Role of Hippo Signaling in Cancer Stem Cells

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Cancer stem cells (CSCs) have been proposed and evidenced as the initiator of tumor formation and the seeds of metastases. Thereby, the molecular mechanisms regarding modulation of CSCs have been widely explored, aimed to improve treatment for cancer patients. Recent progress has highlighted the effects of Hippo signaling in tumorigenesis and cancer development, including its crucial role in CSC regulation. Although the kernel Hippo signaling cascade has been well studied, its upstream inputs and downstream transcriptional regulation still remain elusive. In this review, we summarize the current understanding of the mechanism and regulatory function of Hippo signaling in CSCs, with emphasis on its possible roles in regulation of CSC self-renewal, differentiation and tumorigenesis.


Cancer has always remained as one of the leading causes of mortality all over the world (Jemal et al., 2011). About one-third of cancer patients die within 5 years of diagnosis. Its complex pathophysiology has made it an almost unconquerable challenge for scientists and clinicians for decades. One of the greatest concerns of cancer disease is the rapid progression from a localized lesion to systemic metastatic sites. Therefore, many researches have been and are still being done for establishing optimal treatment strategies to postpone cancer progression and metastasis.

Over the last decade, enormous evidence suggests that only a small but distinct subset of cells in some tumors hold the capacity to differentiate, self-renew and initiate tumor formation both in vivo and in vitro (Bao et al., 2011). Such cells are also named as cancer stem cells (CSCs). CSCs have the ability to detach from the primary site and invade the surrounding tissues, which is believed to be the main cause of death in cancer patients. CSCs are also found to be highly resistant to both radiation and chemotherapy, and capable of escaping targeted treatment (Yu et al., 2007). Thus, complete cure of cancer cannot be achieved unless and until all CSCs are totally ablated (Li et al., 2012). In this context, further exploration of the characteristics and regulatory mechanisms of CSCs is necessary for better-targeted treatment, which will contribute significantly to cancer therapy.

The Classical Concept and Functional Properties of CSCs

CSCs, which arose more than a decade ago, have been proposed as the driving force of tumorigenesis and metastases (Reya et al., 2001). However, the existence and role of CSCs remain a topic of intense debate (Magee et al., 2012). Over the past decade, CSC-enriched populations have been identified and characterized in several distinct cancer entities (Reya et al., 2001). Recently, the identification of CSCs in three independent studies involving mouse models of brain, skin, and intestinal tumors has provided further support for this concept (Chen et al., 2012; Driessens et al., 2012; Schepers et al., 2012). Those CSC models indicate that tumors are organized into aberrant cell populations with a subset of tumor cells, which are capable of self-renewal and differentiation.

Like normal stem cells, CSCs are defined by their functional properties as well. First, CSCs have unlimited self-renewal potential. Although human CSCs have only been found in xenografts, mouse CSCs have been detected in several immunocompromised mouse models and their syngenic models of leukemia (Deshpande et al., 2006), breast cancer (Cho et al., 2008), and skin cancer (Malanchi et al., 2008). Moreover, some CSCs have shown remarkable resistance to conventional therapies, which may provide cues why cancer diseases are hard to cure. These studies suggest that CSCs do exist and initiate tumorigenesis, tumor recurrence, and metastases in intact organs. Thus, accurate targeting of the CSCs is thirstily required for more effective cancer therapies.

The self-renewal and differentiation of CSCs have been reported to encompass multiple signal pathways. One of the therapeutic strategies to target CSCs involves inhibition of self-renewal or survival pathways in stem cells, like Notch, Hedgehog, and Wnt (Korkaya and Wicha, 2007). However, interfering such intrinsic pathways may affect normal stem cell functions and result in systemic disorders. In addition to intrinsic pathways, CSCs can be regulated by extrinsic pathways.
pathways as well. A recent in vitro study showed that modulating the interactions between CSCs and the microenvironment could represent a rational approach to target breast CSCs selectively without affecting normal stem cells (Ginestier et al., 2010). It provided a new insight into the therapeutic strategy aiming at interactions between CSCs and microenvironment, which might greatly increase the treatment efficacy compared with current therapies.

