

REVIEW

The Hippo pathway regulates stem cell proliferation, self-renewal, and differentiation

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ABSTRACT

Stem cells and progenitor cells are the cells of origin for multi-cellular organisms and organs. They play key roles during development and their dysregulation gives rise to human diseases such as cancer. The recent development of induced pluripotent stem cell (iPSC) technology which converts somatic cells to stem-like cells holds great promise for regenerative medicine. Nevertheless, the understanding of proliferation, differentiation, and self-renewal of stem cells and organ-specific progenitor cells is far from clear. Recently, the Hippo pathway was demonstrated to play important roles in these processes. The Hippo pathway is a newly established signaling pathway with critical functions in limiting organ size and suppressing tumorigenesis. This pathway was first found to inhibit cell proliferation and promote apoptosis, therefore regulating cell number and organ size in both *Drosophila* and mammals. However, in several organs, disturbance of the pathway leads to specific expansion of the progenitor cell compartment and manipulation of the pathway in embryonic stem cells strongly affects their self-renewal and differentiation. In this review, we summarize current observations on roles of the Hippo pathway in different types of stem cells and discuss how these findings changed our view on the Hippo pathway in organ development and tumorigenesis.

KEYWORDS Hippo pathway, stem cell, YAP, Lats, differentiation, self-renewal

INTRODUCTION

Stem cells have the capacity to proliferate and differentiate

into multiple cellular lineages, also defined as pluripotency. There are different classifications of stem cells that reflect the range of possible cell types they can produce and the ways from which the stem cells are derived. These include embryonic stem cells (ESCs), progenitor cells, mesenchymal stem cells (MSCs), and induced pluripotent stem cells (iPSCs). ESCs are derived from inner cell mass of blastocysts and can undergo extensive self-renewal *in vitro* and have the ability to undergo differentiation into all cell lineages, also called totipotency. Therefore ESCs are the cells of origin for multi-cellular organisms. Progenitor cells, which are derived from more developed fetal or adult tissues, are multipotent, meaning they give rise to more restricted lineages than ESCs. These potential lineages are usually determined by the tissue of origin. For example, liver progenitor cells or liver stem cells are capable of differentiating into cell types within a liver. MSCs are multipotent stem cells derived from tissue of mesoderm origin that can differentiate into a variety of cell types, including but not limited to: osteoblasts, chondrocytes, and adipocytes. However, MSCs do not have the capacity to reconstitute an entire organ. iPSCs are a type of pluripotent stem cells artificially derived from non-pluripotent cells, typically an adult somatic cell, for example by expression of a defined combination of transcription factors (Takahashi and Yamanaka, 2006). Due to the bypass of blastocyst as starting material, iPSCs are promising sources of stem cells for future medical use. More recently, studies of neoplastic tissues have provided evidence of self-renewing, stem-like cells within tumors, which have been called cancer stem cells (CSCs) (Reya et al., 2001). Despite rapid progress in generating stem-like cells from different sources and efforts in translating these cells to practical medical use, the underlying molecular mechanism of stem cell self-renewal, differentiation, and proliferation is not clear. Recently, the newly established Hippo signaling pathway was found to play important roles in each of these different types of stem cells.

These findings will be summarized and discussed below.

THE *DROSOPHILA* HIPPO PATHWAY

Defined organ size is one of the most visible features of multi-cellular organisms. The regulation of organ size is a highly coordinated process governed by both physiological cues and an intrinsic mechanism. The underlying mechanism of organ autonomous size determination has remained largely unknown until the past decade. Extensive research has led to the identification of the Hippo tumor suppressor pathway as a key regulator of organ size in *Drosophila* and mammals (Pan, 2010). By genetic mosaic screens, mutation of the first batch of Hippo pathway genes *warts* (*wts*) (Justice et al., 1995; Xu et al., 1995), *hippo* (*hpo*) (Harvey et al., 2003; Jia et al., 2003; Pantalacci et al., 2003; Udan et al., 2003; Wu et al., 2003), and *salvador* (*sav*) (Kango-Singh et al., 2002; Tapon et al., 2002) were found to dramatically promote tissue overgrowth and organ size enlargement. These genes belong to the hyperplastic group of *Drosophila* tumor suppressors wherein mutations of these genes result in robust tissue overgrowth without alterations of cell fate determination or cell polarity (Hariharan and Bilder, 2006). Biochemical studies further revealed that Hpo directly interacts with Sav to phosphorylate and activate the complex formed by Wts and another core Hippo pathway protein, Mats (Wu et al., 2003; Lai et al., 2005; Wei et al., 2007) (Fig. 1A). The kinase activity of Hpo is antagonized by a newly identified PP2A phosphatase complex, dSTRIPAK (Ribeiro et al., 2010). By yeast two-hybrid, the Pan group identified the transcription co-activator Yorkie (Yki) as a Wts-interacting protein and a potent effector of the Hippo pathway (Huang et al., 2005). Subsequent biochemical studies showed that Wts directly phosphorylates and inhibits Yki (Dong et al., 2007). Scalloped (Sd), the homolog of mammalian TEAD family transcription factors, were found to be critical partners of Yki in the regulation of gene expression (Goulev et al., 2008; Wu et al., 2008; Zhang et al., 2008; Zhao et al., 2008; Zhang et al., 2009a). Several transcriptional targets of the Hippo pathway have been identified, including *cyclin E*, which directly promotes cell cycle progression and cell proliferation, and *diap1*, which inhibits apoptosis (Tapon et al., 2002; Jia et al., 2003; Udan et al., 2003; Wu et al., 2003; Lai et al., 2005). The Hippo pathway also inhibits expression of a microRNA *bantam* to affect organ size, although the downstream effector of *bantam* is not clear. Furthermore, Yki-Sd was shown to transcriptionally induce *dMyc*, a potent promoter of ribosome biogenesis and cell growth (Neto-Silva et al., 2010; Ziosi et al., 2010), which may mediate the cell competition phenomenon observed in tissues with imbalance of Hippo pathway activity. In addition, Yki induces *E2F1* (Goulev et al., 2008), which may be involved in cell-autonomous regulation of cell proliferation; the EGFR ligands *Vein*, *Keren*, and *Spitz* (Zhang et al., 2009b; Ren et al., 2010); the Jak/Stat pathway ligands

