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REVIEW ARTICLE

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Physiological and pathological roles of the Hippo-YAP/TAZ signaling pathway in liver formation, homeostasis, and tumorigenesis

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Abstract

The liver plays central homeostatic roles in metabolism and detoxification, and has a remarkable capacity to fully recover from injuries caused by the various insults to which it is constantly exposed. To fulfill these functions, the liver must maintain a specific size and so must regulate its cell numbers. It must also remove senescent, transformed, and/or injured cells that impair liver function and can lead to diseases such as cirrhosis and liver cancer. Despite their importance, however, the mechanisms governing liver size control and homeostasis have resisted delineation. The discovery of the Hippo intracellular signaling pathway and its downstream effectors, the transcriptional coactivators Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), has provided partial elucidation of these mechanisms. The Hippo-YAP/TAZ pathway is considered to be a cell's sensor of its immediate microenvironment and the cells that surround it, in that this pathway responds to changes in elements such as the ECM, cell–cell tension, and cell adhesion. Once triggered, Hippo signaling negatively regulates the binding of YAP/TAZ to transcription factors such as TEAD and Smad, controlling their ability to drive gene expression needed for cellular responses such as proliferation, survival, and stemness. Numerous KO mouse strains lacking YAP/TAZ, as well as transgenic mice showing YAP/TAZ hyperactivation, have been generated, and the effects of these mutations on liver development, size, regeneration, homeostasis, and tumorigenesis have been reported. In this review, I summarize the components and regulation of Hippo-YAP/TAZ signaling, and discuss this pathway in the context of liver physiology and pathology.

KEYWORDS
Hippo pathway, homeostasis, liver cancer, liver size, regeneration, YAP/TAZ

Abbreviations: AA, arachidonic acid; COL17A, collagen XVII; Dox, doxycycline; FLSPC, fetal liver stem/progenitor cell; LATS, large tumor suppressor homolog kinase; Hpo, *Drosophila melanogaster* kinase Hippo; Mob, Mps1 binder kinase activator; MST, mammalian STE20-like protein kinase; PGE_2, prostaglandin E_2; Sav1, Salvador homolog-1; TAZ, transcriptional coactivator with PDZ-binding motif; TEAD, transcriptional enhanced associate domain; TF, transcription factor; VGLL4, transcription cofactor vestigial-like protein 4; YAP, Yes-associated protein.

1 | INTRODUCTION

During mammalian embryogenesis, the liver develops from the foregut derived from the endoderm and functions as a site of hematopoiesis (Figure 1).[1,2] In the adult, this hematopoietic function is lost and the liver instead plays a central role in metabolism that involves the synthesis, storage, and redistribution of nutrients. The liver is also a major detoxifying organ, removing waste and xenobiotics through metabolic conversion and biliary excretion. Undesirable substances in the gastrointestinal tract enter the liver by way of the portal vein and diffuse through its structure through small blood vessels known as hepatic sinusoids. These sinusoids wind among the several different cell types composing the liver mass, including hepatocytes, which metabolize and detoxify substances, liver sinusoidal endothelial cells, which form the walls of sinusoids and cover the hepatocytes, and Kupffer cells, which are sinusoid-resident macrophages.

For the liver to properly function and maintain homeostasis, it must achieve and preserve a specific size. To this end, the liver must regulate its cell numbers while removing senescent, transformed, and/or damaged cells that can impair function and lead to liver diseases such as cirrhosis and cancer. The liver has a striking capacity to recover from injuries caused by various insults, such as surgical resection, viral infection, metabolic disorders, and chemical or toxic stresses. Interestingly, the processes underlying liver recovery differ depending on the type of injury. In the case of a partial hepatectomy in which 70% of the liver tissue is surgically removed, the remaining 30% returns the liver to near-original size through cell hypertrophy and cell proliferation.[3,4] However, the precise mechanisms that the liver uses to control its size and maintain everyday homeostasis have been difficult to resolve.

The discovery of the Hippo-YAP/TAZ pathway has provided clues that could solve some of the above mysteries. Genetic studies using KO and transgenic mice have revealed that the Hippo-YAP/TAZ pathway is a key regulator of organ size, regeneration, and homeostasis in many tissues, and that perturbations in the Hippo-YAP/TAZ pathway can lead to the development of various cancers. Many excellent review articles have been written about these strains and their phenotypes.[5-11] So as not to repeat these outstanding efforts, I focus in this review on the roles of the Hippo-YAP/TAZ pathway in mammalian liver physiology and pathology, specifically with respect to this organ's development, regeneration, mechanisms of size control and cell competition, and tumorigenesis.

