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Project Titel: Lung Fibrosis Resolution: Mechanims and Therapeutics

Background: Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease characterized by excessive extracellular matrix deposition, irreversible destruction of alveolar architecture, and a median survival of only 3–4 years[1]. Although the currently approved antifibrotic therapies can slow disease progression, they do not halt or resolve fibrosis[2]. A major unmet clinical need is the development of therapies capable of promoting the resolution of established fibrosis. Evidence from our lab suggests that lipofibroblasts—a specialized fibroblast population that stores lipid droplets and supports alveolar epithelial regeneration—play a key role in lung repair and fibrosis resolution[3,4]. Cysteine cathepsins have been implicated in profibrotic signalling but also in adipocyte-related metabolic pathways[5,6], making them promising therapeutic targets to simultaneously suppress fibrogenesis and enhance the lipogenic phenotype of reparative lipofibroblasts. To exploit this, an inhalable formulation of the cysteine cathepsin inhibitor, aloxistatin, has been developed, providing improved pulmonary specificty together and representing a promising candidate for regenerative antifibrotic therapy.

Hypothesis and Objectives: We hypothesize that cathepsin inhibition could not only attenuates extracellular matrix (ECM) deposition and the profibrotic phenotype of lung fibroblasts but also promotes their differentiation towards a pro-regenerative lipofibroblast, thereby facilitating the resolution of established fibrosis. The aims of this PhD project are to 1) Determine the antifibrotic and pro-resolution effects of inhaled aloxistatin in relevant in vitro and preclinical models of pulmonary fibrosis; 2)Elucidate the molecular and cellular mechanisms by which aloxistatin modulates fibroblast activation, lipogenic differentiation, and tissue repair and 3) evaluate the therapeutic potential of aloxistatin in combination with current standard-of-care antifibrotic therapies to determine whether combination treatment provides additive or synergistic benefits.

Methodology: This project combines cutting-edge cell culture, ex vivo, and in vivo models. Students will study human lung fibroblasts and endothelial cells exposed to pro-fibrotic conditions and assess the effects of aloxistatin, alone or in combination with nintedanib. Using molecular (qPCR, Western blotting, immunofluorescence), functional (collagen contraction, barrier integrity, metabolic flux), and Omics (e.g RNA-seq) approaches, the project will uncover mechanisms driving fibrosis resolution. Key findings will be validated in precision-cut lung slices and preclinical models of pulmonary fibrosis, providing hands-on experience with advanced disease models, multi-omics, and translational drug discovery.

References:

1. Podolanczuk AJ et al. Idiopathic pulmonary fibrosis: state of the art for 2023. Eur Respir J. 2023.

2. Kou M, et al. Real-world safety and effectiveness of pirfenidone and nintedanib in the treatment of idiopathic pulmonary fibrosis: a systematic review and meta-analysis. Eur J Clin Pharmacol. 2024

3. Wojcik G, et al. Enhanced fatty acid availability rewires fibroblasts metabolism and decreases collagen deposition in lung fibrosis. Cell Mol Life Sci. 2026 Jun 1.

4. Kheirollahi V, et al. Metformin induces lipogenic differentiation in myofibroblasts to reverse lung fibrosis. Nat Commun. 2019

5. Chen, M. et al. Cathepsin K Aggravates Pulmonary Fibrosis Through Promoting Fibroblast Glutamine Metabolism and Collagen Synthesis. Adv. Sci. 12, e13017 (2025).

6. Mizunoe, Y. et al. Cathepsin B overexpression induces degradation of perilipin 1 to cause lipid metabolism dysfunction in adipocytes. Sci. Rep. 10, 634 (2020).

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Project Title: Defining neuroanatomical and endocrine alterations in a mouse model of *CACNA1D* channelopathy using quantitative 3D tissue imaging

Background: Activity-enhancing de novo missense variants in *CACNA1D*, the gene encoding the pore-forming α1 subunit of Cav1.3 voltage-gated Ca^{2+} channels, cause a rare neurodevelopmental and endocrine disorder[1,2]. Affected individuals present with a broad clinical spectrum that includes developmental delay, autism spectrum disorder (ASD), epilepsy, hypotonia and endocrine abnormalities such as hypoglycemia and hyperaldosteronism[2]. Although altered Ca^{2+} signaling is thought to drive these clinical manifestations, the underlying structural and cellular changes in affected organs remain largely unknown. In addition, increasing evidence implicates neuroinflammatory processes and altered glial function in ASD and related neurodevelopmental disorders[4], suggesting that activity-enhancing *CACNA1D* variants may also induce changes in microglial and astrocyte abundance, morphology and activation.

