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Fresh Perspectives on the Functions of the Hippo Signaling Pathway in Stem Cell Biology

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RESEARCH ARTICLE

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Abstract: The Hippo signaling pathway involves a highly conserved kinase cascade, featuring mammalian STE20-like protein kinases (MSTs), large tumor suppressors (LATSs), and downstream transcription coactivators such as YES-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ). TAZ and YAP, critical transcriptional coactivators, are negatively regulated by the Hippo signaling pathway. These coactivators play a crucial role in inducing organ regeneration and the expression of genes promoting cell growth and division by binding to transcription factors, particularly TEA domain transcription factors (TEADs). The Hippo pathway is well-documented in controlling organ size, maintaining homeostasis, regulating cell proliferation, and influencing tumor development. Additionally, research underscores the pathway's significance in cancer stem cell biology, impacting processes like self-renewal and drug resistance. Aberrant activation of YAP and TAZ due to Hippo pathway dysregulation or increased expression can expedite tumorigenesis. Consequently, the pharmacological inhibition of YAP and TAZ emerges as a promising strategy in treating tumors with elevated YAP/TAZ activity.

Keywords: *Hippo signaling pathway, Hippo signaling pathway, Hippo signaling pathway*

1.Introduction:

The Hippo signaling pathway (HSP), an evolutionarily conserved pathway, plays pivotal roles in governing organ size, tissue regeneration, and suppressing tumors (1, 2). Within the HSP, the YES-associated protein (YAP) and the transcriptional coactivator with a PDZ-binding motif (TAZ) function as transcriptional coactivators (3). Upon activation, YAP and TAZ instigate a transcriptional program that fosters cell proliferation and augments the self-renewal of stem cells, crucial for stimulating tissue regeneration. However, the improper activation of YAP and TAZ can lead to the formation of malignant tumors (4). This review provides an overview of the latest developments in the HSP within the context of stem cell biology and explores potential therapeutic targets.

2. The Hippo Signaling Pathway (HSP)

The HSP comprises a highly conserved cascade of serine/threonine kinases that exert negative regulation on the expression and activity of YAP and TAZ. Key members of this pathway in mammals include mammalian STE20-like protein kinase 1 (MST1) and MST2, as well as large

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tumor suppressor 1 (LATS1) and LATS2 (5, 6). The activity of these kinases is influenced by interactions with various scaffolding proteins. Salvador homolog 1 (SAV1) forms complexes with MST1/2, while MOB kinase activator 1A (MOB1A) and MOB1B interact with LATS1/2 (7-9). Upon HSP activation, MST1/2 phosphorylates and activates LATS1/2 and MOB1A/1B (10, 11). Subsequently, the HXRXXS motifs of YAP and TAZ are directly phosphorylated by LATS1/2 (12). Two main phosphorylation mechanisms function to inhibit the activity of YAP and TAZ. Firstly, phosphorylation of YAP (on serine 127) and TAZ (on serine 89) creates a binding site for 14-3-3, leading to the sequestration of YAP and TAZ in the cytoplasm by the 14-3-3 complex (13, 14). In the second mechanism, phosphorylation of YAP serine 381 and TAZ serine 311 promotes casein kinase-1 mediated phosphorylation. This additional phosphorylation induces a motif targeted by β-transducin repeat-containing protein (β-TrCP), resulting in the degradation of YAP and TAZ (15). Accumulated YAP and TAZ in the nucleus promote the expression of specific genes when the HSP is deactivated (Figure 1). YAP and TAZ lack a DNA-binding domain, regulating the expression of target genes by forming complexes with other transcription factors. In mammals, YAP and TAZ frequently bind to TEA domain transcription factors (TEAD1 to TEAD4). Interactions have also been reported with other transcription factors, including p73, T-box transcription factor 5 (TBX5), and RUNT-related transcription factor 1 (RUNX1), RUNX2 with YAP/TAZ (16).

