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REVIEW

The Hippo pathway in tissue homeostasis and regeneration

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ABSTRACT

While several organs in mammals retain partial regenerative capability following tissue damage, the underlying mechanisms remain unclear. Recently, the Hippo signaling pathway, better known for its function in organ size control, has been shown to play a pivotal role in regulating tissue homeostasis and regeneration. Upon tissue injury, the activity of YAP, the major effector of the Hippo pathway, is transiently induced, which in turn promotes expansion of tissue-resident progenitors and facilitates tissue regeneration. In this review, with a general focus on the Hippo pathway, we will discuss its major components, functions in stem cell biology, involvement in tissue regeneration in different organs, and potential strategies for developing Hippo pathway-targeted regenerative medicines.

KEYWORDS Hippo, YAP, regeneration

INTRODUCTION

Tissue damage, such as traumatic or surgical injury, infection, or aging, results in the loss of cells and tissue. To maintain their physiological functionality and morphology, damaged organs must be repaired or regenerated. Some lower organisms, such as planarian and salamanders, can effectively regenerate following injury. However, most mammals have limited regenerative potential, and only a few organs, such as the liver, skin, and intestine, have some regenerative capability (Whyte et al., 2012). In some organs, such as the skin, the limited regenerative capability is compensated by excess fibrosis, which results in tissue scaring (Gurtner et al., 2012).

Tissue regeneration is a complex process involving multiple cell types. First, the tissues surrounding the damaged sites need to induce cell proliferation and differentiation to supply necessary tissue-specific cells acting as building blocks for regeneration. Second, the vascular, nervous, and immune systems as well as the extracellular matrix (ECM) need to be restored to maintain functionality of the new tissue. Thus, different cell populations are required to work in a cooperative manner to support a successful tissue regeneration (Carlson, 2007).

The origin of these "new" cells during regeneration remains controversial. At least four different mechanisms have been suggested: 1) proliferation of terminally differentiated cells (usually polyploid cells); 2) dedifferentiation of mature cells; 3) expansion and differentiation of resident progenitor cells; and 4) influx of stem cells from other tissues. It is likely that different organs employ a unique mechanism in a tissue-specific manner (Carlson, 2007).

Tissue regeneration also involves diverse cellular signaling pathways (Stoick-Cooper et al., 2007). For example, Wnt signaling plays a vital role in intestinal regeneration (Barker, 2014), hepatocyte growth factor (HGF) signaling is required for liver regeneration (Borowiak and Wigler, 2004; Huh et al., 2004), and bone morphogenetic protein (BMP) signaling is critical in digit tip regeneration (Han et al., 2003). Following injury, multiple signaling pathways are coordinated spatiotemporally, in a tissue and context-dependent manner, to ensure a successful regeneration program.

The Hippo pathway is a relatively new signaling pathway involved in tissue homeostasis, organ size control, and tumorigenesis (Yu et al., 2015b). Here, we will review what is currently known about the role of the Hippo pathway in modulating tissue regeneration.

THE HIPPO PATHWAY

The Hippo pathway has been established in *Drosophila melanogaster* as an important regulator of organ size, and this pathway is highly conserved in mammals (Pan, 2010; Halder and Johnson, 2011; Yu and Guan, 2013). The core Hippo pathway consists of a kinase cascade (Fig. 1). MST1/2 and MAP4Ks phosphorylate LATS1/2, leading to LATS1/2 activation (Full names of Hippo pathway components are shown in legends of Fig. 1). Activated LATS1/2 then phosphorylate YAP/TAZ, which results in YAP/TAZ inactivation. As the major downstream effectors of the Hippo pathway, unphosphorylated YAP/TAZ translocate to the nucleus and induce target gene transcription by interacting with the transcription factors TEADs (TEAD1–4). In addition, SAV1 and MOB1 are scaffold proteins for MST1/2 and LATS1/2, respectively, and upstream regulators such as NF2 can also induce LATS1/2 activity. Furthermore, the interaction between YAP/TAZ and TEADs is antagonized by VGLL4. The *Drosophila* orthologues of these Hippo pathway components are also shown in Fig. 1.

