# Chapter 13
# Hippo Signaling and Stem Cells

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**Abstract** The normal growth and development of an organ is dependent on the precise balance of stem cell self-renewal and differentiation. Slightest aberrations in signals stem cells receive can cause growth abnormalities and cancer. Emerging data suggest that the highly conserved Hippo signaling pathway can directly regulate stem cell proliferation and maintenance to control organ size. Furthermore, deregulation of the pathway promotes cancer stem cell-like properties and leads to tumor formation. Together, these findings implicate that the Hippo pathway modulates the dynamic activity of stem cells in tissue repair, regeneration, and development. Here, we summarize the latest findings that establish the role of Hippo pathway in stem cell biology.

**Keywords** Stem cell • Cancer • Hippo pathway • Organ size • Cancer stem cell

## 13.1 Introduction

Stem cells are unique cell types that can differentiate to produce diverse cells in the body. During development, the regulation of stem cell number is key in determining the final size of an organ (Depaepe et al. 2005; Stanger et al. 2007). For instance, depletion of forebrain neural progenitors in developing mice leads to reduced cortical

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size. Conversely, increasing the progenitor number causes increased cortical size leading to exencephalic forebrain overgrowth (Depaepe et al. 2005). A similar phenomenon has been described in the mouse pancreas where the size of the progenitor cell pool, set aside in the developing pancreatic bud, determines the size of the organ (Stanger et al. 2007). Clearly, proper regulation of organ size requires that stem cells integrate the surrounding environmental cues and respond appropriately; however the mechanism by which this occurs is poorly understood.

The Hippo Signaling pathway has emerged as a key regulator of organ size control. The pathway was first characterized in *Drosophila melanogaster* where deregulation of the pathway was shown to cause a strong overgrowth phenotype (Justice et al. 1995). The *Drosophila* Hippo signaling pathway is highly conserved through evolution having mammalian orthologues of all pathway components. Consistent with its role in organ size regulation in *Drosophila*, the mammalian Hippo pathway (Fig. 13.1) is also linked to organ size regulation mainly by controlling cell proliferation and apoptosis. The core mammalian Hippo pathway consists of the STE20 family kinases, MST1 and MST2 together with their regulatory protein SAV1. MST1/2 form an activated complex when bound to SAV1 (Pan 2010; Zhao et al. 2011). Interaction with the RASSF family of proteins also activates these kinases (Khokhlatchev et al. 2002; Oh et al. 2006) which then phosphorylate the NDR family kinases LATS1 and LATS2 (Dong et al. 2007; Chan et al. 2005; Hirabayashi et al. 2008). MST1/2 also phosphorylate the MOB1 complex (MOBKL1A and MOBKL1B) to enhance the interaction with the LATS1/2 kinases. MOB1 acts as a regulator of LATS1/2 activity (Praskova et al. 2008).

LATS1/2 kinases phosphorylate the paralogous transcriptional coregulators Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) (Hao et al. 2008). Phosphorylation of YAP/TAZ promotes their interaction with the 14-3-3 family members thereby keeping them localized in the cytoplasm (Dong et al. 2007; Hao et al. 2008; Oh and Irvine 2008; Zhao et al. 2007; Lei et al. 2008; Oka et al. 2008). The binding of 14-3-3 is primarily mediated by Ser89 residue in human TAZ (Ser 87 in mouse TAZ) and Ser127 in human YAP (Ser112 in mouse YAP) (Kanai et al. 2000; Basu et al. 2003). Low LATS1/2 activity allows the unphosphorylated YAP/TAZ to localize into the nucleus and execute their transcriptional functions. In the nucleus, YAP/TAZ can act as coactivators for several transcription factors (Mauviel et al. 2012) (Sudol and Harvey 2010) although preferentially coregulating the members of the TEAD family of transcription factors (Zhao et al. 2008; Schlegelmilch et al. 2011). There are four members of the mammalian TEAD family transcription factors that share the same TEA DNA-binding domain (Kaneko and DePamphilis 1998; Jacquemin et al. 1998). At least one member of the TEAD transcription factor family is expressed in almost all adult tissues (Jacquemin et al. 1998; Kaneko et al. 1997). The upstream regulators of the pathway, potentially sensing the environmental cues, are not well established. The Neurofibromatosis2 gene product NF2 (also known as Merlin) is the only upstream component that is functionally validated in vivo (Hamaratoglu et al. 2006; Zhang et al. 2010). However, it is still unclear how the cytoskeleton and membrane-associated NF2 protein signals to the MST kinases. A more thorough description of these and other pathway components can be found in other chapters of this book.

