

## Review Article



# Hippo Signal Transduction Mechanisms in T Cell Immunity

Antoine Bouchard <sup>1,2</sup>, Mariko Witalis <sup>1,2</sup>, Jinsam Chang <sup>1,2</sup>, Vincent Panneton <sup>1,3</sup>,  
Joanna Li <sup>1,4</sup>, Yasser Bouklouch <sup>1</sup>, Woong-Kyung Suh <sup>1,2,3,4,\*</sup>

<sup>1</sup>Institut de Recherches Cliniques de Montréal (IRCM), Montreal, QC H2W 1R7, Canada

<sup>2</sup>Molecular Biology Program, Department of Medicine, University of Montreal, Montreal, QC H3T 1J4, Canada

<sup>3</sup>Department of Microbiology, Infectiology, and Immunology, University of Montreal, Montreal, QC H3T 1J4, Canada

<sup>4</sup>Department of Microbiology and Immunology, McGill University, Montreal, QC H3A 0G4, Canada

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### \*Correspondence to

Woong-Kyung Suh

Institut de Recherches Cliniques de Montréal (IRCM), 110 Avenue des Pins Ouest, Montreal, QC H2W 1R7, Canada.

E-mail: woong-kyung.suh@ircm.qc.ca

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### ORCID iDs

Antoine Bouchard

<https://orcid.org/0000-0002-4292-5015>

Mariko Witalis

<https://orcid.org/0000-0001-6855-0226>

Jinsam Chang

<https://orcid.org/0000-0002-6827-3756>

Vincent Panneton

<https://orcid.org/0000-0001-7621-8178>

Joanna Li

<https://orcid.org/0000-0001-6746-8722>

Yasser Bouklouch

<https://orcid.org/0000-0003-1583-318X>

Woong-Kyung Suh

<https://orcid.org/0000-0002-6420-3283>

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## ABSTRACT

Hippo signaling pathways are evolutionarily conserved signal transduction mechanisms mainly involved in organ size control, tissue regeneration, and tumor suppression. However, in mammals, the primary role of Hippo signaling seems to be regulation of immunity. As such, humans with null mutations in *STK4* (mammalian homologue of *Drosophila Hippo*; also known as *MST1*) suffer from recurrent infections and autoimmune symptoms. Although dysregulated T cell homeostasis and functions have been identified in *MST1*-deficient human patients and mouse models, detailed cellular and molecular bases of the immune dysfunction remain to be elucidated. Although the canonical Hippo signaling pathway involves transcriptional co-activator Yes-associated protein (YAP) or transcriptional coactivator with PDZ motif (TAZ), the major Hippo downstream signaling pathways in T cells are YAP/TAZ-independent and they widely differ between T cell subsets. Here we will review Hippo signaling mechanisms in T cell immunity and describe their implications for immune defects found in *MST1*-deficient patients and animals. Further, we propose that mutual inhibition of Mst and Akt kinases and their opposing roles on the stability and function of forkhead box O and  $\beta$ -catenin may explain various immune defects discovered in mutant mice lacking Hippo signaling components. Understanding these diverse Hippo signaling pathways and their interplay with other evolutionarily-conserved signaling components in T cells may uncover molecular targets relevant to vaccination, autoimmune diseases, and cancer immunotherapies.

**Keywords:** Hippo; Signal transduction; T cells

## INTRODUCTION

The gene *Hippo* was discovered as a regulator of organ size in *Drosophila* (1). In the canonical Hippo signaling pathways, upstream sensors of cellular environment initiate a cascade of protein phosphorylation events that ultimately suppress the key transcriptional co-activators Yes-associated protein (YAP) and transcriptional coactivator with PDZ motif (TAZ), thereby slowing cell proliferation (1). Consistent with this, liver-specific deletion of Hippo genes (*Mst1* and its homolog *Mst2*) in mice leads to hepatomegaly and hepatocellular carcinoma through hyperactivation of YAP (2-4). Also, germline deletion of *Mst1* and *Mst2* genes (but not each single gene) causes embryonic death due to developmental defects (2,4). However,

**Abbreviations**

FoxO, forkhead box O; GSK-3, glycogen synthase kinase 3; Lats, large tumor suppressor kinase; LFA-1, lymphocyte function-associated Ag 1; LRP, low-density lipoprotein receptor-related protein; Mob, Mps One Binder; Mst, mammalian sterile 20-like kinase; NDR, nuclear Dbf2-related; RapL, regulator of cell adhesion and polarization enriched in lymphoid tissues; SKAP1, Src kinase-associated phosphoprotein 1; SMAC, supramolecular activation cluster; TAZ, transcriptional coactivator with PDZ motif; TEAD, TEA domain family member; Tfh, T follicular helper cell; Wnt, Wingless-related integration site; WW45, 45 kDa WW domain protein; YAP, Yes-associated protein.

