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REVIEW

Role of Hippo signaling in regulating immunity

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The Hippo signaling pathway has been established as a key regulator of organ size control, tumor suppression, and tissue regeneration in multiple organisms. Recently, emerging evidence has indicated that Hippo signaling might play an important role in regulating the immune system in both *Drosophila* and mammals. In particular, patients bearing a loss-of-function mutation of MST1 are reported to have an autosomal recessive primary immunodeficiency syndrome. MST1/2 kinases, the mammalian orthologs of *Drosophila* Hippo, may activate the non-canonical Hippo signaling pathway via MOB1A/B and/or NDR1/2 or cross-talk with other essential signaling pathways to regulate both innate and adaptive immunity. In this review, we present and discuss recent findings of cellular mechanisms/functions of Hippo signaling in the innate immunity in *Drosophila* and in mammals, T cell immunity, as well as the implications of Hippo signaling for tumor immunity.

Keywords: The Hippo pathway; MST1/2; innate immunity; adaptive immunity

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INTRODUCTION

The Hippo signaling pathway defines a novel signaling cascade regulating cell contact inhibition, cell proliferation, apoptosis, and cancer development.[1–9] While originally identified in *Drosophila*, the pathway is highly conserved in mammals. The core components of the canonical Hippo pathway in mammals consist of mammalian Ste20-like kinases 1/2 (MST1/2, orthologs of Hippo in *Drosophila*) and their adapter protein, Sav family WW domain-containing protein 1 (WW45, ortholog of Salvador), large tumor suppressor 1/2 (LATS1/2, orthologs of Warts), and their adapter protein, Mps one binder 1A and B (MOB1A/B, orthologs of Mats), two Yorkie orthologs, Yes-associated protein (YAP), and transcriptional co-activator with PDZ-binding motif (TAZ). The MST1/2-WW45 complex phosphorylates and activates the LATS1/2-MOB1A/B complex, which in turn phosphorylates YAP/TAZ and prevents their nuclear localization. As a transcriptional co-activator, nuclear YAP or TAZ binds to their key partners, the TEAD family transcription factors (TEAD1-4, orthologs of Sd), to enhance cell proliferation and survival through regulating the transcription of target genes. More recently, several new members, such as Ras association domain family member 5 (Rasff5, also called RAPL or Nore1B), nuclear Dbf2-related 1 (NDR1/2, also known as STK38/STK38L), and mitogen-activated protein kinase kinase kinase kinase (MAP4Ks), were identified and added to the Hippo-centered network. Many functional studies on canonical Hippo signaling currently focus on the well-known YAP-dependent role in the regulation of development, growth, and tumorigenesis.

The immune system protects the host from diseases by recognizing and responding to antigens from either outside or inside of the body. Recently, increasing evidence has revealed that Hippo signaling also plays a crucial role in regulating the immune system. The first few studies that attracted researchers' attention to the indispensable role of Hippo signaling in the immune system were in vitro studies demonstrating that the MST1/RAPL complex was required for Rap1-GTP-dependent integrin lymphocyte function-associated antigen-1 (LFA-1) activation/clustering-mediated T-cell adhesion and migration, following T-cell receptor (TCR) or chemokine stimulation.[10, 11] Later, in vivo animal studies, as well as the discovery of loss-of-function (LOF) *MST1* mutations in human patients, demonstrated that Hippo signaling components act as important regulators in regulating both innate and adaptive immune responses. To date, various knockout mice of major components of the Hippo pathway have been generated and studied to elucidate the role of this pathway in the immune system (Table 1).

MST1 DEFICIENCY AND HUMAN PRIMARY IMMUNODEFICIENCY SYNDROME

In 2012, the Klein group in Germany and the Basile group in France independently reported that LOF mutations of *MST1* in patients resulted in autosomal recessive primary immunodeficiencies.[12, 13] MST1-deficient patients showed recurrent bacterial and viral infections and autoimmune manifestations, as well as clinical signs of T- and B-cell lymphopenia and a progressive loss of naive T cells, which were also observed in MST1-deficient mice.[12–15] More recently, the Sanal group found another novel *MST1* mutation in a group of patients, whose clinical symptoms were similar to those of dedicator of cytokinesis 8 (DOCK-8) deficiency, a form of autosomal recessive hyperimmunoglobulin E syndrome.[16] Overall, all the patients carry nonsense mutations in the *MST1* gene, and the majority of these patients have symptoms that onset at a very early age and encounter bacterial or virus infections. Immunologic studies showed significant lymphopenia, especially T-cell lymphopenia, and revealed a possible neutrophil

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Table 1. Mouse models to study the Hippo pathway in immunity

Gene	Mice	Phenotypes of the immune system	Ref.
Mst1; Mst2	$Mst1^{-/-}$	T-cell lymphopenia, fewer naive peripheral T cells, and a high proportion of effector/memory T cells in liver and lung	Zhou et al.[15]
	$Mst1^{-/-}$; $Mst1^{fl/-}$*Lck*-Cre	Splenomegaly; autoimmune diseases	Du et al.[30]
	$Mst1^{-/-}$	Impaired thymocyte selection and thymic Treg generation	Ueda et al.[33]
	$Mst1^{fl/fl}$*CAG*-Cre;	Autoimmunity; leukocyte infiltrates in multiple organs	Ueda et al.[33]
	$Mst1^{fl/fl}$*Lck*-Cre	Severe leukocyte infiltration and autoantibody production	
	$Mst1^{fl/fl}$*CD11c*-Cre	Enhanced Th17 differentiation and more severe EAE; enhanced anti-fungal immunity	Li et al.[41]
	$Mst1^{fl/fl}$*Lysm*-Cre	Chronic inflammation-related hepatocellular carcinoma	Li et al.[21]
	$Mst1^{-/-}Mst2^{fl/fl}$*Vav*-Cre	Impaired thymocytes egress, lymphopenia; recurrent infections; inflammation	Mou et al.[15]
	$Mst1^{fl/fl}Mst2^{fl/fl}$*Lyz2*-Cre	Enhanced susceptibility to CLP-induced sepsis; impaired phagocytosis and clearance of bacteria	Geng et al.[21]
	$Mst1^{fl/fl}Mst2^{fl/fl}$*Ox40*-Cre	Normal numbers of T cells in peripheral lymphoid tissues; enhanced induction of Th17 cells and decreased numbers of Treg cells in draining lymph nodes after KLH immunization	Geng et al.[27]
Ndr1;Ndr2	$Ndr1^{-/-}$	Susceptible to CLP-induced sepsis; susceptible to *Escherichia coli* infection	Wen et al.[23]
	$Ndr1^{-/-}Ndr2^{fl/fl}$*Lck*-Cre	Peripheral T-cell lymphopenia; impaired thymic emigration	Tang et al.[35]
Yap	$Yap^{fl/-}$*EIIa*-Cre	Resistance to VSV/HSV-1 infection	Wang et al.[25]
	$Yap1^{fl/fl}$*Lyz2*-Cre	Enhanced viral clearance and diminished morbidity	
Taz	$Taz^{fl/fl}$*Lck*-Cre	Impaired Th17 differentiation and enhanced Treg differentiation; resistance to EAE	Geng et al.[27]