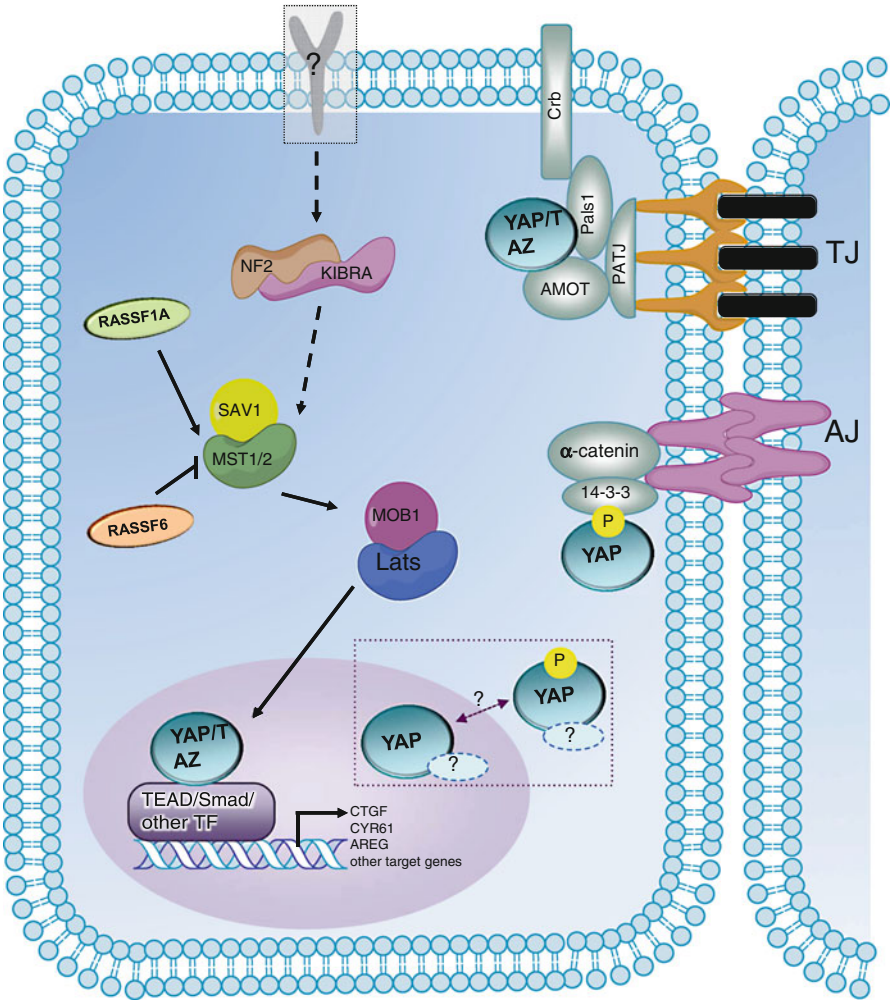


**Fig. 13.1** The mammalian Hippo signaling pathway. Blunted or arrowed end depicts inhibition and activation, respectively. *Dashed line* depicts unknown mechanisms *solid line* depicts known interactions

## 13.2 Hippo Signaling in Adult Stem and Progenitor Cells

### *13.2.1 Liver Progenitor Cells*

The first characterization of the roles of YAP in vivo demonstrated a critical importance of the Hippo pathway in controlling liver size (Dong et al. 2007; Camargo et al. 2007). The regenerative capacity of the liver is remarkable and of significant clinical importance. Following partial hepatectomy, the regenerative process in the

liver is mostly mediated by the cell cycle reentry and proliferation of the terminally differentiated hepatocytes. However, in some cases where the proliferation of hepatocytes is suppressed, regeneration of the liver can occur via expansion and proliferation of a putative periportal liver stem cell population, the oval cells (Avruch et al. 2011).

