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Invited Mini Review

Emerging role of Hippo pathway in the regulation of hematopoiesis

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In various organisms, the Hippo signaling pathway has been identified as a master regulator of organ size determination and tissue homeostasis. The Hippo signaling coordinates embryonic development, tissue regeneration and differentiation, through regulating cell proliferation and survival. The YAP and TAZ (YAP/TAZ) act as core transducers of the Hippo pathway, and they are tightly and exquisitely regulated in response to various intrinsic and extrinsic stimuli. Abnormal regulation or genetic variation of the Hippo pathway causes a wide range of human diseases, including cancer. Recent studies have revealed that Hippo signaling plays a pivotal role in the immune system and cancer immunity. Due to pathophysiological importance, the emerging role of Hippo signaling in blood cell differentiation, known as hematopoiesis, is receiving much attention. A number of elegant studies using a genetically engineered mouse (GEM) model have shed light on the mechanistic and physiological insights into the Hippo pathway in the regulation of hematopoiesis. Here, we briefly review the function of Hippo signaling in the regulation of hematopoiesis and immune cell differentiation. [BMB Reports 2023; 56(8): 417-425]

INTRODUCTION

Hippo signaling was originally uncovered in *Drosophila melanogaster* via a genetic mosaic screen to find novel regulators of cell proliferation and apoptosis, which led to the discovery of an evolutionary conserved organ-size control mechanism from insect to human (1-3). Similar to other signaling pathways, the crucial molecular circuits are transduced by a kinase cascade composed of two serine/threonine kinases (*hippo* and *warts* in fruitfly; their mammalian homologues are MST1/2 and LATS1/2, respectively). In response to various intrinsic and extrinsic stimuli, like cell contact, polarity, mechanical stress, and growth factors, the Hippo pathway can be initiated by stimulating MST1/2 kinases to activate LATS1/2 kinases (4). The active LATS1/2 kinases phosphorylate YAP and TAZ (Yorkie in fruitfly) to induce cytoplasmic retention by interaction with 14-3-3, and subsequent phosphorylation-dependent proteolysis by β-TrCP E3 ubiquitin ligase (5). Genetic variation or cytoskeleton rearrangement inhibits LATS1/2-mediated YAP/TAZ phosphorylation, directing YAP/TAZ to enter into the nucleus, where they interact with TEA domain transcription factor (TEAD) to activate transcriptional program (6).

In addition to primary function, such as organ size determination and homeostasis, recent research efforts have revealed that the Hippo pathway regulates immunity, autophagy, stemness, and miRNA biogenesis (7-11). Because dysregulation of the Hippo pathway causes a wide range of human diseases, such as developmental disorders and cancer, the precise and coordinated mechanism of the Hippo pathway in maintaining organ physiology is well-established (12). However, the role of Hippo signaling in lymphoid organs, such as bone marrow and thymus, is relatively less studied. Furthermore, the Hippo signaling pathway may also play a role in the development of hematologic malignancies, although there is limited evidence. Based on studies that show Hippo components are ablated in leukemia and lymphoma, the Hippo pathway may suppress hematologic malignancy in general (13). However, Hippo pathway component appears to have a context-dependent effect in the regulation of hematologic malignancy. YAP acts as a tumor-suppressor by regulating the Abl1-dependent DNA damage response (14), whereas TEADs promote the transcriptional stimulation during B-cell transformation (15). Furthermore, the patients with chronic leukemia frequently show hepatosplenomegaly (16). The transcription co-activators YAP/TAZ play a central role in organ size control, regeneration, and the expansion of stem cells by activating their target gene expression (17, 18). Likewise, the physiological role of Hippo signaling and YAP/TAZ in the immune system is still largely unknown.

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Table 1. Genetically engineering mouse models of the Hippo pathway in hematopoiesis