granulocyte deficiency, which may lead to a primary immunodeficiency effect on both innate and adaptive immunity. It is unknown whether these MST1-deficient patients will be more susceptible to tumor development. Studies in genetically modified mice showed that mice deficient in both MST1 and MST2 exhibit early embryonic lethality, while MST1 and MST2 conditional double-knockout mice develop spontaneous tumors in corresponding tissues, such as the liver and colon. However, *Mst1* or *Mst2* single-gene knockout mice are viable and do not exhibit obvious organ overgrowth or tumor development, suggesting a functional redundancy of MST1 and MST2. As has been observed in mice, MST1 expression is highest in the patients' lymphoid tissues, which strongly suggests that MST1 has a major role in the immune system. Thus, it is perhaps not surprising that MST1 deficiencies in either mice or humans result in multiple defects in the immune system.

HIPPO SIGNALING IN THE INNATE IMMUNE SYSTEM

The innate immune system constitutes the first critical line against microbial infections by discriminating self- from non-self-components.[17] Phagocytic cells, such as neutrophils, macrophages, and dendritic cells (DCs), can utilize pattern recognition receptors to detect, engulf, and kill extracellular pathogens. These receptors include the Toll-like receptor (TLR) family, the C-type lectin-like family, scavenger receptors, and complement receptors. Innate immune cells can also recognize viruses and other intracellular pathogens using receptors inside the cells, such as retinoic-acid inducible gene I (RIG-I) and cyclic GMP-AMP synthase (cGAS). The recognition of pathogens triggers the synthesis and release of various kinds of cytokines and chemokines, which in turn recruit more immune cells to the site of infection and bring the infection under control. Different members of the Hippo pathway have been implicated in the particular functions of the innate immune system (Fig. 1).

Hippo signaling and the innate immune response in *Drosophila*

Although the vertebrate immune response can be divided into innate and adaptive immunity, invertebrates only have innate immunity responses, which are powerful and effective to protect them from infections. The Toll-NF-κB pathway was found to control innate immunity in *Drosophila* two decades ago, which facilitated the discovery of the TLR family and of cross-talk between innate and adaptive immunity. Recently, an elegant study by Liu et al.[18] reported a role for the Hippo pathway in Toll receptor-mediated innate immunity in *Drosophila*. The Toll pathway function is required for host defense against Gram-positive bacterial and fungal infections in *Drosophila*. In the absence of Hippo function, Gram-positive bacteria and fungi, but not Gram-negative bacteria, resulted in increased lethality in flies. This immune phenotype was very similar to that observed in Toll signaling-deficient *Drosophila*. Without inhibition by Hippo, Yorkie directly increased the transcription of the *Drosophila* IκB factor, *Cactus*, which prevented the nuclear translocation of the NFκB transcription factor(s) dorsal and the dorsal-related immunity factor (Dif), as well as the expression of anti-microbial peptides. Upon activation by Gram-positive bacteria, the Toll-Myd88-Pelle cascade led to phosphorylation and degradation of the Cka subunit of the Hippo inhibitory complex, releasing Hippo to achieve Yorkie blockage and induction of anti-microbial effects. Thus, Hippo signaling enhances NF-κB signaling and promotes anti-microbial peptide expression in *Drosophila* (Fig. 1). Similar results were obtained from experiments with *Warts* knockdown and *Yorkie* overexpression flies. Consistent with these results, Dubey and Tapadia[19] showed that Yorkie reduced anti-microbial peptides and polyglutamine-mediated neurodegeneration by negatively regulating the IMD and Toll pathways. All these results indicate that the canonical Hippo pathway in fat bodies functions as a regulator of innate immunity. However, with regard to the regulation of NF-κB signaling by Hippo signaling, results are inconsistent between mammalian macrophages and *Drosophila* fat cells. The phosphorylation levels of IκB kinase (IKKα/β) in response to LPS stimulation, as well as the phosphorylation levels of IκBα, were higher in MST1/2-deficient bone marrow-derived macrophages (BMDMs) compared to that of wild-type control cells, suggesting that the loss of Hippo signaling might enhance