2 | HIPPO-YAP/TAZ SIGNALING PATHWAY

2.1 | Components of Hippo-YAP/TAZ signaling

The Hippo signaling pathway is an evolutionarily conserved regulator of cell proliferation and organ size control during embryogenesis, tissue regeneration, stem cell self-renewal, and tumorigenesis.[5-11] In mammals, the core components of the Hippo pathway are MST1/2, which are mammalian homologues of Hpo, the MST adaptor protein Sav1, LATS1/2, and their adaptor proteins Mob1a/1b (Figure 2A). The major effectors downstream of the Hippo core are the transcriptional coactivators YAP and its paralog TAZ. Activation of the Hippo core components results in the phosphorylation of conserved serine residues in YAP/TAZ. Phosphorylated YAP/TAZ proteins either undergo proteasomic degradation in the cytoplasm, or are retained in the cytoplasm by binding to the phosphoserine/phosphothreonine-binding protein 14-3-3. Thus, Hippo activation negatively regulates YAP/TAZ activity. Conversely, a lack of triggering of Hippo signaling, or inactivation of a pathway element, allows unphosphorylated YAP/TAZ to translocate into the nucleus. These coactivators then bind to various TFs, including TEAD1/2/3/4, Smad1/2/3, p73, KLF5,

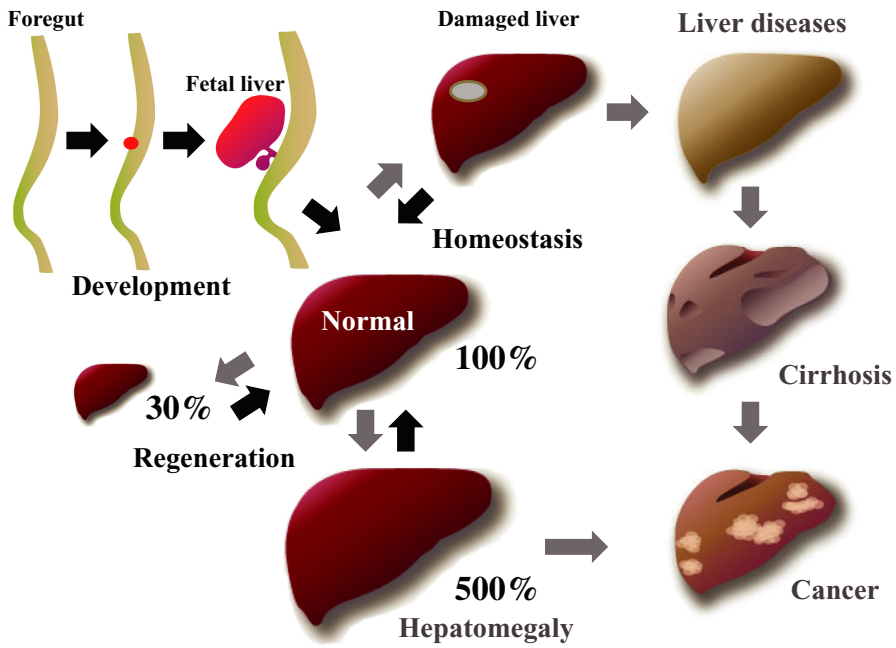

FIGURE 1 Liver development, regeneration, and homeostasis. In the mammalian embryo, the endoderm gives rise to the foregut, which in turn gives rise to the fetal liver. In the adult, the liver plays a central role in metabolism and detoxification. The normal adult liver can successfully regenerate even after 70% of its mass is removed by partial hepatectomy. When there is a failure to regulate liver cell number or remove senescent, transformed, and/or damaged cells (gray arrows), abnormalities like hepatomegaly (increased liver size) can impair function and lead to liver diseases, including cirrhosis and cancer

