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Hippo signaling in the liver: role in development, regeneration and disease

Jacquelyn O. Russell^{1,2},

Fernando D. Camargo^{1,2,*}

¹Stem Cell Program, Boston Children's Hospital, Boston, MA, USA.

²Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA.

Abstract

The Hippo signaling pathway has emerged as a major player in many aspects of liver biology, such as liver development, cell fate determination, homeostatic function, and regeneration from injury. The regulation of Hippo signaling is complex, with activation of the pathway coming from diverse upstream inputs including signals from cellular adhesion, mechanotransduction, and crosstalk with other signaling pathways. Pathological activation of the downstream transcriptional co-activators yes-associated protein 1 (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ), which are negatively regulated by Hippo signaling, has been implicated in multiple aspects of chronic liver disease, such as the development of liver fibrosis and tumorigenesis. As such, development of pharmacological inhibitors of YAP/TAZ signaling has been an area of great interest. In this review, we summarize the diverse roles of Hippo signaling in liver biology and highlight areas where outstanding questions remain to be investigated. Greater understanding of the mechanisms of Hippo signaling in liver function should help to facilitate the development of novel therapies for the treatment of liver disease.

Introduction

Chronic liver disease (CLD) is a global epidemic, with deaths from CLD continuing to rise annually. Cirrhosis and liver cancer together account for approximately 2 million deaths each year¹ despite the fact that the liver has immense regenerative potential. The differentiated epithelial cells of the liver, hepatocytes and cholangiocytes (also known as biliary epithelial cells (BECs)), are capable of exiting quiescence and proliferating to repair hepatic damage². It is theorized that repeated bouts of hepatic damage, such as from alcoholic hepatitis, viral hepatitis, non-alcoholic fatty liver disease (NAFLD), cholangiopathies, etc., can over time exhaust the liver's regenerative potential, leading to the progression of fibrosis to cirrhosis and potentiating the development of liver cancer³. Currently, therapies which prevent progression or reverse CLD are extremely limited, and the only treatment for end-stage liver disease is a liver transplant. However, the demand

*corresponding.

Competing interests

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for donor organs far exceeds the supply¹. Therefore, there is a vast unmet clinical need for effective therapies for CLD.

Over the past decade, it has become apparent that the Hippo signaling pathway has major roles in many aspects of hepatic function, including regulation of liver development, homeostasis, regeneration, and cancer. First identified as a regulator of tissue growth in *Drosophila*^{4,5}, the corresponding mammalian pathway was quickly identified and found to regulate organ size, as inhibition of Hippo signaling led to a dramatic overgrowth phenotype in the liver^{6,7}. Hippo signaling has since been found to regulate a variety of key cellular processes such as cell fate, metabolism, tumorigenesis, and regulation of the immune system. Much research has been devoted to identifying the complex upstream regulators of this pathway and have demonstrated that Hippo signaling is modulated by a vast array of biochemical and biophysical inputs. In this review, we will provide an overview of the role of Hippo signaling in many aspects of liver function and discuss the potential development of novel therapies targeting components of the Hippo signaling pathway.

Canonical Hippo Signaling Pathway

This conserved pathway was first identified in *Drosophila*, where deletion of the serine/threonine protein kinase *hippo* led to a dramatic overgrowth phenotype⁴. The corresponding mammalian proteins (Figure 1) are mammalian STE20-like protein kinase 1 (MST1) and MST2^{8,9}, which form a complex with the scaffolding protein salvador homolog 1 (SAV1, also called WW45)¹⁰. MST1/2 phosphorylate and activate large tumor suppressor kinase 1 (LATS1) and LATS2 (homologues to the *Drosophila* protein Warts)^{4,11}. Neurofibromatosis type II (NF2, homolog to *Drosophila* Merlin) facilitates the activation of LATS1/2 by MST1/2 through recruitment of these proteins to the plasma membrane^{12,13}. LATS1/2, together with the regulatory proteins MOB kinase activators 1A (MOB1A) and MOB1B, phosphorylate yes-associated protein 1 (YAP, homolog to the *Drosophila* protein Yorkie¹⁴) at multiple serine residues¹⁵, including S127 in the human protein⁶ or S112 in the mouse protein. Transcriptional co-activator with PDZ-binding motif (TAZ), a paralog of YAP, is also phosphorylated by this complex¹⁶. The phosphorylation of YAP/TAZ causes the scaffolding protein 14-3-3 to bind and sequester them in the cytoplasm and eventually target them for proteasomal degradation via β -Transducin Repeat Containing E3 Ubiquitin Protein Ligase (β -TRCP)^{15,17} (Figure 1A). When YAP/TAZ are hypophosphorylated, they can translocate to the nucleus and interact with the TEAD family of transcription factors (TFs) to mediate target gene expression^{18,19} (Figure 1B). Thus, the canonical Hippo signaling pathway functions to suppress the activity of YAP/TAZ.

Upstream Regulators

Although Hippo signaling regulates many aspects of hepatic function, much remains unknown about the upstream signals which regulate the activity of the pathway. In this review we will summarize the currently known upstream regulators of Hippo signaling, but more research is required to fully elucidate these mechanisms.

Cellular Polarity and Adhesion

A major function of Hippo signaling is to restrict cell proliferation, such as during contact inhibition. During *in vitro* culture, cells at low density exhibit nuclear YAP, whereas YAP is predominately cytoplasmic in high density cell culture^{17,20,21}. Mechanistically, this regulation of Hippo signaling is in part mediated by protein complexes involved in junction formation and cell polarity. Assembly of the tight junction-associated Crumbs complex in high density epithelial cells was found to increase YAP/TAZ phosphorylation, leading to their cytoplasmic sequestration²⁰. Scribble, a protein regulating apical-basal polarity, was also required for Hippo activity in mammalian cells by promoting the formation of the membrane complex of MST, LATS, and TAZ proteins²². The tumor suppressor Liver Kinase B1 (LKB1) was found to promote the proper localization of Scribble to this membrane complex through activation of proteinase-activated receptor-1 family proteins, thus activating Hippo signaling (Figure 1A)²³, although other groups have reported LKB1 inhibition of YAP in a LATS1/2-independent manner²⁴. Importantly, liver-specific deletion of LKB1 resulted in increased hepatocyte proliferation and hepatomegaly. This phenotype was rescued by co-deletion of YAP²³, demonstrating a role of cell polarity complexes in regulating Hippo signaling in the liver.

Components of the adherens and tight junctions themselves have also been shown to regulate Hippo signaling. In human keratinocytes the adherens junction protein α -catenin inhibits YAP activity by forming a cell membrane complex with S127 phosphorylated YAP and 14-3-3, which also reduces the accessibility of YAP to protein phosphatase 2A²¹. Indeed, liver-specific knockdown of α -catenin prior to two-thirds partial hepatectomy increased YAP activation, hepatocyte proliferation, and triggered hepatomegaly, disorganization of the actin cytoskeleton, disrupted tight junctions, and dilated bile canaliculi²⁵. In breast cancer cell lines, E-cadherin promoted contact inhibition through regulation of Hippo signaling components, and depletion of E-cadherin, β -catenin, or α -catenin promoted YAP nuclear localization²⁶. In kidney epithelial cells, mechanical strain triggered rapid YAP nuclear translocation and cell cycle reentry, and E-cadherin homotypic cell-cell adhesion was found to maintain quiescence and prevent nuclear accumulation of YAP²⁷. Interestingly, YAP can also regulate junction formation, as overexpression of transgenic YAP in mouse hepatocytes antagonized E-cadherin-mediated adherens junction formation and inhibited hepatocyte cell-cell adhesion *in vitro*²⁸. These data suggest crosstalk between cell polarity and junctional complexes as key regulators of Hippo/YAP signaling.

Biomechanical Regulation

Mechanotransduction has also been identified as an important regulator of Hippo/YAP signaling and can operate in both a Hippo component-dependent and independent manner (reviewed in²⁹). YAP/TAZ activity is governed by both stiffness and cell geometry: YAP/TAZ underwent nuclear translocation and increased transcriptional activity in cells grown on extracellular matrix (ECM) of high stiffness or during cell spreading^{30,31}. This activation required the formation of stress fibers and the activity of the small GTPase Rho independently of the Hippo cascade^{30,31} (Figure 1B). However, other groups have reported that the regulation of YAP by the actin cytoskeleton requires Hippo signaling,

as actin cytoskeleton disruption-induced YAP inhibition required LATS1/2 activity^{32,33}. Importantly, a recent study demonstrated that mechanical tension and formation of stress fibers triggered YAP activation in cultured primary hepatocytes, triggering hepatocyte dedifferentiation and loss of hepatocyte function. Restricting cell spreading, deletion of *Yap1* from hepatocytes, or treatment with a cocktail of drugs targeting actin/actomyosin dynamics maintained hepatocyte function during long term culture³⁴, demonstrating the crucial role of mechanotransduction in the regulation of YAP activity in hepatocytes.