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despite the ubiquitous expression of Mst kinases, global deletion of *Mst1* and *Mst2* genes in adult mice leads to organ outgrowth and cancer only in the liver but not in other organs (2,4). These findings confirmed that Mst1 and Mst2 in mammals have redundant roles in the control of embryonic development, organ size, and tumor suppression. Moreover, these results suggest that Mst kinases in adult mammals may play biological roles beyond the control of organ size or cancer. Indeed, tissue-specific Mst-deficiency leads to a wide spectrum of defects in cell survival, proliferation, migration, and differentiation in tissues such as heart, brain, and the immune system (5-8).

Humans harboring autosomal recessive null-mutations in the *MST1* gene suffer from recurrent infections by viruses, bacteria, and fungi at early ages (1-10 years) (9-13). Common immune defects in Mst1-deficient patients include T cell and B cell lymphopenia due to increased apoptosis of naïve T cells (9,10). Both in mice (14) and humans (9), Mst1-deficient naïve T cells were susceptible to apoptosis induced by oxidative stress (14) or Fas ligation (9). In some patients, immunodeficiency is accompanied by autoimmune symptoms (9-12). Mouse studies suggest that this is likely driven by defects in the thymic development, maintenance, and suppressive function of Mst1-deficient Treg cells (15-18).

In spite of reduced numbers of T and B cells, serum IgG, IgA, and IgE titers are moderately higher in Mst1-deficient patients (9-13). However, the fact that patients who received prophylactic Ab replacement therapy were protected from infections (10-12) suggests that the apparently normal levels of class-switched Abs may not provide protection against infections. In support of this, mice lacking Mst1 cannot produce long-lived high-affinity Abs even when they had enough numbers of T cells and B cells to support initial Ab production (19). This appears to be due to hyperactive T follicular helper cells supporting premature transition of germinal center B cells into plasma cells, which fail to sustain themselves in the bone marrow (19).

Accumulating evidence indicates that, in most cases, T cells utilize Hippo (Mst) kinase signaling without relying on YAP/TAZ pathways to regulate apoptosis, differentiation, migration, and function (7). At the molecular level, Mst1/2 kinases have been shown to be activated by T cell receptor as well as cytokine/chemokine receptors in T cells. Once activated, Mst kinases control diverse signaling components such as YAP/TAZ, small GTPases, lymphocyte function-associated Ag 1 (LFA-1), STAT5, forkhead box O (FoxO), and  $\beta$ -catenin. Importantly, Hippo signaling pathways appear to interact with Akt pathways to counterbalance signaling input to common downstream signaling nodes such as FoxO and  $\beta$ -catenin.

In this review, we will categorize different modes of Hippo signaling in T cells, their biological functions in T cell immunity, and their potential implications in immunological disorders.

