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Targeting the Hippo pathway in cancer, fibrosis, wound healing and regenerative medicine

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Abstract

The Hippo pathway is an evolutionarily conserved signalling pathway with key roles in organ development, epithelial homeostasis, tissue regeneration, wound healing and immune modulation. Many of these roles are mediated by the transcriptional effectors YAP and TAZ, which direct gene expression via control of the TEAD family of transcription factors. Dysregulated Hippo pathway and YAP/TAZ–TEAD activity is associated with various diseases, most notably cancer, making this pathway an attractive target for therapeutic intervention. This Review highlights the key findings from studies of Hippo pathway signalling across biological processes and diseases, and discusses new strategies and therapeutic implications of targeting this pathway.

The Hippo pathway was first discovered and delineated in Drosophila, in which tumour-suppressive roles for the pathway were identified, as mutations in genes encoding key effectors resulted in tissue overgrowth[1–8]. Conservation of the pathway has guided studies that have described its dysregulation in various human cancers and other diseases, as well as essential functions in development and regeneration. Accordingly, interest in understanding the molecular functions, the regulation and the therapeutic targeting of this pathway has come to the forefront of research across numerous fields.

The Hippo pathway consists of a network of signals that culminate to direct the function of the transcriptional regulators YAP and TAZ (orthologues of Yorkie in Drosophila) (Fig. 1). These paralogous factors (hereafter described together as YAP/TAZ) share conserved structural domains and regulatory mechanisms, and when localized in the nucleus direct the activity of a range of transcription factors. The best-characterized transcription factors regulated by YAP/TAZ are the TEAD family, which includes four members in mammals (TEAD1–TEAD4) and a single orthologue in flies, known as Scalloped. Nuclear YAP/TAZ

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Author contributions

All authors contributed equally to all aspects of the article.

Competing interests

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direct gene expression programmes that promote cell proliferation and survival signals, and control cell fate decisions. YAP/TAZ binding to the TEAD transcription factors has been linked to most, if not all, YAP/TAZ-directed biological functions, including pro-tumorigenic phenotypes that arise from dysregulated Hippo pathway signalling. Recent efforts have therefore been devoted to disrupting interactions between these factors with the hope that such a strategy may offer therapeutic benefits.

YAP/TAZ are predominantly regulated by phosphorylation, which promotes cytoplasmic localization and, therefore, inhibition of YAP/TAZ transcriptional activity. Two members of the NDR family of kinases, LATS1 and LATS2 (hereafter described as LATS1/2) and their single homologue in flies, known as Warts, are major regulators of YAP/TAZ. They phosphorylate YAP/TAZ and lead to their destabilization and restriction from accessing the nucleus[9]. Numerous upstream signals direct YAP/TAZ activity, including those activated by the extracellular matrix (ECM), mechanical forces, cell adhesion, cell polarity, mitogens, tyrosine kinase receptors, G protein-coupled receptors (GPCRs) and alterations in cellular metabolism[9]. Many of these signals are relayed via LATS1/2, although LATS1/2-independent regulation has also been reported[10]. Thus, complex networks of intrinsic and extrinsic signals have the potential to direct the localization and activity of YAP/TAZ. Understanding these cues in different cells, tissues and diseases has become the focus of many recent studies. This Review summarizes our current understanding of Hippo–YAP/TAZ signalling in diseases such as cancer and outlines the key roles for the pathway in tissue and organ development, homeostasis, tissue regeneration and immune modulation. We discuss current strategies that are being developed to manipulate or block Hippo pathway signalling, with the hope that such strategies may offer promise for disease therapy and/or directions of tissue regeneration.

Dysregulated Hippo signalling in cancer

Advances in next-generation sequencing approaches have afforded unprecedented amounts of genomic information on cancers. Although there are no classical hotspot mutations in Hippo pathway effectors, there is emerging evidence of a large number of cancers with aberrant levels of YAP/TAZ and gene expression associated with dysregulated Hippo pathway activity (also reviewed elsewhere[11]). Below, we outline what is known about dysregulated YAP/TAZ in cancers and the potential paths for this Hippo pathway dysregulation.

Elevated YAP/TAZ levels and activity in cancer.

Analyses of various tumours and cancer cell lines indicate a prominent role for YAP/TAZ in cancer (Table 1). Data from The Cancer Genome Atlas for over 9,000 tumours show that the Hippo pathway is one of the eight signalling pathways that are frequently altered in human cancer[12]. Notably, the *YAP1* and *WWTR1* genes, encoding YAP and TAZ, respectively, are amplified in ~14% of head and neck squamous cell carcinomas, ~16% of lung squamous carcinomas, ~17% of cervical squamous cell carcinomas and ~15% of oesophageal squamous cell carcinomas[12]. Increased levels of YAP/TAZ and TEAD protein expression in the nucleus correlate with poor prognosis and increased therapeutic resistance in various

cancers arising from thoracic, gastric, genitourinary, gynaecological, skin, bone and brain tissues[11]. Some of these prognostic outcomes relate to increased YAP/TAZ levels that result from gene amplification, but other mechanisms that account for elevated YAP/TAZ levels remain largely unknown. Some aberrant YAP/TAZ levels can be explained by the intersection of Hippo pathway signalling with the activation of pro-tumorigenic signalling pathways. For example, YAP nuclear localization and activation are induced by phosphoinositide 3-kinase (PI3K) and 3-phosphoinositide-dependent protein kinase 1 (PDK1), which concomitantly inhibit the Hippo pathway and lead to poor prognosis in patients[13,14]. Altered mechanotransduction or signalling through pathways such as Wnt, transforming growth factor-β (TGFβ), MAP kinase, GPCRs or metabolic networks12 likely promotes aberrant YAP/TAZ activity in different contexts.

YAP/TAZ are also implicated in metastatic progression - the leading cause of cancer-related death - which involves several events that participate in enhancing the migratory potential of aggressive cancers. Early studies indicated that ectopic expression of YAP has potent pro-metastatic activity, particularly nuclear localized mutants of YAP[15]. This activity relies on TEAD binding, suggesting that disruption of this interaction may offer therapeutic potential in aggressive cancers. YAP promotes a metabolic shift in cancers that metastasize to lymph nodes by inducing the expression of genes that promote fatty acid oxidation[16]. Deletion of Yap1 or pharmacological inhibition of fatty acid oxidation suppressed lymph node metastasis in mice. Metabolic vulnerability of cancer cells with elevated nuclear YAP/TAZ levels may therefore offer potential therapeutic potential in treating metastatic disease. Further supporting this idea are observations that metastatic triple-negative breast cancer cells with high YAP/TAZ levels are distinctly sensitive to inhibitors of glutamine transaminases, such as aminooxyacetate (AOA), which results from YAP/TAZ-induced expression of transaminases, such as GOT1[17].

Disruption of upstream YAP/TAZ regulators. The mechanisms contributing to altered YAP/TAZ activity in human cancers seem to be diverse. Although not frequent or conserved across many cancers, loss-of-function mutations in negative regulators of YAP/TAZ or activating mutations that have the potential to promote nuclear YAP/TAZ activity have been observed. Consistent with this, the presence of nuclear YAP is strongly associated with mutations in NF2 (encoding Merlin) in tumours that arise from the nervous system, such as schwannomas, meningiomas and ependymomas[11]. Mutations in NF2 and LATS2 have also been observed in malignant mesothelioma[12,18,19]. However, whether aberrant YAP/TAZ activity in these cancers contributes to disease phenotypes has not yet been revealed. Interestingly, activating mutations in genes coding for guanine nucleotide-binding proteins, G(q) subunit α (GNAQ) and GNA11 - which are downstream of GPCRs and found in more than 80% of uveal melanomas - drive uncontrolled nuclear YAP activation, which directly promotes tumorigenesis[20, 21]. These identifiable alterations may offer an opportunity to predict responsiveness to therapeutics targeting YAP/TAZ activity.

Gene fusions in cancer related to the Hippo pathway.—Gene fusions are structural chromosomal aberrations that can often involve the exchange of regulatory regions of genes, and can act as strong drivers of tumorigenesis. Core effectors of Hippo signalling can contain such anomalies. For example, a fusion between the genes *WWTR1* and *CAMTA1*

(encoding calmodulinbinding transcription activator 1) accounts for more than 90% of a type of vascular cancer called epithelioid haemangioendotheliomas[22]. In this type of tumour, the encoded TAZ–CAMTA1 fusion protein has similar transcriptional activity and tumorigenic functions to TAZ. The TAZ portion of the fusion protein retains an amino-terminal region that includes LATS phosphorylation sites as well as the TEAD binding domain, but has replaced the carboxy-terminal transactivation domain with a C-terminal domain from CAMTA1[22]. Interestingly, TEAD4 is required for the transforming activity of TAZ–CAMTA1, indicating that the TEAD binding interface is conserved[23]. Therefore, patients with epithelioid haemangioendothelioma may benefit from potential therapeutics that target TAZ–TEAD binding. Fusion of the *YAP1* and *TFE3* (encoding transcription factor E3) genes has been observed in other vascular cancers[24], and additional gene fusions involving *WWTR1*, *NF2*, *LATS1* and *LATS2* have been found in lung cancer[25]. Thus, it seems that genetic alterations that activate YAP/TAZ function may be enriched in some cancers and additional study of these diseases will offer a greater understanding of YAP/TAZ function in cancer.