Additionally, YAP/TAZ have been shown to activate Hippo signaling in a negative feedback loop through increased transcription and activation of LATS1/2, NF2, and the angiominin (AMOT) protein family^{35–37}. The AMOT protein family consists of AMOT130, AMOTL1, and AMOTL2 which bind F-actin, and disruption of the actin cytoskeleton frees AMOT proteins to bind and sequester YAP/TAZ in the cytoplasm^{35,38}. LATS1/2 can additionally phosphorylate and stabilize AMOT proteins to enhance YAP/TAZ inhibition^{35,38}. However, AMOT proteins may also promote YAP activity. NF2 is a known tumor suppressor, and mice with liver-specific deletion of NF2 develop liver cancer^{40,41}. Dual deletion of NF2 and Angiominin reduced liver tumorigenesis, and in NF2-deficient cells Angiominin bound to YAP and promoted its nuclear translocation and transcription of target genes⁴¹. Thus, in the context of the liver, AMOT proteins may function to promote YAP activity⁴².

Input from Other Signaling Pathways

In addition to its regulation by biomechanical cues, many other inputs are known to regulate Hippo signaling. For example, Hippo signaling is modulated by diverse aspects of cell function, such as responses to oxidative stress⁴³ and many aspects of cellular metabolism⁴⁴. GPCR signaling can modulate Hippo signaling; lysophosphatidic acid or sphingosine 1-phosphate activated Gα_{12/13}- or Gα_{q/11}-coupled receptors which in turn inhibited LATS1/2 and increased YAP activation (Figure 1B). Subsequent studies have shown that non-canonical Wnt ligands (Wnt3a, Wnt5a, and Wnt5b)⁴⁶ and thrombin⁴⁷ can signal through Gα_{12/13}-coupled receptors to activate Rho GTPase, which in turn inhibit LATS1/2 and promote YAP/TAZ activation. However, the role of these pathways in physiological activation of YAP activity remains to be determined

Signaling pathways involving tyrosine kinase receptors such as epidermal growth factor receptor (EGFR)⁴⁸, fibroblast growth factor receptor (FGFR)⁴⁹, and insulin signaling⁵⁰ have been shown to crosstalk with Hippo signaling. These pathways are especially relevant in the context of liver cancer and will be discussed in more detail later in this review. The nonreceptor tyrosine kinases Src and focal adhesion kinase (FAK) can regulate Hippo signaling through multiple mechanisms. In keratinocytes, Src has been shown to activate YAP through direct phosphorylation of residues Y341/357/394⁵¹. Src has additionally been shown to phosphorylate and inhibit LATS1⁵². Nemo-like kinase has been found to phosphorylate YAP on S128 in response to osmotic stress, which increases YAP activity through reduced proteasomal degradation^{54,55}. YAP activity is also regulated by lysine methyltransferases. Methylation of YAP K494 by Set7 inhibited YAP activity by promoting cytoplasmic retention⁵⁶, while methylation of YAP K342 by SET1A promoted YAP activity through increased nuclear accumulation⁵⁷. Nuclear receptors have also been shown to

regulate YAP activity. Stimulation of the pregnane X receptor (PXR) induced hepatomegaly, as PXR bound YAP and promoted its nuclear translocation and subsequent hepatocyte proliferation⁵⁸. The nuclear receptors farnesoid X receptor and small heterodimer partner regulate bile acid (BA) levels within the liver, and pathologically elevated BA levels activated YAP through increased levels of the scaffolding protein IQ Motif Containing GTPase Activating Protein 1 (IQGAP1)⁵⁹. Interestingly, overexpression of IQGAP1 inhibits cell-cell adhesion by promoting dissociation of α -catenin from E-cadherin and β -catenin⁶⁰, linking BA regulation of YAP to regulation of adherens junctions. Collectively, these studies demonstrate the complex regulation of YAP activity by myriad upstream inputs.

Downstream targets of YAP/TAZ

YAP/TAZ promote the activation of a wide variety of signaling pathways in a cell type and context-dependent manner. As will be discussed in more detail in the following sections, YAP/TAZ are known to regulate NOTCH signaling⁶¹, anti-apoptotic pathways⁶, transforming growth factor β (TGF β) signaling⁶², actin polymerization⁶³, Hedgehog signaling⁶⁴, mammalian target of rapamycin complex 1 (mTORC1) activity⁶⁵, nucleotide biosynthesis⁶⁶, AKT signaling⁶⁷, and more.

Hippo Signaling in Liver Development

During mouse embryonic development, the liver is derived from the foregut endoderm. The activity of the TFs hepatocyte nuclear factor 1 β (HNF1 β), forkhead box A1 (FOXA1), FOXA2, and GATA binding protein 4 (GATA4) are crucial for hepatic specification, which occurs at approximately E8.25 within the foregut endoderm⁷³ (Figure 2A). At E9.0, the liver diverticulum forms from the ventral domain of the foregut endoderm and subsequently develops into the liver bud containing hepatoblasts, the bipotent embryonic precursors to both hepatocytes and BECs (Figure 2B). As the mechanisms underlying hepatoblast differentiation are complex, for a more in-depth review of this topic we direct the reader to references^{73,74}. In general, activation of TGF β , Notch, Wnt/ β -catenin, and other signaling pathways promote a cholangiocyte fate within hepatoblasts⁷⁴, whereas the hepatocyte fate depends on the activity of a core regulatory network of TFs consisting of HNF1 α , HNF1 β , FOXA2, HNF4 α , HNF6, and liver receptor homolog-1⁷⁵ (Figure 2C). Interestingly, single cell RNA sequencing (scRNA-seq) analysis of isolated hepatoblasts from multiple time points during fetal mouse liver development found that hepatoblast differentiation to hepatocytes represents the default cell fate, with differentiation occurring from E13.5 to E15.5. In contrast, cholangiocytes were specified as early as E11.5 but more prominently by E13.5, with cholangiocyte maturation being driven by activation of MAPK signaling⁷⁶.

Although the mechanisms of hepatoblast specification to hepatocytes or BECs remains incompletely understood, several studies have implicated a role for YAP signaling in this process. scRNA-seq of hepatoblasts undergoing differentiation to BECs identified *Tead1* and *Tead4* as significantly upregulated during this process⁷⁶. The TEAD1 TF motif was enriched in an analysis of enhancer regions co-bound by the master regulators of liver development HNF4 α and FOXA2 in hepatoblasts from E14.5 livers. *Tead2* was highly expressed in embryonic liver, and expression of TEAD2 and YAP1 increased *in vitro* luciferase reporter

expression driven by enhancers regulated by HNF4 α and FOXA2, suggesting a role for YAP in regulating hepatoblast differentiation⁷⁷. Mice with deletion of *Lats1/Lats2* in hepatoblasts via *Albumin-Cre* died prior to weaning due to lack of functional hepatocytes, as their livers consisted of large numbers of immature cholangiocytes. Mechanistically, this aberrantly enhanced cholangiocyte differentiation was due to increased TGF β signaling through YAP-driven expression of *Tgf β 2*. Furthermore, expression of constitutively-active YAP in hepatoblasts decreased *Hnf4a* expression, suggesting YAP can function to suppress hepatocyte differentiation^{62,78}. Similarly, liver-specific deletion of *Mst1/Mst2*⁶⁸, *Ww45* (SAV1)⁷⁹, or *Nf2*⁴⁰ via *Albumin-Cre* results in liver overgrowth due to expansion of cells resembling immature BECs (Figure 2F). Simultaneous deletion of *Nf2* and *Notch2* partially rescued the *Nf2* mutant phenotype, suggesting the Notch signaling pathway may act downstream of YAP signaling during hepatoblast differentiation⁸⁰. However, none of these studies have assessed the role of Hippo signaling in early hepatoblast differentiation, as *Albumin-Cre* activity peaks during late gestation (Figure 2D)⁴⁰ and hepatoblast differentiation begins as early as E13.5⁷⁶. Thus, the specific timing of YAP activation and the molecular mechanisms driving this process remain to be completely elucidated.

While the above studies demonstrate that YAP activity must be carefully regulated during early liver development, recent work has demonstrated that YAP may not be required for late-gestational or early postnatal hepatocyte proliferation. Mice with *Albumin-Cre*-mediated deletion of *Yap/Taz* developed normally and did not display a reduction in liver size or hepatocyte proliferation^{81,82}. However, hepatocytes from YAP-null livers displayed reduced viability⁴⁰, consistent with the observation that YAP promotes resistance to apoptosis⁶. Furthermore, YAP/TAZ-null livers failed to form mature bile ducts^{81,82}, triggering progressive development of hepatic steatosis, fibrosis, and liver enlargement during adulthood⁴⁰ (Figure 2E). As will be discussed further in the following sections, these studies suggest that YAP/TAZ activity play a more pronounced role in BEC development and function compared to hepatocytes.