Unpaired1/2/3 (*Upd1/2/3*) (Karpowicz et al., 2010; Ren et al., 2010; Shaw et al., 2010; Staley and Irvine, 2010), which could mediate non-cell-autonomous function of the Hippo pathway; and the Hippo pathway genes *Ex*, *Kibra*, *Crb*, and *Fj* (Cho et al., 2006; Hamaratoglu et al., 2006; Genevet et al., 2009; Genevet et al., 2010), which may constitute a signal feedback loop.

Upstream regulators of the Hippo pathway have been intensively pursued in the past years, which led to identification of many new Hippo pathway components. Two apical cytoskeleton-binding proteins, Merlin (Mer) and Expanded (Ex) (Hamaratoglu et al., 2006), and Kibra, which interacts with Mer and Ex (Baumgartner et al., 2010; Genevet et al., 2010; Yu et al., 2010), were found to activate the Hippo pathway. Double mutations of these genes produce phenotypes closely resembling those caused by mutations of Hippo pathway components. In addition, Pez may activate the Hippo pathway by binding to Kibra (Poernbacher et al., 2012). The Fat protocadherin, a cell surface molecule, was also identified as an upstream regulator of the Hippo pathway (Bennett and Harvey, 2006; Cho et al., 2006; Silva et al., 2006; Willecke et al., 2006; Tyler and Baker, 2007). Removing one copy of *yki* dramatically suppressed the *fat* mutant overgrowth phenotype (Bennett and Harvey, 2006; Silva et al., 2006; Willecke et al., 2006), indicating that *yki* is an important mediator of *fat* function. Fat activity is regulated by binding to another protocadherin, Dachsous (Ds) (Matakatsu and Blair, 2006), and is modulated by several proteins such as the casein kinase Discs overgrown (Dco) (Feng and Irvine, 2009; Sopko et al., 2009), the Golgi-resident kinase Four-jointed (Fj) (Rogulja et al., 2008; Willecke et al., 2008; Simon et al., 2010), and the Fat/Ds-interacting protein Lowfat (Lft) (Mao et al., 2009). A LIM domain-containing protein Zyxin (Zyx) also inhibits Hippo signaling possibly by physical interaction with Wts and Dachs downstream of Fat (Rauskolb et al., 2011). dJub, another LIM domain-containing protein that physically interacts with Wts and Sav, was shown to negatively regulate Hippo signaling, although the detailed mechanism has not been delineated (Das Thakur et al., 2010). A number of cell polarity and cell adhesion proteins were also found to regulate the Hippo pathway. These include the Scribble (Scrib)-Discs large (Dlg)-Lethal giant larvae (Lgl) complex, atypical protein kinase C (aPKC), Crumbs (Crb), and Echinoid (Ed) (Chen et al., 2010a; Grzeschik et al., 2010; Ling et al., 2010; Robinson et al., 2010; Yue et al., 2012), indicating a role of cell polarity and cell adhesion in Hippo pathway regulation. Furthermore, two studies identified Tao-1, a STE20 family protein kinase as a positive regulator of the Hippo pathway, possibly by directly phosphorylating and activating the Hpo kinase (Boggiano et al., 2011; Poon et al., 2011). In addition, homodimerization was also found to regulate Hpo kinase activity (Jin et al., 2012). Another exciting finding is that accumulation of F-actin *in vivo* by loss of actin capping proteins or expression of active formin leads to tissue over

