Review



# Targeting the Hippo-YAP/TAZ Signaling Pathway in Primary Liver Cancer Therapy

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*Review*

# Targeting the Hippo-YAP/TAZ Signaling Pathway in Primary Liver Cancer Therapy

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**Simple Summary:** The Hippo signaling pathway, an evolutionarily conserved cascade, plays a crucial role in regulating organ size, tissue regeneration, and tumorigenesis. Dysregulation of this pathway leads to activation and nuclear translocation of YAP/TAZ, the pivotal downstream regulators. The Hippo/YAP/TAZ pathway promotes cell proliferation and tumorigenesis, notably in primary liver cancer. The Hippo/YAP/TAZ cascade has emerged as a significant therapeutic target in liver cancer. This review provides a comprehensive summary of recent advances in the Hippo-YAP/TAZ signaling pathway in primary liver cancer, highlighting its therapeutic potential as a target for intervention.

**Abstract:** Liver cancer imposes a pervasive global health challenge, ranking among the most prevalent cancers worldwide. Its prevalence and mortality rates are on a concerning upward trajectory and exacerbated by the dearth efficacious treatment options. The Hippo signaling pathway, originally discovered in Drosophila, comprises four core components: MST1/2, WW45, MOB1A/B, and LATS1/2. This pathway regulates the cellular localization of the transcriptional coactivator YAP/TAZ (Yes-associated protein/PDZ-binding motif) through a series of enzymatic reactions. The Hippo/YAP/TAZZ pathway maintains a balance between cell proliferation and apoptosis, regulates tissue and organ sizes, and stabilizes the internal environment. Abnormalities of any genes within the Hippo signaling pathway, such as deletion or mutation, disturb the delicate balance between cell proliferation and apoptosis, creating a favorable condition for tumor initiation and progression. Mutations or epigenetic alterations in the Hippo signaling pathway components can lead to its inactivation. Consequently, YAP/TAZ becomes overexpressed and activated, promoting excessive cell proliferation and inhibiting apoptosis. This dysregulation is closely associated with the development of liver cancer. This review discusses the pivotal role of the Hippo signaling pathway in the pathogenesis and progression of liver cancer. By elucidating its mechanisms, we aim to offer new insights into potential therapeutic targets for effectively combating liver cancer.

**Keywords:** Hippo signaling; YAP/TAZ; liver cancer; therapy targets

## 1. Introduction

Global liver cancer incidence increases rapidly, and liver cancer is one of the leading causes of cancer-related death worldwide, with a 5-year survival rate as low as 30–40% [1,2]. Primary liver cancer is a prevalent clinical malignancy encompassing hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA). In 2020, primary liver cancer had a global incidence of 4.7% and a mortality rate of 8.3%, ranking the fifth among common malignancies and the third among cancer-related causes of death worldwide [3]. Liver cancer is often diagnosed at advanced stages due to difficulties in early diagnosis, high metastatic potential, and frequent recurrence, resulting in a poor prognosis with low 5-year survival rate [4]. Therefore, understanding the pathogenesis of and

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influencing factors for primary liver cancer, as well as exploring new therapeutic targets, are current challenging and become a focal point in research. The Hippo-YAP/TAZ signaling pathway is well-established to regulate cell proliferation, embryonic development, tissue and organ sizes, liver development, and liver regeneration [5,6]. As research progresses, its role in tumor development has emerged, particularly in regulating liver cancer cell proliferation, metastasis, and the tumor microenvironment (TME) [7]. This review summarizes mechanisms by which the Hippo-YAP/TAZ pathway regulates primary liver cancer development and discusses the advances in targeting this pathway to hinder cancer progression. We aim to explore novel effective therapies for primary liver cancer.