Camargo et al. generated transgenic mice carrying a doxycycline (Dox)-inducible YAP allele targeted to the collagen1a1 locus. The YAP construct used in these studies carries a mutation in residue 127 (Ser->Ala) mimicking a constitutively active form of YAP (Camargo et al. 2007). Using a tetracycline transactivator (rtTA) under the control of the hepatocyte-specific liver activator protein (LAP) they created a model in which YAPS127A expression could be temporally and spatially controlled in hepatocytes upon Dox induction. Remarkably, administration of Dox to postnatal animals resulted in a fourfold in total liver mass (Camargo et al. 2007). It was shown that this overgrowth resulted from proliferation of mature hepatocytes, and not oval-like cells, that were also less resistant to Fas-mediated apoptosis (Camargo et al. 2007). Another independent study described a similar YAP-induced liver overgrowth phenotype using a transgenic mouse carrying the wild-type human YAP cDNA also under the tetracycline-response element and using an rtTA driver under control of ApoE promoter (Dong et al. 2007). In both studies, the overgrowth phenotype could be completely reversed upon withdrawal of Dox to discontinue YAP expression (Dong et al. 2007; Camargo et al. 2007). Activating YAP for longer periods of time led to the development of hepatocellular carcinoma in adult livers (Dong et al. 2007; Camargo et al. 2007). The YAP overexpression phenotype in the liver can be fully rescued by expressing a dominant-negative form of TEAD2 that lacks its DNA-binding domain (Liu-Chittenden et al. 2012). This rescue of the increased liver size suggests that YAP's proliferative function in the liver is dependent on the TEAD transcription factors (Liu-Chittenden et al. 2012).

The inducible deletion of upstream factors of the pathway such as SAV1, MST1/2, and NF2 has confirmed their role in regulating YAP in vivo. Livers carrying mutations in these genes display elevated levels of nuclear localized YAP and also display dramatic increases in liver size. And in similar fashion as the YAP overexpression experiments, tumors develop in these models, which resemble both hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) malignancies (Zhang et al. 2010; Zhou et al. 2009; Song et al. 2010; Lee et al. 2010; Lu et al. 2010). Intriguingly, the cellular phenotype in these mutants has been described as an oval cell-like expansion, and the fact that tumors display mixed biliary and hepatocytic morphologies supports the hypothesis that a bipotential progenitor was expanded. It is still a bit unclear why the cellular phenotype differs between the YAP overexpression models and the other mutants. It will be essential to perform hepatocyte or oval cell-specific gene manipulations to fully understand the cell types that are endogenously more sensitive to Hippo signaling requirements.

Intriguingly, the connection of NF2 with YAP was disputed by a study that credited the NF2 mutant phenotype to aberrant activity of the epidermal growth factor receptor (EGFR) rather than to the Hippo pathway (Benhamouche et al. 2010). Nevertheless, the rescue of the NF2 mutant phenotype by deletion of only one allele

of YAP constitutes strong functional evidence that the main tumor suppressive mechanism of NF2 is through YAP inactivation (Zhang et al. 2010). Collectively, these studies suggest that the Hippo pathway plays a crucial role in maintaining the quiescence of the postnatal liver and that its misregulation can lead to overgrowth and tumorigenesis. A big question in the field is the nature of the transcriptional program that YAP activates, as there is no functional evidence that the described targets are important for the YAP-driven phenotypes.

### *13.2.2 Intestinal Stem Cells*

The intestinal epithelium renews continuously to maintain tissue homeostasis, turning over entirely every 4–5 days. The rigorous process of repair and renewal relies on the continuous proliferation of the intestinal stem cells (ISCs) located at the base of the intestinal crypt. Using an inducible mouse model that expressed YAP-S127A ubiquitously, it was first reported that YAP activation caused a reversible expansion of undifferentiated progenitor-like cells in the small intestine. This population expanded from the bottom of the crypt upwards and ended up replacing the mature cell types along the intestinal villi (Camargo et al. 2007). Within 5 days of YAP activation, the entire intestinal epithelium became highly proliferative and showed absence of differentiated enterocytes, mature goblet cells, and Paneth cells. Upon interruption of YAP expression by Dox withdrawal, the intestinal progenitors were able to resume their differentiation program, indicating a requirement for YAP expression in the progenitor expansion phenotype (Camargo et al. 2007). Similarly, Mst1/2-deficient intestines exhibit marked expansion of an undifferentiated progenitor population accompanied by a dramatic decrease of secretory lineages. In this model the enterocytes were better preserved compared to the Yap overexpression model (Zhou et al. 2011). It was further reported that ablation of a single YAP allele can suppress the hyperproliferative phenotype in MST1/2 deleted intestines. Similarly, deletion of Sav in the intestine leads to a hyperproliferative phenotype in the colon with a very long latency (Fre et al. 2009). This phenotype was also fully rescued by the concomitant loss of YAP (Fre et al. 2009).