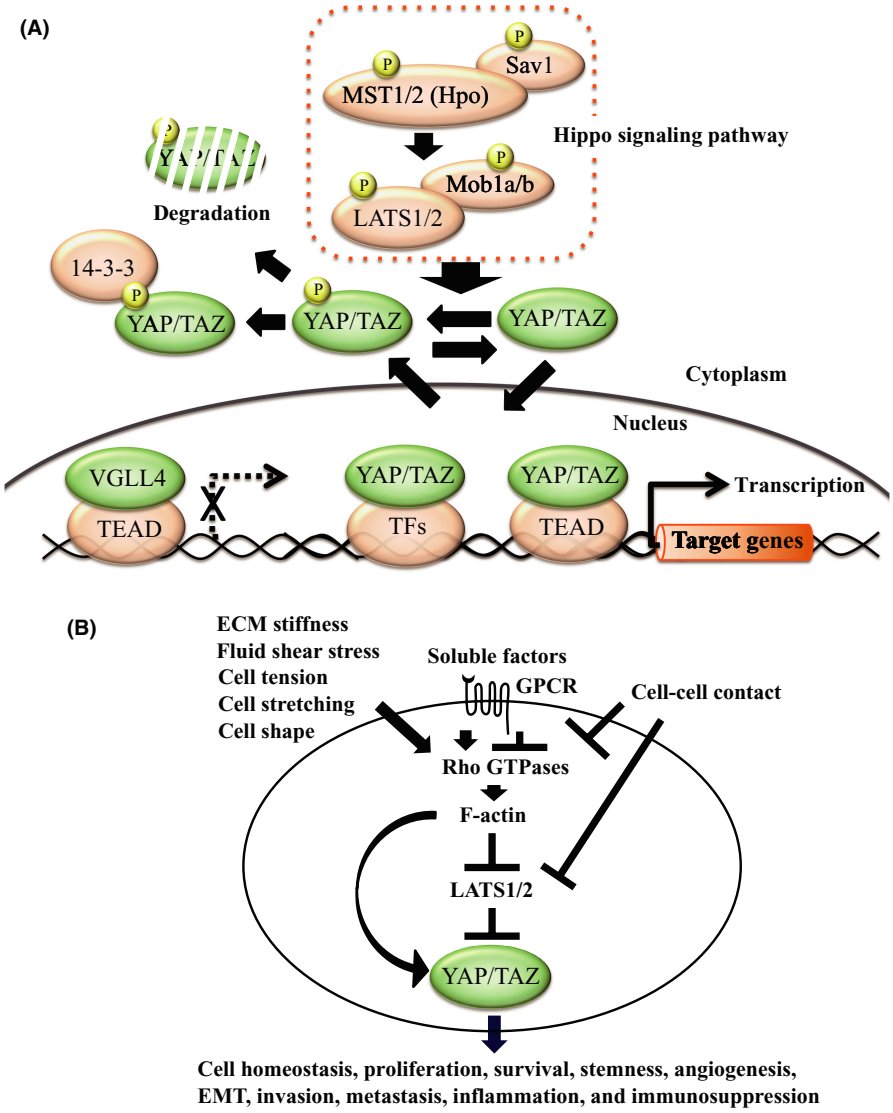

FIGURE 2 Hippo signaling and the regulation of Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ)-dependent gene expression. (A) Once triggered, the Hippo pathway initiates the activation of the mammalian STE20-like protein kinases (MST1/2; Hpo in Drosophila) supported by their adaptor protein Salvador homolog-1 (Sav1). MST1/2 phosphorylate the large tumor suppressor homolog kinases (LATS1/2) kinases to activate them, supported by their adaptor proteins Mob1a/b. Activated LATS1/2 phosphorylate YAP/TAZ, negatively regulating their translocation from the cytoplasm to the nucleus by either inducing binding to 14-3-3 protein or promoting degradation. In the absence of Hippo signaling, unphosphorylated YAP/TAZ bind to many transcription factors (TFs) and coactivate them to launch transcription of their target genes. In the liver, the main targets of YAP/TAZ binding are the transcriptional enhanced associate domain (TEAD) TFs. Transcription cofactor vestigial-like protein 4 (VGLL4) competes with YAP/TAZ for binding to TEADs and represses TEAD target gene expression. (B) The indicated mechanical cues regulate the Hippo-YAP/TAZ pathway by activating, or inactivating, Rho GTPases. These Rho GTPases promote F-actin formation, which blocks LATS1/2 activity. Rho GTPase-independent inhibition of LATS1/2 activity also exists. In the absence of LATS1/2-mediated phosphorylation (and sometimes independently of LATS1/2), YAP/TAZ are free to enter the nucleus and coactivate transcription by TEADs, inducing the indicated diverse cellular responses. EMT, epithelial–mesenchymal transition; GPCR, G protein-coupled receptor

Runx1/2, ErbB4, TBX5, and FoxO1, and enable them to drive expression of their target genes. As a result, YAP/TAZ control a myriad of cellular responses such as cell proliferation, apoptosis, competition, and contact inhibition, as well as the epithelial–mesenchymal transition. In the liver, the dominant TFs binding to YAP/TAZ are the TEADs, and the main target genes activated by TEADs in the liver include *Ctgf*, *Cyr 61*, and *Birc5*, also known as *Survivin*.[12-15] The expression of these TEAD target genes is balanced by VGLL4, which competes with YAP/TAZ for binding to the TEADs and represses TEAD target gene expression.[16,17]

