Hippo Pathway Regulation of Gastrointestinal Tissues

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Abstract

The Hippo pathway plays a crucial role in regulating tissue homeostasis and organ size, and its deregulation is frequently observed in human cancer. Yap is the major effector of and is inhibited by the Hippo pathway. In mouse model studies, inducible Yap expression in multiple tissues results in organ overgrowth. In the liver, knockout of upstream Hippo pathway components or transgenic expression of Yap leads to liver enlargement and hepatocellular carcinoma. In the small intestine or colon, deletion of upstream Hippo pathway components also results in expansion of intestinal progenitor cells and eventual development of adenomas. Genetic deletion of Yap in the intestine does not change the intestinal structure, but Yap is essential for intestinal repair upon certain types of tissue injury. The function of the Hippo pathway has also been studied in other gastrointestinal tissues, including the pancreas and stomach. Here we provide a brief overview of the Hippo pathway and discuss the physiological and pathological functions of this tumor suppressor pathway in gastrointestinal tissues.

INTRODUCTION

Gastrointestinal (GI) tissues include both the GI tract (esophagus, stomach, small intestine, and colon) and other functionally related tissues (liver, gallbladder, pancreas, salivary glands, and the oral cavity) that are derived mostly from the endoderm during human development (1). Together, these tissues are responsible for food uptake, digestion, absorption, and disposal in mammals. Abnormalities in GI tissues may result in acute disorders (such as colitis and diarrhea) and in chronic diseases (such as fibrosis and cancer). The GI tract is constantly exposed to hostile environments, which increase the chance of tissue injury. Therefore, these tissues must have an efficient repair/regenerative mechanism to maintain proper structure and function. Two examples are the epithelial lining of the intestine, which is replaced every 4–5 days under physiological conditions (2), and the liver, which in mammals can regenerate back to its original size following partial hepatectomy (surgical removal of a large chunk of the liver's mass) (3). Due to these regenerative properties, the intestine and liver are two attractive model systems for studying the molecular mechanisms of tissue regeneration and homeostasis.

The development, repair, and regeneration of GI tissues are complex physiological processes that are fine-tuned by multiple cellular signaling pathways, and these pathways must be precisely regulated and integrated. In this article, we review the Hippo pathway, a relatively newly described signaling pathway that plays a critical role in organ size control and tissue growth, with a major focus on the intestine and liver. For a more comprehensive view of the Hippo pathway, please refer to several recent reviews (4–7).

THE HIPPO PATHWAY

The Core Kinase Cascade

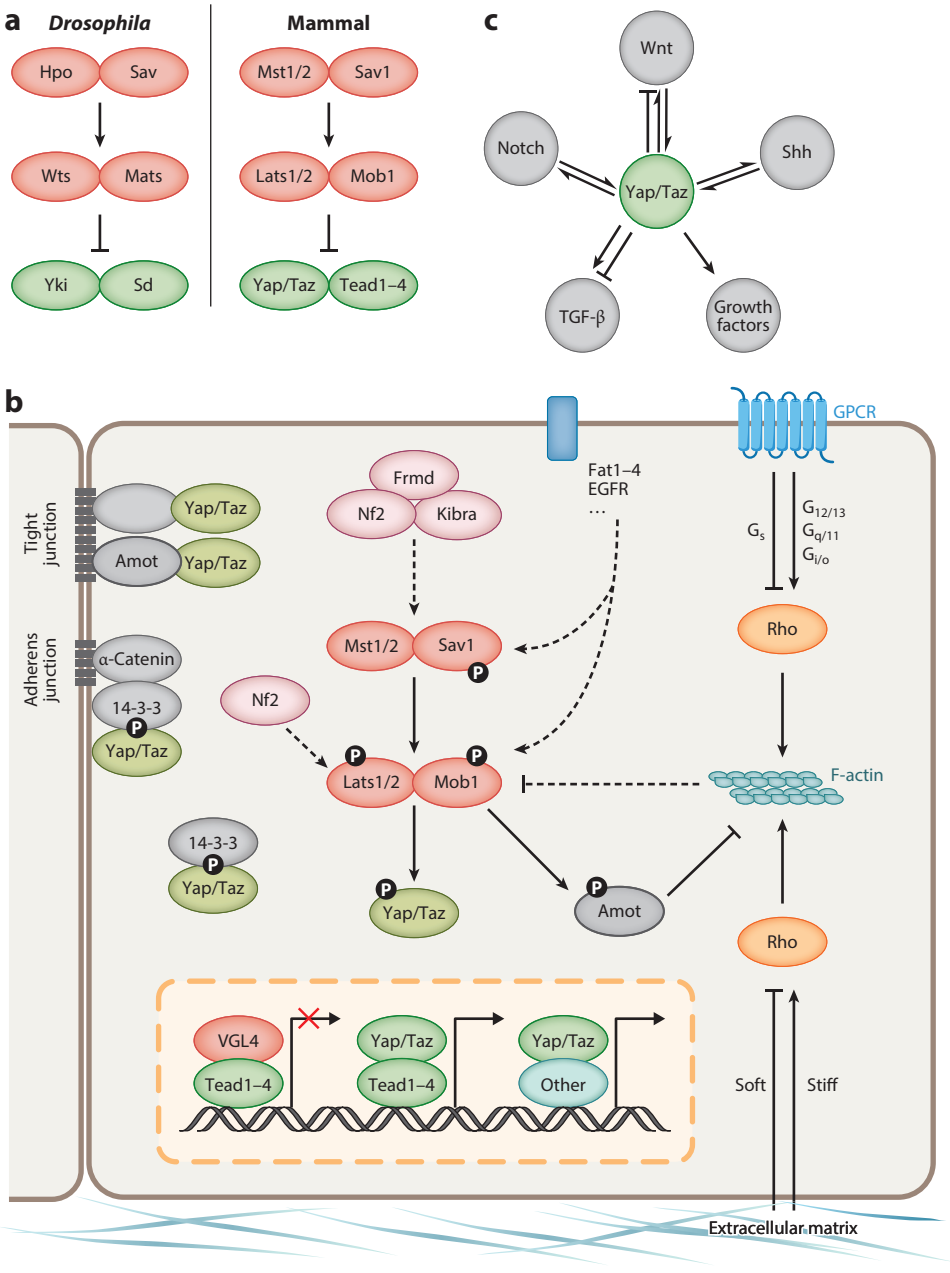
The Hippo pathway is an evolutionarily conserved signaling pathway first discovered in *Drosophila*. The core components of the Hippo pathway are Hippo (Hpo), Warts (Wts), Salvador (Sav), Mats, Yorkie (Yki), and Scalloped (Sd) (**Figure 1*a***) (8–22). For most Hippo pathway components with the exception of Yki and Sd, genetic inactivation results in tissue overgrowth of the eye, wing, and/or limbs. In contrast, inactivation of Yki reduces tissue growth. In addition to its role in these peripheral organs, the Hippo pathway also plays a role in GI tissues, including the *Drosophila* midgut.

The Hippo pathway is highly conserved in mammals. Core components of the Hippo pathway form a kinase cascade that is responsible for the inhibition of downstream effectors (**Figure 1*a***)

Figure 1

The Hippo pathway. (*a*) Comparison between *Drosophila* and mammalian Hippo pathway core components. (*b*) Regulation of the Hippo pathway. Yap and Taz, when localized in the nucleus, interact with Tead1–4 and other transcription factors to induce gene expression. Tead1–4 also interact with VGL4, a transcription corepressor. Yap and Taz compete with VGL4 for Tead binding. Yap and Taz localization is regulated mainly by upstream components of the Hippo pathway, which can be represented by a kinase cascade: Mst1 and -2 phosphorylate Lats1 and -2, and Lats1 and -2 in turn phosphorylate Yap and Taz. Upon phosphorylation, Yap and Taz interact with 14-3-3 and are sequestered in the cytoplasm. Yap and Taz also interact with cell junction components such as Amot and α-catenin, and these interactions may contribute to Yap and Taz localization at cell junctions. Hippo pathway kinases are also regulated by other mechanisms, including cell polarity, GPCR signaling, and mechanical cues. Changes in the actin cytoskeleton (by Rho GTPase modulation) are a key in Lats1 and -2 regulation. In addition, Amot is a substrate of Lats1 and -2 and may regulate the actin cytoskeleton. (*c*) Interactions between the Hippo pathway and other signaling pathways.

