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Review

Review

The non-canonical Hippo/Mst pathway in lymphocyte development and functions

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Abstract

The canonical Hippo/Mst pathway, originally discovered in *Drosophila*, is famous for its function in promoting apoptosis, inhibiting cell proliferation and tumorigenesis, and regulating tissue regeneration. However, emerging evidence shows that multiple non-canonical Hippo signaling pathways are also implicated in the regulation of various other biological processes. Recent studies have revealed that Mst1/2, the core kinases of Hippo/Mst pathway are required for T cell development, function, survival, trafficking, and homing, and also involved in regulation of autoimmunity. In this review, we discuss the roles of non-canonical Hippo/Mst signaling pathways in lymphocyte development and functions.

Key words: Mst1/2, lymphocyte, development, function

Introduction

The canonical Hippo/Mst pathway, which is evolutionally conserved from *Drosophila* to mammals, plays critical roles in organ size control during animal development and regeneration [1]. The Hippo/Mst pathway components were first identified in *Drosophila* by genetic screens. Mammalian sterile 20-like kinase 1 and 2 (*Mst1/2*), large tumor suppressor 1 and 2 (*Lats1/2*), Salvador (*Salv,* also known as *WW45*), Mps one binder 1A and B (*Mob1A/B*), and Yes-associated protein (*Yap*)/transcriptional coactivator with PDZ-binding motif (*Taz*) are the mammalian homologs of *Drosophila hpo,* wts, *sav*, *mats*, and *yki*, respectively [2]. Mst1/2 and Lats1/2 are the core kinases of the Hippo/Mst pathway. Mst1/2 proteins interact with Salv, a WW domain-containing protein, to phosphorylate and activate Lats1/2 kinases. Lats1/2 then phosphorylate and inactivate the transcriptional coactivator YAP/TAZ by sequestering phosphorylated YAP/TAZ in the cytoplasm by 14-3-3 proteins [1,2]. Inhibition or loss of function of Hippo/Mst pathway core components such as *Mst1/2* and *Lats1/2* results in the nuclear translocation of YAP/TAZ, and subsequently, YAP/TAZ in conjunction with TEADs (Scalloped orthologs) mediates transcription of target genes to promote cell proliferation and inhibit apoptosis. The above mentioned *Mst–Lats–Yap* signal pathway is a canonical signaling transduction process of the Hippo pathway in regulating cell proliferation, apoptosis, tumorigenesis, and tissue regeneration. Plenty of excellent reviews have already been published on this aspect [1–6].

In addition to their role in the regulation of cell proliferation and differentiation through YAP1, the Hippo pathway core components are also involved in multiple non-canonical Hippo signaling pathways, which are implicated in the regulation of other biological processes. *Mst1* can also induce apoptosis in a number of different ways: by phosphorylating Foxo1/3 and enhancing their nuclear entry in primary granule neurons [7,8], by phosphorylating histone H2B [9], by promoting phosphorylation of Runx3 and Mst2–Sav1–Runx3 complex formation [10], or by antagonizing cell survival signals through interacting with AKT and suppressing its activation [11] in various tumor cell lines. MST1 can impair insulin secretion by phosphorylating and destabilizing the β-cell transcription factor PDX1 [12]. *Mst2–Sav1* complex promotes adipocyte differentiation by stabilizing and activating PPARγ [13]. *Mst1–Ndr1* signaling promotes stable kinetochore–microtubule attachment by restraining Aurora B activity and centrosome duplication, whereas the Mst1–Sav1 complex regulates centrosome disjunction via Nek2A [14–16]. Lats–Mob1 complex has an evolutionarily conserved role in mitotic exit and centrosome maintenance [17,18].

The immune system is a complex system of cells and biological reactions that constitute our body's first line of defense for fighting off invaders and preventing its own cells from self-deterioration and mutation. The immune system is composed of both innate immunity and adaptive immunity in mammals. Several recent studies have revealed

[20,21]. The results reported so far about apoptosis, proliferation, and cytokine production of *Mst1*-deficient peripheral T cells vary dramatically and are sometimes contradictory. This is probably due to different methods used for generating the *Mst1*-deficient mice such as gene trapping versus gene targeting, and differences in genetic background of the mutant mice used by different research groups.

There is enhanced apoptosis of peripheral T cells in *Mst1*$^{-/-}$ mice [22,23,30] and human patients [28,29]. Zhou *et al*. [22] reported that ongoing apoptosis of peripheral *Mst1*-deficient T cells was enhanced. However, it was not observed in *Mst1*$^{-/-}$ mice generated by other groups [20,30]. Ongoing apoptosis of peripheral *Mst1*$^{-/-}$ T cells is significantly increased only when CD62^{high} CD44^{low} naïve T cells and CD62L^{low}CD44^{high} effector/memory T cells are analyzed separately [20]. Apoptosis is significantly increased when *Mst*$^{-/-}$ T cells are cultured *in vitro* with CD3/CD28 activation [30] or oxidative stresses [23]. Furthermore, increased apoptotic cells were found to be restricted to *Mst1*

