes in liver cell proliferation, apoptosis, and transformation. Loss of YAP in liver results in spontaneous liver necrosis as early as 4 weeks of age [8]. Liver-specific YAP transgenic mouse exhibits apporoximately a 4-fold increase in liver size within a few weeks [9,10]. Furthermore, ablation of Mst1/2, Nf2 and WW45 leads to YAP-dependent hepatomegaly and eventually liver cancers [11–16]. The Hippo signaling pathway has emerged as a critical regulator of liver homeostasis and tumorigenesis.

The Hippo Signaling Pathway in Liver Regeneration and Homeostasis

Adult liver cells are largely quiescent, and the turnover of these cells is estimated to be between 180 and 400 days, which means that liver cells divide once or twice a year [17]. However, the mammalian liver has a tremendous regenerative capacity. Seventy percent of the liver mass lost in a partial hepatectomy (PH) can be restored by hepatocyte proliferation of the remaining liver lobes within a few days. Our study has revealed that the inactivation of Hippo signaling and enhanced YAP expression and activity are critical for the process of liver regeneration [8]. After the PH in mouse, the expression level and activity of YAP were increased within 24 h, before the onset of hepatocyte proliferation, and remained elevated for 72 h. This result was confirmed by a study using a 70% partial PH rat model showing the increased activation of YAP and the decreased activation of Mst1/2 and Lats1/2 one day following the PH, and when the liver size restored, Mst1/2 kinase activation returned to levels observed in quiescent livers [18]. The regulation of dynamic changes in the Hippo signaling pathway and YAP activation might be responsible for the liver homeostasis and maintain the liver-to-body weight ratio.

Hippo signaling is required to maintain the differentiated hepatocytes state and is essential for the cell fate determination in liver [12–14,19]. Over-expression of YAP resulted in hepatocyte hyperproliferation and liver enlargement [2,5], while inactivation of YAP led to loss of hepatocytes and biliary epithelial cells and eventually liver damage [4,9]. Unlike the skin or the intestinal epithelial cells which are renewed by the multipotent stem cells, the differentiated adult hepatocytes are the source of tissue replenishment of cell turnover under physiological conditions in the liver. When the proliferation of hepatocyte is blocked under conditions of extreme stress or chronic injury, a population of small cells emerges from the bile ducts, classically referred to as 'oval cells' or 'atypical ductal cells' and thought to participate in liver repair [20–23]. Oval cells normally are not present in healthy liver. As putative hepatic stem cells, oval cells are able to give rise to both hepatocytes and cholangiocytes as convinced by lineage tracing studies after injury [22,23].

Oval cell expansion is a common phenotype associated with liver size enlargement and liver cancer formation in the liver-specific Mst1/2, WW45 or Nf2 knockout mice, as well as in YAP transgenic animals [24]. These observations suggest that oval cell expansion has resulted from an intrinsic genetic defect rather than from hepatocyte damage or impaired hepatic regeneration and the Hippo pathway is required to repress the oval cell activation in liver. Recent work from Camargo's group showed that hepatocytes might participate in liver repair not only by self-duplication but also by dedifferentiation into progenitor cells or oval cells [19]. In other words, adult hepatocytes have the potential to give rise to not only cells with ductal characteristics, but also cells that molecularly and functionally resemble liver stem cells. The differential transcriptional output of YAP between hepatocytes and progenitor cells indicated that different YAP levels/activities could determine different hepatic cell fates. Combined with the finding of cholangiocyte hypoplasia in mice with a developmental deletion of YAP in the liver, intermediate YAP levels might specify a differentiated ductal cell or cholangiocyte fate. Previous work showed that the absence of differentiated cells in the Mst1/2 null intestine or the YAP over-expressing intestine was associated with the activation of the Notch signaling pathway, which has also been shown to be important for ductal specification during development [25,26] and liver regeneration [27]. Consistently, Camargo's group also demonstrated that YAP/TEAD could directly control the expression of the Notch2 receptor and other Notch pathway genes to modulate Notch signaling, suggesting that there are multiple layers of signaling crosstalk existing during the progenitor/ductal specification in the liver [19], although earlier study from Pan's group showed that Jagged 1, Notch2, and Hes1 expression was not affected in either YAP- and Nf2-deficient livers, indicating that Hippo signaling might regulate the bile duct development independent of Notch signaling [16].

