

CMMC Symposium 2026

September 28-30, 2026



Metabolic Control of Tissue Regeneration and Degeneration

Poster Abstract Book

Information

Welcome to Cologne



Welcome to the CMMC Symposium 2026

Dear Colleagues, Students and Friends

It is our great pleasure to welcome you to the CMMC Symposium 2026 in Molecular Medicine, entitled “Metabolic Control of Tissue Regeneration and Degeneration”. The symposium takes place September 28–30, 2026, at the Medical Campus of the University of Cologne in Cologne, Germany.

Recent advances in molecular medicine have highlighted the central role of cellular metabolism in regulating tissue regeneration and degeneration. Metabolic programs and their associated metabolites not only support energy production and biosynthesis but also regulate cell proliferation, differentiation, cellular reprogramming, and immune responses. Understanding the mechanistic links between these cellular processes and metabolism of the various building blocks of life is critical to addressing major challenges in human health and disease.

The symposium aims to foster interdisciplinary exchange between basic and clinical research, bringing together scientists from molecular biology, biochemistry, genetics, metabolism research, immunology, stem cell biology, and clinical medicine.

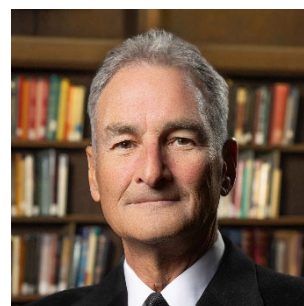
We are honored to welcome three distinguished external scientific coordinators and keynote speakers:



Elly Tanaka
IMBA, Vienna, AT



Axel Roers
Univ. Hospital Heidelberg, DE



Gerald I Shulman
Yale School of Medicine, US

Together with our external scientific coordinators, we have developed a wide-ranging and interdisciplinary program that brings together leading experts across these complementary fields. We sincerely thank them for their valuable scientific contributions and close collaboration.

We also extend our sincere appreciation to all invited speakers contributing to the six scientific sessions. We are grateful that they have accepted our invitation and will share their expertise, latest findings, and scientific perspectives with us.

We are particularly pleased to welcome students, early-career researchers, and junior group leaders, and we greatly value their active participation. With more than 60 poster presentations, the poster session is a central component of the symposium and provides a dynamic forum for presenting ongoing research, exchanging ideas, receiving valuable feedback, and establishing new scientific collaborations.

The scientific program is further complemented by a BioTech Exhibition and social events, offering additional opportunities for scientific exchange and networking.

We gratefully acknowledge the generous support of the Fritz Thyssen Foundation, our local supporters at the University of Cologne, the Cologne Convention Bureau – KölnTourismus, and our industry partners. Their support has been instrumental in making this symposium possible.

We look forward to sharing three days of stimulating science, lively discussion, and new collaborations with all of you in Cologne.

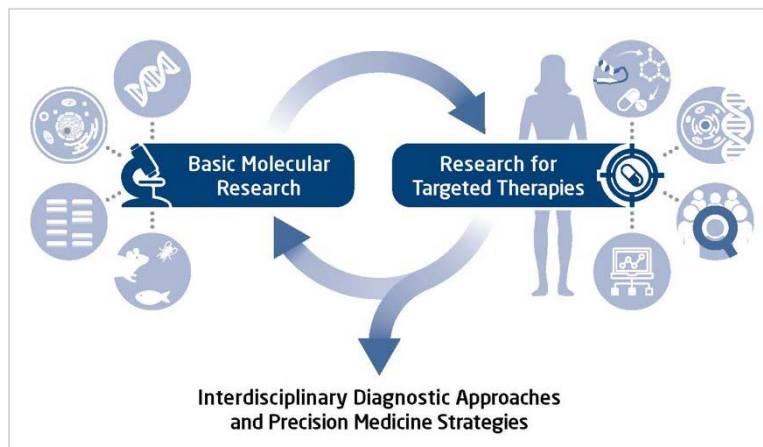
Sincerely,

Sabine Eming¹ - Guenter Schwarz² - Jens Brüning³ – The Cologne Scientific Organizers

¹ Dept. of Dermatology, Univ. Hospital Cologne - ² Inst. of Biochemistry, Univ. of Cologne - ³ MPI for Metabolism Research

The Center for Molecular Medicine Cologne (CMMC)

The Center for Molecular Medicine Cologne (CMMC) is an interdisciplinary biomedical research and education center at the University of Cologne, jointly supported by the Faculty of Medicine and the Faculty of Mathematics and Natural Sciences. Its mission is to advance the understanding of molecular and cellular mechanisms underlying human disease and to translate scientific discoveries into improved approaches to prevention, diagnosis, and treatment.



By bringing together basic scientists, clinician-scientists, and physicians, the CMMC connects fundamental discovery with clinically relevant research questions and provides a strong foundation for translational biomedical research. The center fosters scientific collaboration, supports emerging research fields and methodological innovation, and contributes to the continued development of Cologne's life sciences campus.

Research at the CMMC is organized into three main areas:

- Mechanisms of Tumor Development: Intrinsic and Extrinsic Control of Cell Proliferation and Tissue Invasion
- Principles of Immunity, Inflammation and Infection
- Molecular Mechanisms and Metabolic Control of Tissue Degeneration and Regeneration

Through strategically focused funding programs, the CMMC supports researchers and clinicians at different career stages, with a particular emphasis on early- and mid-career researchers and clinician-scientists establishing scientific independence. The CMMC community comprises more than 300 researchers, clinician-scientists, professional staff members, and students from nearly 26 countries.

Since its foundation, the CMMC has developed into an important platform for internationally visible research initiatives as well as successful DFG- and ERC-funded projects. Its scientific productivity and visibility are reflected in a strong publication record, with CMMC members publishing 253 peer-reviewed publications in 2025, including 204 original research articles and 49 reviews. The center's impact is also reflected in the appointment of CMMC members to professorships and other leadership positions in academic and non-academic research institutions, highlighting its role in fostering scientific careers and academic leadership. Since July 2026, Professor Sabine Eming has served as CMMC Chairperson, with Professor Günter Schwarz and Professor Jonathan Jantsch serving as Vice Chairs. For more information, please visit www.cmmc-uni-koeln.de.



About the CMMC Symposium

Since 2005, the CMMC has organized an annual international symposium as a forum for cutting-edge biomedical research and scientific exchange. The symposium was originally established by the late Professor Wilhelm Stoffel as the **Ernst Klenk Symposium**, in honor of Ernst Klenk, the first Director of the Institute of Biochemistry at the Faculty of Medicine, University of Cologne. Over the years, the meeting has become one of the University of Cologne's premier international biomedical research events.

Renamed the **CMMC Symposium** in 2021 to mark the CMMC's 25th anniversary, the symposium continues the tradition of the Ernst Klenk Symposia. The 2026 CMMC Symposium incorporates the **38th Ernst Klenk Symposium** and continues to serve as a major international forum for biomedical research and scientific exchange at the University of Cologne.

 UNIVERSITY OF COLOGNE  UNIKLINIK KÖLN

certified with
16 CME credits

- Almudena Chaves Perez - New York, US
- Ahmed Mahmoud - La Jolla, US
- Mónica M Sousa - Porto, PT
- Elly Tanaka - Vienna, AT



- Jens Brüning - Cologne, DE
- Arvid Sandforth - Berlin, DE
- Roy Taylor - Newcastle, GB

- Can Aztekin - Tübingen, DE
- Ruma Banerjee - Ann Arbor, US
- Hozumi Motohashi - Sendai, JP

- Kenji Kabashima - Kyoto, JP
- David E Sanin - Baltimore, US
- Jane Reznick - Cologne, DE

- Ana Martin-Villalba - Heidelberg, DE
- Min Ae Lee-Kirsch - Dresden, DE
- Hendrik Poeck - Regensburg, DE
- Axel Roers - Heidelberg, DE

- Triantafyllos Chavakis - Dresden, DE
- Kitt Petersen - New Haven, US
- Gerald I Shulman - New Haven, US
- Gregory Steinberg - Hamilton, CA

- Elly Tanaka - Vienna, AT
- Axel Roers - Heidelberg, DE
- Gerald I Shulman - New Haven, US

- Sabine Emling
- Guenter Schwarz
- Jens Brüning

Organized by the CMMC Office
cmmc-symposium@uni-koeln.de

We gratefully acknowledge support by:



Monday – September 28, 2026

12.45 - 1.00 p.m. Welcome Address

Carien Niessen, Vice Rector for Research, University of Cologne

Sabine Eming, Chair - Center for Molecular Medicine Cologne

Elly Tanaka - Axel Roers - Gerald Shulman

International Scientific Coordinators

Sabine Eming - Guenter Schwarz - Jens Brüning

Local Scientific Coordinators

Session 1 Regeneration and Metabolism

1.00 - 2.30 p.m. Chairs

Elly Tanaka (Vienna) and Matthias Hammerschmidt (Institute for Zoology)