Intriguingly, loss of YAP in the intestine does not lead to any major phenotype during development or normal homeostasis of the intestine (Fre et al. 2009). Given that YAP is normally expressed in the crypts of the intestine and presents a robust nuclear localization there (Zhang et al. 2010), it was quite surprising that YAP loss did not lead to a phenotype in intestinal development or renewal. It remains to be seen whether TAZ plays a compensatory role or simply YAP is not necessary for normal homeostasis. A role for YAP in tissue repair was demonstrated following challenging with the colitis-inducing agent dextran sulfate sodium (DSS). YAP-deficient mice were not able to recover from a DSS challenge due to an inability to jumpstart a proliferative response to the injury (Zhang et al. 2010). It will be interesting to see if this phenotype is observed following other regimes that induce regeneration such as irradiation or chemotherapy-induced intestinal injury. An

intriguing thought is that YAP is part of a regeneration-specific molecular response in the intestine.

Recently, work has begun to provide insight in to the mechanisms used by YAP to control intestinal progenitor biology. YAPS127A-induced expansion of the intestinal progenitor compartment was partially credited to Notch pathway activation given the upregulation of the Notch target gene Hes1 in mutant intestines. Additionally, short-term chemical inhibition of the Notch pathway using γ-secretase inhibitors reduced the dysplastic phenotype in intestine (Camargo et al. 2007). This will be better addressed by genetic studies that utilize mutants for the Notch pathway. MST1/2 null intestine also display strong activation of the Wnt and the Notch signaling pathway (Zhou et al. 2011). Interestingly, the mRNA levels of a Notch ligand, Jagged 1, was significantly increased while the mRNA level of the Notch receptor, Notch1, remained unchanged in MST1/2 mutant intestine. Since Jagged 1 is not known to be a direct target of YAP, but is a direct target of the Wnt pathway, upregulation of the Notch Pathway was hypothesized to be likely consequence of YAP-induced Wnt activation (Zhou et al. 2011). Given these potential functional interactions, several studies have explored the mechanism of cross talk between these pathways in the intestine. It is known that the Wnt and Notch signaling pathway synergistically play an important role in progenitor maintenance and proliferation (Fre et al. 2009). In 2010, a study demonstrated that the Hippo pathway restricts Wnt signaling by sequestering Dishevelled (DVL) in the cytoplasm by promoting its interaction with cytoplasmic TAZ (Varelas et al. 2010a). More precisely, TAZ controls Wnt signaling by inhibiting the CK1delta/epsilon-mediated phosphorylation of DVL (Varelas et al. 2010a). The unphosphorylated DVL induces activation of the Axin-APC-GSK3 destruction complex that leads to the degradation of the Wnt pathway transcriptional regulator, β-catenin (Clevers 2006; Nusse 2005). It has also been shown that cytoplasmic YAP could directly bind to β-catenin to mediate its cytoplasmic retention (Imajo et al. 2012). While these observations indicate a potential Wnt repressive role for cytoplasmic YAP, it is still unclear how nuclear YAP might activate progenitor proliferation. Still these studies indicate that the Hippo pathway plays a crucial role in the tight control of intestinal proliferation and differentiation partially or entirely via other signaling pathways.