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The Hippo Signaling Pathway in Liver Tumorigenesis

Tumor suppressors of the Hippo pathway in liver cancer

The Mst1/2 are core kinases in the Hippo signaling pathway. Ablation of *Mst1/2* genes in mouse livers was found to result in remarkable liver enlargement at 4–5 weeks age and eventually liver tumors showing features of both hepatocellular carcinoma (HCC) and mixed hepatocellular and cholangiocellular carcinoma (HCC/CC) [1,10,11] (**Table 1**). Acute inactivation of Mst1/2 in the liver is associated with rapid loss of YAP (Ser127) phosphorylation, increased YAP nuclear localization and protein expression. Knockdown of YAP reversed the transformed phenotype of HCC-derived cells from those Mst1/2 knockout mice, and deleting one copy of YAP gene in these mice completely rescued the animal from developing HCC [11]. Previous study showed that YAP was regulated by the ubiquitin-proteosome machinery via interaction with the E3-ligase β-TRCP, and YAP (Ser381) phosphorylation promoted YAP degradation [28], thus the loss of this phosphorylation likely contributed to the rise of YAP protein levels in the Mst1/2 double knockout (DKO) liver. More recently, we identified an Ets family transcription factor called GA-binding protein (GABP) as a critical regulator of YAP expression, and found that the transcriptional activities of GABP are negatively regulated by the Hippo signaling pathway [8]. As a result, the deletion of Mst1 and Mst2 from the mouse liver was accompanied by an increase at the *YAP* mRNA level. In contrast, the GABP-dependent transcriptional activity of the *YAP* promoter was strongly suppressed in an Mst1/2 DKO HCC cell line with reconstitution of Mst1 expression. Furthermore, enhanced YAP expression was correlated with increased nuclear expression of GABP in human liver cancers.

Compared with Mst1/2 mutants, the Sav1/Ww45 liver specific deficient mouse exhibited a milder phenotype by inducing only 1.5-fold liver enlargement at early age and showing a great latency of liver tumor development after 12 months [13,14]. Hyperactivation of YAP in oval cells but not in hepatocytes underlined the only expansion of oval cells but not over-proliferation of hepatocytes in WW45-deficient liver. Thus, almost all of liver tumors presented in heterozygous *Ww45* deficient, the *Ww45*$^{fl/fl}$/*Albumin-Cre* or *Ww45*$^{fl/fl}$/*CAGGS–CreERT* mice exhibited histology characterized of mixed HCC/CC. WW45 inhibits the accumulation and activation of YAP in oval cells to suppress their expansion and prevents liver tumorigenesis.

Nf2 is required for the initiation of gastrulation during embryogenesis and is a well-known tumor suppressor, especially in the nervous system, before identified as an upstream factor of the Hippo pathway [29]. Heterozygous Nf2 deficiency (*Nf2*$^{+/-}$) in mice was sufficient to develop a variety of malignant tumors including HCC in life (10–30 months) [30]. The HCC tumors in the *Nf2*$^{+/-}$ mice showed loss of function at the *Nf2* locus and high metastatic potential. Liver-specific deletion of Nf2 led to marked abdominal enlargement in mice beginning at 6–8 weeks of age due to massive hepatomegaly, with livers representing up to one-third of the total body weight, and eventually resulted in both HCC/CC [15,16]. Consistent with the phenotype observed in the WW45-deficient mice, Nf2-deficient animals yielded a vivid, progressive expansion of oval cells throughout the liver without affecting differentiated hepatocytes. With regard to whether YAP mediates the over-proliferation of Nf2-deficient liver oval cells, two research groups provided controversial results. Pan's group showed that the Nf2–deficient liver phenotypes were largely suppressed by heterozygous deletion of YAP, suggesting that YAP was a major effector of Merlin/Nf2 in growth regulation. Their studies linked Nf2 to mammalian Hippo signaling and implicated YAP activation as a mediator of pathologies relevant to Neurofibromatosis 2 [16]. In contrast, McClatchey's group suggested that Nf2 was not a major regulator of YAP, and YAP did not mediate the over-proliferation of oval cells in Nf2 deficient liver, which was driven by aberrant epidermal growth factor receptor (EGFR) activity [15]. They demonstrated that the liver phenotype of the Nf2–deficient mice was effectively suppressed by treatment with erlotinib, an inhibitor of EGFR kinase, indicating that EGFR activity may be the driver of the oval cell over-proliferation in Nf2–deficient mice. Whether Nf2 downregulation of EGFR signaling is an independent, parallel output of Nf2 in oval cells or involves YAP as an intermediate remains to be defined.