Metabolic Interventions in Regeneration and Cancer

[Almudena Chaves Perez](#) - New York, US

From Scar to Regeneration - Defining the Roadmap for Mammalian Spinal Cord

Regeneration using Spiny Mice

[Mónica M Sousa](#) - Porto, PT

Metabolic Regulation of Heart Regeneration

[Ahmed I Mahmoud](#) - La Jolla, US

2.30 - 3.00 p.m. *Coffee Break incl. Biotech Exhibition*

Session 2 Carbohydrate Metabolism and Diabetes

3.00 - 4.30 p.m. Chairs

Gerald Shulman (New Haven) and Carien Niessen (Dept. of Cell Biology of the Skin)

CNS-Control of Liver Metabolism

[Jens Brüning](#) - Cologne, DE

Prediabetes Remission – An Emerging Target in Type 2 Diabetes Prevention

[Arvid Sandforth](#) - Berlin, DE

The Claude Bernard Approach to Scientific Proof: The Aetiology of Type 2 Diabetes

[Roy Taylor](#) - Newcastle, GB

4.30 - 5.00 p.m. *Coffee Break incl. Biotech Exhibition*

5.00 - 6.00 p.m. Keynote Lecture I

Reprogramming Fibroblasts to Make Bone

Elly Tanaka - Vienna, AT

Laudatory Introduction by Sabine Eming - Cologne, DE

6.00 - 9.00 p.m. CMMC Poster Session with Welcome Get-Together (with free finger food and beverages)

moderated by Nathalie Schmidt und Sebastian Theobald (Chairs of the Poster Evaluation Committee)

More than 70 posters have been submitted across the following Research Areas (RA):

RA 1 Cancer Biology and Tumor Mechanisms

RA 4 Metabolic Control and Disease

RA 2 Immunity, Inflammation and Infection

RA 5 Other topics in Molecular Medicine

RA 3 Tissue Degeneration, Regeneration & Repair

Please note; the Poster Session will be organized by odd & even poster numbers, not by research area.

Tuesday – September 29, 2026

Session 3 Redox Metabolism and Regeneration

9.00 - 10.30 a.m. Chairs
Guenter Schwarz (CMMC Vice Chair) and Jan Riemer (Inst. of Biochemistry, MNF)

Sulfide Signaling: From Biomolecules to Behavior
[Ruma Banerjee](#) - Ann Arbor, US

CARS1-Mediated Supersulfidation in Skeletal Muscle
[Hozumi Motohashi](#) - Sendai, JP

Oxygen Sensing Bridges Amphibian and Mammalian Regeneration
[Can Aztekin](#) - Tübingen, DE

10.30 - 11.00 a.m. *Coffee Break incl. Biotech Exhibition*

Session 4 Immunity and Metabolism

11.00 - 12.30 p.m. Chairs
Hamid Kashkar (Inst. for Molecular Immunology) and Esther von Stebut (Dept. of Dermatology)

Young at Heart: Neoteny and Regeneration in the Naked Mole-Rat
[Jane Reznick](#) - Cologne, DE

Identification of HEV-Like Endothelial Cells and CD4+ Resident Memory T Cells in Atopic Dermatitis
[Kenji Kabashima](#) - Kyoto, JP

Persistent Arginase 1 Expression drives Enhanced Mitochondrial Function in Tissue Resident Macrophages
[David E Sanin](#) - Baltimore, US

12.30 - 2.30 p.m. *Lunch Break with Poster Presentation and Biotech Exhibition (free lunch for all participants)*

Session 5 Nucleic Acid Metabolism

2.30 - 4.00 p.m. Chairs
Axel Roers (Heidelberg) and Henning Walczak (Institute of Biochemistry, MF)

Perils and Promise of Stemness in Adult Regeneration: What the Methylome Reveals
[Ana Martin-Villalba](#) - Heidelberg, DE

Genetic Insights into Nucleic Acid Immunity
[Min Ae Lee-Kirsch](#) - Dresden, DE

Immunomodulatory Microbial Metabolites in Immune-Based Therapies
[Hendrik Poeck](#) - Regensburg, DE

4.00 - 4.05 p.m. The Poster Prize Winners – CMMC Symposium 2026 announced by
Nathalie Schmidt and Sebastian Theobald (Chairs of the Poster Evaluation Committee)

4.05 - 4.30 p.m. *Coffee Break incl. Biotech Exhibition*

4.30 - 5.30 p.m. Keynote Lecture II
Immunotherapy by Modulation of Nucleic Acid Driven Innate Immunity
Axel Roers - Heidelberg, DE
Laudatory Introduction by Thomas Krieg - Cologne, DE

Wednesday – September 30, 2026

9.00 – 9.30 a.m. Short Talks by the three Poster Awardees – CMMC Symposium 2026
moderated by Nathalie Schmidt & Sebastian Theobald (Chairs - Poster Evaluation Committee)

Session 6 Lipid and Fatty Acid Metabolism

9.30 - 11.00 a.m. Chairs
Thomas Benzing (Dept. II for Internal Medicine) and Michal-Ruth Schweiger (Inst. for Translational Epigenetics)

Role Immunometabolic and Epigenetic Mechanisms in Innate Immune Memory
[Triantafyllos Chavakis](#) - TU Dresden, DE

Regulation of Hepatic Lipid Metabolism
[Gregory Steinberg](#) - Hamilton, CA

Role of Muscle Insulin Resistance in the Development of MASLD
and Cardiovascular Disease
[Kitt Petersen](#) New Haven, US

11.00 - 11.30 a.m. *Coffee Break incl. Biotech Exhibition*

11.30 - 12.30 p.m. Keynote Lecture III
Mitochondrial Protonophores to Treat MASLD/MASH, Obesity
and Cardiometabolic Disease
[Gerald Shulman](#) - New Haven, US
Laudatory Introduction by Guenter Schwarz - Cologne, DE

12.30 - 12.45 p.m. Summary, open questions, concluding remarks and farewell
Sabine Eming – Guenter Schwarz – Jens Brüning

We gratefully acknowledge support by the following partners:



Sponsorship funds cover venue, technology, speaker travel, conference materials, and event management. Sponsors have no influence over the symposium's program, speakers, content, or presentation formats.



Aztekin, Can

The Friedrich Miescher Laboratory
of the Max Planck Society, Tübingen, DE

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Session 3: Redox Metabolism and Regeneration



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Session 3: Redox Metabolism and Regeneration



Brüning, Jens

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Session 2: Carbohydrate Metabolism and Diabetes



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Session 6: Lipid and Fatty Acid Metabolism



Chaves Perez, Almudena

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Session 1: Regeneration and Metabolism



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Session 4: Immunity and Metabolism

Overview – Invited Speakers



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Session 5: Nucleic Acid Metabolism



Mahmoud, Ahmed I

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Session 1: Regeneration and Metabolism



Martin-Villalba, Ana

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Session 5: Nucleic Acid Metabolism



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Session 3: Redox Metabolism and Regeneration



Petersen, Kitt

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Session 6: Lipid and Fatty Acid Metabolism



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Session 5: Nucleic Acid Metabolism



Reznick, Jane

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Session 4: Immunity and Metabolism



Roers, Axel

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Session 5: Nucleic Acid Metabolism | Keynote Speaker



Sandforth, Arvid

Dept. of Endocrinology and Metabolic Diseases,
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Session 2: Carbohydrate Metabolism and Diabetes



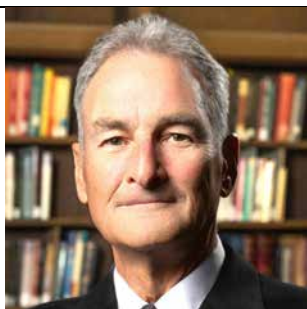
Sanin, David E

Bloomberg-Kimmel Inst. for Cancer Immunotherapy,
Johns Hopkins University School of Medicine - Baltimore, US

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Session 4: Immunity and Metabolism

Overview – Invited Speakers



Shulman, Gerald I

Dept. of Internal Medicine, Yale School of Medicine - New Haven, US

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Session 6: Lipid and Fatty Acid Metabolism | Keynote Speaker



Sousa, Mónica M

Nerve Regeneration Group, Instituto de Investigação e Inovação em Saúde - Porto, PT

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Session 1: Regeneration and Metabolism



Steinberg, Gregory

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Session 6: Lipid and Fatty Acid Metabolism



Tanaka, Elly

Institute of Molecular Biotechnology (IMBA) - Vienna, AT

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Session 1: Regeneration and Metabolism / Keynote Speaker



Taylor, Roy

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Session 2: Carbohydrate Metabolism and Diabetes

