Hippo–YAP/TAZ signalling in organ regeneration and regenerative medicine

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Abstract

The Hippo pathway and its downstream effectors, the transcriptional co-activators YAP and TAZ, regulate organ growth and cell plasticity during animal development and regeneration. Remarkably, experimental activation of YAP/TAZ in the mouse can promote regeneration in organs with poor or compromised regenerative capacity, such as the adult heart, and the liver and intestine of old or diseased mice. However, therapeutic YAP/TAZ activation may cause serious side effects. Most notably, YAP/TAZ are hyperactivated in human cancers and prolonged activation of YAP/TAZ triggers cancer development in mice. Thus, can the power of YAP/TAZ to promote regeneration be harnessed in a safe way? Here we review the role of Hippo signaling in animal regeneration, examine the promises and risks of YAP/TAZ activation for regenerative medicine, and discuss strategies to activate YAP/TAZ for regenerative therapy while minimizing adverse side effects.

[H1] Introduction

Trauma, disease, or ageing induce tissue damage, which requires the activation of regenerative responses to restore organ function[1]. Unfortunately, however, most organs in adult humans have little or no potential to regenerate, and injury triggers scaring and fibrosis that ultimately lead to organ malfunction[1,2]. Thus, is it possible to therapeutically activate repair, prevent or reverse scaring, and restore organ function?

Regenerative potential of a tissue is endowed either by the presence of resident stem cells, such as in the skin and intestine, or by the ability to reactivate cell proliferation in terminally differentiated cells, such as in hepatocytes in the liver[1,3]. One approach of regenerative medicine aims to mimic such repair mechanisms in organs with poor regeneration. This is attempted, for example, by activating endogenous repair mechanisms or by transplanting stem or progenitor cells. However, simply triggering cell proliferation of differentiated cells is generally not sufficient to endow a measurable regenerative potential onto a tissue, as exemplified by the heart[4-7]. Similarly, clinical trials for stem cell transplantation approaches showed only limited efficacy[8]. Then, what mechanisms engage cells to regenerate tissue damage, and can they be applied for therapy in non-regenerating organs?

The discovery of the Hippo pathway and its fundamental role in organ growth and regeneration identified a novel approach to incite regeneration. The downstream effectors of the Hippo pathway YAP (Yes-associated protein, also known as YAP1) and TAZ (transcriptional co-activator with PDZ-binding motif; also known as WWTR1) are required for regeneration in different organs, and their ectopic activation in adult mice can drive overgrowth of some organs (liver), promote dedifferentiation of mature cell types (lung secretory cells, hepatocytes), and trigger the expansion of stem and progenitor cell compartments (skin, intestine)[9-12]. These findings prompted

investigations of the potential of artificial YAP/TAZ activation (for simplicity we will treat YAP and TAZ as equivalent proteins as they are structurally similar, have largely redundant functions, and cause similar effects when overexpressed in many contexts, although some differences exist that need further investigation) in stimulating organ repair and regeneration in non-regenerating organs, with exciting results in mice[13-17]. The mouse heart is currently the most prominent example where experimental activation of YAP/TAZ had beneficial regenerative effects, but activation of YAP/TAZ also aided regeneration of several other organs in adult mice including the liver[18], muscle, and intestine[13-17,19]. These studies raise the possibility of manipulating Hippo pathway activity as a means to stimulate endogenous mechanisms of regeneration in injured human organs. However, the therapeutic activation of YAP/TAZ for regenerative purposes may bear notable risks, because YAP/TAZ hyperactivation is well established to promote cancer development [20,21].

Here, we first provide a synopsis of the Hippo pathway and its function during tissue repair and organ regeneration and then review experiments indicating that YAP/TAZ activation can stimulate regeneration in different mouse tissues. We also survey the risks of artificial YAP/TAZ activation associated with their potential to disrupt organ function and cause cancer, and discuss how it may be possible to safely activate YAP/TAZ for regenerative medicine.

[H1] The Hippo pathway and its regulation

The first components of the Hippo pathway were identified in *Drosophila melanogaster*[22-30] and the founding member, the Hippo (Hpo) kinase, was named after its overgrown and darkened (hyperpigmented) mutant cuticle phenotype in adult flies. Soon after, the conserved nature of the pathway and its role in mammalian organ size regulation was revealed by the characterization of the mammalian core kinase cascade and the generation and analysis of mutant alleles [31-38].

The Hippo pathway is a highly conserved signal transduction pathway that regulates gene expression (Fig. 1). The core of the pathway is a kinase cascade that in mammals comprises the Ste20-like kinases 1 and 2 (MST1 and MST2 (also known as STK4 and STK3), the homologues of the

D. melanogaster Hpo kinase), the large tumor suppressor kinases 1 and 2 (LATS1 and LATS2, Warts in *D. melanogaster*), the adaptor proteins Salvador1, MOB1A/B (SAV1 and Mats in *D. melanogaster)*, the homologous transcriptional co-activators YAP and TAZ (Yorkie in *D. melanogaster)*, and the TEAD transcription factors (TEAD 1-4, Scalloped in *D. melanogaster*) (Fig.1)[9,12,39,40].

Mechanistically, YAP/TAZ in complex with a TEAD transcription factor bind to gene enhancers, interact with chromatin remodelling factors, and modulate RNA Polymerase II (Pol II) to drive or repress the expression of target genes, which prominently include cell cycle, cell migration and cell fate regulators (see also below) [40-48]. Of note, although TEAD transcription factors are required for YAP/TAZ target gene expression, YAP-TEAD complexes alone may not be sufficient to activate the different genetic programs. Indeed, bioinformatics analyses of the regulatory regions that are bound by YAP/TAZ–TEAD complexes identified cooperation between YAP/TEAD and other transcription factors[45,49-52] (Fig. 1). In addition, although TEAD factors are their main interaction partners, YAP/TAZ can interact with other DNA binding transcription factors such as p73[53], RUNX[54], and TBX5[55,56]. Therefore, YAP/TAZ cooperate with various transcription factors to regulate target gene expression.

Activation of MST1/2 induces the phosphorylation of SAV1 and MOB1A/B [57,58], which assist MST1/2 in the recruitment, phosphorylation, and activation of LATS1/2[58-60]. LATS1/2 can also be phosphorylated and activated by the MAP4K1-7 family kinases[61-63]. Subsequently, LATS1/2 phosphorylate YAP and TAZ[32,39,40,46,64-66]. YAP/TAZ phosphorylation by LATS1/2 causes their cytoplasmic sequestration by 14-3-3 proteins and triggers further phosphorylation by Casein kinase 1δ/ε as well as ubiquitylation by the SCF E3 ubiquitin ligase complex and proteasomal degradation[67-70]. Thus, the core Hippo kinases inhibit YAP/TAZ activity and suppress the transcriptional output of the Hippo pathway.

The activity of the Hippo pathway is regulated by a multitude of upstream inputs, many of which relay signals from the plasma membrane[9,12,39]. However, unlike other classical signal transduction pathways, such as the epidermal growth factor (EGF), transforming growth factor-β (TGFβ), or WNT signalling pathways, the Hippo pathway does not appear to have dedicated receptors and extracellular ligands. Rather, the Hippo pathway is regulated by a network of upstream components that have roles in other processes such as the establishment of cell adhesion[71-75], cell morphology[76-78], and cell polarity[79-90](Fig. 1). The activity of the Hippo pathway is thus modulated in response to mechanical strains, changes or defects in cell–cell and cell–extracellular matrix (ECM) adhesion but also nutrient availability[91,92] and other cellular stresses[93,94]. Therefore, the Hippo pathway constitutes a sensor for tissue and cellular integrity rather than responding to dedicated extracellular signalling molecules.