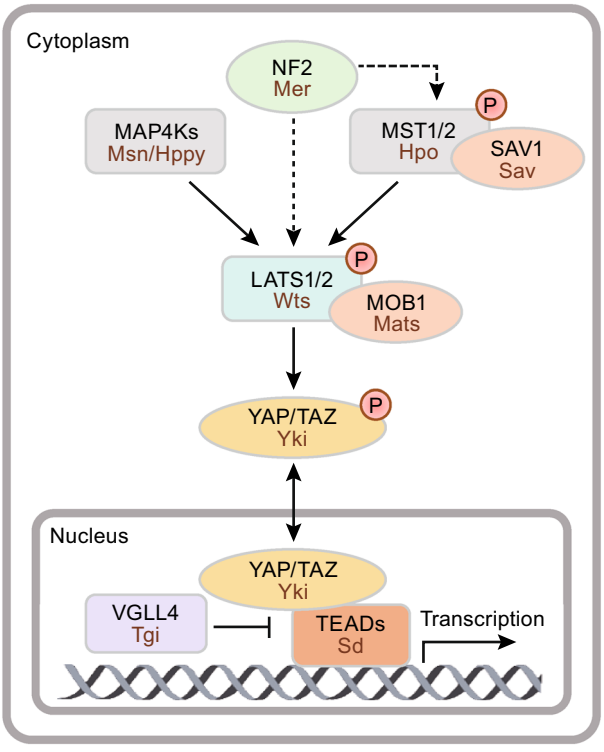

Figure 1. The Hippo signaling pathway. Major mammalian Hippo signaling pathway components and their *Drosophila* orthologues are also shown. Abbreviations: Yes Associated Protein (YAP), Transcriptional Co-Activator With PDZ-Binding Motif (TAZ, also known as WWTR1), TEA Domain Transcription Factor (TEAD), Vestigial Like Family Member 4 (VGLL4), Large Tumor Suppressor Kinase 1/2 (LATS1/2), Mammalian STE20-Like Protein Kinase 1/2 (MST1/2, also known as STK4/3), MOB Kinase Activator 1 (MOB1), Salvador (SAV1), Mitogen-Activated Protein Kinase Kinase Kinase Kinase (MAP4K), Neurofibromin 2 (NF2, also known as Merlin), Yorkie (Yki), Hippo (Hpo), Warts (Wts), Merlin (Mer), Misshapen (Msn), Happyhour (Hppy), Salvador (Sav), Marts (Mats), Scalloped (Sd), Tondu-domain-containing Growth Inhibitor (Tgi).

The Hippo pathway is regulated by a variety of signals including cell polarity, cell-cell contact, cell-ECM interaction, mechanical cues, and diffusible signals including a variety of G-protein-coupled receptor (GPCR) ligands (Yu et al., 2015b). These upstream signals of the Hippo pathway are important constituents of the stem cell niche, and undergo dynamic changes upon tissue injury. Thus, in response to injury-derived signals, the Hippo pathway may function as an immediate mechanism to mobilize tissue resident progenitor cells and initiate tissue regeneration.

THE HIPPO PATHWAY IN STEM CELL BIOLOGY

The proliferation, differentiation, and migration of stem cells are crucial during tissue regeneration, and the Hippo pathway has been shown as an important regulator in stem cell function. The first cell lineage specification during embryonic development is the emergence of the inner cell mass (ICM) and trophectoderm (TE), and the Hippo pathway plays an essential role in this process (Sasaki, 2015). High YAP activity is required for TE specification, and in mice, the cell fate of trophoblasts (TE) and embryoblasts (ICM) can be interconverted by manipulating *Yap*/*Taz* or their upstream regulators (Cockburn et al., 2013; Hirate et al., 2013; Lorthongpanich et al., 2013). This suggests that the Hippo pathway plays a key role in regulating stem cell biology at early embryonic stage.

The function of the Hippo pathway has been well studied in both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). YAP is highly expressed in self-renewing ESCs but is inactivated during differentiation (Lian et al., 2010; Tamm et al., 2011). YAP may induce the expression of pluripotency-associated genes which promote ESC self-renewal. Overexpression of *Yap* inhibits ESC differentiation and maintains stem-like properties and self-renewal even under differentiation conditions, while *Yap*/*Taz* knockdown is sufficient to result in the loss of the ESC phenotype (Varelas et al., 2008; Lian et al., 2010; Tamm et al., 2011; Beyer et al., 2013). Likewise, knockdown of *Lats2* increases the reprograming efficiency of iPSCs (Qin et al., 2012). Deletion of *Mst1/2* in ESCs causes enhanced cell proliferation and impaired differentiation (Li et al., 2013). Moreover, stem cells overexpressing *YAP* reveal naïve state-like properties (identical to stem cells from pre-implantation embryos), and the YAP activator lysophosphatidic acid (LPA) can partially substitute for YAP to promote the transition to naïve state (Qin et al., 2016).

Several recent studies suggest that YAP is dispensable for self-renewal but required for differentiation of ESCs. Knockdown or knockout of *Yap* does not alter ESC self-renewal but impairs their differentiation (Azzolin et al., 2014; Chung et al., 2016). TAZ and the TEADs are also

dispensable for ESC self-renewal (Chung et al., 2016). In addition, deletion of *Lats2* in ESCs impairs both their pluripotency and ability to differentiate (Aylon et al., 2014). This discrepancy between these studies is likely due to the high sensitivity of the Hippo pathway to different cell culture conditions. Indeed, most experiments on pluripotent stem cells are performed on cultured cells, and experimental settings may differ. Thus, further investigations are required to gain a better understanding of the function of YAP/TAZ in PSCs. Nevertheless, the mechanisms regulating the Hippo pathway in PSCs may be shared by tissue-resident progenitor cells involved in tissue regeneration.