(6). The mammalian Hpo orthologs mammalian sterile 20-like 1 and 2 (Mst1 and -2) are two serine/threonine kinases belonging to group II germinal center kinases (11). Mst1 and -2 form heterodimers with Sav1 (a Sav ortholog) between their C-terminal SARAH (Sav/Rassf/Hpo) domains, and this interaction is required for Mst1 and -2 to phosphorylate Hippo pathway components Sav1, Mob1 (Mobkl1a/b, Mats orthologs), and large tumor suppressor 1 and 2 (Lats1 and -2, Wts orthologs) (10, 11, 23–25). Phosphorylation of the hydrophobic motif of Lats1 and -2 by



Mst1 and -2, and the interaction between Lats1 and -2 and phosphorylated Mob1, leads to Lats1 and -2 activation (11, 24, 25). A recent report shows that Sav1 and neurofibromatosis 2 (Nf2) are responsible for recruiting Mst1 and -2 and Lats1 and -2, respectively, to the plasma membrane, thereby promoting phosphorylation and activation of Lats1 and -2 by Mst1 and -2 (26). Lats1 and -2 directly phosphorylate Yki orthologs Yes-associated protein 1 (Yap, also known as Yap1 or Yap2, representing alternative splicing products) and WW domain–containing transcription regulator 1 (Wwtr1, or Taz) (27–30). Yap and Taz are transcriptional coactivators and, upon phosphorylation by Lats1 and -2, are sequestered and inactivated in the cytoplasm. Unphosphorylated Yap and Taz accumulate in the nucleus and interact with DNA-binding transcription factors TEA domain family members 1–4 (Tead1–4, orthologs of Sd) to induce a transcriptional program important for cell proliferation, cell death, and cell differentiation, thus regulating tissue homeostasis and organ growth (**Figure 1*b***) (18–21, 28, 31).

In addition, Angiomotin (Amot) family proteins are also substrates of Lats1 and -2. Phosphorylation increases Amot protein stability and decreases binding of Amot to actin filaments (F-actin) (**Figure 1*b***) (32–35). The regulation of Amot proteins by the Hippo pathway is critical for cell fate determination at the early blastocyst stage and for cell migration (34–36). However, the link between Amot family proteins and the Hippo pathway is not evolutionarily conserved, as no Amot ortholog has been identified in *Drosophila*.

The Regulation of Yap and Taz, Major Hippo Pathway Effectors

Yap and Taz lack a DNA-binding domain and therefore cannot interact with DNA directly. In the nucleus, Yap and Taz interact primarily with Tead1–4, which recognize and occupy a consensus DNA sequence (a Tead-binding site) present in the promoter regions of many genes (37). Additionally, Yap and Taz regulate gene transcription by interacting with other transcription factors or by recruiting DNA- or histone-modifying enzymes. For example, Yap and Taz interact with SWI/SNF chromatin-remodeling complexes (38, 39). In *Drosophila*, Yki interacts with the Brahma complex, the GAGA factor, and the Mediator complex (40, 41), all of which are important for gene transcription. In mammals, VGL4 (an ortholog of *Drosophila* vestigial) competes with Yap for Tead binding, resulting in repression of gene transcription (**Figure 1*b***) (42, 43). VGL4-mediated gene repression may not apply to other vestigial homologs, because vestigial and VGL1 activate, as opposed to repressing, gene transcription (44–46). The interaction between Yap/Taz and Tead is critical for the Hippo pathway, and small molecules and peptides able to disrupt this interaction have been discovered (47, 48). These molecules therefore have clinical potential as Yap inhibitors. In addition to the transcription-dependent functions of Yap, a transcription-independent role of Yap was recently revealed: Nuclear Yap can interact with microRNA processor components and inhibit global microRNA processing (49).

Yap and Taz activity is regulated primarily at the levels of protein stability and subcellular localization. Lats1 and -2 phosphorylate Yap and Taz on multiple HXRXXS (the substrate consensus sequence for Lats kinases) sites, which are important for regulating Yap and Taz stability and localization (27, 28, 30, 50) (here, H denotes histidine, R denotes arginine, S denotes serine, and X denotes any amino acid). Yap phosphorylation at serine 127 (S127) creates a binding site for 14-3-3 proteins and results in Yap cytoplasmic localization (**Figure 1*b***) (27, 30). In addition, the amino acid sequence around serine 381 (S381) of Yap contains a phosphodegron; Lats1 and -2 phosphorylation of S381 primes a subsequent phosphorylation event at serine 384 (S384) by CK1ε and -δ, and this coordinated phosphorylation results in binding of the SCF E3 ubiquitin ligase, leading to Yap ubiquitination and degradation (50). Taz is also regulated by Lats1 and -2

and CK1ε and -δ in a similar fashion (28, 51, 52), although this mechanism is not conserved in *Drosophila* Yki. Taz is less stable than Yap due to an additional phosphodegron site in its N terminus, which contains GSK3 and Lats1 and -2 phosphorylation sites (53). In addition, Yap is also phosphorylated by Cdk1 and Hipk2, and these phosphorylation events may positively contribute to the Yap function of promoting tissue growth and tumorigenesis (54–57).

Signals Upstream of the Hippo Pathway

Most cell types exhibit polarity, which is important for their specialized cellular functions. Epithelial cells show apical-basal polarity and planar cell polarity (PCP), and both regulate the Hippo pathway (**Figure 1*b***). The apical domain of a cell usually faces the surface of the body or internal cavity. In *Drosophila* epithelium, apically localized Merlin (Mer), Expanded (Ex), and Kibra form a complex and activate Wts in a cooperative manner (58–61). The function of this apical protein complex in the Hippo pathway is also conserved in mammals. Nf2 (a Mer ortholog), Kibra, and Willin (also known as Frmd6, a potential Ex ortholog) repress Yap activity (58, 62–64). PCP indicates the positional and directional information of a cell within an epithelial layer and plays a critical role in development. In *Drosophila*, the Fat/Dachsous (Ft/Ds) system is a molecular network of PCP and modulates Wts activity (7, 65). The effect of the Ft/Ds system in the mammalian Hippo pathway is less clear, although multiple orthologs (Fat1–4 and Hchs1 and -2 for Ft and Ds, respectively) are present in mammals (65).

Yap and Taz are regulated through interactions with multiple proteins localized at cell junctions. Using tandem purification coupled with mass spectrometry, several groups discovered that many tight junction (TJ) and adherens junction (AJ) components can strongly interact with Yap (**Figure 1*b***). For instance, Amot proteins interact with Yap regardless of Yap phosphorylation status and localize Yap to TJs or the actin cytoskeleton. Moreover, Amot proteins may also induce Lats1 and -2 activity. Therefore, Amot proteins restrict Yap activity via both Lats1 and -2–dependent and Lats1 and -2–independent mechanisms (66–69). An AJ component, α-catenin, forms a trimeric complex with 14-3-3 and phosphorylated Yap, leading to Yap inactivation (70). Taken together, the evidence indicates that interaction with proteins at cell junctions is a common strategy for Yap regulation (inhibition in most cases), and cell junctions behave like a magnet to sequester Yap and prevent its nuclear localization. However, unlike the case for β-catenin, another important transcription coactivator, we rarely see a strong localization of Yap at cell junctions under most conditions.