The CMMC gratefully acknowledges the support by

- the Fritz Thyssen Stiftung,



- the research institutions at the University of Cologne,

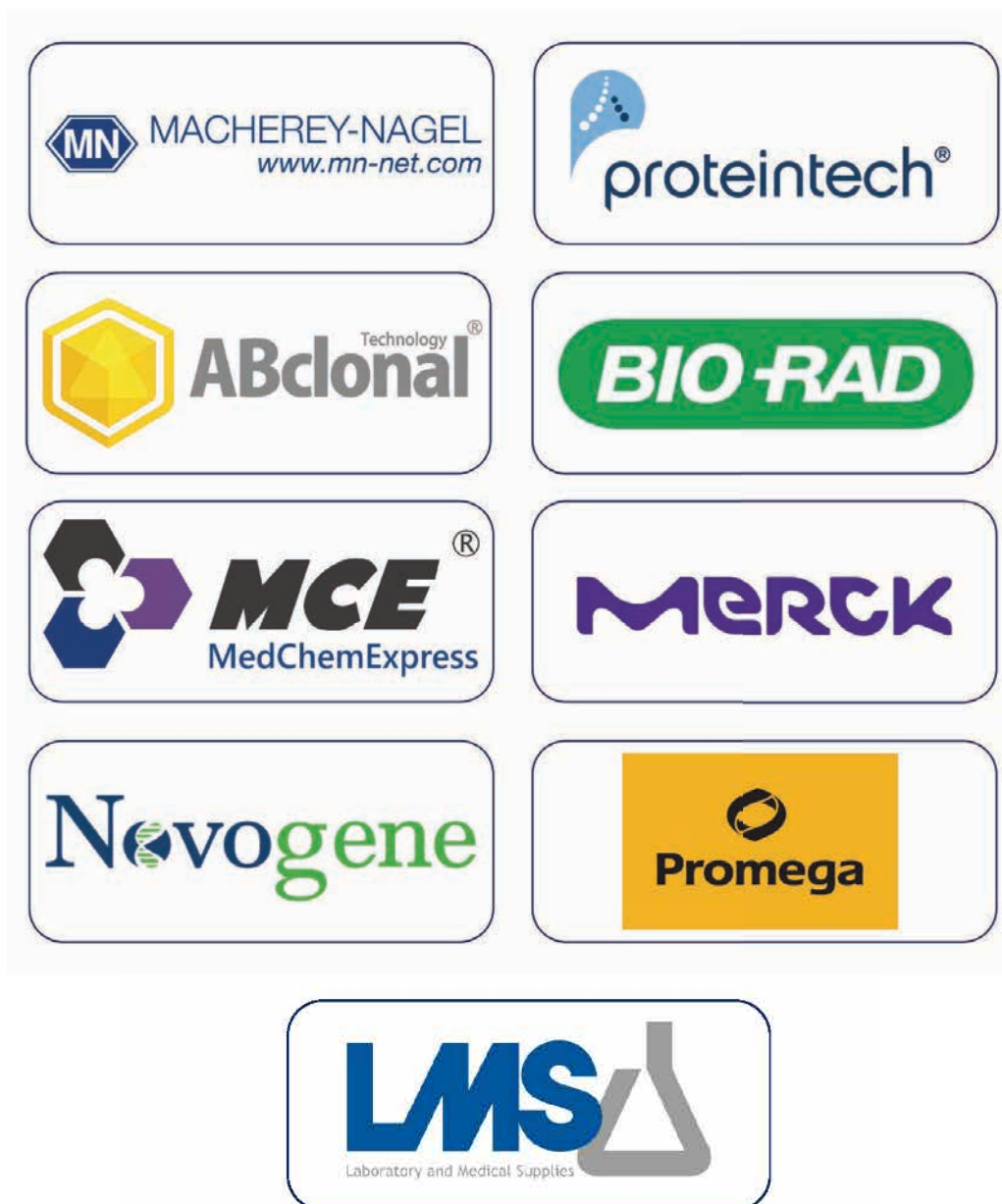


- and our local partner



The CMMC gratefully acknowledges the support by

- following Biotech Companies



Sept. 28, 2026 Conference Check-In

Upon arrival all Participants are requested to come to the Reception Desk to check in for the conference and to receive the name badge and conference bag.

Poster Number and Poster Display

Participants presenting a poster will receive their poster board number at the Reception Desk.

To promote scientific exchange throughout the meeting, we kindly ask all presenting authors to mount their posters on the assigned poster boards upon arrival and to leave them on display until the end of the symposium. This will provide additional opportunities to discuss the posters during coffee and lunch breaks.

The CMMC Symposium 2026 ends on Wednesday, Sept. 30, 2026, at approximately 1.00 p.m.

Sept. 28, 2026 Time Plan - Poster Session

- 6.15 - 7.00 p.m. Posters with even numbers
- 7.00 - 7.45 p.m. Posters with odd numbers
- 7.45 - 8.30 p.m. all poster numbers
- 8.30 - 9.00 p.m. ongoing discussions

During the Poster Session, members of the Poster Evaluation Committee will visit the poster boards, discuss the presented work with the authors, and evaluate the contributions as part of the selection process for the Poster Prize winners.

Sept. 29, 2026 Announcement of the Poster Prize Winners

- 2.30 - 4.05 p.m.
Session 5 Nathalie Schmidt and Sebastian Theobald, Chairs of the Poster Evaluation Committee, will announce the Poster Prize Winners of the CMMC Symposium 2026 – nominated by the Poster Evaluation Committee.

The presenting authors of the three most outstanding poster contributions will be honored with the Poster Awards of the CMMC Symposium 2026 and will receive a voucher worth EUR 250.

Sept. 30, 2026 Short Talks of the Poster Prize Winners – CMMC Symposium 2026

- 9.00 - 9.30 a.m. The three poster prize winners will be invited to present their work before Session 6 on Wednesday, Sept. 30, 2026 from 9.00 - 9.30 a.m.
Each presentation will consist of a seven-minute talk followed by a three-minute discussion.
The Poster Awards will be presented in conjunction with the short talks.

No.	RA	1 st / Presenting Author(s)	1 st Affiliation	Poster Title
P 01	RA 3	Abdo JM	College of Medicine, University of Zakho, IQ	Apolipoprotein E (ApoE) Genetic Polymorphisms and Susceptibility to Type 2 Diabetes Mellitus: A Case–Control Study in Duhok, Iraq
P 02	RA 5	Al Kabbani A	Inst. of Human Genetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Building an AAV Core Facility at the University of Cologne: From Vector Design to Translational Research
P 03	RA 3	Alabed L	Inst. for Human Genetics, MF - Univ. of Cologne / Univ. Hospital Cologne, DE	Laser-Targeted Optogenic Activation of Channelrhodopsin in iPSC Derived Cells Using High-Resolution Microscopy
P 04	RA 5	Amanzadeh Jajin E	Inst. of Human Genetics, MF - Univ. of Cologne / Univ. Hospital Cologne, DE	Integrative Multiomics Analysis Reveals a Coordinated Molecular Signature of Neuroresilience in Human MAPT-Knockout hiPSC-Derived Neurons with Relevance for Alzheimer's and Neurodegenerative Disease
P 05	RA 1	Badawy H	Inst. for Translational Epigenetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Functional Consequences of Cancer-Associated CREBBP Loss-of-Function Mutations on the Cellular Stress Response
P 06	RA 1	Barati Z	Inst. for Translational Epigenetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Functional Implications of SF3B1 Splice Variants in Hematological Malignancies Identified by Long-Read Transcriptomics
P 07	RA 4	Baspinar B	Erzincan Binali Yıldırım University, TR	Central Adiposity and Metabolic Dysfunction in Women with Breast Cancer: Associations with HOMA-IR and the Triglyceride-Glucose Index
P 08	RA 5	Bassett F	Developmental Biology, Institute of Zoology, Univ. of Cologne, DE	Genetic Abrogation of Palmitate Synthesis in Cells of the Nucleus Pulposus Causes Intervertebral Disc Degeneration and Platyspondyly in Zebrafish
P 09	RA 5	Benitez Alvarez, D	Inst. of Human Genetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Physiological Role and Mediation of TAU-Based Neuronal Dysfunction via Specific TAU Isoforms <i>in vivo</i>
P 10	RA1	Bergmann L-A	Inst. for Translational Epigenetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Dysregulation of the Nuclear Stress Response in HPV-Associated Cancer and Mechanisms of Viral Genome Integration

Overview - Poster Abstracts

No.	RA	1 st / Presenting Author(s)	1 st Affiliation	Poster Title
P 11	RA 1	Berikkhanova K	National Laboratory Astana, Nazarbayev Univ., Astana, KZ	Red Blood Cell-Derived Carriers for Liver-Targeted Delivery of Ademethionine
P 12	RA 3	Budash G	National University Kyiv-Mohyla Academy, Kyiv, UA	Extracellular Vesicles as Modulators of Tissue Recovery after SARS-CoV-2-Associated Injury
P 13	RA 5	Chmes H	Inst. of Human Genetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	The Role of MARK Isoforms in Neuronal Structure and Function: Implications for Neurodegenerative Diseases
P 14	RA 1	Dasarathan L	Center for Molecular Medicine Cologne, Univ. of Cologne, DE	SMARCA Chromatin Remodelers Coordinate MYC Recruitment to G-Quadruplex DNA during Cellular State Transitions
P 15	RA 4	Eh, M	Dept. of Chemistry - University of Cologne, DE	Exploring the Roles of the Moco-Containing Mitochondrial ARC Enzymes in Lipid Metabolism
P 16	RA 1	Faili A	Dept. of Pathology, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Development of a SIX1-specific PROTAC for the Treatment of Esophageal Adenocarcinomas
P 17	RA 4	Fischer D	Dept. II of Internal Medicine, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Delineating Cysteine Catabolism as a Mediator of Renal Stress-Resistance
P 18	RA 4	Guo Q	Inst. of Human Genetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Adeno Associated Virus (AAV) Based Gene Therapy Approach for the Treatment of L-2-Hydroxyglutaric Aciduria
P 19	RA 3	Halwas K	Developmental Biology, Institute of Zoology, Univ. of Cologne, DE	Investigation of the Molecular Mechanisms of Scar Free Cutaneous Wound-Healing in Adult Zebrafish
P 20	RA 3	Hegazy B	Dept. of Dermatology, FM – Univ. of Cologne / Univ. Hospital Cologne, DE	Mitochondrial Metabolism Controls Macrophage Transcriptional and Epigenetic Reprogramming during Tissue Repair
P 21	RA 1	Heinze CE Zweerink SE	Dept. of Gastroenterology and Hepatology, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	The Role of Nope in Hepatocellular Carcinogenesis under Western Diet Conditions
P 22	RA 1	Heydarzadeh Heris S	Dept. of Pathology, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Development of a Biomarker-Based Therapeutic Approach for Pediatric Rhabdomyosarcomas