THE HIPPO PATHWAY IN MAMMALIAN TISSUE REGENERATION

The Hippo pathway has been shown to be involved in the regeneration of several organs following tissue damage. In this section, we will review the current understanding of the functions and molecular mechanisms of the Hippo pathway in regulating tissue regeneration.

Intestine

The intestinal epithelium undergoes rapid turnover, and most differentiated cells are replaced with newer ones in less than a week (Barker, 2014). This self-renewal capability is dependent on intestinal stem cells (ISCs)—the crypt base columnar (CBC) cells marked with the leucine-rich repeat-containing GPCR5 (Lgr5) (Fig. 2). $Lgr5^+$ ISCs are actively cycling, and a single cell can grow a complete minigut comprised of all types of intestinal epithelial cells, including enterocytes, goblet cells, enteroendocrine, and Paneth cells. A population of quiescent ISCs (stem cells at +4 positions relative to the crypt bottom) may give rise to additional $Lgr5^+$ cells in response to tissue damage to promote regeneration (Li and Clevers, 2010).

To maintain stemness, $Lgr5^+$ stem cells require niche factors provided by surrounding cells such as nearby Paneth cells and myofibroblasts underneath the epithelial lining, and the function of these niche factors regulate diverse signaling pathways such as Wnt, BMP, and EGF (Crosnier et al., 2006). Wnt signaling is instrumental in intestinal homeostasis, as indicated by the essential role of Wnt3, R-spondin, and downstream β-Catenin/TCF transcription regulators in stem cell maintenance, and Lgr5 is actually a target gene of Wnt signaling (Korinek et al., 1998; Kim et al., 2005; Sato et al., 2011). Recently, the Hippo pathway has also been reported to play an important role in intestinal stem cell self-renewal and regeneration (Yu et al., 2015a). YAP is mainly expressed in $Lgr5^+$ stem cells in adult intestine (Barry et al., 2012), suggesting a role for the Hippo pathway in regulating ISC function (Fig. 2). Indeed, overexpression of *Yap*, suppression of *Lats1*/*2*, deletion of *Mst1*/*2*, or deletion of *Sav1* specifically in the intestine all lead to expansion of ISCs and defective cell differentiation, as evidenced by the loss of Paneth cells and goblet cells in the small intestine (Camargo et al., 2007; Lee et al., 2008; Cai et al., 2010; Zhou et al., 2011; Imajo et al., 2014). Surprisingly, mice with conditional knockout of *Yap* and *Taz* exhibit no visible abnormalities (Cai et al., 2010; Zhou et al., 2011; Azzolin et al., 2014). Thus, this suggests that in adult intestine, YAP is not absolutely required for normal tissue homeostasis, and high YAP activity results in a hyperplasia phenotype mainly due to the accumulation of immature cells.

However, the Hippo pathway appears indispensable for intestine tissue regeneration. In mice, tissue injuries such as dextran sodium sulfate (DSS) treatment and gamma radiation represent acute colitis and radiation enteritis, respectively. Following injuries, the intestinal epithelium undergoes an ordered regenerative program. YAP protein levels are dramatically induced following DSS treatment, and YAP is distributed in both cytoplasm and nuclei of all cells in regenerating crypts (Cai et al., 2010). Similarly, YAP is also activated following gamma irradiation, and YAP shows predominant nuclear localization (Gregorieff et al., 2015). In *Yap* cKO or *Yap*/*Taz* dcKO mice following DSS treatment or irradiation, the intestinal epithelium regeneration is defective and loss of crypts is profound (Cai et al., 2010; Gregorieff et al., 2015). *Yap* deficient mice exhibit a dramatic reduction of crypt proliferation, and the ISCs marker Olfm4 is strongly downregulated. Furthermore, transient activation of YAP may reprogram $Lgr5^+$ ISCs by partial inhibiting the Wnt pathway, which prevents ISCs from differentiating into Paneth cells and drives a pro-regenerative program through activation of the EGFR signaling pathway (Gregorieff et al., 2015), indicating an essential role for YAP (and TAZ) in intestinal regeneration.