Mob1A/B are the only Mst1/2 substrates identified thus far whose phosphorylation does not require, nor is facilitated *in vivo* or *in vitro* by a scaffold molecule [31,32]. Although the ability of phospho-Mob1A/B to bind and activate Lats1/Lats2 is indisputable, this outcome does not always accompany Mst1/2 activation and Mob1 phosphorylation. Deletion of Mst1/2 from mouse liver resulted in a complete loss of Mob1 phosphorylation, but minimal alteration of Lats1/2 phosphorylation [11]. Recently, Nishio *et al.* revealed the critical roles for Mob1 in development and proliferation control using Mob1A/1B knockout mice [33]. Single-mutant mice bearing a null mutation of *Mob1a* (*Mob1a*$^{\Delta/\Delta}$) or a trapped mutation of *Mob1b* (*Mob1b*$^{tr/tr}$) were viable and fertile and did not exhibit organ

Table 1. Mouse studies of the Hippo pathway deregulation in livers

Gene	Mice	Liver defects and tumorigenesis	Reference
Mst1/2	*GAGGS-CreERT Mst1*$^{-/-}$*; Mst2*$^{fl/-}$ *Mst1*$^{-/-}$*; Mst2*$^{fl/-}$*/Ad-Cre* *Mst1*$^{-/-}$*; Mst2*$^{fl/-}$*/Albumin-Cre* *Mst1*$^{-/-}$*; Mst2*$^{fl/fl}$*/Albumin-Cre*	HCC and CC; hepatomegaly; hepatocytes and oval cell expansion; resistant to Fas/TNFα-induced apoptosis	[11–13]
Ww45	*Ww45*$^{fl/fl}$*/CAGGS–CreERT* *Ww45*$^{fl/fl}$*/Albumin-Cre*	Hepatomegaly; HCC/CC; proliferation of oval cells	[13,14]
Nf2	*Nf2*$^{+/-}$ *Nf2*$^{fl/fl}$*/Albumin-Cre*	HCC; HCC/CC; proliferation of oval cells	[15,16,30]
Mob1	*Mob1a*$^{\Delta/\Delta}$*1b*$^{tr/+}$ *Mob1a*$^{\Delta/+}$*1b*$^{tr/tr}$	50% HCC None	[33]
Yap	*LAP1/tTA-Yap(S127A)* *ApoE/rtTA-Yap* *Yap*$^{fl/fl}$*/Albumin-Cre*	HCC; hepatomegaly in a reversible manner; impaired bile duct development	[10,34]

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overgrowth or tumor development. $Mob1a^{\Delta/\Delta}Mob1b^{tr/tr}$ double knockout mice exhibited early embryonic lethality, while $Mob1a^{\Delta/+}1b^{tr/tr}$ and $Mob1a^{\Delta/\Delta}1b^{tr/+}$ mice were born healthy and fertile, but 52% of them had dental malocclusion. They monitored tumorigenesis in $Mob1a^{\Delta/+}1b^{tr/tr}$, $Mob1a^{\Delta/\Delta}1b^{tr/+}$, and $Mob1a^{\Delta/+}1b^{tr/+}$ (control) mice over 70 weeks and found that various tumor types arose spontaneously in 100% of $Mob1a^{\Delta/+}1b^{tr/tr}$ and $Mob1a^{\Delta/\Delta}1b^{tr/+}$ mice, but in only 4% of controls. Loss of the wild-type *Mob1* allele was confirmed in all tumors. The liver cancers were found only in $Mob1a^{\Delta/\Delta}1b^{tr/+}$ mice with 50% incidence, but not in $Mob1a^{\Delta/+}1b^{tr/tr}$ mice. This observation implied that Mob1A might play a more vital role in liver. However, the molecular mechanism of Mob1A/B regulation in liver, the role of YAP in *Mob1a/1b* mutant liver and the redundancy role of Mob1A and Mob1B remain to be further investigated.

