**Institutionen för mikrobiologi, tumör- och cellbiologi**

# The zebrafish as a model to elucidate human diseases and development

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av

**Samantha Lin Chiou LEE**

*Huvudhandledare:*
Professor Birgitte Lane
Karolinska Institutet
Institutionen för mikrobiologi, tumör- och cellbiologi
Sweden
Institute of Medical Biology,
A*STAR, Singapore

*Bihandledare:*
Professor Philip Ingham
Institute of Molecular and Cell Biology,
A*STAR, Singapore

*Fakultetsopponent:*
Professor Peter Currie
Monash University
Australian Regenerative Medicine Institute
Australia

*Betygsnämnd:*
Professor Lorenz Poellinger
Karolinska Institutet
Institutionen för cell- och molekylärbiologi
Sweden

Professor Sven Pettersson
Karolinska Institutet
Institutionen för mikrobiologi, tumör- och cellbiologi
Sweden

Docent Christoph Winkler
National University of Singapore
Department of Biological Sciences
Singapore

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# ABSTRACT

This thesis explores the zebrafish as a model organism to illuminate our understanding of processes that orchestrate the progression of cancer metastasis and myogenesis.

First, I describe the successful establishment of a cancer metastasis model in the zebrafish, specifically to study intravasation - one of the earliest steps in the metastatic cascade. Fluorescently labeled tumour cell are transplanted into pervitelline space of 2 days post fertilisation (dpf) zebrafish embryos before they are exposed to normoxic or hypoxic conditions, allowing us to study the effect of hypoxia on tumour-induced angiogenesis and metastasis. Hypoxia elicited an enhanced angiogenic response and neovascularisation to the transplanted tumour and escalated the extent of metastasis in the living zebrafish embryo. Loss of function experiments such as vascular endothelial growth factor (VEGF) blockade using a clinically available drug – Sunitinib or VEGF morpholino knockdown attenuated tumour-induced angiogenesis and metastasis. The *in vivo* metastasis assay in zebrafish delivers numerous unique advantages over conventional *in vitro* cell-based or biochemical chemotaxis assays. The transparent embryo facilitates the tracking of the entire intravasation process in real time within a living organism, allowing us to explore the dynamic interplay of tumour cells and the environmental cues that drive metastasis. This model can be further employed to discriminate between tumour cells of different metastatic potential and to identify novel factors that present as impetus for the earliest step of the metastatic cascade.

Next, I demonstrate the analysis of one class of motility mutants originally identified in the 1996 Tübingen genetic screen that potentially serve as models for human myopathies and dystrophies. Two of these mutants have previously been cloned and shown to encode proteins involved in muscle fibre attachment whilst a third has been found to encode the molecular chaperone Heat shock protein (HSP) 90. I describe the phenotypic and molecular characterization of another of these zebrafish motility mutants, *frozen (fro)*. I present evidence that the $fro^{t027c}$ mutation disrupts the locus encoding the autophagy pathway component Atg10. This analysis implicates Atg10 in the assembly of both skeletal and cardiac muscle fibers suggesting a previously uncharacterized role for the autophagy pathway in this process.

Together, my findings illustrate the utility of the zebrafish as a model organism that complements established mammalian and invertebrate models. The fecundity and amenability of the zebrafish to genetic manipulation together with the rapid development and translucency of its embryos combine to provide a powerful system with which to unravel and inform the underlying mechanisms that govern fundamental biological processes and shed light on our understanding of human development and diseases.


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