YAP/TAZ have been shown to induce both proliferation of crypt cells and differentiation of ISCs into goblet cells, in which YAP and the TEADs regulate ISCs proliferation, while YAP and Kruppel-like factor 4 (Klf4) regulate goblet cell differentiation (Imajo et al., 2014). However, an inhibitory role for YAP in intestinal regeneration has been observed in another study: overexpression of a constitutively-active *Yap* (S127A mutant) in the mouse intestine leads to the loss of proliferative crypts, and *Yap* knockout results in hyperplastic crypts after whole-body irradiation. In the *Yap* KO intestine, Wnt target genes are upregulated which leads to expansion of ISCs and Paneth cells (Barry et al., 2012). Considering the pivotal role of Wnt signaling in ISCs, the discrepancy between this study and others may be due to the differences in the extent and duration of Wnt inhibition by YAP/TAZ. In addition, intestinal epithelial cells consistently communicate with resident immune cells, and a role of the Hippo pathway in immune response has been revealed recently (Moroishi et al., 2016), thus the differences in mouse immune background and immune-epithelial interaction may also contribute to inconsistent results. Nevertheless, further investigation is required to fully understand the function of YAP/TAZ in intestinal regeneration.

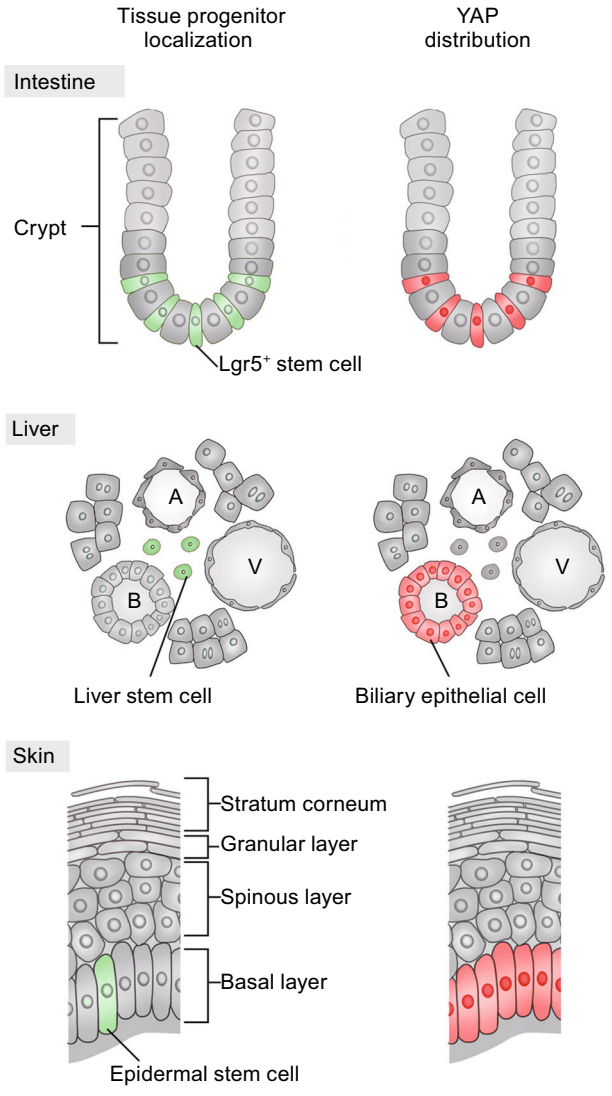

Figure 2. Localization of tissue resident stem cells and YAP expression in intestine, liver, and skin. The cellular organization of intestine crypt, portal area of liver, and epidermis are depicted. Cells shaded in green indicate tissue resident stem cells, and red paint indicates YAP expressing. Abbreviations: A, hepatic artery; V, portal vein; B, bile duct.

Liver

The liver has a remarkable regenerative capacity following chemical injury or partial hepatectomy. In response to liver injury, mature hepatocytes proliferate to compensate for cell loss, and tissue resident progenitors also emerge and participate in the regenerative process (Fig. 2). Different populations of cells around the portal area have been suggested as liver progenitor cells, such as bipotent oval cells which give rise to both hepatocytes and cholangiocytes (Miyajima et al., 2014). Recently, the Hippo pathway has been recognized as an essential regulator for regulating liver homeostasis and regeneration.

The Hippo signaling is a crucial regulator in controlling liver development and tumorigenesis. YAP upregulates TGF-β signaling to trigger proliferation of biliary epithelial cells (BEC), and reduces Hnf4α expression to inhibit hepatocyte differentiation (Lee et al., 2016). Liver-specific deletion of *Yap* leads to the loss of biliary epithelial cells, and the liver failed to develop bile ducts (Zhang et al., 2010; Lee et al., 2016). On the other hand, YAP activity is decreased during hepatocyte differentiation, and mature hepatocytes have low YAP expression and nuclear accumulation (Yimlamai et al., 2014; Yi et al., 2016). Conditional activation of YAP leads to liver overgrowth and cancer (Camargo et al., 2007; Dong et al., 2007). Similarly, liver-specific deletion of *Mst1*/*2*, *Lats1*/*2*, *Sav1*, or *Nf2* results in expansion of progenitors, liver enlargement, and liver cancer. The tumor nodules display oval cell accumulation and characteristics of hepatocellular carcinoma (HCC) and cholangiocarcinoma (Zhou et al., 2009; Benhamouche et al., 2010; Lee et al., 2010; Lu et al., 2010; Song et al., 2010; Zhang et al., 2010; Lee et al., 2016; Yi et al., 2016). Surprisingly, Yap^{S112A} knock-in mice are phenotypically normal despite that YAP shows prominent nuclear accumulation (Chen et al., 2015). Further analysis reveals that YAP/TAZ could induce a negative feedback regulation of the Hippo pathway by inducing the expression of LAST1/2 and NF2, which in turn leads to decrease of YAP/TAZ protein level (Chen et al., 2015; Moroishi et al., 2015).