**CSCs and Its Microenvironment**

Like normal stem cells, CSCs require signals transduced from their surrounding environment to achieve an optimal balance between activation, self-renewal, and differentiation (Borovski et al., 2011; Sheshopalov and Zon, 2012). However, the current understanding of such regulatory mechanism is still limited. Part of the reason lies in that most studies used isolated CSCs and studied them in vitro, leading to the view that this regulation is intrinsic to CSCs. Nevertheless, several researchers propose the role of microenvironment, termed CSC niche, in the regulation of CSCs (Medema and Vermeulen, 2011). In glioma, some of the surrounding endothelial-like cells near CSCs are tumor-derived, indicating that CSCs in glioma generate their own niche (Ricci-Vitiani et al., 2010; Wang et al., 2010). In breast cancer, CSCs are thought to be supported by mesenchymal cells through cytokine networks dependent on interleukin 6 (IL-6) and chemokine C-X-C motif ligand 7 (CXCL7; Liu et al., 2011). It can be implied that more malignant tumors depend less on their niche, as they can regenerate new microenvironment. Thus, the microenvironment might be a crucial factor that dictates CSC qualities. And the fact that CSCs are always found near stromal cells supports such intimate connection (Fedde and Brabletz, 2007; Christensen et al., 2011).

Recent studies have also highlighted the role of such extrinsic signals generated in the microenvironment, like extracellular matrix (ECM), in regulating CSC functions (Brizzi et al., 2012; Lu et al., 2012; Hao et al., 2013). Such regulation might provide new insights into the therapeutics of cancer disease. The Hippo pathway is an evolutionarily conserved signaling cascade, which has been found to be linked to the transduction of signals from the microenvironment (Zhao et al., 2012). Recent research has also indicated that CSC-like properties are induced following deregulation of Hippo pathway activity (Bhat et al., 2011; Cordenoensi et al., 2011), indicating the association between CSCs and Hippo signaling. These fascinating findings may provide new possibilities of creating novel CSC-interfering methods for cancer therapy. Thus, we review the regulatory mechanisms of the Hippo pathway in cancer biology and discuss the potential implications in the regulation of CSCs.

**Core Hippo Signaling Pathway in Mammals**

The microenvironment affects CSC activity, differentiation, and self-renewal process through mechanotransduction pathway, which is believed to take the center stage during tumor formation. And recent findings suggest a key role of Hippo signaling in mechanotransduction. In addition, the Hippo signaling has been accepted to play important roles in the regulation of cell proliferation, differentiation, migration, and apoptosis. It is essential both during development of organs and in maintenance of their homeostasis. And when deregulated, it can cause tumorigenesis (Pan, 2010). The core of the Hippo pathway in mammals comprises mammalian STE 20-like protein kinase 1 (Mst1) and Mst2 (Harvey et al., 2003; Pantalacci et al., 2003; Udan et al., 2003; Wu et al., 2003), as well as Salvador 1 (Sav1; or WW45) (Kango-Singh et al., 2002; Tapon et al., 2002), MOBKL1A and MOBKL1B (collectively referred to as Mob1), and Large tumor suppressor homologues 1 (Lats1) and Lats2 (Justice et al., 1995; Xu et al., 1995; Pan, 2010). Under nonproliferating conditions, Lats1/2 phosphorylate the homologous transcriptional coactivators YAP (Yes-associated protein) and TAZ (transcriptional coactivators with PDZ-binding motif, encoded by WWTR1) (Huang et al., 2005; Oh and Irvine, 2008; Oh and Irvine, 2009) to repress growth, leading to the sequestration of YAP/TAZ in the cytoplasm by interacting with 14-3-3 proteins and resulting in proteasomal degradation afterward (Codelia and Irvine, 2012). When YAP and TAZ are not phosphorylated, they accumulate in the nucleus, leading to gene expression that promotes proliferation (Hong and Guan, 2012; Staley and Irvine, 2012). TEA domain family member (TEAD) emerged as the main DNA-binding protein that YAP/TAZ associate with in the nucleus (Goulev et al., 2008; Wu et al., 2008; Zhang et al., 2008; Chan et al., 2009; Peng et al., 2009; Zhang et al., 2009). The key components of Mst1/2 are pro-apoptotic kinases, and Sav1 interacts with Mst1/2 through the SARAH domains, which exist in both Sav1 and Mst1/2 (Callus et al., 2006). Mst1 and Mst2 have been shown to be activated by Sav1, but the underlying mechanism remains elusive (Lee et al., 2008). And they are also activated by binding to Ras association domain family (RASSF), possibly by the alteration of subcellular localization (Khokhlatchev et al., 2002). Activation of Mst1/2 results in activation and phosphorylation of Lats1/2 (Chan et al., 2005). Mob1, which forms a complex with Lats1/2, is phosphorylated by Mst1/2 as well, leading to an enhanced Lats1/2-Mob1 interaction (Praskova et al., 2008).