2.2 | Regulation of Hippo-YAP/TAZ signaling

The Hippo-YAP/TAZ pathway is a sensor of the mechanical properties of the extracellular environment surrounding a cell and a

regulator of cellular integrity. Cellular stresses that impinge on the cell membrane, such as stiffness of the ECM, fluid shear stress, cell tension, cell stretching, altered cell shape, and cell–cell contact, initiate a chain of events that leads to activation or inactivation of Hippo signaling and thus inactivation or activation of YAP/TAZ, followed by specific effects on cell behavior (Figure 2B). In particular, cell stresses trigger the activation of intracellular Rho GTPases, which may also be stimulated (or inhibited) following the binding of soluble extracellular factors, such as lysophosphatidic acid and sphingosine-1 phosphate, to surface G protein-coupled receptors.[18,19] Once activated, these Rho GTPases induce the remodeling of F-actin within the cell, which in turn can control the coactivation function of YAP/TAZ in both LATS1/2-dependent and -independent ways.[20,21] When F-actin inactivates LATS1/2, YAP/TAZ avoid phosphorylation, translocate to the nucleus, and enable TEAD-mediated target gene expression. The functions of these genes then allow the cell to take action to alleviate the stress. For example, consider "cell contact inhibition", which is a well-known phenomenon in which cells in monolayer culture stop proliferating when they reach confluence. In response to cell–cell contact, the angiomotin complex at the tight junction directly binds to YAP/TAZ. Angiomotin then stimulates LATS-dependent phosphorylation of YAP/TAZ that inhibits their activity.[22] The cells can no longer transcribe the genes driving proliferation and culture overgrowth is prevented. Conversely, when Hippo signaling is inactivated by loss of function of a kinase or adaptor protein, YAP/TAZ activity in the nucleus is uncontrolled, cell proliferation is rampant, and tumorigenesis can initiate. Thus, although the Hippo-YAP/TAZ pathway has properties shared by many other existing signaling pathways, it also displays unique regulatory mechanisms.

3 | FUNCTIONS OF HIPPO-YAP/TAZ SIGNALING IN THE LIVER

Tissues and organs undergo stress that can lead to damaged, senescent, and/or transformed cells requiring elimination. The loss of these cells must then be compensated for by cell proliferation, which maintains the size and functionality of the tissues and organs. The liver functions normally over a long period of time despite being exposed to more stress than most tissues, suggesting the existence of a variety of mechanisms that maintain liver homeostasis starting from birth. Some of these mechanisms involve Hippo signaling and YAP/TAZ regulation.[23]

3.1 | Early embryogenesis and liver development

Various animal-based methods of studying the Hippo-YAP/TAZ pathway have been reported (Table 1). *Yap* KO ($Yap^{-/-}$) mouse embryos are lethal around embryonic day 8.5 (E8.5).[24] $Taz^{-/-}$ mice are viable but develop glomerulocystic kidney disease and pulmonary

TABLE 1 Functions of YAP/TAZ in mouse embryos and adult livers

Mouse strain	Phenotype	References
$Yap^{-/-}$ embryos	Developmental arrest around E8.5	24
$Taz^{-/-}$ mice	Viable; some adults develop kidney disease and pulmonary disease	25–27
$Yap^{-/-}Taz^{-/-}$ embryos	Embryos die before the morula stage (16–32 cells)	28
ApoE/rtTA; TetO-*Yap* mice	Massive hepatomegaly (5-fold increase), liver cancer	32
LAP/tTA; TetO- *Yap (S127A)* mice	Greater than 4-fold increase in liver size	33
$Mst1^{-/-}$ $Mst2^{-/f}$; *Albumin-Cre*	Increased liver size and liver cancer	15
$Mst1^{-/-}$ $Mst2^{-/f}$; *Adenovirus Cre* mice	Increased liver size and liver cancer	34
Sav (WW45)$^{f/f}$; *Albumin-Cre* mice	Increased liver size and liver cancer	35
$Lats1^{-/-}$ $Lats2^{f/f}$; *Adenovirus Cre* mice	Massive hepatomegaly	36
$Mob1a^{-/-}$ $Mob1b^{-/-}$ embryos	Defective primitive endoderm formation	29
$Mob1a^{-/-}$ $Mob1b^{+/-}$ mice	Liver cancer, skin cancer, and exostosis	29
$Yap^{f/f}$ $Taz^{f/f}$; *Albumin-Cre* mice	Enlarged liver in neonates and adults, defective liver regeneration	37
$Yap^{f/f}$; *FoxA3-Cre* mice	Absence of intrahepatic biliary network	30
siRNA against MST1/2	Improved liver regeneration in aged WT mice	38
Plasmid *Albumin-Yap (S127A)* by HTVi	Enhanced elimination of injured hepatocytes	54
$Yap^{f/f}$ $Taz^{f/f}$; *AAV-Cre* mice	Liver cancer suppression due to peritumoral YAP/TAZ activation	39
MST1/2 inhibitor, XMU-MP–1	Promotion of liver repair and regeneration	56
siYAP-Lipid Nanoparticles	Restoration of hepatocyte differentiation in liver cancer and tumor regression	57

