University of Basel

DBM Basel
Department of Biomedicine

TWO OPEN MASTER THESIS PROJECTS @ DEVELOPMENTAL GENETICS GROUP – FROM MESENCHYMAL PROGENITORS TO SKELETAL TISSUES: COMBINING GENETIC AND ORGANOID MODELS TO STUDY CARTILAGE AND BONE DEVELOPMENT.

Understanding how organs arise from undifferentiated progenitor cells is a fundamental question in developmental biology, with far-reaching implications for tissue repair and regeneration. We address this question by investigating how mesenchymal progenitors differentiate into cartilage and bone structures with distinct identities during limb formation — a process that exemplifies the complex orchestration of organ development. To gain mechanistic insight into this process, we combine in vivo genetic approaches in mouse embryos with in vitro limb organoids derived from mouse limb bud progenitor cells.

We offer two complementary ~9 month Master's research projects, each examining chondrogenic and osteogenic differentiation through a distinct experimental framework: **Project 1** addressing the question using *mouse embryology* while **Project 2** approaches it through *organoid-based cell culture*. These projects will give the involved students a unique opportunity to explore the strengths of mouse embryonic and organoid models in discovery research.

Both projects provide the opportunity to explore fundamental mechanisms of skeletal development using state-of-the-art tools in either mouse embryology or organoid-based systems.

Project 1: Genetic ablation of limb mesenchymal progenitors and its consequences on cartilage and bone development

The team has recently identified previously uncharacterised populations of limb mesenchymal progenitor cells, each defined by distinct molecular signatures uncovered through single-cell RNA sequencing. These progenitor populations appear to have complementary and potentially non-redundant roles in digit development. The goal of this project is to functionally assess these roles by selectively ablating the descendants of specific progenitor populations during embryonic limb development.

As part of ongoing research in the lab, we are mapping the lineage trajectories of these progenitor cells to better understand their contributions to limb development. This project will build on that foundation by using a genetic ablation strategy in *in vivo* mouse models. Specifically, we will employ the diphtheria toxin A (DTA) system, in which a Cre-dependent expression of the DTA gene leads to targeted cell death. By crossing mouse lines that express Cre recombinase in specific progenitor lineages with a conditional DTA allele, defined cell populations will be selectively eliminated during development.

The project will address the following key questions:

1. What are the consequences of ablating specific progenitor lineages on skeletal development?

Embryos will be analysed using classical skeletal staining techniques to assess digit morphology and identify any cartilage and bone defects.

2. To what extent can the developing limb compensate for the loss of an entire progenitor lineage?

We have previously shown that limb buds exhibit extraordinary developmental robustness. This question will address whether other progenitor populations can compensate for the ablated lineage and whether the targeted cells fully account for a specific fate.

To address these questions, the student will use a combination of genetic lineage tracing and molecular analysis. Cell lineages will be marked using fluorescent reporters, and changes in gene and protein expression will be examined using state-of-the-art whole-mount HCR (hybridisation chain reaction) RNA in situ hybridisation and immunofluorescence microscopy. These methods allow spatially resolved analysis of developmental processes at cellular resolution.

This project is expected to provide fundamental insights into the functional contributions and potential plasticity of limb mesenchymal progenitors during digit development.

Project 2: Limb Organoids as a Platform to Study Developmental Programs and Skeletal Disorders

Limb organoids will be used to investigate the cellular and molecular mechanisms underlying key developmental and disease-related processes in the following three contexts:

The same analytical strategy will be applied across all conditions: organoids will be generated from mouse limb bud mesenchymal progenitor cells and molecular and cellular changes will be analysed at defined stages using whole-mount fluorescent detection of transcripts and proteins. Imaging will be performed using state-of-the-art confocal microscopy, providing high-resolution insight into spatial organisation and tissue architecture. This approach enables spatial and temporal tracking of chondrogenic (cartilage) and osteogenic (bone) tissue development, allowing direct comparisons between *in vitro* and *in vivo* differentiation dynamics.

1. Normal development:
Limb organoids will be derived from mouse limb bud progenitor cells and their progression into cartilage and bone development will be compared to *in utero* limb development. This will reveal similarities and potential differences between embryonic and in vitro organoid-based development.

2. Greig's Synpolydactyly syndrome:
This congenital disorder, caused by the Gli3 gene deficiency, leads to the formation of extra digits. While the molecular changes have been well-characterized in mouse embryos, the cellular mechanisms remain poorly understood. Limb organoids will be generated from *Gli3-deficient* mouse limb bud progenitor cells to explore how Gli3 loss alters chondrogenic and osteogenic to generate extra digits — insights not accessible from embryos analysis or human-derived samples

3. Fibrodysplasia Ossificans Progressiva (FOP):
FOP is a severe, incurable juvenile-onset disorder marked by progressive ectopic bone formation and caused by activating mutations in the TGFβ superfamily receptor ACVR1. Using the conditional *Acvr1*^{R206H} mouse model, we previously found that chondrogenesis is already altered during mouse embryonic development, yet ectopic ossifications does not occur prior to birth. To understand this discrepancy, we will compare the bone-forming potential of wildtype and *Acvr1*^{R206H} mutant mouse limb bud progenitor cells using the limb cartilage organoids. This approach will not only allow direct comparison of skeletal differentiation dynamics and help uncover early cellular mis-regulation that predisposes to pathological ossification but in the longer term, it could also help develop strategies for preventing abnormal bone formation in FOP.

Both projects require scientific and project-oriented planning and analysis skills, innovative and critical thinking the students will benefit from interactions with several group members. Presentation and scientific communication skills in English will be furthered as part of the weekly research seminars, journal clubs and writing of the master thesis.

Interested?
Drop us an email indicating your preferred project, along with your CV, to arrange an interview and discuss the project in more detail. We look forward to hearing from you!

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More information on the group can be found here: https://www.devgenbasel.com