Yap activity is also sensitive to extracellular matrix (ECM) stiffness, and Yap has been proposed to be a sensor for mechanotransduction (71). Cell tension, cell geometry, cell spreading, and cell attachment/detachment are also able to modulate the Hippo pathway (72, 73), and in all cases, changes in Yap and Taz activity are associated with Rho GTPase activity and rearrangement of the actin cytoskeleton (**Figure 1*b***). Interestingly, Yap phosphorylation and subcellular localization correlate with cell density (30); Yap may perceive the cell density signals by extensive interactions with TJ/AJ proteins (see above) or by a mechanosensing mechanism. In *Drosophila*, increasing F-actin levels in different ways (such as deletion of actin-capping proteins, inactivation of Capulet, or expression of a constitutively active Diaphanous) results in high Yki activity and tissue outgrowth (74, 75). In mammalian cells, knockdown of actin-capping or severing proteins also leads to Yap and Taz activation (76). These results indicate that actin cytoskeleton dynamics play an important role in regulating Hippo pathway activity.

Recently, many extracellular diffusible signals, most of which are ligands for G protein–coupled receptors (GPCRs), have been shown to regulate Yap and Taz activity. GPCR ligands can either

positively or negatively regulate Yap/Taz, depending on the class of G proteins activated (**Figure 1*b***). $G\alpha_{12/13}$-, $G\alpha_{q/11}$-, and $G\alpha_{i/o}$-coupled ligands, such as lysophosphatidic acid, sphingosine 1-phosphate, and peptide agonists for thrombin receptors, can induce Yap and Taz activity (77–79). In contrast, $G\alpha_s$-coupled ligands, such as epinephrine and glucagon, can repress Yap and Taz activity through cAMP (cyclic adenosine monophosphate) and protein kinase A (79–81). Again, dynamic modulations of Rho GTPases and the actin cytoskeleton are required for Yap and Taz regulation by different GPCR ligands (**Figure 1*b***). In addition to GPCR signaling, mitogenic growth factors such as epidermal growth factor (EGF) can regulate the Hippo pathway (82, 83), although additional studies are needed to confirm the role of mitogenic growth factors in Yap regulation.

Rho GTPases and the actin cytoskeleton play important roles in Yap and Taz activity regulated by mechanical cues and by GPCR signaling (**Figure 1*b***). Lats1 and -2 most likely mediate the effect of Rho GTPases and actin on Yap and Taz because the phosphorylation status and kinase activity of Lats1 and -2 are clearly regulated by GPCR ligands, cell density, cell geometry, and cell attachment in a RhoA-dependent manner (30, 72, 73, 78–81). However, Lats1 and -2–independent Yap and Taz (all known Lats1 and -2 sites are mutated) are still regulated by matrix stiffness, and knockdown of Lats1 and -2 (although this is not definitive due to incomplete depletion) failed to block soft matrix–induced Yap and Taz phosphorylation (76). Therefore, Yap and Taz may be regulated by upstream cues independently of Hippo pathway kinases. Nevertheless, Rho GTPases are key regulators of Yap and Taz activity, and there is a tight positive correlation between RhoA and Yap/Taz, a relationship analogous to that of Ras and ERK1/2.

Investigators recently discovered many regulators for the Hippo pathway that regulate either the Mst-Sav1 complex or the Lats-Mob1 complex or directly modulate Yap and Taz activity. Johnson & Halder (84) summarized these regulators in detail in a recent review article.

Signaling Cross Talk

Yap and Taz may exert their growth-promoting activities via cell-autonomous or non-cell-autonomous approaches. Detailed mechanisms linking Yap and Taz activity to their diverse functions are currently not available. The story behind Yap and Taz is rather complex because the Hippo pathway cross talks with multiple signaling pathways involved in development and regeneration (**Figure 1*c***), and some of these pathways, such as the Wnt pathway, play critical roles in GI tissues (2).

Many connections between the Hippo and Wnt pathways have been reported. Taz is directly phosphorylated by GSK3 (which is repressed by Wnt), resulting in Taz degradation (53). In addition, Taz interacts with β-catenin and is degraded together with β-catenin when GSK3 phosphorylates β-catenin (85). Multiple studies have also indicated that the Hippo pathway can regulate Wnt signaling. Cytoplasmic Yap and Taz interact with Dishevelled (Dvl), inhibiting Dvl phosphorylation and β-catenin activation (86, 87). Cytoplasmic Yap and Taz also interact with β-catenin directly and restrict β-catenin nuclear translocation (88). Conversely, nuclear Yap seems to cooperate with β-catenin to boost Wnt target gene expression (89, 90). Therefore, Wnt signaling depends on the status of the Hippo pathway: High Mst1 and -2 or Lats1 and -2 activity (which leads to cytoplasmic localization of Yap and Taz) restricts Wnt signaling, whereas mutation or deletion of upstream components of the Hippo pathway (which leads to nuclear localization of Yap and Taz) results in increased and probably pathological β-catenin activation. On the basis of the current literature, the relationship between Wnt and Yap/Taz is very complex. Additional studies may be needed to clarify the interplay between these two pathways.

The Hippo pathway has extensive cross talk with transforming growth factor beta (TGF-β) signaling. The effect of Yap and Taz on TGF-β signaling also depends on Yap and Taz phosphorylation and subcellular localization, similar to the case for β-catenin. Upon TGF-β stimulation, nuclear Yap and Taz retain Smad proteins in the nucleus, facilitate assembly of transcription machinery, and induce transcription. In contrast, cytoplasmic Yap and Taz sequester Smad proteins in the cytoplasm, restricting TGF-β signaling–induced transcription (91–93). Yap and Taz can also directly induce BMP4 (a protein of the TGF-β superfamily) expression, and BMP4 is involved in Yap/Taz-induced cell migration and differentiation (94, 95).

The Hippo pathway also modulates Notch, Sonic Hedgehog (Shh), and mitogenic growth factor pathways. In the mouse intestine, Yap overexpression or Mst1/2 deletion results in Notch activation (96, 97), and the Notch ligand Jagged-1 is induced by Yap (98). In Shh-induced medulloblastomas, Yap expression is induced and important for tumorigenesis; conversely, the expression of Gli2, a downstream effector of Shh signaling, may be induced directly by Yap (99). Amphiregulin (AREG, a ligand for EGF receptors), insulin-like growth factor (IGF)-binding proteins, and the IGF receptor are also Yap target genes (100, 101), suggesting that the Hippo pathway can modulate growth factor–coupled receptor tyrosine kinase signaling.

The interplay between the Hippo pathway and other signaling pathways may contribute to both cell-autonomous and non-cell-autonomous functions of the Hippo pathway. Stimulation of Yap and Taz activity by Wnt and Shh, and the induction of Shh, BMP4, and AREG expression and secretion by Yap and Taz, may constitute a paracrine and/or autocrine signaling network.

The Hippo Pathway in Stem Cell Biology

In mice, when Yap is systematically deleted, the embryo stops developing at embryonic day 8.5 (E8.5), whereas Taz single-knockout mice are viable (102, 103). Yap and Taz double-knockout embryos die before the morula stage (16–32 cells) and embryo implantation (104). These results suggest that Yap and Taz play overlapping yet distinct roles during early development. Yap is critical for the first lineage specification in the preimplantation mouse blastocyst, when Yap shows dominant nuclear localization in the trophectoderm and cytoplasmic localization in the inner cell mass (104). Inhibition of Yap activity is essential for inner cell mass specification, as depletion of Lats1/2 (104, 105), Mob1a/b (106), Nf2 (107), or Amot/Amotl2 (35) results in nuclear Yap localization and aberrant lineage specification in inner cells.

The Hippo pathway is important for maintaining pluripotency in both human and mouse embryonic stem cells in vitro (91, 93, 108, 109). Downregulation of Yap or Taz activity leads to spontaneous differentiation of embryonic stem cells (91, 108, 109). In contrast, overexpression of Yap or Lats2 knockdown can enhance the reprogramming of differentiated cells into induced pluripotent stem cells (108, 109). These results indicate that high Yap or Taz activity can promote stem cell pluripotency and inhibit differentiation. Yap and Taz are involved in cell differentiation controlled by GPCR signals and mechanical cues (71, 81). Yap and Taz likely play a role in stem cell renewal and differentiation in vivo because both mechanical cues and GPCR ligands are important constituents of the stem cell niche.