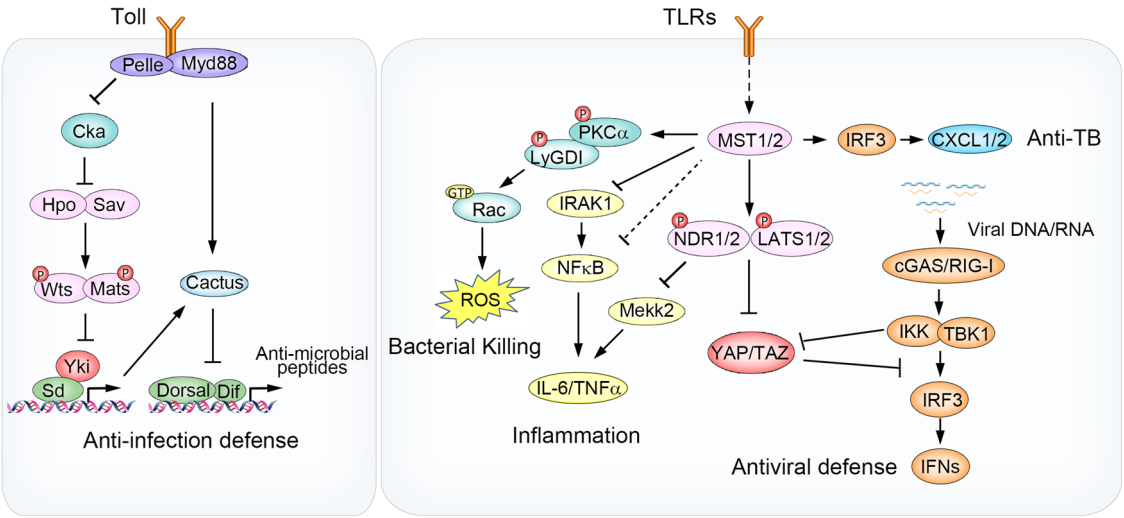

Fig. 1 The Hippo pathway plays critical roles in the innate immune regulation. The Toll or TLR signaling pathway activates Hippo signaling in innate immune cells. Left: upon activation by gram-positive bacteria, the Toll-Myd88-Pelle cascade leads to the activation of Hippo signaling, which causes Yorkie (Yki) blockage and induction of Dorasl/Dif-mediated anti-microbial peptide expression in *Drosophila*. Right: upon TLR ligation, activated MST1/2 induces TRAF6-ECSIT complex assembly through PKCα and Rac, promoting ROS generation and bacterial killing. MST1/2 also regulates the expression and secretion of chemokines (such as CXCL1/2) through IRF3 for anti-mycobacterial immunity. On the other hand, MST1/2 and its downstream effectors LATS/NDR limit pro-inflammatory cytokine (IL-6 and TNFα) production by inhibiting IRAK1/NF-κB and Mekk2, respectively. During viral infection, virus-activated kinase IKK phosphorylates YAP and targets it for degradation, while YAP/TAZ negatively regulates anti-viral immunity by blocking either TBK1 or IRF3 activity. Collectively, the Hippo pathway plays important roles in innate immunity against pathogens and protects the host from inflammatory injury during infection

the LPS/TLR4-mediated activation of NF-κB, which could be responsible for increased induction of pro-inflammatory cytokines, such as interleukin (IL)-6 and TNFα, in MST1/2-deficient BMDMs upon stimulation with LPS.[20] Whether downstream effectors, such as YAP or TAZ, regulate the transcription of IκBα in mammalian macrophages remains to be investigated.

Hippo signaling modulates bactericidal activity and immune responses in mammals

Human patients bearing LOF mutation of *MST1* have recurrent bacterial or viral infections.[12, 13, 16] Consistently, our group showed that *Mst1/2* knockout mice had enhanced susceptibility to cecal ligation and puncture (CLP)-induced bacterial sepsis.[20] TLR1, TLR2, and TLR4 are the major sensors of bacteria and fungi infections in mammals. LPS (an agonist of TLR4), Pam_3CSK_4 (an agonist of TLR1/2), or LTA (an agonist of TLR2) induced MOB1A/B phosphorylation in BMDMs, suggesting the activation of MST1/2 by TLR signaling. Further study showed that TLR/MyD88-mediated activation of MST1 and MST2 in phagocytes was required for phagocytosis and efficient clearance of bacteria. Upon TLR activation, MST1/2 activated the small GTPase Rac to induce TRAF6-ECSIT complex assembly, which is required for recruitment of mitochondria to phagosomes and generation of reactive oxygen species (ROS) to kill engulfed bacteria. This study demonstrated that TLR-MST1/2-Rac-TRAF6 signaling is essential for ROS production and bactericidal activity.[20] In addition, Li et al.[21] showed that MST1 phosphorylated and degraded the interleukin-1 receptor-associated kinase 1 (IRAK1) to regulate the TLR signaling pathway and protect against chronic inflammation-associated hepatocellular carcinoma (HCC). Similarly, *Mycobacterium tuberculosis* was shown to utilize the TLR2-IRAK1/4 axis to activate MST1/2, which in turn modulated C-X-C motif chemokine ligand 1/2 (CXCL1/2) expression and host immune responses in an interferon regulatory factor 3 (IRF3)-dependent but LATS1-independent manner.[22] In addition, NDR1, another component of the Hippo signaling pathway, which is downstream of MST1/2 kinases, was shown to interact with the E3 ubiquitin ligase Smurf1 and mediate Mekk2 ubiquitination and degradation, which was required for TLR9-induced inflammatory cytokine production. As a result, $Ndr1^{-/-}$ mice were more susceptible to *Escherichia coli* infection and CLP-induced sepsis.[23] These results, including the above-mentioned study in *Drosophila*, all suggested that the major innate sensors, TLRs, or Toll receptors, can either directly activate or interact with the Hippo signaling pathway for modulating immune responses and host defense during infections.

Hippo signaling in innate anti-viral responses

In general, intracellular viral nucleic acids are recognized by innate immune sensors such as RIG-I and cGAS. Upon recognition, they activate kinases IKK and TANK-binding kinase 1 (TBK1), which then recruit IRF3 and promote the phosphorylation, dimerization, and nuclear translocation of IRF3 to induce the expression of its effector genes, such as IFNs. Recent studies demonstrated that YAP negatively regulated an anti-viral immune response by preventing IRF3 dimerization and its translocation to the nucleus after viral infection.[24, 25] Knockout of YAP resulted in enhanced innate immunity and a reduced viral load. Notably, the viral-triggered innate immune signaling induced IKKε-mediated, but not MST1/2-LATS kinase-mediated, phosphorylation of YAP at Ser403 and thereby promoted lysosome-mediated degradation of YAP, leading to enhanced cellular anti-viral responses. Similarly, Zhang et al.[4] showed that YAP/TAZ inhibited host anti-viral immunity by blocking the function of TBK1, which is central to cytosolic nucleic acid sensing and anti-viral defense. Briefly, YAP/TAZ interacted with TBK1 directly and prevented Lys63-linked ubiquitination of TBK1 and its association with the adapters/substrates, and therefore, abolished virus-induced TBK1 activation. Thus, loss of YAP/TAZ or Lats1/2 kinases-mediated inactivation of YAP/TAZ relieved TBK1 suppression and enhanced anti-viral responses. It is well known that Mst1/2 kinases negatively regulate YAP activation and that loss of MST1/2 results in activation of YAP. Interestingly, the role of Mst1 in host anti-viral defense seems to be controversial, since Meng et al.[26] showed that MST1 negatively regulated nucleic acid sensing and anti-viral immunity by inhibiting IRF3 and TBK1 activation, which was also inhibited by YAP activation as mentioned above.[4, 24, 25] Excessive reactions to