Role of the Hippo pathway in resistance to targeted therapy.

Targeted therapies are an attractive approach to selectively treat cancer with mutations in key oncogenic drivers. However, intrinsic and acquired resistance to targeted therapies is common and presents a barrier to the efficacy of such treatments. Elevated YAP/TAZ levels contribute to resistance to chemotherapy as well as to therapies that target activating mutations in EGFR, RAS and BRAF[26]. For instance, in EGFR-mutant lung cancer cells, YAP activation induces the expression and activation of the tyrosine kinase receptor AXL, which mediates intrinsic and acquired resistance, although the prevalence of AXL-driven resistance in patients is largely unknown[27, 28]. YAP is also a key driver in enabling compensation and bypassing of KRAS dependency in pancreatic and colon cancer models[29, 30]. In pancreatic ductal adenocarcinoma, YAP–TEAD complexes direct cell cycle progression, DNA replication and a shift to a mesenchymal phenotype to compensate for the loss of oncogenic KRAS function[29]. In colon and lung cancers, the interaction between YAP and transcription factor FOS induces an epithelial–mesenchymal transition programme to bypass KRAS dependence. *YAP1* amplification was also found to bypass inhibition of RAF and MEK in lung, skin, colorectal and thyroid cancers by promoting survival by upregulating the anti-apoptotic gene BCLX (also known as Bcl-2-like protein 1, BCL2L1)[31]. Interestingly, YAP and TAZ were among the top candidates from a large-scale functional genetic study that aimed to identify mechanisms of resistance to ALK inhibition in ALK-addicted lung cancer[32]. In most of these cases, a combinatorial regimen of YAP/TEAD inhibition and targeted therapy against these other pathways would be expected to overcome intrinsic and acquired resistance. Thus, elucidating the mechanism of YAP and TAZ activation during drug therapy resistance is of significant interest when considering targeted therapies in cancer [33,34].

The Hippo pathway in immune modulation

Hippo pathway components have prominent roles in immune regulation. The best studied is MST1 (encoded by STK4), as early studies showed that mutations in STK4 can lead to

immunodeficiency in both human and mouse[35–37]. MST1 positively participates in T cell development, adhesion and migration as well as key dendritic cell functions[38–42]. Although studies do support a role for YAP/TAZ in T cell biology and innate immunity, MST1 seems to modulate immune functions independently of canonical Hippo pathway YAP/TAZ regulation.

Early evidence for the role of MST1 and MTS2 (encoded by STK3) in immune responses came from the study of regulator for cell adhesion and polarization enriched in lymphoid tissue (RAPL; also known as Ras association domain family member 5, RASSF5), which is required for immune cell trafficking. RASSF5 interacts with and stimulates MST activity[43]. MST1 overexpression in the mouse pro-B lymphocyte cell line BAF stimulated cell adhesion, and MST1 knockdown inhibited cell polarization and adhesion in vitro. Germline STK4 knockout mice display a significant reduction of naive T cells - but not of effector or memory T cells - in circulation, supporting a role for MST1 in the maintenance of circulating naive T lymphocytes[44]. In this context, loss of MST1 did not dramatically affect the phosphorylation of LATS1/2 or YAP in T cells[44], suggesting that the essential MST1 functions in T cells may be independent of conventional Hippo pathway signalling. Subsequent studies showed that deletion of STK3 and STK4 does not affect T cell development, but dramatically reduces the number of circulating CD4+ and CD8+ T cells[45]. This is likely owing to a severely compromised T cell ingress from lymph nodes, revealing a specific role for MST in lymphocyte trafficking and migration[45].

Systematic deletion of the Hippo pathway components in dendritic cells - which are essential for antigen presentation to T cells and serve as messengers between the innate and adaptive immune response - has shown that MST1/2 are selectively required for CD8+, but not CD8−, dendritic cells to orchestrate CD8+ T cell-mediated immune response in vivo[41]. Mechanistically, deletion of STK3 and STK4 compromises mitochondrial function and bioenergetics, and MST1/2-deficient dendritic cells have low interleukin 12 (IL-12) production and defects in crosstalk with non-canonical nuclear factor NF-κB signalling[41]. Notably, deletion of LATS1/2 or YAP1/WWTR1 did not produce similar phenotypes[41], supporting the observation that conventional Hippo pathway signalling mediated by MST1/2 is not involved in the regulation of dendritic cells. Homozygous mutations in *STK4* can, extremely rarely, cause immunodeficiency in patients[35,36]. These patients are characterized by a progressive loss of circulating naive T cells, impaired survival of T cells in vitro, increased viral and bacterial infections and autoimmune defects [35,36]. These observations establish a pathological significance of MST1 in the human immune system, particularly in maintaining normal T cell functions.

Both YAP and TAZ have also been identified as key effectors of T cell biology. TAZ was shown to promote CD4+ helper T (T_H) cell differentiation towards the T_H17 cell fate, which are key adaptive immune cells that produce IL-17. Interestingly, the role for TAZ in promoting T_H17 cells is described as independent of the TEAD transcription factors[46]. YAP levels are increased upon T cell activation, having immunosuppressive functions that blunt CD4+ and CD8+ T cell activating signals and T_H cell differentiation[47]. YAP has also been identified as being required for proper CD4+CD25+ regulatory T (T_{reg}) cell function[48]. T cell-specific deletion of *YAP1* results in increased antitumour T cell responses, which

include an increase in T cell tumour infiltration and local activation47, as well as in dysfunctional T_{reg} cells that are unable to suppress antitumour immunity[48]. Activation of CD8+ T cells induces expression of several Hippo pathway effectors, including MOB1A, LATS1 and TEAD1/2[49]. It was proposed that cell–cell contact upon clonal expansion of activated T cells leads to Hippo activation and YAP inhibition, offering a potential mechanism for coupling CD8+ T cell terminal differentiation with clonal expansion.

Interestingly, YAP/TAZ have also been implicated in innate immunity[50, 51]. Cytosolic RNA and DNA sensing and signalling is strongly enhanced under Hippo activating conditions, in which YAP/TAZ are inactive in the cytoplasm[51]. YAP/TAZ can bind and directly inhibit TBK1 kinase, which is activated and has a key role in innate response[51]. YAP/TAZ deletion or inactivation by overexpression of Hippo pathway kinases relieved their inhibition of TBK1 to boost innate immune response and increase antiviral response[51]. The function of YAP in TBK1 regulation is independent of its TEAD-dependent transcription co-activator activity. The interferon regulatory factor IRF3 is a key transcription factor in immune response by inducing cytokine expression. YAP inhibits IRF3 by blocking its dimerization and nuclear translocation in response to viral infection[50]. Inhibitor of NF-κB kinase subunit ε (IKKε), which is the closest TBK1 paralogue with similar functions and is also activated by infection, phosphorylates YAP and promotes its lysosomal degradation[50]. Collectively, these studies establish an inhibitory function of YAP/TAZ in innate immune response, but future studies are needed to clarify their mechanisms of action.

Hippo pathway in cancer immunity and interplay with the immune system.

Besides directly acting in immune cells as described in the above section, alteration of the Hippo pathway in cancer cells can impact the interplay between cancer cells and the host immune system. In a mouse model of liver cancer, high YAP activity in cancer-initiating cells promotes the recruitment of type II macrophages, which are immunosuppressive, to inhibit the host immune response and likely contribute to protection of cancer cells from immune surveillance[52]. This is mediated by YAP-TEAD-dependent expression of cytokines to recruit macrophages. In a mouse model of prostate cancer driven by loss of *Pten* and *Smad*, high YAP activity in tumour cells stimulated the recruitment of myeloid-derived suppressor cells to inhibit host immune response[53]. At the molecular level, YAP–TEAD induced expression of cytokines, such as C-X-C motif chemokine 5 (CXCL5), to recruit myeloid-derived suppressor cells in the tumour microenvironment, thus allowing immune tolerance and tumour progression[53]. These studies indicate that high YAP activity in cancer cells contributes to the immunosuppressive niche.