YAP activity also influences liver cell fate during regeneration. In adult liver, YAP is mainly localized to the bile ductal epithelium (Yimlamai et al., 2014) (Fig. 2). Upon liver injury and inflammation, YAP is transiently activated, which promotes proliferation of progenitors and represses hepatocyte differentiation. Deletion of *Yap* in the adult liver causes inhibition of hepatocyte and bile duct proliferation after cholestatic injury (Bai et al., 2012; Su et al., 2015). On the contrary, acute deletion of *Lats1*/*2* in adult mice leads to rapid immature BEC expansion, hepatomegaly, and lethality (Lee et al., 2016). Hepatocyte-specific activation of YAP causes the emergence of cells sharing a similar identity with ductal cells, which is likely due to hepatocyte dedifferentiation as a result of Notch activation (Yimlamai et al., 2014). Taken together, appropriate YAP protein levels are crucial for controlling hepatoblast proliferation and differentiation.

Skin

The skin is the largest organ that protects the organism from external lesions. The epidermis is continuously renewed to maintain skin homeostasis. Epidermal tissue self-renewal and wound healing are mainly dependent on epidermal stem cells (Solanas and Benitah, 2013; Goodell et al., 2015).

YAP is highly expressed and predominantly nuclear in the early embryonic epidermal progenitors, and is essential for the proliferative capacity of progenitors and the development of the epidermis (Fig. 2). Epidermis-specific deletion of *Yap* at early embryonic stage causes lethality, the skin of these mice is thinner and deficient in epidermal tissue, a

notable reduction of both progenitor cells and proliferative basal cells are also observed (Schlegelmilch et al., 2011). On the other hand, the mice carrying a constitutively-active form of YAP (S127A) shows significantly increased proliferation of basal epidermal progenitors, a thicker epidermis, and hyperkeratinization of skin (Schlegelmilch et al., 2011). MST1/2 are activated during keratinocyte differentiation, deletion of their scaffold protein encoded by *Sav1* also causes perinatal lethality and the embryonic epidermal hyperplasia (Lee et al., 2008). Previous work has identified GPCRs as crucial upstream regulators of Hippo signaling (Yu et al., 2012; Yu et al., 2014). Epidermal-specific deletion of *Gnas* leads to expansion of stem cell compartments and basal-cell carcinoma-like lesions due to PKA inactivation and in part, YAP activation (Iglesias-Bartolome et al., 2015).

High YAP activity also affects differentiation of epidermal progenitors and wound healing. Deletion of *Yap* leads to a reduction of cell growth, inhibition of keratinocyte differentiation, and delay in wound healing (Elbediwy et al., 2016). In contrast, terminal differentiation of keratinocytes is blocked in *Yap* (S127A) transgenic mice (Schlegelmilch et al., 2011; Zhang et al., 2011). Thus, these studies demonstrate that YAP activity is crucial to maintain epidermal homeostasis and is indispensable for the wound healing process.

Heart

Heart growth is strictly restricted and it can be generally divided into two phases: 1) fetal heart growth is mainly due to the proliferation of cardiomyocytes; 2) soon after birth, cardiomyocytes stop proliferating, and heart size is principally controlled by the size of cardiomyocytes. Cardiomyocyte loss is a major pathogenic mechanism leading to heart failure. However, unlike other organs, the heart has been considered as a non-regenerative organ. Recently, some studies have reported that cardiomyocytes also maintain limited regenerative capacity, and cardiac progenitors which may contribute to cardiac regeneration have also been identified at different developmental stages (Laflamme, 2011; Porrello and Olson, 2014; Zhou et al., 2015). Moreover, several studies have shown that the Hippo pathway plays a crucial role in maintaining basal heart homeostasis and regulating cardiomyocyte proliferation and cardiac regeneration. It's noteworthy that the regenerative potential of adult heart is very low, and the limited recovery of morphology and function of heart following injury may be better referred as tissue repair.