Cre line	Target genes and types	Phenotype and function	References
-	*Mst1*$^{-/-}$	Defect of the thymic egress and impairment of chemotactic responses to chemokines in T cell	(40)
-	*Mst1*$^{-/-}$	Splenomegaly and lymphadenopathy, skin lesion; autoimmune disease	(45)
-	*Mst1*$^{-/-}$	Deficiency of B cell recirculation to the bone marrow and B cell migration to the splenic red pulp	(47)
-	*Mst2*$^{-/-}$	Normal phenotype	(47)
CD11c-Cre	*Mst1*$^{loxp/loxp}$	Normal dendritic cell homeostasis Enhanced Th17 cell differentiation and autoimmune phenotypes	(44)
CD11c-Cre	*Mst1*$^{loxp/loxp}$*Mst2*$^{loxp/loxp}$	Reduction of the cellularities of lymphoid organs and CD8+ T cell population	(46)
CD11c-Cre	*Lats1*$^{loxp/loxp}$*Lats2*$^{loxp/loxp}$	Normal T cell homeostasis	(46)
CD11c-Cre	*Yap*$^{loxp/loxp}$*Taz*$^{loxp/loxp}$	Normal T cell homeostasis	(46)
Lck-Cre	*Mst1*$^{loxp/-}$	Defect of regulatory T cell development	(45)
Lck-Cre	*Mst1*$^{loxp/-}$	Reduction of peripheral T cells and accumulation of mature SP T cells in the thymus	(40)
Lck-Cre	*Ndr1*$^{-/-}$*Ndr2*$^{loxp/loxp}$	Lymphopenia and impaired thymic egress	(41)
Lck-Cre	*Taz*$^{loxp/loxp}$	Decrease of T_H17 population and increase regulatory T cells after KLH immunization	(43)
Ox40-Cre	*Mst1*$^{loxp/loxp}$*Mst2*$^{loxp/loxp}$	Normal T cell development in the thymus and normal numbers of T cells in peripheral lymphoid tissues Increase of T_H17 cell population and decrease regulatory T cell population after KLH immunization	(43)
Mx1-Cre	*Nf2*$^{loxp/loxp}$	Increase of vascular structures in bone marrow and HSC egress from BM to bloodstream	(35)
Mx1-Cre	*Mst1*$^{loxp/loxp}$*Mst2*$^{-/-}$	Increase of HSC pools, B cell lymphopenia, erythropenia in the BM and myeloid expansion Defect of HSC engraftment and homing ability	(31)
Mx1-Cre	*Yap*$^{loxp/loxp}$*Taz*$^{loxp/loxp}$	Mild erythropenia in old mice	(25)
Vav1-Cre	*Mst1*$^{-/-}$*Mst2*$^{loxp/loxp}$	T cell lymphopenia and defect of follicular B cells' recirculation	(47)
Vav1-Cre	*Yap1*$^{loxp/loxp}$*Taz*$^{loxp/loxp}$	Embryonic lethality	(17)

Hematopoiesis is a continuous process that generates all types of blood cells. After birth, hematopoiesis occurs in the bone marrow (BM), which is derived from hematopoietic stem cells (HSCs). Since its discovery in 1961 through bone marrow transplantation to lethally irradiated recipient mice, the spectrum of HSC differentiation has been extensively studied (19). The progenies of hematopoietic stem and progenitors (HSPCs), which are various blood cells and immune cells, are rapidly and expansively produced in adults. These processes may be associated with the Hippo pathway, because it has been shown to regulate extensive cellular proliferation and differentiation in other tissues. For example, new red blood cells (RBCs) are produced at approximately 2.4×10^6/s to maintain 5 L of blood in adult (20). Notably, Althoff and colleagues generated genetic ablation of *Yap* and *Taz* in hematopoietic stem cells using *Vav1-Cre* line to evaluate their function in lineage specification and differentiation, and they observed that the deficiency of *Yap*/*Taz* results in nonviable pups (21). Another study looked at the phenotypic association of *MST1* deficiency with three related people, and discovered that all three had severe lymphocytopenia and sporadic neutropenia (22). Furthermore, LATS2 was highly expressed in patients with chronic myeloid leukemia than in healthy controls. TAZ expression also higher in chemo-resistant than chemo-sensitive cells (23). These results indicate that the Hippo pathway or its core components are involved in hematopoiesis and immune responses.

HSCs give rise to nearly all immune cells in the body. Immune cell differentiation, activity, and cell-to-cell interactions are fundamental to comprehending immunology in the context of physiology and disease. Because of its pathophysiological importance, the roles of the Hippo pathway in hematopoiesis have recently gained much attention. Here, we discuss the emerging role and mechanistic insights of the Hippo pathway in hematopoiesis. In addition, for better understanding, Table 1 lists genetically engineered mouse models of the Hippo pathway to study hematopoiesis and briefly summarizes phenotypic results, while Fig. 1-3 depict the molecular mechanisms in hematopoiesis.

