

Lkb1 takes different paths to morphogenesis

Study uses chemical genetics and embryonic explants to reveal kinase's tissue-specific functions.

FOCAL POINT

The protein kinase Lkb1 is a tumor suppressor that controls many different cellular processes. Determining which of Lkb1's many functions are most important during development and disease has proven difficult; the complex phenotypes of Lkb1-knockout mice provide little mechanistic insight, and the physiological relevance of Lkb1-deficient tissue culture cells is unclear. Lo et al. bridge the gap between animal models and cell culture by acutely inhibiting Lkb1 in embryonic tissue explants, revealing that the kinase regulates distinct pathways in different organs (1).

Lkb1 is best known for its role in regulating cellular energy levels and promoting cell polarization, though the kinase has also been implicated in cell adhesion and proliferation (2). Mutations in Lkb1 cause the multi-organ human disease Peutz-Jeghers syndrome (3, 4), and, says senior author Ira Mellman from Genentech in San Francisco, California, "Lkb1 is one of the most common tumor suppressor genes mutated in lung and, to a lesser extent, pancreatic cancer."

Though conditional knockout mice have confirmed the importance of Lkb1 for both normal development and oncogenesis, how the protein functions in vivo is unclear, in part because gene knockouts permanently inactivate the entire protein. "We needed to have a way of acutely and reversibly inactivating Lkb1's kinase activity to complement and extend the conventional knockout

approach," Mellman explains. Mellman and colleagues, led by Bryan Lo and Geraldine Strasser, therefore generated mice expressing a mutant version of Lkb1 that retains its kinase activity but is susceptible to inhibition by a bulky ATP analog called 1NMPP1 (5). In addition, to allow better imaging and biochemical analyses of the effects of Lkb1 inhibition, Lo et al. developed improved methods of culturing embryonic organs explanted from the Lkb1-mutant mice. "So we could reproduce interesting phenotypes that could be turned on and off with the inhibitor and were amenable to study," Mellman says.

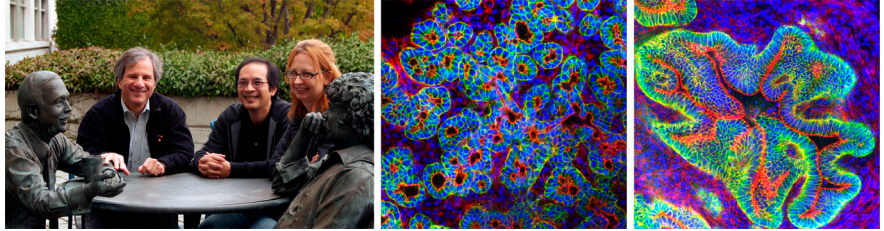


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A team of researchers led by Ira Mellman, Bryan Lo, and Geraldine Strasser (pictured, left to right, with statues of Genentech founders Bob Swanson and Herb Boyer) examine how the kinase Lkb1 contributes to tissue morphogenesis and the early stages of cancer development. The researchers generated mice expressing a mutant version of Lkb1 that can be reversibly inhibited by the ATP analog 1NMPP1. By culturing organs from these mice, Lo et al. reveal that Lkb1 regulates branching morphogenesis in the lung via an AMPK-dependent pathway, whereas, in the pancreas, Lkb1 inhibition induces the formation of epithelial cysts and precancerous lesions independently of AMPK. In both tissues, loss of Lkb1 activity fails to disrupt cell polarity. Pancreatic lesions induced by Lkb1 inhibition (right) show the same pattern of apical actin (red) and basolateral EpCAM (green) as the acini from a control pancreas (center).

Lo et al. focused on ex vivo cultures of embryonic lung and pancreatic tissues (1). Inhibiting Lkb1 disrupted branching morphogenesis in the lung epithelium. In the pancreas, on the other hand, morphogenesis at first proceeded normally in the presence of 1NMPP1, but, at later stages of development, Lkb1 inhibition induced the formation of numerous pancreatic cysts. Surprisingly, however, the individual cells in both tissues remained polarized, suggesting that Lkb1's

kinase activity isn't required to polarize lung and pancreatic cells and that the morphogenetic defects induced in these tissues didn't arise from a loss of polarity.

To determine what might underlie these developmental defects, Lo et al. investi-

gated Lkb1's best known substrate, AMP kinase (AMPK). Lkb1 phosphorylates and activates AMPK, and AMPK's activity was reduced in embryonic tissues upon Lkb1 inhibition. An allosteric activator of AMPK rescued branching morphogenesis in lung explants lacking Lkb1 activity but was unable to suppress the formation of pancreatic cysts. "So the downstream effectors of Lkb1 that are important for morphogenesis in these two tissues seem to be different," Mellman says. "Just because you find one mechanism in one tissue, it doesn't mean it's going to be applicable to something else."

It remains to be seen how Lkb1 regulates the development of the lung and pancreas, but Lo et al.'s ex vivo approach has also opened the door to understanding how the loss of Lkb1 activity contributes to oncogenesis. The pancreatic cysts induced by Lkb1 inhibition eventually collapse into structures that closely resemble pancreatic intraepithelial neoplasias (PanINs), disorganized tissue lesions that characterize the early stages of pancreatic cancer.

"The loss of a single tumor suppressor creates a morphology that is highly reminiscent of what you might expect to see if you expressed activating oncogenes such as K-Ras," Mellman says. His group is now studying the effects of other tumor suppressors and oncogenes using the same ex vivo strategy in both pancreas and lung and investigating whether the lesions produced by inhibiting Lkb1 in pancreatic cultures also show the same transcriptional profile as PanINs. "If they are similar," Mellman continues, "it gives us a way to look at the very earliest stages of oncogenesis under controlled conditions."

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2. Ollila, S., and T.P. Mäkelä. 2011. *J. Mol. Cell. Biol.* 3:330–340.
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4. Jenne, D.E., et al. 1998. *Nat. Genet.* 18:38–43.
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