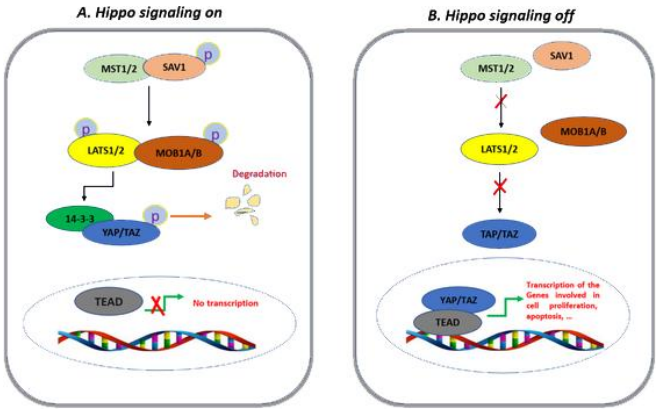


Figure-1 *Hippo Signaling Pathway: The Core Components and Signal Transduction*

A. Activation of the Hippo signaling pathway (HSP) occurs in high cell density conditions. Upstream signaling events, as yet unidentified, lead to the activation of mammalian STE20-like protein kinase 1 (MST1)/2. Activated MST1/2 then phosphorylates Salvador homolog 1 (SAV1), which, in turn, phosphorylates large tumor suppressor (LATS) and MOB kinase activator 1 (MOB1). The activated LATS/MOB complex phosphorylates YES-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ), resulting in their cytoplasmic retention by the 14-3-3 protein and subsequent proteasomal degradation of YAP/TAZ.

B. In low cell density conditions, when Hippo signaling is inactive, the kinases MST1/2 and LATS remain inactive, preventing the phosphorylation of YAP and TAZ. Stabilized YAP/TAZ translocate into the nuclei, where they interact with TEA domain transcription factor (TEAD), enhancing the transcription of target genes associated with anti-apoptosis and proliferation.

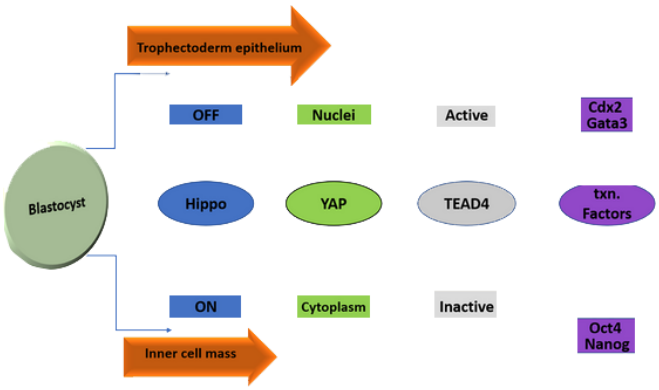
Recent evidence suggests that YAP/TAZ regulation is also influenced by extracellular cues, such as mechanical forces and hormonal signals. The extracellular matrix and neighboring cells impact YAP/TAZ activity through mechanical cues. F-actin stability promotes YAP/TAZ

activation, while F-actin disruption leads to their deactivation. Additionally, various extracellular hormones, including epinephrine, glucagon, lysophosphatidic acid (LPA), and thrombin, have been identified as potent regulators of the HSP. The actin cytoskeleton serves as a mediator for both mechanical and hormonal cues. G-protein-coupled receptors (GPCRs) transduce extracellular hormonal cues through the actin cytoskeleton and RHO GTPases, modulating YAP/TAZ activity. The activation of G12/13 or Gq/11 stimulates YAP/TAZ, while Gs inhibits their activity. Beyond the core members, numerous additional proteins have been reported to regulate the HSP, including thousand-and-one amino acid protein kinases (TAOK1-3), MAP/microtubule affinity-regulating kinases (MARK1-4), salt-inducible kinases (SIK1-3), RAS association domain-containing family protein (RASSF), multiple ankyrin repeats single KH domain-containing protein (MASK), and homeodomain-interacting protein kinase 2 (HIPK2).

3. Hippo Signaling Pathway in Embryogenesis and Embryonic Stem Cells

The Hippo signaling pathway (HSP) is a crucial regulator that constrains tissue and organ growth. Human embryonic stem cells (hESCs), characterized by their ability to self-renew and maintain pluripotency in culture, are derived from the inner cell mass of the blastocyst. The initial cell fate determination occurs during the morula to blastocyst stages before implantation into the uterus (preimplantation). The outer cells of the morula acquire apicobasal polarity and differentiate into a cyst-like epithelial tissue in the blastocyst called trophectoderm (TE). The TE plays a vital role in implantation and subsequent formation of placental tissues. The inner cell mass (ICM), composed of nonpolar inner cells adhering to one side of the TE, gives rise to the embryo proper and various extraembryonic tissues.