Adherens junctions and tight junctions are major hubs of Hippo pathway regulation[36,60,75,79,81-83,88,90,95-98]. These cell-cell junctions contain protein complexes that establish apico-basal cell polarity, such as the Crumbs and aPKC complex, and they directly link to core components of the Hippo pathway through the angiomotin (AMOT)-family proteins[96,97,99], E-cadherin and its adaptor protein α-catenin that interacts with YAP–14-3-3 complex [73-75], LIM domain proteins that interact with LATS kinases (such as AJUBA)[100,101], and various scaffolding components (such as neurofibromin 2 (NF2; also known as Merlin), kidney and brain protein (KIBRA; also known as WWC1)) that regulate the activity of the core complex[60,84-86,90]. Thus, disruption of cell–cell adhesion can have strong effects on Hippo pathway activity and lead to activation or repression of YAP/TAZ.

The mechanical properties of the extracellular environment and cell shape are other profound regulators of Hippo activity[76-78]. Mechanical stress, such as that caused when cells are grown on stiff surfaces or exposed to fluid shear stress triggers YAP and TAZ nuclear translocation[76-78,102,103], whereas detachment from the ECM causes YAP/TAZ nuclear export[72]. The effects of the

mechanical properties of the ECM on the Hippo pathway are mediated by integrin complexes at cell–ECM adhesion sites and changes in the actomyosin cytoskeleton induced by integrin signalling in response to physical ECM properties [72,76-78,104,105]. Although the exact mechanisms are not known, mechanical forces may further regulate YAP/TAZ through modulating the structure of nuclear pores and hence the nuclear translocation of YAP/TAZ[106].

The Hippo pathway is also modulated by crosstalk with other signalling pathways. In particular these are G-protein coupled receptors (GPCRs) that are activated by lipids (lysophosphatidic acid and sphingosine 1-phosphophate or hormones (glucagon or epinephrine) and signal through F-actin to regulate YAP/TAZ[107,108]; the WNT pathway which regulates YAP/TAZ through direct interaction with the β-catenin destruction complex **[G]**[109] and through destruction complex independent mechanisms[110,111]; Src family kinases and c-Abl kinase that phosphorylate tyrosine residues on YAP and promote YAP nuclear localization and activity in transcription[56,112,113]; and the phosphoinositide 3-kinase (PI3K)pathway [114]. Various other signaling mechanisms that modulate YAP/TAZ localization, degradation, activity, and their ability to interact with TEAD have also been described. Detailed descriptions of these mechanisms can be found in other reviews[9,39,98].

In summary, YAP and TAZ activation–inactivation is a dynamic process that integrates multiple cellular and extracellular signals through a variety of molecular mechanisms. Thus, the Hippo pathway is a complex network of different inputs that offers many potential targets to pharmacologically modulate YAP/TAZ activity.

[H1] Roles in development and regeneration

The Hippo pathway is heralded as a master regulator of organ growth. This is because loss of function of the core kinases or hyperactivation of YAP/TAZ in mice or Yki in developing *D. melanogaster* causes overgrowth of multiple organs such as liver and heart in mice or imaginal discs **[G]** in flies[9-12,39,115-117]. Hippo signalling is often also involved in organ regeneration and tissue

repair where it regulates not only cell proliferation but also other processes that are important for regeneration such as cell survival, cellular dedifferentiation, and expansion of progenitor cell and stem cell compartments (Table 1)[9,11,12,39,115-117]. Unfortunately, these downstream effects are also hallmarks of cancer and YAP/TAZ are indeed often hyperactivated in different types of human cancers where they contribute to tumour development[20,21].

[H2] YAP/TAZ in growth control and development

The functions of the Hippo pathway in organ growth are exemplified in studies where YAP/TAZ were hyperactivated by loss of function mutations in the core kinases or overexpression of constitutive active phosphosite-mutant YAP or TAZ where the major LATS phosphorylation site, a serine residue, is mutated to an alanine (S127 in human YAP and S109 in mouse YAP, referred as YAP-1SA) (Fig. 2). Conditional deletion of the MST1/2 kinases or YAP-1SA overexpression during mouse embryogenesis caused extensive liver and heart overgrowth by driving the proliferation of hepatocytes and cardiomyocytes beyond normal organ size[23,31,32,34-38,118-120]. In adult mice YAP hyperactivation caused overgrowth of the liver but not the heart[15,31,32]. In the adult intestine, lung, and skin, hyperactivation of YAP triggered stem cell hyperproliferation and blocked terminal differentiation (Table 1) [31,73,121-123]. These results thus show that the activity of the Hippo core kinases is normally required to restrict YAP/TAZ activity and to prevent overgrowth and ectopic cell proliferation. On the other hand, the YAP/TAZ gain-of-function phenotypes demonstrate the power of YAP/TAZ to trigger cell proliferation and to promote cell stemness (Table 1).

In contrast to the dramatic overgrowth phenotypes caused by YAP/TAZ gain of function in a number of organs, requirements for *Yap/Taz* for normal growth are surprisingly limited. During development, YAP and TAZ have partially redundant functions and are essential for a variety of processes that include growth and proliferation but also cell type specification and differentiation (Table 1). Homozygous *Yap* mutant mouse embryos arrest development around E8.5 and have defects in trophectoderm **[G]** specification, yolk sac vascularization, body axis extension, and

neuroepithelium formation[124]; homozygous *Taz* mutant mice are viable although some adults suffer from pulmonary and kidney disease[125-127]. *Yap* and *Taz* double-null embryos die prior to the morula stage, suggesting functional redundancy of these transcription co-activators during early embryonic development[128]. Conditional deletion of *Yap* and *Taz* revealed prominent roles in many different tissues and developmental stages. For example, in the developing cardiovascular system, deletion of *Yap* in the embryonic heart impeded cardiomyocyte proliferation and caused myocardial hypoplasia[17,129], and deletion of *Yap* and *Taz* in endothelial cells caused defects in sprouting angiogenesis resulting in impaired vascularization and embryonic lethality[130,131]. Similarly, deletion of *Taz* in the lung epithelium resulted in hypoplastic lung tissue and disruption of branching morphogenesis **[G]**, and *Yap* deletion resulted in loss of basal airway stem cells, and lung hypoplasia[121,125,126,132]. Single deletion of *Taz* or double deletion of *Yap* and *Taz* in the ureteric bud led to cyst-like branching during kidney development[125,127,133]. Notably, deletion of *Yap* and *Taz* was inconsequential for the development and homeostasis in the adult intestine, even though YAP expression is enriched in the stem cell compartment located at the base of normal intestinal crypts **[G]** [109,134,135]. Simiarly, deletion of *Yap* and *Taz* during liver development did not affect adult liver size but caused defects in bile duct differentiation, where YAP is normally expressed at high levels[19,36,136].

Altogether, the *Yap*/*Taz* loss-of-function phenotypes demonstrate that YAP and TAZ have pleiotropic and tissue specific functions. They support and promote growth of some organs, but this role is not ubiquitous.