disease.[25-27] *Yap*$^{-/-}$*Taz*$^{-/-}$ embryos die before the morula stage (16–32 cells).[28] *Mob1a*$^{-/-}$*Mob1b*$^{-/-}$ blastocysts are normal at E3.5 but fail to form primitive endoderm.[29] Thus, YAP/TAZ play essential roles in mouse early embryogenesis.

With respect to the liver, development of this organ in mice initiates around E9, when epithelial cells of the foregut endoderm interact with the cardiogenic mesoderm and commit to becoming the liver primordium. Around E14.5, bipotential hepatoblasts give rise to both hepatocytes and bile duct epithelial cells. The hepatocytes become major elements of the postnatal liver and this organ's main function switches from hematopoiesis to metabolism.[1,2] Deletion of YAP in bipotential hepatoblasts achieved in *FoxA3-Cre* mice led to a complete loss of the intrahepatic biliary tree in adult mutant mice but did not impair hepatoblast differentiation into hepatocytes.[30] Thus, YAP is a key regulator of bile duct development. There are several pediatric diseases that affect the bile ducts, such as Alagille syndrome and biliary atresia. These mouse findings imply that perturbations in YAP function could contribute to the pathogenesis of these human disorders.[31]

3.2 | Liver size control and regeneration

The Hippo-YAP pathway controls liver size, as evidenced by studies showing that YAP overexpression in mouse liver induced hepatomegaly.[32,33] Interestingly, this increase in liver mass was completely reversible since cessation of YAP hyperactivation resulted in a normal-sized liver without any gross abnormalities. To explore YAP's role in this process in detail, Dong et al. generated transgenic mice carrying a tetracycline (Tet)-On system that induced conditional YAP activation.[32] When provided with Dox-containing drinking water, these transgenic mice developed massive hepatomegaly and showed YAP hyperactivation in hepatocytes. The elevation in liver mass was detectable as early as 3 days after induction, and whereas a normal liver constitutes approximately 5% of body weight, livers in these mutants reached 25% of total body weight after 4 weeks of Dox treatment. The increase in liver mass was caused by an expansion in cell numbers (hyperplasia) as opposed to an increase in cell size (hypertrophy). Strikingly, by 2 weeks after Dox withdrawal, the enlarged livers had returned to near-normal size due largely to hepatocyte apoptosis. However, when the mice were exposed to Dox for over 8 weeks, they developed hepatocellular carcinoma. This study dramatically demonstrated the tight regulation of liver size. In addition to YAP mutants, mice with liver-specific deficiencies of Mst1/2, Sav1, Lats1/2, or Mob1a/1b all show sustained YAP activation leading to hepatomegaly.[15,29,34-36] These results reveal a direct link between dysregulation of Hippo-YAP/TAZ signaling in liver size control, and hepatomegaly and tumorigenesis.

In *Yap*$^{f/f}$*Taz*$^{f/f}$;*Albumin-Cre* mutant mice, YAP and TAZ are specifically depleted in the liver, and consequently liver mass was increased in neonates and adults.[37] However, hepatocytes of these YAP/TAZ-deficient livers showed profound defects in processes needed to support liver regeneration, including the coordination of cell cycle entry. This apparent discrepancy could be due to the loss of suppression of other cell proliferation signals caused by inactivation of the Hippo-YAP/TAZ pathway, which could result in an increase in mass of YAP/TAZ-deficient livers. A similar link exists for liver regeneration, in that YAP/TAZ activation induced by siRNA-mediated inactivation of MST1/2 provoked hepatocyte proliferation in quiescent livers of aged WT mice subjected to two-thirds partial hepatectomy.[38] Thus, YAP/TAZ are required in more than one way in the mouse liver, with their regulatory roles in liver formation during fetal development differing from their multiple influences on liver regeneration in the adult.

3.3 | Liver cancer formation and suppression

There is now much evidence that dysregulation of the Hippo-YAP pathway promotes liver cancer formation. The overexpression of YAP in mouse liver caused by any one of numerous defects in the Hippo pathway induced not only hepatomegaly but also eventually liver cancer.[15,29,32-36] Liver-specific Mob1a/1b double-deficient mice also developed liver cancer, which could be suppressed by inactivation of the YAP gene in these animals.[29] In general, the liver phenotypes arising from impairment of any step of the Hippo pathway are strongly dependent on YAP. Thus, hyperactive YAP functions as an oncogene, promoting liver overgrowth and liver cancer formation.