The Hippo signaling pathway primarily consists of upstream core kinases and downstream transcriptional coactivators. In mammals, the upstream kinases include ste20-like kinases 1/2 (MST1/2) and large tumor suppressor 1/2 (LATS1/2). Downstream transcriptional coactivators primarily comprise YAP and TAZ [8]. MST1/2 phosphorylate activate LATS1/2, and LATS1/2 in turn phosphorylate and inhibit YAP/TAZ. 14-3-3 protein binds to phosphorylated YAP/TAZ and remains YAP/TAZ in the cytoplasm; additionally, phosphorylation promotes ubiquitination and degradation of YAP/TAZ [7,8]. When cells detect signals such as cell-to-cell contact, cell polarity, energy levels, and stress, the MST1/2 and LATS1/2 kinase cascade is activated to suppress YAP/TAZ. In contrast, inhibition of the Hippo signaling pathway suppresses YAP/TAZ phosphorylation, and non-phosphorylated YAP/TAZ enter the nucleus and bind to TEA domain 1-4 (TEAD1-4), stimulating transcription of target genes. This process promotes cell proliferation, suppresses apoptosis, and regulates tissue growth [9]. Mutation or deletion of genes associated with the Hippo signaling pathway is linked to apoptosis suppression, uncontrolled cell proliferation, and accelerated tumor progression, thus impacting patient prognosis [10,11]. The Hippo signaling pathway has been established to play a crucial role in tumorigenesis across various organs, including the liver. YAP/TAZ transcriptional co-factors are a pivotal downstream effector of the Hippo pathway to govern migration, proliferation, and survival. Aberrant activation of YAP is critical for proliferation of both HCC and iCCA cells [12]. The Hippo-YAP/TAZ signaling pathway has emerged as a promising new target for clinical prevention and treatment of cancer. This article provides a comprehensive review of the Hippo-YAP/TAZ pathway in the initiation and progression of liver cancer.

## 2. Hippo/YAP Signaling Pathway

The Hippo/YAP signaling pathway, originally discovered in Drosophila, comprises a cascade of protein kinases (MST1/2, LATS1/2) and transcriptional regulators (YAP, TAZ, TEAD) [13]. Since the beginning of the 21st century, scientists have conducted extensive research on the Hippo signaling pathway and uncovered numerous related genes. It became evident that this pathway constitutes a protein kinase cascade and transcriptional coactivators and pivotally regulates liver tissue homeostasis [14,15]. Active YAP/TAZ, central to the pathway, undergo nuclear translocation and then activate transcription of target genes [16]. YAP/TAZ's target genes regulate development, cell proliferation, migration, survival, and other critical functions. YAP/TAZ is upregulated in solid tumors and exhibits oncogenic characteristics [17,18]. Hayashi et al. discovered that expression of both TAZ and YAP is elevated in liver cancer [19]. Interestingly, knocking down TAZ results in compensatory upregulation of YAP [19]. Wu et al. observed that in patients with liver cancer, high YAP expression is associated with large tumors, multiple foci, liver cirrhosis, vascular infiltration, and intrahepatic metastasis [20].

Under physiological conditions, the Hippo signaling pathway maintains a dynamic balance between cell proliferation and apoptosis. This balance regulates the development and maturation of tissues and organs, suppresses tumorigenesis, and ensures the stability of the internal environment [21]. The primary function of the Hippo signaling pathway (i.e. MST1/2, LTAS1/2) is to deactivate key effector molecules (YAP/TAZ), thereby rendering the transcription co-factors inactive. This action blocks the expression of genes, such as cyclin E, cyclin D1, β-catenin, AXL receptor tyrosine kinase

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(AXL), cysteine rich 61 (CYR61) and connective tissue growth factor (CTGF), which are involved in cell proliferation, adhesion, migration, and invasion [22,23].