No.	RA	1 st / Presenting Author(s)	1 st Affiliation	Poster Title
P 23	RA 5	Hiluf D	Center for Molecular Medicine Cologne, Univ. of Cologne, DE	Consequences of Age-Related G-Quadruplex Formation for the Fidelity of Mammalian Transcription
P 24	RA 5	Hou M	Inst. of Human Genetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Roles and Mechanisms of Specific Tau Isoforms in Neuronal Development and A β -Induced Pathology in MAPT-KO Human 2D/3D Neurons and Mapt-KO Mice
P 25	RA 3	Injarabian L	Dept. of Dermatology, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Macrophage Necroptosis Disrupts Tissue Architecture during Skin Repair
P 26	RA 3	Jayachandran J	Dept. of Pediatrics and Adolescent Medicine, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	GNAS Dysfunction Alters ECM Secretion and Deposition, Causing Ectopic Skin Mineralization
P 27	RA 2	Khan A	Dept. of Biotechnology, International Islamic University, Islamabad, PK	Targeting SARS-CoV-2 Papain-Like Protease: Structure-Based Discovery of Paraherquamide E as a Selective Inhibitor
P 28	RA 2	Kietzmann K	Dept. I of Internal Medicine, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	The Impact of Obesity and GLP-1 Agonists on T Cell Phenotype and Function
P 29	RA 3	Klein A	Inst. of Translational Pediatrics, Univ. Hospital Würzburg, DE	Age-Dependent Metabolic Programming is Associated with Regenerative Capacity in Airway Epithelial Cells
P 30	RA 5	Koch AE	Center for Molecular Medicine Cologne, Univ. of Cologne, DE	Age-Associated G-Quadruplexes Affect DNA Stability in Regulatory Regions
P 31	RA 5	Köhler L	Inst. of Human Genetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Microtubule Surface Regulation by Polyglutamylating TTL Enzymes in Human Tauopathy Models: Implications for Neurodegeneration and Healthy Brain Aging
P 32	RA 4	Lopes F	Cluster of Excellence Cellular Stress Responses in Aging-associated Diseases, Univ. of Cologne, DE	Diet-induced Kidney Protection is Mediated by a Cell-Type-Specific Modulation of Cysteine Metabolism
P 33	RA 4	Makwana H	Institute for Pathology, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Chromatin-Guided Metabolic Reprogramming in Liver Disease

Overview - Poster Abstracts

No.	RA	1 st / Presenting Author(s)	1 st Affiliation	Poster Title
P 34	RA 3	Manastireanu DM	School of Health Sciences, Universidad Cardenal Herrera-CEU, Alfara del Patriarca, ES	Aging Compromises Cellular Plasticity and Autophagic Adaptation to Changes in Oxygen Levels via Hippo Pathway
P 35	RA 2	Milnes BL	Dept. of Biochemistry, University of Washington, US	Defining how the Pentose Phosphate Pathway Supports Innate Immune Responses during Regeneration
P 36	RA 5	Molinié T	Institute for Genetics, University of Cologne, DE	uORF Regulates the Subcellular Localization and Expression of the NADase Prodegenerative Protein SARM1
P 37	RA 1	Molodetska D	Dept. of Biological Chemistry, Kharkiv National Medical Univ., Kharkiv, UA	Shared and Divergent Transcriptional and Metabolic Programs of Glioblastoma Invasion and 5-ALA Detectability
P 38	RA 2	Narayan S	Heart Center Cologne, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Establishing a Suitable Murine Model to Study Innate Immunological Aspect of Aortic Valve Stenosis (AVS)
P 39	RA 4	Naylor AS	Inst. of Human Genetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Restoring Mitochondrial Resilience: From Patient-Derived Preclinical Models to Therapeutic Intervention and Setup of Clinical Trials for POLG-associated Mitochondriopathies
P 40	RA 3	Nega MH	College of Health Sciences, Mekelle University, ET	Redox-Modulating and Hepatoprotective Effects of <i>Calpurnia aurea</i> Seed Extract Against HAART-Induced Toxicity in a Wistar Rat Model
P 41	RA 4	Nguyen TTM	Dept. of Molecular Genetics of Ageing, MPI for Biology of Ageing, Cologne, DE	Ether Lipids Are Conserved Mediators of Diapause Across Species
P 42	RA 3	Oehlert F Nüchel J	Center for Biochemistry, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Abundance Regulation of the Selective ECM Cargo Receptor TANGO1 via the mTORC1/ULK1 Axis
P 43	RA 1	Ostendorp J	Center for Molecular Medicine Cologne, Univ. of Cologne, DE	Uncovering SLUG's Role in Shaping Drug Tolerance in Non-Small Cell Lung Cancer (NSCLC)
P 44	RA 2	Özoglan E	Dept of Gastroenterology and Hepatology, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Impact of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) on Immune Control of Chronic Hepatitis B Virus Infection

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P 48	RA 5	Propp J Balke-Want H	Dept I of Internal Medicine, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Characterizing B-Cell Lymphoma-Induced T-Cell Dysfunction to Design CRISPR-Engineered CAR T Cells
P 49	RA 3	Rane KT	Center for Regenerative Therapies Dresden, Technische Universität Dresden, DE	PEAR1 Signaling Regulates Fibroblast Phenotype and Is Necessary for Spinal Cord Regeneration in Zebrafish
P 50	RA 2	Raveendran A	Dept. of Ophthalmology, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Dendritic Cell Plasticity: How the Cytokine Environment Shapes Phenotype and Tolerogenic Responsiveness
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P 52	RA 4	Sander L	Dept. of Neurology, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Unlocking the Temporal Sequence of Sensory Dysfunction Diabetic Neuropathy
P 53	RA 3	Sankova A	Dept. of Biological Chemistry, Kharkiv National Medical University, Kharkiv, UA	Identification and Analysis of Differentially Expressed Genes during Wound Healing
P 54	RA 2	Schatz V	Inst. for Medical Microbiology, Immunology & Hygiene, FM - Univ. of Cologne / Univ. Cologne, DE	Interferon- γ -Signaling Regulates Local Tissue Perfusion, Tissue Oxygenation and Antimicrobial Control
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P 57	RA 3	Sharma L	Center for Molecular Medicine Cologne, Univ. of Cologne, DE	Hedgehog – Gli2 Signalling Determines Lineage Fate and Metabolic Programming of Progenitor Cells during Skin Morphogenesis
P 58	RA 1	Stylianakis E	Institute for Molecular Medicine Mainz, University Mainz, DE	Constitutive B Cell-Specific Overexpression of the Akt Kinase Initiates Immunometabo-Epigenetic Alterations Leading to Macrophage-Like CD11b ⁺ CD68 ⁺ B Cells
P 59	RA 4	Tamura N	Max Planck Institute for Biology of Ageing, Cologne, DE	Ratiometric Fluorescent Citrate Biosensors Reveal Citrate Dynamics and Cellular Heterogeneity in Cancer and Mouse Embryonic Stem Cells
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P 61	RA 5	Torabi M	Inst. of Human Genetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Establishing an Inducible HEK293 Suspension Cell Platform for Controlled AAV Vector Production
P 62	RA 5	Torres R	Center for Molecular Medicine Cologne, Univ. of Cologne, DE	Co-Translational Quality Control of Transmembrane Proteins
P 63	RA 4	Tsotoulidis P	Dept. of Biology, University of Crete, Heraklion, GR	Investigation of the Importance of Metabolic Rewiring in a Neural Tumor Model in <i>Drosophila melanogaster</i>
P 64	RA 1	Walder R	Dept. II of Internal Medicine, FM - Univ. of Heidelberg / Univ. Hospital Heidelberg, DE	Targeting Serine Metabolism Rescues Contractile Dysfunction across Genetic and Acquired Heart Failure
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P 68	RA 4	Wrobel J	Dept. III for Internal Medicine, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	The Role of Fumarate Hydratase 1 (FH1) in Atherosclerosis
P 69	RA 5	Wunderlich L	Inst. of Human Genetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Domain-Specific Tau Mechanisms and Tau Suppression as Therapeutic Strategies Supported by PET-Based Microtubule Imaging in Alzheimer's Disease
P 70	RA 3	Xia X	Inst. of Human Genetics, FM - Univ. of Cologne / Univ. Hospital Cologne, DE	Mitochondrial Control of TAU-Based Neurodegeneration and Gene-Therapy Approaches
P 71	RA 4	Zakirov Y	National Laboratory Astana, Nazarbayev University, Astana, KZ	Pharmacokinetic Evaluation of Ademethionine- Loaded Red Blood Cell-Based Drug Delivery Systems

Apolipoprotein E (ApoE) Genetic Polymorphisms and Susceptibility to Type 2 Diabetes Mellitus: A Case–Control Study in Duhok, Iraq

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Objective:

Apolipoprotein E (ApoE) is a major regulator of lipid transport, fatty acid metabolism, and lipoprotein clearance, all of which are required for metabolic stability. Disruptions in these pathways lead to dyslipidemia, insulin resistance, and the development of type 2 diabetic mellitus (T2DM). This study looked into the relationship between ApoE genetic polymorphisms and T2DM susceptibility, with an emphasis on the function of altered lipid metabolism in disease development.