B Cell

In contrast to the extensive researches on the roles of *Mst1* and *Mst2* in T cell development and function, there are few studies focusing on the function of *Mst1* and *Mst2* in B cells. The number of peripheral B cells is reduced in *Mst1*-deficient mice [20–22]. Splenic marginal zone B cells are dramatically reduced and B cell adhesion and trafficking are impaired in *Mst1*-deficient mice [21,22]. A similar mechanism of T cell trafficking was proposed to explain the defects of B cell adhesion and trafficking [21]. When stimulated by BCR, the response of *Mst1*-deficient B cells was reported to be similar to controls [21]. However, Salojin *et al.* [30] found that *Mst1*-deficient B cells showed decreased responsiveness to B cell mitogens *in vitro* and displayed deficient antigen-specific IgE production *in vivo* when compared with the controls. More experiments are needed to further explore and confirm the function of *Mst1* and *Mst2* in B cells.

Eosinophil Apoptosis

Eosinophils and neutrophils are two types of innate immune cells that play important roles in fighting multicellular parasites and infections. They are also involved in the pathogenesis of allergies and asthma. Both Mst1 and Mst2 kinases are expressed in eosinophils, but not in neutrophils. However, only Mst1 is activated by caspase-mediated cleavage during spontaneous or Fas-induced apoptosis of human eosinophils, suggesting that *Mst1*, but not *Mst2*, plays a role in the regulation of eosinophil apoptosis [35]. However, whether *Mst1* can affect eosinophil development and function has not been investigated yet.

Immune System-related Disease

The Mst1 and Mst2 kinases emerge as critical regulators of lymphocyte function and autoimmunity. Loss-of-function mutations of *Mst1* in human patients have been identified in clinical studies [28,29,36]. MST1 deficiency in humans leads to T cell lymphopenia with a low proportion of naïve T cells and high proportion of effector T cells, impairment of T cell response to stimulation with anti-CD3, various mitogens and recall antigens [28]. It is also associated with neutropenia and heart malformations in some patients [29]. *Mst1*-deficient patients also display recurrent pulmonary infections, susceptibility to candidiasis and non-regressing cutaneous warts caused by multiple types of human papillomavirus infections [28,29,36]. These phenotypes have not been reported or observed in *Mst1*-deficient mice, probably as a result of the mice under SPF conditions. Autoimmune antibodies, which usually occur in autoimmune diseases, are also detectable in *Mst1*-deficient patients [28,29]. The protein levels of FOXO1 and FOXO3, IL-7 receptor and BCL2 are significantly lower in T cells from *Mst1*-deficient patients than those in the control [28,29]. Conversely, FAS expression and the FAS-mediating apoptotic pathway are up-regulated [28].

Similar to *Mst1*-deficient patients, *Mst1*-deficient mice, generated separately by Dr Kinashi's lab and Dr Tao's lab, have been shown to be prone to autoimmune diseases [24,25]. Lymphocyte infiltration, T cell over-activation, and autoantibody production are observed in young [25] and aged *Mst1*-deficient mice [24,25]. The autoimmune phenotypes were more severe when both *Mst1* and *Mst2* genes were deleted, indicating that the functions of *Mst1* and *Mst2* are redundant in preventing autoimmunity, although *Mst2* does not display any autoimmune phenotypes [25]. Although $Mst1^{-/-}$ bone marrow is sufficient for inducing colitis as well as over-activation of naïve T cells and splenomegaly in recipient mice, these *Mst1*-deficiency-mediated mutant phenotypes are all suppressed in recipients with co-transplanted wt Tregs [25]. $Mst1^{-/-}$ Tregs fail to inhibit colitis induced by wt naïve T cells transplanted into recipient mice [27]. These results suggested that the impaired function of $Mst1^{-/-}$ Tregs is a major cause of autoimmune diseases in $Mst1^{-/-}$ mice. However, it has also been reported that deletion of *Mst1* in mice reduces the severity of experimental autoimmune encephalomyelitis (EAE) with a lower number of infiltrated CD4 T cells in the spinal cord and protects the mice from the development of collagen-induced arthritis (CIA) [30]. It was found that the severity of EAE was alleviated in mice treated with an Mst1 inhibitor [30]. One explanation is that *Mst1*-deficient mice are prone to autoimmune diseases in normal conditions but are protected from autoimmune EAE and CIA induction. Further detailed studies are required to determine the exact role of *Mst1* in autoimmunity.

In addition to the role of *Mst1/2* in the regulation of autoimmune diseases, *Mst1* is also implicated in preventing leukemia. Mst1-null mice are highly susceptible to the development of ENU-induced T-ALL [37], probably through its role in the maintenance of chromosome integrity [38].

Concluding Remarks

Recent studies about the roles of non-canonical Hippo/Mst signaling in lymphocyte development and functions have revealed that at least *Mst1/2*, core components of Hippo/Mst pathway, can play important but different or opposite roles in a cell or tissue-dependent manner. *Mst1/2* are required for T cell survival, whereas the canonical Hippo/Mst signaling promotes apoptosis of non-lympho0.3-323-7.9(on-the)1-8.6T[(0.

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