The specification of TE and ICM cell fates is under the regulation of the HSP. In the outer cells, the HSP remains inactive, allowing nuclear accumulation of YAP. The binding of TEAD4 and nuclear YAP forms a complex, turning TEAD4 into a transcriptional activator. This complex activates transcription factors Cdx2 and Gata3 specific to TEs, driving TE development. In contrast, the HSP is active in the inner cells, preventing nuclear accumulation of YAP. This inactivation of TEAD4 and deficiency of nuclear YAP limit the production of transcription factors unique to TE, facilitating the elevated expression of Oct3/4, Nanog, and Sox2. These transcription factors promote cell differentiation into the ICM and are likely involved in auto-activation mechanisms.



Differential activation of the Hippo signaling pathway specifies distinct cell fates in the preimplantation embryo. The inner cells initiate Hippo signaling, enabling the expression of inner cell mass (ICM)-specific transcription factors and the adoption of the ICM fate. Conversely, Hippo signaling is repressed in the outer cells, facilitating the differentiation of outer cells into trophectoderm (TE).

Recent studies on YAP and TAZ have unveiled their role as transcriptional coactivators in regulating embryonic stem cell (ESC) self-renewal and differentiation (39). Investigations linking the Hippo signaling pathway (HSP) to ESC biology

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have indicated that TAZ plays a crucial role in controlling the subcellular localization of transcriptional regulators SMAD2/3-4, which mediate transforming growth factor (TGF)-signaling. The interaction between transcriptional coactivator with PDZ-binding motif and SMAD2/3-4 proteins promotes their nuclear clustering and association with the mediator complex, thereby enhancing their transcriptional activity following TGF stimulation. Recent studies have reported that YAP is inactivated during mouse ESC (mESC) development, and knock-down of YAP or TEAD leads to the loss of pluripotency (40, 41). Conversely, ectopic overexpression of activated YAP in induced pluripotent stem cells (iPSCs) enhances reprogramming efficiency and prevents differentiation in mESCs (40). These investigations have also revealed that YAP-TEAD promotes the transcription of well-known stemness genes in mESCs, including Oct3/4, Sox2, Nanog, BMP signaling, and LIF targets, but not in mature cells. These findings support the hypothesis that YAP/TAZ mediates BMP/TGF transcriptional activity and directly upregulates the expression of essential stemness genes, contributing to the maintenance of ESC pluripotency in vitro (42, 43).

4. Hippo Signaling and Somatic Stem Cells

4.1. Liver Progenitor Cells

The Hippo Signaling Pathway (HSP) plays a pivotal role in various somatic stem cells, particularly in the context of liver regeneration. The liver, a vital metabolic organ with remarkable regenerative capabilities, relies on the activation of hepatic progenitor cells to initiate regeneration. Despite hepatocytes, the primary cell type in the adult liver, being non-mitotic, the liver contains oval cells (OCs) capable of generating a transit precursor compartment. The intricate mechanisms underlying liver regeneration, particularly how the liver determines its return to its original size, remain unknown.

Past studies demonstrated the physiological significance of HSP in the mammalian liver using mouse models with conditional YAP overexpression in hepatocytes. This led to significant but reversible liver hyperplasia, marked by a four-fold increase in total organ mass. The primary contributor to hyperplasia was the excessive proliferation of mature hepatocytes. These findings highlight the impact of the human homolog of the Drosophila HSP on tissue size, laying the groundwork for further investigations.

Additional HSP elements have been implicated in inhibiting liver development. Mice with deleted Mst1/2 genes developed liver tumors exhibiting characteristics of both cholangiocarcinoma (CC) and hepatocellular carcinoma (HCC), originating from bipotential liver progenitor cells. Cell lines derived from MST1/2 null livers exhibited growth inhibition and extensive apoptosis upon YAP depletion, reinforcing the idea that YAP activation is a primary driver of liver enlargement associated with MST1/2 deficiency. This suggests a significant regulatory role of HSP components in organ size and hepatocyte quiescence, with dysregulation potentially leading to stem cell proliferation, enlargement, and cancer through various mechanisms. Further research is necessary to fully comprehend the functions and modes of action of these HSP components in regulating liver progenitor cells.

4.2. Neural Progenitor Cells

Neural progenitor cells, located throughout the ventricular zone of the neural tube in developing vertebrates, give rise to various cell types constituting the mature central nervous system (CNS). The YAP protein, colocalizing with Sox2, is expressed in neural tube progenitor zones across species, including mice, frogs, and chicks.