## CANONICAL PATHWAY: YAP AND TAZ IN T CELL DIFFERENTIATION

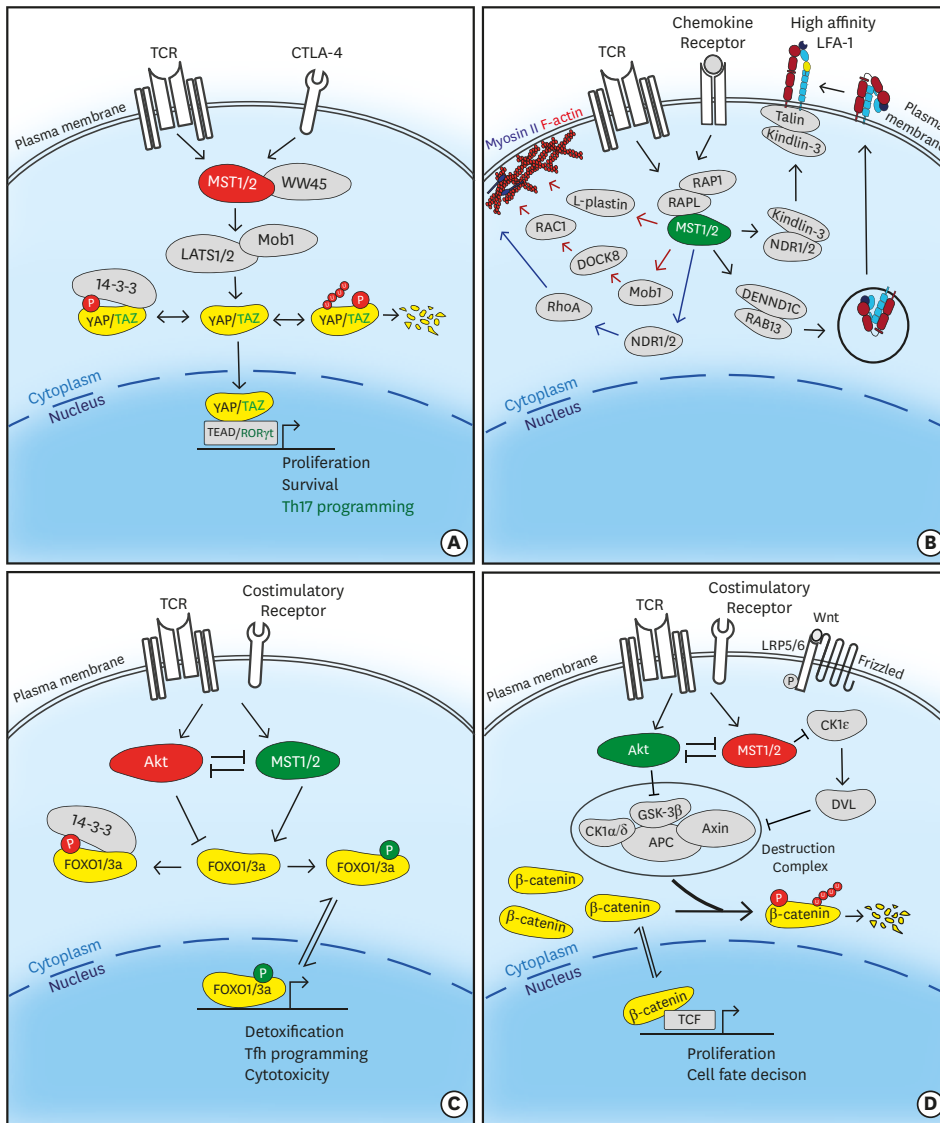
In the “canonical” Hippo signaling pathway, environmental cues (such as cell-cell contact) activate kinases Mst1 and Mst2 (1). Mst kinases normally exist as inactive homodimers (20), which require the release of inhibitory phosphates and intermolecular autophosphorylation of key activation motifs to initiate kinase cascades (20). Activated Mst1/2 can phosphorylate and activate large tumor suppressor kinase 1 (Lats1) and Lats2, which in turn phosphorylate transcriptional co-activators YAP (1). Phosphorylated YAP is either retained in the cytoplasm

by binding to 14-3-3 or degraded by proteasomes after further phosphorylation and ubiquitination (1). YAP can translocate into the nucleus where it binds to the transcription factor TEA domain family member (TEAD) to induce expression of TEAD target genes (1) (Fig. 1A). In general, YAP target genes promote cellular proliferation and survival, fitting with the oncogenic role of YAP and tumor suppressor function of Mst kinases (1,8). In the immune system, naïve murine CD4<sup>+</sup> and CD8<sup>+</sup> T cells express little amounts of Yap protein but increase protein levels when stimulated *in vitro* (21,22). In depth analysis using OT1 TCR transgenic T cells indicated that in addition to TCR signaling (which is sufficient to increase mRNA levels), IL-2 is required to increase Hippo pathway proteins 45 kDa WW domain protein (WW45), Lats1, Mps One Binder 1 (Mob1), TEAD1, and TEAD3 (22). Although Mst1 protein is equally expressed in naïve and stimulated OT1 T cells, its activation presumably depends on the ligation of CTLA-4 by CD80 which is highly upregulated in CD8<sup>+</sup> T cells upon TCR ligation in the presence of IL-2 (22) (Fig. 1A). The cell-cell contact between activated CD8<sup>+</sup> T cells in the presence of Ag and IL-2 leads to degradation of Yap proteins and concomitant BLIMP1 expression and terminal differentiation of CD8<sup>+</sup> T cells (22). Consistently, either lack of CTLA-4 or expression of non-degradable Yap protein inhibited generation of KLRG1<sup>+</sup> CD127<sup>lo</sup> effector CD8<sup>+</sup> T cells during viral infection (22). These results support the view that clonally expanded CD8<sup>+</sup> T cells utilize the canonical Hippo pathway (Mst-Lats-Yap axis) for quorum sensing to initiate terminal differentiation only after clonal expansion has passed a threshold level.

