YAP and TAZ in Lung Development: The Timing Is Important

The Hippo signaling pathway was first whimsically named (as big as a hippo) based on its loss-of-function screen phenotype in *Drosophila* larvae (1). In other words, when Hippo is missing or its downstream kinase cascade is not working, *Drosophila* larvae grow to be too big and some even grow tumors. Thus, it may be deduced that Hippo has important tissue growth–suppression functions that limit the eventual size of tissues and organs, including the induction of apoptosis, as well important tumor-suppression functions.

The Hippo signaling pathway consists of a kinase cascade containing YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif) (1). YAP and TAZ are also key evolutionarily conserved downstream components of the Hippo signaling kinase pathway that mediate Hippo-like functions. Hippo, YAP, and TAZ thus all have numerous important growth-modulating functions in the lung, including such processes as early airway branching morphogenesis, epithelial lineage differentiation, cellular transition to air breathing, carcinogenesis and cancer progression, injury repair, and tissue regeneration (2–10). Mechanotransduction, sensing, and integration of the cell's mechanical environment are also now recognized as important functions of the Hippo YAP and TAZ signaling genes (11).

In this issue of the *Journal*, Isago and colleagues (pp. 256–266) report that sequential timing of YAP and TAZ inactivation has functional consequences for normal lung development (12). They discovered this by making inducible epithelium-specific YAP or TAZ knockout mice using an epithelial-specific SP-C-Cre driver line to test the respective roles of timed YAP and TAZ knockouts in lung development. YAP deficiency induced early on caused severe branching morphogenesis defects in the mouse lung. However, later TAZ deficiency produced an alveolar simplification phenotype during postnatal alveogenesis that progressed into an emphysema-like phenotype in adult mice. They also confirmed that YAP and TAZ function in complex signaling networks that impinge on other factors and signal transduction processes known to be important in lung development, including Sonic Hedgehog, FGF, Wnt, β-catenin, and TGF-β signaling.

When the Hippo kinase cascade is inactive, YAP and TAZ are unphosphorylated and thus preferentially stay within the nucleus, where they are available to compete with transcriptional effectors such as VGLL4 (vestigial-like family member 4) for binding to TEAD (TEA domain transcription factor). An interaction among YAP, TAZ, and VGLL4 thus facilitates TEAD DNA binding so that TEAD can then mediate the transcriptional activation of many downstream genes involved in suppression of tissue growth and induction of apoptosis (1).

On the other hand, TAO (thousand-and-one amino acid) serine threonine kinases phosphorylate and activate MST1/2 (mammalian serine/threonine kinases 1/2), which are the mammalian functional orthologs of *Drosophila* Hippo kinase (1). MST1/2 in turn phosphorylate LATS1/2 (large tumor suppressor kinases 1/2), a reaction that is facilitated by binding with the scaffold proteins SAV1 (Salvador family WW domain containing protein 1), MOB1A/B (mammalian homolog of yeast kinase activator MOB gene), and NF2 (neurofibromatosis type 2 gene). The core of this pathway is thus composed of a kinase cascade wherein MST2 and MST1, in a complex with the regulatory proteins SAV1, MOB, and NRF2, phosphorylate and activate LATS1/2, which in turn are in a complex with their regulatory protein MOB1. Phosphorylation of YAP1 by LATS2 then inhibits YAP translocation into the nucleus, preventing it from being available to bind TEAD and thus downregulating the TEAD-mediated transcriptional induction of genes that are important for suppression of cell proliferation, cell migration, and induction of apoptosis. Thus, the activated Hippo signaling pathway plays a pivotal role in negatively controlling cell growth and thereby limiting organ size and tumor suppression. Hence, the balance between activation and inactivation of Hippo, YAP, and TAZ is a key factor in maintaining tissue homeostasis, especially with regard to tissue and tumor growth.

The temporospatial clock timing mechanisms that in turn govern Hippo function via YAP and TAZ expression and function are as yet unknown. Nor is it known precisely how YAP and TAZ functionally interact with the growing list of molecules that control lung development, injury, repair, regeneration, and carcinogenesis. Therefore, the control and timing of YAP and TAZ relative expression, translation, abundance, activity, and nuclear translocation become even more important to explore. The timing of sequential events in lung development has previously been linked with various pathways, including clock genes, smooth muscle contractions, calcium waves, cycles of intraluminal pressure, and endocrine events such as cortisol secretion, to name but a few. It will be interesting to see in the future whether any of these pathways—or indeed other novel ones—predominate in influencing the Hippo YAP/TAZ pathway. Moreover, because YAP and TAZ have become potential therapeutic targets for growth suppression in lung and other cancers, molecular probes have been generated that can target and inhibit them and their respective kinases. It will be interesting to see whether any of these potential YAP- and TAZ-targeting cancer drugs can be repositioned to correct defects involving hypoplastic lung development or alveolarization. However, the potential for untoward side effects from injudicious pharmaceutical dysregulation of the Hippo YAP/TAZ pathway is obvious. Moreover, as the current paper shows, temporal- and spatial-specific modulation of Hippo YAP and TAZ will be another interesting challenge. In addition, inhibiting or eliminating YAP and TAZ functions altogether may have untoward consequences

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that could limit tissue growth and differentiation in the developing lung, and could potentially cause involution of alveoli in adult lungs. On the other hand, because YAP and TAZ also play key roles in cancer suppression, inhibiting them may stimulate the emergence and progression of lung cancers.

It is interesting that the fundamentally important genes in the Hippo pathway, YAP and TAZ, exert markedly different effects depending on the timing of their functional expression and activation during lung development. What controls this timing and whether it presents a tractable therapeutic window of opportunity remains an open question. ■

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