Although hyperactivated YAP/TAZ are most often function as drivers of tumor growth, there are some reports of YAP/TAZ also exerting a tumor-suppressive function. In one study of mouse liver cancer development, some normal hepatocytes surrounding a liver tumor were found to exhibit activated YAP/TAZ, and deletion of YAP/TAZ in these peritumoral hepatocytes accelerated tumor growth.[39] Conversely, hyperactivation of YAP in peritumoral hepatocytes triggered the regression of primary liver cancers. In this model, the survival of the tumor cells clearly depended on the relative levels of YAP/TAZ activity in the tumor cells compared to the surrounding normal hepatocytes. These results indicate that, when YAP/TAZ act to eliminate tumor cells, they do so through a mechanism of cell competition and not by direct regulation of target genes in cancer cells.

3.4 | Cell competition

"Cell competition" is a type of cell–cell interaction that was originally discovered in the imaginal wing disc of *Drosophila melanogaster*.[40] During cell competition, a cell compares its fitness to that of its neighboring cells. Cells that are less fit than their neighbors are "losers" and are eliminated by either apoptosis or apical extrusion; cells that are more fit are the "winners" and survive. For example, within the *Drosophila* wing disc, cells that were heterozygous for the *Minute* gene, which encodes a ribosomal protein, underwent apoptosis as losers when they were confronted with WT cells (Figure 3A).[41,42] Activation of the Src oncogene also turns *Drosophila* cells into losers,

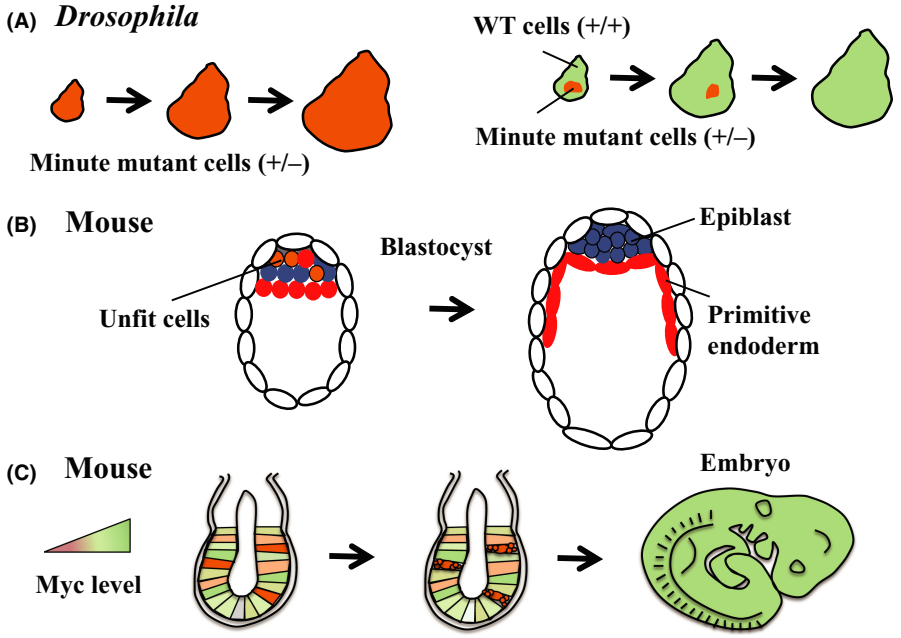

FIGURE 3 Principles of cell competition in *Drosophila* and mice. (A) Left: In a *Drosophila* wing disc in which all cells are heterozygous for the *Minute* gene encoding a ribosomal protein, the cells proliferate as usual and the disc reaches normal size without incident. Right: In a *Drosophila* wing disc where most cells are WT but a small fraction are Minute$^{+/-}$, the Minute heterozygous cells surrounded by WT cells are the "losers" of a cell competition and are forced to undergo apoptosis. The wing disc reaches normal size but contains only WT cells. (B) In mouse epiblasts, Yes-associated protein (YAP)–transcriptional enhanced associate domain (TEAD) activity regulates the expression of pluripotency factors in the blastocyst. Any cells that fail to specify their lineage are deemed "unfit" and are eliminated through cell competition. (C) In mouse embryonic stem cells or epiblasts, cells expressing lower levels of Myc lose in a competition with Myc^{hi} cells and are eliminated by apoptosis. All cells in the developing embryo thereafter express high levels of Myc

whereas increased activity of the Myc oncogene or the YAP ortholog *Yki* turns *Drosophila* cells into winners. These studies set the stage for the study of Hippo-YAP/TAZ signaling in the mammalian context.

