Mst Out and HCC In

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Mst1 and Mst2 are key components of the Hippo tumor suppressor pathway. In this issue, Zhou et al. (2009) reported that Mst1/2 ablation leads to hepatocellular carcinomas. Unexpectedly, Mst1/2 may activate another kinase besides Lats1 and Lats2 to phosphorylate YAP, and the role of Mst1/2 in YAP regulation is cell type dependent.

Mst1 and Mst2 are STE20 family kinases homologous to the *Drosophila* Hippo, a founding member of the Hippo pathway. Mst1/2 in association with an adaptor protein SAV1 phosphorylates and activates Lats1 and Lats2 kinases, which associates with another adaptor, Mob (Figure 1A). The Hippo pathway proteins inhibit cell proliferation and promote apoptosis by directly phosphorylating and inhibiting a transcription coactivator, Yes-associated protein (YAP) (Zhao et al., 2007), which is also a candidate human oncogene (Overholtzer et al., 2006; Zender et al., 2006). Genetic manipulations of the Hippo pathway in *Drosophila* revealed critical roles of these genes in organ size control (Kango-Singh and Singh, 2009). Several of the Hippo pathway proteins are mutated in cancers and are potential tumor suppressors. Therefore, this pathway regulates a fundamental aspect of normal development and plays an important role in human cancers, so mammalian genetic models of this pathway are highly anticipated.

Previous genetic manipulation of the Hippo pathway in mouse provided limited information because of genetic redundancy or early lethality. For example, *Mst1* knockout mice develop normally probably because of redundant functions of *Mst2*, although they carry some immunological defects (Katagiri et al., 2009; Zhou et al., 2008). The one exception is a liver-specific *Yap* transgenic mice model that showed a reversible and dramatic overgrowth of liver and the development of hepatocellular carcinoma (HCC), confirming the role of YAP in organ size regulation and tumorigenesis (Camargo et al., 2007; Dong et al., 2007).

In this issue, by using both germline and liver-specific conditional *Mst1/2* double knockout mouse models, Bardeesy, Avruch, and colleagues confirmed the regulation of YAP phosphorylation in mediating Mst1/2 functions (Zhou et al., 2009). Ablation of both *Mst1* and *Mst2* largely abolished YAP phosphorylation in liver, increased YAP nuclear localization and target gene expression, and caused liver tumorigenesis. Knockdown of YAP reversed the transformed phenotype of HCC-derived cells from these mice. Strikingly, liver-specific $Mst1^{-/-}Mst2^{-/-}$ mice developed enlarged liver phenotypes similar to *Yap* transgenic mice and their livers are more resistant to FAS ligand-induced apoptosis. These in vivo experiments further supported the physiological function of Mst1/2 in YAP inhibition.

The *Mst1/2* double knockout mice also unequivocally established the role of the Hippo pathway in tumorigenesis. Interestingly, the germline $Mst1^{-/-}Mst2^{+/-}$ mice mainly developed HCC because of *Mst2* loss of heterozygosity. This result suggests that the Hippo pathway may be particularly important in liver and is consistent with previous observations that elevated YAP is most frequently observed in human liver cancers (Zhao et al., 2007). Moreover, tissue-specific ablation of both *Mst1* and *Mst2* in liver leads to massive HCC with a mean latency of 10 weeks, very similar to what is observed with YAP overexpression. Strikingly, 70% of human HCC samples show a markedly reduced Mst1/2 activity as determined by Mob phosphorylation and most of them are also confirmed by loss of the presumably active cleaved form of Mst1. It is worth noting that in all but three of those samples with attenuated Mst1/2 activity, YAP phosphorylation is also clearly decreased. Together with previous observations of YAP genomic amplification (Overholtzer et al., 2006; Zender et al., 2006) and elevated nuclear localization in human HCC (Dong et al., 2007; Zhao et al., 2007), one may conclude that YAP activation plays important roles in human HCC, and an impaired Hippo pathway might be a common mechanism for YAP activation.

One unexpected finding in this report is the cell-type-dependent function of Mst1/2 in YAP regulation. Although Mst1/2 are ubiquitously expressed, their activity was shown to be differentially regulated in different cell types. For example, Mst1/2 could be cleaved into a 34 kDa presumably active N-terminal fragment in liver but not in spleen or MEF cells (Figures 1B and 1C). Although the caspase-dependent cleavage of Mst1/2 is known to activate the kinase in other contexts, this regulation has not been examined in the Hippo pathway nor shown to be tissue specific. It is worth noting that the cleaved form of Mst1/2 lost the SAV1-interacting SARAH domain. SAV1 is required for Hippo pathway activity in *Drosophila* and for Mst1 phosphorylation and translocation under differentiation signal in mammalian keratinocytes (Lee et al., 2008). So in liver, SAV1 is either not required for Hippo pathway activity or functions prior to Mst1/2 cleavage. Either way, there likely exist cell-type-specific variations of the Hippo pathway. As shown in this report, Mst1/2 are not required for Lats1/2 phosphorylation and cell density-stimulated YAP nuclear-cytoplasmic translocation