YAP and TAZ can both stimulate the expression of programmed cell death 1 ligand (PDL1)[54–56], a ligand for the PD1 receptor, which creates an immunosuppressive environment. TAZ promotes the expression of PDL1 in lung and breast cancer cells[55, 57], whereas YAP promotes the expression of PDL1 in melanomas and head and neck squamous cell carcinoma cells[56,58]. Therefore, targeting YAP/TAZ may offer an attractive approach to reduce PDL1 levels and, thus, enhance the effect of immunotherapy using neutralizing antibodies against PDL1 and/or PD1. Deletion of *Yap1* in T cells using a CD4-cre murine model increased antitumour immune responses, as *Yap1* knockout mice could not support

growth of the syngenic B16 melanoma xenograft[47,48]. Notably, combination of YAP inhibition using verteporfin, which disrupts YAP nuclear activity, with an antibody against PD1 led to synergistic reduction of tumour growth, supporting the notion that YAP inhibition promotes the efficacy of immunotherapies[48]. An immunosuppressive function of YAP has been observed in other cell types[59]. Conditional deletion of *Yap1*/*Wwtr1* in mouse epicardium reduced the expression of interferon-γ (IFNγ), an important regulator of T_{reg} cells. Consequently, reduced recruitment of T_{reg} cells was observed, which led to profound post-myocardial infarction, pericardial inflammation and myocardial fibrosis.

The function of the Hippo pathway in cancer immunity is rather complex. Several syngeneic mouse xenograft model experiments indicate that LATS1/2 exhibits strong inhibitory activity towards cancer cell immunity[60]. LATS1/2 deletion in cancer cells blocked xenograft tumour formation in immune-competent syngeneic mice, which interestingly was a phenotype not observed in immunodeficient mice[59]. Further characterization showed that LATS1/2 deletion caused stronger immunogenic exosome secretion and type I interferon response, leading to tumour suppression. YAP/TAZ activation seems to partially contribute to the increased cancer cell immunity in the *LATS1/2* knockout cells, indicating that LATS1/2 autonomously suppresses cancer cell immunity.

The data from the xenograft study apparently contradict the data from LATS1/2 genetically engineered mouse models, which collectively support that loss of LATS1/2 promotes tumorigenesis. However, such differences could be attributed to experimental settings. In xenograft tumour model experiments, the mouse hosts receive many tumour cells at once, whereas tumours in genetically engineered mouse models presumably originate from a single or few cancer-initiating cells. High YAP activity in one cancer-initiating cell may not induce a strong immune response in the genetically engineered mouse models, but may confer a growth advantage to such a cancer-initiating cell. By contrast, many cancer cells with high levels of YAP in the xenograft model may be sufficient to induce a strong immune response, leading to elimination of tumour cells by the host immune system. Future studies are therefore needed to clarify the context of Hippo pathway signalling in immune cells and the interplay between cancer and immune surveillance.

Tissue regeneration and repair

Multicellular organisms have the ability to replace damaged or injured tissues or organs, which in some cases can include complete regeneration. Some examples of this complete regeneration include accurate regeneration of complete tail fins in fish, replacement of an amputated limb in frogs and regeneration of the liver following partial hepatectomy in humans. Hippo pathway signalling has emerged as central to such regeneration, playing key parts in stem cell proliferation, survival and specification, ultimately determining organ patterning and size.

Liver regeneration.

Early insight into the involvement of Hippo pathway signalling in regeneration came from observations in *Drosophila* intestines, which showed that the YAP/TAZ homologue Yorkie is critical for intestinal stem cell expansion following injury[61–63]. Studies in mammalian

animal models, such as mice, revealed similar importance for YAP/TAZ in promoting the regenerative capacity of intestinal stem cells[64–68], with recent single-cell RNA-sequencing studies highlighting YAP as a central factor in the regeneration of distinct intestinal stem cell populations[69,70]. A prime example of the importance of Hippo pathway signalling in regeneration is the involvement in liver recovery following partial hepatectomy. YAP/TAZ activity is essential for liver regeneration following damage, and increased levels and activity of nuclear YAP are observed in the liver following partial hepatectomy in rat models[71,72]. Conditional deletion of *Yap1* and *Wwtr1* in mice results in defective regeneration following partial hepatectomy[73]. Similarly, deletion of *Yap1* compromises the regenerative response of hepatocytes following cholestatic liver injury[74]. Interestingly, transient ectopic expression of YAP[75] or deletion of upstream Hippo pathway effectors, such as *Stk3*, *Stk4* [76–78], *Nf2* [79,80] or *Sav1* [76] (encoding WW45, a regulator of MST1/2 expression that restricts proliferation and promotes apoptosis), drives strong pro-proliferative signals that result in liver overgrowth. Ectopic expression of YAP can also drive hepatocyte plasticity, as hepatocytes convert into a hepatic progenitor state that enables ex vivo expansion and transplantation of such cells into animals. Remarkably, these cells have the ability to revert back to hepatocytes if YAP levels are brought back to basal levels[81]. Such a strategy is effective in liver repopulation following partial hepatectomy in mice[82]. Similarly, transient depletion of MST1/2 kinases facilitates regeneration in aged mice following partial hepatectomy[83]. Given that deletion of *Yap1*/*Wwtr1* in the liver of mice seems to have no overt consequences on homeostasis[84], manipulating YAP/TAZ levels in the liver may prove to be an effective strategy for regeneration. However, given the pro-tumorigenic roles of YAP/TAZ in the liver and other organs, such an approach would require caution.

Lung regeneration.

YAP is also involved in the regeneration of the lung following pneumonectomy. Pneumonectomy in mice results in elevated levels of YAP in expanding alveolar type II cells, a cell population that has stem cell potential85, and conditional deletion of *Yap1* and/or *Wwtr1* in this cell population results in defective lung regeneration in response to injury[86–88]. In the context of the severe lung injury of pneumonectomy, YAP activation is mediated by mechanical tension-regulated signals that are relayed by the GTPase CDC42[86]. Thus, controlled regulation of YAP/TAZ activity in alveolar type II cells may offer a mechanism for distal lung damage. YAP also contributes to the maintenance of basal stem cells of the airway epithelium, which has an important function in regenerating damaged airway tissue. Ectopic expression of YAP is sufficient to drive basal airway cell expansion [89–91], raising the possibility that controlled activation of YAP could provide a strategy for airway epithelium regeneration.

Wound healing.

Roles for YAP/TAZ in basal stem cell control are apparent in epidermal development and skin wound repair. Nuclear YAP and TAZ localization is observed in the basal layer of skin and these nuclear levels are elevated upon wound healing[92]. Ectopic expression of an activated YAP mutant or dysregulation of upstream regulators of YAP localization - such as the cell adhesion regulator α-catenin[93,94] - results in the expansion of epidermal basal cells, which resembles an uncontrolled epidermal damage response. Skin-specific deletion of both

Yap1 and *Wwtr1* in adult mice severely impairs regeneration after wounding [92,93,95–97]. YAP/TAZ activity is essential in skin homeostasis as deletion of *Yap1*/*Wwtr1* slows proliferation of basal layer cells and leads to hair loss and tissue damage[92]. Thus, strategies directed at inhibiting YAP/TAZ activity would have to take this important homeostatic role into account.

Cardiac regeneration.

Cardiomyopathies make up a group of heart muscle diseases that result in heart failure that is linked to the loss of cardiomyocytes. The maintenance and regeneration of cardiomyocytes have been linked to YAP/TAZ activity. For example, ectopic expression of YAP in the heart is sufficient to stimulate the proliferation of postnatal cardiomyocytes and cardiac regeneration in mice, and improve contractility after myocardial infarction [98–100]. Conversely, cardiacspecific deletion of *Yap1* represses mouse neonatal heart regeneration [98–100]. Interestingly, recent screening efforts have identified TT-10 (C11H10FN3OS2) as an activator of YAP that promotes cardiomyocyte proliferation and improves cardiac function after myocardial infarction in mice[101,102]. Mechanistically, TT-10 inhibits the phosphorylation of GSK3β, which leads to the activation of Wnt/β-catenin signalling and nuclear accumulation of YAP[101,102]. Microenvironmental changes, tensional forces and actin remodelling have been linked to the activation of YAP in regenerating cardiomyocytes [103–105], and therefore offer research directions for heart regeneration strategies.

Other tissue regeneration.