HEMATOPOIETIC STEM CELL

During mouse embryonic development, hematopoiesis begins in the yolk sac at embryonic day 7.5, and cells migrate from the yolk sac to the fetal liver, thymus, and finally bone marrow (24). HSCs reside in the bone marrow, and are at the top of the hematopoiesis hierarchy; all blood cells are produced by HSCs (25). The cell cycle of HSCs is activated in fetal liver, whereas most HSCs are dormant in adult bone marrow (26). There are at least three types of HSC populations: long-term (LT)-HSC, short-term (ST)-HSC, and multipotent progenitor (MPP). LT-HSCs are extremely rare, dormant, and have a long-term self-renewal reconstituting capacity (more than 34 months), whereas ST-HSCs have a short-term reconstituting capacity (less than one month). LT-HSCs differentiate into ST-HSCs, which then give rise to MPPs.

As MPPs develop, they gradually lose their capacity for self-renewal, and acquire a more diverse range of differentiation potentials (27). The first branch point occurs when MPPs divide into common lymphoid progenitors (CLPs, potentially differentiating into lymphoid lineage; B cell, T cell, Natural killer cell, and dendritic cells) and common myeloid progenitors (CMPs, potentially differentiating into myelocyte, erythrocyte, and megakaryocyte). CMPs can further differentiate into megakaryocyte-erythrocyte progenitors (MEPs) and granulocyte-macrophage progenitors (GMPs) (19, 28).

Hippo pathway is strongly linked to embryonic or adult stem cell self-renewal and differentiation, so it can be hypothesized that HSCs may be regulated by Hippo-YAP/TAZ signaling. Unexpectedly, YAP is expressed at very low levels in LT-HSCs, and even additional expression of ectopic wild-type or hyperactive YAP constructs (Hippo-insensitive form) in HSCs does not alter hematopoietic lineage distribution (29). Similarly, genetic double homozygous ablation of *Yap* and *Taz* in the adult hematopoietic system using *Mx1-Cre* recombinase results in no significant difference in cell number, including white blood cells (WBCs) and RBCs in the blood, and HSCs in the BM (30). As opposed to this dispensable role in adult HSCs, the genetic deletion of *Yap/Taz* at early embryonic development leads to miscarriage (21). Mechanistically, Scribble, known to act as a scaffold protein in various signaling pathways, forms complex with Cdc42 GTPase and Yap in the cytoplasm of HSC. In HSCs, the Scribble-Cdc42-Yap complex co-polarizes to control quiescence, fate, and fitness. Interestingly, even active YAP is preferentially localized in cytoplasm, and formed the Scribble-Cdc42-Yap complex (Fig. 1) (21).

Embryonic stem cells (ESCs) sequentially differentiate into mesoderm cells, hemangioblasts (HB), and hemogenic endothelium (HE) cells. HE cells have both hematopoietic and endothelial potential, and ultimately differentiate into HSCs via a process known as the endothelial-hematopoietic transition (EHT). The subcellular localization pattern of YAP is of interest during cellular lineage specification. At the HB stage, YAP is mostly found in the nucleus, while after EHT, is translocated to the cytoplasm. In addition, TEAD binding element region is more accessible at HB stage than at other stages, and the expression of both YAP and TEAD is downregulated. These results indicate that Hippo-YAP signaling is dynamically regulated, and plays a pivotal role in transcriptional reprograming during hematopoietic specification and differentiation (31).

Accumulating evidence suggests that mechanical stress acts as a molecular mechanism regulating stem cell fate, and cell behavior and the microenvironment (also known as niche). In vertebrates, blood flow is generated when the heart beats, which then induces mechanical forces, such as wall shear stress (WSS) and circulatory elongation (CE) on the endothelial cells of the dorsal aorta. This mechanical stress stimulates HSC differentiation through expression of the transcription factor RUNX1, a critical regulator of hematopoiesis (32, 33). The Hippo-YAP pathway serves as an important molecular mediator in responding to mechanical stimuli and adopting environmental changes, such as shear force, cell density, stretch, and matrix stiffness (33, 34). Recent study has shown that YAP-mediated mechanotransduction influences HSC fate. In HE cells, CE is sufficient to promote YAP nuclear localization and target gene activation, whereas WSS is not, despite the fact that both CE and WSS induce RUNX1 expression (Fig. 1). Additionally, YAP is required for the maintenance or progression of the hematopoietic lineage in HE, but not for the initiation of HE specification. Further experiments provide convincing evidence that Rho-GTPase functions as an upstream mechanotransducer of YAP in HSC differentiation induced by blood flow-mediated CE (Fig. 1) (35).

