TAZ-Mediated Crosstalk between Wnt and Hippo Signaling

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Previously, research addressing Hippo signaling focused on the inactivation of the proto-oncoproteins TAZ and YAP caused by the sequestration of supposedly inactive phospho-TAZ/YAP in the cytoplasm. In this issue of *Developmental Cell*, the Attisano laboratory now shows that cytoplasmic TAZ inhibits canonical Wnt signaling, thereby highlighting that the Hippo pathway can control other signaling cascades.

The precise coordination of organ growth and cell patterning ensures the correct development of organ architecture and size in multicellular organisms. Over the past decade, *Drosophila* geneticists have successfully defined a tumor suppressor cascade controlling organ growth (Harvey and Tapon, 2007; Hergovich et al., 2006; Pan, 2007; Saucedo and Edgar, 2007). This conserved kinase pathway is called Hippo signaling after its core component, the *Drosophila* Hippo kinase. Hippo activates the Warts/Lats kinase by phosphorylation, which then phosphorylates the transcriptional coactivator Yorkie (Yki), resulting in the cytoplasmic retention of inactive phospho-Yki. Hippo signaling has also been delineated for mammals (Hergovich and Hemmings, 2009; Zhao et al., 2008). MST1/2 activate LATS1/2 (the mammalian kinase counterparts of Hippo and Warts/Lats, respectively), which in turn phosphorylate two mammalian Yki homologs, the transcriptional coactivator with PDZ-binding motif (TAZ) and Yes-associated protein (YAP) proto-oncoproteins. The subsequent accumulation of inactive phospho-TAZ/YAP in the cytoplasm results in decreased cell proliferation and increased apoptosis. In summary, Hippo tumor suppressor signaling accurately coordinates cell death and proliferation by phosphorylation and consequent cytoplasmic retention of Yki/TAZ/YAP.

Reporting in this issue of *Developmental Cell*, Varelas et al. (2010) have now discovered that cytoplasmic TAZ functions as a negative regulator of the canonical Wnt pathway. Normally, in canonical Wnt signaling (MacDonald et al., 2009) Wnt ligands (e.g., Wnt3A) stimulate the cell surface receptors Frizzled and LRP5/6.

Consequently, the intracellular signal mediators Dishevelled (DVL) and Axin are recruited to these receptors, resulting in the disassembly of the β-Catenin destruction complex composed of Axin, adenomatous polyposis coli, casein kinase 1δ/ε (CK1δ/ε), and GSK3. This disassembly leads to the accumulation of β-Catenin and subsequent activation of Wnt target genes. Intriguingly, depletion of endogenous TAZ in human cells is sufficient to increase nuclear accumulation of β-Catenin and thus expression of Wnt target genes. In some tissues of TAZ null mice, inappropriate levels of nuclear β-Catenin could be observed (Varelas et al., 2010). These results indicate that TAZ is a negative regulator of β-Catenin signaling in mammals.

How does TAZ regulate the canonical Wnt/β-Catenin pathway? Varelas et al. (2010) approached this question via an elegant high-throughput protein-protein interaction assay, revealing that DVL2 strongly associates with TAZ. The authors then go on to show that knockdown of TAZ increased basal and Wnt3A-induced DVL2 phosphorylation by CK1δ/ε. Pharmacological inhibition of CK1δ/ε as well as depletion of CK1δ/ε blocked increased DVL2 phosphorylation upon TAZ depletion. By analyzing TAZ mutants, they further show that (1) the TAZ/DVL2 interaction interferes with DVL2/CK1δ/ε binding, and (2) cytoplasmic TAZ is required to downregulate β-Catenin signaling (Varelas et al., 2010). However, it still remains to be determined whether the interaction of TAZ with DVL2 in the cytoplasm is sufficient to block the phosphorylation of DVL2 by CK1δ/ε. Furthermore, the role of DVL2 phosphorylation in canonical Wnt signaling is yet to be

completely understood (Gao and Chen, 2010). The regulatory roles of TAZ phosphorylation on Ser66, Ser117, and Ser311 by LATS2 (Lei et al., 2008) are also yet to be studied in this context.

