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PERSPECTIVE

Redemystifying MST1/hippo signaling

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Since the Mammalian Ste20-like kinases (MST) 1/2 were first indentified in 1995, our knowledge about MST1/2 and its *Drosophila* ortholog Hippo has expanded to diverse biologic process ranging from cell survival and death, organ size control, to proliferation and tumorigenesis.

MST1 (also known as *Stk4* and *Krs2*) and MST2 (also known as *Stk3* and *Krs1*) were first identified as homologs of the ste20 kinase from *Saccharomyces cerevisiae* (Creasy and Chernoff, 1995). Subsequently, these proteins were also identified in 'in gel' kinase assays as kinases that respond to extreme cellular stress (Taylor et al., 1996). Though a number of apoptotic or stress stimuli have been reported to activate MST, proteolytic cleavage of MST by caspase 3 remains the only and best understood mechanism that regulate MST kinase activity to date (Kakeya et al., 1998). Upon proapoptotic stimuli, the N-terminal proteolytic fragment of MST translocates into the nucleus and phosphorylates histone H2B, which leads to the chromatin condensation and mammalian cell death (Cheung et al., 2003). Interestingly, histone H2B phosphorylation also occurs in *S. cerevisiae*, through the phosphorylation on Ser10, a residue distinct from mammalian histone H2B Ser14 (Ahn et al., 2005). However, growing evidence suggests that full length MST also promotes cell death independently of proteolysis or nuclear translocation (Ahn et al., 2005; Lehtinen et al., 2006). In primary mammalian neurons, oxidative stress-activated-MST phosphorylates the transcription factor FOXO, which could translocate into the nucleus and upregulate the transcriptional activity of pro-death genes, including BIM. Moreover, the characterization of the *C. elegans* ortholog *CST-1* broadens MST functions beyond the control of cell death to the regulation of life span in nematodes (Lehtinen et al., 2006). Despite the flurry of interest in the MST, both the upstream regulators and downstream targets of MST in different scenarios are still in a puzzle.

In contrast to the limited progress of study in mammals, genetic screens for flies with defects in organ size lead to the identification of Hippo complex and the upstream regulators that modulate cell growth and survival. As a tumor suppressor, The Hippo/MST kinase cooperates with adaptor protein Salvador (Sav)/WW45, phosphorylates and activates the downstream kinase Warts/Lats, which in turn leads to the phosphorylation and inhibition of the transcriptional activator Yorkie (Yki)/YAP through promoting its nuclear exportation in 14-3-3 dependent and-independent manners. Two membrane-associated FERM domain proteins, Merlin (Mer) and Expanded (Ex), have been suggested to function in parallel but differentially regulate the Hippo pathway since Ex predominantly regulates proliferation whereas Mer regulates apoptosis (Pellock et al., 2007). However, McNeill and colleagues showed a physical interaction between Ex and Yki that may directly inhibit Yki activity independent of Hippo (Badouel et al., 2009), implying that there is more complicated cross-regulation in the Hippo/Yki signaling. Recently, the atypical cadherin Fat (Ft) and its ligand Dachsous (Ds) appear to signal through Ex to activate the Hippo pathway (Bennett and Harvey, 2006). Crumbs (Crb) were identified as a novel Hippo pathway regulator via modulating Ex levels and localization, indicating that Hippo/MST might be involved in the establishment of the cellular polarity (Grzeschik et al., 2010; Robinson et al., 2010). Richardson and colleagues also proposed that cell polarity regulators giant-larvae (Lg) l and atypical protein kinase C (aPKC) feed into Hippo pathway. Lgl acts antagonistically to aPKC to regulate Hippo and Ras association family member (RASSF) localization (Grzeschik et al., 2010). Most recently, genetic analysis revealed that WW domain containing protein Kibra physically interacts with Mer and acts upstream of Mer to regulate Hpo activity (Baumgartner et al., 2010). The core components of the Hippo pathway are well conserved, and the understanding of Hippo functions in flies may shed light on the study of MST function in mammals.