The roles of MST1/2 in regulating hematopoiesis have been investigated using the conditional knockout mice models. Hematopoietic cell-specific deficiency of *Mst1* in combination with whole-body knockout of *Mst2* results in expansion of the HSC pool via the promoted proliferation and inhibition of HSCs apoptosis (Fig. 1) (36). The *Mst1/2* double-knockout (DKO) also causes severe B cell lymphopenia, mild erythropenia, and expansion of the myeloid cell population. *Mst1/2*-deficient BM cells represent impaired engraftment, suggesting reduced homing activity of HSCs by the loss of *Mst1/2* (36). Similarly, in *Xenopus* primitive hematopoiesis model, Mst1/2 play significant roles in cell differentiation from hematopoietic and endothelial progenitors (37).

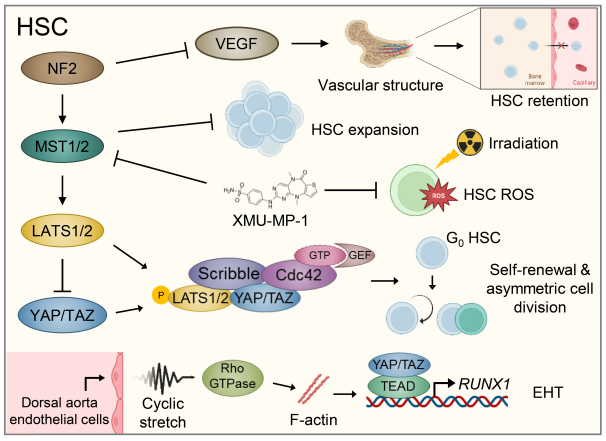

Fig. 1. The Hippo pathway is a signaling pathway that regulates the self-renewal and differentiation of hematopoietic stem cells (HSCs). Mechanical stresses and the presence of VEGF activate the pathway, which promotes HSC retention in the bone marrow. MST1/2 and XMU-MP-1, respectively, suppress HSC expansion and ROS signaling. LATS1/2 and YAP/TAZ copolarize and form a complex in the cytosol with Scribble-Cdc42 GTPases, which controls HSC self-renewal and asymmetric cell division. Mechanical cyclic stretch influences embryonic aortic endothelial cell differentiation into HSCs by activating Rho GTPase, an upstream regulator of YAP-mediated mechanotransduction, which leads to RUNX1 expression for endothelial-hematopoietic transition (EHT).

On the other hand, XMU-MP-1, a potent MST1/2 inhibitor, is sufficient to rescue irradiation-induced bone marrow damage (38). In mice, a significant decrease in blood cell counts and reduction of immune cell portion in the BM are observed from 4 Gy of total body irradiation. Pre-treatment with XMU-MP-1 seven days prior to irradiation ameliorates the hematopoietic defects by inhibiting NOX4/ROS/p38 signaling in HSCs and bone marrow nucleated cells (Fig. 1).

Overall, it is unclear whether MST1/2 is coupled to Hippo signaling in the hematopoietic system, although certain phenotypes are correlated. Since MST1/2 appears to be important in development, and the cell-to-cell interaction is critical in regulating hematopoiesis, MST1/2 could potentially provide a combining mechanism of action. For example, Nf2, an upstream regulator of MST1/2 (39), can regulate HSCs mobilization and generation in a non-cell-autonomous manner by maintaining the bone marrow microenvironment (40).

LYMPHOPOIESIS

There are two types of immune systems in vertebrate: innate immunity, and adaptive immunity. The majority of lymphocytes associated with adaptive immunity are B cells, T cells, and dendritic cells, which cooperate minutely with the innate immune system. During B and T cell development, DNA rearrangements and clonal selection of antigen receptor genes occur, resulting in a wide range of antigen recognition receptors. Through a process of negative selection, each lymphocyte can avoid recognizing self-antigens during development. Positive selection of T cells in the thymus also allows for the formation of single positive mature T cells (41).

The thymus is a specialized tissue for T cell development, which begins with the arrival of lymphoid precursors to the thymic cortex from the BM (42). Thymocytes then differentiate into double negative (DN; $CD4^{-}CD8^{-}$), double positive (DP; $CD4^{+}CD8^{+}$), and finally, single positive (SP; $CD4^{+}CD8^{-}$ or $CD4^{-}CD8^{+}$) thymocytes. As mentioned above, antigen receptor gene rearrangements, as well as negative and positive selection, occur concurrently. The thymus sheds mature $CD4^{+}$ or $CD8^{+}$ T cells into the bloodstream. Then, T cells circulate between secondary lymphoid organs and the bloodstream to monitor infections (43). An earlier study discovered that Mst1/2 expression was higher in SP thymocytes than in DP thymocytes (Fig. 2) (44). Therefore, the authors speculate that Mst1/2 is associated with late stages of differentiation. Similarly, Mst1 deficiency results in an abundance of SP thymocytes in the thymus, but no significant difference in DP thymocytes (45). Interestingly, Mst2 does not alter the lymphocyte cellularity, while an additional deletion of *Mst2* genes on *Mst1*-deficient mice exacerbate the Mst1 knockout phenotype. Mst1 and Mst2 also play roles in thymic degeneration and the homing of mature thymocytes (44). CCL19 is a chemokine that promotes thymocyte migration by activating the Rac1 guanyl nucleotide exchanger Dock8, which requires Hippo components Mob1A/B