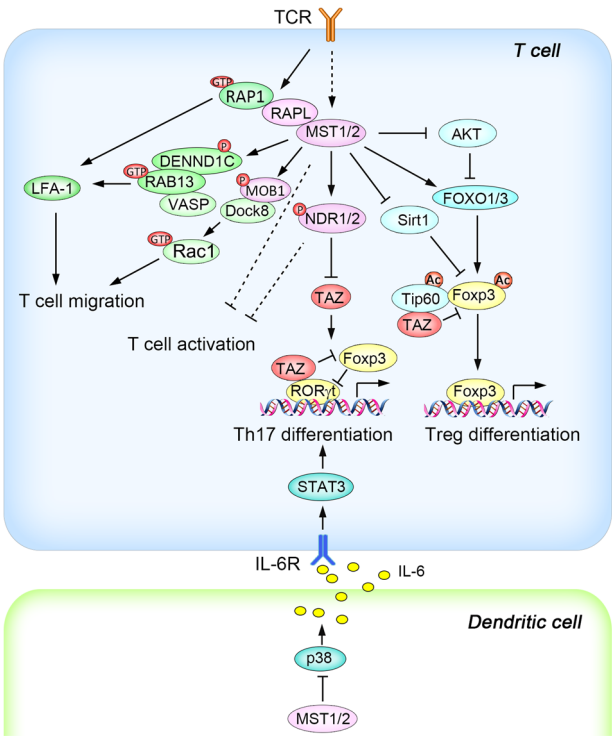

Fig. 2 Hippo signaling plays vital roles in T cell proliferation, migration and differentiation. The TCR-Rap1-RAPL axis activates MST1/2 in T cells. The core kinase MST1/2 promotes T cell migration via the MST1-MOB1-DOCK8-Rac1 axis or by activating and clustering LFA-1though DENND1C-RAB13, RIAM-Kindlin-3-Talin or VASP signaling. MST1/2 enhances Treg differentiation via promoting Foxp3's acetylation and activity though multiple mechanisms. Consistent with this, TAZ binds RORγt for Th17 differentiation, meanwhile isolating Tip60 from Foxp3 and decreasing the Tip60-mediated acetylation of Foxp3, which leads to impaired Treg differentiation. Moreover, MST1/2 in dendritic cells modulates Th17 differentiation in an MST1/2-p38-IL6-IL-6R-STAT3-dependent manner.

host nucleic acids and subsequent interferon production are associated with inflammatory and autoimmune disorders, therefore these results may explain the autoimmune manifestations in MST1-deficient patients. However, they fail to explain the recurrent viral infections in these patients. One possibility is that MST1 deficiency results in a failure of the adaptive immunity against the viral pathogens, since these patients also experienced T- and B-cell lymphopenia.

HIPPO SIGNALING IN THE ADAPTIVE IMMUNE SYSTEM

Adaptive immunity refers to antigen-specific immune responses, consisting of cell-mediated responses and antibody responses, which are carried out by different lymphocytic cells—T cells and B cells, respectively. The function of the Hippo signaling pathway in lymphocytes, especially T cells, is widely studied, and emerging results show that MST1/2 is critical for T-lymphocyte development, migration, homing, and differentiation.[10, 11, 14, 15, 27–31] MST1 also regulates B-cell adhesion and trafficking; the number of peripheral B cells, as well as splenic marginal zone B cells, was significantly reduced in MST1-deficient mice. However, there are few studies focusing on the functions of Hippo signaling in B cells.[14, 28, 32] Here, we summarize and discuss the recent updates of the regulatory role of Hippo signaling in T lymphocytes (Fig. 2).

T-cell development and activation

As a critical component of the adaptive immune system, T lymphocytes develop in the thymus via three-step selection from double-negative thymocytes (DN, $CD4^-CD8^-$) to double-positive thymocytes (DP, $CD4^+CD8^+$), and finally mature to single-positive (SP, $CD4^+CD8^-$, or $CD4^-CD8^+$) thymocytes, which are then released from the thymus to peripheral tissues to orchestrate and initiate effective adaptive immune responses. During the T-cell development process in thymus, the expression levels of MST1 and MST2 progressively increased, from detectable in DP thymocytes to high levels in SP thymocytes.[15] Compared to their wild-type counterparts, *Mst1*$^{-/-}$*Mst2*$^{fl/fl}$*Vav*-Cre mice exhibited an increased abundance of SP thymocytes with slightly, but not significantly, decreased total numbers of thymocytes, and severely reduced numbers of peripheral $CD4^+$ and $CD8^+$ T cells in blood, spleen, and lymph nodes. In general, T-cell development in the *Mst1*$^{-/-}$*Mst2*$^{fl/fl}$*Vav*-Cre mice occurred successfully, but thymus egress of MST1/2-null SP thymocytes was severely blocked.[15] In addition, Ueda et al. showed that MST1 might also be involved in regulating negative selection of thymocytes, since loss of MST1 resulted in inefficient migration and antigen recognition mediated by adhesion molecules within the medulla, such as lymphocyte function-associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1), but had little or no effect on positive selection, since the trafficking patterns of DP cells and the number of immature selected $CD69^+$ DP thymocytes were not affected in *Mst1*$^{-/-}$ mice.[33]