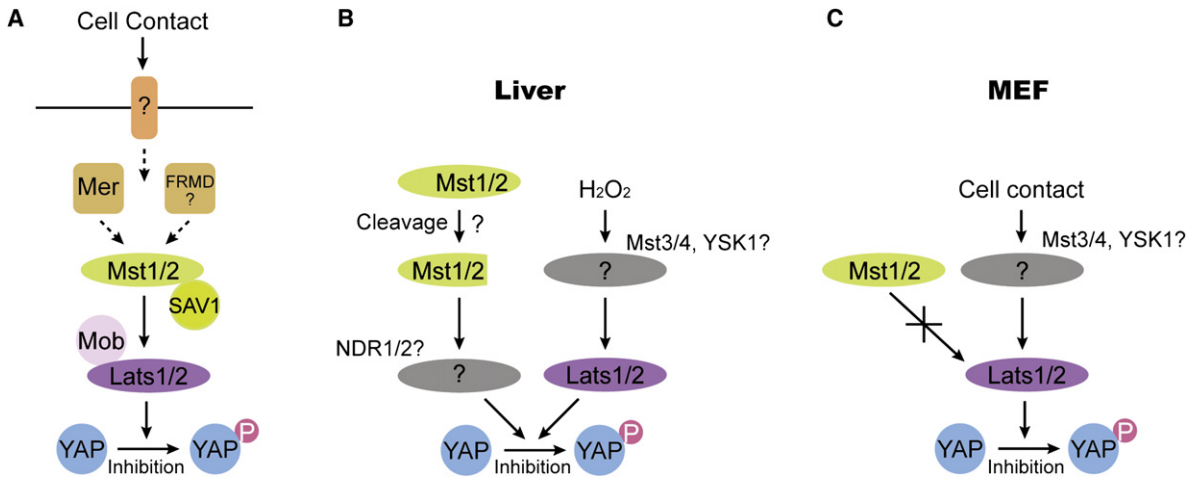

Figure 1. Cell Type Dependent Variation of the Hippo Pathway

(A) A generally accepted paradigm of the Hippo pathway.

(B) Hippo pathway in liver and cultured hepatocytes. Cleaved Mst1/2 activate an unknown kinase of YAP, and Lats1/2 may phosphorylate YAP once activated by a kinase distinct from Mst1/2.

(C) Hippo pathway in MEF cells. High cell density activates an elusive kinase to activate Lats1/2 and stimulate phosphorylation of YAP. Mst1/2 are not required for YAP phosphorylation in MEFs.

in MEFs (Figure 1C). Lats1/2 are NDR family kinases that require phosphorylation of the hydrophobic motif by upstream kinases for activation. Therefore, a key question is what is the upstream activating kinase of Lats1/2 in MEF cells in response to cell contact? Potential candidates are Mst3, Mst4, or YSK1, three kinases most similar to Mst1 and Mst2 with identical residues in their substrate-binding pockets. It is also possible that a totally unrelated kinase phosphorylates Lats1/2.

The possible existence of a YAP kinase other than Lats1/2 downstream of Mst1/2 in liver is the other interesting finding of this work (Figure 1B). Two pieces of evidence support this prediction: first, Mst1/2 ablation in liver reduced YAP phosphorylation but had little effect on Lats1/2 phosphorylation; second, fractionation of liver lysates showed Mst1/2-regulated YAP kinase activity distinct from Lats1/2, whereas Lats1/2 activity is unresponsive to Mst1/2 deficiency. Noteworthy, the decrease of NDR1/2 phosphorylation in Mst1/2-deficient liver is consistent with the possibility of them being the elusive kinases. It should also be noted that in the kinase assay with fractionated liver lysates, distribution of the phosphorylated endogenous YAP strictly correlated with Lats1, suggesting that Lats1 may form a complex with endogenous YAP. To unequivocally clarify the role of Lats1/2 or other kinases in YAP phosphorylation, mouse models with *Lats1* and *Lats2* double deletion are needed.

This report confirmed the inhibition of YAP by Mst1/2 as an important mechanism in tumor suppression, especially in HCC. And as of many other interesting studies, more questions have been raised than been answered. To what extent could the Hippo pathway differ in different cell types? In MEF cells, what is the kinase in response to cell contact signal upstream of Lats1/2? What is the YAP kinase acting downstream of Mst1/2 in liver? And what are the mechanisms of Mst1/2 inactivation and YAP dysregulation in human cancers? Answers to these questions will significantly advance our understanding of the Hippo pathway in normal organ size control and pathological tumor development.

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