YAP is expressed in the myocardium of both the fetal and postnatal mouse heart (von Gise et al., 2012; Lin et al., 2016). From neonatal to adult stage, the expression of YAP decreases, whereas the expression of VGLL4 (a competitive inhibitor of YAP, Fig. 1) increases gradually, suggesting a decrease in YAP activity with age (Lin et al., 2016). YAP simulates cardiomyocyte proliferation in a TEAD-dependent manner (von Gise et al., 2012; Morikawa et al., 2015; Lin et al., 2016), and YAP is crucial for the development of the embryonic heart, as deletion of *Yap* in the embryonic heart leads to lethality at E10.5 (Xin et al., 2011). Postnatal deletion of *Yap* also leads to increased myocardial fibrosis, cardiomyocyte apoptosis, and decreased cardiomyocyte proliferation, thereby resulting in dilated cardiomyopathy and premature death (Del Re et al., 2013; Xin et al., 2013). Embryonic deletion of *Sav1* leads to the enlargement of the heart and excessive cardiomyocyte proliferation (Heallen et al., 2011). Overexpression of *Lats2* in the mouse heart represses cardiac hypertrophy and reduces ventricle size without influencing myocardial apoptosis (Matsui et al., 2008). Thus, the Hippo pathway is indispensable for regulating embryonic heart development and maintaining basal heart homeostasis.

Recently, several groups have reported that the Hippo pathway plays a role in cardiac regeneration. Activated YAP could reduce myocardia injury and promote cardiac function (Lin et al., 2014). Inhibition of endogenous *Lats2* reduces myocardial apoptosis under stress (Matsui et al., 2008). Overexpression of *Mst1* in mice induces apoptosis and leads to lethal cardiomyopathy (Yamamoto et al., 2003; Delre et al., 2014). Suppression of endogenous *Mst1* prevents cardiomyocyte apoptosis, cardiac dysfunction, and fibrosis in the remodeling heart without influencing cardiomyocyte hypertrophy (Odashima et al., 2007). Cardiac-specific deletion of *Mst2* does not affect cardiomyocyte proliferation in the neonatal or adult heart, but reduces the pathological cardia hypertrophic response under pressure overload (Zi et al., 2014). Loss of *Sav1* in adult mouse cardiomyocytes promotes cell cycle entry and cytokinesis and enhances cardiomyocyte regeneration after myocardia injury (Heallen et al., 2013; Morikawa et al., 2015). Thus, YAP activation represents an attractive approach for promoting heart regeneration.

YAP activity may promote heart regeneration by multiple mechanisms. Expression profiling analysis shows that YAP induces expression of genes related to cell proliferation, DNA synthesis, and cytoskeletal remodeling (von Gise et al., 2012; Morikawa et al., 2015). In addition, YAP stimulates IGF-1 and Akt signaling to reduce cardiomyocyte apoptosis (Xin et al., 2011; Del Re et al., 2013; Xin et al., 2013). Moreover, YAP also binds with different transcriptional factors in the heart, such as FoxO1 and Pitx2, and promotes the expression of genes involved antioxidant response (Shao et al., 2014; Tao et al., 2016).

Together, all these studies demonstrate that appropriate YAP activity is crucial for embryonic heart development and basal heart homeostasis, and YAP could stimulate cardiomyocyte proliferation and cardiac regeneration in response to heart injury such as myocardial ischemia. However, recent studies have principally focused on the effect of the Hippo pathway on cardiomyocytes but not on cardiac fibroblasts or potential cardiac stem cells. Cardiac fibroblasts form one of the largest pools of cells in the heart and contribute to the normal structure and function of the myocardium (Souders et al., 2009). It is reported that cardiac

fibroblasts could be directly reprogrammed into adult cardiomyocyte-like cells (Qian et al., 2012). Therefore, it will be important to study the relationship between Hippo pathway and non-myocyte cells in the heart.

Nervous system

Neural stem cells (NSCs) are capable of self-renewal and generate multiple neuronal and glial lineages, which exist in both the fetal and adult nervous system in mammals. The cell cycle is strictly coordinated in NSCs to ensure precise neurogenesis (Bond et al., 2015). Recent findings indicate a crucial role for Hippo signaling in controlling NSC proliferation, fate determination, differentiation, and maturation.