Human patients bearing LOF mutations in MST1 exhibited a naive $CD4^+$ and $CD8^+$ T-cell lymphopenia. Consistently, compared to wild-type littermates, MST1-deficient mice exhibited increased numbers of SP thymocytes, but dramatically decreased numbers of peripheral T cells in spleen or lymph nodes, which are composed of many fewer naive T cells ($CD62^{high}CD44^{low}$) and a higher proportion of effector/memory T cells ($CD62^{low}CD44^{high}$).[14, 28, 29, 34] In wild-type animals, the expression levels of MST1 were much lower in effector/memory T cells than those in naive T cells, while the expression levels of MST2 had no significant difference between these T-cell subsets. Together with the observation fewer naive T cells and higher proportion of effector/memory T cells in the spleen or lymph nodes of MST1-deficient mice, these results suggested that MST1 might serve as a molecular brake in limiting T-cell activation and proliferation, and T cells may only be activated when the MST1 level is downregulated to certain levels. Interestingly, a global deletion of MST2, which shares 76% identity in amino acid sequence with MST1, in mice had little effect on lymphocyte development. However, *Mst1*$^{-/-}$*Mst2*$^{fl/fl}$*Vav*-Cre mice, in which both *Mst1* and *Mst2* genes were deleted in the hematopoietic lineage, exhibited much more severe lymphopenia than MST1-deficient mice, suggesting a redundant role of MST2 in T-cell development and homeostasis in the absence of MST1.[15]

In response to TCR/anti-CD3 and anti-CD28 stimulations, MST1-deficient $CD4^+$ T cells exhibited more vigorously proliferative responses, higher production of the cytokines IL-2, IFN-γ, and IL-4, and enhanced activation-induced cell death responses when compared to that of wild-type control cells. Loss of MST1 in T cells did not affect the classical TCR signaling pathway, since the phosphorylation levels of CD3ε, ZAP70, Lck, PLCγ, and the MAP kinases (except the JNK) in response to anti-CD3/CD28 stimulation were comparable in splenic T cells isolated from wild-type and MST1-deficient mice. TCR stimulation was able to activate MST1 and stimulate phosphorylation of the downstream substrates MOB1A/B, whose phosphorylation was barely detectable in the MST1-deficient T cells. Interesting, elimination of MST1 had little effect on the phosphorylation of LATS1/2 and YAP, which are major downstream effecters of the canonical Hippo signaling pathway in non-immune cells.[14] More recently, instead of LATS1/2, the kinases NDR1/2, members of the same family of kinases as LATS, were reported to play an important role in regulating T-cell

function. Compared with wild-type littermates, matured naive T cells were dramatically reduced in peripheral lymphoid tissue in NDR1/2-deficient mice, suggesting that loss of NDR1/2 phenocopied the deletion of MST1 in thymocytes.[35] In addition, Comils et al. reported that both intrinsic and extrinsic proapoptotic stimuli, such as anti-Fas antibody or DNA damage, induced thymocyte NDR1 activation, and NDR1 ablation mice were more prone to the development of T-cell lymphomas than wild-type mice, indicating that NDR1 may play an important role in regulation of T-cell homeostasis.[36, 37] Taken together, these results suggest that MST1/2 may activate the YAP-independent non-canonical Hippo signaling pathway via MOB1A/B and/or NDR1/2 to regulate $CD4^+$ T-cell activation and proliferation.

T-cell adhesion and migration

Unlike the canonical Hippo-LATS-YAP signaling pathway to restrain cell proliferation in non-immune cells, MST1 and MST2 kinases play distinct roles in T cells by regulating their adhesion and migration and eventually affect T-cell immune function. Integrin-mediated cell adhesion and migration of T cells has been implicated in many contexts, such as T-cell maturation in thymus, T-cell trafficking in immune surveillance, and immune synapse formation between T cells and antigen-presenting cells (APCs). Kinashi and colleagues demonstrated that MST1 is required for the activation and clustering of the leukocyte-specific integrin LFA-1 during T-cell polarization and adhesion, in which MST1 was activated through the small GTPase RAP1-GTP recruitment of a RAPL/MST1 complex to the immunological synapse in response to anti-CD3/CD28 and chemokine stimulation.[10, 31, 33, 38] In addition, MST1 phosphorylated DENN domain-containing 1C (DENND1C), a GEF for a member of the RAS oncogene family (RAB13), to activate RAB13, or promoted F-actin polymerization by phosphorylation and activation of the actin regulatory protein vasodilator-stimulated phosphoprotein, which was critical for RAB13-dependent spatial distribution of LFA-1 and the trafficking of lymphocytes.[39] As a result, MST1-deficient, or MST1 and MST2 conditional knockout mice exhibited defective self-tolerance, impaired thymocyte egress and lymphocyte migration and homing, which led to an accumulation of mature SP thymocytes in thymus and a decreased number of lymphocytes in circulation.

In addition to the RAP1-RAPL-MST1 complex regulating LFA1 function, other downstream regulators of MST1/2 that mediate T-cell migration were reported. Kinashi's group further identified that NDR1 kinases, after being activated by the RAP1-RAPL-MST1 signaling cascade, associated with and recruited kindling-3 to the immune synapse, which was required for high-affinity LFA-1/ICAM-1 binding and immune synapse formation.[40] Meanwhile, we demonstrated that MST1/2-deficient thymocytes lacked the ability to activate rho family GTPases such as Rac and RhoA, and then exhibited defects in cytoskeletal regulation processes that are necessary for thymic egress of mature thymocytes.[15] Thymocytes express multiple guanine nucleotide exchange factors (GEFs) for Rac, including DOCK8. T cells from DOCK8-deficient mice shared some commonalities with the MST1/2-deficient SP thymocytes, especially a failure to polarize LFA-1 or actin to the immune synapse. In addition to regulating the LFA-1 function, our result showed that MST1 or MST2 directly phosphorylated MOB1A/B and promoted the activation of DOCK8, followed by the increased Rac1 GTP charging and subsequent cytoskeleton remodeling and cell migration.[16] Interestingly, our recent work showed that MST1/2 might activate Rac1 through the MST1/2-PKC-LyGDI pathway in myeloid cells,[20] suggesting that the regulatory mechanism and effects of Hippo signaling on small GTPase activation profoundly depend on the cellular context.