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Loss of Mst1/2 or Lats1/2, or activation of YAP-TEAD, results in a substantial increase in neural progenitors, attributed to the overexpression of cell cycle re-entry and stemness genes, impeding differentiation by inhibiting critical genes. Conversely, YAP deficiency increases cell death and premature neuronal differentiation.

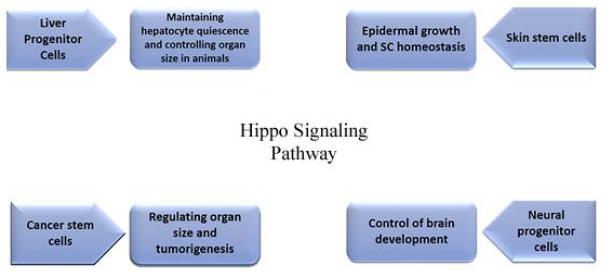
In the perivascular niche of the cerebellum, high concentrations of endogenous YAP are observed in cerebellar granule neural precursors (CGNPs) and tumor-repopulating cancer stem cells (SCs). YAP is also highly expressed in medulloblastomas, common childhood brain tumors, suggesting a potential link between the Hippo and sonic hedgehog (Shh) pathways. Activation of Shh signaling in CGNPs induces the expression and nuclear localization of YAP, promoting the development of these cells. Recent research proposes a new model wherein YAP acts as a nexus between neural stem cell proliferative pathways, including Notch and Shh, enhancing neural stem cell proliferation, challenging previous notions of how these pathways cooperatively regulate brain development.

4.3. Skin Stem Cells

The skin, being the largest organ in animals, acts as a crucial barrier against environmental threats and plays a vital role in maintaining the body's moisture balance. Epidermal stem cells (eSCs) located in the basal layer possess the remarkable ability for self-renewal, enabling the continuous regeneration of the skin while preserving its structural and functional integrity. Recent investigations have underscored the significance of YAP in epidermal development and the homeostasis of stem cells. Activation of YAP, as demonstrated in mouse models with skin-inducible YAP expression, leads to a significant thickening of the epidermal layer. Interestingly, this hyperplasia results from the expansion of undifferentiated interfollicular stem and progenitor cells. Studies also revealed that the classical Hippo kinases do not regulate YAP in the skin, with β-catenin, a component of adherens junctions, identified as a downstream negative regulator of YAP. This suggests that adherens junctions may function as molecular biosensors, regulating cell density and position. Disruption of this molecular network can lead to hyperproliferation and cancer in the skin.

4.4. Cancer Stem Cells

The Hippo Signaling Pathway (HSP) plays a crucial role in controlling organ size and inhibiting carcinogenesis by modulating cell proliferation, inducing apoptosis, and regulating the growth of stem/progenitor cells. Unphosphorylated YAP/TAZ in the nucleus promotes tumor and cell proliferation, while phosphorylated YAP/TAZ in the cytoplasm inhibits tumor growth. Elevated expression and activity of YAP/TAZ are associated with numerous malignant cancers, including breast cancer stem cells (CSCs) regulated by TAZ. Medulloblastoma CSCs also exhibit high expression of YAP and TEAD. Cancer stem cells, a small subset with self-renewal capabilities, differentiation potential, and the ability to drive tumor growth, have been linked to tumor development and resistance to chemotherapy, contributing to cancer spread and recurrence. Dysregulation of HSP has been implicated in various human malignancies, and inhibiting YAP/TAZ is considered a potential therapeutic strategy. Recent studies indicate that HSP controls the transcriptional networks of YAP and TAZ, offering potential avenues for treating cancers, such as prostate cancer.



Role of the Hippo signaling pathway in somatic stem cells

5. Drug Resistance

Drug resistance remains a significant challenge in current cancer treatments, predominantly relying on chemotherapy and targeted therapy. Unfortunately, these conventional approaches often fall short in eliminating cancer cells that transition into cancer stem cells (CSCs), leading to clinical recurrence. Chemotherapeutic resistance is particularly evident in tumor cells exhibiting heightened YAP/TAZ activity, contributing to CSC characteristics. For instance, YAP activation transforms prostate epithelial cells into androgen-insensitive cells with castration resistance, underscoring its role in treatment resistance. Recent investigations have identified miR-302/367-mediated LATS2 inhibition as a mechanism leading to YAP activation and conferring CSC status in prostate cancer cells.