We established a novel Cav1.3 knock-in mouse model of the ASD-associated *CACNA1D* p.A749G variant, which recapitulates key aspects of the human disease[3], including behavioral abnormalities, endocrine dysfunction and altered neuronal and endocrine cell activity. However, **whether enhanced Cav1.3 activity also induces structural remodeling of affected organs remains unknown**.

Recent advances in tissue clearing and light-sheet fluorescence microscopy now enable quantitative three-dimensional imaging of intact organs, allowing us to investigate tissue architecture, cellular composition and spatial organization in their native anatomical context.

Hypothesis and Objectives: We hypothesize that enhanced Cav1.3 channel activity induces structural and/or cellular remodeling of the brain and adrenal gland that contributes to the neurological and endocrine manifestations of *CACNA1D*-related disorders.

The **objectives** of this PhD project are to:

- establish and optimize quantitative 3D tissue imaging workflows for murine brain and adrenal gland tissue;
- identify disease-associated structural alterations in the brain and adrenal gland associated with enhanced Cav1.3 activity;
- investigate how these alterations differ between sexes and change during disease progression;
- generate novel insights into disease mechanisms that will support the future development of precision medicine approaches for *CACNA1D* channelopathy while establishing a broadly applicable imaging platform for future research.

Methodology: **The project combines method development with translational disease research.**

Building on successful pilot studies performed in our laboratory, the PhD candidate will further develop and optimize workflows for tissue clearing, multiplex immunolabeling and quantitative 3D imaging of murine brain and adrenal gland tissue.

The project will involve systematic comparison of tissue clearing strategies, commercial tissue-clearing kits and multiplex immunolabeling protocols to establish robust workflows for different tissues and cellular markers. The candidate will learn tissue dissection and preparation, perform high-resolution light-sheet fluorescence microscopy in collaboration with the local Bioimaging Core Facility, and develop quantitative image analysis pipelines (e.g. using Imaris) to extract morphometric and cellular information from large three-dimensional imaging datasets.

The optimized workflows will subsequently be applied to compare wildtype and Cav1.3 p.A749G mutant mice across genotypes, sexes and developmental stages (young, adult and aged animals). Imaging findings will be integrated with complementary molecular, electrophysiological and physiological data generated within the research group to provide a comprehensive understanding of disease mechanisms and identify structural correlates of *CACNA1D* channelopathy.

References:

1. Scholl, U.I., et al., *Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism.* Nat Genet, 2013. **45**(9): p. 1050-4.

2. Ortner, N.J., et al., *De novo CACNA1D Ca(2+) channelopathies: clinical phenotypes and molecular mechanism.* Pflugers Arch, 2020. **472**(7): p. 755-773.

3. Ortner, N.J., et al., *The human channel gating-modifying A749G CACNA1D (Cav1.3) variant induces a neurodevelopmental syndrome-like phenotype in mice.* JCI Insight, 2023. **8**(20).

4. Hughes, H.K., R.J. Moreno, and P. Ashwood, *Innate immune dysfunction and neuroinflammation in autism spectrum disorder (ASD).* Brain Behav Immun, 2023. **108**: p. 245-254.

Peter Ulz, PhD

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Project Titel:

Integrating DNA Methylation and Nucleosome Positioning for Enhanced Liquid Biopsy Analyses

Background:

Liquid biopsy based on the analysis of cell-free DNA (cfDNA) in plasma has emerged as a powerful non-invasive tool for cancer diagnostics, monitoring, and molecular profiling. Previously we've established inference of gene expression and transcription factor (TF) activity from nucleosome positioning patterns embedded in cfDNA fragmentation profiles [1, 2]. In parallel, DNA methylation has emerged as a complementary and highly informative epigenetic layer in cfDNA: tissue- and cancer-specific CpG methylation patterns enable the deconvolution of cfDNA tissue-of-origin, early cancer detection, and molecular subtyping [3, 4]. Despite the clear biological interplay between CpG methylation, chromatin accessibility, and nucleosome positioning these two layers of cfDNA information have to date been analyzed largely in isolation.

Hypothesis and Objectives:

We hypothesize that integrating genome-wide DNA methylation information with nucleosome positioning signals derived from cfDNA fragmentation profiles will synergistically improve the accuracy and resolution of functional liquid biopsy analyses, including inference of gene expression states, TF activity, and tissue-of-origin deconvolution. The objectives of this project are: (i) to establish robust, validated methylation analysis workflows at the Institute of Human Genetics using bisulfite-based and enzymatic cfDNA methylation sequencing; (ii) to develop computational frameworks that jointly model methylation and nucleosome positioning features from matched plasma samples; and (iii) to apply these integrated models to patient cohorts for enhanced prediction of cancer-specific gene expression programs, TF activation states, and tumor tissue-of-origin.