Hippo Signaling in Liver Homeostasis

Hippo signaling plays a crucial role in maintaining adult hepatic quiescence by limiting cell proliferation, as overexpression of YAP in the liver is sufficient to induce dramatic hepatomegaly in mice and zebrafish^{6,7,66}. Importantly, Hippo signaling has recently emerged as a master regulator of liver cell fate. Doxycycline (Dox)-inducible expression of S127A-YAP or acute deletion of *Nf2* in adult hepatocytes triggered their dedifferentiation to cells expressing BEC markers such as SRY-box 9 (Sox9) and cytokeratin 19 (CK19). Notch signaling was identified as a crucial downstream pathway in the dedifferentiation process. Cessation of S127A-YAP expression allow cells to re-differentiate into hepatocytes, highlighting the plasticity of liver cell fate⁶¹. YAP signaling also plays major roles in BEC homeostasis. Numerous groups have described active YAP signaling in adult cholangiocytes^{61,80,83,84}. A recent study from our group demonstrated that YAP is required for maintenance of adult BECs, as long-term deletion of *Yap* throughout the entire liver led to bile duct paucity and development of hepatic necrosis⁸⁵. Similarly, lack of bile ducts is observed in adult *Albumin-Cre Yap* or *Yap/Taz* knockout mice^{40,81} or mice with

conditional BEC-specific *Yap/Taz* knockout⁸². YAP activity in BECs was found to be driven by exposure to hydrophobic BAs^{59,85}, and BA sequestration prevented loss of bile ducts in long-term *Yap* deleted mice⁸⁵, suggesting active YAP signaling functions to prevent cell death in adult BECs (Figure 3A). In contrast to its vital role in BECs, YAP appears to play a minimal role in hepatocytes during homeostatic conditions. Deletion of YAP from adult hepatocytes resulted in no discernable phenotype in the liver^{84,85}, although compensatory upregulation of TAZ may confound this observation³⁷. YAP appears to be minimally expressed in adult hepatocytes. In mice with knock-in of enhanced green fluorescence protein (EGFP) in the YAP target gene connective tissue growth factor (*Ctgf*) locus, no EGFP expression was observed in hepatocytes⁶¹. scRNA-seq of hepatocytes isolated from homeostatic livers failed to detect hepatocytes with active YAP signaling gene signatures⁸⁵.

Despite seemingly negligible expression of YAP in hepatocytes, YAP has been proposed to regulate aspects of hepatic metabolism such as metabolic zonation, which is the compartmentalization of opposing metabolic processes in zones of hepatocytes based upon their localization within the hepatic lobule⁷³. YAP protein staining was reported as a gradient, with nuclear localization of YAP in periportal hepatocytes and reduced staining with nuclear exclusion in pericentral hepatocytes⁸³. Acute deletion of *Yap1* in adult hepatocytes led to expansion of pericentral glutamine synthetase (GS)-positive hepatocytes, while acute deletion of *Mst1/Mst2* reduced the number of GS-positive pericentral hepatocytes⁸³. Similarly, inducible liver-specific deletion of the F-actin capping protein CapZ β resulted in YAP hyperactivation and reduced expression of pericentral metabolic genes such as GS⁴². However, a study by Verboven *et al.* reported no alterations in hepatic zonation in liver-specific *Yap/Taz* knockout mice⁸². Thus, the role of YAP in hepatic zonation remains to be clearly defined.

Hippo Signaling in Liver Regeneration

The liver possesses dramatic regenerative capacity, as both hepatocytes and BECs are capable of exiting quiescence and proliferating to restore liver mass after injury^{87–90}. In cases of extreme liver injury, BECs are capable of transdifferentiation into hepatocytes^{91–94} or vice versa^{95,96}. Due to its well-known role in regulating cell proliferation, the role of Hippo signaling in liver regeneration has been extensively studied. A common acute liver injury model is acetaminophen (APAP)-overdose, which induces centrilobular necrosis and subsequent hepatocyte proliferation. Mice with liver-specific hyperactive YAP signaling were protected from APAP-induced liver damage^{42,97}, although this may be due to decreased expression of the genes required to metabolize APAP into its cytotoxic metabolite, which displayed reduced expression in livers with YAP hyperactivation^{42,83}. Excitingly, pharmacological inhibition of *Mst1/2* after exposure to APAP reduced hepatocyte death and improved overall survival, suggesting YAP activation may be a novel therapeutic approach to treat APAP overdose⁹⁸.

One of the most common models to study hepatocyte-driven liver regeneration is the two-thirds partial hepatectomy (PHx) model, where two-thirds of the liver is surgically removed, inducing hepatocyte proliferation until the liver returns to its normal size. Decrease in phosphorylated (inactive) YAP and increase in nuclear YAP localization in

hepatocytes was evident as an early event post-PHx in mice^{97,99}, and pharmacological inhibition of Mst1/2 promoted hepatocyte proliferation and enhanced liver regeneration in mice post-PHx⁹⁸. Interestingly, YAP signaling in hepatic stellate cells (HSCs) was found to promote liver regeneration post-PHx. Hedgehog signaling was identified as an upstream activator of YAP in HSCs, and inhibition of Hedgehog signaling in HSCs reduced YAP nuclear accumulation in hepatocytes post-PHx, suggesting a YAP-dependent crosstalk between HSCs and hepatocytes during liver regeneration¹⁰⁰ (Figure 3B). Finally, mice with *Albumin-Cre*-mediated deletion of both YAP and TAZ displayed major delays in liver regeneration post-PHx due to decreased hepatocyte proliferation⁸¹. However, these results may be confounded by the fact that YAP and TAZ were deleted during embryonic liver development.

A recent study sought to carefully assess the cell type-specific role of YAP/TAZ in liver regeneration. Mice were subjected to PHx or acute liver injury via carbon tetrachloride (CCl₄) administration, which results in centrilobular hepatocyte death. Surprisingly, while hepatocytes displayed robust activation of YAP/TAZ signaling 48 hours post-CCl₄ exposure, deletion of *Yap/Taz* from hepatocytes did not impair liver regeneration either post-PHx or after CCl₄-induced liver injury. Instead, deletion of *Yap/Taz* from BECs resulted in delayed liver regeneration via cholestasis-induced delays in the recruitment of phagocytic macrophages and clearance of dead hepatocytes⁸². These results demonstrate that YAP/TAZ activation in hepatocytes is not required for liver regeneration from acute hepatic injury. This is not necessarily surprising, as the liver is able to compensate for the inhibition of a specific signaling pathway by activating alternative pathways during hepatocyte proliferation¹⁰¹ and YAP/TAZ are not required for hepatocyte proliferation in postnatal liver development^{81,82}. However, as will be discussed in the next section, YAP/TAZ activity are essential for models of BEC-driven liver regeneration.

Hippo Signaling during Chronic Liver Injury

YAP signaling has also been implicated in regeneration during chronic liver injury. A common feature of CLD of nearly any etiology is the ductular reaction (DR), or expansion of cells expressing BEC markers. Depending on the injury context, the cells of the DR may originate from proliferation of BECs or dedifferentiation of hepatocytes¹⁰². Hippo signaling has been recently identified as a crucial regulator of this process. Increased YAP activity is observed in BECs isolated from the livers of mice subjected to bile duct ligation (BDL)⁸⁵ and deletion of *Yap1* in the liver via *Mx1-Cre* impaired the DR, decreased hepatocyte proliferation, and increased hepatic necrosis post-BDL⁸⁴. Recently, a CRISPR-Cas9 loss of function screen in liver biliary organoids demonstrated that YAP was essential for organoid growth *in vitro*. Furthermore, mice with *Yap1* deletion in both hepatocytes and BECs placed on the 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC) diet displayed a dramatically reduced DR due to decreased proliferation and increased apoptosis of CK19+ cells⁹⁰. Interestingly, a recent report identified alternative splicing of Hippo pathway components to neonatal isoforms in hepatocytes as a mechanism of increased YAP activity and hepatocyte proliferation in response to DDC diet-induced liver injury¹⁰³. Our group has shown via scRNA-seq increased numbers of YAP-active BECs as well as increased YAP activity in hepatocytes isolated from DDC diet fed animals (Figure 3C). Furthermore, deletion of

Yap1 specifically in hepatocytes reduced the extent of the DR in DDC diet fed animals, implying hepatocyte dedifferentiation as a major source of DR cells in DDC diet-induced liver injury⁸⁵. A study by Li *et al.* identified *Arid1a*, a component of the ATP-dependent SWI/SNF chromatin remodeling complex, as a key player in this process. Deletion of *Arid1a* specifically from hepatocytes reduced the number of Hnf4 α + Sox9+ hepatocytes in the livers of animals fed DDC diet for two weeks. Furthermore, chromatin accessibility studies revealed that a significant number of genes were co-bound by YAP and Arid1a. YAP binding to target sites and target gene expression were significantly reduced in *Arid1a*-null hepatocytes isolated from DDC diet fed animals, leading the authors to conclude that Arid1a facilitates YAP-mediated transcription during liver regeneration¹⁰⁴. However, a separate study performed by Chang *et al.* described exactly the opposite phenotype. Mice with hepatocyte-specific deletion of *Arid1a* administered DDC diet for 6 weeks displayed an increased DR, and this phenotype was abrogated in *Arid1a/Yap1/Wwtr1* triple mutant livers. Furthermore, hepatocyte-specific deletion of both *Nf2* and *Arid1a* triggered dramatic liver overgrowth, strong induction of YAP/TAZ transcriptional activity, and the development of liver cancer¹⁰⁵. Arid1a seems to be dispensable for YAP regulation during homeostasis, as both groups found that *Arid1a*-deleted livers appeared normal during resting conditions^{104,105}. A possible explanation for this phenomenon is the different duration of DDC diet, which may influence the extent of liver injury and the DR. More research is necessary to clarify the role of Arid1a in the regulation of YAP activity during liver regeneration.