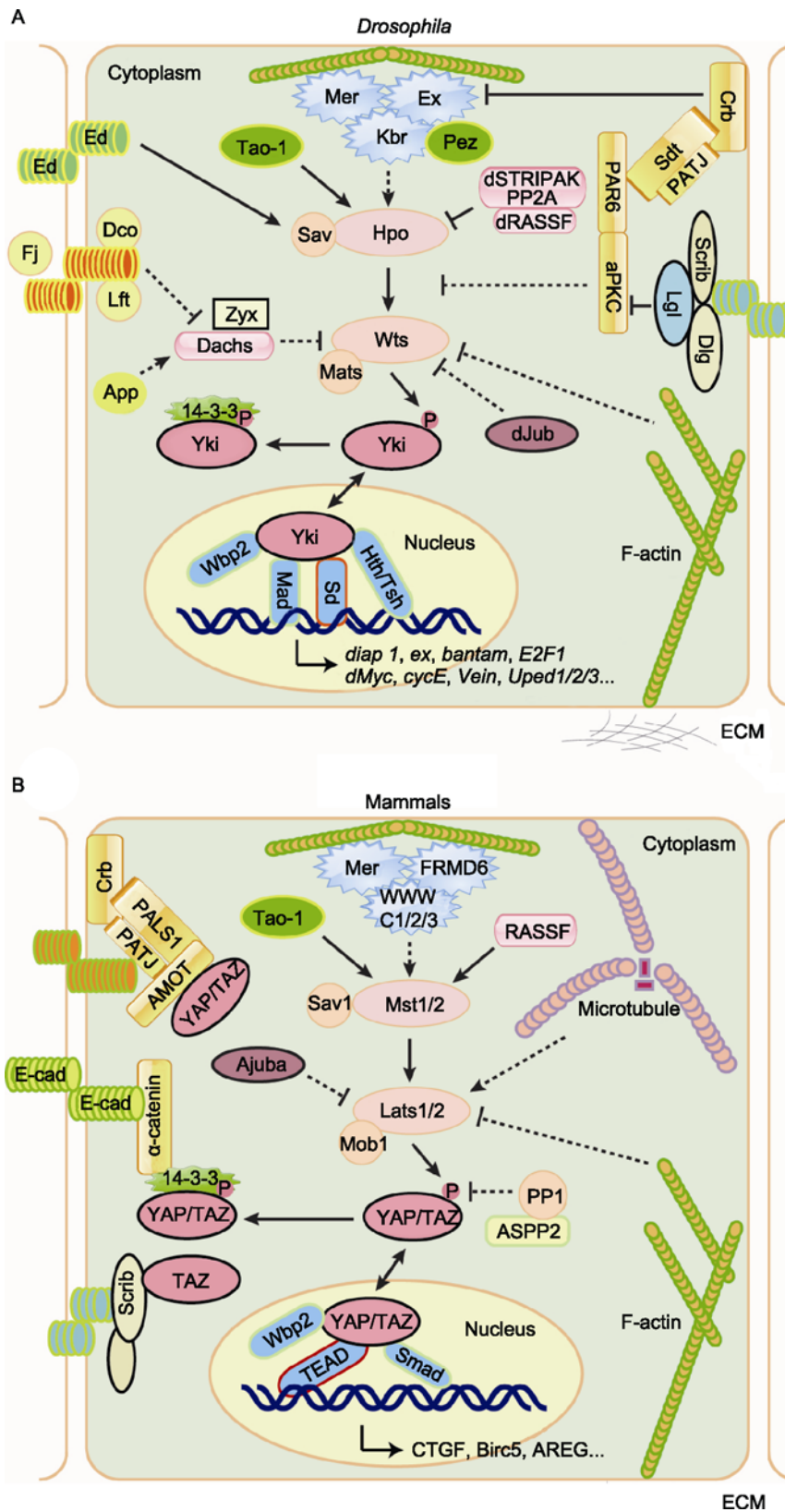


Figure 1. The Hippo pathway in *Drosophila* and mammals. Corresponding proteins in *Drosophila* (A) and mammals (B) are indicated by matching colors. Arrowed or blunted ends indicate activation or inhibition, respectively. Dashed lines indicate unknown mechanisms.

growth due to inhibition of the Hippo pathway (Fernández et al., 2011; Sansores-Garcia et al., 2011). Such a regulation may mediate signal from epithelial architecture and external mechanical stress.

THE MAMMALIAN HIPPO PATHWAY

The core components of the *Drosophila* Hippo pathway are highly conserved in mammals such as Mst1/2 for Hpo, Sav1 for Sav, Lats1/2 for Wts, MOBKL1A and MOBKL1B (collectively referred to as Mob1) for Mats, and YAP and its paralog TAZ for Yki (Fig. 1B). It has been demonstrated that human YAP, Lats1, Mst2, and Mob1 can rescue the phenotypes of their corresponding *Drosophila* mutants *in vivo* (Tao et al., 1999; Wu et al., 2003; Huang et al., 2005; Lai et al., 2005), suggesting the functional conservation of these proteins. The core components Mst1/2 are known to be pro-apoptotic kinases that are activated by caspase cleavage under apoptotic stress (Graves et al., 1998). Sav1 is known to interact with Mst1/2 through the SARAH domains present in both Sav1 and Mst1/2 (Callus et al., 2006). While Sav1 has been shown to activate Mst1/2, the underlying mechanism is unclear. It has been suggested that Sav1 plays a role in the nuclear translocation of Mst1 (Lee et al., 2008). In mammalian cells, Mst1/2 are also activated by binding to Ras association domain family (RASSF) proteins (Oh et al., 2006; Guo et al., 2007) possibly due to alteration of Mst1/2 subcellular localization (Khokhlatchev et al., 2002; Praskova et al., 2004). Recently, Mst1/2 were reported to partially co-localize with actin cytoskeleton, disruption of which leads to mild activation of the kinase (Densham et al., 2009). The activation of Mst1/2 leads to phosphorylation and activation of their direct substrates, Lats1/2 (Chan et al., 2005). Mob1, which forms a complex with Lats1/2 (Chow et al., 2010), is also phosphorylated and activated by Mst1/2, resulting in enhanced interaction between Lats1/2 and Mob1 (Hirabayashi et al., 2008; Praskova et al., 2008). Similar to that in *Drosophila*, MST1/2/Sav1 and Lats1/2/Mob1 form a physical and functional core of the Hippo pathway. Activated Lats1/2 in turn phosphorylate YAP/TAZ transcription co-activators on several residues (Dong et al., 2007; Zhao et al., 2007; Hao et al., 2008; Lei et al., 2008; Oka et al., 2008). Phosphorylation of S127 in YAP promotes 14-3-3 binding, resulting in cytoplasmic sequestration and therefore inactivation of YAP (Dong et al., 2007; Zhao et al., 2007; Hao et al., 2008; Lei et al., 2008; Oh and Irvine, 2008). Indeed, mutation of S127 and disruption of 14-3-3 binding lead to activation of YAP (Zhao et al., 2007), confirming the inhibitory nature of this phosphorylation. YAP phosphorylation by Lats also leads to YAP inhibition through protein degradation. Phosphorylation on YAP S381 primes subsequent phosphorylation by another kinase, possibly casein kinase 1 (CK1 δ/ϵ), thereby activating a phosphorylation-dependent degradation motif termed phosphodegron. Subsequently, the activated phosphodegron recruits