In another study, Yap is shown to be a negative regulator of T cell responses especially in the tumor microenvironment (21). T cells lacking YAP showed increased levels of activation markers, and key T helper cytokines such as IFN- $\gamma$  upon *in vitro* stimulation (21). Importantly, mice with T cell-specific YAP-deficiency showed slower growth of transplanted tumors with increased infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> T cells (21). YAP-deficient T cells that infiltrated into tumors showed greatly increased expression of genes involved in T cell activation, migration, and adhesion (21). Interestingly in multiple human cancers, YAP-low gene signatures were highly correlated with better patient survival (21). However, it remains to be clarified how the Mst-Lats-YAP signaling axis contributes to the overall YAP function since YAP can be regulated by other signaling pathways such as MAP4K (1).

Unlike YAP, another Hippo effector molecule TAZ has been shown to be highly expressed in Th17 cells and to a lesser extent in Treg cells but is not found in Th1 or Th2 cells (23). Functionally, TAZ is required to promote Th17 differentiation as a co-activator of ROR $\gamma$ t, the master Th17 lineage defining transcription factor (23). In addition, TAZ promotes degradation of Foxp3 by interrupting Foxp3 acetylation and dampens the ability of Foxp3 to inhibit ROR $\gamma$ t function (23). Consistently, mice with T cell-specific TAZ knockout showed reduced disease severity in mouse models of Th17-driven inflammatory diseases whereas transgenic overexpression of TAZ in T cells did the opposite (23). Of note, Mst-deficient CD4<sup>+</sup> T cells induced stronger colitis symptoms and Th17 responses when adoptively transferred to lymphopenic host mice and this was reversed by eliminating the TAZ gene in T cells (23). Collectively, these findings indicate that under inflammatory conditions, Mst kinases normally suppress Th17 responses and promote Treg differentiation by dampening TAZ activity.

Thus, the core elements of conventional Hippo signaling pathways are expressed in T cells in a highly regulated manner. YAP appears to control CD8<sup>+</sup> T cell differentiation whereas TAZ influences CD4<sup>+</sup> T cell differentiation between Th17 and Treg fates. Available evidence



**Figure 1.** Hippo signal transduction mechanisms in T cells. Currently identified Hippo signaling mechanisms can be grouped into 4 modes. Three of them involve the abundance and activity of transcriptional regulators in the nucleus; one of them involves actin remodeling and LFA-1 function. Symbols for Mst, Akt, phosphorylation, and ubiquitination are color coded in red (inhibitory) or green (stimulatory) to reflect their impact on the effector molecules. (A) Inhibition of YAP/TAZ transcriptional activity through canonical Hippo pathway. Upstream stimuli (e.g., CTLA-4 ligation through cell-cell contact between highly expanded CD8<sup>+</sup> T cells) can lead to YAP degradation. In this model, Mst kinases get activated through phosphorylation in association with the scaffold protein WW45. Activated Mst kinases subsequently phosphorylate and activate Lats kinases that are in complex with Mob1. Ultimately, Lats kinases phosphorylate transcription co-activator YAP. Depending on specific serine or threonine residues that become phosphorylated, YAP proteins can be either retained in the cytoplasm by binding to 14-3-3 proteins or undergo ubiquitin/proteasome-dependent degradation. In the absence of Mst activation, YAP can accumulate in the nucleus to transactivate TEAD-target genes that favor cellular proliferation. Under Th17 differentiation conditions, TAZ is similarly regulated by Mst kinases but it works as co-activator of RORγt instead of TEAD. (B) Regulation of cytoskeleton and LFA-1 through Mst-RAPL complex. In response to TCR or chemokine receptor ligation, Mst1 becomes part of the Rap1/RapL complex. On the one hand, Mst kinases phosphorylate Mob1 and NDR1/2 (Lats-related Ser/Thr kinases), which activate small GTPases Rac1 and RhoA to induce actin remodeling to polarize T cells. Mst1 also phosphorylates an actin-binding protein L-plastin which stabilizes F-actin. On the other hand, Mst kinases phosphorylate guanine nucleotide exchange factor DENND1C, which activates Rab13 to promote trafficking of LFA-1-containing vesicles to the plasma membrane. Mst-mediated NDR activation increases binding of kindlin-3 and talin to the cytoplasmic tail of LFA-1, a key “inside-out” signaling mechanism stabilizing high affinity LFA-1. (C) Counterregulation of FoxO activity by Mst and Akt. Akt-mediated phosphorylation leads to cytoplasmic retention of FoxO proteins by increasing FoxO binding to 14-3-3 proteins. This is antagonized by Mst-mediated phosphorylation on a different phosphorylation site. Nuclear FoxO proteins control genes that are involved in ROS detoxification, Tfh programming, and cytotoxicity. In theory, the cell-type specific biochemical milieu and nature of upstream stimuli that shifts the balance between Akt and Mst activities have the potential to influence the outcome of FoxO signaling. (D) Opposing roles of Mst and Akt in β-catenin stabilization. Akt can stabilize β-catenin by inactivating GSK-3β, a key component of the Wnt signaling “destruction complex” in T cells. On the other hand, Mst kinases may inhibit CK1ε, a positive regulator of Wnt signaling, thereby dampening Wnt-induced accumulation of β-catenin. Accumulated β-catenin goes into the nucleus to promote expression of TCF-1 target genes, which are known to play important roles in T cell development, differentiation, and function. P, phosphorylation; U, ubiquitination; DENND1C, differentially expressed in normal and neoplastic cells domain 1C.