In the Hippo signaling pathway, YAP is subjected to negative regulation through a phosphorylation cascade of MST1/2 and LATS1/2 [24]. The kinase activity of MST1/2 can be activated through binding to Salvador family WW domain-containing protein 1 (WWC1), a scaffold protein that also forms complexes with LATS1/2. MST1 also interacts with the members of the Ras association domain family, further enhancing kinase activity [25,26]. MST1/2 activates LATS1/2 by phosphorylating LATS1/2 as well as Mps-one binder kinase activator-like 1 (MOB1), a LATS1/2-binding protein [27]. Besides MST1/2, mitogen-activated protein kinase kinase kinase (MAP3K) family members also can phosphorylate and activate LATS1/2 [28]. Activated LATS1/2 phosphorylates YAP/TAZ, which are bound to 14-3-3ζ proteins and sequestered in the cytoplasm, thereby inhibiting transcriptional activity of YAP/TAZ [29,30]. Additionally, phosphorylation of YAP can trigger ubiquitination and degradation mediated by β-transducing repeat-containing protein (β-TrCP) [31,32]. YAP can also be inactivated by binding to tight junction protein 2 (TJP2) and non-receptor protein tyrosine phosphatase 14 (PTPN14) [33,34]. G protein-coupled receptors can activate YAP via the rho-GTPase signaling pathway [35].

In addition to phosphorylation, multiple post-translational modifications play a crucial role in regulating YAP/TAZ activity. YAP can undergo methylation by a methyltransferase complex containing SET domain 1A, thereby boosting its oncogenic activity by preventing nuclear translocation [36]. O-GlcNAc glycosylation of LATS2 activates YAP, promoting tumor growth [37]. Acetylation of LATS1 inhibits YAP phosphorylation and degradation, fostering the invasion and proliferation of cancer cells [38,39]. Thus, post-translational modifications serve as an important mechanism for fine-tuning YAP signaling pathways. The Hippo signaling pathway activation leads to the removal of inactive YAP/TAZ factors from the cell nucleus, transforming this information into a signal that inhibits growth and development. Consequently, it effectively regulates cell number and organ size [40].

## 3. Role of the Hippo/YAP Signaling Pathway in Primary Liver Cancer

The mammalian liver exhibits robust regenerative capability and rapidly grows to the original size after 70% of hepatectomy [41]. The Hippo signaling pathway not only regulates liver size but also promotes primary liver cancer primarily by influencing cell proliferation, autophagy, invasion and metastasis, tumor resistance, and the tumor microenvironment. Current research is predominantly centered on YAP and TAZ.

### *3.1. Cell Proliferation*

Hepatocytes are highly regenerative, and liver homeostasis is maintained mainly by a balance between proliferation and apoptosis. When the Hippo pathway is inactivated (activation of YAP/TAZ), hepatocytes undergo uncontrolled proliferation, leading to abnormal liver growth and HCC [42]. Aberrant activation of hepatic YAP results in liver enlargement and HCC in MST1/2 double knock out (DKO) mice, whereas inhibition of YAP expression restores normal liver growth [43]. In mice with c-Myc-induced HCC, inactivation of the Hippo pathway significantly increases TAZ expression, and TAZ in turn inhibits apoptosis through the TAZ-BCL2L12 axis, thereby promoting c-Myc-dependent hepatocellular carcinoma [44]. Grijalva et al. performed 70% of liver resection and observed that expression of YAP target genes is increased within 24 hours when MST1/2 and LATS1/2 are inhibited. YAP Levels and expression of target genes return to the baseline level in approximately 7 days when the liver grows to a normal size [45]. Ba et al. found that overexpression of YAP is closely associated with oncogenic features, such as epithelial-mesenchymal transition (EMT) and anchorage-independent growth. YAP acts in concert with TEAD1 to stimulate expression of EMT transcription factor Zeb1, proliferation, and EMT while weakening intercellular tight junctions, thereby enhancing the migratory and invasive capabilities of tumor cells [46].

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### *3.2. Autophagy*

Autophagy deficiency impedes YAP degradation, resulting in its nuclear accumulation. YAP serves as a pivotal factor for tissue remodeling and HCC induced by autophagy deficiency. YAP in the cytoplasm and the nucleus in the liver is considerably increased in mice lacking autophagy-related protein 7 (Atg7), contributing to liver enlargement and tumorigenesis [47]. YAP is markedly upregulated in chemotherapy-resistant liver cancer cell lines and enhances drug resistance by suppressing autophagy-related cell death.