Methods:

A case-control study was conducted on 141 participants recruited from the Duhok Diabetes Center, including T2DM patients and healthy controls. A standardized questionnaire was used to collect demographic and clinical data, as well as to determine and compare ApoE genotypes between groups. Statistical analyzes looked at genotype distributions and their relationships with demographic data. Furthermore, published case-control studies on ApoE polymorphisms and T2DM were thoroughly evaluated and analyzed with R software (version 3.4.3).

Results:

$\epsilon 3/\epsilon 3$ genotype was the most prevalent in both controls (78.9%) and patients with T2DM (82.5%), followed by $\epsilon 2/\epsilon 3$ (12.3% vs. 15.0%). Only patients were found to have the $\epsilon 2/\epsilon 2$ genotype, while controls had the $\epsilon 3/\epsilon 4$ genotype. ApoE genotype distribution showed significant relationships with age ($P = 0.016$) and gender ($P = 0.033$). While the regression model has modest explanatory power (Nagelkerke $R^2 = 0.449$), it was not statistically significant ($P = 0.383$).

Conclusions:

ApoE polymorphisms may affect T2DM susceptibility by affecting lipid transport and fatty acid metabolism, resulting in insulin resistance and metabolic dysfunction. These findings lend support to additional research into ApoE as a potential biomarker for T2DM risk and precision therapy in bigger, more well-characterized populations.

Building an AAV Core Facility at the University of Cologne: From Vector Design to Translational Research

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Adeno-associated viruses (AAVs) are widely used as gene delivery tools in biomedical research and are increasingly important for therapeutic applications. To support the growing demand for reliable and standardized AAV production, we established an AAV Core Facility at the Center for Molecular Medicine Cologne (CMMC). The facility supports researchers throughout the AAV workflow, from project design and cloning to vector production, purification, and quality control.

So far, we have supported seven research groups and produced more than 15 AAV vectors, including AAV1, AAV6, AAV7, AAV8, AAV9, PHP.eB, PHP.S, rh10, B10, and B22, enabling targeted expression in skin, liver, heart, CNS and PNS cells, with successful expression in in vitro and in vivo models validated via Western blotting and qPCR.

We provide different purification grades suitable for both in vitro and in vivo transduction and also offer cloning and project design services. In parallel, we are expanding our production platform to include suspension-adapted cell systems and more scalable production processes and welcome collaborative efforts in this regard. These developments are intended to provide a transition from research-grade AAV production towards future GMP-compatible manufacturing. The facility aims to support AAV-based research across the University of Cologne and associated research facilities while contributing to the development of future translational and gene therapy programs.

Laser-Targeted Optogenic Activation of Channelrhodopsin in iPSC Derived Cells Using High-Resolution Microscopy

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Introduction: Mutations in the mitochondrial DNA polymerase gamma (POLG) gene are among the most common causes of inherited mitochondrial disorders and are characterized by impaired mitochondrial DNA replication, defective oxidative phosphorylation, and progressive neurodegeneration [1]. Neurons are particularly vulnerable to mitochondrial dysfunction because of their high energy demands. However, the mechanisms by which neuronal activity influences mitochondrial function in POLG patient-derived neurons remain incompletely understood. Optogenetics enables precise spatiotemporal control of neuronal stimulation and, when combined with live-cell imaging of mitochondrial membrane potential, provides a powerful approach to investigate activity-dependent mitochondrial responses.

Methods: Human induced pluripotent stem cell (iPSC)-derived neurons generated from POLG patients and healthy controls were used as an in vitro model of mitochondrial disease. Neurons were transfected with a Channelrhodopsin-ABCB-eYFP construct, enabling optical stimulation and visualization of transfected cells. Mitochondrial membrane potential ($\Delta\Psi_m$) was monitored using tetramethylrhodamine methyl ester (TMRM), a potentiometric fluorescent dye [2]. Live-cell imaging was performed on a Leica fluorescence microscope equipped with a 488 nm laser, allowing localized optogenetic stimulation of neuronal compartments, including axons and the soma, while recording dynamic changes in TMRM fluorescence.

Results: Preliminary experiments demonstrated successful expression of the Channelrhodopsin-ABCB-eYFP construct in wild-type iPSC-derived neurons, confirmed by eYFP fluorescence. Targeted illumination with the 488 nm laser was successfully integrated with live-cell imaging, establishing the feasibility of combining optogenetic stimulation with fluorescence microscopy in this experimental system

Discussion: This experimental approach combines human disease-specific neuronal models with optogenetic stimulation and real-time mitochondrial imaging to investigate activity-dependent mitochondrial function. We hypothesize that POLG neurons will exhibit impaired regulation of mitochondrial membrane potential in response to localized stimulation, reflecting reduced bioenergetic adaptability. Characterizing these functional differences may improve our understanding of how mitochondrial dysfunction contributes to neuronal degeneration in POLG-related disease.

Perspectives: Future work will quantify changes in mitochondrial membrane potential within distinct neuronal compartments and correlate these responses with mitochondrial distribution and morphology. Establishing this optogenetic imaging platform will facilitate mechanistic studies of mitochondrial dysfunction and may provide a framework for evaluating therapeutic strategies aimed at improving mitochondrial resilience in POLG-associated neurodegeneration.

References:

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2. Valdebenito GE, Duchon MR. Monitoring Mitochondrial Membrane Potential in Live Cells Using Time-Lapse Fluorescence Imaging. *Methods Mol Biol*. 2022;2497:319–324.

Integrative Multiomics Analysis Reveals a Coordinated Molecular Signature of Neuroresilience in Human MAPT-Knockout hiPSC-Derived Neurons with Relevance for Alzheimer's and Neurodegenerative Disease

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Background:

Transcriptome data provides a robust analytical viewpoint about cellular processes in Alzheimer's Disease (AD). Integrative multiomics analysis including transcriptome and proteome data elucidates the underlying mechanisms of this progressive disease. The microtubule-associated protein Tau (*MAPT*) is central to Alzheimer's disease (AD) pathogenesis. We demonstrated that Tau loss confers resistance to amyloid-beta (A β) toxicity in AD-relevant human glutamatergic neurons, but the active molecular reprogramming driving this neuroresilience remains poorly defined.

Methods:

We employed an integrative multiomics approach, combining temporal transcriptomics (3h and 24h post-A β exposure) with newly processed proteomic layers from terminally differentiated and electrophysiologically active human iPSC-derived neurons (WT vs. *MAPT*-KO). Analysis included DESeq2, WGCNA, and GSEA, followed by cross-layer integration to identify convergent signatures and master regulators.

Results:

Our analysis revealed a striking transcriptomic stagnation in WT neurons, which failed to mount an adaptive response to A β stress. In contrast, *MAPT*-KO neurons exhibited a pre-established resilience signature characterized by profound baseline reprogramming. WGCNA identified a co-expression gene cluster (turquoise module, $r=0.74$) strongly associated with the *MAPT*-KO phenotype and enriched in mitochondrial transport, autophagy, and synaptic homeostasis. Key hub genes including *SIM1*, *INPP5F*, and *AKR1C2* established a mitochondrial metabolic defense mechanism. Multiomics integration identified 27 core consensus hits (e.g., *COL17A1*, *PER3*, *ST3GAL6*) consistently preserved across both layers. Furthermore, transcription factor enrichment identified *KLF4* (Kruppel-like Factor 4), a key regulator of neuronal differentiation and metabolic adaptation, as the master regulator orchestrating this protective network.

Conclusion:

These findings demonstrate that Tau deletion induces an active, multilevel gain-of-resilience rather than passive resistance. By identifying a *KLF4*-driven molecular signature, this study highlights novel therapeutic targets that could be leveraged to induce synthetic resilience in AD patients, protecting neurons from amyloid-induced bioenergetic and structural collapse.