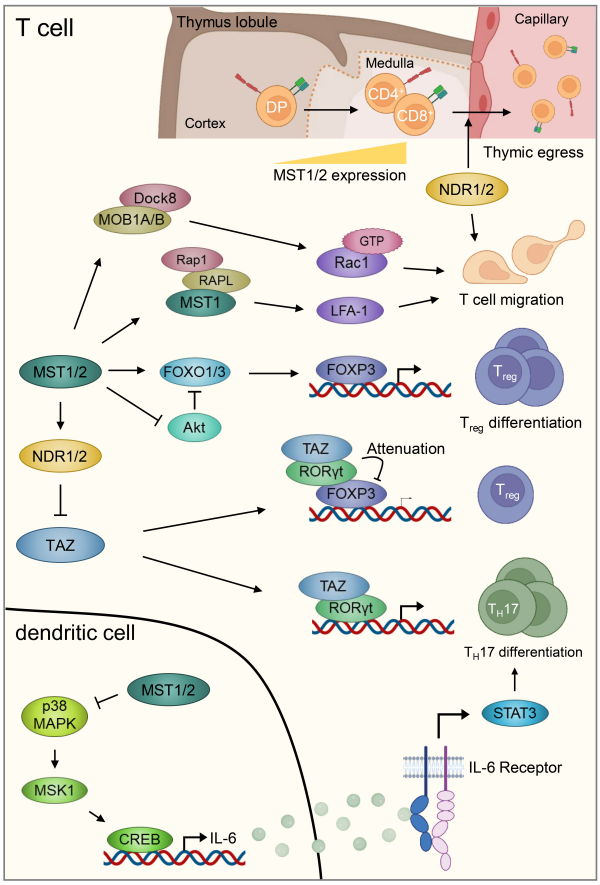

Fig. 2. The Hippo pathway is involved in T cell development. In the thymus, MST1/2 are more expressed in single-positive (SP) thymocytes than in double-positive (DP) thymocytes in the thymus. NDR1/2 regulate the egress of naive T cells from the thymus and migration to the periphery. MST1/2 regulates T cell migration by activating MOB1A/B, which is required for the activation of the guanyl nucleotide exchanger Dock8, and by promoting LFA-1 clustering through direct interaction with the small GTPase Rap1-binding effector protein RAPL. MST1/2 also enhances regulatory T (T_{reg}) cell differentiation by stabilizing FOXO1/3 or attenuating TCR-stimulated AKT activity. TAZ suppresses T_{reg} differentiation by inhibiting FOXP3 via the TAZ-RORγt complex. TAZ stimulates Th17 cell differentiation through a complex with RORγt. MST1/2 in dendritic cells affect Th17 cells differentiations by inhibiting p38MAPK-MK2/MSK1-CREB signaling, which is implicated in IL-6 production.

that are regulated by Mst1/2 (Fig. 2). Similarly, NDR1/2, members of the same kinase family as LATS1/2, act as downstream effectors of MST1 to mediate thymic egression and the interstitial migration of naive T cells (Fig. 2) (46). Mst1 also plays a role in T-cell polarity and adhesion by interacting directly with the small GTPase Rap1-binding effector protein RAPL and promoting LFA-1 clustering (Fig. 2) (47). Taken together, the Rap1/RapL-MST/MOB1-NDR axis regulates T cell development via promoting thymocyte egression and lymphocyte migration.

Naïve $CD4^+$ T cells mature to effector T cells, which in response to stimuli then differentiate into helper T cell subsets. TAZ promotes the development of T_H17 cells among effector $CD4^+$ T cell subsets by acting as a co-activator of the key transcription factor RORγt. TAZ interacts with RORγt directly to induce IL-17 for T_H17 differentiation (Fig. 2). Moreover, TGF-β-IL-6 signaling promotes TAZ expression together with the activation of downstream transcription factors SMAD3 and STAT3 that promote early T_H17 cell differentiation (48). MST1 activity can also play a role in T_H17 cell differentiation via modulating IL-6 production by dendritic cells (DCs) (Fig. 2). In mice with DC-specific *Mst1* deletion, the number of DCs and other immune cells is unaffected, which includes DC generation, as well as the apoptosis or proliferation of DCs (49). On the other hand, *Mst1*-deficient mice DCs represent p38MAPK-MK2/MSK1-CREB signaling activation, resulting in IL-6 cytokine production (Fig. 2). One of the key mechanisms for T_H17 cell development from effector $CD4^+$ T cells is IL-6-dependent STAT3 activation (49).