Differentiation of Th17 cells and Treg cells

Upon the stimulation of TCR and particular cytokines in the microenvironment, naive $CD4^+$ T cells are activated and differentiate into specific subtypes, including helper T cells 1 (Th1), Th2 cells, Th17 cells, or regulatory T cells (Treg). IL-17 producing-$CD4^+$ Th17 cells represent a pro-inflammatory subset, while $CD4^+Foxp3^+$ Treg cells are immunosuppressive. Some MST1-deficient patients exhibit autoimmune manifestations. Consistently, mice with the elimination of MST1, or both MST1 and MST2, were prone to autoimmune diseases such as Sjögren's syndrome and colitis. Recent studies revealed that Hippo signaling plays important roles in regulating the differentiation and function of Treg cells and Th17 cells in many aspects.[27, 41–43]

Our recent work showed that compared with $Mst1^{fl/fl}Mst2^{fl/fl}$ littermates, $Mst1^{fl/fl}Mst2^{fl/fl}$ *Ox40*-Cre mice, in which *Mst1* and *Mst2* genes were deleted in activated T cells, exhibited a substantial higher frequency of Th17 cells and a modestly lower frequency of Treg cells after immunization with keyhole limpet hemocyanin.[27] Further studies revealed that TAZ, the downstream transcriptional cofactor of the Hippo pathway, acted as a molecular switch for reciprocal differentiation of Th17 and Treg regulation. The expression levels of *RORC* and *TAZ* in memory $CD4^+$ T cells isolated from peripheral blood of patients with rheumatoid arthritis or Sjögren's syndrome were positively correlated and were significantly upregulated compared with those from healthy individuals. Mice with TAZ-deficiency in T cells had more Treg cells and were resistant to the induction of Th17 cell-dependent inflammatory diseases. The messenger RNA levels of *Taz*, but not *Yap*, were significantly increased by TGF-β alone and further enhanced by stimulation with a combination of TGF-β and IL-6, dependent on the transcription factor SMAD family member 3 (SMAD3) and signal transducer and activator of transcription 3 (STAT3). Under Th17-inducing conditions, TAZ was highly induced by TGF-β and IL-6 stimulation and acted as a critical co-activator of RAR related orphan receptor gamma T (RORγt) for Th17 differentiation; meanwhile, TAZ attenuated the effect of Tat interactive protein 60 kDa (Tip60) and p300 on the acetylation and stabilization of forkhead box P3 (Foxp3) and then targeted Foxp3 for proteasomal degradation and blocked its inhibitory effect on RORγt. On the other hand, under Treg-inducing conditions, both TAZ and TEAD1, a transcription factor downstream of Hippo signaling that has high-affinity binding to TAZ, were induced by TGF-β alone. Thus, TAZ was sequestered from RORγt and Foxp3 by the highly expressed TEAD1, which resulted in enhanced Treg cell differentiation. Taken together, these results indicate that TAZ, a key downstream transcriptional co-activator of MST1/2 signaling, promoted the development of inflammatory Th17 cells but decreased the differentiation of immune suppressive Treg cells, supporting the likely physiopathologic relevance of the Hippo signaling pathway it engages.

More recently, MST1 signaling from DCs has also been shown to negatively regulate Th17 differentiation.[41] Mechanistically, MST1-deficient DCs promoted p38 MAPK signal-dependent IL-6 secretion and regulated the activation of IL-6 receptor α/β and signal transducer and STAT3 in $CD4^+$ T cells in the course of inducing Th17 differentiation. Furthermore, MST1 mediated LFA-1 activation is essential for efficient antigen-specific contacts with DCs, thus inefficient conjugate formation between DCs and $Mst1^{-/-}$ Treg cells impaired the latter's immunosuppressive activity.[43] Regarding the regulation of Treg cells by Hippo signaling, earlier studies also demonstrated that MST1 kinase might stabilize forkhead box O1/3 (Foxo1/3) by phosphorylating Foxo1/3 directly or by phosphorylating serine/threonine kinase AKT and then inhibiting the phosphorylation of Foxo1/3 by AKT indirectly, subsequently promoting the expression of the transcriptional factor Foxp3.[30] MST1 can also improve the stability and transcriptional activity of Foxp3 by inhibiting Sirt1's deacetylation

activity and accordingly promoting Foxp3 acetylation and enhancing its activity for Treg cell development.[42]

HIPPO SIGNALING AND CANCER IMMUNOLOGY

The Hippo signaling pathway has been convincingly established as a tumor suppressor pathway for cellular transformation and tumorigenesis. In recent years, much attention has been drawn to the effects of the Hippo signaling pathway on tumor growth in the context of reciprocal interactions between tumor cells and the host anti-tumor immune responses. Wang et al.[44] reported a cancer cell non-autonomous function of the Hippo-YAP pathway in the regulation of C-X-C motif chemokine ligand 5 (CXCL5), a ligand for CXCR2-expressing myeloid-derived suppressor cells (MDSCs). Hyperactivation of YAP in cancer cells promoted CXCL5 secretion, which enhanced recruitment of MDSCs to promote cancer progression. Consistently, clinicopathological studies revealed that the YAP1 signature was enriched in a subset of human primary prostate tumor samples with stronger expression of MDSC-relevant genes. More recently, another study showed that in pancreatic ductal adenocarcinoma, YAP drove the differentiation and accumulation of MDSCs and regulated the immunosuppressive microenvironment. Deletion of YAP or depletion of MDSCs promoted macrophage reprogramming and reactivation of T cells to enhance anti-cancer immunity.[45] In liver cancers, YAP activation has also been shown to recruit M2 macrophages for suppressing immune clearance and promoting tumorigenesis.[46] In contrast, recent work from Guan's group revealed a surprising role of the Hippo pathway kinases LATS1/2, which were long known to act as tumor suppressors, in suppressing anti-tumor immunity.[47] They demonstrated that loss of LATS1/2 in tumor cells inhibited tumor growth in the inoculation sites using several different murine syngeneic tumor models. Tumor regression by LATS1/2 deletion required adaptive immune responses, and loss of LATS1/2 increased tumor vaccine efficacy. Mechanistically, LATS1/2 deletion in tumors improved tumor immunogenicity by releasing nucleic-acid-rich extracellular vesicles from LATS1/2-null tumors to induce type I IFNs production via the TLRs-MyD88-TRIF pathway in host cells and enhance the anti-tumor immune response, leading to tumor destruction. These findings indicated that altered canonical Hippo-YAP signaling in cancer cells can affect the microenvironment to regulate cancer immune responses, although more regulatory details remain to be explored. As a tumor suppressor pathway, as well as an immune function modulating pathway, the Hippo signaling pathway is a promising therapeutic target for the treatment of cancer, however, its effects on either inhibiting cancer growth or enhancing tumor immunity need to be considered for safety reasons.