The regulations of oncogene YAP in liver cancer

YAP coordinates regulation of cell proliferation and apoptosis and plays an essential role in liver cancer development. The first indication that YAP is important in liver cancer development came from studies using a mouse liver cancer model initiated from p53-null liver progenitor cells over-expressing c-Myc [34]. Recurrent amplification of the chromosomal locus 9qA1 harboring *YAP* gene was found in HCC developed from the above-mentioned engineered cells. In parallel, similar analyses on human HCC samples were conducted which revealed a focal amplification chromosome 11q22, a region that is syntenic to 9qA1 locus in the murine, revealing consistent over-expression of YAP associated with human HCC. Meanwhile, Haber's group also showed that YAP was amplified in human and mouse tumors and YAP over-expression induced epithelial–mesenchymal transition (EMT) in mammary epithelial cells and colony formation on soft agar and inhibited apoptosis [35]. Extension and striking confirmation in the animal level were provided by the generation of transgenic mice engineered to over-express YAP with the doxycycline-inducible promoter [9,10]. YAP expression induced in adult mice, either ubiquitously or specifically in the liver, led to immediate and pronounced liver overgrowth. Removal of doxycycline to stop YAP induction after 8 weeks resulted in a reversion to normal size and architecture within 2 weeks, while extended YAP induction led to the development of numerous discrete nodules throughout the livers which displayed many characteristics of HCC. Furthermore, clinical studies showed that YAP was an independent prognostic marker for overall survival and disease-free survival for HCC patients and that it was associated with tumor differentiation and serum AFP levels [36]. Currently, regulation of the expression and activation of YAP has been one of the major focuses in the field of liver cancer study (**Fig. 2**).

Regulation of the YAP protein level is a very important aspect of its oncogenic function. Many studies have been carried out to study the regulation of the YAP expression. Danovi *et al.* showed that YAP is a critical component of c-Jun-mediated induction of apoptosis and YAP expression is c-Jun-dependent [37]. Konsavage *et al.* [38] demonstrated that β-catenin/TCF4 complexes bind a DNA enhancer element within the first intron of the *YAP* gene to drive YAP expression in colorectal cancers cells. More recently, our group identified an Ets family transcription factor GABP that specifically binds to multiple Ets-binding sequences (GGAAG) of the *YAP* promoter and promotes the transcriptional expression of YAP [8]. Furthermore, the Hippo signaling pathway suppresses GABP transcriptional activity via a mechanism that Lats1 binds and promotes the phosphorylation of GABPβ to inhibit the homodimerization and nuclear localization of GABP. Hepatitis B virus (HBV) plays critical roles in the development of HCC. Recent work showed that the expression of YAP was dramatically elevated in clinical HCC samples, HBV-infected hepatoma HepG2.2.15 cell line and liver cancer tissues of HBV X protein