[H2] YAP/TAZ directly control cell cycle progression and cell survival genes.

YAP/TAZ can promote cell proliferation in different cell types in vivo and in vitro. Chromatin immunoprecipitation and RNA sequencing data showed that YAP/TAZ directly regulate the expression of genes involved in cell cycle progression such as components of the DNA replication, mitosis, cell growth, and DNA repair machineries (Fig. 1; Table 1)[43,45,137-139]. In addition, YAP/TAZ

promote cell survival by upregulating the expression of several inhibitors of apoptosis, including members of the BCL-2 (B cell lymphoma 2) and IAP (inhibitor of apoptosis) families, such as *survivin*, *cIAP1* and *MCL1*[32,43] (Table 1). Thus, YAP and TAZ promote an increase in cell numbers by driving cell proliferation and suppressing apoptosis. YAP/TAZ also regulate various aspects of cellular metabolism to promote cell proliferation[39].

[H2] YAP promotes dedifferentiation and stemness.

Accumulating evidence indicates that YAP/TAZ can promote pluripotency programmes (Fig. 1; Table 1). Genome-wide analysis of YAP/TAZ-binding targets revealed that they bind to the promoters and drive the expression of genes important for stem cell potency[140]. Among these target genes are the four Yamanaka pluripotency factors *SOX2*, *NANOG*, *OCT4*, and *MYC*, and several components of the Polycomb group (PcG) proteins **[G]**[140]. YAP expression is indeed enriched in diverse stem cell populations in vivo and in vitro[11]. In human embryos, for instance, YAP is localized to the nucleus of inner cell mass cells of the developing blastocysts[141], which contains the embryonic stem (ES) cells. Surprisingly, however, YAP localized to the nucleus of trophoblast **[G]** cells and not in the inner cell mass of the mouse embryo[128]. The trophoblast, however, is also composed of stem cells which give rise to extraembryonic tissues[142]. Human and mouse embryonic stem cells cultured in non-differentiating conditions had high levels of YAP and endogenous YAP was re-activated in somatic cells during reprogramming into inducible pluripotent stem (iPS) cells[140]. In contrast, YAP levels were downregulated during differentiation[140], and LATS2 levels increased in differentiating mouse ES cells, primordial germ cells, and iPS cells[143]. However, while YAP and TAZ were essential for maintenance of ES cell stemness in some conditions[140], others reported that they are dispensable indicating that YAP and TAZ are not absolutely required for ES cell fate and that their requirement may depend on tissue and culture conditions[109,144].

Experimental hyperactivation of YAP or TAZ expanded stem cell compartments, induced cell dedifferentiation, and potentiated cell reprogramming into stem or progenitor cells. In vitro,

overexpression of YAP or deletion of the MST kinases allowed the expansion of naive pluripotent mouse ES cells even in pro-differentiation conditions[140,145]. Furthermore, knockdown of LATS2 or overexpression of YAP accelerated the reprogramming of human fibroblasts into iPS cells[140,143], and overexpression of YAP reprogrammed somatic cells in vitro and allowed the formation of different types of tissue specific stem cells[146]. In vivo, activation of YAP induced expansion of existing stem cell compartments in the skin[73,122,147,148], dental epithelium[149], embryonic brain[150], intestine[31,134], lung[121], and trachea[151]. Importantly, YAP hyperactivation could reprogram somatic cells in organs that lack resident stem cells, such as in the liver where YAP overexpression transdifferentiated mature hepatocytes into ductal/progenitor cells[136,152]. Thus, YAP functions as a reprogramming factor and its activation is sufficient to overrule differentiation-inducing cues in vitro and in vivo.

[H2] Requirement for YAP/TAZ in regeneration.

Although YAP and TAZ play pivotal roles during development, they are largely dispensable for homeostasis of many adult organs. However, YAP and TAZ activity is important for regeneration of multiple tissues in adults. In the adult mouse liver YAP is expressed in bile duct cells and endothelial cells, but its expression is low or absent in hepatocytes[36,136]. Upon liver injury after partial hepatectomy, YAP was activated in regenerating hepatocytes and repressed again once regeneration was completed[153]. Deletion of *Yap* and *Taz* in the liver delayed, but did not prevent, liver regeneration after partial hepatectomy due to reduced hepatocyte proliferation[19]. Similarly, YAP was activated after bile duct ligation, a model for cholestatic liver injury, and deletion of *Yap* compromised hepatocyte and bile duct cell proliferation by augmenting hepatocyte necrosis[154]. Thus, YAP and TAZ are not required for hepatocyte maintenance but are activated during and contribute to liver regeneration.

The level and activity of YAP also change dynamically from homeostasis to injury and regeneration in the intestine, skin, and heart. Ionizing radiation or administration of dextran sulphate sodium (DSS) salts caused injury to the intestinal epithelium and induced an initial reduction of YAP levels

during the injury phase[155]. Upon stopping DSS administration, however, the intestine initiated regeneration and the levels of YAP dramatically increased, exceeding the levels observed during homeostasis[134,155,156]. Deletion of *Yap* in the intestinal epithelium impaired crypt cell proliferation and regeneration in a number of reports[134,135,155], but others found that *Yap* deletion accelerated crypt cell proliferation after ionizing radiation[156]. The reason for this discrepancy is currently unknown. In the skin, depletion of *Yap* and *Taz* by siRNA-mediated knockdown or by Cre mediated KO slightly reduced proliferation of basal epithelial cells and delayed wound closure in mice[122,148,157]. Finally, nuclear YAP was absent in normal adult cardiomyocytes, but enriched in epicardium and myocardium of infarcted hearts[158,159]. Cardiomyocyte-specific deletion of Yap impaired neonatal heart regeneration and resulted in fibrotic scaring after myocardial infarction[160].

Althogether, these results indicate that YAP/TAZ are specifically activated and contribute to regeneration but not to homeostasis in the intestine, liver, and heart, whereas in the skin YAP/TAZ contribute to homeostasis and regeneration. Thus, YAP and TAZ are generally activated during organ regeneration, but their specific functions may differ in different organs.

***[H1]* Organ regeneration by YAP activation**

Most adult organs have a limited ability to regenerate properly patterned and functional organs after injury, but recent experiments in mice show that ectopic YAP activation can promote regeneration of several organs (Figs. 2-4).

[H2] YAP activation promotes heart regeneration.