YAP is selectively expressed in NSCs and astrocytes, but not neurons. In astrocytes, YAP is required for astrocytic proliferation. Deletion of *Yap* in NSCs or astrocyte leads to impaired astrogliogenesis and increased neocortical neurodegeneration (Huang et al., 2016). In the neural tube of the mouse, chicken, and frog, YAP is expressed in the ventricular zone progenitor cells and co-localizes with the neural progenitor cell marker Sox2. YAP activation leads to decreased neuronal differentiation and expansion of the neural progenitor cell population, which in part is due to the upregulation of stemness genes such as cyclin D1 (Cao et al., 2008). On the contrary, repression of either YAP or TEADs in the neural tube causes a significant increase in cell death, cell cycle exit, and differentiation of neuronal cells (Cao et al., 2008). In addition, YAP is necessary for proliferation of ependymal progenitor cells, apical attachment of progenitor cells, and maintaining the integrity of the ventricular lining of the aqueduct. Nervous-specific deletion of *Yap* in the brain obstructs the rostral aqueduct and leads to hydrocephalus (Park et al., 2016). Moreover, YAP/TAZ are also important for the morphogenesis of peripheral nerves, Schwann cells specific knockout of YAP/TAZ in mice leads to reduced cell proliferation, impaired radial sorting, and defective myelination (Poitelon et al., 2016). NF2 is localized in the apical region of NPCs and plays a crucial role in restricting NPC expansion by negatively regulating YAP/TAZ activity. NF2 promotes corpus callosum development and hippocampal morphogenesis, and deletion of *Nf2* in the mouse dorsal telencephalon causes a significant expansion of the NPCs at hippocampus and neocortex, resulting in dysgenesis of the corpus callosum and malformation of the hippocampus (Lavado et al., 2013; Lavado et al., 2014).

THE HIPPO PATHWAY IN *DROSOPHILA* TISSUE REGENERATION

The function of the Hippo pathway in tissue regeneration has also been studied in *Drosophila*. The *Drosophila* midgut is equivalent to the small intestine in mammals. In *Drosophila*, the Hippo pathway is also critical for maintaining midgut homeostasis. The Hippo pathway restricts proliferation of ISCs under normal physiological conditions. Suppression of Wts or Hpo, mutation of the Kibra binding partner Pez, or loss of Msn increases ISC proliferation and causes a significant hyperplasia phenotype in the midgut (Ren et al., 2010; Shaw et al., 2010; Staley and Irvine, 2010; Poernbacher et al., 2012; Li et al., 2014). However, high Yki activity is required for injury-related proliferation of ISCs (Karpowicz et al., 2010; Ren et al., 2010; Staley and Irvine, 2010; Poernbacher et al., 2012). The activation of Yki leads to the production of unpaired (Upd) family cytokines and EGFR ligands, which then activate Jak/Stat and EGFR signaling and accelerate ISC division during intestine regeneration (Karpowicz et al., 2010; Ren et al., 2010; Shaw et al., 2010).

In *Drosophila* nervous system, NSCs remain quiescent at early larval stages and proliferate at embryonic and adult phases, and the Hippo pathway has been shown to control the quiescence of NSCs. Yki is inactive and localized in the cytoplasm when NSCs are quiescent, and Yki will relocate to the nucleus to regulate NSC proliferation and growth during NSC reactivation. Suppression of Hippo pathway upstream regulators such as *Wts* leads to premature exit from quiescence and reactivation of NSCs (Ding et al., 2016). Poon et al. also demonstrated that the Hippo pathway restricts proliferation of neural stem cells, controls neuroblast reactivation from quiescence during postembryonic neurogenesis of *Drosophila*, and perturbation of *Tao*, *Hpo*, or *Wts*, or overexpression of *Yki*, leads to brain overgrowth (Poon et al., 2016). Moreover, the Hippo pathway can modulate asymmetric cell division of NSCs (Keder et al., 2015) and regulate glial cell proliferation (Reddy and Irvine, 2011). In the future, it will be interesting to study the function of the Hippo pathway in neural degenerative diseases or neural regeneration following injuries.

THE HIPPO PATHWAY AND REGENERATIVE MEDICINE

Regenerative medicine refers to medical approaches which promote functional regeneration of damaged tissues or organs, such as stimulation of intrinsic regenerative/repair mechanisms by molecular therapy, or transplantation of tissues or stem/progenitor cells cultured in laboratories (Lane et al., 2014). Due to the shortage of donors compared to the increasing needs of tissue/organ transplantation, there is an urgent need for the development of novel regenerative medicines.

YAP/TAZ activity is generally high during embryonic development, but soon declines to a basal level after birth. During tissue injury, YAP/TAZ activity is immediately reactivated in a transient manner, and transient activation of YAP/TAZ can promote expansion of progenitors or dedifferentiation of mature cells to facilitate tissue regeneration (Fig. 3A). Thus, activation of YAP/TAZ is a potential strategy to promote tissue regeneration.