Broad functions for YAP/TAZ have been described in a number of contexts of tissue regeneration, including essential roles in repairing damaged mammalian corneal epithelium[106], Schwann cells[107], neuromuscular junctions[108], skeletal development and bone repair[109]. YAP/TAZ are also involved in the homeostatic turnover of tissues in the mammalian tooth[110]. Notably, the regeneration of amputated limbs in animal models with high regenerative capacity relies on YAP/TAZ activity. For example, *Xenopus* limb bud and tail regeneration requires proper *Yap1* activity[111, 112], and the control of tissue growth in regenerating zebrafish fins relies on the activation of YAP[113]. Planarian flatworms, which have a tremendous regenerative capacity, also rely on YAP for the proper growth and patterning of stem cell populations following damage[114]. Several conserved themes are evident from studies of YAP/TAZ in regeneration, which include essential roles for YAP/TAZ in somatic stem cell regulation, alterations in the microenvironment and responses to mechanical and tensional forces in damaged tissues. The relationship between YAP/TAZ and altered mechanical forces is of particular interest, as the opportunity for therapeutic intervention that can either activate the regenerative programme or inhibit aberrant wound healing responses may be accessible from gaining better knowledge into these cues. Interestingly, the activity of YAP/TAZ does not seem to be essential for development of some tissues. For example, YAP is dispensable in the homeostasis of the intestinal epithelium and liver hepatocytes under normal physiological conditions. Therefore, better understanding of the signals that control the regenerative capacity of YAP/TAZ versus the developmental roles for YAP/TAZ may hold promise for regenerative therapies.

YAP/TAZ in fibrotic disease

A hallmark of fibrotic disease is the excessive deposition of the ECM, including cross-linked collagen fibres, which results in the stiffening of tissues and eventual dysfunction of affected organs. YAP/TAZ act as effectors of mechanical cues, which has led to the investigation of these factors in a range of fibrotic diseases. ECM stiffening promotes the nuclear activity of YAP/TAZ in cancer-associated fibroblasts, and fibroblasts of the liver[115,116], kidney[117], lung[118] and skin[119], as nuclear YAP/TAZ promote fibrotic cellular phenotypes, such as myofibroblast differentiation and increased matrix remodelling potential. Several genes that encode key secreted factors implicated in fibrosis are direct YAP/TAZ targets in various cell models in vitro, most of which are also targets of the TEAD family of transcription factors. These genes include well-characterized pro-fibrotic factors, such as connective tissue growth factor (CTGF), plasminogen activator inhibitor 1 (PAI-1) and the lysyl oxidase (LOX) family of collagen cross-linking enzymes[118,120]. Therapeutic strategies that target these YAP/TAZ-regulated pro-fibrotic factors are being explored for fibrotic diseases, including a monoclonal antibody (FG-3019 from FibroGen) that interferes with CTGF function[121], small-molecule inhibitors of PAI-1 (such as TM5275)[122] and inhibitors of LOX and LOX-like proteins (such as the non-selective LOX inhibitor β-aminoproprionitrile[123] and the specific LOXL2 blocking antibody AB0023[124].

Several lines of evidence support YAP/TAZ as contributors to fibrotic disease in vivo. These include reports of elevated YAP/TAZ levels and transcriptional activity in fibroblasts as well as in alveolar and respiratory epithelium of patients with idiopathic pulmonary fibrosis[118,125]. Elevated TAZ levels correlate with local tissue stiffness in idiopathic pulmonary fibrosis, supporting the idea that increased mechanical forces may drive YAP/TAZ activity. Increased nuclear YAP has also been observed in patients with primary sclerosing cholangitis and primary biliary cirrhosis, which are chronic fibrotic disorders of liver injury[74]. Ectopic expression of YAP or TAZ in immortalized mouse fibroblasts that have been adoptively transferred, and that accumulate in mouse lungs, is sufficient to drive pro-fibrotic phenotypes[118]. Expression of YAP or TAZ in the duct cells of the liver drives fibrosis progression that parallels fibrosis in non-alcoholic fatty liver disease[126–128]. Renal fibrosis induced in mice by unilateral ureteral obstruction exhibits elevated YAP levels[129]. Interestingly, unilateral ureteral obstruction-induced myofibroblast accumulation, matrix deposition and fibrosis can be suppressed following deletion of *Yap1*/*Wwtr1* in *Gli1*$^{+}$ cells[129], which are recruited mesenchymal cells thought to function as progenitors in the kidney[130]. Furthermore, mice lacking one allele of *Wwtr1* are more resistant to bleomycin-induced fibrosis[131]. Likewise, knockdown or pharmacological inhibition of YAP/TAZ delays wound healing[96] and prevents TGFβ-induced renal fibrosis[117]. Collectively, these studies suggest that targeting aberrant YAP/TAZ activity in fibrotic diseases may hold promise for therapy.

YAP/TAZ converge with pro-fibrotic signalling pathways, such as TGFβ signalling. Indeed, YAP/TAZ are essential for pro-fibrotic cues induced by TGFβ in a stiff microenvironment[117, 118, 132]. As with YAP/TAZ activity, TGFβ-induced signalling is increased by matrix stiffness and the synergistic activation of these signals in fibroblasts strongly promotes myofibroblast activation, matrix production and fibroblast proliferation

and contractility. Depletion of YAP/TAZ blocks TGFβ-induced myofibroblast transformation and production of the ECM, whereas ectopic expression of activated YAP/TAZ promotes these events. YAP/TAZ bind and directly regulate TGFβ-activated SMAD transcription factors [133, 134], offering a mechanism for pro-fibrotic signalling cross-talk. However, YAP/TAZ can induce pro-fibrotic effects in response to matrix stiffness in the absence of exogenous TGFβ, suggesting that they also play a broader part in fibrosis [115,118]. Consistent with this idea, nuclear YAP/TAZ activity correlates with increased phosphorylated PTEN, phospho-S6 ribosomal protein (p-S6) and total S6 in fibrotic lungs [125], all of which suggest increased PI3K–AKT–mTOR signalling in cells with activated YAP/TAZ. As such, elevated nuclear YAP/TAZ levels may function to establish and coordinate a fibrogenic signalling network that is necessary for relaying and responding to microenvironment signals[135]. Targeting this dysregulated network or the mechanical/matrix signals that drive elevated nuclear YAP/TAZ activity would therefore have strong therapeutic potential. One recent such strategy that has shown promise is activation of the Gα-coupled dopamine receptor D1 (DRD1) through selective agonists, which results in selective inhibition of YAP/TAZ in lung and liver mesenchymal cells[136]. DRD1 activation reversed ECM stiffening in vitro and tissue fibrosis in animal models, highlighting a potentially interesting therapeutic avenue for selectively targeting YAP/TAZ activity in fibrotic disease.

Targeting the Hippo pathway

Given that several Hippo pathway members are bona fide tumour suppressors and aberrant YAP/TAZ activity is implicated in a range of cancers and other diseases, targeting this pathway offers potential opportunity for therapy. Blocking the activity of the YAP/TAZ–TEAD transcriptional complex is one potential approach. Indeed, recent in vivo tumour studies with preliminary compounds that disrupt YAP/TAZ-TEAD interactions suggest that this approach may be tenable. Inhibiting proteins encoded by YAP/TAZ-activated genes have also shown promise for therapy[121, 137, 138], and thus understanding these gene products in specific disease contexts may offer translational opportunity. Furthermore, given the roles of the Hippo pathway in regeneration and wound repair, harnessing the ability to conditionally alter Hippo pathway activity with therapeutics may also prove successful in the clinic. Below, we highlight current avenues for therapeutic intervention (Fig 2), some of which are showing promise with initial studies.

Inhibition of YAP/TAZ–TEAD-dependent transcription.—Initial efforts to identify inhibitors of YAP/TAZ–TEAD activity relied on screening for inhibitors of TEAD-regulated transcriptional reporters. The first such inhibitor, verteporfin (porphyrin compound), was identified from a small library of FDA-approved compounds, and was reported to block the interaction between YAP and TEAD and suppress tumour growth in mice [139]. Several reports have since indicated that verteporfin has the ability to block YAP-TEAD activity in various contexts, but this inhibition may not be specific to only inhibiting YAP-TEAD complexes as verteporfin has also been reported to have proteotoxic effects[140–142]. These caveats, along with poor pharmacokinetics, suggest that this compound may not translate well to the clinic. However, identification of verteporfin as an inhibitor of YAP-TEAD activity has initiated a wealth of screening efforts, with other less well tested compounds also emerging. As with verteporfin, the compounds CA3 and C19 have been identified in

transcriptional reporter screens as inhibitors of YAP–TEAD activity[143,144], with CA3 exhibiting in vivo efficacy. In silico modelling of compound libraries led to the identification of CPD3.1, which disrupts YAP association with TEADs and represses TEAD transcriptional activity, cell proliferation and cell migration[145]. Similarly, MYF-01–37 was recently identified as a covalent binder of TEADs that disrupts binding with YAP/TAZ. MYF-01–37 was shown to be effective in targeting EGFR-mutant non-small-cell lung cancer cells when used in combination with EGFR and MEK inhibitors[146]. Details of additional compounds proposed to target YAP/TEAD activities in various cancers by impacting their interaction, activity or localization have been reviewed elsewhere[147,148]. Although these compounds provide useful research reagents to investigate pathway biology, their target specificity and selectivity remain to be determined. Therefore, whether these screening methods are useful for targeted therapy remains to be confirmed.