### *3.3. Tumor Invasion and Metastasis*

YAP/TAZ are frequently overexpressed in liver cancer tissues, positively correlating tumor vascular invasion and EMT, thereby accelerating liver cancer progression [48]. Knockout of *TAZ* in liver cancer cell lines increases E-cadherin expression and decreases N-cadherin expression. Additionally, the activities of matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase-9 (MMP9) are diminished, suppressing EMT and invasiveness of liver cancer cells [49].

### *3.4. The Tumor Microenvironment*

The TME refers to the tissue milieu in which tumor cells proliferate and interact and plays a crucial role in promoting HCC growth, invasion, and immune evasion [50]. Key TME components include tumor-associated macrophages (TAMs), other immunosuppressive cells, and their secreted inflammatory factors. MST1/2 inactivation increases production of proinflammatory cytokines, leading to infiltration of a significant number of M1 or mixed M1/M2 macrophages into the liver and liver inflammation. This process creates the TME that contributes to liver cancer development [43].

## 4. The Hippo/YAP Pathway in Liver Cancer Drug Resistance

Drug resistance in hepatocarcinoma cells is highly intricate, operating by various mechanisms including altering drug distribution and metabolism, influencing cell proliferation, and regulating the DNA damage response [51–53]. The Hippo signaling pathway plays a crucial role in governing cancer cell proliferation, metastasis, and drug resistance, and YAP activation may confer resistance to chemotherapy, radiotherapy, or immunotherapy [54,55].

DNA-damaging drugs, such as doxorubicin, target DNA replication to induce cytotoxic effects and eliminate tumor cells and are extensively used for liver cancer treatment. Research indicates that liver cancer resistance to DNA-damaging drugs is closely linked to YAP. In hepatocellular carcinoma cells, overexpression of YAP enhances resistance to doxorubicin by activating the mitogen-activated protein kinase (MAPK) pathway [56]. Sophora japonica, a traditional Chinese medicine, increases the sensitivity of hepatocellular carcinoma cells to oxaliplatin by both promoting YAP phosphorylation and inhibiting YAP expression [57]. A hypoxic microenvironment can induce YAP nuclear translocation and activate YAP via the HMG-CoA reductase signaling pathway, thereby increasing the sensitivity of hepatocellular carcinoma cells to irinotecan [58]. Sorafenib is a primary chemotherapy agent for HCC treatment; however, tumor resistance diminishes its effectiveness in promoting apoptosis and inhibiting liver cancer cell proliferation and compromises patient survival. Research has established a close relationship between the expression level of YAP and the sensitivity of liver cancer cells to sorafenib. YAP can induce tumor recurrence and resistance by suppressing the ability of sorafenib to stimulate liver cancer cell apoptosis and ferroptosis while promoting liver tumor stem cell the self-renewal [59].

In conclusion, a growing body of research indicates that activation of the Hippo/YAP signaling pathway contributes to chemotherapy resistance of liver cancer cells. Conversely, inhibiting this pathway has been shown to enhance cancer cell sensitivity to anticancer drugs. For example, Hao et al. found that dihydroartemisinin reduces lipid droplets through YAP to promote the effect of anti-PD-1 antibody on hepatocellular carcinoma [30]. Therefore, targeting the YAP signaling pathway represents a promising therapeutic strategy to counter chemotherapy resistance.

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YAP is closely linked to various malignant behaviors of hepatocarcinoma cells, including proliferation, invasion, and drug resistance. Growing evidence indicates that YAP contributes to tumor resistance against anticancer therapies. YAP represents a promising target for the development of anti-hepatocarcinoma therapies by disrupting drug resistance. A combination of YAP inhibition and anti-hepatocarcinoma drugs may offer a novel strategy for tackling drug-resistant liver cancer.

## 5. Targeting the Hippo/YAP Signaling Pathway in Primary Liver Cancer

Inhibitors, which target the upstream kinases, are expected to activate YAP/TAZ. Several agents have been developed to alter YAP/TAZ expression, subcellular distribution, and activities, ultimately impeding tumor initiation and progression. Targeting the Hippo signaling pathway serves as a promising therapeutic strategy for treating primary liver cancer.