### *13.2.3 Neural Progenitor Cells*

Multipotent neural progenitor cells that generate the central nervous system reside along the ventricular zone in the developing vertebrate neural tube (Merkle and Alvarez-Buylla 2006). In 2008, it was reported for the first time that the Hippo pathway could regulate the neural stem cell population. The overexpression of YAP in the chick neural tube resulted in an increased number of neural progenitor cells (Cao et al. 2008). Inhibiting upstream Hippo kinases, LATS1/2 and MST2, also led to an increase in the neural progenitor pool. This result demonstrated that the MST-LATS kinase cascade was important in mediating the YAP function in neural stem

cells (Cao et al. 2008). Akin to the findings in the intestine, Hippo inactivation or YAP expression led to increased self-renewal and reduced differentiation of neural progenitor cells. The proliferative effect of YAP could be rescued by disrupting the YAP–TEAD interaction, suggesting TEAD transcription factors mediate YAP activity in neural stem cells (Cao et al. 2008).

Cerebellar granule neuron precursors (CGNPs) are speculated to be the cell of origin for some medulloblastomas (Provias and Becker 1996). Sonic hedgehog (Shh) pathway regulates the proliferation of CGNPs and its aberrant activation can lead to the formation of medulloblastoma (Dahmane and Ruiz i Altaba 1999; Raffel et al. 1997; Reifenberger et al. 1998). Fernandez et al. indicated that YAP was highly expressed in a subset of medulloblastomas that were driven by Shh deregulation (Fernandez et al. 2009). Shh induces YAP expression and promotes YAP nuclear localization in CGNPs leading to increased proliferation (Fernandez et al. 2009). Ectopic expression of YAP in CGNPs grown without Shh in culture demonstrated higher mRNA level of known Shh effector, Gli2. Further, ChIP analysis revealed that YAP binds two of the four putative TEAD-binding sites on Gli2 promoter region. A possible mechanism of YAP-mediated CGNP proliferation could be via Gli2 induction, which then activates other downstream mediators of Shh signaling in CGNPs. In mouse medulloblastomas, YAP localizes to cells in the perivascular niche (PVN) that are proposed to be the tumor-repopulating cells (Fernandez et al. 2009). These YAP expressing PVN cells are resistant to irradiation and contribute to tumor regrowth (Fernandez et al. 2009). These findings indicate that YAP is an effector of the Shh pathway and a potential therapeutic target for medulloblastoma.

Recently, Li et al. (2012) reported that Notch activation leads to symmetric neural stem cell division by studying a new transgenic mouse model in which activated form of NOTCH1 receptor can be conditionally expressed in maturing neuroepithelium (Li et al. 2012). ChIP-seq and transcriptome analysis revealed that the transcription factors of the Hippo, Wnt, and Shh pathways are direct targets of the Notch pathway in neural stem cells in vivo (Li et al. 2012). Furthermore, Li et al. (2012) showed that YAP is selectively expressed in the stem cell compartment in the developing forebrain. Ectopic YAP expression rescues the effect of Notch inhibition suggesting that YAP is an effector of the Notch pathway in neural stem cells. The existing studies in neural progenitor cells implicate the complicated cross talk between the Notch, Shh, Hippo, and other pathways that may be required to maintain the proper regulation of the NSCs. Future studies should validate these observations in transgenic mice with stem cell-specific deletions of Hippo signaling molecules.

### *13.2.4 Epidermal Progenitor Cells*

Similar to the intestinal epithelium, the skin regenerates continuously and relies on the proper balance between quiescence and differentiation of the epidermal progenitor cells that reside in the basal layer to maintain homeostasis. The development of the mammalian skin starts as a single-layered multipotent embryonic progenitors that

differentiate to generate epidermis, sebaceous glands, and hair follicles (Fuchs 2007). On embryonic day 14, mouse epidermis exists as single-layered basal epidermal progenitors that express nuclear YAP (Zhang et al. 2011). The nuclear expression of YAP progressively declines as the proliferative capacity of the basal epidermal progenitor is reduced. By postnatal day 11, nuclear expression of YAP in basal epidermal cells is restricted to very few cells in the basal cell layer (Zhang et al. 2011).