supports the view that Mst kinases transmit upstream signals to downregulate the amounts of YAP and TAZ in the nucleus.

## REGULATOR OF CELL ADHESION AND POLARIZATION ENRICHED IN LYMPHOID TISSUES (RapL) COMPLEX: CYTOSKELETON AND LFA-1

Although T cell lymphopenia in MST1-null human patients was mainly attributed to increased apoptosis of Mst1-deficient naïve T cells in the periphery, mouse studies suggest that there is an additional problem in thymic output (24-26). Mst1-deficient thymocytes normally mature into single positive T cells but fail to efficiently migrate out of the thymus (24). In addition, those T cells have difficulties entering secondary lymphoid organs resulting in reduced cellularity in spleens and lymph nodes (24,25). *In vitro* experiments demonstrated that Mst1-deficient T cells display reduced migration towards chemokines such as CCL19/21 (ligands for CCR7) and S1P (ligand for S1PR1) (24,27). Mst1 deficient T cells were initially able to adhere to the endothelium but could not establish stable contact during *in vitro* adhesion assays under shear flow (25). Further, they showed reduced ability to go through narrow passages (28). Migration and passage through blood vessels require coordinated actin remodeling and dynamic changes in the distribution and the affinity of integrins such as LFA-1 (29,30). Mst kinases (Mst1 being dominant and Mst2 supplementary) play central roles in these processes in collaboration with RapL, an effector molecule of the small GTPase Rap1 (27,31) (Fig. 1B). Importantly, under conditions where Mst kinases affect T cell migration and adhesion, the amount and phosphorylation status of Lats and YAP proteins did not change implicating non-canonical pathways (24,26).

Although Hippo pathways started gaining more attention from immunologists after the discovery that *STK4* mutation causes human immunodeficiency (9,10), Mst1 was first described as a binding partner of RapL in murine T cells (27). Upon TCR ligation or chemokine exposure, Mst1 binds to RapL and becomes activated (27), presumably through intermolecular autophosphorylation (20). Activated Mst kinases promote F-actin polymerization by phosphorylating Mob1 (24), which in turn activates Dock8 leading to activation of Rac1 (24). In parallel, Mst1 can directly phosphorylate L-plastin, a protein known to stabilize F-actin (32). In addition to F-actin polymerization and stabilization, Mst1 also regulates contractility of cytoskeletons by regulating the location and activity of myosin IIa although detailed molecular mechanisms are yet to be uncovered (28).

Another key role of the RapL-Mst complex is to control the affinity and avidity of LFA-1 on the T cell surface. First, Mst1 promotes the transport of LFA-1-containing vesicles to the leading edge of polarized T cells (33). Upon exposure to chemokines, Mst1 specifically phosphorylates and activates differentially expressed in normal and neoplastic cells domain 1C, a guanine nucleotide exchange factor for Rab13 (33). GTP-bound Rab13, in complex with RapL and Mst1 binds to the cytoplasmic tail of LFA-1 and promotes the delivery of LFA-1-containing vesicles to the front edge of T cells (33). This directional transport is aided by Mst1-mediated phosphorylation of vasodilator-stimulated phosphorylation, an adaptor protein that facilitates extension of actin filaments to the cell periphery (33). Second, Mst kinases regulate LFA-1 affinity in activated T cells through nuclear Dbf2-related (NDR) kinases, another group of Ser/Thr kinases related to Lats (31,34). Normally LFA-1 exists in a low-affinity conformation on

the cell surface until TCR or chemokine receptors provide “inside-out” signals (30). Binding of talin and kindlin-3 to the cytoplasmic tail of CD18 ( $\beta$ 2 integrin subunit of LFA-1) is key for “inside-out” signaling mechanisms (30). Through phosphorylation and activation of NDR-1, Mst1 promotes kindlin-3 binding to LFA-1 (31). Also, NDR-2 can be rapidly activated upon TCR ligation and subsequently phosphorylates filamin A (34). This triggers the release of filamin A from low affinity LFA-1 allowing binding of talin and kindlin-3 to the tail of CD18, key steps for increasing LFA-1 affinity (34). In addition, Mst1 has been detected as part of another “inside-out” signaling mediator adhesion and degranulation-promoting adaptor protein (ADAP) / Src kinase-associated phosphoprotein 1 (SKAP1) complex that binds to LFA-1 cytoplasmic tails upon TCR or CCR7 ligation (35,36). Although the exact role of Mst1 in this context has not been clarified, the association of SKAP1 and Mst1 bridged by RapL seems important to support LFA-1 function in T cells (35).