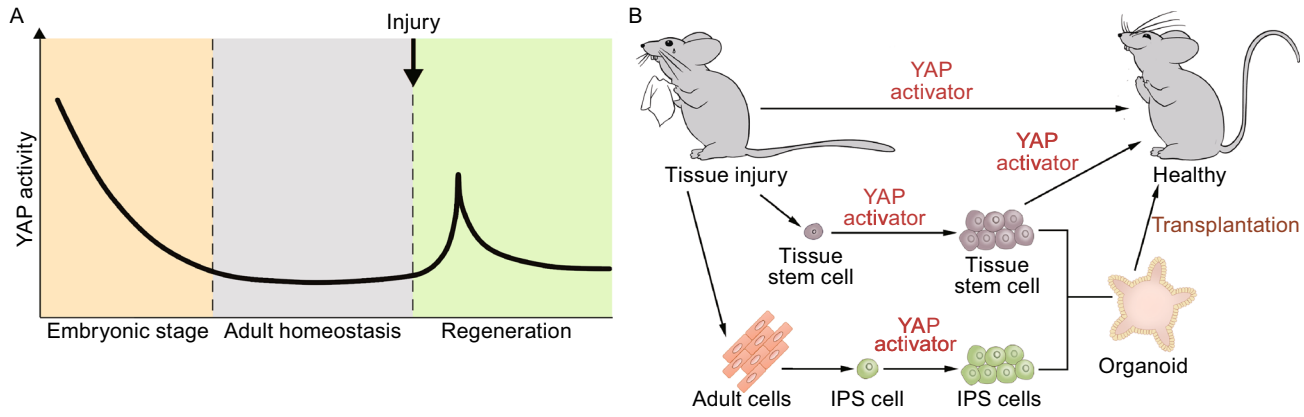

Figure 3. The Hippo signaling in tissue regeneration and regenerative medicine. (A) YAP/TAZ activity is declined to a base line level after birth, and immediately reactivated in a transient manner following tissue injuries. (B) The potential of YAP/TAZ activation in regenerative medicine. YAP/TAZ activators or effectors (either small or macro molecules) directly induce tissue regenerative program *in vivo*; in addition, YAP/TAZ activation may promote expansion of tissue resident stem cells or iPSCs *in vitro*, which in turn can be transplanted back to injury sites to facilitate tissue regeneration.

The Hippo pathway consists of a kinase cascade, and therefore, inhibiting upstream kinases represents an ideal approach to activate YAP/TAZ. Systematic or local delivery of Hippo pathway kinase inhibitors could, in principle, induce this regenerative program (Fig. 3B). Recently, an MST1/2 inhibitor has been discovered and has shown good efficacy in promoting liver and intestinal regeneration (Fan et al., 2016). Inhibitors for MAP4Ks or LATS1/2 may have a similar effect in promoting regeneration. Gene therapy is an effective approach in regenerative medicine (Ruiz and Regueiro, 2012), introducing small interfering RNA or microRNA mimics targeting the pathway components or YAP target genes by viral- or viral free- approaches may benefit tissue regeneration (Yin et al., 2014).

An alternative approach is to deliver macromolecules to damaged tissues to facilitate tissue regeneration. Some YAP/TAZ targeting genes encode secretory proteins, and these proteins may have regenerative potential. Indeed, YAP targets Epiregulin and CTGF have been shown to promote tissue repair in the mouse intestine and zebrafish spinal cord, respectively (Gregorieff et al., 2015; MH et al., 2016).

In addition to *in vivo* reprogramming of regenerative process using a molecular approach, regeneration may also be promoted by transplantation of *in vitro* expanded progenitors, organoids, or tissues (Fig. 3B). In recent years, a variety of organoids have been cultured successfully *in vitro*, including the stomach, liver, kidney, lung, gut, brain, and retina (Clevers, 2016). However, it is still difficult to control the complicated biological parameters such as the cell type, organization, and cell-cell or cell-matrix interactions within an organoid system (Yin et al., 2016). In a recent study, intestinal organoid formation has been fine-tuned by differential YAP activity associated with matrix stiffness (Gjorevski et al., 2016). Moreover, YAP/TAZ transient activation can efficiently convert differentiated mammary, neuronal, and pancreatic cells into a progenitor cell state, and these cells can form organoids and be used for transplantation (Panciera et al., 2016). Thus, modulating the Hippo pathway may represent a useful approach for enrichment of progenitor cells or differentiated organoids for regenerative medicine.

Given the importance of the Hippo pathway in cell plasticity, novel and specific activators of YAP/TAZ may be a powerful tool for promoting tissue regeneration. While the Hippo field is largely focused on developing YAP/TAZ inhibitors for treating cancer (Gong and Yu, 2015), it might be equally important to develop YAP/TAZ activators for regenerative medicine. Moreover, long term activation of YAP/TAZ may lead to tumorigenesis, thus caution should be taken when using YAP/TAZ activators in regenerative medicine.

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ABBREVIATIONS

BEC, biliary epithelial cells; BMP, bone morphogenetic protein; CBC, crypt base columnar; DSS, dextran sodium sulfate; ECM, extracellular matrix; ESCs, embryonic stem cells; GPCR, G-protein-coupled receptor; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; ICM, inner cell mass; iPSCs, induced pluripotent stem cells;

ISCs, intestinal stem cells; LPA, lysophosphatidic acid; NSCs, neural stem cells; TE, trophectoderm.

COMPLIANCE WITH ETHICS GUIDELINES

Yu Wang, Aijuan Yu, and Fa-Xing Yu declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by the any of the authors.

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