**Hippo Signaling and Cancer**

What is the relationship between such complex signaling cascade and tumor formation? Functions of the Hippo signaling in tumor suppression have been confirmed in genetically engineered mouse models. Mutations of Merlin, a protein that binds to multiple upstream of Hippo to promote phosphorylation of Lats1/2, lead to neurofibromatosis type 2 (NF2). Merlin−/− mice develop malignant tumors including lymphomas, osteosarcomas, lung adenocarcinomas, fibrosarcomas, and hepatocellular carcinomas (McClatchey et al., 1998). In addition, sustained overexpression of YAP leads to tumor formation (Dong et al., 2007). In human cancers and a mouse model of breast cancer also imply genomic amplification of YAP (Zender et al., 2006). Moreover, up-regulated YAP levels and the nuclear localization have been found in various cancer diseases (Zender et al., 2006; Zhao et al., 2007; Steinhardt et al., 2008). Overexpression of TAZ has also been observed in cell lines of non-small cell lung cancer and breast cancer cells (Chan et al., 2008; Zhou et al., 2011). The Lats1/2 promoters are hypermethylated in almost 50% of breast cancers and 60–70% of astrocytomas (Takahashi et al., 2005; Jiang et al., 2006). Mst1 and Mst2 promoters are hypermethylated in nearly 37% and 20% of soft tissue sarcomas, respectively (Seidel et al., 2007). In breast cancer cells, activation of YAP or TAZ induces epithelial-mesenchymal transition (EMT) and increases the invasiveness (Lei et al., 2008; Zhao et al., 2008). A recent study has found that mechanotransduction and YAP expression are required for the maintenance and generation of cancer-associated fibroblasts (CAF; Calvo et al., 2013). It revealed that the function of actomyosin cytoskeleton is requisite for remodeling ECM and then promoting cancer cell invasion, while the YAP has taken part in regulating several cytoskeletal regulators. Therefore, the down-regulation of Lats1/2, the increased nuclear localization of YAP and the reduced cytoplasmic expression of Mst1 in cancer are highly related to its malignant properties and
a poor prognosis (Takahashi et al., 2005; Minoo et al., 2007; Xu et al., 2009). Taken together, these findings highlight a significant role of the Hippo signaling in tumorigenesis.

**Hippo Signaling and CSCs**

Considering that CSCs are the leading cause of tumorigenesis, and the role of Hippo signaling in CSCs has been evidenced by several researches, we then focus on the regulatory mechanisms of Hippo signaling in CSCs. Although CSCs represent only a small population of cells in tumors, they have the capacity to seed new tumors as described above. Distinguishing the differences between CSCs and other cancer cells might help develop novel therapeutic strategies for cancer. Recent studies using gene expression profiling have found that high CSC content tissues in breast cancer overlap with YAP/TAZ-induced gene expression, suggesting the importance of YAP/TAZ in CSCs (Cordenonsi et al., 2011). Earlier studies had also found that the increased nuclear activity of YAP and TAZ could drive cell transformation and tumorigenesis (Overholtzer et al., 2006). Such oncogenic activity is correlated to an acquisition of mesenchymal properties, like EMT in epithelial tissues. The combined abilities of self-renewal and invasive properties are suggested as the driving force to develop aggressive cancers (Mani et al., 2008; Radisky and LaBarge, 2008; Hanahan and Weinberg, 2011).