Keywords:

Alzheimer's Disease, MAPT knockout, Multiomics Neuroresilience, Proteomics, Transcriptomics

Functional Consequences of Cancer-Associated CREBBP Loss-of-Function Mutations on the Cellular Stress Response

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Introduction:

CREBBP is a lysine acetyltransferase and transcriptional co-activator that modifies histone and non-histone proteins involved in proliferation, differentiation, apoptosis, reprogramming and DNA repair. Loss-of-function mutations in CREBBP, particularly within its histone acetyltransferase (HAT) domain, occur in lung cancer and several hematological malignancies. Under cellular stress, heat shock factor 1 activates non-coding RNAs, including pericentromeric human satellite III (HSat3), and promotes the formation of nuclear stress bodies that recruit transcriptional regulators such as CREBBP. We hypothesize that cancer-associated HAT-domain mutations impair CREBBP activity and disrupt this stress-response pathway.

Methods:

Stable isogenic HeLa and H1339 cell models expressing FLAG-HA-tagged wild-type CREBBP will be generated using CRISPR-Cas9-based genome editing. Cancer-associated missense variants, including p.R1446H and p.Q1491K, will be introduced into the HAT domain by CRISPR-Cas9 or prime editing. Correct tag insertion and mutagenesis will be validated by PCR and sequencing, and protein expression will be confirmed by western blotting.

Wild-type and mutant cells will be compared under basal conditions and after heat shock at 42°C for one hour. CREBBP abundance and nuclear localization will be assessed by western blotting and immunofluorescence, while HSat3 transcription will be quantified by qPCR. Genome-wide CREBBP binding and associated chromatin changes will be examined by ChIP-seq together with H3K27ac, H3K4me1 and H3K4me3 profiles.

Expected outcomes and significance:

This study will determine whether recurrent cancer-associated CREBBP mutations alter protein expression, nuclear stress-body localization, HSat3 induction and genome-wide chromatin occupancy. Integrating functional and epigenomic analyses will clarify how impaired CREBBP acetyltransferase activity reshapes the cellular stress response and may identify mutation-dependent regulatory pathways relevant to tumor development and therapeutic vulnerability.

Functional Implications of SF3B1 Splice Variants in Hematological Malignancies Identified by Long-Read Transcriptomics

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Introduction: SF3B1 is a core component of the U2 spliceosome and is frequently mutated in chronic lymphocytic leukemia (CLL) and myelodysplastic syndromes (MDS), with K700E being the most common hotspot mutation. Interestingly, SF3B1 mutations are associated with favorable prognosis in MDS but poor prognosis in CLL. Our group previously identified three novel SF3B1 splice variants by long-read RNA sequencing of CLL (n=19) and MDS (n=25) samples: SF3B1-ΔEx24, SF3B1-InsIn4, and SF3B1-InsIn12. Among these, SF3B1-ΔEx24 was MDS-specific and became the focus of the present study [1].

Methods: AlphaFold2 modelling was used to assess structural changes in the SF3B1 isoforms. Their functional effects were then tested using minigene assays and knockdown/rescue experiments. In addition, protein-interaction changes between SF3B1-ΔEx24 and WT were analyzed by immunoprecipitation (IP) followed by mass spectrometry (IP-MS).

Results: SF3B1-InsIn4 retains 45 nucleotides from intron 4, introducing a premature stop codon and resulting in a highly truncated protein that was not detectable by Western blot. SF3B1-InsIn12 results from alternative 3' splice-site usage, leading to the inclusion of 24 nucleotides from intron 12 and an eight-amino-acid insertion within the H3 HEAT repeat [2]. While SF3B1-ΔEx24 lacks the penultimate exon and is predicted to lose HEAT repeats 18–20 and the C-terminal anchor domain. Functionally, SF3B1-K700E induced aberrant 3' splice site usage in the minigene assay, whereas SF3B1-K700E-ΔEx24 did not. Following SF3B1 knockdown, the tested variants showed only limited restoration of normal splicing. In addition, IP-MS showed different interaction patterns between ΔEx24 and WT.

Discussion and Perspectives: These findings suggest that SF3B1 splice variants differ functionally and that exon 24 deletion may alter SF3B1 complex interactions. Ongoing work aims to validate ΔEx24-associated candidate proteins, uncover the underlying molecular mechanisms and better define its interaction network and potential role in MDS.

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Central Adiposity and Metabolic Dysfunction in Women with Breast Cancer: Associations with HOMA-IR and the Triglyceride–Glucose Index

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Introduction:

Metabolic dysfunction and excess adiposity are common in women with breast cancer and may contribute to adverse metabolic health. HOMA-IR is widely used to estimate insulin resistance but requires fasting insulin measurement, whereas the triglyceride–glucose (TyG) index can be derived from routinely available fasting triglyceride and glucose concentrations. We examined whether central adiposity was associated with both HOMA-IR and TyG and evaluated the relationship between these two surrogate markers of metabolic dysfunction in women with breast cancer.

Methods:

This cross-sectional analysis included 74 women with histologically confirmed breast cancer. Anthropometric measurements and fasting biochemical parameters were obtained using standardized procedures. HOMA-IR was calculated from fasting glucose and insulin concentrations, and TyG as $\ln[\text{fasting triglycerides (mg/dL)} \times \text{fasting glucose (mg/dL)} / 2]$. Associations of waist circumference with HOMA-IR and TyG were examined using age-adjusted regression models with heteroscedasticity-consistent (HC3) standard errors. HOMA-IR was log-transformed because of its skewed distribution. The relationship between TyG and HOMA-IR was additionally examined using Spearman correlation and multivariable regression.

Results:

HOMA-IR was available for 70 participants. Greater waist circumference was associated with higher log-transformed HOMA-IR after adjustment for age (standardized $\beta=0.259$, $p=0.017$) and with higher TyG (standardized $\beta=0.187$, $p=0.010$). Both associations persisted after additional adjustment for metastatic and active-treatment status (HOMA-IR: $\beta=0.254$, $p=0.023$; TyG: $\beta=0.190$, $p=0.011$). TyG was positively correlated with HOMA-IR (Spearman $\rho=0.583$, $p<0.001$) and remained associated with log-transformed HOMA-IR after adjustment for age and BMI (standardized $\beta=0.493$, 95% CI 0.304–0.681; $p<0.001$).

Discussion:

Central adiposity showed consistent associations with two complementary surrogate markers of metabolic dysfunction in women with breast cancer. The concordant associations observed for HOMA-IR and TyG suggest that central adiposity is closely related to the metabolic phenotype in this population.

Perspectives:

The substantial association between TyG and insulin-based HOMA-IR supports further investigation of TyG as an accessible marker for metabolic phenotyping in women with breast cancer. Prospective studies are needed to determine its clinical utility and prognostic relevance.

Genetic Abrogation of Palmitate Synthesis in Cells of the Nucleus Pulposus Causes Intervertebral Disc Degeneration and Platyspondyly in Zebrafish

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From a forward genetic screen for recessive mutations causing malformed skeletons in full-grown zebrafish, we isolated a mutant with an obviously compressed antero-posterior body axis. Subsequent μ CT analysis revealed that lengthwise flattened vertebral bodies (centra) mainly account for the short stature of the mutants, while all other bones like the ones of the head and the fins appear rather unaffected. Also, a progressive collapse of intervertebral disc (IVD) tissue was detected along the entire spine already during juvenile stages in the mutants. This IVD degeneration is particularly characterized by a loss or mislocalization of collagen 2 expressing cells within the discs, cells that have been described to be homologous to the human IVD's Nucleus pulposus [1].

Via a combination of genetic mapping and whole exome sequencing, we identified the causative mutation in the fatty acid synthase (*fasn*) gene. FASN is a multidomain enzyme that catalyzes the synthesis of the C16 saturated fatty acid palmitate, the endogenous founder molecule of a cell's fatty acid pool [2]. According to our current phenotypic analysis, the IVDs of the zebrafish *fasn* mutants collapse due to disrupted plasma membrane integrity within the Nucleus pulposus. In turn, this progressive IVD degeneration appears to have an impact also on the longitudinal growth of the centra themselves. Noteworthy, patients with impaired IVDs due to mutations in collagen 2 also show such platyspondylic vertebrae [3].

Given the late-onset phenotype of the zebrafish *fasn* mutant, we hypothesize that in regular axial skeleton development, from a certain time point onwards, the growing bone of the centra entraps the Nucleus pulposus to a degree that exogenous diet-derived lipids cannot sufficiently reach that tissue anymore. Instead and from that stage on, that rather insulated tissue depends on its endogenous lipid synthesis starting from the founder fatty acid palmitate. We propose that our zebrafish *fasn* mutant might serve as a valuable model for comparable situations in human pathologies.

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Physiological Role and Mediation of TAU-Based Neuronal Dysfunction via Specific TAU Isoforms *in vivo*

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Introduction:

Alzheimer's disease (AD) and related tauopathies are age-associated neurodegenerative disorders characterized by TAU pathology, neuronal dysfunction, and cognitive and motor decline. In the adult human brain, alternative splicing of *MAPT* generates six TAU isoforms based on N-terminal inserts (0N, 1N, or 2N) and microtubule-binding repeats (3R or 4R). Although these isoforms are differentially expressed during development and adulthood, their specific roles in neuronal function and disease remain unclear. Here, we investigate isoform-specific TAU functions using complementary *in vitro* and *in vivo* approaches.