In multiple tissues, the Hippo pathway can also regulate tissue-specific stem cells (84). The renewal, proliferation, and differentiation of tissue-specific stem cells are critical for tissue homeostasis under normal physiological conditions and tissue regeneration following tissue damage. High Yap activity expands progenitor cell populations in the liver, intestine, nervous system, and skin, although not in the heart (89) or hematopoietic system (110). We discuss the functions of the Hippo pathway in GI tissue–specific stem cells below.

FUNCTIONS AND REGULATION OF THE HIPPO PATHWAY IN THE LIVER

The liver plays a central role in the synthesis of plasma proteins and hormones, in the metabolism of carbohydrates and lipids, in the detoxification of xenobiotics, and in the secretion of bile acids (111). The liver is populated by two types of cells: epithelial cells, which include hepatocytes and cholangiocytes, and mesenchymal cells, which include Kupffer cells, stellate cells, and sinusoidal endothelial cells (112). Hepatocytes constitute approximately 60% of the cells in the liver and 80% of the total liver volume, and they are responsible for the liver's main metabolic functions (111, 112). Hepatocytes are derived from the endoderm, which gives rise to bipotential hepatoblasts during liver development. The hallmarks of mature hepatocytes specified from hepatoblasts include the appearance of a highly polarized morphology and glycogen storage. Cholangiocytes, which form the intrahepatic bile duct, also arise from hepatoblasts. Both hepatocytes and cholangiocytes are normally in mitotically quiescent states. The liver does not need to frequently renew hepatocytes and cholangiocytes due to their long life span (more than 200–300 days) and low rate of spontaneous apoptosis (2–4/10,000) (113, 114). Our current knowledge suggests that the Hippo pathway can modulate organogenesis of the liver and turnover of hepatocytes and cholangiocytes (26, 115).

Genetic Studies in Mouse Liver

The first reported physiological significance of the Hippo pathway in mammals was from two independent mouse transgenic studies. Liver-specific Yap transgenic mice displayed significant hepatomegaly soon after Yap expression was induced, and they showed hepatocellular carcinoma (HCC) following sustained Yap expression (27, 96). The pathogenesis was attributed to abnormal hepatocyte proliferation and to the loss of apoptosis in the liver. These two reports provided key evidence for an essential role of Hippo signaling in controlling mammalian organ size and tissue expansion.

Subsequent mouse models with conditional deletions of upstream Hippo pathway regulators provided additional evidence for a critical role of the Hippo pathway in regulating liver homeostasis. Although hepatic deletion of either Mst1 or Mst2 alone does not lead to liver pathogenesis, the combination of homozygous deletion of one Mst with heterozygous deletion of the other Mst is sufficient to cause spontaneous HCC and death (116). Similar phenotypes have been observed in other studies using slightly different conditional Mst1- and Mst2-deficient mouse strains (117, 118). Moreover, some Mst1- and Mst2-deficient mice also develop cholangiocarcinoma, a type of cancer that originates from bile duct epithelial cells. These studies support a role for Mst1 and -2 in liver organ size control and tumorigenesis by restricting liver cell proliferation and maintaining hepatocyte quiescence. Whether Yap regulation requires Lats1 and -2 in Mst1 and -2 double-knockout mice is not clear. Zhou et al. (116) observed minimal defects in Lats1 and -2 phosphorylation in Mst1- and Mst2-deficient mouse models, whereas Yap phosphorylation is reduced. These authors speculated that Mst1/2 deletion may affect other Yap kinases distinct from Lats1 and -2 (116). However, Lu et al. (118) showed that Lats1 and -2 phosphorylation is almost completely abolished in Mst1 and -2 double-knockout mice. Our experience indicates that the commercial phospho-specific Lats1 and -2 antibodies do not have high specificity or affinity, which may contribute to the contradictory results in these studies.

Hepatic deletion of Sav1 results in liver tumors with morphologies different from those of the Mst1 and -2 knockout mice (118, 119): These liver tumors contain a mixed population of hepatocyte-derived cancer cells and progenitor-like oval cells. However, neither Yap

overexpression nor Mst1- and Mst-2-deficient mice show an increased population of oval cells (27, 116). Thus, Sav1 may have additional roles beyond mediating Mst1 and -2 kinase activity. Inactivation of Nf2 in the mouse liver results in spontaneous HCC and bile duct hamartomas, and this Nf2 deficiency phenotype can be largely rescued by heterozygous deletion of Yap (62). Deletion of either Sav1 or Nf2 does not result in an immediate increase in liver size. However, deletion of both Sav1 and Nf2 induces Yap activation, liver enlargement, and massive overgrowth of biliary epithelial cells (26). These results suggest that Sav1 and Nf2 synergistically mediate Lats1 and -2 activation by Mst1 and -2.

Global Lats2 knockout mice are embryonic lethal due to defective cardiac growth (120). Lats1 knockout mice develop soft-tissue sarcomas and ovarian tumors, but not liver cancer (121). No conditional Lats1 and -2 double knockouts in the liver have been studied thus far. However, homozygous deletion of Mob1a with the heterozygous trapped mutation of Mob1b leads to a 50% incidence of liver cancer during the life span of the mice (106). Lats1 and -2 also regulate cytokinesis (122, 123), so their downstream signaling mediated by Yap and Taz may be masked by a dominant cytokinesis defect in Lats1 or Lats2 knockout mice. As Mob1a and -b are critical cofactors for Lats1 and -2 kinase activity, the results from Mob1 knockout mice suggest an important role for Lats1 and -2 in liver growth.

Efforts to generate transgenic mice with deletions of peripheral components of the Hippo pathway also contribute to our growing knowledge of the complexity and subtlety of Hippo pathway regulation. For example, Amot knockout mice, rather than showing enhanced proliferation in biliary epithelial cells, unexpectedly display a significant decrease in ductal cell proliferation and hepatocarcinogenesis in response to toxin-induced liver injury (124). Moreover, deletion of different Hippo pathway regulators in mice also results in tissue-specific phenotypes. For instance, conventional deletion of Rassf1 in mice leads to carcinogenesis in lung and mammary tissues, but not in liver (125). Moreover, a tamoxifen-inducible Mst1 and -2 double-knockout mouse strain shows abnormal growth in the liver and stomach but no abnormality in either the lung or breast (117).

In most, if not all, knockout mouse models, changes in the phosphorylation and cellular localization of Yap have been observed, demonstrating the importance of these components in regulating Yap phosphorylation and the role of Yap in mediating the functions of upstream regulators of the Hippo pathway in the liver. Yap inactivation leads to the formation of abnormal tubular structures during development due to the loss of mature hepatocytes and of bile duct epithelial cells (62). Therefore, the Hippo pathway must tightly regulate the appropriate timing and intensity changes in Yap activity to maintain liver homeostasis, especially during liver development. In the future, more efforts are still needed to characterize the roles of the Hippo pathway in different mouse models, especially those with inducible and more liver cell type–specific deletions. In addition, lineage tracing of different Hippo pathway components during cell differentiation and/or transformation by reporter mouse models may reveal critical new insights into the Hippo pathway during liver development and carcinogenesis.

The Hippo Pathway in Liver Cancer

In the past decade, research has uncovered a detrimental role for the uncontrolled, compensatory proliferation of hepatocytes and/or cholangiocytes resulting from severe liver cell death and chronic inflammation due to hepatitis viral infection, xenobiotic insults, or alcohol overconsumption (126, 127). Hepatocyte necrosis and apoptosis activate hepatic mesenchymal cells, mainly Kupffer cells and stellate cells, which release inflammatory cytokines and growth factors into the liver microenvironment to induce hepatocyte proliferation (112, 128). The compensatory

proliferation of hepatocytes occurs and continues unless the appropriate antimitotic signals, such as TGF-β family growth factors, stop this process (112). Rapid cell turnover and replication are believed to be essential steps in liver cancer initiation (128).