Proliferation of mammalian cardiomyocytes has long been thought to be restricted to embryonic stages, with little or no capacity to regenerate after birth[161](Fig. 2). However, it was recently discovered that not only embryonic but also neonatal mouse hearts can regenerate heart muscle damage after myocardial infarct, although this capability progressively declines and is lost in 7-day old animals[161](Fig. 2a). This discovery put forward the idea that reactivating embryonic and

297 neonatal pathways of cell proliferation and organ growth may trigger adult cardiomyocytes to
298 proliferate and regenerate an injured heart.
299
300 Recent findings pointed to YAP as a prime candidate to stimulate heart regeneration in adults. The
301 idea of activating YAP comes from studies in mice showing that cardiomyocyte-specific
302 hyperactivation of YAP by overexpression of a constitutive active YAP-1SA or by conditional
303 deletion of *Sav1,* a upstream negative regulator of YAP/TAZ, caused increased proliferation of
304 embryonic cardiomyocytes[13,17,120,160]. Indeed, such hyperactivation of YAP starting in embryonic
305 cardiomyocytes caused continuous proliferation of cardiomyocytes in adults and enabled heart
306 regeneration and restored heart function after infarction (Fig. 4). However, these experiments
307 induced adult heart regeneration by prolonging the embryonic capability of cardiomyocyte
308 proliferation into adults, which is of course not a clinically relevant strategy. Therefore, can acute
309 activation of YAP provoke heart regeneration in adults?
310
311 To test whether acute activation of YAP stimulates heart regeneration in a more therapeutic
312 setting, YAP was activated after infarction in adults mice[13]. Remarkably, activation of YAP by
313 conditionally deleting *Sav1* or *Lats1/2* or by using an adeno-associated virus subtype 9 (AAV9)
314 expressing human YAP-1SA or *Sav1 siRNA* (Table 2) after heart infarction triggered heart repair,
315 improved cardiac function, and increased mouse survival[13,15,162]. This was possible even though the
316 extent of cardiomyocyte proliferation was 20-fold less than the proliferation induced in wild-type
317 regenerating neonatal cardiomyocytes. Notably, long term overexpression (four and a half
318 months) of YAP-1SA induced by AAV9 transduction into adult hearts did not induce cardiac
319 hypertrophy, compromise gross heart architecture, or induce tumour formation in the heart[15] (see
320 section Risks of YAP/TAZ activation below for more discussion).
321
322 Artificial activation of YAP was also beneficial in another mouse model for heart disease, namely
323 muscular dystrophy. There, YAP activation ameliorated the symptoms of dilated cardiomyopathy

in dystrophin **[G]** loss-of-function hearts (Mdx mice), a developmental model for Duchenne muscular dystrophy **[G]** [16]. *Mdx* mutant mice showed decreased heart function and increased fibrosis, but *Sav1*;*Mdx* double mutant hearts showed less severe dilation, reduced fibrosis, and maintained cardiac function in adults[16]. These studies thus suggest that YAP activation can awaken the regeneration potential of post-mitotic cardiomyocytes in mice and may be used to treat a number of human heart maladies.

Several lines of evidence indicate that YAP drives the embryonic programme of heart growth and that its artificial activation can partially reactivate this program in adult cardiomyocytes. As mentioned above, YAP is required for heart development[17] and neonatal heart regeneration[160]. YAP activity is then repressed a few days after birth by an increase in the activity of Hippo pathway kinases, coincident with the loss of regenerative capacity (Fig. 2)[13]. Experimental YAP activation in adult cardiomyocytes upregulated genes such as *ACTA1* (encoding smooth muscle α-actin), *CTNNB1* (encoding β-catenin), *Snai2, and Sox2,* which are only expressed in foetal hearts, during neonatal cardiac repair or in cardiomyocyte reprogramming[14,120]. In addition, the insulin-like growth factor and WNT signalling pathways, which are active in embryonic hearts, are reactivated by YAP-1SA overexpression in the infarcted adult heart[129]. In addition, YAP activation in the heart of transgenic mice caused an increase in the number of small and mononuclear cardiomyocytes, which resemble the immature cardiomyocytes found during early development[13].

In conclusion, YAP activation in adult cardiomyocytes does not only trigger ectopic proliferation but induces cardiomyocyte reprogramming and rejuvenation. However, whether these effects are translated to humans is currently not known. Also, our understanding of how YAP aids heart regeneration is still rudimentary, and further research is required to reveal the therapeutic potential and risks of activating YAP in the infarcted human heart.

[H2] YAP activation and liver regeneration.

While young and healthy livers have a supreme regenerative capacity, old and chronically diseased livers affected by diabetes, infection, alcoholic liver disease, and cholestasis cannot regenerate efficiently[163-166]. Thus, being able to counteract the effects of chronic liver disease and induce liver regeneration has great clinical value.

Like in the heart, YAP is also a prime candidate to activate an endogenous regeneration programme in the liver. As discussed above, YAP is activated and required in regenerating livers and experimental activation of YAP enhanced liver regeneration in mice[18,154,167]. Knock down of MST1/2 by liposomal vesicles **[G]** containing siRNAs targeting MST1/2 (Table 2) triggered hepatocyte proliferation and accelerated liver regeneration after partial hepatectomy in young and old mice[18,19,167]. Similarly, pharmacological activation of YAP using an MST1/2 inhibitor called XMU-MP-1 (Table 2) augmented liver repair and reduced liver fibrosis after acute and chronic injury induced by paracetamol and carbon tetrachloride intoxication or by bile duct ligation[167]. Notably, even though mice were treated daily with XMU-MP-1 for 1 week after paracetamol-induced injury, they did not develop cancerous phenotypes for at least 10 months after initiating the treatment[167]. Thus, YAP seems to be a driving force that enhances the regeneration capacity of the young and healthy liver in mice and suggest that therapeutic activation of YAP may improve regeneration in diseased and aged livers in humans.

Overexpression of activated YAP in adult mouse hepatocytes triggered their transdifferentiation into progenitor-like cells that could differentiate into biliary epithelial cells **[G]** or re-differentiate into hepatocytes[136]. Cells with progenitor cell-like traits and elevated levels of YAP expression were also found in humans, where cholestatic livers show YAP upregulation in ductular reactions **[G]** [154,168]. In summary, ectopic activation of YAP may promote liver regeneration by activating two distinct cellular mechanisms: hepatocyte proliferation and transdifferentiation into stem-cell-like progenitor cells.

[H2] The Hippo pathway in the regulation of intestinal stem cells.

The healthy intestine is an organ that has an enormous regenerative capacity owing to the presence of a designated stem cell compartment, but fails to efficiently regenerate when affected by disease, such as ulcerative colitis **[G]** and Crohn's disease **[G]** [169]. Interestingly, the intestine is also highly sensitive to Hippo pathway manipulations: gain of YAP/TAZ activity by YAP-1SA overexpression or by conditional deletion of *Mst1/2* or *Sav1* in adult mice led to extensive expansion of undifferentiated intestinal progenitor cells and intestinal crypt hyperplasia (Fig. 3A)[31,32,38,134]. Thus, YAP activity also affects cell proliferation and stemness in the intestine, suggesting that its hyperactivation, if controlled, might have beneficial effects in promoting intestinal regeneration. Indeed, artificial YAP hyperactivation by administering the MST1/2 inhibitor XMU- MP-1 in a mouse model of colitis, markedly suppressed the colitis symptoms [167]. Notably, even though mice were treated daily with XMU-MP-1 for 1 week after DSS-induced injury, they did not develop cancerous phenotypes for at least 10 months after initiating the treatment[167]. Thus, inhibition of MST kinases or direct activation of YAP may be used to restore the regeneration potential of injured intestinal epithelia.

How does YAP activate regeneration in the intestine? As mentioned above, YAP is enriched in the stem cell compartment (characterized by the expression of LGR5) of the intestinal crypt during homeostasis and is highly upregulated throughout the intestinal epithelium during regeneration[134]. Surprisingly, however, rather than simply promoting stem cell proliferation during regeneration, the activation of YAP temporarily supressed the normal homeostatic programme of intestinal stem cells and drove a more embryonic stem cell programme instead. The WNT signalling and the adult stem cell programme were suppressed, while foetal markers such as *AnxA1*, *Trop2* and *Sca1* were induced[135,155]. Thus, YAP activation rejuvenated the intestinal epithelium by inducing embryonic cell phenotypes and the expression of a more primitive stem cell programme. The induction of these embryonic phenotypes are transient and adult cell fates are restored once the injury is resolved.