Moreover, metastatic activity and drug resistance, observed in breast CSCs against chemotherapeutic drugs like paclitaxel and doxorubicin, have been associated with YAP/TAZ activation. Numerous studies emphasize that inhibiting the Hippo signaling pathway (HSP) or activating YAP/TAZ in various tumor types enhances cancer cell survival against DNA-damaging drugs such as cisplatin, taxol, and fluorouracil (5-FU). Additionally, YAP/TAZ plays a role in promoting resistance to targeted therapy. A notable clinical challenge is the observed YAP-induced resistance to agents targeting RAF and MEK in tumor cells harboring mutations in BRAF, KRAS, or NRAS. YAP's involvement in actin remodeling is a mechanism contributing to resistance in melanoma cells against BRAF inhibitors.

Crucially, YAP/TAZ-TEAD target genes, particularly secreted ligands, have been implicated in drug resistance. Connective tissue growth factor (CTGF), one of these target genes, has been identified as promoting chemotherapeutic drug resistance in various cancers, including glioblastoma multiforme (GBM), breast cancer, and osteosarcoma. These findings suggest that non-cell autonomous processes may play a pivotal role in targeting YAP/TAZ-induced secreted ligands for more effective interventions in drug resistance.

6. Pharmacological Interventions

Cancer stands as a leading global cause of mortality, with cancer stem cells exhibiting epithelial-mesenchymal transition (EMT) capabilities that contribute to metastasis and the transmission of drug resistance. In the realm of cancer cells displaying characteristics of cancer stem cells (CSCs), the Hippo-YAP/TAZ pathway has emerged as a potent oncogenic driver. When exploring therapeutic avenues for small-molecule treatments, targeting protein kinases that act as oncogenes becomes a promising strategy. Unfortunately, the pursuit of therapeutics has faced challenges due to the dual role of MST1/2 and LATS1/2, the core kinases of the Hippo pathway, serving as tumor suppressors.

As illustrated in Figure 4, researchers have been compelled to explore alternative therapeutic modalities to bypass the limitations posed by the tumor-suppressive nature of MST1/2 and LATS1/2. Subsequently, the following section outlines the most notable inhibitors of the Hippo-YAP/TAZ pathway.

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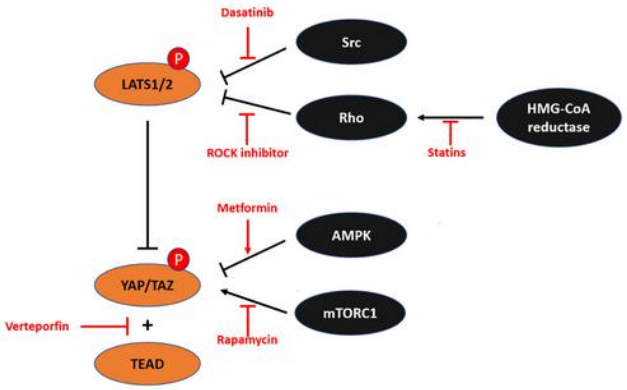

Strategies for Therapeutic Targeting of the Hippo Pathway are depicted in this figure, focusing on the modulation of YES-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) activity within the pathway. Several substances are identified for their inhibitory effects on YAP/TAZ activity:

1. Dasatinib, ROCK inhibitors, and statins: These compounds act by activating large tumor suppressor (LATS), resulting in the inhibition of YAP/TAZ activity.
2. Rapamycin and metformin: These substances function by inhibiting LATS-independent YAP/TAZ activity.
3. Verteporfin: This compound inhibits the interaction between YAP/TAZ and TEA domain transcription factor (TEAD).

10. Conclusions

Although the majority of components in the Hippo signaling pathway (HSP) were first identified in Drosophila, recent studies involving humans and animal models have revealed the pathway's crucial role in stem cell development, cancer initiation, and tissue homeostasis. YAP/TAZ, as vital downstream effectors of the HSP, play roles in embryonic stem cells, the self-renewal of tissue-specific stem cells, tissue regeneration, and maintenance of homeostasis in various organs such as the liver, colon, pancreas, heart, skin, and CNS. Strong evidence also suggests that YAP/TAZ contributes to the acquisition of traits associated with cancer stem cells. Consequently, targeting elements of the HSP holds promise for therapeutic interventions in diseases like cancer and degeneration. Despite the significance of the HSP in cancer development, oncogenic driver mutations in its main components are rare. The identification of the YAP/TAZ oncogenic signaling network specific to cancer stem cells provides a potential avenue for eliminating these cells and treating the progression of cancer.

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