The Hippo pathway is modulated by xenobiotics, metabolites, and hepatitis viral proteins. A compound named TCPOBOP, which mimics the xenobiotics acetaminophen and phenobarbital to activate the constitutive androstane receptor (a nuclear receptor) (129), causes hepatomegaly and increases Yap protein levels in the liver (130). The hepatitis B virus (HBV) X protein, which mediates many of the tumor-promoting functions of HBV, upregulates Yap expression levels by binding to the Yap promoter and enhancing Yap gene transcription (131). Ethanol enhances tissue overgrowth from the loss of upstream components of the Hippo pathway in *Drosophila* (132). However, whether ethanol regulates the Hippo pathway in the mammalian liver is unknown. Due to the important roles of these insults in liver cancer, it would be important to investigate the role of the Hippo pathway in liver carcinogenesis resulting from inflammation or hepatitis infection.

A series of studies have demonstrated a close association between cholestasis and Hippo signaling (133–135). Cholestasis is usually caused by the disruption of enterohepatic circulation and overload of bile acids, which results in diseases such as primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC). In fact, increased Yap expression and nuclear localization have been reported in the bile duct epithelial cells of samples from patients with PSC and PBC (133). Furthermore, in an animal model for obstructive cholestasis (bile duct ligation), Yap is essential in preventing apoptosis and inflammation in the bile ducts. A recent study showed that Yap expression is associated with persistent neonatal cholestatic liver disease and can be used as a diagnostic marker (134). All these results suggest that bile acids may be external activators of Yap, at least in bile duct epithelial cells. Indeed, a recent study provided direct evidence that elevated bile acid levels increase YAP expression in mouse liver (135). Nevertheless, more studies are needed to elucidate the mechanisms by which bile acids affect the Hippo pathway and to clarify the role of the Hippo pathway in hepatocytes and bile duct epithelial cells. Mutations in the gene encoding the $G\alpha_s$ protein are frequently observed in neoplasms of the bile duct (136). Therefore, dysregulation of the Hippo pathway by GPCR signaling may contribute to neoplastic growth of the bile duct, and several hydrophobic bile acids are also GPCR ligands (137, 138).

Enhanced liver carcinogenesis in mouse models defective in Hippo signaling core components (e.g., Mst1 and -2, Nf2, Mob1a and -b, and Sav1) suggests that the Hippo pathway effectors Yap and Taz may contribute to liver tumor initiation (see above). For humans, genetic studies have also revealed that the Yap gene is amplified in liver cancer (139), and frequent nuclear stains of Yap are observed in HCC tissue, in contrast to negative or cytoplasmic stains in peripheral nontumor tissues (27, 30). A later study with a much larger cohort of HCC patients with definitive pathological and follow-up data not only confirmed Yap overexpression in liver tumors but also suggested that Yap expression levels are correlated with the stages of tumor progression, with levels of serum α-fetoprotein (a diagnostic marker for liver cancer), and with the patients' overall prognosis (140).

Although Yap hyperactivation has been observed in HCC and can be correlated with patient prognosis, there is relatively limited direct evidence that Yap actually contributes to adult HCC. Nevertheless, even if Yap is merely a secondary response of hepatocyte malignant transformation that helps tumor cells proliferate and escape from quiescence or apoptosis, it could still be a cancer cell–specific target. Specifically, verteporfin, a chemical that disrupts Yap binding to Tead1–4, prevents Yap overexpression–induced hepatomegaly and hepatocellular carcinogenesis and may be a promising agent for liver cancer therapy (48). More preclinical studies with additional Yap inhibitors will provide key insights into the role of Yap in HCC.

The Hippo Pathway in Liver Homeostasis and Regeneration

Since it was first discovered that the Hippo pathway could regulate liver size, researchers have asked whether the Hippo pathway is also involved in liver regeneration. The role of the Hippo pathway in regulating organ size is almost exclusively based on an albumin-Cre system to conditionally knock out genes in the liver in the early developmental stages (E12.5) (62, 116–119). In contrast to what we know about the role of the Hippo pathway in early development, these models provide us limited information about the function of the Hippo pathway in the adult liver. The rate-limiting step of liver regeneration is priming the hepatocytes out of deep quiescence, for which the activities of Mst1 and -2 are indispensable (115). There are also indications that Yap mRNA and protein levels are differentially regulated during mouse liver regeneration (141), which suggests that the Hippo pathway is dynamically modulated during liver regeneration. However, no studies with animal models of partial hepatectomy or chemical-induced liver injury have been reported in Yap transgenic or knockout mice. Whether the Hippo pathway is critical to maintaining adult liver homeostasis following liver damage is still relatively unclear.

Characterization of the Hippo pathway during liver regeneration has significant clinical relevance. Failure in liver transplantation is usually caused by insufficient growth during the initial stage after implantation (142), which may result from the slow priming of implanted liver cells in the recipients. One approach to improve survival may be to inhibit Mst1/2 or Lats1/2 in donor livers prior to transplantation. Alternatively, recipients may receive medications to activate Yap, such as TCPOBOP. Testing these hypotheses in mouse models may provide promising therapeutics for liver transplantation.

INTESTINAL REGULATION BY THE HIPPO PATHWAY

Intestinal Homeostasis and Regeneration

A single layer of epithelial cells facing the intestinal lumen is responsible for both nutrient absorption and enzyme secretion (2, 143). In the small intestine, the epithelial lining is organized into crypts of Lieberkühn, which are small invaginations, and villi, which are relatively larger finger-like protrusions (**Figure 2**). This organization efficiently increases the small intestine's surface area and its capability to absorb nutrients. All epithelial cells in the intestinal lining are derived from intestinal stem cells (ISCs) that reside at the base of each crypt. ISCs first migrate toward the lumen and differentiate into transient-amplifying (TA) cells. TA cells then differentiate further to become absorptive cells [enterocytes (ECs)] and secretory cells [goblet cells and enteroendocrine cells (EEs)]. The mature cells migrate toward the tip of each villus along the crypt-villus axis and cover the entire villus surface. Some TA cells can further differentiate into secretory Paneth cells, which migrate toward the crypts' base and reside beside the ISCs. In addition to their role in intestinal immunity, Paneth cells are thought to be critical for maintaining ISC stemness. The intestinal epithelium undergoes constant self-renewal: When mature cells reach villi tips, they undergo programmed cell death and are shed into the lumen, and they are replaced by the next generation of migrating cells. In the colon, the mucosa has a flat surface without villi, and ISCs also reside at the base of crypts and give rise to all cell types of the epithelial lining (**Figure 2**).