[H2] YAP/TAZ in skin and wound healing.

The skin epithelium is constantly being renewed by a population of basal layer stem cells and has a tremendous potential to heal wounds, although this progressively declines with age[170]. In young mice, YAP expression is high in the basal layer and hair follicle of the skin, two compartments that harbor stem cells (Fig. 3b), but YAP levels decrease with age in correlation with the decline in proliferative potential of basal layer stem cells[122]. Deletion of *Yap* and *Taz* in the skin of adult mice slightly impaired proliferation of basal layer cells, and caused hair loss[157].

Upon wounding, YAP and TAZ are activated and translocate to the nucleus of differentiated epidermal cells and promote their proliferation[157]. Downregulation of *Yap* by siRNA-mediated knockdown[148] or double conditional knockout of *Yap* and *Taz*[157] in adult mice reduced the proliferation rate of skin epithelial cells and delayed skin wound closure in mice. Conversely, conditional hyperactivation of YAP in basal cells in embryos and new born pups induced expansion of the basal cell compartment by promoting proliferation of basal cells and inhibiting terminal differentiation[122,147]. Accordingly, ectopic YAP activation in the developing skin caused a decrease in the expression of differentiation markers (*Krt1*, *Ivl*, *Lor*) and upregulation of progenitor cell markers[74]. Thus, YAP and TAZ are required for skin homeostasis by maintaining proper levels of proliferation of basal progenitor cells, which need to be expanded during regeneration. However, whether ectopic activation of YAP can accelerate wound healing and skin regeneration in mice or in humans has not been reported.

In conclusion, the induction of tissue repair and regeneration mediated by the activation of YAP/TAZ in different tissues of animal models suggest that YAP/TAZ activation may also aid in the regeneration of injured organs in humans, particularly, the heart. However, whether and how YAP/TAZ can be activated in a safe and effective in human patients still needs to be investigated.

[H1] Risks of YAP activation

While the idea of activating YAP to promote tissue regeneration is exciting, it also raises concerns regarding safety. This is because sustained activation of YAP in adult mice can result in aberrant cell proliferation, tissue fibrosis, and tumorigenesis (Fig. 5)[12,20,21,171]. Surprisingly, however, while ectopic activation of YAP is sufficient to induce hyperplasia in many organs, only a few organs develop tumours after YAP activation in adult mice[20]. Indeed, hyperactivation of YAP/TAZ alone is insufficient to trigger tumour formation in pancreas, heart, lung, mammary gland, and nervous system in genetically manipulated mice[73,120,172-175]. Even in organs where YAP activation induces cancer, not all YAP-activated cells initiate tumour formation. For example, only a few tumour nodules develop in livers with ubiquitous YAP activation throughout the parenchyma **[G]** [33-37]. This indicates that YAP alone is not sufficient to induce tumour formation and that cooperation with other pro-tumorigenic events is required for cancer development.

[H2] YAP activation contributes to cancer development in humans.

Analysis of YAP/TAZ function in human cancer cells in vitro and in vivo by xenotransplants into mice showed that YAP/TAZ regulate many of the hallmarks of cancer, including the promotion of cancer cell proliferation, cancer stem cell fate, chemoresistance, and metastasis[12,20,21]. In line with this, elevated levels and nuclear localization of YAP are observed with appreciable frequency in many human cancers, such as liver[176-180], lung[181-186], breast[187,188], skin[74] and colorectal cancer[189,190] where they are correlated with poor prognosis.

Interestingly, the activation of YAP or TAZ in the vast majority of these cancers is not associated with mutations in currently known Hippo pathway components[12,20,21], except for some oral cancers[191], hepatocellular carcinomas[192], uveal melanoma[193,194], mesotheliomas **[G]** [195], neurofibrosarcomas **[G]** [196], pancreatic ductal adenocarcinoma[197], and schwannomas **[G]** [198] that can have genomic amplifications of YAP or TAZ and somatic mutations in components of the Hippo pathway. Thus, although YAP is highly upregulated in many human cancers, there is no strong

selective pressure for mutations in Hippo pathway components and the activation of YAP is likely due to epigenetic events (such as DNA methylation) and/or defects in the many molecular mechanisms that regulate the activity of the Hippo pathway.

[H2] Pro-tumorigenic activity of YAP/TAZ requires other oncogenic events.

In the mouse liver, tissue-wide overexpression of YAP-1SA or deletion of *Mst1/2*, *Sav1*, *Nf2*, or *Mob1A/B*, caused increased liver size and eventually lead to the development of liver tumours that resembled human mixed HCC/ICC **[G]** [31,32,34,35]. In addition, ectopic expression of a constitutively active version of YAP where all five Lats phosphorylation sites have been mutated (YAP5SA) mice triggered liver tumorigenesis in adult even when YAP was expressed only in a subpopulation of sparse hepatocytes [199]. Thus, YAP is a powerful driver of hepatocyte proliferation and its uncontrolled hyperactivation eventually culminates in the formation of liver tumours. However, only a few YAP expressing cells transformed into tumour initiating cells, suggesting that YAP must cooperate with other tumour inducing stimuli to trigger tumorigenesis, at least in the time frame of the life span of a mouse.

Activated YAP can synergize with oncogenic mutations to trigger tumorigenesis or increase cancer cell malignancy. In the lung, activation of YAP was not sufficient to trigger tumour formation and caused only hyperplasia[200]. However, YAP activation promoted progression of small adenomas to high-grade lung adenocarcinomas when it was combined with overexpression of an oncogenic mutant of the *Kras* gene (*Kras*^{G12D}) that activates the RAF-MAPKinase pathway [200]. Another example is the mammary gland where YAP overexpression alone impaired terminal differentiation of secretory cells during lactation but did not induce tumour formation[172]. However, YAP/TAZ overexpression transformed benign human and mouse breast cancer cells into high-grade and metastatic tumours[187,188,201]. Similarly, TAZ overexpression in normal brain cells was insufficient to induce glioma formation but increased the malignancy of such tumours[202].

YAP can also synergize with injury and inflammation to trigger tumour initiation. Chronic injury and inflammation can potentiate the neoplastic transformation of proliferative cells because of the abundance of growth factors, activated stroma, and DNA damage promoting agents in the inflammatory milleu[203]. Indeed, artificial YAP activation synergizes with tissue injury and inflammation to promote cancer cell hallmarks in the liver and intestine[204,109,134]. Single hepatocytes expressing YAP-1SA were eliminated and did not expand clonally, unless hepatotoxins or the inflammatory cytokine IL-6 were provided[204] . Thus, YAP-1SA overexpressing hepatocytes only survived and hyperproliferated when YAP expression was combined with liver injury that caused inflammation. In the colon, sustained overexpression of YAP-1SA or deletion of *Sav1* only caused sporadic formation of benign adenomas (Fig. 5)[109,134], but combining *Sav1* deletion with acute injury caused by DSS resulted in the development of multiple neoplastic colonic polyps prone to transform into invasive adenocarcinomas [134]. Similarly, in the skin, activation of YAP in basal stem cells induced cell proliferation but did not cause cancer[73]. However, YAP overexpressing skin cells produced tumours resembling squamous cell carcinoma after they were transplanted onto immunocompromised mice — a procedure that causes inflammation and activates a wound healing response[73]. Thus, ectopic YAP activity alone potently induces hyperplastic growth but is inefficient in causing cancer in several organs. However, the ectopic activation of YAP can synergize with pro-inflammatory cues to cause tumorous growth in injured organs.