### *5.1. Targeting Upstream Kinases MST/LATS*

MST or LATS can be activated by epithelial splicing regulatory protein-2 (ESRP2)/neurofibromin 2 (NF2), long non-coding RNA LOC107985656, WW and C2 domain-containing protein (WWC) and T-box transcription factor 3 (TBX3)/phospholipase D1 (PLD1) [60–63]. Activation of the MST/LATS cascade increases phosphorylation and subsequent proteasomal degradation of YAP/TAZ, which in turn inhibits the occurrence of liver cancer. In contrast, RACGTPase activating protein 1 (RACGAP1) and GTP-binding protein family members Septin 6 (SEPT6) inhibit LATS phosphorylation, thus increasing YAP nuclear translocation and expression of target genes and promoting liver cancer progression [64,65]. Agents, which target MST/LATS to regulate YAP/TAZ, are listed in Table 1.

**Table 1.** The molecules involved in indirect regulation of YAP/TAZ by targeting the MST/LATS.

| Status | Molecules | Targets | Function |
|---|---|---|---|
| Hippo on | ESRP2/NF2 | MST1/2 | Loss of ESRP2/NF2 function permits sustained YAP/TAZ activity that drives hepatocyte proliferation, advantaging growth of cells with mutations that enable them to survive chronic oncogenic stress [60]. |
| | lncRNA LOC107985656/ miR-106b-5p | LATS1 | LOC107985656 regulated the expression of LATS1 by acting as a sponge for absorbing miR-106b-5p in HCC cells [61]. |
| | WWC | LATS1/2 | WWC proteins positively regulate the Hippo pathway via the activation of LATS1/2 kinases and the subsequent cytoplasmic accumulation of phosphorylated YAP [62]. |
| | TBX3/ PLD1 | LATS2 | TBX3 inhibited HCC cell growth as well as YAP/TAZ activation by promoting overexpression of LATS2 via suppressing transcriptional target PLD1 [63]. |
| Hippo off | RACGAP1 | LATS1/2 | RACGAP1 promotes proliferation of HCC cells by reducing activation of the LATS1/2 [64]. |
| | SEPT6 | LATS1 | SEPT6 facilitates F-actin formation, which induced LATS1 dephosphorylation, inhibited Hippo signaling, upregulated YAP expression and nuclear translocation [65]. |

### *5.2. Direct Regulation of YAP/TAZ*

Expression levels and subcellular localization of YAP/TAZ govern target gene expressions, cell proliferation, apoptosis, and EMT, among others. These factors are linked to the onset and progression of tumors. Thus, elucidating targets, which regulate YAP/TAZ transcription, post-

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translational modifications, and subcellular localization, holds a promise for developing targeted therapies for primary liver cancer.

The transcriptional activity of YAP/TAZ can be regulated by many molecules and stress. High mobility group box 1 (HMGB1)/GA-binding protein alpha (GABPα), miR-1224/cyclic AMP (cAMP)-response element binding protein (CREB), lysine acetyltransferase 6A (KAT6A), microRNA-590-5p and AT-rich interaction domain 1A (ARID1A) can increase YAP levels [66–70]. MicroRNA-9-3p and MicroRNA-125b are involved in the hepatocellular carcinoma tumorigenesis by suppressing TAZ translation [71,72]. C-X-C chemokine receptor type 4 (CXCR4)/ubiquitin domain-containing protein 1 (UBTD1) is involved in the proteasome-dependent degradation of YAP [73]. Ubiquitin-specific peptidase 10 (USP10) and Josephin domain-containing protein 2 (JOSD2) promote proliferation of primary liver cancer by deubiquitinating and stabilizing YAP/TAZ [74,75]. Pectrin beta, non-erythrocytic 1 (SPTBN1)/variegation 3-9-enhancer of zeste-trithorax domain containing lysine methyltransferase 7 (SETD7) promote YAP methylation, leading to YAP degradation and inactivation [76]. Insulin like growth factor 1 receptor (IGF1R), histone lysine methyltransferase SET domain containing 1A (SETD1A) and programmed cell death 10 (PDCD10) are involved in YAP phosphorylation [77–79]. The piezo type mechanosensitive ion channel component 1 (Piezo1)/mitogen-activated protein kinase (MAPK) pathway and fluid shear stress (FSS) can directly induce translocation of YAP into the nucleus, enhancing hepatocellular carcinoma progression [80,81]. TNF receptor II/heterogeneous nuclear ribonuclear protein K (TNFR2–hnRNPK) directly stabilize YAP to regulate YAP target genes, therefore promoting HCC progression [82]. The molecules and stress involved in direct regulation of YAP/TAZ are shown in Table 2.