Methodology:

Methylation assay establishment. In the first phase, genome-wide DNA methylation profiling of cfDNA will be established at the Institute of Human Genetics using whole-genome bisulfite sequencing (WGBS). Using existing low-pass whole-genome sequencing (WGS) data as well as public data from patient cohorts, nucleosome positioning profiles at transcription start sites and TF binding sites, building on prior published methodologies [1, 2] will be established. Moreover, we are currently generating genome-wide methylation data as a pilot dataset using Illuminas 5-base sequencing technology that can help guide method establishment.

Integrative computational modeling. Machine learning models — including regularized regression, gradient boosting, and deep learning architectures — will be trained to predict gene expression levels,

TF activity scores, and tissue-of-origin fractions using either single-modality or jointly encoded methylation plus nucleosome positioning features.

Clinical cohorts and validation. Existing plasma samples from patients with solid tumors (prostate, breast, colorectal cancer) already present at the Institute will serve as the primary discovery cohort. For each patient, longitudinal plasma samples will be used where available to assess dynamic changes in integrated methylation and nucleosome profiles during treatment. Clinically annotated outcome data will be used to evaluate the prognostic and predictive value of integrated liquid biopsy signatures.

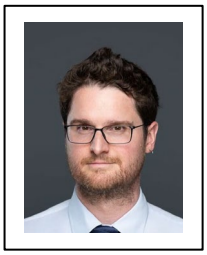
References:

[1] Ulz P, Thallinger GG, Auer M, et al. Inferring expressed genes by whole-genome sequencing of plasma DNA. Nat Genet. 2016;48(10):1273–1278. doi:10.1038/ng.3648

[2] Ulz P, Perakis S, Zhou Q, et al. Inference of transcription factor binding from cell-free DNA enables tumor subtype prediction and early detection. Nat Commun. 2019;10:4666. doi:10.1038/s41467-019-12714-4

[3] Moss J, Magenheim J, Neiman D, et al. Comprehensive human cell-type methylation atlas reveals origins of circulating cell-free DNA in health and disease. Nat Commun. 2018;9:5068. doi:10.1038/s41467-018-07466-6

[4] Loyfer N, Magenheim J, Peretz A, et al. A DNA methylation atlas of normal human cell types. Nature. 2023;613:355–364. doi:10.1038/s41586-022-05580-6



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Project Titel: Exploring how the interaction between immune and non-immune cells in the joint underscores the pathogenesis of osteoarthritis

Background:

Osteoarthritis (OA) was thought to exclusively be a degenerative disease of the joints. Dysfunctional chondrocytes within the OA cartilage have a role in the pathogenesis of the disease, driven by the disequilibrium between their anabolic and catabolic functions. Inflammation, however, is now acknowledged as a key culprit of OA pathogenesis. Immune cells infiltrating the OA joint secrete effector molecules that directly cause tissue damage, as well as influencing chondrocytes to promote cartilage damage.

Hypothesis and Objectives:

This PhD project aims at dissecting the complexity of the immune infiltrate within the OA joint, as well as investigating how immune cells interact with, and affect the function of, fibroblast-like synoviocytes (FLS) in the synovium, and chondrocytes in the cartilage.

Methodology:

A. To explore the functional complexity of the immune infiltrate in the OA joint. Using spectral flow cytometry, the PhD candidate will assess the *function* of immune and non-immune cells isolated from OA synovium and cartilage. Precisely, we will functionally characterize: 1. T cells and NK cells; 2. B cells; 3. Myeloid cells and FLS; and 4. Chondrocytes and their subsets.

B. To assess in vivo the interaction of immune and non-immune cells in the OA joint. To obtain spatial resolution of the immune response in the OA joint, we will evaluate physical interactions between immune cells and: 1. FLS in the synovium; 2. Chondrocytes in the cartilage. Using multiplex immunofluorescence imaging, we will assess which immune cells interact with FLS and with chondrocytes in the OA joint.

C. To test how the interaction between immune and non-immune cells in the OA joint affects their functions. The results of aim B will guide the choice of immune cells to use in *in vitro* co-cultures with FLS and/or chondrocytes, to mimic what happens in the OA joint. Precisely, we will assess how immune cells and their effector molecules impinge on the functional characteristics of FLS and chondrocytes.

References:

Moulin M, The role of the immune system in osteoarthritis: mechanisms, challenges and future directions. *Nat Rev Rheumatol* 2025.

Linda Waldherr, PhD

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Project Titel: Deciphering the role of tumor-derived PGE_2 as a driver of cancer-associated cachexia

Background: Cancer-associated cachexia is a severe wasting syndrome characterized by involuntary body weight loss, skeletal muscle depletion, adipose tissue remodeling, systemic inflammation, reduced treatment tolerance, and poor survival. Despite its major clinical relevance, effective mechanism-based therapies remain limited. Current approaches mainly target downstream metabolic consequences, while the tumor-derived signals that initiate and maintain systemic wasting remain insufficiently understood (Ferrer et al., 2023).