In conclusion, sustained YAP activation can induce tumour formation in cells which are facing different environmental stresses, such as inflammation, or that already have premalignant oncogenic mutations, such as in *RAS*. This is likely because YAP can induce ectopic cell proliferation, confer cancer stem cell traits, and induce metastatic behaviour of normal cells within damaged or diseased organs, thus increasing the risk of malignant transformation. However, the tissue overgrowth and tumour formation resulting from transient activation of YAP are often reversible upon cessation of YAP activation[31,32]. Thus, although the activation of YAP can trigger undesired

side effects, there might be a therapeutic window of YAP activation that can be used to induce organ regeneration while avoiding excessive organ growth or tumorigenesis.

[H2] YAP/TAZ activation promotes tissue fibrosis.

YAP/TAZ activation can promote tissue fibrosis by regulating the activation of myofibroblasts. Human and mouse myofibroblasts and stellate cells **[G]** showed prominent nuclear YAP accumulation in fibrotic lungs, kidneys, and livers[171,205,206]. In mouse models of liver and renal fibrosis, administration of carbon tetrachloride or unilateral ureteral obstruction resulted in rapid cytoplasmic to nuclear translocation of YAP and induction of YAP target genes in hepatic stellate cells and renal fibroblast[171,206]. Injection of human fibroblasts that overexpressed constitutive active versions of YAP or TAZ into immunocompromised mice caused accumulation of ECM components and lung fibrosis[205]. Conversely, inhibition of *Yap* expression in hepatic stellate cells or in renal fibroblast impeded fibrogenesis in livers and kidneys, indicating that YAP activation is essential for myofibroblast activation and fibrosis [171,206]. Thus, because YAP/TAZ drive stellate cell and myofibroblast activation and tissue fibrosis in different organs, therapeutic activation of YAP/TAZ for regenerative medicine could be hampered by the induction of fibrosis.

[H1] Approaches to activate YAP/TAZ

The power of YAP/TAZ to provoke regeneration opens new opportunities for clinical applications in regenerative medicine. However, it is not clear how YAP/TAZ action can be harnessed therapeutically in a way that avoids its deleterious side effects (Table 2). In the following sections we provide an overview of different strategies that may help accomplish using Hippo pathway modulations to aid regenerative medicine.

[H2] Transient YAP/TAZ activation and the reversibility of YAP/TAZ driven phenotypes.

Although organ overgrowth and tumorigenesis can arise after sustained YAP/TAZ hyperactivation, short-term activation of YAP/TAZ may avoid these problems. In the liver of adult mice, for example,

539 doxycylin-inducible YAP-1SA overexpression caused massive hepatomegaly with a 5-fold increase
540 in liver size after 4 weeks of induction[31,32]. Strikingly, however, termination of YAP overexpression
541 by withdrawing doxycycline induced cell death in these enlarged livers, which returned to near
542 normal size in only two weeks[31,32,207]. Furthermore, YAP downregulation, induced by liposome
543 encapsulated siRNA (Table 2) in MST1/2 mutant livers, caused regression of hepatic tumours
544 associated with long term YAP hyperactivation and reactivated a hepatocyte differentiation
545 signature[152]. Thus, YAP induced liver overgrowth is not permanent and can be reverted upon YAP
546 inactivation.
547
548 YAP-driven hyperplasia and cell dedifferentiation phenotypes are also reversible in other organs.
549 Halting YAP-1SA overexpression restored normal intestinal structure and led to the rapid
550 reappearance of differentiated enterocytes **[G]**, goblet cells **[G]** and Paneth cells **[G]** at the expense
551 of stem and progenitor cells induced by YAP overactivation (Fig. 3a) [31]. Similar observations were
552 also made in the skeletal muscle. Here, transient hyperactivation of YAP-1SA induced regenerative
553 myogenesis and cell dedifferentiation characterized by the induction of regenerative myogenesis
554 markers [208], which, unexpectedly, was followed by muscle degeneration(Fig. 5)[208]. Nonetheless,
555 the muscle atrophy and deterioration phenotype was largely reversible upon cessation of YAP-1SA
556 expression [208]. These observations suggest that halting YAP activation after a desired therapeutic
557 time window required to induce regenerative programmes, may avoid or revert many side effects
558 observed after long-term YAP activation (Fig. 5). However, additional investigations need to test
559 the reversibility of abnormal growths and carcinogenesis induced by YAP hyperactivation,
560 especially in unhealthy tissues affected by oncogenic mutations, inflammation, or fibrosis.
561
562 ***[H2] Hypomorphic deregulation of Hippo signaling.***
563 In addition to temporally restricted activation, hypomorphic (partial) activation of YAP/TAZ may be
564 sufficient to drive tissue regeneration while minimizing adverse side effects. Deletion of different
565 upstream Hippo regulators resulted in different strengths of YAP/TAZ activation[209]. Deletion of

LATS1/2 resulted in the strongest activation of YAP owing to the complete inability to phosphorylate YAP/TAZ[209]. MST1/2 deletion caused moderate activation of YAP owing to a decrease in the activation of LATS1/2, whereas deletion of SAV1 resulted in an even weaker activation of YAP because the lack of SAV1 only reduces MST1/2 activity. Accordingly, in the heart, for example, the number of dividing cells was larger in *Lats1/2* mutant hearts compared to *Sav1* mutants[13], but *Sav1* mutant cardiomyocytes still re-entered the cell cycle and were able to promote heart regeneration in adult mice[13]. Thus, YAP can be activated to different strengths depending on which upstream regulator is targeted by a therapeutic agent. One good example is the MST1/2 inhibitor XMU-MP-1 (Table 2) whose administration increased YAP activity enough to promote organ repair, but caused only mild liver and intestinal overgrowth phenotypes, much weaker than those caused by genetic deletion of *Mst1/2*[167]. Similarly, knockdown of MST1/2 by liposome encapsulated siRNAs (Table 2) only partially activated YAP, but stimulated liver regeneration[18]. Overall, partial activation of YAP/TAZ might be sufficient to reach therapeutic efficacy without causing adverse effects such as tissue overgrowth and tumorigenesis.

[H2] Tissue-specific activation of YAP/TAZ.

An additional possibility to avoid adverse side effects is to activate YAP/TAZ only in the tissue or cell type of interest. In the heart, for example, an adeno-associated virus serotype 9 (AAV9) (Table 2) was used to express human YAP-1SA to stimulate cardiomyocyte proliferation and heart regeneration in adult mice after myocardial infarction[15]. Notably, overexpression of YAP-1SA in the adult heart did not induce tumour formation or other overt phenotypes, suggesting that the use of AAV9-YAP-1SA may be a safe strategy to activate YAP in the heart[15]. Notably, different AAV vectors and serotypes **[G]** have been approved for use in humans and can be used to deliver genes to different cell types[210,211,212].