**Table 2.** The molecules involved in direct regulation of transcriptional coactivators YAP/TAZ.

| Regulation level | Molecules | Function |
|---|---|---|
| Transcription | HMGB1/GABPα | The binding of HMGB1 to GABPα promotes the expression YAP in transcriptional level [66]. |
| | miR-1224/CREB | By binding with CREB, miR-1224 could repress the transcription and the activation of YAP [67]. |
| | KAT6A | KAT6A was associated with sorafenib resistance and contributes to progression of HCC by targeting YAP expression [68]. |
| | miR-590-5p | YAP is regulated by microRNA-590-5p and is critical for HCC chemoresistance through regulating expression of stemness markers and ATP-binding cassette transporters [69]. |
| | ARID1A (AT-rich interaction domain 1A) | ARID1A was discovered to bind to YAP, inhibiting its transcriptional output [70]. |
| Translation | MicroRNA-9-3p | MicroRNA-9-3p acts as a tumor-suppressor miR by targeting TAZ expression in HCC cells [71]. |
| | MicroRNA-125b | miR-125b may be involved in the tumorigenesis of HCC at least in part by the suppression of TAZ [72]. |
| Ubiquitination | CXCR4/UBTD1 | CXCR4 to decrease the levels of UBTD1, which is involved in the proteasome-dependent degradation of YAP [73]. |
| | USP10 | USP10 promotes proliferation of hepatocellular carcinoma by deubiquitinating and stabilizing YAP/TAZ [74]. |
| | JOSD2 | Deubiquitinase JOSD2 stabilizes YAP/TAZ to promote cholangiocarcinoma progression [75]. |

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| | | |
|---|---|---|
| Methylation | SPTBN1/ SETD7 | SPTBN1 positively regulated the expression of suppressor of SETD7 to promote YAP methylation, which leads to YAP degradation and inactivation [36]. |
| Phosphorylation | IGF1R | Depletion of IGF1R increased the p-YAP, which denoted the loss of YAP function [76]. |
| | SETD1A | SETD1A deficiency impairs YAP phosphorylation and activation. In contrast, SETD1A enhances YAP activation to induce sorafenib primary resistance in HCC [77]. |
| | PDCD10 | PDCD10 directly binds to the catalytic subunit of protein phosphatase 2A (PP2Ac) and increases its enzymatic activity, leading to dephosphorylation of the YAP, which contributes to YAP nuclear translocation and transcriptional activation [78]. |
| Nucleus translocation | Piezo1/MAPK | Piezo1 activates the mitogen-activated protein kinase (MAPK) pathway, and then integrates with YAP signaling to control the nuclear translocation of YAP and regulation of its target genes [79]. |
| | FSS | FSS induces translocation of YAP from the cytomembrane to the nucleus, contributes to epithelial–mesenchymal transition (EMT) and enhances metastasis in hepatocellular carcinoma [80]. |
| Stabilization | TNFR2–hnRNPK | TNFR2–hnRNPK acted downstream of TNF$\alpha$–TNFR2 signaling to directly interact with and stabilize YAP on target gene promoters genome-wide, therefore coregulating the expression of YAP target genes [81]. |

## 6. Drugs Targeting Hippo/YAP Signaling in Primary Cancer Therapy

Currently, many drugs and small molecule inhibitors have been developed to target the Hippo/YAP signaling pathway and display anti-liver cancer potential.