Since LFA-1 is a critical adhesion molecule for the formation of the supramolecular activation cluster (SMAC), Mst-deficiency also affected T cell interaction with Ag presenting cells (17,31). In response to TCR ligation, complexes between phospho-NDR-1 and phospho-Mob1 form in an Mst kinase-dependent manner (31). In turn, NDR-1 forms complexes with kindlin-3 surrounding the TCR-enriched central SMAC. Stable LFA-1-intercellular adhesion molecule-1 interaction coincides with kindlin-3 accumulation in the SMAC consistent with the role of kindlin-3 in maintaining high affinity LFA-1 through binding to the cytoplasmic tail of the  $\beta$ 2 integrin subunit (CD18) of LFA-1 (31,36). Impaired SMAC formation in Mst1-deficient T cells partially explains why Mst1-deficient Treg cells display defects in thymic selection (15) and suppressive function (17).

Although detailed mechanisms are not clear, activation of Rac1 through Mst-Mob1-Dock8 complexes can enhance the sensitivity of Treg cells to IL-2 (37). Treg cells lacking Mst1 and Mst2 had decreased levels of STAT5 activation in response to low dose IL-2 and consequently had reduced amounts of Foxp3 (37). These defects were substantially rescued by forced expression of a constitutively active form of RAC1 (37).

Taken together, Mst kinases play a pivotal role in directing T cell motility towards chemokines and migration between organs. In this case, Mst kinases are relaying signals emanating from chemokine receptors into directional movement of T cells by inducing dynamic actin remodeling and changes in LFA-1 affinity/avidity. This same machinery can be utilized to promote intimate interactions between T cells and Ag presenting cells upon Ag recognition and enhance IL-2-STAT5 signaling in Treg cells.

## FoxO-DEPENDENT PATHWAYS

FoxO family proteins are evolutionarily conserved transcription factors that control longevity, metabolism, and immunity (38). They are highly regulated by differential phosphorylation (39). Depending on the specific serine or threonine residues that are phosphorylated, FoxO can be retained in the cytoplasm or migrate into the nucleus to promote transcription of target genes (40) (Fig. 1C). Importantly, Akt-mediated phosphorylation inhibits the function of FoxO proteins whereas Mst1 has been shown to do the opposite (39). Moreover, Akt and Mst kinases can be activated in a context-dependent manner by T cell costimulatory receptors such as ICOS (41) and CTLA-4 (22), respectively. In T cells, FoxO1 and FoxO3a are highly expressed and they regulate T cell survival and differentiation (39).

### ROS detoxification in naïve T cells

Mst1-deficiency in patients and mice results in lymphopenia in T cells and B cells (9,10). Mouse knockout studies have shown that apoptotic death of naïve CD4<sup>+</sup> and CD8<sup>+</sup> T cells is the main driving force of T cell lymphopenia whereas B cell lymphopenia seems to be caused by loss of helper T cells (14). At the molecular level, both in mice and human T cells, Mst1-deficiency led to reduced FoxO1 and FoxO3a (10,14) concomitant with increased ROS and reduced mitochondrial membrane potential (10,14). This is because FoxO1 and FoxO3a are required to upregulate ROS-detoxifying enzymes such as superoxide dismutase and catalase, a mechanism conserved from nematodes to mammals (38). The subsequent inability to detoxify ROS was highly correlated with increased apoptosis in naïve T cells (9,10,14). However, the triggering mechanisms of cell death could be different between humans and mice. Mst1-deficiency in human T cells increased sensitivity to Fas-mediated cell death (10), whereas genetic abrogation of Fas-mediated cell death mechanisms could not rescue lymphopenia in Mst1-deficient mice (14).

