Two proteins, one model organism: On the functional characterization of Lkb1 and Ring1b in zebrafish
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Germline mutations in the serine-threonine kinase LKB1 cause Peutz-Jeghers syndrome (PJS). PJS is a dominantly inherited disorder that causes polyps in the gastrointestinal tract, pigmentation of mucous membranes and an increased risk for sporadic cancers. At the molecular level, LKB1 functions as kinase, a protein that regulates the function of other proteins by the addition of a phosphate group (phosphorylation). LKB1 is the kinase for AMP-activated protein kinase (AMPK) as well as 12 AMPK-related kinases. LKB1 is, through phosphorylation of these proteins, involved in a variety of cellular processes, including metabolism and cell polarity.

Cell polarity is a term used to describe the spatial differences of a cell in form, structure and function. The role of LKB1 in regulating cell polarity is complex and depending on the organ, organism and even energetic state of the cell. However, LKB1 function is in general often required for the induction and/or maintenance of cell polarity. More is known about the defects in cell polarity due to loss of LKB1 function in lower organisms, such as the roundworm *Caenorhabditis elegans* and the fruit fly (*Drosophila melanogaster*), than in vertebrates. Although it is clear that LKB1 regulates polarity of neuronal cells in vertebrates, it remains to be determined whether LKB1 is generally involved in the regulation of cell polarity of non-neural tissues in vivo. It is therefore also not clear whether a defect in cell polarity is directly causing the formation of polyps in PJS patients.

The other well-established function of LKB1 is the regulation of energy homeostasis via activation of AMPK, a protein which is considered as the “energy sensor” of the cell. Energy deprivation causes a reduction of adenosine triphosphate (ATP), the universal energy carrier of cells. To restore the energy balance, ATP is converted to adenosine monophosphate in order to free the energy locked in the energy-rich phosphate bond. As consequence, AMP can now bind to AMPK because of the increase in AMP:ATP ratio. For complete activation, AMPK must also be phosphorylated. Although several kinases can phosphorylate AMPK, LKB1 is by far the most physiologically relevant kinase during energetic stress. Activation of AMPK then ensures that energy-consuming anabolic processes are inhibited and energy-generating catabolic processes become activated, to further restore the energy balance.

To gain more insight into how LKB1 regulates energy metabolism and cell polarity in vertebrates, we examined the role of Lkb1 in zebrafish. We were able to investigate the consequence of loss of Lkb1 function in the zebrafish as mutations in zebrafish *lkb1* were identified previously. We showed that Lkb1 deficiency does not cause visible morphological defects during embryonic development in the zebrafish. In more detail, we examined whether Lkb1 deficiency results in polarization defects of the intestinal epithelium. Our research indicated that this was not the case. However, we did reveal a metabolic defect caused by loss of Lkb1. At day 5 post fertilization, the yolk is absorbed and zebrafish larvae become dependent on external energy sources. We observed that lkb1 mutants did not eat and died within 3 days, at day 7 or 8 post fertilization. We showed lkb1 mutants rapidly exhausted their energy resources and failed to down-regulate their metabolic rate. 7 dpf lkb1 mutants looked phenotypically similar to 11 days old wild-type larvae that were food-deprived for 6 days: the larvae were emaciated, obtained a “dark” liver as well as a flattened epithelium without visible villi. This “starvation” phenotype of lkb1 mutants could be suppressed
by attenuation of metabolism through both genetic and pharmacological ways, suggesting that severe energetic stress is causing the \textit{lkb1} “starvation” phenotype. In summary, our research has shown that loss of Lkb1 function in zebrafish does not lead to polarity defects in the intestinal epithelium, but that Lkb1 function is essential for energy metabolism control during energetic stress in vivo.