Hippo signaling has also been implicated in the development of liver fibrosis. During acute liver injury, activation of HSCs (widely considered the main source of liver myofibroblasts) and deposition of ECM is an intrinsic component of the wound healing response. However, during CLD, iterative cycles of hepatic damage and repair triggers continuous activation of HSCs and eventually exhausts the regenerative potential of the liver, leading to massive deposition of ECM and loss of organ function as fibrosis progresses to cirrhosis³. A common mouse model used to study fibrosis progression in mice is chronic CCl₄ administration (Figure 3D). A recent study found that expression of S127A-YAP in hepatocytes of mice exposed to CCl₄ promoted expression of Cyr61, a macrophage chemoattractant, and increased inflammation, myofibroblast expansion, and fibrosis. Furthermore, deletion of YAP/TAZ from hepatocytes reduced CCl₄-induced liver fibrosis¹⁰⁶. A separate group identified increased expression of TAZ in hepatocytes in samples from human patients with fibrosis due to non-alcoholic steatohepatitis (NASH), a condition marked by accumulation of fat in the liver accompanied by inflammation, hepatocyte death, and fibrosis. These findings were confirmed in multiple mouse models of NASH, where elevated expression of TAZ in hepatocytes increased expression of TAZ target Indian hedgehog, which increased activation of HSCs and promoted fibrosis⁶⁴. Interestingly, a separate group found that YAP activation was protective against fibrosis in a mouse model of ischemia-reperfusion injury in a Nrf2-dependent manner. Mechanistically, YAP activation reduced oxidative stress, apoptosis, innate immune activation, and HSC activation¹⁰⁷. The discrepancy between these studies may be explained by the fact that YAP activation prior to the onset of ischemia-reperfusion injury reduced overall hepatocyte death¹⁰⁷ and therefore

reduced the subsequent injury response. These results demonstrate that, depending on the liver injury context, YAP/TAZ activation can be either pro- or anti-fibrotic.

Recently, YAP, but not TAZ⁶⁴, has been identified as a driver of HSC activation. Nuclear YAP staining is not detected in HSCs from healthy livers but is evident in the fibrotic livers of both humans and mice¹⁰⁸. Isolated primary HSCs are known to undergo transdifferentiation to myofibroblasts when grown under conventional 2D culture conditions. Treatment with the YAP inhibitor verteporfin prevented upregulation of *Acta2* and *Col1a1* in cultured HSCs and reduced fibrogenesis after chronic CCl₄ treatment *in vivo*, suggesting YAP signaling is required for HSC activation^{108,109}. Mechanistically, activation of HSCs is partly driven by the stiffness of the surrounding environment, as HSCs grown in 3D spheroids *in vitro* remained quiescent and did not upregulate expression of YAP target genes¹⁰⁸. As described earlier in this review, YAP is a well-known mechanotransducer which is activated in cells grown on high-stiffness substrates²⁹. Consistent with these reports, loss of integrin β 1 in cultured HSCs inhibited induction of fibrotic markers and prevented increases in YAP expression and nuclear localization¹⁰⁹.

In addition to HSCs, Hippo signaling in other non-parenchymal cell (NPC) types have been found to influence chronic liver disease. A recent study described a role for YAP activation in Kupffer cells, the resident liver macrophage, in promoting NASH progression. Mice fed a high fat diet (HFD) for 12 weeks displayed increased YAP expression and nuclear accumulation in Kupffer cells. Macrophage/monocyte-specific YAP deletion reduced pro-inflammatory cytokine expression and lobular inflammation without affecting steatosis in HFD-fed mice, and expression of constitutively-active YAP in primary Kupffer cells increased pro-inflammatory cytokine expression¹¹⁰. Functions of YAP in liver sinusoidal endothelial cells (LSECs) are only just beginning to emerge. YAP has been reported to promote vessel maintenance in response to laminar shear stress in zebrafish endothelial cells¹¹¹. A study by Zhang *et al.* found that YAP stabilized hypoxia-inducible factor 1 α levels in LSECs, which promoted angiogenesis in CCl₄-induced liver fibrosis¹¹². Thus, YAP activity in NPCs may facilitate disease progression, although more research is necessary to fully elucidate the role of Hippo signaling in these cell types.

Hippo Signaling in Liver Cancer

Liver cancer is the 6th leading cause of cancer worldwide and has a 5-year survival rate of only 18%¹. The lack of effective treatments for liver cancers has prompted intense investigation into the molecular mechanisms promoting tumorigenesis that represent potentially druggable targets. To this end, the Hippo signaling pathway has been extensively studied, as chronic overexpression of YAP⁶ or loss of *Mst1/2*^{8,113,114}, *Nf2*⁴⁰, or *Sav1*¹¹³ in the mouse liver triggers the eventual development of liver cancers including hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC).

Hippo Signaling in Hepatocellular Carcinoma

HCC is the most common primary liver cancer, and nuclear accumulation of YAP is frequently observed in human HCC samples^{6,17,115,116}. Activation of YAP is associated with more aggressive subtypes of HCC^{83,117}, including a concordance of a silenced

Hippo pathway gene signature with a hepatic stem cell gene signature¹¹⁸. These patients exhibited significantly reduced overall survival, demonstrating that YAP activation is an independent prognostic marker in HCC^{117,118}. However, mutation of components of the Hippo pathway are rarely observed in liver cancer¹¹⁹, although focal amplification of human chromosome 11q22, which contains the *YAP1* gene, has been reported as a low-frequency event in HCC¹²⁰. The lack of mutations suggests inhibition of the Hippo pathway or activation of YAP occurs through crosstalk with other oncogenic signaling pathways during tumorigenesis.

The upstream mechanisms which activate YAP in liver cancer have not been fully elucidated, but several likely candidates have emerged (Figure 4A). BA overload has been shown to promote liver tumorigenesis^{121,122}, and BA-mediated activation of IQGAP1 has been shown to promote YAP activity⁵⁹. Importantly, expression of YAP and IQGAP1 was minimal in healthy livers but detected in cholestatic diseased livers and in HCC⁵⁹, suggesting BAs may promote YAP activation as a mechanism of tumorigenesis. Tyrosine kinase receptors are major oncogenic pathways in liver cancer and have been shown to interact with Hippo signaling. EGFR signals through PI3K-PDK1 to activate YAP in HCC, and treatment of HCC cell lines simultaneously with EGFR and YAP inhibitors synergistically induced cytotoxicity¹²³. Mice with liver-specific deletion of *Sav1* and phosphatase and tensin homolog initially developed fatty liver, inflammation, and liver tumors by 15 weeks of age. Mechanistically, YAP/TAZ increased expression of insulin receptor substrate 2, which in turn activated AKT and promoted tumorigenesis⁶⁷. These studies highlight the role of crosstalk between YAP/TAZ and other oncogenic signaling pathways in the development of HCC.

As discussed in an earlier section, YAP is a mechanotransducer and is activated in cells grown on stiff substrates^{30,31}. As liver cirrhosis is a major risk factor for HCC³, this raises the question of whether increased liver stiffness during cirrhosis activates YAP signaling and potentiates tumorigenesis. A recent study reported that increased expression of the ECM proteoglycan Agrin in liver cancer was associated with reduced overall survival. Increased ECM stiffness activated Agrin, which in turn activated YAP activity to promote oncogenesis in liver cancer cell lines¹²⁴. Furthermore, HCC cell lines grown on substrates mimicking the stiffness of cirrhotic livers displayed increased resistance to sorafenib in a YAP-dependent manner¹²⁵, suggesting the mechanotransduction functions of YAP may play a role in promoting HCC. Multi-drug resistance is common in HCC, and intratumor hypoxia has been shown to promote HCC resistance to sorafenib¹²⁶. Hypoxia has been shown to trigger YAP activation through inhibition of Lats2¹²⁷, which led to increased sorafenib resistance in HCC cells¹²⁸.