The base of the intestinal crypts is a niche for ISCs. Two distinct ISC populations may coexist in the crypt: Crypt base columnar (CBC) cells [marked with leucine-rich repeat–containing GPCR5 (Lgr5)] represent actively cycling stem cells, and +4 cells above Paneth cells represent quiescent stem cells (144). $Lgr5^+$ stem cells can differentiate into all kinds of mature intestinal epithelial cells in vivo (145), and a single $Lgr5^+$ stem cell can grow an entire crypt-villus-like structure mimicking that of the small intestine (146), demonstrating a critical role for $Lgr5^+$

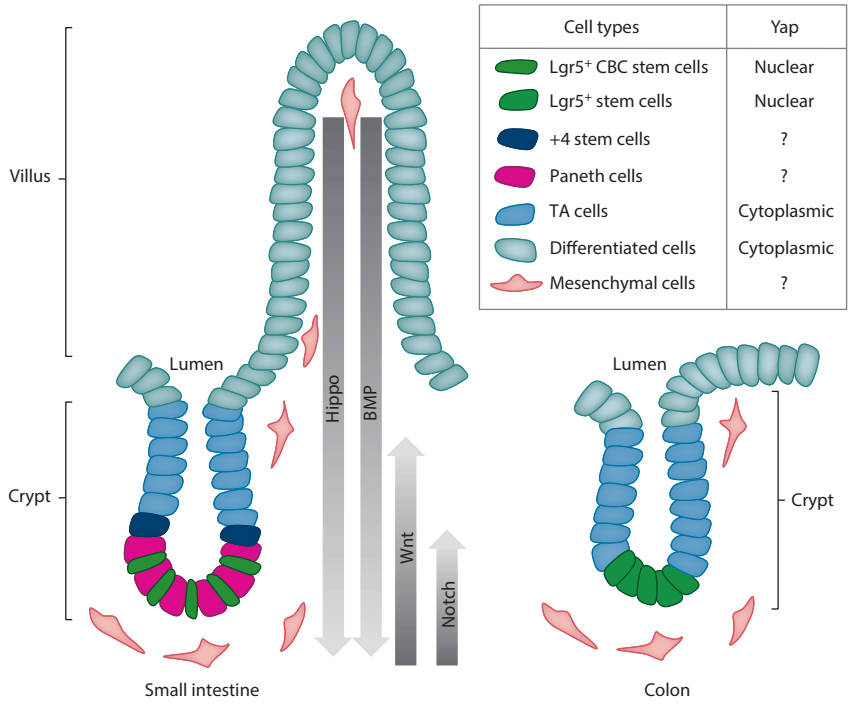


Figure 2

Homeostatic regulation of the intestinal epithelium. The epithelial lining of the small intestine and colon is depicted. The structure of the crypts and villi and their corresponding cell types are also indicated; Paneth-like cells in the colon are not shown. Yap localization of these different cell types is shown in the table at upper right. Hippo pathway activation (which has a pattern opposite to that of Yap activity) and BMP signaling show a descending gradient from the villi to the crypt base, whereas Wnt signaling and Notch signaling are stronger near the crypt base and decrease along the crypt-villus axis. Abbreviations: CBC, crypt base columnar; Lgr5, leucine-rich repeat–containing GPCR5; TA cells, transit-amplifying cells.

cells as ISCs. Although there are currently no well-accepted markers for +4 stem cells, Bmi1 and Musashi-1 were recently proposed (147, 148). Following depletion of Lgr5$^+$ cells, Bmi1$^+$ cells can give rise to Lgr5$^+$ cells and support crypt-villi regeneration (149). It has also been proposed that Lgr5$^+$ cells are important for homeostatic self-renewal, whereas Bmi1$^+$ cells are responsible for injury-induced regeneration (150).

Multiple signaling pathways, including Shh, BMP, Wnt, Notch, and Eph/ephrin, are involved in intestine homeostasis and regeneration (**Figure 2**) (151). Shh signaling (from intestinal epithelium to the mesenchyme) is important for the formation of the crypt-villus axis (152). Wnt signaling is critical for the proliferation of ISCs and TA cells, as revealed by the lack of proliferative crypts in mice with deletion of Tcf4 (a transcription factor that is a binding partner of β-catenin) and by dramatic crypt hyperplasia following treatment with exogenous R-spondin 1 (a ligand for Lgr5 that can potently enhance the Wnt response) in mice (153, 154). Ligands and inhibitors of the BMP signaling pathway are expressed in the mesenchyme of villi and crypt, respectively, and BMP signaling negatively regulates cell proliferation (155, 156). Notch signaling in the intestinal epithelium is involved in cell proliferation, and inhibiting Notch signaling results in the conversion of proliferative cells into secretory cell types, including predominantly goblet cells (157, 158). Paneth cells are important sources of mitogenic signals, and they can express Wnt3,

Dll1/4 (Notch ligands), and Noggin (a BMP signaling inhibitor) to maintain the proliferation of proximal ISCs (159, 160). Taken together, the evidence suggests that multiple signaling pathways are orchestrated to regulate the proliferation and differentiation of stem cells and the renewal of the intestinal epithelium (**Figure 2**).

The *Drosophila* Midgut

The *Drosophila* midgut and hindgut are equivalent to the small intestine and large intestine in mammals, respectively (161). Similar to the mammalian intestines, the *Drosophila* midgut is derived from the endoderm during development. However, the morphology of the *Drosophila* midgut is different from that of its mammalian counterpart in that it lacks any crypt-like or villi-like structure. The inner lining of the midgut consists of pseudostratified epithelium containing large polyploid ECs and diploid EEs. The midgut ISCs are located at the basal position of the epithelium. During renewal and regeneration, ISCs can maintain the stem cell pool and give rise to progenitor cells (enteroblasts), which can further differentiate into ECs and EEs. Wnt signaling is essential for stem cell maintenance and proliferation in the *Drosophila* gut, where Wingless (a *Drosophila* Wnt homolog) is released from muscle cells underneath the epithelium in the midgut (162). Notch signaling is responsible for cell differentiation in the midgut; the exclusive expression of Delta (a Notch ligand in *Drosophila*) in ISCs guides the maturation of surrounding daughter cells (163). Injury or bacterial infection activates JNK in ECs, which leads to the production of Unpaired (Upd) family cytokines. Upd cytokines activate Jak/Stat signaling in ISCs and induce ISC proliferation and differentiation into progenitor cells (164, 165).

Multiple studies have focused on the role of the Hippo pathway in the midgut's self-renewal and regeneration. Overexpression of Yki or inactivation of upstream components of the Hippo pathway in ISCs or ECs induces ISC expansion in either a cell-autonomous or a non-cell-autonomous manner. Activation of Yki in the midgut epithelium results in Upd production, which activates Jak/Stat signaling and drives cell proliferation (166–169). Dextran sodium sulfate (DSS) feeding or *Pseudomonas entomophila* infection also activates Yki to increase midgut regeneration (167, 168). In this case, JNK most likely mediates Yki activation (168, 169). In addition, two Yki targets, Bantam microRNA and the Brahma chromatin-remodeling complex, are required for ISC proliferation driven by high Yki activity in the midgut (40, 170). Together, these results suggest that Yki activation drives expansion of the *Drosophila* midgut epithelium and is indispensable for midgut regeneration following tissue injury.

The Hippo Pathway in Mammalian Intestine

Yap is expressed in both the small intestine and the colon (97). In the small intestine, Yap is primarily cytoplasmic in upper crypts (97) and villi and nuclear in the $Lgr5^+$ ISCs at the crypt base (86), suggesting a descending gradient of Hippo activity (ascending Yap activity) from the villi to the crypt base (**Figure 2**).

Inducible and ubiquitous expression of Yap in the mouse intestine results in reversible dysplasia of the intestinal epithelium: The dysplasia phenotype becomes significant 2 days after Yap induction, with proliferative cells occupying the entire epithelial lining. However, upon withdrawal of ectopic Yap expression, the intestine regenerates and becomes structurally normal (96). Following induction of Yap expression, both Notch signaling and Wnt signaling are induced in the intestine, which may be responsible for the disappearance of all differentiated cell types in the small intestine (96). This Yap-induced dysplasia is largely blocked by a γ-secretase inhibitor (a Notch signaling inhibitor) (96), suggesting a critical role for Notch signaling downstream of

Yap activation. However, intestine-specific Yap deletion has no significant effects on intestinal cell proliferation, structure, or function, indicating that Yap is dispensable for normal intestinal homeostasis (97, 171). Taz may have a role in the intestine in compensating for the loss of Yap.

During DSS-induced colon regeneration, Yap protein levels increase in the early stages of regeneration but return to normal once the intestinal structure is fully recovered. This result suggests that Yap may play a positive role during intestinal regeneration. Indeed, deletion of Yap in the colon blocks DSS-induced crypt regeneration and results both in a dramatic decrease in body weight and in increased mortality (171). Similar results have been observed in *Drosophila* (see above), suggesting that the involvement of the Hippo pathway in DSS-induced intestinal regeneration is evolutionarily conserved.