Tadalafil, a PDE5 inhibitor, can inhibit YAP/TAZ by activating MST1/LATS1 [82]. $\alpha$-hederin, a monodesmosidic triterpenoid saponin isolated from Fructus akebiae, inhibits the proliferation of HCC cells by activating MST1/LATS1 [83]. Evodiamine and homoharringtonine exert anti-tumor effects in HCC through activating MST1/2 and upregulating LATS1 phosphorylation, leading to phosphorylation and inactivation of YAP [84,85]. Zhao et al. found that metformin directly activated MST1/2, phosphorylated YAP1 in vitro, suggesting that metformin activates Hippo signaling pathway to regulate interleukin-22 (IL-22) mediated HCC progression [86]. Liu et al. found that bioactive components of poplar propolis obtained from north China can inhibit proliferation of human hepatocellular carcinoma HepG2 cells in a LATS1-dependent manner [87]. Zhu et al. found that CT-707, a multi-kinase inhibitor approved by the China FDA and currently in clinical trials, potently inhibits YAP. CT-707 demonstrates significant cytotoxicity against HCC cells, particularly under hypoxic conditions. Mechanistically, CT-707's inhibition of YAP signaling is attributed to its disruption of hypoxia-activated insulin like growth factor 1 receptor (IGF1R) [76]. Zhang et al. also found that corosolic acid can suppress liver cancer growth by decreasing the level of CDK19/YAP-mediated O-GlcNAcylation [88]. Trametes robiniophila Murr, a traditional Chinese herbal medicine, restrains the proliferation and migration of HCC cells via downregulation of YAP, and has been widely used in adjuvant therapies of HCC [89]. In addition, statin such as fluvastatin and simvastatin have been shown to reduce TAZ expression levels in Huh1 cells, suppress the expression of TAZ target genes CYR61 and CTGF, as a result, attenuating hepatocellular carcinoma cell proliferation [90]. Clinical studies demonstrated that statin prolongs recurrence-free survival in patients following HCC surgery. Drugs targeting Hippo/YAP signaling in primary cancer therapy are shown in Table 3.

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**Table 3.** Drugs targeting Hippo/YAP signaling in primary cancer therapy.

| Target | Drug | function |
|---|---|---|
| MST1/LATS1 | Tadalafil | Tadalafil blocks YAP/TAZ protein expression by activating Hippo pathway and enhances the therapeutic efficacy of BET inhibitors in hepatocellular carcinoma treatment [82]. |
| | $\alpha$-Hederin | $\alpha$-Hederin treatment effectively enhanced MST1 and LATS1 gene expression while downregulated YAP gene expression in HepG2 and SMMC-7721 cells [83]. |
| MST1/2 and LATS1 | Evodiamine | Evodiamine activates MST1/2 and upregulates LATS1 phosphorylation, leading to phosphorylation and decreased nuclear translocation of YAP [84]. |
| | Homoharringtonine | Homoharringtonine treatment increased the phosphorylation levels of MST1/2 and LAST1, significantly inhibit HCC cell growth by suppressing cell proliferation and colony formation [85]. |
| MST1/2 and LATS1/2 | Metformin | Metformin directly inhibits LATS1/2 and activates MST1/2, phosphorylates YAP1, as a result, suppressing IL-22 mediated HCC progression [86]. |
| LATS2 | Poplar propolis | Poplar propolis obviously up-regulated the levels of LAST2 and decreased the expression of YAP, TAZ, and their target protein in the nucleus [87]. |
| YAP | CT-707 | CT-707 has remarkable inhibitory activity against YAP function and exhibits prominent cytotoxicity under hypoxia on HCC cells [76]. |
| | Corosolic acid | Corosolic acid can reduce YAP expression and O-GlcNAcylation by inhibiting the activity of CDK19 [88]. |
| | Trametes robiniophila Murr | Trametes robiniophila Murr treatment translocated YAP from nucleus to cytoplasm, and further promoted phosphorylation of YAP to be degraded by ubiquitination [89]. |
| TAZ | Statin (fluvastatin and simvastatin) | TAZ expression was suppressed in HCC cells by fluvastatin and simvastatin treatment, which have the anti-proliferative effects and induced apoptosis in HCC cells and improved the prognosis of HCC patients [90]. |

The most effective approach to reduce YAP protein expression is by directly targeting YAP protein [91]. RNA interference technology has also be used to lower *YAP* mRNA levels in MST1/2-deficient mice, thereby decreasing YAP protein expression. Reduction in YAP protein has been shown to substantially decrease liver tumor burden in mice [92]. It's important to note that for YAP to be oncogenic, it must interact with downstream regulatory factors. The Notch signaling pathway is known to promote abnormal proliferation in liver cancer. Inhibitors for the Notch signaling pathway represent potential treatments for YAP-based liver cancer [93].