YAP transcriptional activity in HCC has been shown to promote tumorigenesis through multiple mechanisms. We recently identified the kinase NUA2 as a direct transcriptional target of YAP. NUA2 activity triggered actin polymerization and increased actomyosin tension to further promote YAP activity, and NUA2 inhibition reduced tumorigenesis in a YAP-driven liver cancer mouse model⁶³. A separate group found that YAP activity promoted cell viability, migration, and invasion in HCC cell lines through induction of *Jagged1* and activation of Notch signaling in a TEAD4-dependent manner. Furthermore, nuclear

expression of YAP, Jagged1, and Notch intracellular domain (NICD) were associated with poor prognosis in human HCC patients¹¹⁵. Interestingly, YAP induction of *Jagged1* was regulated by Mst1/2 but not β -catenin^{115,129}. Activating mutations in β -catenin (encoded by *CTNNB1*) are present in approximately 30% of HCC samples, but expression of YAP is negatively correlated with expression of β -catenin in HCC tumors^{83,130}, suggesting antagonism between the two pathways in the context of HCC. Indeed, liver-specific deletion of *Mst1/2* increased tumorigenesis through YAP/TAZ activation, which formed a positive feedback loop with Notch signaling as increased NICD expression prevented TAZ protein degradation and further promoted YAP/TAZ activity. Co-deletion of *Ctnnb1* and *Mst1/2* dramatically increased tumorigenesis, as increased β -catenin activity promoted expression of dimerization partner 1, which in turn promoted NICD degradation and inhibition of Notch signaling¹²⁹.

Hippo Signaling in Hepatoblastoma

Paradoxically, while YAP and β -catenin appear to have an antagonistic relationship in HCC, they appear to cooperate in the promotion of hepatoblastoma (HB), the most common pediatric liver cancer. Nuclear accumulation of YAP and β -catenin was observed in approximately 80% of HB samples, and sleeping beauty transposon system hydrodynamic tail vein injection (SB-HDTVI)-mediated delivery of S127A-YAP and constitutively-active β -catenin into hepatocytes led to the rapid development of HB in mice¹³⁰. Later studies found that in HB cells YAP/TAZ promoted expression of the amino acid transporter SLC38A1, triggering activation of mTORC1 and tumorigenesis⁶⁵ (Figure 4B). Interestingly, YAP/TAZ-driven upregulation of amino acid transporters¹³¹ or enhanced nucleotide biosynthesis⁶⁶ triggering downstream mTORC1 activation has also been identified in HCC, suggesting crosstalk between the YAP and mTOR signaling pathways as a common driver of liver carcinogenesis. Importantly, a recent study showed that silencing of S127A-YAP in the S127A-YAP/constitutively-active β -catenin-driven HB mouse model resulted in dramatic tumor regression, driven by apoptosis in a subset of tumor cells and partially restoring hepatocyte differentiation in surviving tumor cells¹³². These results suggest targeting YAP may be an effective therapy for HB.

Hippo Signaling in Cholangiocarcinoma

YAP has also been hypothesized to be an oncogenic driver of CC, the second most common type of primary liver cancer. Nuclear YAP accumulation is observed in 85–98% of human CC tissue samples^{116,133,134} and is associated with poor prognosis^{133,135}. YAP activation is also associated with increased CC proliferation^{133,134}, invasion¹³³, angiogenesis¹³⁴, chromosomal instability¹³⁶, and chemoresistance^{133,134}. Dual SB-HDTVI-mediated expression of NICD and AKT in hepatocytes^{137,138} or S127A-YAP and oncogenic AKT in hepatocytes¹³⁹ or BECs¹⁴⁰ led to rapid CC formation (Figure 4C). FGFR signaling was found to upregulate YAP expression in CC cell lines, and treatment with a pan-FGFR inhibitor reduced tumor burden in a YAP/AKT-driven CC mouse model⁴⁹. Furthermore, deletion of *Yap* and *Wwtr1* from established CC dramatically reduced tumor burden¹³⁸, suggesting an important role for YAP in driving CC. In addition to the tumor cells themselves, activation of YAP in peritumoral hepatocytes has been observed in both HCC and CC. Surprisingly, deletion of *Yap* and *Wwtr1* specifically in peritumoral hepatocytes

led to increased tumor burden. Activation of YAP in peritumoral hepatocytes reduced tumor burden in a NICD/AKT-driven CC as well as in models of HCC and melanoma liver metastasis¹³⁸. However, deletion of *Yap* and *Wwtr1* in both tumor cells and peritumoral hepatocytes caused no change in tumor burden. Although the exact mechanism remains unclear, increased cell death was detected in tumor cells surrounded by YAP-active hepatocytes, leading the authors to speculate that these cells displayed increased fitness during cell competition with tumor cells¹³⁸. More studies will be necessary to elucidate the mechanisms of YAP activation in peritumoral hepatocytes on the suppression of liver cancer.

Hippo Signaling and Cancer Immunity

Hippo signaling has additionally gained attention in recent years for its emerging role in cancer immunity. The field of cancer immunotherapy has made great advances in treatment strategies, such as anti-PD1/PD-L1 therapy to reactivate T cells to promote tumor cell killing, although resistance or relapse remains a major clinical problem¹⁴¹. YAP/TAZ have been shown to induce expression of PD-L1 in human melanoma, lung, and breast cancer cell lines¹⁴²⁻¹⁴⁵, suggesting a role for YAP/TAZ in suppressing anti-tumor immune responses. However, these results could not be replicated in murine cell lines¹⁴⁴, suggesting species-specific differences in the regulation of PD-L1. Despite the knowledge that PD-L1 expression in HCC is correlated with poor prognosis¹⁴⁶, the relationship between YAP/TAZ and PD-L1 in the context of liver cancer remains to be investigated. In contrast to these studies, YAP/TAZ expression in tumor cells has also been found to promote adaptive immunity anti-cancer responses. Deletion of *LATS1/2* from melanoma, head and neck squamous cell carcinoma, and breast cancer cell lines promoted tumor growth *in vitro* but dramatically inhibited tumor growth *in vivo* during subcutaneous tumor cell transplantation into immunocompetent syngenic mice. It was found that *LATS1/2* deletion increased production of extracellular vesicles rich in nucleic acids which stimulated the type I interferon response and enhanced anti-tumor immunity¹⁴⁷. In the context of the liver, YAP/TAZ activation in hepatocytes have consistently been shown to promote inflammation. Deletion of *Lats1/2* or *Mst1/2* or YAP activation in hepatocytes rapidly triggers immune cell recruitment^{62,106,148,149}. Oncogenic YAP activation in hepatocytes induced *Ccl2* expression, which promoted macrophage recruitment, immune evasion, and tumorigenesis^{148,149}. These results suggest that the inflammation triggered by YAP activation is a key mechanism of tumorigenesis. Although the role of YAP-induced inflammation in tumor maintenance and progression is less well defined, it seems logical that targeting YAP/TAZ could potentially synergize with immunotherapy, although the efficacy of this approach remains to be determined.

Pharmacological Targeting of the Hippo Signaling Pathway

Due to its putative roles as an oncogene and in promoting the progression of CLD, there has been great interest in developing small molecule inhibitors of YAP activity (Figure 5A). Classically, verteporfin was utilized as an inhibitor of YAP-TEAD interactions¹⁵⁰. However, recent work has called into question this mechanism of action, precluding the use of verteporfin as a specific inhibitor of YAP activity¹⁵¹. Much research has been devoted to developing a specific inhibitor of the YAP-TEAD protein-protein

interaction, although this is difficult due to its unusually large interface and lack of a defined binding pocket¹⁵². Recent strategies include targeting the TEAD palmitate-binding pocket, resulting in allosteric inhibition of the YAP-TEAD interaction^{153,154}. Similarly, a covalent TEAD inhibitor promoted apoptosis in non-small cell lung cancer cells with YAP-dependent resistance to EGFR/MEK inhibition¹⁵⁵. Another strategy involves exploitation of endogenous proteins which also bind to TEAD or manipulation of regulators of YAP activity. One group demonstrated that Vestigial Like Family Member 4 (VGLL4) competed with YAP for binding to TEADs, and a VGLL4-mimicking peptide inhibited YAP activity and reduced gastric cancer growth both *in vitro* and *in vivo*¹⁵⁶. Inhibitors targeting positive regulators of YAP activity such as tankyrases^{157,158} or transcriptional regulators such as bromodomain-containing protein 4 (BRD4), which mediates YAP/TAZ-driven transcription¹⁵⁹, have also been considered, although these inhibitors lack specificity to the Hippo signaling pathway. Another approach has been to target disease-specific pathway components rather than YAP/TEAD directly. For example, our group identified NUA2 as a critical downstream target of YAP during liver tumorigenesis, and pharmacological inhibition of NUA2 suppressed YAP-driven tumor growth *in vivo*⁶³. This type of approach may reduce potential off-target effects rather than directly targeting components of such a pleiotropic signaling pathway.

Despite the abundant evidence of the oncogenic role of YAP/TAZ in liver cancer, strategies for targeting the Hippo pathway must be designed with great care. For example, inhibiting YAP/TAZ in homeostatic hepatocytes would probably be well tolerated while inhibition in BECs would most likely have deleterious effects on liver function^{82,85}. Furthermore, Moya *et al.* recently demonstrated genetic deletion of YAP/TAZ specifically in tumor cells reduced tumor burden in a CC mouse model, but YAP/TAZ activity in peritumoral hepatocytes suppressed tumor growth¹³⁸. These results suggest that indiscriminate YAP/TAZ inhibition may be of limited efficacy. An exciting strategy to circumvent these problems is to target YAP/TAZ inhibition to specific cell types. For example, siRNA against *Yap1* or components of the Hippo pathway can be formulated into lipid nanoparticles which target hepatocytes¹⁶⁰. However, it will be important to develop technology to deliver siRNA or small molecules specifically to tumor cells to promote the greatest efficacy and minimize off-target effects.