However, unlike YAP, knockdown of TAZ alone inhibits the self-renewal ability of CSCs in breast cancer (Cordenonsi et al., 2011). And overexpression of TAZ promotes mammosphere formation and CSC marker expression in non-CSC cancer cell populations (Cordenonsi et al., 2011). TAZ is also the leading factor to sustain CSC-like properties, and the expression of a nuclear localized TAZ-S89A mutant is sufficient to confer the self-renewal process of breast cancer populations (Cordenonsi et al., 2011). These findings imply a more crucial role of TAZ protein in certain CSC populations. In addition, TAZ has also been thought to function as a downstream effector of other transcriptional regulators known to sustain CSC-like properties. Transcriptional repressors Snail and Twist are such regulators, which function to promote EMT and CSC-like properties by regulating the expression of genes for cell polarity and adhesion. Crumbs and Scribble protein complexes, which are cell polarity-regulating proteins, control TAZ activity and localization as well (Varelas et al., 2010; Cordenonsi et al., 2011). Therefore, the induction of CSC-like and metastatic properties in breast cancer by Snail and Twist may reflect their direct influence on cell polarity and indirect influence on the activity of nuclear TAZ.

Like in breast cancer, TAZ expression also correlates with glioblastoma aggressiveness. Glioblastomas with up-regulated TAZ expression levels exhibit mesenchymal properties (Bhat et al., 2011). However, hypermethylation of TAZ promoter and reduced TAZ levels are found in low-grade glioblastomas, suggesting that epigenetic regulation mechanisms are also involved in TAZ expression and the nature of the disease (Bhat et al., 2011). Knockdown of TAZ expression in mesenchymal-like stem cells in glioma decreases the gene expression for mesenchymal properties and limits the capacity of these cells to self-renew and initiate tumors (Bhat et al., 2011). In other studies, YAP and TEAD expression was observed to be much higher in CSCs of certain type of medulloblastomas (Fernandez-L et al., 2009). And knockout of Hippo signaling proteins also induced accumulation of tumorigenic stem cells in mouse liver (Zhou et al., 2009).

Recently, the crosstalk between Hippo signaling and PI3K-mTOR pathway has been elucidated (Strassburger et al., 2012; Tumaneng et al., 2012; Ye et al., 2012; Fig. 1). YAP regulates the main effects of Hippo signaling by regulating miRNA-29 family, which inhibits the tumor suppressor PTEN. PTEN is a key antagonist of PI3K, which is known as an upstream activator of mTOR. Therefore, Hippo signaling may affect CSC functions by inactivating PI3K-mTOR pathway, and it gives cues that multiple upstream signals function together to regulate Hippo signaling in cancer development and CSC control. Enormous evidence indicates that YAP/TAZ is a crucial regulator in cancer development and progression. Considering the connections between Hippo signaling, cell polarity, and other cancer-promoting signals, we are still far from fully understanding the role of YAP/TAZ in tumorigenesis and metastases. However, their role in promoting CSC-like properties has been elucidated, making them potential targets for therapeutics of cancer disease.

**Conclusion and Future Perspectives**

Over the past 10 years, many researches have revealed the critical role of Hippo signaling in organ size control and tumorigenesis, as well as in stem cell biology. Recent findings regarding the functions of Hippo signaling and its downstream effector YAP/TAZ in promoting self-renewal ability of CSCs and tumor initiation further raised the importance of the pathway. However, the mechanism how YAP and TAZ are regulated by Hippo and other unknown signaling remains unclear, and our understanding to the detailed regulatory mechanism of the Hippo and other signaling crosstalk in CSC functions is still incomplete. Although recent studies suggest that the microenvironment of CSCs can regulate the Hippo signaling pathway (Dupont et al., 2011), it will be crucial to understand the detailed regulatory mechanism of the Hippo and other signaling crosstalk in CSC functions is still incomplete. Although recent studies suggest that the microenvironment of CSCs can regulate the Hippo signaling pathway (Dupont et al., 2011), it will be crucial to
uncover the precise molecular mechanisms of how the physical signals or physiological cues initiate the signaling cascade and how these signals are sensed and then transduced to control tumor formation and progression. Furthermore, the mechanism of YAP/TAZ in regulating CSC self-renewal and differentiation should be investigated and the correlation of such mechanism with tumorigenesis and metastasis should also be studied. In addition, since new crosstalk of Hippo with other signaling pathways have been identified, it is important to examine potent crosstalk between these two signaling cascades, such as TGFβ, Wnt, and Notch (Mauvel et al., 2012).

Undeniably, any insight into those key questions surrounding the Hippo signaling model will be pertinent to our knowledge of cancer diseases, and it would be exciting to explore the new possibility of therapeutic intervention of the Hippo signaling for cancer therapy.

Literature cited