In particular, TAZ/RORγt can interact with FOXP3, which suppresses FOXP3 activity and the development of regulatory T cell (T_{reg}) (48). Accordingly, FOXO1/3 is stabilized by MST1/2 by either directly phosphorylating it, or by attenuating TCR-stimulated AKT signaling, which can cause FOXP3 activity in T cells (Fig. 2) (50). There is evidence that hippo signaling has an impact on $CD8^+$ T cells as well. $CD8\alpha^+$ DCs are a distinct subset that preferentially present antigen to prime $CD8^+$ T cells. It is interesting to note that while the deletion of *Mst1/2* in DCs leads to the disordered homeostasis and function of $CD8^+$ T cells, the deletion of *Lats1/2* or *Yap/Taz* in DCs does not, suggesting that an uncoupled Hippo signaling may play a role in either $CD8^+$ T cell proliferation, or antigen presentation process by $CD8\alpha^+$ DCs (51).

Mice with HSC-specific *Mst1/2* deletion have fewer B cells in the lymph node, and during B cell differentiation, less blood is circulated back to the bone marrow. Early B cell development can be studied using the Hardy fraction sorting method, which divides B cell precursors into subpopulations; however, neither *Mst1*-null mice nor HSC-specific *Mst1/2* deficient mice exhibit obvious changes in follicular B cells recirculating in the BM (52).

MEGAKARYOPOIESIS/ERYTHROPOIESIS

Megakaryopoiesis mainly occurs in the BM, and produces platelets. Platelets are critical for hemostasis and blood coagulation. In adult human, approximately 10^{11} platelets are produced daily at a steady state, but at times, the rate of production can increase 10-fold (53). Mature megakaryocytes (MKs) are large BM cells that undergo endomitosis, leading to polyploidy. This can result in a ploidy level of up to 128 N and visible cell enlargement. Cell division stops during endomitosis at late anaphase. It is known that 64 N MK is 56 ± 8 μm, otherwise 2 N MK is 21 ± 4 μm, and MKs representing more than 4 N can produce platelets. Furthermore, large MKs have abundant mRNA and protein in the granules (54, 55).

Previous study discovered that the Hippo-p53 axis regulates the tetraploid checkpoint in response to decreased Rho activity (56). LATS1/2 interact with MDM2 to inhibit its E3 ligase activity. As a result, activation of Hippo pathway leads to an increase of p53 stability while reducing YAP activity (57). Although RhoA activity is downregulated during MK differentiation and polyploidization, mRNA and protein levels of Hippo pathway components, such as YAP, TAZ LATS1, and LATS2, remain mostly unchanged (Fig. 3, upper panel). In addition, inactivation of RhoA in human umbilical cord blood (CB)-derived MK by a ROCK inhibitor (Y27632) did not induce Hippo-p53 signaling activity. On the other hand, Y27632 induces Hippo-p53 signaling during erythropoiesis via the *in vitro* differentiation of human primary HSC/HSPC-derived erythrocytes, implying that the RhoA and Hippo-p53 pathways are not coupled in MKs (Fig. 3, upper panel). Furthermore, depletion of YAP does not significantly affect the megakaryocytic phenotype. Nevertheless, whole platelet formation is reduced by YAP inhibition (58).

The effect of LATS1/2 and YAP levels in MK was partially investigated using MEG-01 cells, a human megakaryoblastic leukemia cell line (59). Overexpression of YAP in MEG-01 cells increases the number of $CD41^+$ cells, which represents MK precursors. Although there are limitations to fully understanding hematopoiesis *in vivo*, this study shows that YAP activity may be involved in regulating some anti-apoptotic genes like *BCL-XL* and maintaining blood cell precursors (60, 61).

