

THE DEVELOPMENTAL ROLES OF ONCOGENES AND TUMOR SUPPRESSORS IN ZEBRAFISH

Our research focuses on understanding how vertebrates sense and respond to energetic stress, using zebrafish as our central experimental model. We aim to provide insights into how tumors adapt their metabolism to survive, proliferate and grow under nutrient and oxygen-poor conditions. We are following two main lines of research:

Analysis of energy metabolism and hypoxia during development and cancer

During development of an organism, mechanisms that sense nutrient availability are intimately linked with growth control pathways in order to coordinate energy conditions with organ growth and tissue homeostasis. Tumor cells employ several of the same mechanisms that coordinate nutrient availability with growth during development to ensure tumor cell proliferation. To improve cancer therapy, we need a better understanding of the molecular pathways that link energy metabolism with growth control during development and how are those affected in cancer.

Zebrafish (*Danio rerio*) is an ideal system to dissect molecular mechanisms and pathways, largely owing to the rapid development and optical clarity of the embryos. Furthermore, the large progeny size (females typically lay 200 eggs at a time), and availability of readily accessible transparent embryos combine to render the zebrafish an excellent model for high-throughput genetic and chemical screens in the intact organism. The major metabolic pathways are very well conserved between humans and zebrafish.

Mutations in the serine-threonine kinase LKB1 in humans lead to a gastrointestinal polyposis disorder with highly increased predisposition to cancer. Recently, somatic mutations in LKB1 have been found in about 30% of lung carcinomas as well as in endometrial cancer. LKB1 activates AMP-activated kinase (AMPK) the “energy switch” of the cell and that leads to growth suppression through several pathways including inhibition of the mTOR pathway. Since mouse models of *lkb1* deficiency are embryonic lethal, the role of energy metabolism during development has not been explored.

To study control of metabolism during development, we generated and characterized *lkb1*-deficient zebrafish. Importantly, the zebrafish *lkb1* mutants are embryonic viable -unlike their mouse counterparts- and exhibit deregulated metabolism.

We demonstrated that zebrafish LKB1 is required for AMPK activation and found that *lkb1* mutants are unable to sense and respond to energetic stress. *Lkb1* mutant zebrafish display a fast metabolic rate and exhaust their energy reserves prematurely. They exhibit hallmarks of premature starvation such as abnormal lipid accumulation in the liver (figure 7). We showed that attenuation of metabolic rate in *lkb1* mutants, by application of the TOR inhibitor rapamycin, suppresses key aspects of the *lkb1* phenotype.

We are currently exploring the connection between LKB1, energy metabolism and hypoxia. This investigation will lead to a better understanding of the adaptation of metabolic processes in order to meet energy demand that tumor cells employ in order to cope with decreasing nutrient and oxygen supply within the tumor. We aim to gain insight into these processes at the organism level during normal development and during cancer formation.

Analysis of the developmental roles of the Polycomb group proteins (collaboration with Maarten van Lohuizen)

Polycomb group (PcG) protein complexes, which function in the epigenetic regulation of gene expression, control numerous developmental processes. Epigenetic silencing mediated by PcG is implicated in stem-cell fate maintenance and cancer. The PcG member *Ring1b* is an E3 ubiquitin ligase that ubiquitinates histone H2A. This mark correlates with the repression of gene expression of PcG target genes. Targeted inactivation of *Ring1b* in mice leads to very early lethality precluding analysis of the role of *Ring1b* in embryonic development. We have recently succeeded in generating a stable mutant in *Ring1b* by using Zinc Finger Endonuclease technology. We have established that *Ring1b* protein levels and ubiquitination of H2A are dramatically reduced in the mutants. *Ring1b* mutant embryos lack fins and all jaw cartilage elements. We are currently characterizing the mechanism underlying these specific defects.



Group leader Anna-Pavlina Haramis

Anna-Pavlina Haramis PhD Group Leader

Yme van der Velden MSc PhD student

Liqin (Bruce) Wang Research assistant

Publication

Brennan C, Dosch R, Haramis AP, Luckenbach T, Martinez-Morales JR, Moro E, Polok B, Ramesh TM, Russell C, Argenton F, Strähle U. Report of the European zebrafish principal investigator meeting in Padua, Italy, March 18-22 2010. *Zebrafish*. 2010;7:305-10

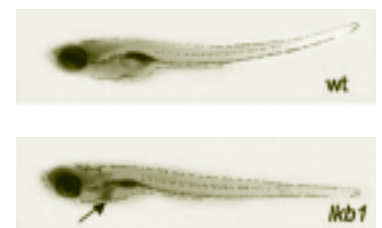


Figure 7: Zebrafish larvae at day 7 post-fertilization stained with Oil Red O, a red dye that binds lipids. No lipids are detected in wt larvae; abnormal lipid accumulation in the liver of *lkb1* mutants (arrow).