the E3 ubiquitin ligase SCF β -TRCP, leading to poly-ubiquitination and degradation of YAP (Zhao et al., 2010). This mechanism is conserved in TAZ but not in Yki (Liu et al., 2010), which lacks a residue equivalent to S381.

Besides phosphorylation, other mechanisms for YAP and TAZ inhibition have been reported. YAP could interact with angiomin (AMOT) family proteins (Varelas et al., 2010; Wang et al., 2010; Chan et al., 2011; Zhao et al., 2011), which results in YAP localization to tight junction and YAP inhibition through phosphorylation-dependent and -independent mechanisms (Zhao et al., 2011). YAP and TAZ also interact with another tight junction protein ZO-2, which was reported to increase nuclear localization of YAP and tight-junction localization of TAZ, respectively (Oka et al., 2010; Remue et al., 2010). Interestingly, a major adherens junction protein alpha-catenin could also bind to and inhibit YAP by mediating its cell-cell junction and cytoplasmic localizations (Schlegelmilch et al., 2011; Silvis et al., 2011). Clearly, protein-protein interaction is important in the physiological regulation of YAP. Similar to that in *Drosophila*, the mammalian Hippo pathway is also regulated by cytoskeleton. In response to cell detachment or soft matrix, the Hippo pathway kinases Lats1/2 are activated resulting in YAP and TAZ phosphorylation and inhibition and further leading to anoikis or cell fate decision (Dupont et al., 2011; Wada et al., 2011; Zhao et al., 2012). Such regulation is largely due to the remodeling of cytoskeleton because the effect could be mimicked or blocked by interfering with actin or microtubule cytoskeleton. These studies further demonstrated the possibility of Hippo pathway as a mediator of cell shape and mechanical stress in regulation of cell physiology.

The TEAD family transcription factors were identified to be critical partners of YAP and TAZ in the regulation of gene expression (Goulev et al., 2008; Wu et al., 2008; Zhang et al., 2008; Zhao et al., 2008; Zhang et al., 2009a). Disruption of YAP-TEAD interaction abolishes YAP-dependent gene transcription and largely diminishes YAP-induced cell proliferation, oncogenic transformation, and epithelial-to-mesenchymal transition (EMT) (Zhao et al., 2008). The crystal structure of YAP-TEAD complexes reveals that the N-terminal domain of YAP wraps around the globular structure formed by the C-terminal domain of TEAD (Chen et al., 2010b; Li et al., 2010; Tian et al., 2010). Particularly, a short peptide of YAP from residues 86 to 100 plays the most important role in YAP-TEAD interaction by fitting side chains into a deep pocket formed by TEAD. These residues could be good targets for pharmacological intervention of YAP-TEAD interaction. It is interesting to note that mutation of TEAD1 Y406, which is causal to a human genetic disease Sveinsson's chorioretinal atrophy (Fossdal et al., 2004), results in loss of interaction with YAP due to disruption of a hydrogen bond with YAP residue S94 (Kitagawa, 2007; Li et al., 2010). Precise regulation of YAP-TEAD interaction is therefore important in maintaining normal physiology.

Connective tissue growth factor (CTGF) is the best-characterized direct target gene of YAP-TEAD. It was shown to play an important role in YAP-induced proliferation and anchorage-independent growth (Zhao et al., 2008). CTGF alone, however, does not account for all YAP phenotypes. YAP and TAZ also induce the expression of *AREG* (Zhang et al., 2009b) and *FGF1* (Hao et al., 2008), which may also mediate non-cell-autonomous function of the Hippo pathway. However, the mechanisms underlying the induction of these genes, including the responsible transcription factors, are mostly unclear.

Functions of the Hippo pathway in organ size determination and tumor suppression have been confirmed in genetically engineered mouse models. For instance, liver-specific overexpression of YAP in transgenic mice results in enlarged liver, which is reversible upon cessation of YAP overexpression (Camargo et al., 2007; Dong et al., 2007). However, sustained YAP overexpression eventually leads to formation of tumors characteristic of hepatocellular carcinoma (HCC) (Dong et al., 2007). Furthermore, genomic amplification and elevated expression and nuclear localization of YAP has been observed in human cancers (Overholtzer et al., 2006; Zender et al., 2006; Dong et al., 2007; Zhao et al., 2007; Steinhardt et al., 2008; Hall et al., 2010). Overexpression of TAZ has also been noted in human breast cancer samples and non-small cell lung cancer cell lines (Chan et al., 2008; Zhou et al., 2011b). Similar to YAP overexpression, ablation of the Hippo pathway components Mer and Sav and double knockout of Mst1/2 in mice result in liver enlargement and tumor formation characteristic of HCC and cholangiocarcinoma (CC) (Zhou et al., 2009; Benhamouche et al., 2010; Lee et al., 2010; Lu et al., 2010; Song et al., 2010; Zhang et al., 2010). Aberrant Mst1/2 and Lats1/2 expression is observed in human cancers (Hisaoaka et al., 2002; Jiménez-Velasco et al., 2005; Takahashi et al., 2005; Jiang et al., 2006; Minoo et al., 2007; Seidel et al., 2007; Cho et al., 2009; Zhou et al., 2009). Lats2 is also found mutated in human mesothelioma cell lines and non-small cell lung cancer (Strazisar et al., 2009; Murakami et al., 2011). Additionally, Sav1 and Mob1 mutations have been observed in human cancer cell lines and skin melanomas, respectively (Tapon et al., 2002; Lai et al., 2005). Together, these studies highlight a significant role of the Hippo pathway in organ size regulation and tumorigenesis.