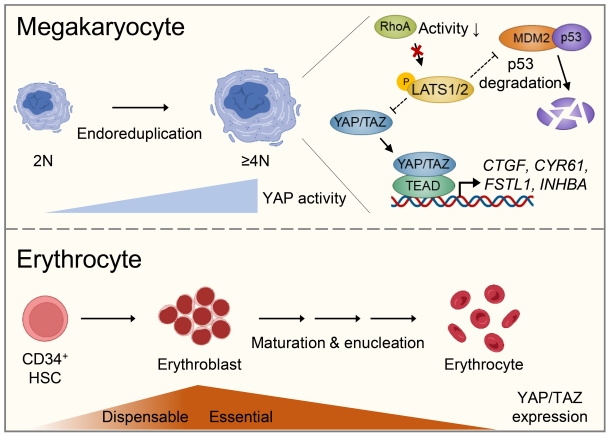

Fig. 3. The Hippo pathway is involved in megakaryopoiesis (upper panel) and erythropoiesis (bottom panel). In megakaryopoiesis, YAP activity is elevated during endoreduplication. Although RhoA activity is reduced, LATS1/2 activity is unaffected. This uncoupling of RhoA and Hippo-p53 signaling is assumed to be essential for megakaryopoiesis (upper panel). YAP/TAZ are required for the maturation and enucleation of erythroblasts at late stages of erythropoiesis. However, they are unnecessary in the early stages (bottom panel).

Erythropoiesis is the process of differentiation that leads to the formation of RBCs. RBCs are abundant in the blood, and are responsible for transporting oxygen from the lungs to all organs via the blood vessels. RBC in adult human has a lifespan of about 120 days. Under normal circumstances, macrophages in the liver and spleen eliminate aged RBCs, while newly formed RBCs are equally released into the bloodstream from the BM. Enucleation is a critical step in the production of reticulocytes during erythropoiesis. Reticulocytes mature into RBCs after losing their reticulum in the BM (62, 63).

During *in vitro*, erythropoiesis derived from peripheral blood (PB) and CB HSCs YAP/TAZ expression is increased (64, 65). The level of YAP/TAZ protein was found to be higher in erythroid progenitors, proerythroblasts, and erythrocytes, than in $CD34^+$ hematopoietic stem cells. The loss of YAP/TAZ function in HSC affects erythropoiesis by impaired enucleation later in the process (Fig. 3, bottom panel) (64). However, gain-of-function has no additional effects on erythropoiesis *in vitro*, suggesting that YAP/TAZ threshold levels in erythroid differentiation may be limited. Furthermore, the absence of YAP/TAZ in human blood-derived HSCs has no effect on myeloid/erythroid lineage determination (64). To maintain a steady state under anemic stress conditions, such as irradiation, robust RBC production can occur. Upregulation of YAP is required under these stress conditions to restore adequate RBC numbers. In lethally irradiated mice, bone marrow transplantation of YAP-depleted BM cells resulted in a significant reduction in proliferative stress erythroid precursors (SEPs), which died within 21 days of transplantation. On the other hand, ectopic expression of YAP-S127A, a constitutively active form, in mouse BM donor cells significantly rescued the anemic phenotype of the recipient mice (66).

GRANULOPOIESIS/MONOCYTOPOIESIS

In addition to megakaryocyte-erythroid precursor, common myeloid progenitor (CMP) differentiates into granulocyte and monocyte. Monocytes are able to differentiate into macrophages or myeloid dendritic cells, known as granulation and monocyte formation. Because of their distinctive cytoplasmic granules and irregular nuclei, granulocytes, such as neutrophils, basophils, and eosinophils, are also known as polymorphonuclear leukocytes. Cytoplasmic granules contain antimicrobial and inflammatory substances (67).

Neutrophils are the most abundant granulocytes that recognize pathogens quickly, and also act as phagocytes. Eosinophils play an important role in innate immune response against helminth and other intestinal parasite infections. Although basophils are known to respond to parasites, their biological function is unknown. Mature granulocytes are continuously produced in large numbers from precursors, averaging (0.5 to 1) $\times$ 10^{11} granulocytes per day in adults (68).

Monocytes circulate in the bloodstream, and can travel to the tissue infection sites. These cells have phagocytic activity, and regulate immune responses. Monocytes mature into macrophages in infected or damaged tissue, where they can ingest not only pathogens, but also apoptotic cells and tissue debris. The function of monocyte-derived macrophages is sometimes divided into two groups: classical and alternative macrophages play important roles in inflammatory response and tissue repair, respectively. The primary biological function of macrophages is to secrete cytokines, which recruit other immune cells and activate innate immunity (69).