## 7. Discussion

It is evident that several commonly used clinical drugs or inhibitors can exert anti-liver cancer effects by modulating the Hippo/YAP/TAZ pathway, including hypoglycemic, lipid-lowering, anti-cancer medications, and certain natural plant ingredients. This offers novel perspectives for clinical drug applications. However, the effectiveness of emerging drugs is still under investigation, and their efficacy repeatability and safety profiles require further exploration. Efficient and reliable methods for clinical translation and safety evaluation of these drugs need extensive research and development.

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The pathogenesis of liver cancer remains incompletely understood. In recent years, roles of cell signaling pathways in cancer formation and progression have received increasing attention. the Hippo signaling pathway is a key one and it was initially hypothesized by international researchers in the 1990s. This pathway has highly conserved across evolutionary contexts in terms of composition, biological function, and molecular mechanisms [94]. The Hippo signaling pathway, which was discovered through fruit fly studies, plays a critical role in regulating cell proliferation, apoptosis, and organ size. YAP, a key component of the pathway, is notably overexpressed in liver tumors as an oncogene candidate. It serves as a significant regulatory factor to promote formation, development, and metastasis of liver cancer, making it a promising target for novel treatments against cancer. The Hippo/YAP signaling pathway plays a pivotal role in regulating various aspects of primary liver cancer, including tumor cell proliferation, autophagy, invasion, metastasis, the tumor microenvironment, and targeted drug resistance. Notably, high expression of YAP/TAZ is a crucial factor in the onset and progression of primary liver cancer. It is worth noting that in addition to the Hippo signaling pathway, matrix stiffening is one of the major risk factors for HCC and drives tumor progression. Ma et al. found that matrix stiffening facilitates liver cancer stem cell (CSC) stemness by increasing YAP activity, and the group also described a novel therapeutic strategy for eradicating CSCs and improving the efficiency of HCC treatment by targeting YAP [95].

Drugs that target the Hippo signaling pathway, particularly inhibitors of YAP and TAZ, have garnered significant attention in recent years. However, their efficacy and safety profiles require further validation through animal experiments and clinical trials. As our understanding of the key regulators of the Hippo signaling pathway expands, the network's regulatory mechanisms diversify, including advancements in upstream kinase inhibition. With ongoing research, more regulatory pathways are anticipated to be elucidated, potentially broadening treatment strategies by targeting the Hippo signaling pathway. This development holds promise for the discovery of new therapeutic agents to enhance the efficacy of liver cancer treatments.

Nevertheless, currently, there are no YAP inhibitors with sufficient specificity. In the future, research and development of YAP-selective inhibitors are essential for expanding options in anti-liver cancer drugs and clinical treatments. The Hippo/YAP pathway may interact with other liver cancer-related signaling pathways, including Notch, Wnt, and UPR, thereby contributing to the onset and progression of liver cancer. Detailed mechanisms linking the Hippo signaling pathway to liver cancer formation require further exploration.

## Conclusion

YAP/TAZ significantly impact initiation, progression, metastasis, invasion, and drug sensitivity of liver cancer. A variety of molecules and pathways can influence the Hippo signaling pathway, thereby affecting how YAP/TAZ regulate liver cancer. Given that YAP/TAZ are a crucial oncogenic factor, dysregulation of the Hippo pathway is a key driver for tumorigenesis and malignant transformation. Therefore, exploring the Hippo-YAP/TAZ pathway offers a valuable opportunity for the development of new targeted therapies for liver cancer.

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