THE HIPPO PATHWAY LIMITS THE POOL OF TISSUE-SPECIFIC PROGENITOR CELLS

The Hippo pathway was originally thought to regulate cell proliferation and apoptosis, thus affecting organ size. However, most organs contain not only differentiated cell types but also a pool of stem or progenitor cells. In some organs, such as pancreas, organ size is known to be regulated by the number of embryonic organ-specific progenitor cells (Stanger

et al., 2007). In adults, these cells may also contribute to organ size homeostasis and regeneration after injury. Later evidence supports that in many tissues the Hippo pathway has a stronger impact on the tissue-specific stem cell compartment, possibly inhibiting their proliferation or self-renewal. It was first observed that intestinal-specific overexpression of YAP or knockout of Mst1/2 in mice caused marked expansion of the stem cell compartment (Camargo et al., 2007; Zhou et al., 2011a). Knockout of Hippo pathway components Mst1/2, Sav1, and Mer in liver also leads to accumulation of liver stem cells (Benhamouche et al., 2010; Lee et al., 2010; Lu et al., 2010; Song et al., 2010). The regulation of liver and intestinal stem cells by the Hippo pathway and its role in regeneration were discussed in great detail elsewhere (Chen et al., 2012).

The effect of the Hippo pathway on neural stem cells (NSCs) was also studied in several species. In a chicken neural tube model, overexpression of YAP markedly expands the neural progenitor cells (Cao et al., 2008). In this case, YAP clearly promotes cell proliferation in these cells as indicated by cell cycle markers. At the same time, differentiation was inhibited as shown by the attenuation of differentiation markers. The activity of YAP in neural progenitor cells depends on TEAD because a TEAD-binding-deficient form of YAP could not promote neural progenitor cell expansion. In contrast to overexpression, knockdown of YAP or introduction of a dominant-negative TEAD leads to apoptosis or differentiation of neural progenitor cells. Loss of FatJ, homolog of the *Drosophila* Fat protocadherin, causes similar phenotypes as YAP overexpression, and the phenotype was reversed by knockdown of YAP (Van Hateren et al., 2011). In *Xenopus*, overexpression or inhibition of YAP also leads to expansion or shrinkage of the pool of neural progenitors respectively (Gee et al., 2011). In *Drosophila*, mutation of *fat* or overexpression of Yki causes delay of neuroblast differentiation (Kawamori et al., 2011). Furthermore, the expression of YAP in developing mouse forebrain is restricted to the stem cell compartment (Li et al., 2012). Such a stem cell-specific expression pattern of YAP has also been observed in mouse intestine (Camargo et al., 2007). In mouse brain, YAP was also found to be a direct target of Notch intracellular domain, which provides a mechanism for Hippo pathway and Notch signaling cross-talk in NSC self-renewal (Li et al., 2012). However, the physiological signals stimulating the Hippo pathway in NSC to regulate self-renewal and proliferation are yet to be identified.

Skin is an organ constantly replenished by dividing progenitors. The role of the Hippo pathway in skin progenitors has also been studied. Similar to intestine and brain, the expression and nuclear localization of YAP is also significantly higher in the basal epidermis progenitor compartment (Schlegelmilch et al., 2011). Nuclear YAP progressively declines with age and correlates with proliferative potential of progenitors (Zhang et al., 2011). K14-Cre-driven expression of YAP caused marked expansion of basal progenitor cells

(Schlegelmilch et al., 2011; Zhang et al., 2011). Staining of specific markers suggested that these cells were actively proliferating. Differentiation was also repressed as indicated by the expression of stem cell markers rather than those of terminal differentiation. Consistently, knockout of YAP or knock-in of a TEAD-binding-deficient form of YAP inhibits progenitor proliferation and leads to failure of skin expansion (Schlegelmilch et al., 2011). Perhaps the most interesting discovery about the Hippo pathway in skin progenitors is the identification of alpha-catenin as a direct inhibitor of YAP. Knockout of alpha-catenin clearly increases nuclear localization of YAP (Schlegelmilch et al., 2011; Silvis et al., 2011). Alpha-catenin is an adherens junction protein in association with E-cadherin and beta-catenin, and plays a key role in epithelium integrity. However, knockdown of E-cadherin and beta-catenin does not exhibit similar effect on YAP localization. It was found that alpha-catenin directly binds to YAP, which may explain the specificity (Schlegelmilch et al., 2011; Silvis et al., 2011). Knockout of alpha-catenin in bulge stem cell also leads to nuclear localized YAP and skin squamous cell carcinoma (Silvis et al., 2011). The role of YAP in alpha-catenin-mediated stem cell expansion and tumorigenesis needs to be further demonstrated by mice with double knockout of alpha-catenin and YAP. Whether the Hippo pathway still plays a role in YAP inhibition with the presence of alpha-catenin is a question not fully addressed. One study suggests that knockout of the Hippo pathway kinases Mst1/2 had no effect on skin progenitor cells (Schlegelmilch et al., 2011). However, another study of Sav1 knockout mice demonstrated a skin phenotype in embryos closely resembles that of YAP overexpression (Lee et al., 2008). Thus the role of classical Hippo pathway in restricting YAP activity in skin progenitors needs to be further clarified.