Another layer to control YAP/TAZ expression is the use of AAV vectors in combination with inducible expression systems, such as a doxycycline inducible system or laser directed activation

using optogenetics **[G]** . This combinatorial approach could take advantage of the tissue specificity of AAV infection and the inducibility of a conditional expression system[213]. Optogenetic techniques, in particular, may allow very accurate control of gene expression in a spatially precise and minimally invasive manner[214]. Such transgenic approaches, however, would still need to consider potential immune responses against the virus and cells expressing foreign proteins before being applied to any clinical setting.

A different approach that does not involve gene therapy or the use of viral vectors is to simply inject recombinant proteins, such as Hippo pathway regulators, directly into the organ of interest (Table 2). Using this approach, intramyocardial injection of recombinant agrin, a component of the neonatal ECM, activated YAP, promoted cardiomyocyte proliferation and cardiac regeneration after myocardial infarction in juvenile and adult murine hearts[215]. Mechanistically, in the muscle tissue, YAP is sequestered out of the nucleus by dystrophin-associated glycoproteins and agrin activates YAP by inducing the disassembly of the dystrophin–glycoprotein complex and the release of YAP [215]. It remains unknown, however, whether agrin has myocardial specificity for YAP activation.

In summary, current technologies already offer a number of options to induce transient and tissue specific expression of YAP/TAZ. Yet, the therapeutic value for the use of YAP/TAZ activation to induce regeneration will depend on developing additional layers of safety measures to control the regenerative potential and adverse effects of these transcription co-activators.

[H2] Activation of select YAP/TAZ target genes.

Activation of YAP/TAZ target genes may be sufficient to mimic the stimulation of regeneration by these transcription factors. *CYR61* and *CTGF*, for instance, are classic YAP/TAZ target genes that belong to a family of secreted cysteine-rich proteins and regulate diverse biological processes, such as cell migration, cell proliferation, and cell adhesion. In lower vertebrates, such as zebrafish, spinal

cord regeneration depends on local secretion of endogenous CTGF and can be enhanced by local delivery of human CTGF recombinant protein[216]. Notably, the beneficial effects of CTGF are not restricted to lower vertebrates, as the delivery of CTGF or transplantation of CTGF overexpressing mesenchymal stem cells augmented the recovery of osteochondral defects **[G]** produced by ligament injury in rabbits [217,218]. Similarly, overexpression of *Cyr61* in hepatocytes or administration of purified CYR61 protein accelerated resolution of injury-induced fibrosis in mice [219]. Administration of purified CCN1 protein also accelerated intestinal epithelial regeneration in a mouse model of colitis[220]. This suggests that CTGF and probably the products of other YAP target genes may be used instead of YAP/TAZ to therapeutically stimulate regeneration. Thus, identification of which YAP/TAZ target genes are the drivers of regeneration may allow the development of therapeutic strategies that avoid the tumorigenic potential of YAP/TAZ.

[H1] Conclusions and perspective

The research thus far shows that YAP/TAZ activation can stimulate stem cell mobilization, induce cell proliferation, and accelerate tissue repair in several organs, including those that are not able to efficiently regenerate. Although these findings are exciting, using YAP/TAZ for regenerative medicine in humans is still far from realization. However, the pioneering mouse studies discussed in this Review provide the groundwork for future research in YAP/TAZ as therapeutic targets to unleash the regenerative potential of non-regenerating tissues and organs. Notably, some organs such as the heart are inherently resistant to cancer formation and may represent ideal targets to explore the use of YAP/TAZ activation to promote tissue regeneration. In other organs, however, sustained YAP/TAZ activity can impair organ function by causing tissue degeneration, hypertrophy or triggering tumour formation. Further studies, thus, need to focus on better understanding tissue-specific effects of YAP/TAZ overactivation and on identifying ways to safely activate YAP/TAZ in different contexts.

There are still crucial open questions that remain to be answered before we can exploit the potential of YAP/TAZ for regenerative medicine. First, because current evidence of YAP/TAZ activation as a cue for regeneration comes from studies of mice and cultured cells, at the moment it is not clear whether primary human cells and organs can respond to YAP/TAZ activation in the same way as cells from current models. Second, it is still necessary to evaluate any potential deleterious effects that could arise in response to transient activation of YAP/TAZ in human tissues and to evaluate their impact on human health. Finally, there are a number or questions open with respect to the molecular mechanisms of tissue regeneration and the role of YAP/TAZ in this process. It remains unknown what triggers the activation of YAP/TAZ in tissues with regenerative potential and why such mechanisms do not take place in organs with no or low regeneration potential. Furthermore, we have only a rudimentary understanding of how YAP/TAZ promote regeneration. Thus, better understanding of genes and processes under their control can inform the development of new approaches for regenerative medicine that are alternative to YAP/TAZ activation, thereby, possibly avoiding the deleterious effects of YAP/TAZ overactivity.

Overall, given the vast potential of YAP/TAZ as promoters of tissue regeneration that emerges from recent studies, we can now look forward to exciting fundamental and translational research on Hippo–YAP/TAZ signalling to pave the way for new clinical approaches in regenerative medicine.

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Figure Legends

Figure 1. Hippo signalling pathway components and regulation

a. To date over thirty components that comprise the Hippo pathway have been identified, but this schematic focuses on the main aspects of the Hippo pathway. The core of the Hippo pathway is defined by an evolutionary conserved kinase cascade composed of the Ste20-like kinases 1 and 2 (MST1 and MST2; Hippo in flies) and the large tumor suppressor kinases 1 and 2 (LATS1 and LATS2 kinases; Warts in flies), their cofactors SAV1 and MOB1A/B, the transcription co-activators YAP and TAZ (Yorkie in flies), and the TEAD1-4 family transcription factors[9,12,39,40]. Activation of the Hippo pathway is associated with the phosphorylation of the core Hippo kinases MST and LATS: MST is autophosphorylated (which is counteracted by STRIPAK–SLMAP protein phosphatase PP2A complex) and subsequently phosphorylates LATS. MST is also activated by TAO kinases, whereas LATS can also be phosphorylated by MAP4 kinases (MAP4Ks). Activation of LATS induces the phosphorylation of YAP and TAZ and inhibits their transcription co-activator function. Phosphorylated YAP/TAZ are exported from the nucleus and degraded in the cytoplasm or sequestered at cellular junctions. When the kinases are not active, YAP/TAZ accumulate in the nucleus, bind to the TEAD transcription factors and promote the expression of target genes. The activity of these core components is regulated by a number of upstream mechanisms that involve: cell junctions through various scaffolding proteins such as angiomotin (AMOT), neurofibromin 2 (NF2; also known as Merlin), kidney and brain protein (KIBRA; also known as WWC1), AJUBA and zonula occludens (ZO) proteins; cell polarity, including Crumbs complex and aPKC–PAR complex; mechanical forces through the actin cytoskeleton and integrin signalling from the extracellular matrix (ECM); and a number of cell surface receptors such as G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs). Hippo also cooperates with WNT signalling: both β-catenin and YAP/TAZ associate with the destruction complex and are targeted for β-TrCP-mediated degradation, and inactivation of the destruction complex upon WNT stimulation drives β-catenin as well as YAP/TAZ nuclear translocation. In addition, metabolic inputs are relayed to Hippo via AMP-activated protein kinase (AMPK). Thus, the Hippo pathway integrates multiple cellular and

extracellular inputs to regulate gene expression and organ growth. **b.** Different transcriptional outputs of YAP/TAZ activity can trigger different cellular processes. Such transcriptional outputs depend on cooperation of YAP/TAZ–TEAD complexes with other transcription factors. CK1, Casein kinase 1δ/ε; GF, growth factor; MARK4, MAP/microtubule affinity-regulating kinase 4; RASSF, Ras association domain family.