Rational design for targeting the YAP/TAZ–TEAD interface.—Very simplistically, the TEAD transcription factors have a DNA-binding domain (DBD) and a YAP-binding domain (YBD). The DBD, as the name suggests, is responsible for DNA binding specificity and harbours a helix–turn–helix homeodomain fold. The YBD has a β-sandwich fold with four helices. Structurally, the interaction between the YBD in TEADs and the TEAD-binding domain in YAP comprises three highly conserved interfaces, with the third interface being the major energetic determinant of high-affinity binding141–143 (Fig. 3a). Crystal structures of YAP–TEAD complexes reveal clear surface pockets at the second and third interfaces that might enable the rational design of TEAD inhibitors. Notably, residues within the TEAD pocket that associate with YAP at interface 3 are 100% identical across all TEAD family members, suggesting that targeting this interface would offer possible pan-TEAD ligands, which could prove useful across many different contexts. Indeed, such efforts to target the YAP-TEAD interface have yielded promising peptides and small molecules that provide proof of concept and could serve as valuable research tools to further validate the strategy[144]. The in vivo impact of inhibitors binding interface 3, however, remains to be determined. Furthermore, given that none of the interfaces is more accessible than the others, whether inhibiting a specific TEAD interface would be more efficacious or provide more specificity remains to be seen. Other key challenges ahead include ensuring permeability, specificity, efficacy and, ultimately, safety for identified compounds, while providing a sufficient therapeutic window. Further, whereas most efforts have focused on disrupting the YAP-TEAD interaction, it will be important to keep in mind other known TEAD-interacting proteins, such as the transcription cofactor vestigial-like protein family members (VGLL1–VGLL4, which are also transcriptional co-activators of TEADs) and p160[145]. VGLL1 and VGLL4 bind TEADs at interfaces 1 and 2, but not at interface 3. How an inhibitor of YAP binding would impact interactions with other TEAD co-activators and how this could translate to phenotypic consequences are all areas of active investigation across various groups.

Post-translational palmitoylation of TEADs at a conserved cysteine extends the palmitoyl group into the hydrophobic cavity in TEADs [149,150]. Unlike other palmitoylated proteins, TEAD palmitoylation does not impact its trafficking and cellular localization, and its influence on YAP/TAZ binding is controversial. Although the regulation and functional

relevance of TEAD palmitoylation remains to be elucidated, it might maintain TEAD folding and stability[149, 150]. Palmitoylation impacts TEAD stability, which has made functional evaluation of the mutants lacking the conserved cysteine technically challenging, as this renders them inherently unstable. Compounds that bind in this pocket could provide valuable insights into the functional consequences and therapeutic potential of targeting this site. It is worth highlighting that this hydrophobic pocket, although 80% conserved across the four TEADs, is not identical and the key residues involved have been highlighted in Fig. 3b,c. This is in contrast to the site 3 pocket on TEADs responsible for the YAP–TEAD interaction, which is 100% identical across all TEADs. Specifically, structural analyses of the residues in the pocket revealed a Tyr230 in TEAD3 relative to a Phe residue in other TEADs, which results in steric hindrance and altered hydrophobicity of the lipid pocket. This observation suggests that any small molecules targeting this lipid pocket could potentially bind TEAD3 differently, thereby making identification of pan-TEAD ligands a potential challenge for this targeting strategy. It also raises the question of whether all TEADs need to be effectively inhibited and whether certain cancers are more dependent on a specific TEAD over others. Flufenamate drugs, which are non-steroidal anti-inflammatory drugs, bind in the TEAD lipid pocket and displace palmitoylation without inhibiting the YAP–TEAD interaction. Treatment with these compounds decreased tumour cell migration and proliferation moderately, thereby providing a promising proof of concept for this strategy as well[151]. Recently, flufenamic acid derivatives comprising chloromethylketone moieties that bind to the conserved cysteine in the lipid pocket have also been shown to inhibit YAP-TEAD interaction, transcriptional activity and glioblastoma growth in vitro[152]. For additional reference, the structures and details of compounds targeting the Hippo pathway have been recently reviewed elsewhere [34] (Fig. 4).

VGLL4 peptide mimetics.—In addition to YAP and TAZ, VGLL1-VGLL4 interact with TEADs via their Tondu (TDU) domains[153–155]. VGLL1-VGLL3 may be transcriptional co-activators of TEADs, but VGLL4 has been identified as a tumour suppressor downregulated in gastric cancer[156]. Mechanistically, VGLL4 competes with YAP for binding to TEADs via a TDU domain and thereby functions as a YAP antagonist[153–155]. Although the mechanistic details of how VGLL4 is dysregulated in cancer remain to be elucidated, a rationally designed ‘super-TDU’ peptide targeted the YAP–TEAD interaction and inhibited growth of gastric cancer cells in vitro and tumour growth in vivo in mice[156] (Fig. 4). The therapeutic path ahead for these specific peptides remains challenging, but this study provides yet another promising proof of concept for targeting YAP-TEAD interactions in cancers. Future studies will address whether this approach would be broadly applicable across cancers or is specific to gastric cancer.

Altering localization and stability of YAP/TAZ–TEADs.—The nuclear–cytoplasmic shuttling of YAP and TAZ has been known for some time and is critically important for their regulation[157]. As transcription co-activators, YAP/TAZ have to be located in the nucleus for their activity. Therefore, blocking YAP/TAZ nuclear localization could be an approach to inhibit YAP/TAZ activity and curtail tumour growth. Indeed, compounds that target a range of upstream YAP/TAZ effectors have been reported. For example, stearoyl-CoA-desaturase 1 (SCD1) has been reported to promote nuclear YAP/TAZ activity in lung cancer cells, and

pharmacological inhibition of SCD1 can reduce YAP/TAZ activity[158]. YAP/TAZ stability is also a potential targeting mechanism, as YAP/TAZ degradation is controlled via multiple mechanisms. For example, YAP/TAZ degradation may be stimulated by activating specific ubiquitin–proteasome pathways or by inhibiting deubiquitination signals. Interestingly, a recent study identified p38 mitogen-activated protein kinase-dependent nuclear–cytoplasmic shuttling of TEADs in response to cellular stress[159]. TEADs also have a highly conserved bipartite nuclear localization signal[160]. TEAD4 has a short splicing form, TEAD4-S - which retains the YAP interaction domain but lacks the DBD[161] - that localizes in both the nucleus and the cytoplasm, and may function as a dominant negative. Given that expression of nuclear TEADs is increased and leads to poor prognosis across multiple cancers, blocking the nuclear entry of TEADs or promoting cytoplasmic shuttling and degradation could be a viable strategy that warrants further investigation.

Targeting core kinases in the pathway.—As discussed in this Review and elsewhere[162], extensive studies have highlighted a key role of the Hippo pathway in tissue repair and regeneration. Specifically, LATS1/2 and MST1/2 inhibitors might be useful in the context of wound healing and regeneration. Recently, pharmacological modulation of MST1/2 kinase activity with XMU-MP-1 demonstrated significant improvement in tissue repair and regeneration; XMU-MP-1 was identified through a high-throughput screening effort as an ATP-competitive inhibitor of both MST1 and MST2, and improved repair and regeneration in multiple mouse models of both acute and chronic liver injury and colitis[163]. Importantly, to address safety concerns when targeting a tumour suppressor, the study showed that treating control mice with XMU-MP-1 daily for up to 2 months did not induce tumours. This could suggest that short-term inhibition of these kinases is a possible therapeutic approach, in contrast to what could be concluded by *Stk3/4* knockout mouse phenotypes across multiple tissues, which cause hepatocellular carcinoma, colonic adenoma or disrupted lung structures and neonatal lethality [78,164,165]. It is worth noting that unlike small molecules that target the kinase activity in adult mice and are reversible, and generally incomplete, genetic deletion in mouse models causes complete inactivation and is not reversible; thus, the latter likely produces stronger effects.