Despite the important role of the Hippo pathway in the stem cell compartment of several organs, not all tissue-specific progenitors are regulated by this pathway. One such example is the hematopoietic system. Transgenic expression of YAP caused unaltered progenitor cell compartment and no changes in the distribution of the hematopoietic lineages compared to control mice (Jansson and Larsson, 2012). Similarly, knockout of Sav1 in mouse heart leads to cardiomegaly due to abnormal proliferation of cardiomyocytes, but the number of cardiac progenitor cells remained normal (Heallen et al., 2011). Therefore, it would be important to further investigate the regulation, function, and specificity of the Hippo pathway in each organ in order to understand the role of the Hippo pathway in both differentiated cells and stem cells in organ development. Such knowledge would be a prerequisite for regenerative medicine use of the Hippo pathway in the future.

THE HIPPO PATHWAY REGULATES ES CELL SELF-RENEWAL AND IPS CELL GENERATION

It is an exciting discovery that the Hippo pathway plays im-

portant roles in differentiation and expansion of tissue-specific progenitor cells. Since then, it becomes a question in the field whether the Hippo pathway could also regulate differentiation, or on the other hand self-renewal, of the more pluripotent ESCs. It was long known that YAP is highly expressed in ESCs (Ramalho-Santos et al., 2002). However, only until recently, it was demonstrated that YAP as well as its transcription factor partner TEAD promotes ES cell self-renewal. When mouse ESCs were induced to differentiate, YAP was inactivated as indicated by decreased protein level and increased phosphorylation (Lian et al., 2010; Tamm et al., 2011). However, YAP overexpression prevents mouse ESCs from differentiation even under differentiation condition such as leukemia inhibitory factor (LIF) withdrawal (Lian et al., 2010; Tamm et al., 2011). In addition, YAP knockdown leads to loss of pluripotency under a condition that would normally support stemness (Lian et al., 2010; Tamm et al., 2011). Chromatin immunoprecipitation (ChIP) experiments demonstrated that YAP-TEAD bind to promoters of many stemness-promoting genes such as Oct4 (Lian et al., 2010; Tamm et al., 2011). Intriguingly, knockdown of TAZ in human ESCs also led to differentiation and loss of pluripotency, although YAP was intact (Varelas et al., 2008). Such a difference in TAZ and YAP requirement by human and mouse ESCs might be explained by the differential requirement of TGFbeta or bone morphogenetic protein (BMP) signaling by human and mouse ESCs respectively. TAZ was shown to promote Smad2/3 nuclear localization in response to TGFbeta signaling and YAP was demonstrated to support Smad1-dependent transcription under BMP signaling (Varelas et al., 2008; Alarcón et al., 2009). However, the mechanism responsible for specificity of YAP and TAZ in BMP and TGFbeta signaling is not known. Another report suggests the regulation of YAP by LIF, which is required by mouse but not human ESCs, which is another possible explanation for the role of YAP and TAZ in the self-renewal of human and mouse ESCs (Tamm et al., 2011).

Separation of trophectoderm (TE), which gives rise to extraembryonic tissue, from inner cell mass (ICM), where ESCs were derived from, is the first differentiation event during development. Recent genetic studies found the Hippo pathway transcription factor TEAD4 as a determinant of TE specification (Yagi et al., 2007; Nishioka et al., 2008). Further studies suggest a model that the Hippo pathway was inactivated in outer cells during blastocyst formation due to cell "crowdness," which activates YAP to drive TEAD4-dependent expression of TE-specific genes such as Cdx2 (Nishioka et al., 2009). In ICM, the Hippo pathway remains active and YAP is inhibited to prevent TE-specific gene expression. If such a model were proved to be correct, the YAP and possibly TAZ transcription co-activators would be dispensable in early ESCs during development, which needs to be demonstrated by concomitant knockout of both YAP and TAZ.

The recent development of iPSC technology is a milestone in stem cell research. It has been demonstrated that

expression of a defined set of transcription factors such as Oct4, Sox2, Klf4 (OSK), and Myc can reprogram differentiated adult cells into pluripotent stem cells (Takahashi and Yamanaka, 2006). Therefore the reprogramming process breaks some kind of barriers to reset cell status. By comparing somatic cells with iPSCs, the Hippo pathway gene *Lats2* was found to be significantly repressed during reprogramming, and may therefore represent such a barrier (Qin et al., 2012). Indeed, knockdown of *Lats2* increased efficiency of human iPSC generation about three fold without accelerating cell proliferation (Qin et al., 2012). The elevated reprogramming efficiency depends on TAZ because concomitant knockdown of TAZ blocked the effect of *Lats2* knockdown. In an independent report, co-expression of YAP was also shown to improve the efficiency of human iPSC generation by OSK (Lian et al., 2010), although in a separate study, YAP did not show any significant effect when all four factors OSKM were used (Chia et al., 2010). Nevertheless, it is still clear that inhibition of the Hippo pathway would improve iPSC generation although the role of TAZ and YAP in different species might be different.