To investigate the physiological functions of MST family proteins *in vivo*, MST1/2 knockout mice were generated (Oh et al., 2009; Zhou et al., 2009). MST1 deletion resulted in decreased numbers of peripheral T cells, mainly naive T cells,

which still proliferated after T cell receptor (TCR) ligation *in vitro* and impaired lymphocyte homing to the spleen and lymph nodes (Zhou et al., 2008; Katagiri et al., 2009). In addition, defective egress of mature thymocytes from Mst1 deficient thymus was also reported (Dong et al., 2009). However, MST2 knockout mice exhibited no developmental or immunological defects. Due to the redundant functions of MST1/2, MST1/2 single knockout model provided limited information. MST1/2 double knockout resulted in embryonic lethality, indicating that MST is essential for the early development in mice. Single copy of either MST1 or MST2 underwent normal organ development. Moreover, by 15 months of age, $MST1^{-/-}$ $MST2^{+/-}$ mice developed hepatocellular carcinoma (HCC) because of MST2 loss of heterozygosity. Correspondingly, tissue-specific ablation of both MST1 and MST2 in liver leads to HCC (Zhou et al., 2009). Yang and colleagues also reported that tumors developed in the liver with a substantial increase of the stem/progenitor cells by 6 months after removing MST1 and MST2 postnatally (Song et al., 2010). Interestingly, apoptosis induced by Fas ligand or TNFα was blocked in the MST1/2 deficient cells *in vivo* (Zhou et al., 2009; Song et al., 2010). These *in vivo* experiments further supported the physiological significance of MST1/2 in apoptosis and tumorigenesis.

It is worth noting that *in vivo* experiment also reveals that MSTs are differentially regulated in various cell types. For example, The cleaved form of MST1 are the major form in livers but absent in spleen or MEF cells, indicating that tissue-specific cleavage may be an important mechanism of MST1/2 regulation *in vivo* (Zhou et al., 2009). Obviously, the upstream regulatory mechanism identified from flies is insufficient for us to understand the variation, especially in mammals. Fortunately, in mammals, we and others have found that Akt directly phosphorylates MST1, which leads to inhibition of MST1 cleavage and kinase activity (Cinar et al., 2007; Yuan et al., 2010). Moreover, this phosphorylation inversely correlates with MST1 auto-phosphorylation/activation and associates with adverse prognosis in human ovarian cancer. Recent study adds a new player PHLPP in this model. As a phosphatase, PHLPP directly dephosphorylates MST1 on Thr387, the same site as phosphorylated by Akt, resulting in the activation of MST1 (Qiao et al., 2010). Though this delicate autoinhibitory triangle cannot explain the cell type-dependent functions of MST1/2, we can conclude that phoshophorylation and cleavage of MSTs, alone or cooperated, play an important role in regulating MST activity in a variety of settings.

According to many elegant experiments, we have learned a lot about MST. However, more questions have been raised than been answered. First, as a tumor suppressor, is mammalian MST dysregulated at pathological state indeed and how? While waiting for the next genetic screen in flies, it is crucial to validate whether Mer, Ex, Crb or those cell polarity regulators really work in mammals. Second, in spite of the redundant roles of MST1/2, do they have distinguished functions in different situations? Why does this happen? Does MST1 or MST2 fall into unique signaling pathway even though they share most of the signaling components? Finally, at present, oxidative stress remains one of the best known stimuli that activate MST kinases, which leading to the phosphorylation of FOXO or histone H2B in different type of cells. How does MST select the substrates? Some evidences from us and others suggest that the c-terminal domain of MST1 is dynamically regulated and can exhibit stimulatory or inhibitory activities with specific substrates (Anand et al., 2008). Therefore, more studies related to the substrate specification through the c-terminal modification and regulation will help to solve this puzzle. Nonetheless, an important goal of future studies is to determine the upstream regulators of MST, possibly including kinases, phophatases or cytoskeleton proteins, in the regulation of the MST function in cell proliferation and apoptosis, organ size control as well as cellular polarity establishment.

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