Recent studies identified a surprising, yet promising, role of LATS1/2 for immunotherapy approaches in Hippo-independent cancers[60]. This study has suggested the value of developing LATS1/2 kinase inhibitors to enhance cancer cell immunogenicity. From a drug discovery standpoint, targeting the pathway using kinase inhibitors is certainly more straightforward and promising than targeting transcription factors and protein-protein interactions. However, the key concern is the wealth of evidence that LATS1/2 kinases have important tumour suppressor roles in a range of tissues - as well as a potential function in suppressing fibrosis - making safety a key liability for such inhibitors. Future studies will address whether this strategy could be safe by enabling intermittent dosing of these compounds, what determines selectivity between normal and damaged tissues, and whether there is a therapeutic window in which these kinases can be inhibited to promote tissue repair without the long-term liability of promoting tumour growth. Undoubtedly, these studies provide valuable research reagents, starting points for medicinal chemistry efforts and, more importantly, proof of concept that targeting the upstream kinases in the Hippo

pathway could provide a great path ahead in the field of repair and regeneration. Further chemical optimization of these inhibitors would be required to completely evaluate this strategy.

Future perspectives

Completion of The Cancer Genome Atlas has revealed frequent alterations of the Hippo pathway - such as activation of YAP/TAZ - in human cancer, thus suggesting the components of the Hippo pathway as targets for cancer treatment. Indeed, numerous companies are developing compounds targeting the Hippo pathway for cancer and other clinical indications. The most obvious molecular targets would be the YAP/TAZ transcription co-activators and the TEAD transcription factors, and the interaction between them. Verteporfin and VGLL4 mimetic peptides can inhibit YAP/TAZ-dependent transcription as well as suppress tumour growth in mouse models, thus validating the proof of concept of targeting this transcription module for cancer treatment. However, therapeutically targeting transcription factors or protein–protein interactions is traditionally challenging. Moreover, using systemic inhibition of YAP/TAZ warrants caution as it may produce severe side effects and toxicity. YAP/TAZ and TEAD might be appealing targets for treatment of fibrotic diseases, which are common pathological complications in the lung, heart, liver and kidney, although additional studies are required to prove these claims.

TEAD might represent a targetable transcription factor owing to its unique biochemical and structural properties. Palmitoylation seems to be important for TEAD function, protein stability and YAP interaction. Therefore, an exciting possibility is to inhibit TEAD palmitoylation to block the YAP–TEAD-dependent transcription. The palmitate moiety on TEAD is buried in a deep hydrophobic pocket inside the YBD of TEAD, which offers an attractive conformation to be inhibited by small molecules.

YAP/TAZ promotes wound healing and tissue regeneration, and, under physiological conditions, YAP/TAZ activity is restricted mainly by the LATS1/2 kinases. Protein kinases are readily druggable and the pharmaceutical industry has a plethora of successful experience in developing protein kinase inhibitor therapy. Therefore, LATS1/2 and their upstream activators MST1/2 kinases are attractive targets for wound healing and tissue regeneration. It is particularly noteworthy that high YAP activity can promote the proliferation of cardiomyocytes, which have low regeneration potential. Thus, LATS inhibitors might be valuable to promote cardiac regeneration. The concern of LATS1/2 inhibition in inducing cancer does exist; however, short-term inhibition for regeneration purposes may not cause tumour development, which could take a long time.

The scientific knowledge gained in the past decade from basic research on the emerging Hippo pathway has revealed the exciting potential of targeting the pathway components for various clinical indications. One major hurdle is that the YAP/TAZ transcription module is not readily druggable. Another key limiting factor is our incomplete understanding of the physiological functions of the Hippo pathway. However, with future advancement in fundamental biology of the Hippo pathway and new technologies, we anticipate with high enthusiasm that targeting the Hippo pathway will lead to fruitful therapeutic treatment.

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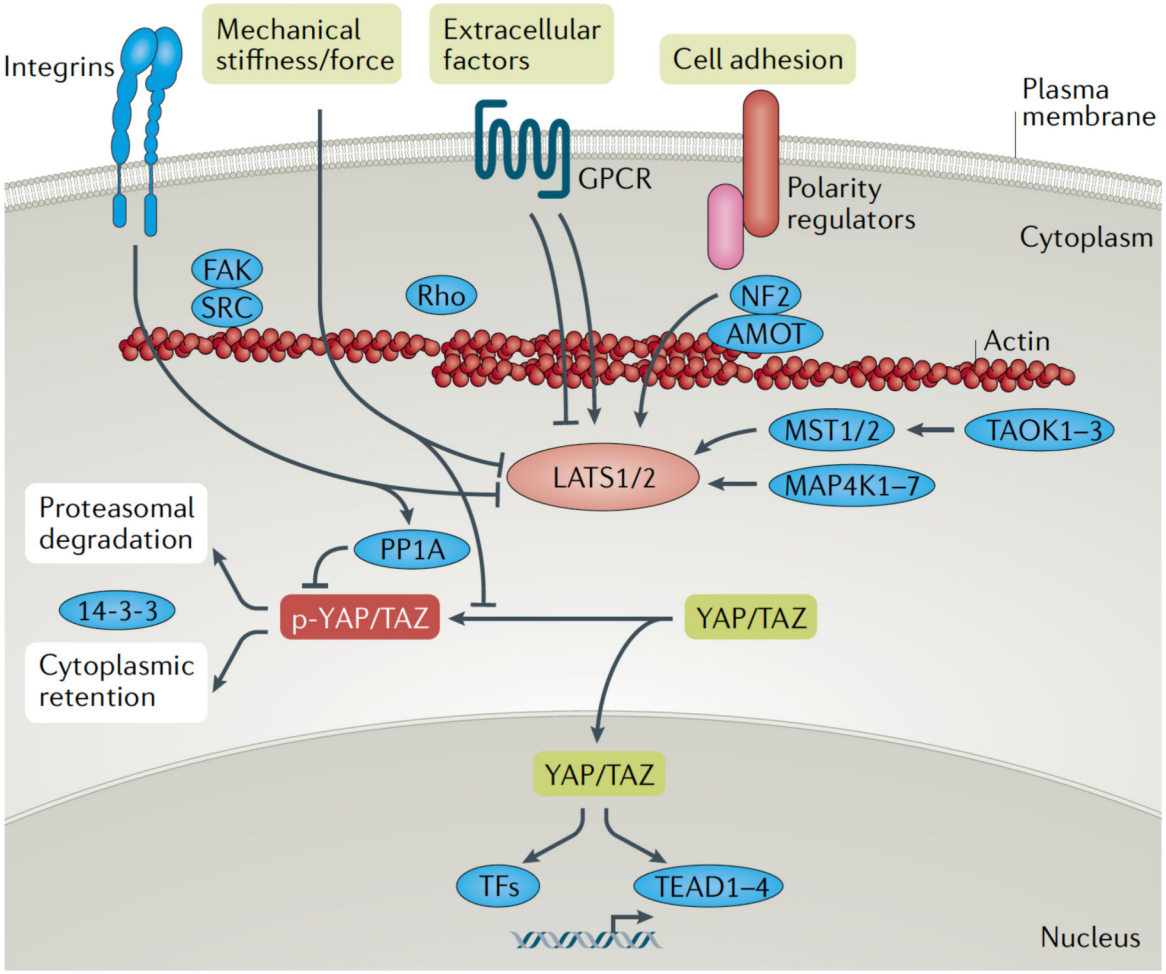

Fig 1. Key signals regulating YAP/TAZ activity.

YAP and TAZ activity is regulated by the LATS1 and LATS2 kinases, which phosphorylate YAP/TAZ on conserved residues. Phosphorylated YAP/TAZ (p-YAP/TAZ) associate with 14-3-3 proteins and are retained in the cytoplasm and targeted for degradation via the proteasome, consequently leading to low nuclear levels. Various upstream effectors of the LATS1 and LATS2 kinases have been identified, including the MST, MAP4K and TAOK families of kinases, which phosphorylate and activate LATS1/2. Cell polarity and adhesion regulators facilitate altered actin dynamics and Hippo pathway effector association to promote LATS1/2-mediated regulation of YAP/TAZ. G protein-coupled receptors (GPCRs), mechanical cues and signals transduced by the extracellular matrix and matrix-binding integrins - such as those transduced by FAK and SRC - can inactivate LATS1/2 or induce the dephosphorylation of YAP/TAZ via PP1A activation, collectively leading to hypo-phosphorylated YAP/TAZ. Hypo-phosphorylated YAP and TAZ accumulate in the nucleus, where they can bind to various transcription factors (TFs), most notably the TEAD family, to direct gene expression changes that control a range of biological events.