While numerous studies have focused on the intersection between the innate immune response and Hippo signaling, the mechanisms involved in granulocyte generation via Hippo signaling are poorly understood (70). The *Mx1*-Cre model is the most widely used transgenic mouse, specifically expressed in HSCs, in which Cre recombinase is controlled by a type I interferon-inducible promoter (Mx1). The genetic loss of *Mst1* and *Mst2* genes using *Mx1*-Cre line increased the number of monocytes and neutrophils. On the other hand, Abdollahpour *et al*. described the clinical phenotype of human *MST1* deficiency, which is associated with lymphopenia, intermittent neutropenia, and arterial septal defects (22). This discrepancy in neutrophils could be due to the patient already being exposed to a number of harmful microbes. Antibodies against herpes simplex virus, herpes zoster virus, EBV, measles, tetanus, diphtheria, and mumps have been discovered in patients. Therefore, because laboratory mice do not normally come into contact with these microbes, the precise role of Hippo signaling in neutrophil development remains to be elucidated.

HEMATOPOIESIS IN *DROSOPHILA MELANOGASTER*

Unlike vertebrates, invertebrates have merely an innate immune system. The Hippo pathway was originally identified in *Drosophila melanogaster*, which has long been used to study innate immunity (71). Thus, the Hippo pathway serves as a critical mechanism that regulates *Drosophila* hematopoiesis, as evidenced by numerous studies. *Drosophila*'s circulating blood cells are referred to as hemocytes, and there are three types of blood cells: plasmatocytes, lamellocytes, and crystal cells. They function similarly to mammalian bone marrow cells. Plasma cells have antibacterial action, as well as the same phagocytic function as macrophages. In the presence of stressful conditions, such as wasp parasitism, injury, or tissue damage, lamellocytes are generated. When bactericidal reactive oxygen species are produced at the site of infection, crystal cells trigger melanization and coagulation. In the larva development and adults, plasmatocytes are the most prevalent (approximately 90-95%) of blood cells, with crystal cells accounting for the remainder (about 2-5%). While lamellocytes are extremely rare, they are induced by parasitic wasps (72).

Yorkie (*drosophila* homologue of YAP/TAZ) and scalloped (*drosophila* homologue of TEAD) are required for crystal cell specification. Yorkie-scalloped in crystal cell progenitors maintain proper cell numbers by regulating serrate that acts as

initiator of crystal cell differentiation. Yorkie, but not scalloped, is still expressed in mature crystal cells, indicating that scalloped was expressed prior to crystal cell formation. Yorkie and scalloped specifically modulate crystal cell differentiation, as aberrant induction of yorkie and scalloped has no effect on plasmatocyte number (73). Down-regulation of scalloped in crystal cell progenitors is critical for maintaining an adequate number of crystal cells, because ectopic expression of scalloped in crystal cell progenitors promotes an increase in the number of crystal cells. Apoptosis is caused by the absence of yorkie and scalloped in crystal cell progenitors (74). In the absence of warts (*drosophila* homologue of LATS1/2), lymph gland cell proliferation is stimulated, and their size increases to that of the wild type. In addition, lozenges, Runx family transcription factors that are key regulators of cell fate, are also regulated by yorkie and scalloped (75). Another group found that ectopic yorkie activation increased plasma cell proliferation, but did not promote lamellocyte differentiation (76).

CONCLUSION

Here, we briefly reviewed how the size-control mechanism of Hippo pathway can be involved in hematopoiesis. Hematopoiesis is an important and essential process for homeostasis and host defense, and is maintained continuously until death. All immune cells are made through hematopoiesis. Despite its continuity and complexity, the hematopoietic lineage has been extensively studied over time, due to its pathophysiological importance. Throughout organismal development from embryo to adult, the Hippo pathway regulates cell identity, stemness, proliferation, growth, organ size determination, and even senescence (18, 77). In embryonic stem cells (ESCs), for example, YAP and TAZ promote self-renewal while inhibiting differentiation (78). Excess Yap causes epidermal stem cell expansion and squamous cell carcinoma in adult mouse skin (79). Similarly, hematopoiesis must occur and continue throughout one's life. It is understandable that to maintain homeostasis, HSCs have to retain pluripotency, self-renewal potential, and bone marrow niche interactions. Despite numerous investigations of Hippo pathway-related hematopoiesis, there is still a dearth of understanding of the precise mechanisms and differentiation processes. YAP/TAZ also acts as a mechanotransducer by sensing cell-cell contacts and matrix rigidity (33, 34). Understanding the physiological relationship between mechanical stress and hematopoiesis has been emerging, as the role of bone marrow niche in hematopoiesis is critical. It would also be interesting to investigate the YAP/TAZ-dependent noncanonical Hippo pathway in hematopoiesis.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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