Manipulation of upstream components of the Hippo pathway can also affect intestinal homeostasis. Deletion of both Mst1 and Mst2 (Mst1 and -2 double knockout) in the mouse intestine leads to crypt dysplasia, impaired differentiation of epithelial cells, and early mortality (97). These phenotypes may be due to increased Yap activity and the consequent induction of the Wnt and Notch pathways (97). Removing one or both Yap alleles prevents premature mortality and restores homeostasis of the intestinal epithelium in these Mst1 and -2 deleted mice, further suggesting that Yap plays a critical role downstream of Mst1 and -2 (97). Intestine-specific deletion of Sav1 in mice also results in Yap activation and in the enlargement of crypts containing more and faster-proliferating cells, and again these phenotypes are completely suppressed by the loss of Yap (171). Sav1 knockout mice may have a milder phenotype with regard to intestinal homeostasis and mortality than do Mst1 and -2 double-knockout mice because of the partial blockage of Lats1 and -2 activation upon loss of Sav1. A combinatory knockout of Sav1 and Nf2 may lead to severe crypt hyperplasia, and this combinatory effect was recently shown in the liver (26). Sav1 was also deleted ubiquitously in mice by using a conventional knockout approach, and multiple epithelial tissues, including the small intestine and colon, display hyperplasia due to impaired proliferation arrest and terminal differentiation of progenitor cells (172). All these data suggest a proproliferation, progenitor cell expansion, and antidifferentiation role for Yap activation in the intestine, which is consistent with the phenotypes following Yap induction observed in other tissues, including mouse liver and skin and chick neural tubes (27, 96, 173).

A recent study revisited Yap function in the small intestine (86). Yap overexpression specifically in the intestine results in suppressed intestinal renewal, as evident by the loss of proliferative crypts, mislocalization and eventual disappearance of Paneth cells, and (at the molecular level) decreased Wnt signaling (86). In these intestine-specific Yap knockout mice following whole-body irradiation, both the small intestine and the colon exhibit overgrowth and crypt hyperplasia, the population of Paneth cells is expanded, and Paneth cell localization is not restricted to the crypt base (86). The overgrowth phenotype is at least partially due to activation of Wnt signaling because Yap deletion results in a massive hyperplastic phenotype following expression of R-spondin 1, a potent Wnt signaling enhancer that induces crypt growth (86). These observations suggest that Yap represses intestinal growth and regeneration, which contradicts the previously reported role of Yap during intestinal homeostasis and regeneration (see above).

Several factors may potentially explain the discrepancies observed in these two transgenic (intestine-specific or ubiquitous) mouse model studies. First, different promoters were used to drive Yap expression, and therefore the Yap expression cell types and levels in these two models may be very different. Such differences may evoke different downstream effectors and result in opposite outcomes with regard to intestinal growth. Second, in the ubiquitous Yap transgenic mice, Yap expression in the mesenchyme or in infiltrated cells may secrete paracrine factors that in turn enhance crypt growth. In support, Yap and Taz are required for cancer-associated fibroblasts to remodel their ECM and to promote cancer cell invasion and angiogenesis (174). Third, as

suggested by Li & Clevers (144), a direct comparison between these two studies is difficult due to the different time points used. In the intestine-specific transgenic Yap model, Yap expression was maintained for 7 days to observe a severe disruption of the intestine epithelium, although the loss of CBC stem cells and Paneth cells had been observed earlier. In contrast, in the ubiquitous transgenic model, Yap expression was maintained for only 4.5 days. Therefore, these two models should be examined side by side under the same experimental conditions and time points, including comparing Yap expression levels, tracing the different types of intestinal epithelial cells expressing Yap, and assessing Yap's effects on cell proliferation and death.

In the intestine-specific Yap knockout mice, the difference in regenerative potential following DSS-induced and irradiation-induced tissue damage was also unexpected (86, 171). Intestinal epithelial cells may respond differentially to different damaging agents. For instance, the mitotically active $Lgr5^+$ ISCs are more sensitive to irradiation, whereas the quiescent $Bmi1^+$ ISCs are resistant to high-dose irradiation (150). Therefore, following irradiation injury the regenerative program may start with the expansion and differentiation of $Bmi1^+$ ISCs in a Yap-independent manner. However, DSS-induced injury may affect different ISC populations equally, and so the transient increase in Yap activity following DSS withdrawal (171) is critical for regeneration. The mechanism underlying Yap activation following DSS withdrawal is not clear, although Yap may be temporally activated by mechanical cues, diffusible signals (such as GPCR ligands, growth factors, or inflammatory cytokines), disrupted cell polarity, or damaged cell junctions (**Figure 1*b***). Moreover, whether irradiation itself leads to changes in Yap activity is unclear. Answers to these questions may help explain Yap-independent enhanced intestinal regeneration following irradiation injury. In *Drosophila*, the Hippo pathway is required for midgut regeneration following DSS feeding, whereas the Jak/Stat and EGF receptor pathways are required for regeneration following tissue damage caused by bleomycin (a DNA-damaging agent) (175). The Hippo pathway's contribution to intestinal regeneration following injury may depend on the injury type.

As discussed above, Yap and Taz may have different roles in the Wnt pathway: Cytoplasmic Yap and Taz inhibit β-catenin, whereas nuclear Yap and Taz promote β-catenin transcriptional activity by directly interacting with Dvl or β-catenin. Therefore, the phenotypes corresponding to Yap overexpression or knockout of components (such as Mst1/2 or Sav1) upstream of the Hippo pathway may be different. Mst1 and -2 double knockout or Sav1 knockout results in Yap (and most likely Taz) hypophosphorylation and nuclear localization. In this situation, Yap and Taz may enhance the mitogenic effects of the Wnt pathway and contribute to crypt hyperplasia. In contrast, in the Yap overexpression model, Yap is localized in both the nucleus and the cytoplasm (86). In this case, the abundant cytoplasmic Yap may restrict Wnt signaling, resulting in crypt loss and intestinal degeneration. Again, the protein levels and localization of overexpressed Yap are critical because they may regulate Wnt signaling differently and result in different phenotypes.

Up to now, most experiments carried out in the mouse intestine have focused on Yap. The possible function of Taz has been neglected, even though Taz is important for the maintenance of breast cancer stem cells and plays a role in cell differentiation in vitro (176). In Yap transgenic or knockout mice, Taz activity has not been extensively examined. Taz expression appears to be increased in intestine-specific Yap knockout mice (86). Due to the high similarity between Yap and Taz, whether Taz can compensate in the case of Yap knockout or overexpression must be further investigated.

The Hippo Pathway in Colorectal Cancer

A large number of colorectal cancer (CRC) cases are linked to environmental factors such as food-borne mutagens, pathogens, and chronic intestinal inflammation (177). Genetic mutations

occur during inflammation or intestinal regeneration and may contribute to neoplastic transformation. The most frequently mutated gene responsible for CRC is adenomatous polyposis coli (APC), a Wnt pathway component (178, 179). APC mutations result in β-catenin stabilization, accumulation, nuclear localization, and ultimately the hyperproliferation of intestinal epithelial cells. Uncontrolled cell proliferation can disrupt the epithelial lining surrounding the lumen and results in the formation of aberrant crypt foci. With additional mutations, these aberrant crypt foci progress to adenoma and later to carcinomas (177).