Methods and Results:

In vitro, human iPSC-derived MAPT knockout neurons were transduced with individual TAU isoforms. Sholl analysis showed that 0N3R, unlike the other isoforms tested, enhanced neurite outgrowth, dendritic complexity, and maximal distance from the soma. EB3-life imaging and multi-electrode array recordings are ongoing to assess microtubule dynamics and neuronal activity. *In vivo*, TAU isoform expression was characterized during development in humanized TAU (hTAU) mice carrying a human MAPT gene replacement. At one week, 0N3R and 0N4R predominated, while all six isoforms were detected in adulthood, resembling the human pattern. Hippocampal proteomics revealed genotype-dependent changes in mitochondrial and synaptic proteins, with MT-CO1 and GRIN1 highest in wild-type and reduced in hTAU mice. Golgi staining was used to assess dendritic complexity and spine density. To investigate TAU in neuronal development, AAV vectors encoding TAU-targeting shRNAs were administered by ICV injection to newborn mice (P1–2). Total TAU knockdown caused a dose-dependent phenotype: 8×10^{10} vg markedly reduced survival, $2.5\text{--}5 \times 10^{10}$ vg caused severe motor deficits and reduced survival, and 1×10^{10} vg produced progressive developmental impairments. In comparison, 2N shRNA caused a milder phenotype, while 1N shRNA and scrambled controls showed no detectable effects. In adult 1-year-old hTAU mice, I.V. administration caused no overt motor abnormalities during three months of monitoring.

Conclusion and Perspective:

These findings support distinct roles of TAU isoforms in neuronal function and suggest that TAU levels differentially affect neuronal development. Ongoing structural, functional, proteomic, and behavioral studies, including APP-Swe/Ind overexpression, will further clarify the contribution of TAU isoforms to tauopathy progression and their potential as therapeutic targets.

Keywords:

AAV-gene therapies, Alzheimer's disease, Neurodegeneration, Neuronal vulnerability, Tau isoforms, Tauopathies

Dysregulation of the Nuclear Stress Response in HPV-Associated Cancer and Mechanisms of Viral Genome Integration

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Almost 5% of all cancers worldwide are associated with an infection of the human papillomavirus (HPV). High-risk HPV infections, caused by HPV 16 and HPV 18, are the main driver of head and neck squamous cell carcinomas (HNSCCs).

Long non-coding RNAs transcribed from pericentromeric repetitive satellite repeats (HSat3) are increased in many cancer types and are upregulated upon heat stress. They accumulate in nuclear stress bodies (nSB) and are transcriptionally regulated by the heat shock factor 1 (HSF1), the bromodomain-containing protein 4 (BRD4) and other cellular proteins, which are all involved in stress-dependent splicing and maintenance of genome stability¹.

BRD4 not only regulates the heat stress response by interacting with HSF1, but also participates in transcriptional regulation of the HPV early protein 2 (E2) during viral transcription. Furthermore, the C-terminal domain of BRD4 binds to the transactivation domain of the HPV E2 protein and thereby ensures an equal distribution of the viral genome to the daughter cells during mitosis². Dysregulation of repetitive regions e.g. satellite regions is frequently associated with epigenetic dysregulation. Satellite RNAs can exert an oncogenic potential and can induce genomic instability, which can promote HPV genome integration after infection. Therefore, the involvement of repetitive regions in HPV integration and the detailed interaction between BRD4 and HPV E2 in this context needs to be investigated.

Deciphering this mechanism will allow for discovery and development of new treatment strategies and therapeutic targets, not only for HPV-associated cancers, but also for any cancer type with increased satellite RNAs.

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Red Blood Cell–Derived Carriers for Liver-Targeted Delivery of Ademethionine

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Introduction: Sepsis-associated liver dysfunction plays a central role in the pathogenesis of abdominal sepsis because the liver regulates key metabolic, detoxification, homeostatic, and immune responses, including bacterial clearance and inflammatory mediator production. Oxidative stress and cytokine/endotoxin-mediated injury are recognized as the major mechanisms of hepatic damage in sepsis.

Although extracorporeal detoxification techniques are used to restore impaired liver barrier, metabolic, and detoxification functions, their effects are short-lived and do not provide sustained correction of toxemia and bacteremia. Therefore, new therapeutic strategies capable of maintaining prolonged drug concentrations in the target organ are needed.

Targeted drug delivery systems are considered a promising approach because they can increase local drug concentration in pathological tissues while minimizing systemic toxicity. Among such systems, red blood cell (RBC)-based carriers are of particular interest due to their biocompatibility, safety, availability, and potential for selective delivery to the reticuloendothelial system (liver, spleen). Ademethionine is a hepatoprotective agent widely used in liver disorders; however, its conventional administration is limited by non-selective distribution and insufficient hepatic accumulation. The present study aimed to develop RBC-derived carriers loaded with ademethionine and to evaluate the in vitro release profile of the encapsulated drug.

Methods: The study was approved by the institutional ethics committee, and blood samples were obtained from healthy volunteers after written informed consent. RBC-derived carriers loaded with 500 mg of ademethionine were prepared using a hypoosmotic hemolysis method with original modifications. To assess drug release from the carriers, equilibrium dialysis through a semipermeable membrane was performed. Dialysate samples were collected after 15 min, 30 min, 1 h, 3 h, 17 h, and 24 h. Ademethionine concentrations in the samples were determined by spectrophotometry.

Results: Ademethionine-loaded RBC-derived carriers demonstrated a gradual release of the drug over 24 h. The highest concentration of released ademethionine was observed at 15 min (17.02 ± 0.21 µg/mL), likely reflecting the release of both superficially adsorbed and encapsulated drug fractions. During the following 3 h, the release profile remained sustained, with ademethionine concentrations maintained within the range of 5–8 µg/mL. By 17 h, the concentration decreased to 1.14 ± 0.13 µg/mL, and by 24 h no detectable release was observed.

Conclusion: RBC-derived carriers successfully encapsulated ademethionine and provided its prolonged in vitro release for up to 24 h, with the most substantial release occurring during the first 17 h. These findings indicate that ademethionine-loaded RBC-derived carriers may represent a promising targeted delivery system for liver-directed therapy. Further in vivo studies are warranted to investigate their pharmacokinetics, hepatic accumulation, and therapeutic potential in abdominal sepsis and sepsis-associated liver dysfunction.

Funding: This research is funded by Nazarbayev University under Collaborative Research Program Grant № 211123CRP1614, A.G.

Extracellular Vesicles as Modulators of Tissue Recovery after SARS-CoV-2-Associated Injury

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Background: SARS-CoV-2-associated pulmonary injury involves disruption of tissue homeostasis and may progress from acute inflammatory damage to impaired tissue recovery. This transition requires coordinated adaptation of injured cells and restoration of functional interactions within the tissue microenvironment. Extracellular vesicles (EVs) released by stromal and immune cells may contribute to these processes by transferring biologically active molecules between cells.

Approach: This study re-evaluated the therapeutic potential of cell-derived EVs described in clinical and translational studies of COVID-19, with particular emphasis on mechanisms relevant to the balance between tissue injury and recovery. The available evidence was assessed to examine how EV cellular origin, molecular cargo, biological activity, clinical safety, therapeutic efficacy and delivery routes may influence their potential for tissue recovery.

Key findings: Most clinical investigations have examined EVs derived from mesenchymal stromal cells obtained from placenta, umbilical cord, amniotic fluid, adipose tissue or bone marrow. These preparations contain regulatory molecules, including proteins, growth factors and RNA species, capable of modifying responses of recipient cells. Their potential relevance to tissue recovery extends beyond attenuation of excessive inflammation: EV-mediated signalling may influence cellular survival, the local tissue environment and processes required for restoration of damaged pulmonary structures. Conversely, the biological effects of EVs are not uniform and may depend on their cellular source, molecular composition and route of administration.

The clinical evidence remains limited and heterogeneous. Differences in EV production, characterization, dosage and delivery complicate comparison of therapeutic outcomes. The review also indicates that systemic administration may provide insufficient targeting, whereas pulmonary delivery by nebulization represents a potentially more direct approach for respiratory injury. These considerations are particularly relevant when EV therapy is viewed not simply as immunomodulation, but as an intervention aimed at supporting recovery of damaged tissue.

Conclusion: In conclusion, cell-derived EVs represent a potential molecular interface between inflammatory injury, cellular adaptation and tissue restoration following SARS-CoV-2 infection. Defining how EV composition, cellular origin and tissue delivery determine biological activity may provide a basis for developing more precise regenerative strategies for post-infectious tissue injury. Further studies should integrate EV characterization with functional indicators of tissue recovery to establish reproducible and clinically relevant therapeutic approaches.