THE HIPPO PATHWAY DIRECTS MESENCHYMAL STEM CELL DIFFERENTIATION

MSCs are cells originating from bone marrow that could contribute to several mesenchyme-derived tissues such as bone, cartilage, fat and muscle. These cells are not pluripotent; however, they are multipotent due to their ability to differentiate into multiple lineages. Deregulation of MSC differentiation underlies human pathological conditions, such as the decreased osteogenesis and increased bone marrow adipogenesis during aging or immobility (Verma et al., 2002). The lineage commitment of MSCs could be governed by specific transcription events in response to soluble factors. For example, activation of peroxisome proliferator activated receptors (PPARs) by rosiglitazone largely promotes adipogenesis of MSCs (Tontonoz et al., 1994). Interestingly, TAZ but not YAP was found to interact with and inhibit the activity of PPAR γ (Hong et al., 2005). Consistently, TAZ inhibits adipogenesis of MSCs. According to this model YAP would not be able to inhibit adipogenesis due to lack of interaction with PPAR γ . This speculation awaits confirmation. TAZ also promotes osteogenesis, possibly due to activation of Runx transcription factors (Hong et al., 2005). YAP also shows potent stimulation of osteogenesis (Dupont et al., 2011). Therefore, the Hippo pathway effectors TAZ and YAP are capable of directing MSC differentiation lineages. It has not been tested whether TEAD family transcription factors play a role in TAZ- and YAP-mediated differentiation of MSCs. Although TEADs seem unnecessary according to the current model of TAZ and YAP in regulation of PPAR γ and Runx activity, it has been reported that TAZ drives aberrant osteoblastic and chondrocytic differentiation of glioma stem cells in a TEAD-

dependent manner (Bhat et al., 2011). Therefore, the ability of YAP and TAZ in directing MSC differentiation may not be an atypical TEAD-independent function, although again it awaits further confirmation.

More interestingly, the recent finding of YAP and TAZ being regulated by matrix stiffness and cytoskeleton may solve another mystery of MSC differentiation. A fascinating feature of MSCs is that they could differentiate into different lineages when cultured on matrixes with different stiffness mimicking their natural tissue environment (Engler et al., 2006). For example, they differentiate into adipocytes on soft matrix and osteoblasts on stiff matrix. However, the key factor regulating differentiation program in response to matrix stiffness and mechanical stress was not known until recently it was found to be YAP and TAZ. It was demonstrated that on stiff matrix these Hippo pathway transcription co-activators localize to cell nuclei (Dupont et al., 2011). However, on soft matrix, they translocate to cytoplasm. Such a regulation depends on actin cytoskeleton remodeling but was reported to be independent of the Hippo pathway (Dupont et al., 2011). Nevertheless, in another report comparing cell attachment on stiff matrix or complete detachment, the Hippo pathway kinases *Lats1/2* were found to be activated by cell detachment, also in a cytoskeleton-dependent manner (Zhao et al., 2012). It is well established that *Lats1/2* directly phosphorylate YAP/TAZ and trigger their cytoplasmic translocation (Zhao et al., 2007; Lei et al., 2008). Therefore it would be important to clarify whether *Lats1/2* are mediating mechanical response of YAP/TAZ, and if not, what is the mechanism underlying YAP/TAZ regulation in this context. It has been long suspected that the Hippo pathway might be regulated by cell shape and cytoskeleton due to the involvement of several cytoskeleton-associated proteins such as Mer and Ex in the pathway. The identification of YAP and TAZ in regulation of MSC differentiation in response to mechanical stress also provides an excellent platform to test these possibilities.

THE HIPPO PATHWAY AND CANCER STEM CELLS

Recent studies of neoplastic tissues have provided evidence of self-renewing, stem-like cells within at least some types of tumors, called cancer stem cells (CSCs) (Reya et al., 2001). Although CSCs represent only a small portion of cells in a tumor, they carry the ability to seed new tumors. The differences that separate CSCs from the rest of cancer cells would therefore be a good therapeutic target in order to eradicate cancer. Interestingly, by gene expression profiling, the gene expression signature of high grade, high CSC content mammary tumors was found to overlap with TAZ/YAP-induced gene expression signature, suggesting an important role of TAZ/YAP in CSCs (Cordenonsi et al., 2011). More importantly, knockdown of TAZ impairs the self-renewal of breast CSCs as indicated by comprised CSC markers, mammosphere formation and tumor seeding *in vivo* (Corde-

nonsi et al., 2011). In contrast, overexpression of TAZ promotes CSC marker expression and mammosphere formation in non-CSC cancer cell populations (Cordenonsi et al., 2011). The EMT program was known to elicit stem cell properties from epithelial cells (Mani et al., 2008). And both TAZ and YAP have been shown to induce EMT in cultured mammary epithelial cells (Overholtzer et al., 2006; Lei et al., 2008). Interestingly, induction of EMT by other transcription regulators of EMT such as Twist also elevated TAZ expression (Cordenonsi et al., 2011). Therefore it was asked whether TAZ promotes CSC self-renewal by inducing EMT or *vice versa*. It was shown that re-expression of E-cadherin could not reverse TAZ-induced CSC self-renewal (Cordenonsi et al., 2011). However, it was unknown whether re-expression of E-cadherin completely reversed TAZ-induced EMT, especially the mesenchymal features, which might be more important to the function of TAZ in promoting CSC self-renewal. It was shown in non-epithelial glioma stem cells that TAZ is associated with the expression of mesenchymal markers and higher-grade gliomas, which are less differentiated (Bhat et al., 2011). One possibility is that TAZ is downstream of EMT-inducing transcription factors and upstream of EMT, as well as EMT-induced CSC self-renewal. To finally resolve this issue requires identification of the molecular mechanisms

mediating TAZ-induced EMT and EMT-induced TAZ expression. Besides breast cancer, YAP and TEAD expression was shown to be much higher in CSC compartment of certain type of medulloblastomas (Fernandez-L et al., 2009). In addition, knockout of Hippo pathway proteins in mouse liver also induces accumulation of tumorigenic liver stem cells, which would be discussed elsewhere (Zhou etc. al., this issue,).