### KLF2 upregulation in T follicular helper cell (Tfh) cells

Despite lymphopenia, Mst1-deficient mice and humans have normal or elevated levels of serum immunoglobulins (9,10,19). At least part of this can be attributed to enhanced T follicular helper cell activities including IL-4, IL-21, and CD40L (19). How Mst1-deficiency augments Tfh function is not clear. One possibility is that Mst1 may downregulate Tfh function through the FoxO1-KLF2 signaling axis during germinal center reactions since KLF2 is known as a negative regulator of Tfh function (42,43). In keeping with this idea, it has been shown that MST1-deficiency in human patients leads to reduced KLF2 mRNA in peripheral blood mononuclear cells (10). Mouse studies showed that, by elevating S1PR1, KLF2 promotes egress of Tfh cells from the germinal center at the later phase of germinal center reaction (42,43). KLF2 also increases BLIMP1 and thereby reduces expression levels of Bcl6, a key Tfh lineage-determining transcription factor (43). However, it remains to be established whether Mst kinases are required for the timely upregulation of KLF2 expression to suppress Tfh function during the natural course of germinal center reaction. Also, it will be interesting to see if the Mst-FoxO-KLF2 signaling axis is temporarily suppressed in Tfh cells at the early stages of germinal center reaction and, if so, whether there are active signaling mechanisms that counteract Mst1-FoxO pathways.

### T-bet downregulation in CTL

In contrast to the compromised immunity against infections in Mst1-deficient patients, CD8<sup>+</sup> T cells lacking Mst1 provide better protection against implanted tumors in animal models (7). Interestingly, Mst1-deficient CD8<sup>+</sup> T cells express elevated levels of T-bet and its target effector molecule IFN- $\gamma$ , suggesting that Mst1 may have a negative regulatory role in anti-tumor CD8<sup>+</sup> T cell responses. The increased T-bet expression in Mst1-deficient CD8<sup>+</sup> T cells was correlated with reduced levels of FoxO1 (44), consistent with the previously established role of FoxO1 in repressing T-bet expression (45).

### Opposing regulatory roles of Akt and Mst1 on FoxO

It has been shown that Akt and Mst1 have the ability to inhibit each other's kinase activity (16,46). In HEK 293 cells, Akt can phosphorylate Mst1 in its conserved inhibitory threonine residue and this reduces Mst1 kinase activity towards FoxO3 (46). However, it remains to be tested whether Akt similarly inhibits Mst kinases in T cells. On the other hand, Treg cells lacking Mst1 (or both Mst1 and Mst2) had elevated levels of activated phospho-Akt in response to TCR ligation (16) indicating that Mst kinases somehow attenuate Akt activation in T cells.

In addition, Akt and Mst kinases have well-established opposing roles on their common substrate FoxO transcription factors. Akt-dependent phosphorylation of FoxO induces cytoplasmic retention of FoxO mediated by 14-3-3 proteins (39,40). This process is prevented by alternative phosphorylation of FoxO by Mst1 (47). Consistently, Mst1-deficient T cells had reduced amounts of functional FoxO proteins in mice and humans (10,14). Since TCR ligation can activate Akt as well as Mst, the FoxO signaling pathway is likely to be counterbalanced by Akt and Mst in activated T cells (Fig. 1C). In this scenario, T cell costimulation such as ICOS, which is known to strongly enhance the PI3K-Akt pathway, may dampen FoxO activity (41). On the other hand, ligation of CTLA-4 or chemokine receptors that are known to induce Mst kinase may create an Mst-dominant signaling environment promoting FoxO activities (22).

## $\beta$ -CATENIN-DEPENDENT PATHWAYS

Counterbalancing acts of Akt and Mst appear to influence another evolutionarily conserved signal transduction pathway, Wntless-related integration site (Wnt) signaling (Fig. 1D). The Wnt gene was originally discovered in *Drosophila* as a coordinator of cell fate decision in developmental programming (48). However, some downstream components of Wnt signaling pathway in mammals are involved in tumor suppression in several tissues particularly in the colon epithelium (48). Further, it has been documented that the genetic ablation of key Wnt signaling components affects T cell development, differentiation, and function (49). Importantly, there is some evidence suggesting that Mst kinases may counteract Akt-induced Wnt signaling in T cells.

The key molecular event in Wnt signaling is regulation of  $\beta$ -catenin, whose protein level is normally kept undetectable in the absence of Wnt ligation due to ongoing degradation by destruction complexes (48). The key components of this destruction complex include the scaffold protein Axin,  $\beta$ -catenin binding protein adenomatous polyposis coli, protein kinases casein kinase (CK) 1  $\alpha/\delta$  and glycogen synthase kinase-3 (GSK-3 $\alpha/\beta$ ) that continuously phosphorylate  $\beta$ -catenin (48). Phosphorylated  $\beta$ -catenin undergoes proteasome-mediated degradation. The destruction complex gets temporarily inactivated when it binds to the phosphorylated cytoplasmic tail of low-density lipoprotein receptor-related protein-5 and 6 (LRP5/6), part of the Wnt receptor complex (48). This process can be facilitated when CK1 $\epsilon$  phosphorylates dishevelled (DVL) (50). As a result of Wnt signaling, accumulated  $\beta$ -catenin then goes into the nucleus and associates with T cell factor-1 (TCF-1) to induce an array of target genes (48).