CONCLUSIONS AND PERSPECTIVES

Increasing evidence shows that the Hippo signaling pathway is critical in regulating immune function and maintaining immune homeostasis. Unlike the canonical MST1/2-LATS1/2-YAP Hippo signaling cassette, which evolutionally controls tissue growth during development and regeneration, most studies regarding Hippo signaling in immune regulation have focused on its core kinases, MST1/2, which cross-talk with and regulate other essential pathways by directly regulating the phosphorylation and activation of critical signaling players such as PKC, FOXO, or AKT in specific immune cell types. In addition, some studies also revealed that alternate effectors downstream of MST1/2 are selectively used in different immune cells. For example, MST1 might signal through NDR1/2, but not LATS1/2 kinases, in a YAP-independent manner to negatively regulate naïve $CD4^+$ T-cell proliferation upon TCR stimulation, as well as to regulate peripheral naive T-cell trafficking and thymus egress. It is clear that the expression levels of transcriptional coactivators YAP and TAZ are extremely low in naive T cells. The expression of TAZ, but not YAP, can be induced in activated $CD4^+$ T cells upon TGFβ stimulation and plays a critical role in regulating reciprocal differentiation of Th17 cells and Treg cells.[27] Interestingly, the expression of YAP can also be induced in activated $CD8^+$ T cells stimulated with antigen and IL-2, and the ligation of cytotoxic T-lymphocyte antigen 4 (CTLA-4) between activated $CD8^+$ T cells induces Hippo pathway-mediated YAP degradation and Blimp-1 expression, which is critical for balancing the clonal expansion during viral infection.[48] In addition, YAP negatively regulates IFNβ signaling in macrophages and YAP deficiency potentiated anti-viral responses.[25] These findings suggest that YAP might be a critical factor in immune system for anti-viral or tumor immunity, and it will be interesting to explore the function of YAP in other cytotoxic cells, such as NK cells. Therefore, the expanded and alternative network of the Hippo signaling pathway that is involved in immune regulation largely remains to be explored. Due to the pace of the extensive research efforts on the function of Hippo signaling in the immune system, we expect that many new insights into this field will be beneficial to the design and development of potential therapeutic agents for infections and autoimmune diseases, as well as tumor immunotherapy, by manipulating the Hippo pathway.

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ADDITIONAL INFORMATION

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REFERENCES

1. Yu, F. X., Zhao, B. & Guan, K. L. Hippo pathway in organ size control, tissue homeostasis, and cancer. *Cell* **163**, 811–828 (2015).
2. Avruch, J. et al. Protein kinases of the Hippo pathway: regulation and substrates. *Semin. Cell Dev. Biol.* **23**, 770–784 (2012).
3. Chen, L., Qin, F., Deng, X., Avruch, J. & Zhou, D. Hippo pathway in intestinal homeostasis and tumorigenesis. *Protein Cell* **3**, 305–310 (2012).
4. Zhang, Q. et al. Hippo signalling governs cytosolic nucleic acid sensing through YAP/TAZ-mediated TBK1 blockade. *Nat. Cell Biol.* **19**, 362–374 (2017).
5. Zhou, D. et al. Mst1 and Mst2 protein kinases restrain intestinal stem cell proliferation and colonic tumorigenesis by inhibition of Yes-associated protein (Yap) overabundance. *Proc. Natl Acad. Sci. USA* **108**, E1312–1320 (2011).
6. Zhou, D. et al. Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. *Cancer Cell* **16**, 425–438 (2009).
7. Wu, H. et al. The Ets transcription factor GABP is a component of the hippo pathway essential for growth and antioxidant defense. *Cell Rep.* **3**, 1663–1677 (2013).
8. Fan, F. et al. Pharmacological targeting of kinases MST1 and MST2 augments tissue repair and regeneration. *Sci. Transl. Med.* **8**, 352ra108 (2016).
9. Zhang, S. et al. Hippo signaling suppresses cell ploidy and tumorigenesis through Skp2. *Cancer Cell* **31**, 669–684 (2017). e667.
10. Katagiri, K. et al. Crucial functions of the Rap1 effector molecule RAPL in lymphocyte and dendritic cell trafficking. *Nat. Immunol.* **5**, 1045–1051 (2004).
11. Katagiri, K., Maeda, A., Shimonaka, M. & Kinashi, T. RAPL, a Rap1-binding molecule that mediates Rap1-induced adhesion through spatial regulation of LFA-1. *Nat. Immunol.* **4**, 741–748 (2003).
12. Abdollahpour, H. et al. The phenotype of human STK4 deficiency. *Blood* **119**, 3450–3457 (2012).
13. Nehme, N. T. et al. MST1 mutations in autosomal recessive primary immunodeficiency characterized by defective naive T-cell survival. *Blood* **119**, 3458–3468 (2012).
14. Zhou, D. et al. The Nore1B/Mst1 complex restrains antigen receptor-induced proliferation of naive T cells. *Proc. Natl Acad. Sci. USA* **105**, 20321–20326 (2008).
15. Mou, F. et al. The Mst1 and Mst2 kinases control activation of rho family GTPases and thymic egress of mature thymocytes. *J. Exp. Med.* **209**, 741–759 (2012).