Previous work showed that phosphorylation of TAZ on Ser89 by LATS/MST signaling is crucial for cytoplasmic retention, since mutating this serine residue traps fully active TAZ in the nucleus (Lei et al., 2008). Significantly, this TAZ(S89A) mutant did not restore the cytoplasmic function of TAZ: the inhibition of Wnt target gene expression that is lost upon TAZ knockdown (Varelas et al., 2010). Varelas et al. (2010) therefore examined the roles of MST1/2 and LATS1/2 kinases upstream of TAZ in canonical Wnt signaling. Single depletion of MST1, MST2, LATS1, or LATS2 increased Wnt3A-stimulated gene expression, with MST2/LATS1 signaling representing the predominant kinase cascade in HEK293 cells. Overexpression of kinase-dead MST2 (which can function as a dominant-negative) also elevated Wnt3A-driven expression, suggesting that the kinase activity of MST2 might be required in this setting. Overexpression of LATS1 suppressed Wnt3A-stimulated expression, while depletion of LATS1 in human cells reduced the TAZ/DVL2 association, increased DVL2 phosphorylation, and elevated nuclear β-Catenin levels. This suggests that MST/LATS signaling can influence the canonical Wnt pathway. However, the role of LATS1/2 kinase activity is yet to be clarified.

The authors (Varelas et al., 2010) propose the following working model: (1) stimulated MST1/2 activate LATS1/2, (2) and active LATS1/2 phosphorylate TAZ on Ser89, which inhibits its transcriptional

coactivator activity due to cytoplasmic retention, (3) cytoplasmic TAZ then binds to DVL2, (4) which negatively affects CK1δ/ε binding and phosphorylation of DVL2, (5) subsequently resulting in increased assembly of the β-Catenin destruction complex, and (6) finally decreasing β-Catenin levels (nuclear accumulation of β-Catenin) and expression of canonical Wnt target genes.

Recent focus in Hippo signaling has been on the nuclear regulation of TAZ/YAP activities (Zhao et al., 2008), and therefore researchers have considered cytoplasmic phospho-TAZ/YAP to be an inactive subcellular fraction. Now, the report by Varelas et al. (2010) shows that cytoplasmic TAZ is crucial for the proper regulation of canonical Wnt signaling. This strongly suggests that Hippo signaling can crosstalk with other important pathways. One key question now is whether cytoplasmic YAP (the paralog of TAZ) has similar functions.

Significantly, Varelas et al. (2010) show that TAZ regulates Wnt signaling by binding to DVL, which has also been called the hub of Wnt signaling (Gao and Chen, 2010). DVL has more than 50 reported binding partners and therefore represents an extremely multifaceted signal mediator (Gao and Chen, 2010). More than 20 proteins associate with the PDZ domain of DVL, thereby either inhibiting or stimulating Wnt signaling (Gao and Chen, 2010). Intriguingly, the authors find that TAZ seems to compete with CK1δ/ε for binding to the PDZ domain of DVL2, thereby decreasing DVL2 phosphorylation by CK1δ/ε (Varelas et al., 2010). Potentially, TAZ could compete for DVL2 binding with various additional DVL-interacting proteins. Therefore, many DVL-controlled processes could be affected by misregulation of TAZ expression/localization. Given that the PDZ domain of DVL has already been reported to mediate crosstalk between the canonical Wnt pathway and redox or TGFβ signaling (Gao and Chen, 2010), one wonders how many pathways could potentially be influenced by accumulation of TAZ in the cytoplasm. Consequently, the findings by Varelas et al. (2010) imply that any alterations in Hippo signaling could not only affect canonical Wnt signaling, but also other essential signal transduction cascades. As we adjust to these novel crosstalk concepts of signaling cascades, many studies on Wnt and Hippo signaling might need to be reanalyzed and expanded in this broader context.

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