Similar to Hippo and FoxO pathways, it has been shown that Wnt signaling plays important roles in T cell development differentiation, and function (49). For example, Wnt effector transcription factor TCF-1 drives thymocyte differentiation by establishing the epigenetic landscape of T cell identity genes (51). TCF-1 and  $\beta$ -catenin together promote GATA-3 expression (therefore, Th2 differentiation); on the other hand, TCF-1 alone can suppress the Th1 cytokine IFN- $\gamma$  (52). TCF-1 also promotes Tfh differentiation by enhancing Bcl6 expression and suppressing BLIMP1 expression (53-56). Further, overexpression of the stable form of  $\beta$ -catenin enhanced Treg survival and induced anergy in non-Treg naïve CD4<sup>+</sup> T cells (57). Lastly, TCF1 is critical for the generation of memory CD8<sup>+</sup> T cells (58-60). There are several reports demonstrating that ligation of TCR or (pre-TCR) increases  $\beta$ -catenin protein levels (61-63). However, it remains to be clarified whether TCR signaling can stabilize



$\beta$ -catenin in the absence of conventional Wnt receptor signaling (e.g., in Lrp5/6-deficient T cells). Despite the link, biochemical events that relay TCR ligation and  $\beta$ -catenin stabilization have not been well documented. One study using primary human T cells indicate that inhibition of GSK-3 activity through phosphorylation is a critical event linking TCR ligation to the accumulation of  $\beta$ -catenin (61). This can be interrupted by pharmacological agents that inhibit phosphoinositide 3-kinase (presumably through reduced Akt activation) or protein kinase C.

Interestingly, *in vitro* experiments using non-hematopoietic cell lines showed that activated forms of Mst1 and Mst2 bind to CK1 $\epsilon$ , a positive regulator of Wnt signaling (64). Through this interaction, active Mst kinases reduced the level of phospho-DVL (64). Thus, treatments that activate Mst kinases dampened Wnt-induced accumulation of  $\beta$ -catenin (64). This process worked through non-canonical Hippo signaling since silencing WW45 expression (and disruption of Mst-YAP phosphorylation signaling branch) did not affect the outcome (64). Whether Mst downregulates Wnt-induced  $\beta$ -catenin stabilization in T cells through CK1 $\epsilon$  inhibition is yet to be tested. Nonetheless, it seems conceivable that Mst-deficiency in T cells might be more responsive to the TCR/Wnt-mediated  $\beta$ -catenin stabilization pathway by 2 different mechanisms: enhanced Akt-dependent inactivation of GSK-3 or elevated Wnt signaling through the CK1 $\epsilon$ -DVL pathway. If this is true, mice harboring Mst-deficient T cells would have increased  $\beta$ -catenin when activated by TCR or Wnt and display opposite phenotypes that are seen in mice lacking TCF1 or  $\beta$ -catenin in T cells. In support of this notion, Th2 and Tfh responses were elevated in Mst1 knockout mice (19,65). On the other hand, there was no gross defect in T cell development in mice lacking Mst1 and Mst2 in the T cell compartment—it was rather a migration defect after maturation.

In summary, available data suggest that Akt may promote whereas Mst kinases could dampen  $\beta$ -catenin stabilization in T cells. This is in contrast to FoxO signaling: Akt inhibits whereas Mst promotes nuclear translocation of FoxO proteins. Therefore, the relative strength of Akt and Mst kinase activities in different T cell subsets may highly influence the outcome of  $\beta$ -catenin and FoxO signaling.

## CONCLUSION

Accumulating evidence indicate that Hippo kinase Mst1 and Mst2 play crucial roles in T cell development, differentiation, and function. In contrast to their role as tumor suppressors, Mst kinases in T cells can work without involving canonical Hippo signaling components such as Lats and YAP/TAZ. Rather, Mst kinases act by interacting with small GTPases, FoxO family transcription factors, or Wnt signaling components. Regulation of FoxO and  $\beta$ -catenin protein stability or function by Mst kinases can be counterbalanced by Akt. These opposing roles could explain the various immune abnormalities observed in mouse models when key elements of Hippo signaling are removed in a cell type-specific manner. Thus, deeper understanding of Hippo signaling mechanisms in the context of cooperating pathways may reveal key partners or downstream effectors that are relevant to humoral immunity, autoimmune disorders, or anti-tumor T cell responses.

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