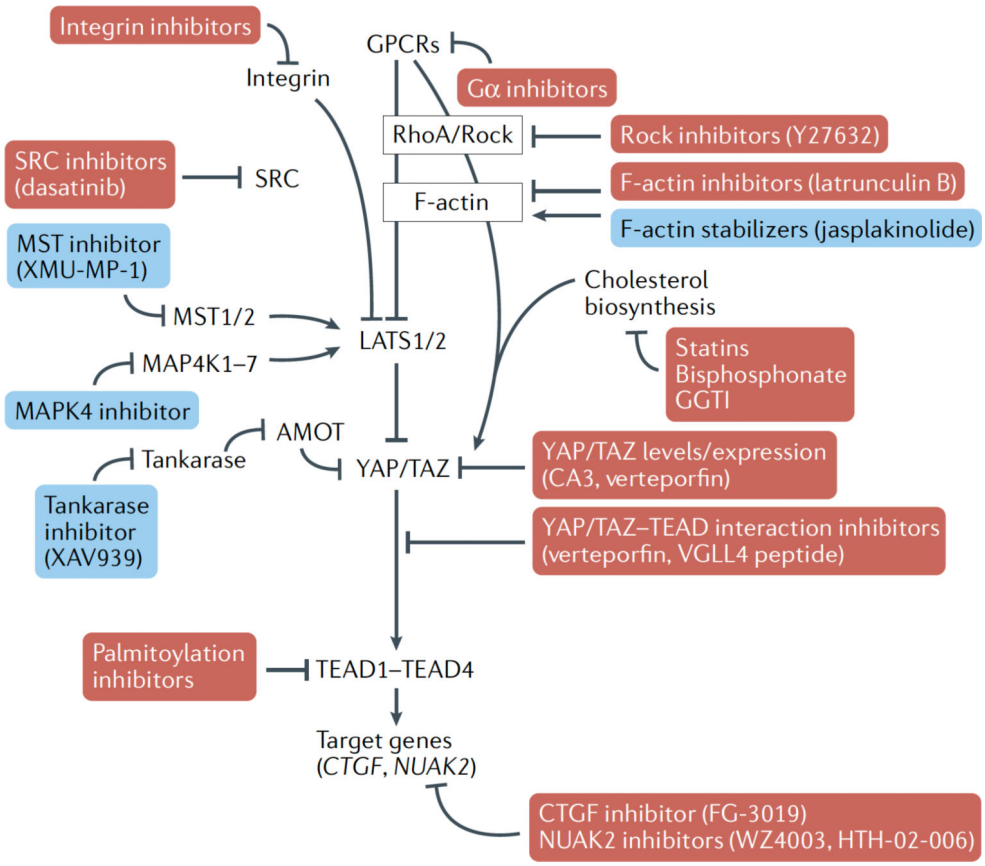

Fig. 2. Proposed strategies and small molecules (direct and indirect) that could promote or inhibit YAP/TAZ activity.

Opportunities exist for small molecules for biological intervention of key YAP/TAZ-regulatory signals that may offer targeted medical promise. These include various targets that would result in repression of YAP/TAZ nuclear activity (shown in red boxes), such as inhibitors of repressors of LATS1/2 activity, inhibitors of YAP/TAZ interacting transcription factors (such as the TEAD family) or inhibitors of important downstream target genes that drive aberrant biological processes. Activation of YAP/TAZ may also be desirable, such as in the context of medical regenerative strategies, and may be achieved by repressing the activity of effectors that promote YAP/TAZ cytoplasmic retention (shown in blue boxes). CTGF, connective tissue growth factor; GPCR, G protein-coupled receptor.

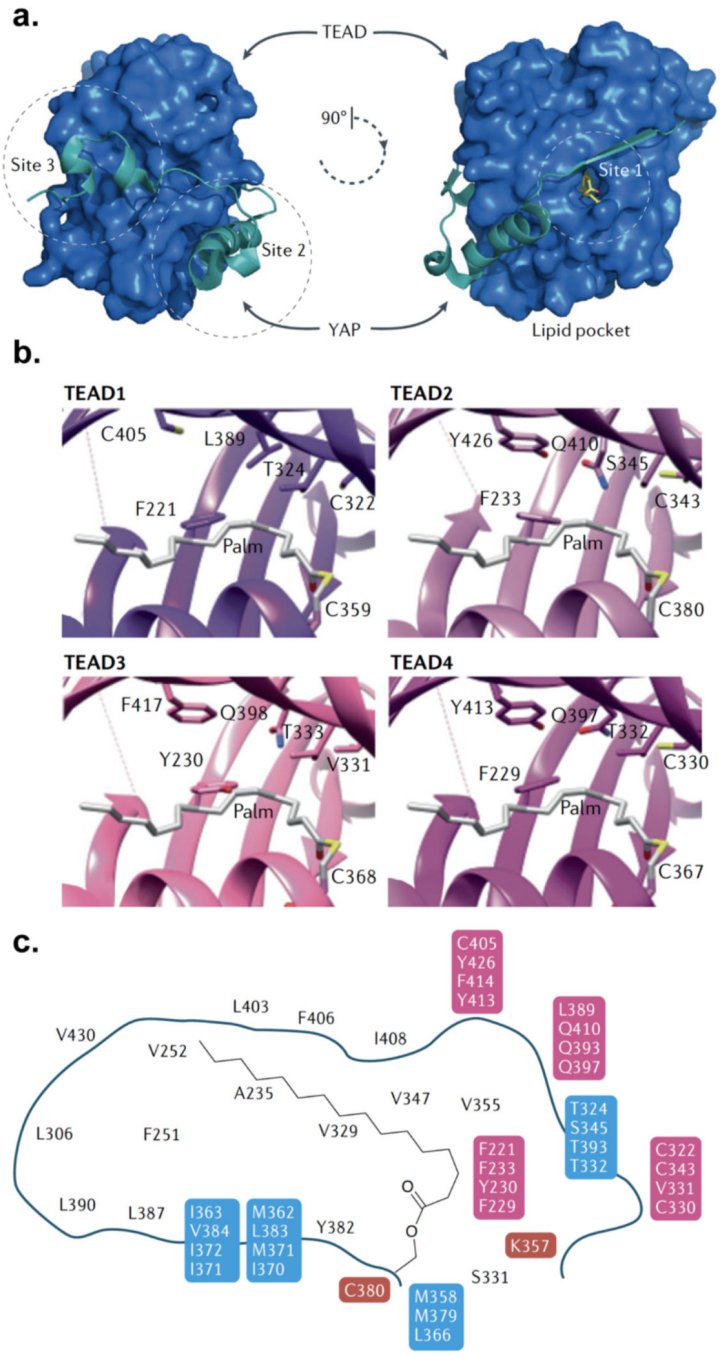

Fig. 3. Structural biology of the TEAD–YAP interface and the TEAD lipid pocket.

(**a)** TEAD proteins contain a highly conserved carboxy-terminal YAP/TAZ binding domain (YBD). The binding interface between the TEAD YBD (blue) and the TEAD-binding peptide of YAP (teal) comprises three highly conserved interfaces (sites 1, 2 and 3) that contribute to high-affinity binding. TEADs also contain a central hydrophobic lipid pocket (shown at the right) with an Spalmitoyl cysteine (yellow sticks) modification at a universally conserved cysteine. (**b)** Snapshots of the lipid pockets of all four TEADs highlight the difficulty in developing pan-TEAD lipid pocket ligands as well as providing a rubric for the

design of isoform-specific ligands. Residues shown are lipid pocket residues that are nonconserved in at least one of the four human TEAD paralogues. **(c)** Schematic representation of the human TEAD lipid pocket. Residues in black as well as those highlighted in red are invariant among human TEADs (residues in red are directly important for lipidation). Those highlighted in blue are non-identical in at least one human TEAD paralogue but still similar. Residues highlighted in pink are non-identical and non-similar in at least one paralogue.