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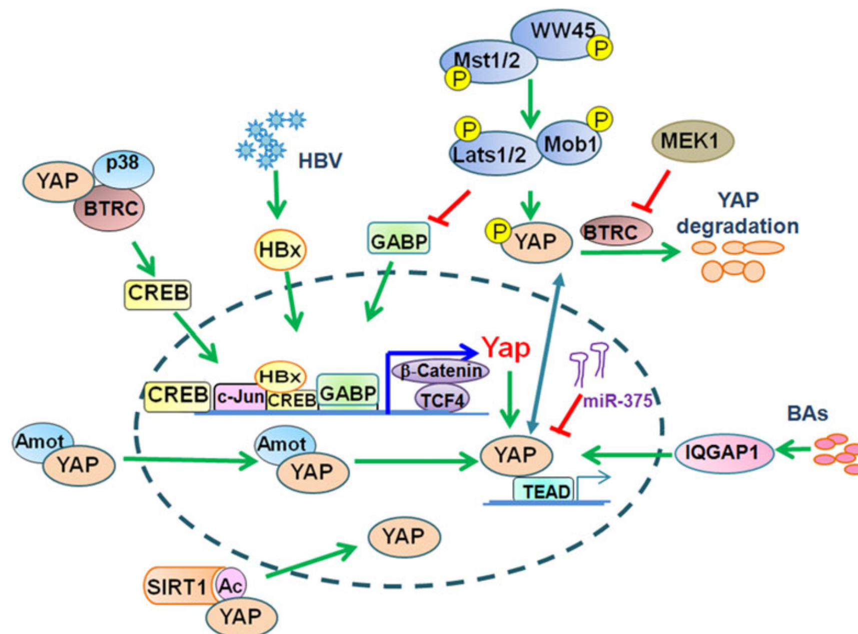

Figure 2. The regulation of YAP oncogene in liver cancer As a key downstream effector of the Hippo pathway, YAP is regulated on the transcriptional and post-translational levels in the liver. Transcription factors, CREB, c-Jun, and/or GABP bind(s) to the promoter of the *YAP* gene, whereas the β-catanin/TCF4 complex binds to the first intron region of the *YAP* gene to regulate the transcription of *YAP*. miR-375 represses the YAP expression. MEK1 inhibits the function of BTRC, an E3 ligase for YAP, to regulate the YAP protein stability. SIRT1-mediated YAP deacetylation enhances the YAP/TEAD association. Certain bile acids positively regulate YAP activity via the scaffold protein IQGAP1. Amot associates with YAP to form Amot/YAP complex and regulates the YAP/TEAD transcriptional activity. Please refer to the text for more details.

(HBx)-transgenic mice [39]. Further experiments revealed that HBx is able to bind to the promoter of *YAP* at −232/+115 region containing cyclic adenosine monophosphate response element-binding protein (CREB) element and activates the *YAP* promoter in a CREB-dependent manner. In addition, miR375 has also been shown to suppress the endogenous YAP protein level and inhibit the proliferation and invasion of HCC cells [40].

Both CREB and YAP proteins are highly expressed in a subset of human liver cancer samples and are closely correlated. Recently, it has been reported that YAP–CREB interaction is critical for liver cancer cell survival and maintenance of transformative phenotypes, both *in vitro* and *in vivo*. CREB promotes YAP transcriptional expression through binding to the −608/−439 region of the *YAP* promoter [41]. Additionally, YAP stabilizes CREB through interacting with mitogen-activated protein kinase 14 (MAPK14/p38) and beta-transducin repeat containing E3 ubiquitin protein ligase (BTRC). Other than MAPK14/P38, MEK/ERK proteins belong to the mitogenic signaling cascade and are linked to hepatocarcinogenesis as well. Sun's group further demonstrated that MEK1 promotes YAP protein expression through BTRC, and MEK1 and YAP are closely correlated in liver cancer samples, suggesting the important role of their interaction in liver tumorigenesis [42].

Bile acids (BAs) have been shown to function as signaling molecules and play important roles in liver regeneration and tumor promotion [43,44]. The elevated BA levels were found in hepatocellular carcinoma [45]. The nuclear receptor farnesoid X receptor (FXR) and the small heterodimer partner (SHP) are two key genes regulating BA homeostasis [46]. Recently, Moore's group showed that mice with a severe defect in BA homeostasis due to the loss of FXR and SHP exhibited enlarged livers, progenitor cell proliferation, and YAP activation and developed spontaneous liver tumorigenesis [47]. The relatively hydrophobic BAs, such as cholic acid (CA) and chenodeoxycholic acid (CDCA), serve as upstream regulators of YAP via a pathway dependent on the induction of the scaffold protein IQGAP1. Patients with diverse biliary dysfunctions exhibit enhanced IQGAP1 and nuclear YAP expression.