Table 1. Cellular responses to YAP activation

YAP/TAZ activation induces the expression of a number of target genes and elicits a variety of cellular responses in a tissue specific manner. The artificial hyperactivation of YAP/TAZ induces increased cell proliferation, cell migration, promotes cell differentiation and stemness, regulates cell fate decisions, induces cell morphology and cytoskeletal changes, and promotes cell survival by counteracting programed cell death.

Figure 2. YAP-mediated heart regeneration

a. Changes of heart regeneration potential and YAP activity during mouse development[13,17]. The potential to repair and regenerate the heart is high in embryos but lost during the first few days after birth[161]. The activity of YAP follows that trend, while the activity of upstream acting large tumor suppressor kinases (LATS1 and LATS2) shows the opposite development. **b.** YAP hyperactivation in adult cardiomyocytes induces the expression of genes associated with an embryonic cardiomyocyte phenotype and increased proliferation [13,17,120,160].

Figure 3. Effects of YAP hyperactivation on regeneration and homeostasis of the intestine and skin a. Although YAP is enriched in crypt stem cells, it is dispensable for intestinal homeostasis. Upon injury, YAP is activated, enters the nucleus (green) and drives the expression of genes associated with a primitive gut stem cell programme that overrules the WNT-driven stem cell programme that occurs in homeostasis and directs low levels of stem cell self-renewal [135,155]. This drives the expansion of the intestinal stem cell (ISC) compartment through increased survival of

ISCs. **b.** YAP is enriched in basal stem cells of the normal skin[9-12]. Upon regeneration, YAP expression is enriched in basal cells, keratinocytes and dermal cells (these cells were marked red). YAP overexpression results in expansion of the basal stem cell compartment, increased proliferation and dedifferentiation of epidermal cells, whereas deletion of *Yap* and *Taz* in mice resulted in thinning of the epidermis and in delayed wound healing.

Figure 4. Effects and benefits of YAP activation in different organs

a. Experimental activation of YAP induces cell proliferation in many organs in a dose dependent manner. In the heart, YAP activation promotes cardiomyocyte proliferation and regeneration after myocardial infarction[13,15,162]. In the liver of old mice, YAP activation restores the regeneration potential after partial hepatectomy (PHx)[18,19,167]. YAP activation in the intestine induces stem cell expansion and regeneration after injury[134,155].

Figure 5. Adverse effects of YAP activation in different organs.

Short and long-term activation of YAP can induce adverse phenotypes[12,20,21,171]. In the liver, sustained activation of YAP by overexpression of constitutive active YAP (YAP-1SA) or by the deletion of upstream Hippo pathway kinases induces overgrowth and eventually hepatocellular carcinoma[33-37]. In the intestine, YAP promotes the expansion of the stem cell compartment resulting in crypt hyperplasia and can result in the formation of benign adenomas if followed by injury[109,134]. In striated muscles, YAP activation triggers inflammation and hypotrophy. In the adult mouse heart, overexpression of YAP-1SA by AAV delivery did not cause overt phenotypes[15].

Table 2 Strategies for therapeutic YAP activation.

Multiple methodologies exist to trigger YAP activation in an organ-specific and transient manner. AAV vectors can drive the expression of YAP in a specific organ or cell type when YAP is expressed under a tissue-specific promoter. Such constructs can be coupled with inducible doxycycline response systems (TetON and variants thereof) or with optogenetics to control the expression or

activation of YAP[213 214]. A different strategy is to deliver extracellular ligands, such as Agrin[215], to activate YAP in the targeted organ or to express CTGF, encoded by a prominent YAP target gene[217,218]. Finally, small molecule inhibitors that target negative regulators of YAP, such as the MST1/2 inhibitor XMU-MP-1, allow hypomorphic (but ubiquitous) activation of YAP[167].

Glossary

***β*-Catenin destruction complex:** β-catenin is degraded by a multiprotein "destruction complex" that includes the tumor suppressors Axin and adenomatous polyposis coli (APC), the Ser/Thr kinases GSK-3 and CK1, protein phosphatase 2A (PP2A), and the E3-ubiquitin ligase β-TrCP. In the absence of Wnt signaling, the complex generates a β-TrCP recognition site by phosphorylation of the β-catenin amino terminus, which targets β-catenin for degradation by the proteasome.

Imaginal discs: A group of undifferentiated cells in an insect larva that will develop into different adult structures such as eyes, antennae, and wings.

Trophectoderm: The trophectoderm or trophoblast is the outer covering of cells that eventually forms the placental interface between mother and offspring.

Branching morphogenesis: Is the growth and branching of epithelial tubules during embryogenesis.

Intestinal crypts: Also known as crypts of Lieberkühn, are glands found in the intestinal epithelium lining the small and large intestine, which contain stem cells and Paneth cells.

Polycomb group (PcG) proteins: A family of chromatin remodelling proteins that induce epigenetic silencing of genes.

Trophoblast: Throphectoderm

Dystrophin: A cytoplasmic component of the dystrophin-associated protein complex in muscle fibres that connects the cytoskeleton to the extracellular matrix.

Duchenne muscular dystrophy: A genetic disorder caused by an absence of dystrophin and characterized by progressive muscle degeneration and weakness.

Liposomal vesicles: Spherical vesicles composed of a bilayer comprising one or more phospholipids and used as vehicles for the administration of nutrients or pharmaceutical drugs.

Biliary epithelial cells: Also known as cholangiocytes, are cuboidal **epithelial cells** that form **bile** ducts in the liver.

Ductular reaction: A pathology associated with as increased number of ductules or fine ramifications of the biliary tree in the liver that are often associated with an injury response.

Ulcerative colitis: is a chronic bowel disease that causes inflammation in the large intestine or colon.

Crohn's disease: Is an inflammatory bowel disease. inflammation can appear anywhere in the digestive tract, from the mouth to the anus.

Parenchyma: Parenchyma is the functional tissue of an organ and does not include any connective or supporting tissue.

Mesothelioma: A type of cancer that develops from the mesothelium, which is the thin layer of tissue that covers many internal organs.

Neurofibrosarcoma: A malignant tumour that develops from the cells surrounding the peripheral nerves. Also known as peripheral nerve sheath tumour.

Schwannoma: A generally benign tumour derived from Schwann cells, which are cells forming part of the nerve sheath.

Mixed HCC/ICC: Rare intrahepatic lesions composed of hepatocellular carcinoma (HCC) and intrahepatic cholangiocellular carcinoma (ICC).

Stellate cells: Pericytes found in the perisinusoidal space of the liver, also known as the space of Disse (a small area between the sinusoids and hepatocytes).

Enterocytes: Simple columnar epithelial cells found in the small intestine which fulfill absorptive functions.

Goblet cells: Mucus producing cells found in the epithelium of the intestinal and respiratory tracts.

Paneth cells: Epithelial cells located at the base of the intestinal crypt, which secrete antimicrobial peptides and produce niche factors that modulate and maintain neighbouring stem cells.