3.4.1 | Mammalian tissue studies

Mammalian cell competition has been reasonably well studied in mice and rats. Mouse epiblasts are a pluripotent cell population first formed in preimplantation embryos, and the normal function of these cells is important for proper embryonic development. It was shown that YAP-TEAD signaling regulated the expression of pluripotency factors and eliminated any cells lacking these factors through cell competition (Figure 3B).[43] Mouse embryonic stem cells or epiblasts showing low Myc levels also underwent apoptosis and were eliminated (Figure 3C).[44] In mouse intestinal epithelium, metabolic changes mediated by pyruvate dehydrogenase kinase 4 promoted the elimination of active Ras-transformed cells.[45] In mouse epidermal stem cells, protein levels of the skin hemidesmosome component COL17A1 fluctuated in response to genomic or oxidative stress.[46] This stress induced COL17A1 proteolysis, and the resulting differing levels of COL17A1 in individual stem cells were a driving force for cell competition. In adult rats, the liver can be repopulated by highly proliferative rat FLSPCs through cell competition.[47] A four- to five-fold increase in liver repopulation occurred when FLSPCs were transplanted into older rats compared with younger rats.[48] Thus, cell competition phenomena have been reported across vertebrate and invertebrate species, and in many tissues. However, the underlying molecular mechanisms remain a mystery in many cases and a better understanding of these phenomena is needed.

3.4.2 | Mammalian cultured cell studies

Madin–Darby canine kidney epithelial cells are useful for studying cell competition in culture and have revealed much about mammalian genes involved in this process.[49,50] When MDCK cells expressing an oncogene, such as *Ras* (G12V) or *v-Src*, and were surrounded by normal MDCK cells, the oncogene-expressing cells were "losers" in cell competitions and underwent apical extrusion. The MDCK cells expressing a constitutively active form of YAP also underwent apical extrusion in the presence of normal MDCK cells through a mechanism that involved TEAD-dependent gene expression (Figure 4A).[51-53] This apical extrusion was regulated by multiple signaling pathways, including those involving PI3K, mTOR, and p70S6 kinase. Also important was the AA cascade, which is related to choline metabolism. Cyclooxygenase-2 catalyzes the conversion of AA to PGE_2. In the presence of active YAP, PGE_2 induced internalization of E-cadherin, leading to apical extrusion of the oncogene-expressing cell. These studies demonstrate that, in vitro, experimental systems