In addition to cancer, there is also interest in inhibiting YAP/TAZ as an antifibrotic therapy (Figure 5B). Again, cell type-specific targeting has been shown to be important. Despite evidence of YAP activity in fibroblasts promoting fibrosis progression, general YAP/TAZ RNA interference in a mouse model of pulmonary fibrosis actually increased lung injury and fibrosis¹⁶¹. Thus, recent research has focused on methods to inhibit YAP specifically in fibroblasts. As YAP activity is known to be regulated by GPCR signaling⁴⁵, one group sought to identify if fibroblast-specific GPCR expression could serve as the basis for novel antifibrotic therapies. Dihydroxidine (DHX), a selective agonist of the G α_s -coupled dopamine D1 and D5 receptors, inhibited YAP nuclear accumulation in a panel of fibroblasts including HSCs. Excitingly, in BDL-induced liver fibrosis treatment with DHX reduced histological staining for collagen and α smooth muscle actin¹⁶¹. A separate group found that the sphingolipid ceramide promoted HSC inactivation through increased YAP/TAZ proteasomal degradation. Targeting the enzyme that metabolizes ceramide, aCDase, which

is expressed in activated HSCs and minimally in other liver cell types, reduced fibrosis in both CCl₄ and dietary NASH mouse models of fibrosis¹⁶². These results suggest that clever targeting of YAP activity specifically in HSCs may hold promise as a therapy to prevent fibrosis progression.

In addition to developing inhibitors, there is also interest in developing drugs which promote YAP/TAZ activity to augment liver regeneration (Figure 5B). Indeed, the MST1/2 inhibitor XMU-MP-1 was well-tolerated in mice and significantly enhanced liver regeneration after APAP-induced liver injury or post-PHx⁹⁸. Furthermore, siRNA-mediated knockdown of Mst1/2 improved liver regeneration post-PHx in aged mice⁹⁹. Importantly, these strategies involve transient inactivation of Mst1/2, reducing the likelihood of oncogenesis. XMU-MP-1 treatment after repeated CCl₄ exposure or bile duct ligation reduced fibrosis compared to vehicle-treated mice⁹⁸, suggesting it may also have therapeutic implications for CLD. However, a recent study demonstrated that hepatocyte-specific deletion of YAP/TAZ reduced liver fibrosis upon exposure to CCl₄ by reducing inflammation and myofibroblast expansion¹⁰⁶. A potential explanation for these contradictory results is that XMU-MP-1 was administered after cessation of CCl₄ treatment (during the liver regeneration phase), while the study by Mooring *et al.* deleted YAP/TAZ from hepatocytes prior to exposure to CCl₄. It is thus important to determine if XMU-MP-1 treatment during ongoing liver injury promotes or inhibits fibrosis development. Nonetheless, activation of YAP/TAZ as a strategy holds promise for regenerative medicine.

Conclusions

The Hippo signaling pathway has complex, cell type and context-specific roles in many aspects of hepatic function including development, regeneration, and carcinogenesis. While incredible progress has been made in unraveling the complex networks of signaling pathways which interact with the Hippo pathway, much remains to be learned. For example, many studies examine perturbation of either YAP or TAZ alone, although a growing body of evidence suggests that these proteins each have non-redundant functions^{22,64,129}. Additionally, much of the research studying the effect of perturbations of Hippo signaling in a disease context have focused on transgenic animals with either deletion of Hippo signaling components or expression of constitutively active forms of YAP. While these studies yield useful insights, they do not allow for the study of Hippo signaling in its native context or provide information on the upstream inputs which trigger pathological inactivation of the Hippo pathway. This is especially important in the context of CLD, as identification of the early triggers of pathological Hippo signaling perturbations may yield novel therapeutic strategies to prevent progression of CLD to cirrhosis and liver cancer.

An unexpected finding from recent studies is the dispensable role of YAP/TAZ activity in hepatocyte proliferation during development and acute regeneration^{81,82}, seemingly at odds with the long-held belief of Hippo signaling as a master regulator of organ size. However, these facts can be reconciled if one adopts the view that Hippo signaling regulates organ size by preventing aberrant YAP/TAZ activity rather than YAP/TAZ activity being essential for liver growth. YAP/TAZ are potent inducers of cell proliferation if unrestrained by the Hippo pathway, as evidenced by the role of Hippo inactivation in the development

of liver cancer^{40,68,113,114}. Although nonessential for hepatocyte proliferation, YAP/TAZ may increase the fitness of hepatocytes, as YAP-null hepatocytes are more prone to apoptosis⁶ and YAP/TAZ activity in peritumoral hepatocytes inhibits tumor progression¹³⁸. Furthermore, recent research has demonstrated that YAP/TAZ activity is essential for normal BEC function^{82,85,90} and promotion of a ductular reaction after liver injury⁹⁰, underscoring the key role of Hippo signaling in liver biology.

Future Directions

Undoubtedly one of the most exciting research areas is development of anti-YAP/TAZ therapies for liver cancer. To this end, future studies should prioritize the ability to inhibit YAP/TAZ specifically in tumor cells. Drugs such as BRD4 inhibitors show promise towards this effect, as they can preferentially inhibit YAP/TAZ transcriptional activity in cancer cells¹⁵⁹. Tailored drug delivery, such as through lipid nanoparticles¹⁶⁰, is another attractive strategy. The role of YAP/TAZ in liver cancer immunity also warrants further research. As YAP/TAZ have been shown to regulate expression of PD-L1 in multiple cancer types^{142–145}, and PD-L1 expression in HCC is correlated with poor prognosis¹⁴⁶, the relationship between YAP/TAZ and PD-L1 in liver cancer is an obvious area of future investigation.

Targeting YAP/TAZ activity to promote liver regeneration is another exciting field of study. In order to pursue these goals, detailed understanding of disease mechanisms is necessary to identify conditions where such therapies will be effective. The roles of YAP/TAZ activity in cell types such as endothelial cells and resident immune cells are understudied and may have important implications. For example, non-specific targeting of YAP/TAZ in a mouse model of pulmonary fibrosis increased lung injury due to putative detrimental effects on endothelial cells¹⁶¹. Thus, further study of the role of YAP/TAZ in NPCs in CLD is warranted, and application of technologies such as scRNA-seq to samples of diseased human liver will prove invaluable in this regard. Timing of therapeutic delivery is also critically important, as suppression of YAP/TAZ during certain types of ongoing injury may prove beneficial¹⁰⁶ but detrimental during liver regeneration^{90,98}. Similarly, inactivation of Hippo signaling to promote liver regeneration must be tightly controlled to prevent tumorigenesis.

Finally, YAP has recently been identified as a master regulator of liver cell fate and an essential regulator of hepatocyte-to-BEC transdifferentiation during liver injury^{61,85,90}, but the mechanisms underlying this process remain poorly defined. Further research into this topic will likely uncover key transcriptional or epigenetic mechanisms that govern hepatocyte/biliary differentiation. This knowledge may prove useful in the effort to maintain primary hepatocytes in culture³⁴ or promote induced pluripotent stem cell differentiation to mature hepatocytes, which may have implications for the *in vitro* study of hepatocyte drug metabolism and the ability to grow mature hepatocytes for transplantation purposes. Undoubtedly, future research will continue to uncover the roles of Hippo signaling in hepatic biology and lead to the development of therapies to combat a wide variety of liver diseases.

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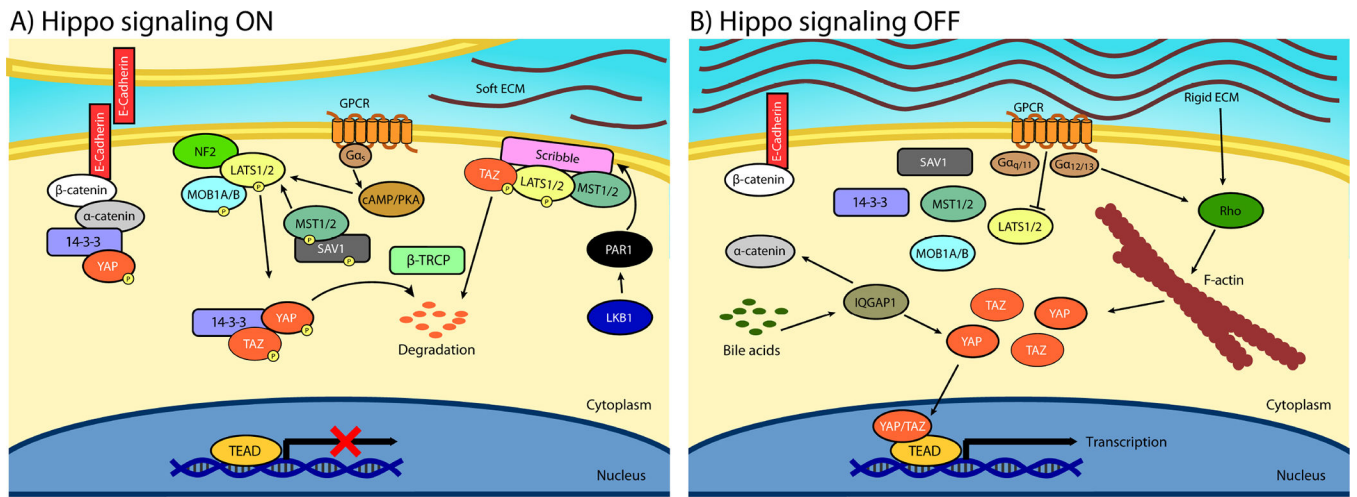