CONCLUSIONS AND PERSPECTIVES

Extensive studies in the last decade have established an important role of the Hippo pathway in organ size control and tumorigenesis. Recent findings regarding functions of the Hippo pathway and its effectors YAP and TAZ in several types of stem cells further raised the significance of the pathway, and possibly complicated the situation, also. YAP and TAZ under regulation by the Hippo pathway and possibly other unknown mechanisms promotes proliferation and self-renewal of tissue-specific progenitors and ESCs, increases iPSC generation efficiency, directs lineage commitment of MSCs during differentiation, and promotes CSC self-renewal and tumor formation and progression (Fig. 2). On the other hand, the Hippo pathway could also function in differentiated somatic cells to promote proliferation, inhibit apoptosis, and

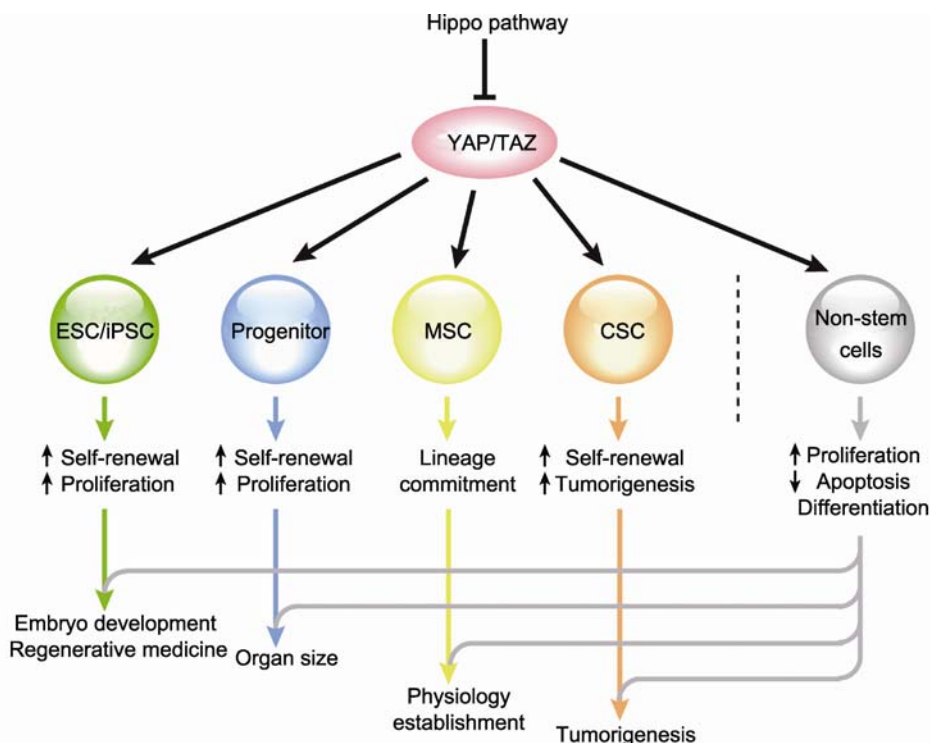


Figure 2. Function of the Hippo pathway in stem cells and differentiated cells. YAP/TAZ under regulation by the Hippo pathway functions in multiple types of stem cells as well as differentiated non-stem cells to regulate proliferation, self-renewal, differentiation, and apoptosis. Depending on cell type and organ context, the functions of the Hippo pathway in stem cells and non-stem cells coordinately control embryo development, organ size, tumorigenesis, and establishment of cell physiology. The ability of YAP/TAZ to promote reprogramming might be useful for regenerative medicine.

regulate differentiation. These mechanisms integrate in an unclear manner to reach delicate regulation of organ size. Disturbance of the balance may result in cancer development and progression. Therefore, although the big picture seems to be on the horizon, key questions remain to be answered. Besides the questions raised above, these still include the mechanism regulating the Hippo pathway in stem cells and how is that different from the mechanisms in differentiated cells. Furthermore, the mechanism of YAP/TAZ in regulation of stem cell self-renewal should also be investigated and what is the relationship of this mechanism with that controlling cell proliferation should also be studied. Examining the cross-talk between the Hippo pathway and other signaling cascades might be important to answer these questions. In addition, it is important to understand the contributions of stem cells and differentiated cells in Hippo pathway-dependent organ size control and tumorigenesis. Eventually, it would be exciting to explore the possibility of pharmacological alteration of the Hippo pathway for cancer therapy or regenerative medicine purposes.

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ABBREVIATIONS

BMP, bone morphogenetic protein; CSCs, cancer stem cells; CTGF, connective tissue growth factor; EMT, epithelial-to-mesenchymal transition; ESCs, embryonic stem cells; ChIP, chromatin immunoprecipitation; iPSCs, induced pluripotent stem cells; LIF, leukemia inhibitory factor; MSCs, mesenchymal stem cells; RASSF, Ras association domain family; PPARs, peroxisome proliferator activated receptors

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