Scaffold proteins angiomotin (Amot) and angiomotin-like proteins (Amotl1 and Amotl2) have been identified as interacting proteins of YAP/TAZ and function as negative regulators of YAP/TAZ by preventing their nuclear translocation [48]. Amot proteins and YAP/TAZ interact directly through the PPXY motifs of Amot proteins and WW domains of YAP/TAZ to either sequester YAP/TAZ in the cytoplasm or target them to the tight junction. Knockdown of Amot proteins increased the expression of YAP target genes such as the connective tissue growth factor gene (*CTGF*) which overcomes cell contact inhibition, and induces EMT [49–52]. In contrast, Yi *et al.* revealed an unexpected and seemingly controversial role of Amot in positively regulating YAP in cell proliferation and cancer development [53]. Liver-specific Amot-deficient mice exhibited reduced hepatic oval cell proliferation and tumorigenesis in response to liver injury induced by porphyrinogenic hepatotoxin 3,5-diethoxycarbonyl-1,4-dihydrocollidine or when crossed with Nf2-deficient mice. The interaction of the p130 splicing isoform of Amot (Amot-p130) and YAP occurred in both the cytoplasm and nucleus compartments. In the cytoplasm, Amot-p130 prevented the phosphorylation of YAP by blocking access of the WW domains to the Lats1 kinase. Within the nucleus, Amot-p130 is associated with the transcriptional complex containing YAP and TEADs and regulates a subset of YAP target genes, many of which are associated with tumorigenesis [53].

Phosphorylation-mediated-YAP activation has been studied extensively. Recent work showed that the acetylation status of YAP is also important for its activity in liver. Hata *et al.* [54] reported that a novel cycle of acetylation/deacetylation of nuclear YAP was induced in response to S_N2 alkylating agents, but not other DNA-damaging stimuli. The YAP acetylation occurred on specific and highly conserved C-terminal lysine residues and was mediated by the nuclear acetyltransferases CREB-binding protein (CBP) and p300. In contrast, the nuclear deacetylase Class III HDAC Sirtuin 1 (SIRT1) was responsible for YAP deacetylation. More recently, Mao *et al*. demonstrated that the expression of SIRT1 was significantly up-regulated in the HCC samples, and *SIRT1* mRNA level was positively correlated with *CTGF*, a target gene of YAP [55]. Furthermore, SIRT1 deacetylated YAP2 protein in HCC cells and SIRT1-mediated deacetylation increased the YAP2/TEAD4 association, leading to enhanced YAP2/TEAD4 transcriptional activation and cell proliferation in HCC cells.

Conclusions and Further Perspectives

Mounting evidence from mouse models, tissue culture assays as well as the clinical sample analysis has clearly established the molecular aspects of the Hippo pathway core components and YAP/TAZ–TEAD effector complex and their critical roles in liver homeostasis and tumorigenesis. However, details of the regulation of the Hippo–YAP pathway in liver tissues remain elusive. For example, the loss of Mst1/2 contributes to the hepatocyte proliferation and promotes hepatic carcinogenesis, but both the upstream regulation of Mst1/2 and the full spectrum of Mst1/2 antiproliferative targets remain to be defined; Mst1 null T cells demonstrate that antiproliferative targets of Mst1/2 (and/or of phospho-Mob1) other than Lats1/2/YAP exist; whether any kinase other than Lats1/2 is able to phosphorylate YAP and what are their functions in liver are still open questions; TAZ, a YAP paralog, shares 50% sequence identity with YAP and is also phosphorylated and inhibited by Lats1/2, however, there has not been any report about TAZ function in the liver; the role of TAZ in the liver remains to be investigated; in addition, investigation of whether and how YAP is regulated independent of the canonical Hippo pathway is also an active and exciting topic. Given the intensive research efforts in the Hippo field, we can expect that many new insights into this pathway will reveal the full potential of manipulations of the Hippo pathway in the prevention and treatment of liver diseases in the near future.

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