Figure 1). Hippo signaling pathway.

a) The Hippo signaling pathway functions to repress activity of the transcriptional co-activators YAP and TAZ. The kinases MST1/2 form a complex with the scaffolding protein SAV1 and phosphorylate the kinases LATS1/2 in a manner facilitated by NF2. Together with the regulatory proteins MOB1A/B, LATS1/2 phosphorylate YAP/TAZ, which are bound by 14-3-3 and targeted for proteasomal degradation by β-TRCP. Other inputs such as LKB1 or GPCR signaling or contact inhibition through adherens junctions can suppress YAP/TAZ activity. **b)** Multiple upstream inputs such as stiff ECM, low cell density, bile acid exposure, GPCR signaling, and increased actomyosin tension can inhibit Hippo signaling. Non-phosphorylated YAP/TAZ are no longer targeted for degradation and translocate to the nucleus where they interact with TEAD transcription factors to promote target gene expression.

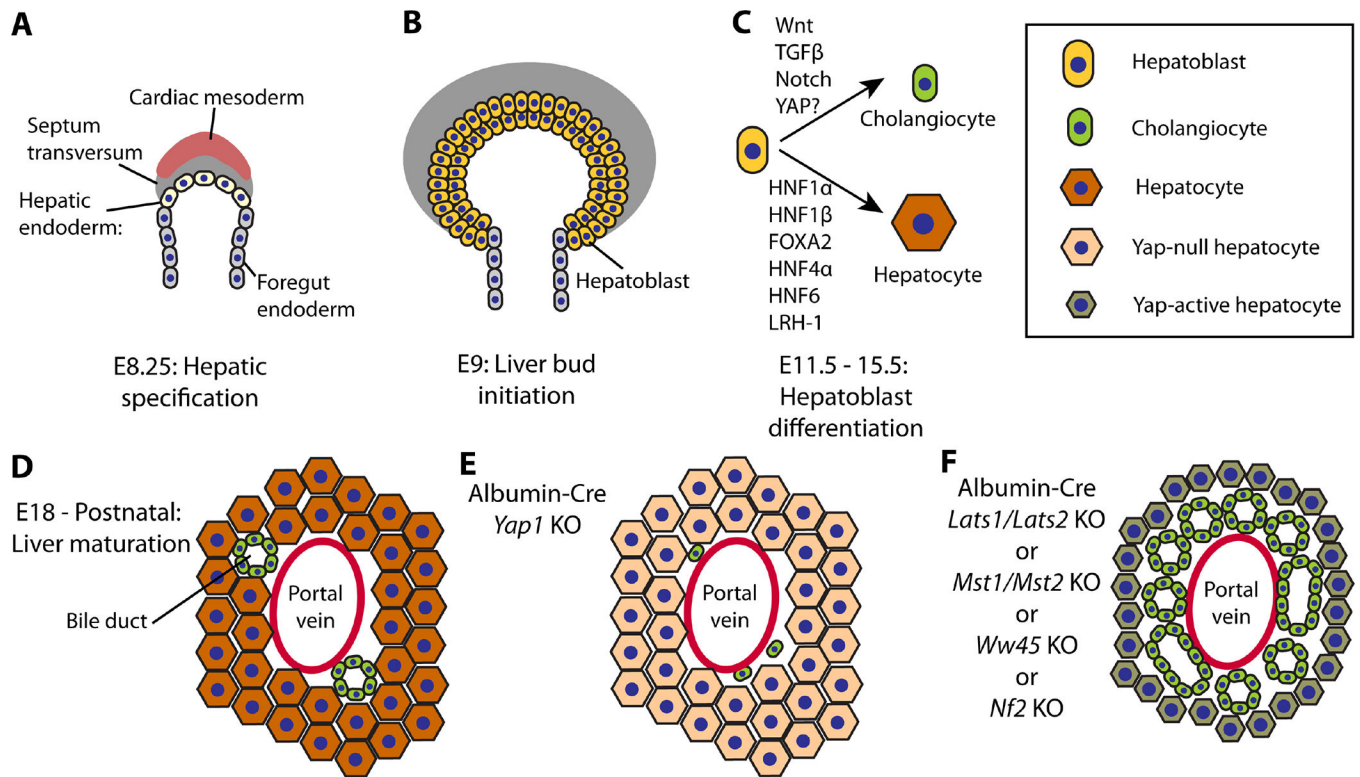


Figure 2). Role of Hippo signaling in liver development.

a) During mouse embryonic development, hepatic specification occurs at E8.25. Interactions of the foregut endoderm with the septum transversum and cardiac mesoderm are required for the specification of hepatic endoderm. **b)** Hepatoblasts, the embryonic precursors to hepatocytes and cholangiocytes, first appear in the liver bud at E9. **c)** Hepatoblast differentiation to hepatocytes or cholangiocytes occurs between E11.5 – E15.5. Cholangiocyte differentiation requires Wnt, TGF β , and Notch signaling, while hepatocyte differentiation requires a regulatory network of transcription factors. **d)** Liver maturation continues after birth to form the mature hepatic architecture. **e)** Deletion of *Yap1* via *Albumin-Cre* impairs bile duct formation and reduces hepatocyte viability. **f)** Hyperactivation of YAP through deletion of upstream Hippo components via *Albumin-Cre* results in failure to form mature hepatocytes and expansion of immature biliary cells.