16. Halacli, S. O. et al. STK4 (MST1) deficiency in two siblings with autoimmune cytopenias: a novel mutation. *Clin. Immunol.* **161**, 316–323 (2015).
17. Liu, J. & Cao, X. Cellular and molecular regulation of innate inflammatory responses. *Cell. Mol. Immunol.* **13**, 711–721 (2016).
18. Liu, B. et al. Toll receptor-mediated Hippo signaling controls innate immunity in *Drosophila*. *Cell* **164**, 406–419 (2016).
19. Dubey, S. K. & Tapadia, M. G. Yorkie regulates neurodegeneration through canonical pathway and innate immune response. *Mol. Neurobiol.* **55**, 1193–1207 (2017).
20. Geng, J. et al. Kinases Mst1 and Mst2 positively regulate phagocytic induction of reactive oxygen species and bactericidal activity. *Nat. Immunol.* **16**, 1142–1152 (2015).
21. Li, W. et al. STK4 regulates TLR pathways and protects against chronic inflammation-related hepatocellular carcinoma. *J. Clin. Invest.* **125**, 4239–4254 (2015).
22. Boro, M., Singh, V. & Balaji, K. N. Mycobacterium tuberculosis-triggered Hippo pathway orchestrates CXCL1/2 expression to modulate host immune responses. *Sci. Rep.* **6**, 37695 (2016).
23. Wen, M. et al. Stk38 protein kinase preferentially inhibits TLR9-activated inflammatory responses by promoting MEKK2 ubiquitination in macrophages. *Nat. Commun.* **6**, 7167 (2015).
24. Kim, N., Park, Y. Y., Joo, C. H. & Kim, H. S. Relief of YAP-mediated inhibition by IKKvarepsilon promotes innate antiviral immunity. *Cell. Mol. Immunol.* **14**, 1–3 (2017).
25. Wang, S. et al. YAP antagonizes innate antiviral immunity and is targeted for lysosomal degradation through IKKvarepsilon-mediated phosphorylation. *Nat. Immunol.* **18**, 733–743 (2017).
26. Meng, F. et al. Mst1 shuts off cytosolic antiviral defense through IRF3 phosphorylation. *Genes Dev.* **30**, 1086–1100 (2016).
27. Geng, J. et al. The transcriptional coactivator TAZ regulates reciprocal differentiation of TH17 cells and Treg cells. *Nat. Immunol.* **18**, 800–812 (2017).
28. Katagiri, K. et al. Mst1 controls lymphocyte trafficking and interstitial motility within lymph nodes. *EMBO J.* **28**, 1319–1331 (2009).
29. Dong, Y. et al. A cell-intrinsic role for Mst1 in regulating thymocyte egress. *J. Immunol.* **183**, 3865–3872 (2009).
30. Du, X. et al. Mst1/Mst2 regulate development and function of regulatory T cells through modulation of Foxo1/Foxo3 stability in autoimmune disease. *J. Immunol.* **192**, 1525–1535 (2014).
31. Katagiri, K., Imamura, M. & Kinashi, T. Spatiotemporal regulation of the kinase Mst1 by binding protein RAPL is critical for lymphocyte polarity and adhesion. *Nat. Immunol.* **7**, 919–928 (2006).
32. Bai, X. et al. Mst1 positively regulates B-cell receptor signaling via CD19 transcriptional levels. *Blood Adv.* **1**, 219–230 (2016).
33. Ueda, Y. et al. Mst1 regulates integrin-dependent thymocyte trafficking and antigen recognition in the thymus. *Nat. Commun.* **3**, 1098 (2012).
34. Choi, J. et al. Mst1-FoxO signaling protects naive T lymphocytes from cellular oxidative stress in mice. *PLoS ONE* **4**, e8011 (2009).
35. Tang, F. et al. The kinases NDR1/2 act downstream of the Hippo homolog MST1 to mediate both egress of thymocytes from the thymus and lymphocyte motility. *Sci. Signal.* **8**, ra100 (2015).
36. Cornils, H., Kohler, R. S., Hergovich, A. & Hemmings, B. A. Downstream of human NDR kinases: impacting on c-myc and p21 protein stability to control cell cycle progression. *Cell Cycle* **10**, 1897–1904 (2011).
37. Cornils, H. et al. Ablation of the kinase NDR1 predisposes mice to the development of T cell lymphoma. *Sci. Signal.* **3**, ra47 (2010).
38. Katagiri, K. et al. Deficiency of Rap1-binding protein RAPL causes lymphoproliferative disorders through mislocalization of p27kip1. *Immunity* **34**, 24–38 (2011).
39. Nishikimi, A. et al. Rab13 acts downstream of the kinase Mst1 to deliver the integrin LFA-1 to the cell surface for lymphocyte trafficking. *Sci. Signal.* **7**, ra72 (2014).
40. Kondo, N. et al. NDR1-dependent regulation of kindlin-3 controls high-affinity LFA-1 binding and immune synapse organization. *Mol. Cell. Biol.* **37**, e00424–16 2017.
41. Li, C. et al. Dendritic cell MST1 inhibits Th17 differentiation. *Nat. Commun.* **8**, 14275 (2017).
42. Li, J. et al. Mammalian sterile 20-like kinase 1 (Mst1) enhances the stability of Forkhead Box P3 (Foxp3) and the function of regulatory T Cells by modulating Foxp3 acetylation. *J. Biol. Chem.* **290**, 30762–30770 (2015).
43. Tomiyama, T. et al. Antigen-specific suppression and immunological synapse formation by regulatory T cells require the Mst1 kinase. *PLoS ONE* **8**, e73874 (2013).
44. Wang, G. et al. Targeting YAP-dependent MDSC infiltration impairs tumor progression. *Cancer Discov.* **6**, 80–95 (2016).
45. Murakami, S. et al. Yes-associated protein mediates immune reprogramming in pancreatic ductal adenocarcinoma. *Oncogene* **36**, 1232–1244 (2017).
46. Guo, X. et al. Single tumor-initiating cells evade immune clearance by recruiting type II macrophages. *Genes Dev.* **31**, 247–259 (2017).
47. Moroishi, T. et al The hippo pathway kinases LATS1/2 suppress cancer immunity. *Cell* **167**, 1525–1539.e17 (2016).
48. Thaventhiran, J. E. et al. Activation of the Hippo pathway by CTLA-4 regulates the expression of Blimp-1 in the CD8+ T cell. *Proc. Natl Acad. Sci. USA* **109**, E2223–2229 (2012).