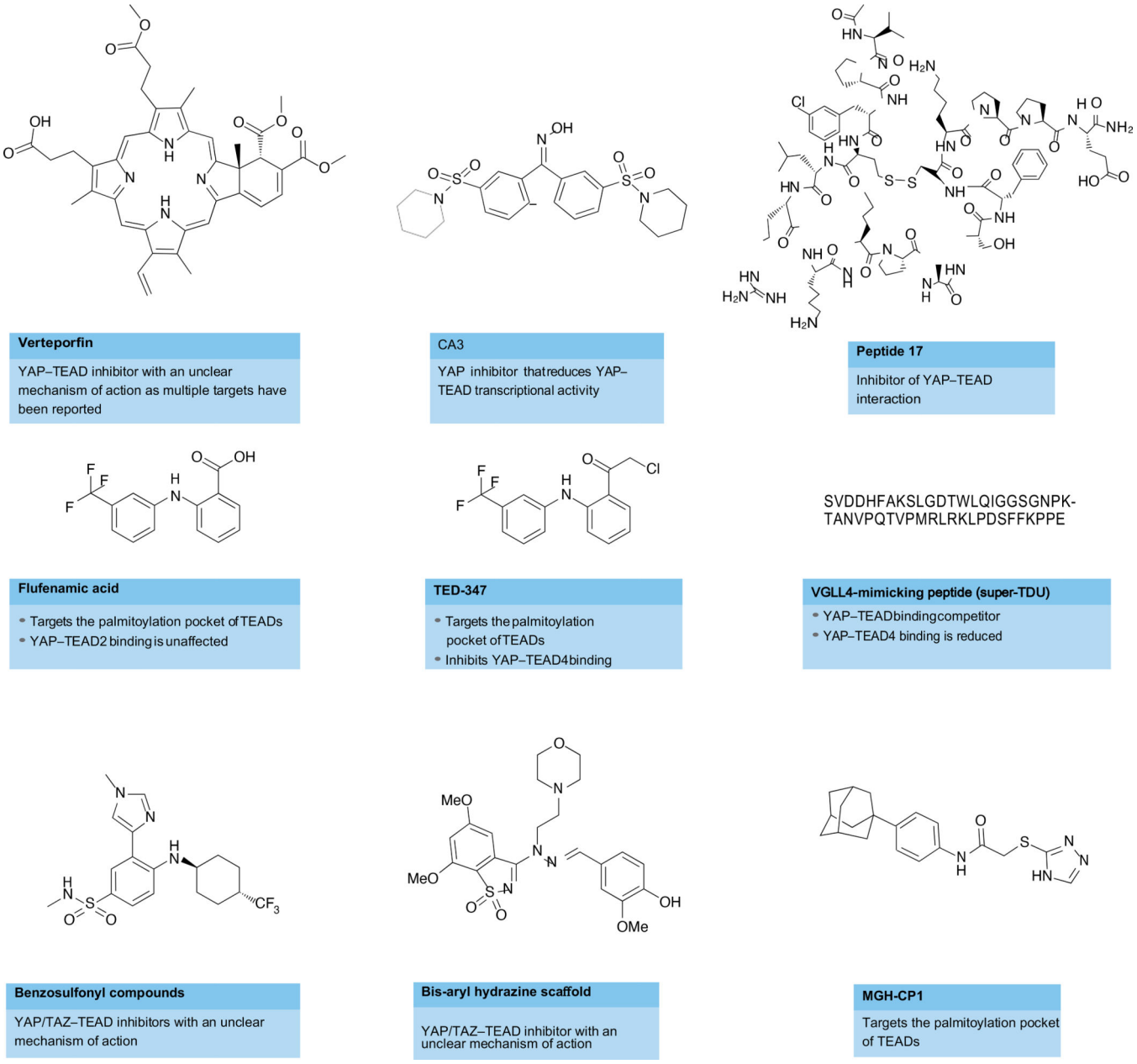

Fig. 4. Representative compounds targeting the Hippo pathway.

Key potentially druggable sites in the protein–protein interaction between YAP/TAZ and TEAD have been identified, as well as a highly conserved palmitoylation pocket in TEADs. Some small molecules that target these interactions are shown, such as Peptide 17 [210,211]. Although compounds such as verteporfin or CA3 successfully inhibit TEAD transcriptional activity and have shown antitumour activity in vivo, the target specificity and selectivity of these compounds remain to be confirmed. Other approaches have focused on disrupting the interaction between TEAD and other transcriptional co-activators, such as vestigial-like protein family members (VGLL1-VGLL4) with VGLL4-mimmicking peptides, such as ‘super-TDU’. Finally, compounds such as flufenamic acid and TED-347, which target the

lipid pocket at the core of all four TEADs and inhibit palmitoylation, have shown antitumour activity in vitro. TDU, Tondu.

Table 1.

WWTR1 and *YAP1* association with cancer

Gene association	Hippo pathway effector expression	Supportive studies
Breast carcinoma		
Hypermethylation of the promoter region of *LATS1* (reF.[166])	High expression of nuclear TAZ in high-grade breast carcinomas and triple-negative breast cancer[167,168]	Overexpression of TAZ in low-expressing non-tumorigenic mammary or breast cancer cells induces transformation, tumorigenicity and migratory activity[169]; loss of TAZ in isolated breast cancer stem cells impairs metastatic colonization and chemoresistance[169]
Cervical cancer		
WWTR1 is amplified in ~10% of cervical cancers; *YAP1* is amplified in ~12% of cervical cancers[170]	YAP levels are elevated in cervical squamous cell carcinomas[171]; LATS1 was downregulated in 45% (36/80) of cervical cancers[172]	High levels of YAP are observed in mouse models induced by transgenic expression of HPV proteins E6/E7 (reF.[173]); YAP knockdown impairs growth and YAP overexpression promotes growth of subcutaneous cervical squamous cell carcinoma xenografts in mice[173]
Colorectal cancer		
Hypermethylation of *STK3*, *WWC1* and *TAOK2* (reF.[174])	YAP expression and nuclear levels are high in colorectal cancer samples[175]; YAP-regulated gene expression is associated with poor prognosis[176]	Hyperactivation of YAP results in widespread early-onset intestinal polyp formation following dextran sodium sulfate treatment in mice[64]; deletion of *MST1*/2 leads to undifferentiated cell expansion in the small and large intestine of mice[177]
Oesophageal carcinoma		
YAP1 (11q22.1) is amplified in oesophageal carcinomas[178]	High nuclear YAP is a predictor of worse prognosis and is associated with therapy resistance[179]	YAP overexpression confers cancer stem cell properties and resistance to chemotherapy to benign oesophageal cells, and enhances subcutaneous tumour formation by the oesophageal adenocarcinoma cell line SKGT-4 (reFs[180,181])
Head and neck cancer		
Mutations in *FAT1* in ~20% of cases, amplification of *WWTR1* in ~14% of cases and amplification of *YAP1* in ~10% of cases	Elevated nuclear staining of YAP or TAZ correlates with tumour recurrence, resistance to radiotherapy, resistance to immunotherapy and poor outcome[182,183]; YAP is a potential biomarker for resistance to cetuximab[183]	YAP/TAZ knockdown impairs primary tumour growth and metastasis formation after orthotopic transplantation[184]
Hepatocellular carcinoma		
Amplification of human chromosome 11q22 (reF.[185])	Elevated YAP and TAZ levels[186]	Amplification at mouse chromosome 9qA1 in a mouse model initiated from progenitor cells; *Yap1* amplification and deletion of *Mst1*/2, *Nf2* and *Sav1* all lead to hepatocellular carcinoma[76,187–189]; YAP inhibition restores hepatocyte differentiation in advanced hepatocellular carcinoma; genetic and pharmacological disruption of YAP–TEAD–YAP activity prevents hepatocellular carcinoma[139]
Malignant mesothelioma		
NF2 alteration (32% of tumours); loss or mutation of *LATS1* (21%), *LATS2* (7%), *MST1* (16%), *MST2* (2%) and *SAV1* (14%)[190,191]	Elevated YAP levels in about 70% of mesotheliomas[192]	YAP knockdown impairs the growth of mesothelioma cell lines[193]
Meningioma		
Associated with *NF2* mutations[194]	Nuclear expression of YAP in 92% of *NF2*-mutant meningiomas[195]	YAP overexpression confers on immortalized human arachnoid cells the ability to form meningiomas[195]
Neuroblastoma		
Mutations of *PTPN14* (negative regulator of YAP) identified at relapse[196]	Elevated *TAZ* mRNA expression is prognostic of poor outcome	*TAZ* knockout impaired the growth of subcutaneous xenografts of the cell line SK-N-AS[197]; pharmacological

Gene association	Hippo pathway effector expression	Supportive studies
		inhibition of YAP/TAZ with verteporfin inhibited metastasis in mice[198]
Lung carcinomas		
Mutations in *FAT1* in ~18.5% of lung carcinomas; *WWTR1* is amplified in ~27% of lung squamous cell carcinomas[199]	Elevated YAP levels in tissues obtained from patients with acquired EGFR inhibitor resistance[54]; TAZ levels are a prognostic factor in non-small-cell lung cancer progression[200,201]	Tankyrase inhibitor sensitizes lung cancer cells to EGFR inhibition via stabilizing angiomotins and inhibiting YAP signalling[202]
Ovarian cancer		
WWTR1 is amplified in ~14% of ovarian carcinomas[203]	Elevated nuclear YAP expression and YAP/TAZ target gene expression correlate with poor prognosis[204–206]	YAP overexpression confers on fallopian tube epithelial secretory cells the ability to form tumours[207]
Undifferentiated pleomorphic sarcomas		
Loss of NF2 (6%), SAV1 (8%) or LATS2 (16%)[208]	High nuclear YAP staining in human samples	Elevated nuclear YAP staining in sarcomas arising by targeted activation of oncogenic KRAS and loss of p53 in fibroblasts; YAP knockdown impairs the growth of subcutaneous xenografts of primary mouse UPS cell lines[209]