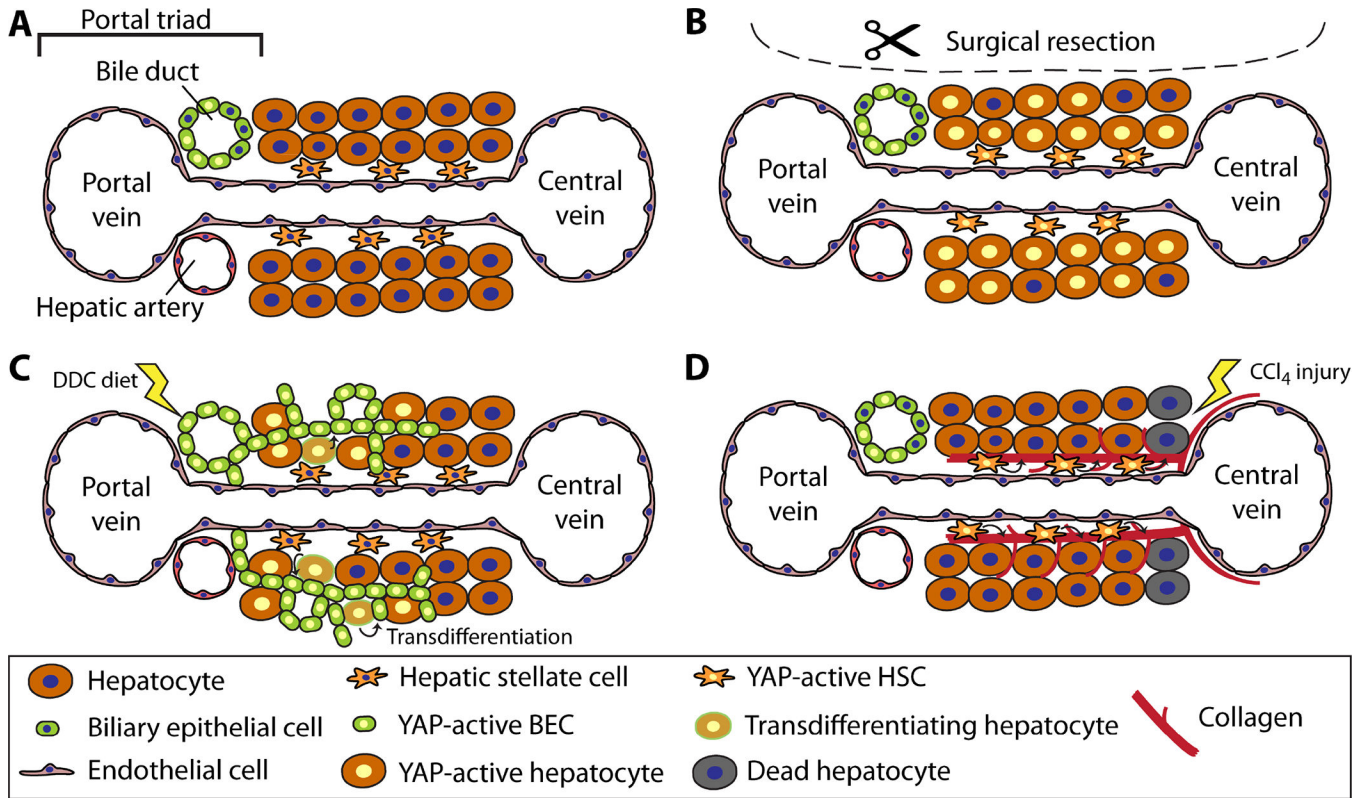


Figure 3). Role of Hippo signaling in liver homeostasis and regeneration.
a) Architecture of the hepatic lobule during homeostasis. The portal triad consists of the portal vein, hepatic artery, and bile ducts. Blood from the portal vein flows through the hepatic sinusoids lined by liver sinusoidal endothelial cells (LSECs) to the central vein. The sinusoids are lined by chords of hepatocytes. Hepatic stellate cells (HSCs) reside in the space of Disse (between LSECs and hepatocytes). During homeostasis YAP is active in a subset of BECs within the bile ducts. **b)** After surgical resection of liver tissue, such as during PHx, YAP signaling is activated in HSCs and hepatocytes. **c)** BEC injury, such as administration of the DDC diet, triggers the ductular reaction. YAP activity in increased in BECs and a subset of hepatocytes. YAP activity in hepatocytes may trigger transdifferentiation to BECs. **d)** Injury induced by CCl₄ administration leads to YAP activation in HSCs and promotes collagen deposition and the development of liver fibrosis.

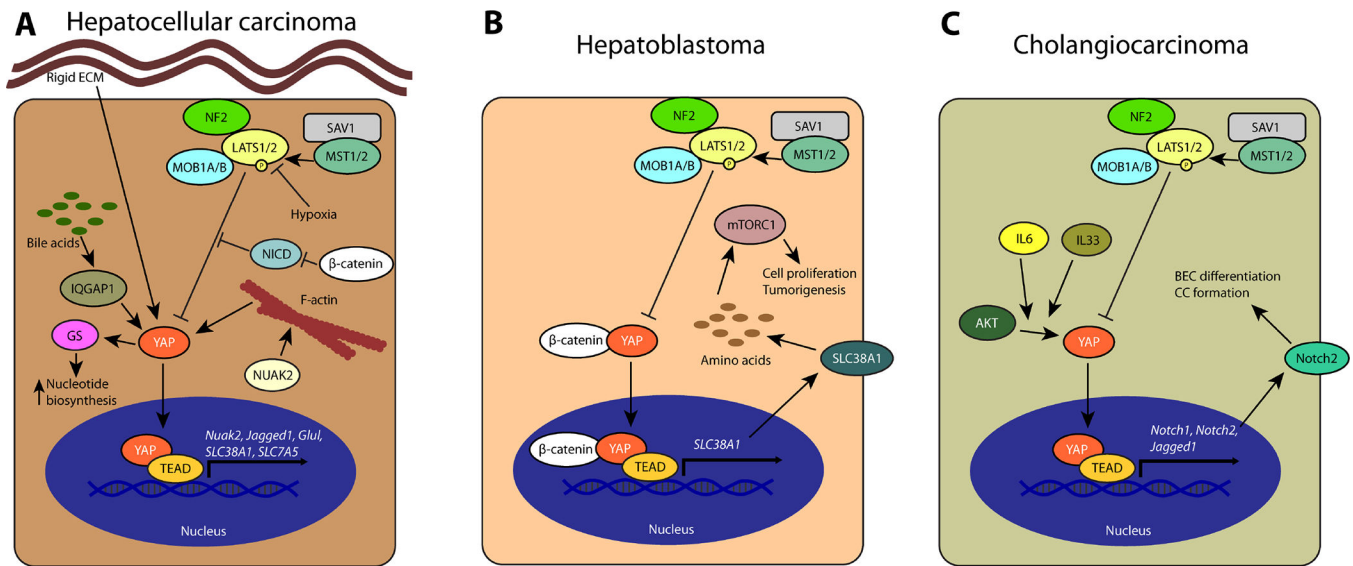


Figure 4). Role of Hippo signaling in liver cancer.

a) In HCC, factors such as rigid ECM, bile acid exposure, hypoxia, and increased actomyosin tension can trigger YAP activation. YAP can promote tumorigenesis through increased nucleotide biosynthesis, enhanced Notch signaling, and expression of amino acid transporters. β -catenin inhibits YAP activity through inhibition of Notch signaling. **b)** In hepatoblastoma, β -catenin and YAP cooperate to promote tumorigenesis through expression of amino acid transporter SLC38A1. Increased accumulation of amino acids activates mTORC1 signaling to promote tumorigenesis. **c)** In cholangiocarcinoma, AKT and YAP can cooperate to enhance Notch signaling, which in turn promotes BEC differentiation and tumorigenesis. The inflammatory cytokines IL6 and IL33 promote this process.

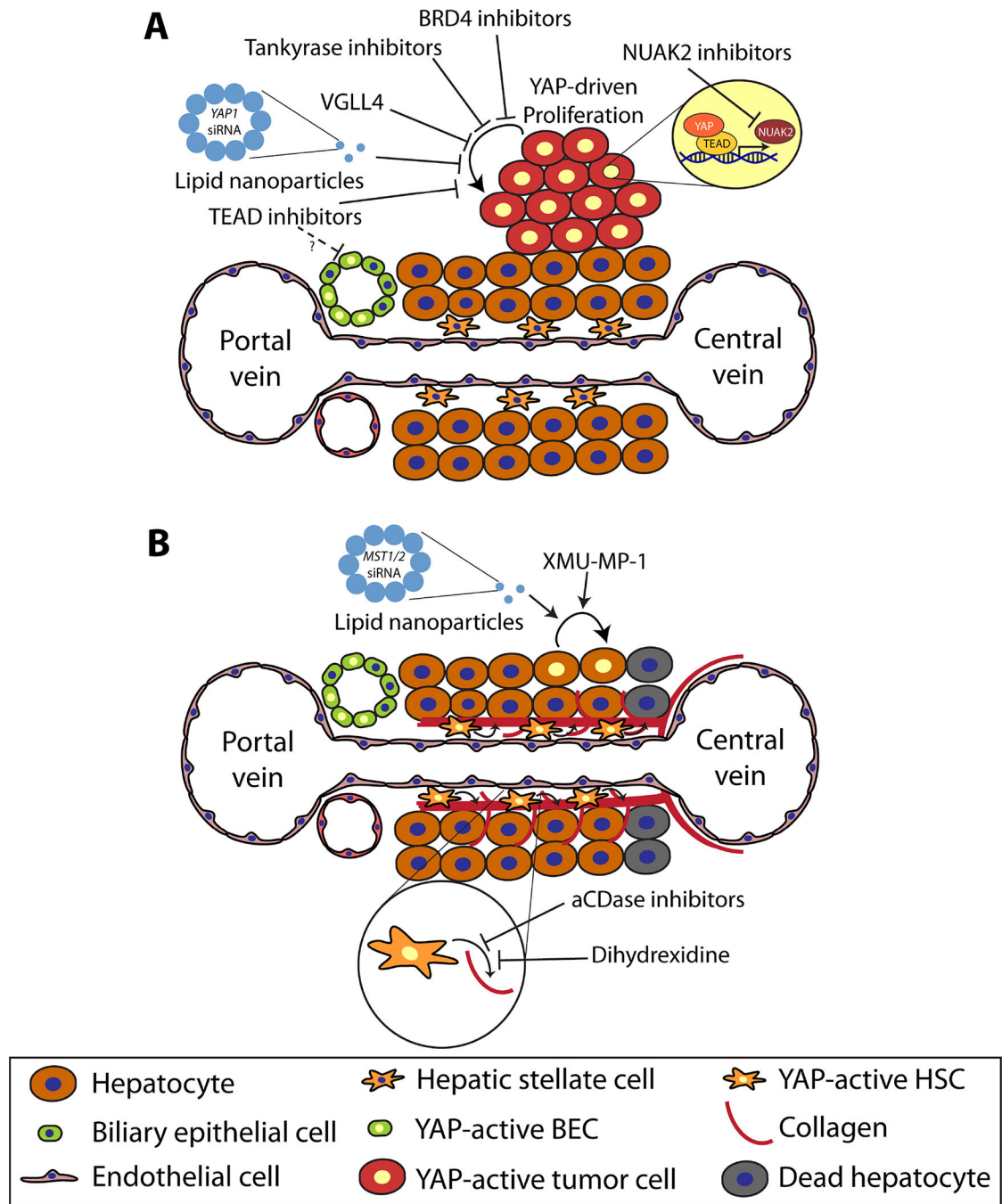


Figure 5). Strategies for targeting the Hippo signaling pathway.

a) Strategies to suppress liver cancer by inhibiting YAP activity in tumor cells. **b)** Strategies to promote liver regeneration by augmenting YAP activity in hepatocytes and to inhibit fibrosis progression by specifically inhibiting YAP activity in HSCs.