Deletion of upstream components of the Hippo pathway in mice results in adenoma formation. For instance, Mst1 and -2 double-knockout mice develop adenomas after 13 weeks (97), and Sav1 knockout mice develop colonic polyps, which resemble a human lesion termed sessile serrated polyps, at 13 months (171). This genetic evidence suggests that the Hippo pathway may also play a role in CRC development. Indeed, Yap and Taz expression is increased in CRC (especially high-grade CRC), and Yap and Taz expression may be used as a CRC prognostic marker (97, 171, 180–182). In addition, Yap can promote resistance of CRC cells to chemotherapy, and high Yap expression levels correlate with CRC relapse (183). Upregulation of Yap and Taz in CRC may be due to the loss of inhibition by upstream kinases. Indeed, decreased Lats1 expression due to promoter methylation has been reported in CRC (184). Induction of Yap and Taz in CRC may also depend on other mechanisms; for instance, β-catenin may directly induce Yap expression or stabilize Taz (185).

The Hippo pathway interacts with the Wnt pathway via several different mechanisms (see above). Some colon cancer cells driven by β-catenin require active Yap for survival and transformation (90). However, whether Yap and Taz induction in CRC depends on Wnt pathway activity is currently unknown. The colonic polyps developed in Sav1 knockout mice are histologically different from the prototypal intestinal adenomas that result from APC mutations (171), suggesting potentially different roles for the Wnt and Hippo pathways in CRC development. Barry et al. (86) also showed that, in a small fraction of aggressive CRC cases, a lack of Yap expression correlates with decreased patient survival, suggesting that Yap may have a tumor suppressor function. Therefore, further in-depth investigations are required to understand the role of the Hippo pathway in the initiation and development of CRC.

HIPPO PATHWAY REGULATION IN OTHER GASTROINTESTINAL TISSUES

The Hippo Pathway in Pancreatic Development and Cancer

The pancreas is a dual-function organ containing both endocrine and exocrine compartments. The exocrine compartment of the pancreas, like the liver, secretes juice containing digestive enzymes into the small intestine to help in the digestion and absorption of nutrients. In contrast, endocrine pancreatic cells secrete insulin, glucagon, and somatostatin to regulate glucose metabolism (186).

In the pancreatic exocrine compartment, Yap is expressed in ductal cells, but not in acinar cells, suggesting that Yap is important for the maintenance and function of ductal cells. Indeed, ectopic Yap overexpression in the pancreas results in the expansion of ductal cells and in disrupted differentiation of acinar cells from E13.5 to E17.5 (187). In addition, mice with deletion of Mst1 and -2 in pancreatic progenitor cells display severe defects in pancreatic architecture. Their pancreas weight is decreased compared with that of their wild-type littermates, and a high ratio of ductal-to-acinar cells is observed, which can be attributed to Yap hyperactivation in the exocrine cells

(187, 188). However, ablation of Mst1 and -2 does not lead to significant defects in pancreas development, as newborn Mst1 and -2 double-knockout mice possess apparently normal acinar and duct cells (187, 188), which is different from the phenotype of Yap overexpression. The discrepancy may be due to a transient disappearance of Mst1 and -2 and Yap expression from E15 to birth—the absence of a target and effector of Mst1 and -2 knockout (187). Nevertheless, these results suggest that Mst1 and -2 are essential for maintaining the balanced differentiation status of the cells in the exocrine compartments of the postnatal pancreas. The Mst1 and -2 double knockout has minimal effects on the endocrine compartment of the pancreas. The islets of these mice are smaller and have an abnormal α/β cell ratio compared with wild-type littermates. However, the knockout mice displayed relatively normal glucose homeostasis (188). Therefore, the major function of Mst1 and -2 in the pancreas seems to be in the exocrine compartments in regulating acinar cell plasticity and differentiation.

Pancreatic cancer has one of the poorest prognoses of all types of cancer due to its resistance to treatment and the difficulties of diagnosis (189). An immunohistochemistry study from Diep et al. (190) demonstrated that the expression and nuclear localization of Yap are increased in pancreatic tumors compared with nontumor tissues. High Yap expression was detected primarily in ductal adenocarcinoma cells, although normal pancreatic cells also showed weak to moderate expression. This study also demonstrated that Yap expression and localization are closely correlated with the patient's prognosis. Another recent study using a Kras mutant mouse model reinforced the role of the Hippo pathway in pancreatic cancer (191). This study reported that Yap is posttranscriptionally regulated by Kras in a MAPK-dependent manner and that pancreatic deletion of Yap in mice carrying the Kras mutant prevents the proliferation and invasion of neoplastic ductal cells. This Kras mutant study suggests that, even though Yap is expressed in normal ductal cells in the adult pancreas, targeting Yap seems to be a good strategy for pancreatic cancer therapies.

The Hippo Pathway in Other Gastrointestinal Tissues

The Hippo pathway is also important in the development of other GI tissues, such as the salivary glands, tongue, esophagus, and stomach. The Hippo pathway is essential for the development of salivary glands in mice, and abnormal activation of Yap and Taz is seen in human Sjogren's syndrome, a chronic autoimmune disease in which white blood cells destroy the exocrine glands (including salivary glands) (192). In the mouse tongue, transgenic expression of Yap leads to epithelial thickening (193), suggesting a role of the Hippo pathway in tongue tissue homeostasis. Yap is expressed in the normal esophageal mucosa and gastric epithelium, and this expression seems restricted to the proliferative epithelial layers (194). However, elevated Yap staining (both cytoplasmic and nuclear) is observed in esophageal squamous cell carcinoma and adenocarcinoma as well as in gastric adenocarcinoma and metastatic gastric cancer, which implies either that Yap protein is stabilized or that Yap mRNA level is abnormally elevated (194–196). As potential mechanisms lead to Yap activation, the expression of VGL4, which antagonizes Yap activity, is decreased in gastric carcinoma (47); in addition, mutations of RhoA, an upstream activator of Yap, have been found in diffuse-type gastric carcinoma (197). Collectively, these studies indicate a role for Yap activation in gastric cancer.

The epithelium of the GI tissues, probably excluding the liver, is active in tissue turnover. However, progenitor cells in the epithelium are also essential for maintaining the normal developmental and postdevelopmental homeostasis of these tissues that maintain rapid turnover due to exposure to harsh environments. Proper activation of the Hippo pathway at different stages or in different contexts appears to be indispensable for maintaining the development and

homeostasis of the digestive organs. Activation of Yap by suppressing Hippo activity clearly plays a role in the regeneration of GI epithelial tissues, particularly after injury. Abnormal regulation of the Hippo pathway, as indicated by Yap and Taz hyperactivation, is shown in many different diseases, especially in the case of carcinogenesis. Our current knowledge supports the notion that Yap and Taz are attractive therapeutic targets for treating some cancer types of the GI tissues.

SUMMARY POINTS

1. The Hippo pathway regulates cell proliferation, cell death, and cell differentiation to modulate tissue growth and homeostasis, mainly through inhibition of Yap and Taz.
2. The Hippo pathway cross talks with multiple signaling pathways, including the Wnt, Notch, TGF-β, and Shh pathways, to regulate GI tissue homeostasis.
3. In the liver, the Hippo pathway kinases are critical for maintaining hepatocyte quiescence. Induced Yap expression or knockout of upstream components of the Hippo pathway causes hepatomegaly, hepatocellular carcinoma, cholangiocarcinoma, and hepatoblastoma.
4. In intestine, inhibition of the Hippo pathway results in stem cell expansion and neoplastic growth. Yap is dispensable for normal tissue homeostasis but is essential for regeneration following certain types of tissue injury.

FUTURE ISSUES

1. The precise signal transduction mechanisms of the Hippo pathway are not fully understood. More studies are needed to address how the Hippo pathway responds to mechanical cues, different diffusible factors, and cytoskeletal changes and how Yap and Taz exert their physiological or pathological functions.
2. The cross talk/functional interplay between Hippo and other signaling pathways is a key to understanding the molecular insights of Hippo and Yap in GI tissue homeostasis and tumorigenesis in vivo.
3. More mouse models should be developed to provide precise spatial and temporal manipulation and intensity control of components of the Hippo pathway.
4. It would be important to study the role of the Hippo pathway under different pathological conditions, such as infection, injury, tissue transplantation, and cancer.

DISCLOSURE STATEMENT

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