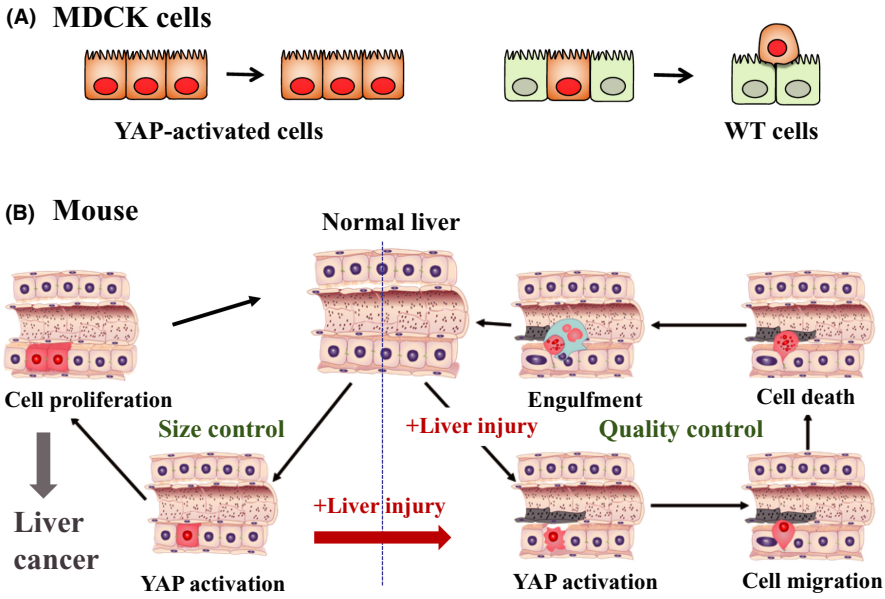

FIGURE 4 Cell competition in MDCK cells and mouse liver cells. (A) Left: MDCK cells expressing active Yes-associated protein (YAP) (red) maintain normal cell numbers in a monoculture. Right: MDCK cells expressing active YAP are forced to undergo elimination by apical extrusion in the presence of WT MDCK cells (green). (B) The normal mouse liver maintains its size and functionality through various mechanisms that control cell proliferation and remove senescent, damaged or abnormal cells. For example, if a hepatocyte sustains a change that causes it to constitutively express YAP (red cells), the cell proliferates. "Size control" mechanisms then act to remove the altered cells and restore normal liver size. If these mechanisms fail, the altered cells proliferate uncontrollably and liver cancer may develop. Similarly, if traumatic liver injury damages a hepatocyte, YAP can become activated and "quality control" mechanisms are triggered to return the liver to normalcy. The injured cell may be eliminated by apical extrusion or induction of apoptosis, followed by Kupffer cell-mediated engulfment

of cell competition can be highly useful for elucidating molecular mechanisms that are difficult to track in vivo.

3.4.3 | Injured hepatocyte elimination studies

Yes-associated protein functions in mouse hepatocytes have been explored using the HTVi method, which introduces foreign genes into cells by hydraulic pressure. The acquisition of plasmids encoding constitutively active YAP creates a mosaic condition in which the liver contains a population of active YAP-expressing hepatocytes among normal YAP-quiescent hepatocytes. Studies of these livers have revealed the dynamics of how the abnormal hepatocytes are dealt with in vivo. It was shown that the YAP-overexpressing hepatocytes were eliminated from the livers of injected mice within 7 days in a manner independent of adaptive immunity.[54] Tracking demonstrated that active YAP-expressing hepatocytes migrated to the hepatic sinusoids where they were engulfed by Kupffer cells (Figure 4B). Clodronate liposome-mediated depletion of Kupffer cells from these mice suppressed the elimination of active YAP-expressing hepatocytes from the liver and increased the presence of TUNEL-positive apoptotic cells. The molecular mechanism underlying this YAP-mediated elimination of abnormal hepatocytes was proposed to proceed as follows: (a) active YAP and TEAD induce *Ect2* and *Fgd3* mRNA expression, (b) Ect2 and Fgd3 proteins activate Cdc42 and Rac, and (c) active Cdc42 and Rac regulate cytoskeletal reorganization and stimulate cell migration. This work concluded that a change in hepatocyte fate from proliferation to migration/apoptosis depended on a mechanism of stress detection involving YAP. In other words, YAP acts as a stress sensor that induces elimination of injured hepatocytes to maintain tissue and liver homeostasis.

4 | THERAPEUTIC PERSPECTIVE

The studies described above raise the tempting possibility of manipulating the Hippo-YAP/TAZ pathway for preventing the development and progression of liver diseases and cancers. Indeed, three groups of drugs and methods manipulating the Hippo-YAP/TAZ pathway are under development. Group I drugs target Hippo components acting upstream of YAP/TAZ, whereas Group II methods target YAP/TAZ or TEAD family molecules, and Group III drugs target downstream targets of YAP/TAZ.[55]

On the flip side, there is the possibility of manipulating the Hippo-YAP/TAZ pathway to boost liver regeneration. Indeed, some such approaches have already been reported. For example, the MST1/2 inhibitor XMU-MP-1 (4-((5,10-dimethyl-6-oxo-6,10-dihydro-5H-pyrimido[5,4-b]thieno[3,2-e][1,4]diazepin-2-yl)amino)benzenesulfonamide) augments mouse liver repair and regeneration in both acute and chronic liver injury mouse models.[56] Conversely, treatment with siRNA-lipid nanoparticles targeting YAP restored hepatocyte differentiation and induced pronounced tumor regression in a genetically engineered mouse model of liver cancer.[57] Although this latter approach might seem to offer proof-of-concept that differentiation

therapy might be used to treat an epithelial tumor, the therapeutic activation of YAP/TAZ for regenerative purposes has significant risks. YAP/TAZ hyperactivation is well established as promoting cancer development, making it imperative to continue detailed research of the Hippo-YAP/TAZ pathway to discover all its functions and regulatory mechanisms before applying any such knowledge to human therapy.

5 | CONCLUSION

The Hippo-YAP/TAZ pathway is a very attractive target of exploration from the point of view of liver physiology and pathology. Tellingly, Hippo pathway mutations are extremely rare in human liver cancers.[58] This observation suggests that nongenetic factors, such as the status of elements in the surrounding microenvironment (e.g., stiff ECM), are vital for the activation of YAP/TAZ linked to cancer initiation. Multifaceted investigation of these issues is sure to yield much helpful information on Hippo-YAP/TAZ signaling in liver biology.

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CONFLICT OF INTEREST

The author declares no conflicts of interest.

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