Conditional deletion of YAP in epidermal progenitor cells of developing mice causes loss of progenitor cells resulting in an overall thinning of the skin and an absence of epidermal tissue in the distal part of the limbs, eyes, and ears in E18.5 mouse embryos (Schlegelmilch et al. 2011). Overexpressing constitutively active YAP in Keratin 14 expressing epidermal cells results in the expansion of the interfollicular stem cell compartment and abnormal thickening of the skin eventually leading to squamous cell carcinoma-like tumors (Schlegelmilch et al. 2011; Zhang et al. 2011). Developmental ablation of SAV1 in mice leads to similar hyperproliferation of skin epithelial progenitors in a manner very reminiscent of the YAP activation model (Lee et al. 2008). However, epidermal-specific ablation of MST1/2 and knockdown of LATS1/2 in HaCaT keratinocytes showed no effect on YAP phosphorylation suggesting that YAP activity is regulated by alternative mechanisms in this cellular context (Schlegelmilch et al. 2011). Two independent studies have shown that an adherens junction component, α-catenin, acts as an upstream negative regulator of YAP and sequesters YAP in the cytoplasm (Schlegelmilch et al. 2011; Silvis et al. 2011). The adherens junctions (AJs) have been speculated to act as molecular biosensors of cell density (Silvis et al. 2011; Lien et al. 2006a; Lien et al. 2006b). Additionally, several studies have demonstrated the association of YAP and TAZ with polarity proteins and cell–cell contact-regulating proteins (Robinson et al. 2010; Ling et al. 2010; Chen et al. 2010; Grzeschik et al. 2010; Skouloudaki et al. 2009; Varelas et al. 2010b; Doggett et al. 2011; Kim et al. 2011). The data linking YAP and α-catenin establishes YAP as a key component of a "crowd control" molecular circuitry in the epidermis.

### *13.2.5 Cardiac and Skeletal Muscle Progenitor Cells*

Maintaining an optimal size is essential for any organ in the body but it becomes even more crucial in cardiac development. The heart must be large enough to pump sufficient volume of blood but not so large that it blocks the outflow of blood from the left ventricle. A recent study inactivated the Hippo pathway by knocking out the upstream regulator, SAV1, in developing mouse hearts; and observed that these mutant embryos had larger hearts with elevated cardiomyocyte proliferation (Heallen et al. 2011). Gene interaction studies uncovered a nuclear interaction between YAP and β-catenin, a well-known promoter of growth in the heart. Loss of β-catenin in SAV1 conditional knockout mouse hearts rescued the overgrowth phenotype caused by Hippo inactivation, implicating that the Hippo pathway restrains cardiomyocyte proliferation and heart size by inhibiting Wnt signaling (Heallen et al. 2011).

Another recent study showed that conditional deletion of YAP in cardiac progenitor cells during cardiogenesis leads to lethal embryonic myocardial hypoplasia while overexpressing constitutively active YAP leads to cardiomyocyte proliferation (Xin et al. 2011). The pro-proliferative activity of YAP in the heart is mediated by its interaction with the TEAD transcription factors (Xin et al. 2011; von Gise et al. 2012). It was suggested that constitutively active YAP promotes cardiomyocyte proliferation and cardiomegaly by coupling with insulin-like growth factor (IGF) and Wnt signaling (Xin et al. 2011). YAP drives cardiac proliferation by activating the IGF pathway followed by glycogen synthase kinase 3b inactivation, which in turn inhibits the β-catenin destruction complex resulting in increased levels of β-catenin. Therefore, YAP promotes cardiomyocyte proliferation by intensifying Wnt signaling directly by interacting with nuclear β-catenin or indirectly via the IGF pathway (Heallen et al. 2011; Xin et al. 2011; von Gise et al. 2012; Shiojima and Walsh 2006; Matsui et al. 2008).

A role for the Hippo pathway in skeletal muscle was also recently reported. Yap overexpression in C2C12 myoblasts and primary mouse muscle stem cells blocks the progression of the myoblasts through the myogenic program and preserves the progenitor-like and proliferative states (Watt et al. 2010; Judson et al. 2012). Interestingly, TAZ, despite the high level of sequence identity with YAP, was shown to promote differentiation of myoblasts by promoting Myod1 activity (Jeong et al. 2010). This opposite effect of the two Hippo pathway effectors, YAP and TAZ, on muscle progenitor fate is a good illustration of the complexity and context-specificity associated with Hippo pathway activation or inhibition and the resulting transcriptional response. Obviously, further studies done in transgenic mice are needed to conclusively determine the role of Hippo signaling in skeletal muscle biology.