This PhD project will investigate the cyclooxygenase-2/prostaglandin E_2 axis as a tumor-intrinsic inflammatory pathway driving cancer-associated cachexia. PGE_2 is a lipid mediator with established roles in tumor inflammation, myeloid immune-cell recruitment, immune suppression, angiogenesis, and tumor progression. Recently published data (Cross et al., 2026), and our own preliminary data indicate that cachexia-inducing tumor cell clones secrete higher levels of PGE_2 than matched non-cachexigenic control clones. In the CHX207 fibrosarcoma model, tumor-bearing mice develop body weight loss, muscle and adipose tissue wasting, systemic inflammation, neutrophilia, lymphocytopenia, and increased IL-6 levels. Importantly, local sustained COX-2 inhibition at the tumor site attenuates cachexia-associated tissue wasting and systemic inflammatory alterations. In addition to that, the tumor innervation and vagal blockade also seem to play major role in cancer cachexia (Garrett et al., 2025). It has been suggested that there is a link between PGE_2 in cancer cachexia, TRPV1+ vagal sensory neurons and changes in the tumor microenvironment. The mechanisms behind cancer cachexia onset and the interplay of tumor innervation and inflammation are yet unclear.

Hypothesis and Objectives:

The central hypothesis of this PhD project is that tumor-intrinsic COX-2-dependent PGE_2 production promotes a pro-cachectic inflammatory secretome, thereby driving systemic inflammation, immune remodeling, skeletal muscle wasting, and adipose tissue loss. The project will test whether genetic or pharmacological inhibition of COX-2/PGE_2 signaling can attenuate cachexia development and will define the

downstream tumor-host mechanisms involved. We will furthermore investigate the role or TRPV1+ vagal sensory nerves in our *in vivo* model and how blockage of the vagal sensory neurons affects the development of cancer cachexia, both *in vitro* and *in vivo*.

Methodology: The PhD candidate will receive training in mammalian cell culture, ELISA, Western blotting, qRT-PCR, multiplex cytokine assays, CRISPR/Cas9 genome editing, preclinical cachexia models (mouse), local drug delivery techniques (*in vitro* and *in vivo*), animal phenotyping, flow cytometry, histological analysis, data integration, scientific writing, and presentation of results at national and international conferences.

References:

Cross, M., Kotschi, S., Wu, W., Luciano-Mateo, F., Kwon, Y.-Y., Dantas, E., Niazi, T., Chen, S., Rashidfarrokhi, A., Pillai, R., Sanford, J., Kim, J., Hsiang, J., Gamallo-Lana, B., Mar, A.C., Hao, Y., Rajalingam, S., Huang, A., Shan, J., Issa, H.A., Gomez, M., Wang, A.R., Zhao, X., Janowitz, T., White, E., Liu, Y., Wong, K.-K., Segal, L.N., Hui, S., Goncalves, M.D., Froemke, R.C., Papagiannakopoulos, T., 2026. A dietary switch promotes sensory neuron–dependent cancer-associated cachexia. Science 393, 90–97. https://doi.org/10.1126/science.adz4196

Ferrer, M., Anthony, T.G., Ayres, J.S., Biffi, G., Brown, J.C., Caan, B.J., Feliciano, E.M.C., Coll, A.P., Dunne, R.F., Goncalves, M.D., Grethlein, J., Heymsfield, S.B., Hui, S., Jamal-Hanjani, M., Lam, J.M., Lewis, D.Y., McCandlish, D., Mustian, K.M., O'Rahilly, S., Perrimon, N., White, E.P., Janowitz, T., 2023. Cachexia: a systemic consequence of progressive, unresolved disease. Cell 186, 1824. https://doi.org/10.1016/j.cell.2023.03.028

Garrett, A., Darzi, N., Deshmukh, A., Rosenfeld, N., Goldman, O., Adler, L., Bab-Dinitz, E., Singer, O., Najafabadi, A.H., Wong, C.W., Bose, S., Randon, P.M., Bustamante, F., Larios, R., Brandis, A., Mehlman, T., Smaglo, B., Chang, P., Oliva, J., Haymaker, C., Nagawekar, L., Wu, S.R., Huang, Y., Shen, A., Vora, A., Padilla, J.F., Pfeffer, A., Sutherland, G., Starr, M., Zimmers, T., Zhu, Y., Morizio, J., Erez, A., Shen, X., 2025. Vagal blockade of the brain-liver axis deters cancer-associated cachexia. Cell 0. https://doi.org/10.1016/j.cell.2025.07.016