## 13.3 Hippo in Embryonic and Induced Pluripotent Stem Cells

Embryonic stem cells (ESCs) are pluripotent cells derived from the inner cell mass of the blastocyst stage of the preimplantation embryo. TAZ and YAP null embryos do not survive past the morula stage because nuclear localization of Yap/Taz in the outside cells of the preimplantation embryo is required to form the trophoectoderm (Nishioka et al. 2009). Coordination of multiple signaling pathways is crucial in maintaining the balance between differentiation and self-renewal of ESCs. Human embryonic stem cells (hESCs) depend on the TGF-β/Activin signaling, BMP signaling, and FGF signaling for self-renewal (Biswas and Hutchins 2007; Darr and Benvenisty 2006; Xiao et al. 2006). The TGFβ/Activin/Nodal signaling is transduced by the SMAD2/3 complex (James et al. 2005; Vallier et al. 2005). Varelas et al. showed that TAZ is responsible for shuttling the SMAD2/3 complex in and out of nucleus in response to the TGFβ signaling (Varelas et al. 2008). Therefore, knockdown of TAZ in hESCs leads to disruption of TGFβ signaling leading to loss of its pluripotent state. Mouse embryonic stem cells (mESCs) rely on the cytokine leukemia inhibitor factor (LIF) signaling and BMP signaling to maintain their

stemness (Evans 2011; Chambers and Smith 2004). The BMP pathway signaling is propagated via the SMAD1/5/8 signal transducer complex (Ying et al. 2003). Chip analysis revealed that YAP and SMAD1 are bound to the BMP-responsive Id gene family during active transcription in response to BMP signaling. This piece of evidence suggests that YAP associates with SMAD1 to enhance BMP-mediated transcription required for hESC maintenance (Alarcon et al. 2009).

Supporting a role for YAP and TEADs in pluripotency, Tamm et al. found that YAP and TEAD2 are highly expressed in ESCs and downregulated when cells undergo differentiation (Tamm et al.). Moreover, YAP/TEAD2 could activate the expression of the ESC master transcriptional regulators Oct4 and Nanog, and TEAD function inhibition resulted in differentiation towards the endoderm lineage (Tamm et al.). Another group reported similar findings and provided additional evidence for the role of YAP in pluripotency using induced pluripotent stem cells (iPSCs) (Lian et al. 2010). The seminal findings of Takahashi et al. demonstrated that mouse somatic cells can be reprogrammed into iPSCs by inducing the activity of four transcription factors, Sox2, Oct3/4, c-Myc, and KLF4. YAP is activated during the reprogramming of human embryonic fibroblasts into iPSCs and addition of YAP to Sox2, Oct4, and KLF4 transcription factors infection in mouse embryonic fibroblasts increases the iPSC reprogramming efficiency (Lian et al. 2010). In conclusion, YAP and TEAD proteins seem to be critical factors for the maintenance of pluripotent properties of both ESCs and iPSCs. Further studies should fully evaluate the role of YAP, TAZ, and other Hippo pathway components using full genetic loss-of-function alleles and should aim to determine how YAP/TEAD interact with known stem cell signaling and transcriptional networks.

## 13.4 Hippo in Cancer Stem Cells

Cancer stem cells (CSCs) are thought to be the tumorigenic cell types in cancer that have stem cell-like properties. These cells constitute only a fraction of the tumor cells, but they have the ability to self-renew and differentiate into other tumor cell types (Visvader and Lindeman 2008). These cells have been shown to be resistant to chemotherapy, and are thought to be responsible for cancer relapse. High-grade tumors are characterized by a higher accumulation of these CSCs (Pece et al. 2010). A screen done in 993 primary human breast tumors revealed that the Hippo signaling gene signature was overrepresented in high-grade (G3) tumors implicating elevated TAZ/YAP activity in high-grade tumors (Cordenonsi et al. 2011).

Studies done using MCF10A-T1k cells (MII) and MCF10A-CA1a cells (MIV) shed light on the role of TAZ in breast cancer cells. Upon injection into mice, the MII cells generate low-grade tumors, whereas the MIV cells generate high-grade tumors. Interestingly, TAZ was highly expressed in MIV cells as compared to the MII cells, while YAP level was comparable. Knocking down endogenous TAZ in MIV cells significantly reduced its potential to produce primary and secondary tumors and caused a 20-fold reduction in the tumor-initiating cells (Cordenonsi

et al. 2011). FACS sorting the MII cells according to CD44 and CD24 expression revealed that the $CD44^{high}/CD24^{low}$ population, that has CSC-like properties, expresses higher levels of TAZ. Knocking down TAZ in $CD44^{high}/CD24^{low}$ cell population led to reduced self-renewal properties (Cordenonsi et al. 2011). Overexpressing constitutively active TAZ in this cell population caused increased cell proliferation, higher ability to form primary, secondary, and tertiary tumors that are more resistant to chemotherapeutic drugs. The TAZ overexpressed MII cells produce invasive carcinoma similar to the ones from MIV cells (Cordenonsi et al. 2011). Collectively, the findings indicate that TAZ is required to sustain self-renewal capacity and tumorigenic potential in breast cancer cells.

Another study done in glioblastomas (GBM) showed that nuclear TAZ is highly expressed in high-grade GBMs. Tumors enriched with a neural development proneural (PN) gene signature display a higher survival rate when compared to tumors with a high mesenchymal (MES) gene expression signature (Bhat et al. 2011). TAZ was hypermethylated in the PN group of tumors compared to the MES group, where at the YAP methylation status was comparable. Silencing TAZ in the MES glioma stem cells (GSCs) led to decreased invasive ability, self-renewal, and tumor-initiating capacity (Bhat et al. 2011). Overexpression of TAZ in PN GSCs induced expression of the MES marker, thus driving aberrant osteoblastic and chondrocytic differentiation. The high-grade breast cancer tumors and glioblastoma tumors present a considerable clinical challenge since they show resistance to chemotherapy as well as radiation. TAZ, thus presents as a novel molecular target for treating these aggressive tumors.

## 13.5 Conclusions

Since its discovery in the fruit fly, much progress has been made in the Hippo signaling field and it is now widely accepted that this pathway and its effector, YAP, play critical roles in mammalian stem and progenitor cells and growth control. Nonetheless, important questions regarding the identity and physiological relevance of upstream Hippo modulators are yet to be answered. As many different components are identified in cell culture experiments, it is important to validate these observations in a physiological context. Recent data implicating cell polarity and adhesion and mechanotransduction as inputs that regulate Hippo kinase activity provide exciting avenues to explore. Another area that is bound to provide important insight into the biology of size control is the definition of the mechanisms by which Hippo and YAP cross talk with other developmental signaling pathways. Current evidence suggests an important relationship between Hippo, Wnt, Hedgehog, Notch, and BMP pathways (Camargo et al. 2007; Zhou et al. 2011; Fernandez et al. 2009; Li et al. 2012; Varelas et al. 2008; Alarcon et al. 2009). These relationships need to be further validated using in vivo animal models and better-defined biochemically.

While the importance of the Hippo pathway in some stem cell populations is well documented, its role in other stem cell populations still remains unknown.

Interestingly, overexpression of YAP in the hematopoietic system revealed no changes in the distribution of the hematopoetic lineages and number/function of hematopoetic stem cells (Jansson and Larsson 2012). Deletion of YAP in the hematopoietic system also does not lead to any major defects (F. Camargo, unpublished data). It is quite intriguing how a pathway that is such a potent regulator of proliferation in epithelial tissues, seems to have absolutely no effect on the proliferation of blood progenitors, even when YAP is overexpressed. It will be interesting to explore what molecular features of blood cells or other tissue-specific progenitors make them insensitive or more sensitive to YAP and TEAD activity.

Organ size is one of the least understood questions in developmental biology. It is now fair to speculate that proper tissue size is achieved through a combination of morphogenic signaling, patterning cues, spatial control of YAP/TAZ localization by cell–cell contact, and mechanical cues dictated by tissue architecture. Further investigation of these processes and how they ultimately converge on Hippo signaling will likely provide insight into molecular mechanisms that regulate development, stem/progenitor renewal, regeneration, and cancer biology.

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