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The Role of MARK Isoforms in Neuronal Structure and Function: Implications for Neurodegenerative Diseases

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Background: PAR-1/MARK kinases (Microtubule Affinity-Regulating Kinases) are serine/threonine kinases known for regulating microtubule dynamics, cell migration, neuronal polarity, and synaptic plasticity. Mammals express four MARK isoforms: MARK1 (Par1c), MARK2 (Par1b/EMK), MARK3 (Par1a/C-TAK1), and MARK4 (MARKL1/Par1d). MARK kinases have significant roles in neuronal function, influencing Tau-mediated microtubule instability, axon/dendrite polarity, and synaptic plasticity. Dysregulation of these pathways is implicated in aging-related neurodegenerative diseases such as Alzheimer's disease (AD), MARK mutations cause neurogenetic developmental diseases. The distinct physiological and pathological functions both for developmental and aging-associated neurodegenerative diseases of individual MARK isoforms remain poorly understood but preliminary data in primary rat neurons show that MARK isoforms differentially regulate Alzheimer-like Tau missorting. In iPSC-derived neurons, MARK isoforms differentially regulate microtubule dynamics, with MARK1 leading to fewer comets per μm , indicating isoform-specific functions.

Objectives: This study aims to dissect the distinct roles of MARK isoforms in neuronal structure and function, with a focus on Tau-dependent MT instability in neurodegenerative diseases.

Methods and Work Plan: Human iPSC-derived neurons (iN) with a MAPT KO line were used to perform overexpression (OE) and knockdown (KD) of individual MARK isoforms. MARK activity was monitored with a FRET-based biosensor. Dendritic complexity and spine formation was quantified via Sholl analysis; microtubule dynamics will be assessed using EB3-GFP live imaging. Calcium imaging will evaluate activity changes following MARK modulation. Fluorescence microscopy with phosphorylation-specific antibodies will assess isoform-specific effects on Tau and PSD-95 phosphorylation, localization, and transport. We will also use Tau pseudophosphorylation mutants (at MARK-targeted KxGS motifs) and APP overexpression as a tauopathy model to study MARK response under disease-relevant conditions.

Results: MARK isoforms show different localization patterns, with MARK2 and MARK3 showing higher somatic localization. Initial results also demonstrate isoform specific activity, with MARK2 and MARK4 OE leading to significantly shorter neurites in neurons as young as day 7. While Sholl analysis in mature neurons didn't show a difference in the number of dendritic intersections, the maximum dendritic distance from the soma was significantly shorter in MARK2 and MARK4. This effect is not observed in Tau KO iN, indicating a dependency on Tau. MARK2 and MARK4 overexpression also induced prominent Tau mislocalisation into the somatodendritic compartment, a key hallmark of aging associated neurodegeneration. Validation of a FRET-based MARK activity reporter in HEK cells proved its reliability as an isoform activity read-out, to be later used in iN.

Conclusion and Perspective: Overall, these findings indicate that MARK isoforms have distinct, Tau-dependent effects on neuronal morphology and Tau localization. In particular, MARK2 and MARK4 promote neurite shortening and somatodendritic Tau mislocalization, suggesting a potential role in tauopathy-related neuronal dysfunction. Further investigation of their distinct effects will help identify isoform-specific MARK pathways and may reveal new therapeutic targets for Alzheimer's disease and other tauopathies.

SMARCA Chromatin Remodelers Coordinate MYC Recruitment to G-Quadruplex DNA during Cellular State Transitions

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Introduction

Drug-tolerant persister (DTP) cells are a reversible population of cancer cells that survive targeted therapy and contribute to disease relapse, and their emergence is associated with cellular plasticity and extensive chromatin remodeling. G-quadruplexes (G4s), non-canonical DNA structures enriched at regulatory genomic regions, are important regulators of transcription and cellular state. MYC is a central regulator of pluripotency and cancer cell plasticity, yet the mechanisms facilitating its recruitment to G4-containing chromatin remain poorly understood. We hypothesized that ATP-dependent chromatin remodelers mediate the MYC–G4 interaction.

Methods

Interactions with G4, G-loop and R-loop structures were investigated using in vitro DNA pulldown assays. G4-associated proteins were identified by in situ TurboID proximity labeling, and MYC–protein interactions by co-immunoprecipitation. Genome-wide occupancy of MYC and candidate remodelers SMARCA4, SMARCA5 and HELLS was mapped by DynaTAG in mESCs and epiblast-like cells (EpiLCs), G4s by BG4 CUT&TAG, and chromatin states by ChromHMM.

Results

MYC strongly associated with G4-containing DNA in vitro, while recombinant MYC showed reduced binding to its canonical motif without nuclear cofactors, suggesting additional proteins aid MYC recruitment. TurboID identified G4-associated helicases and remodelers SMARCA4, SMARCA5 and HELLS, all preferentially associated with G4s over R-loops; HELLS also co-precipitated with MYC in mESCs. Genome-wide analyses revealed extensive co-occupancy of MYC, G4s and accessible chromatin in mESCs and EpiLCs, with MYC enriched at regions containing G4s together with all three remodelers. Changes in SMARCA occupancy during mESC-to-EpiLC differentiation tracked with corresponding changes in G4 occupancy, and mESC-specific MYC sites largely lost their G4-, SMARCA- and active-chromatin-associated state upon differentiation.

Discussion

Our findings identify a coordinated MYC–G4–SMARCA axis linked to cellular state transitions, suggesting chromatin remodeling and G4 formation are coupled to MYC recruitment.

Perspectives

Ongoing work will test causality via depletion of SMARCA4, SMARCA5 and HELLS, pharmacological G4 perturbation, and disruption of selected G4 motif, using MYC DynaTAG, BG4 CUT&TAG, ATAC-seq and RNA-seq to assess effects on MYC occupancy, accessibility and differentiation, establishing whether this axis is a conserved contributor to plasticity and DTP persistence.

Exploring the Roles of the Moco-Containing Mitochondrial ARC Enzymes in Lipid Metabolism

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All mammalian genomes encode two mitochondrial amidoxime reducing component (mARC) proteins: mARC1 and mARC2. Both of them are molybdenum cofactor-containing enzymes that can reduce N-hydroxylated prodrugs. A single nucleotide polymorphism in *MTARC1* leading to p.A165T protects against metabolic dysfunction-associated steatotic liver disease. How mARC proteins impact lipid metabolism and the differences between the two mARC proteins is poorly understood.

In this study we investigated both mARC enzymes in parallel in a human kidney cell line. This revealed distinct interactors and subcellular localizations with both enzymes at the outer mitochondrial membrane but mARC1 more specifically at mitochondria endoplasmic reticulum contact sites.

The loss of *MTARC1* or *MTARC2* led to a unique phenotype. In *MTARC1* knockout cells triacylglycerol and ceramide accumulated while in *MTARC2* knockout cells the lysophosphatidic acid, phosphatidic acid and diacylglycerol axis was depleted. This suggests that mARC1 and mARC2 have non-redundant roles in lipid metabolism. As ceramides and diacylglycerol are both signalling lipids this presents interesting possibilities for how it may modulate metabolism.

In both cases mitochondrial morphology was altered, and the cells proliferated slower indicating the impact on energy metabolism. Both enzymes could bind lipids with free phosphate groups, although the physiological impact of this is unclear, the prospect of a redox reaction at a lipid is intriguing.

Development of a SIX1-specific PROTAC for the Treatment of Esophageal Adenocarcinomas

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Esophageal adenocarcinomas belong to the cancer entities with a sharply increasing incidence. Though multimodal therapies have improved outcomes, prognoses remain poor in advanced stages and the 5-year survival rate is only between 20 to 25%. For this purpose, new therapeutic options are urgently necessary. In this context, this project investigated a SIX1-specific PROTAC in EAC. SIX1 is an embryonic transcription factor, which is required during embryogenesis for the development of various organs and tissues. In contrast, it is no longer or only weakly expressed in adult tissue.

However, many cancers show a reactivation of cancer, which leads to different pro-tumorigenic features, making SIX1 a potentially therapeutic target. We first tested the efficiency of ELX19 in degrading SIX1 via ELISA and flow cytometry. Thereby, a higher SIX1 expression correlated with a better degradation efficiency, leading to a DC50 of below 1 μ M in FLO-1 cells. ELX19 also reduced proliferation and increased apoptosis in FLO-1 cells. Importantly, ELX19 did not affect proliferation and apoptosis in a cell line with low SIX1 expression (OE19) suggesting that the effects caused by ELX19 are indeed SIX1 specific. In conclusion, these preliminary results show the potential of a SIX1-targeting PROTAC for the treatment of esophageal adenocarcinomas.

Delineating Cysteine Catabolism as a Mediator of Renal Stress-Resistance

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Introduction: Acute kidney injury (AKI) is a frequent and severe complication in hospitalized patients and is associated with increased morbidity, mortality, and progression of chronic kidney disease (CKD). Despite its clinical burden, effective preventive therapies are lacking [1]. Previous work from our group demonstrated that dietary preconditioning, including caloric restriction (CR) and sulfur amino acid restriction (SR), markedly protects mice from ischemia-reperfusion injury (IRI). Integrative multi-omics analyses identified cysteine catabolism as one of the most consistently regulated pathways in the AKI-susceptible S3 segment of the proximal tubule, with coordinated regulation of cysteine dioxygenase 1 (CDO1), cystathionine γ -lyase (CTH), and 3-mercaptopyruvate sulfurtransferase (MPST) [2]. We hypothesize that adaptive remodeling of cysteine catabolism is a conserved mechanism of renal stress resistance.

Methods: To define the functional contribution of cysteine catabolism to renal protection, CDO1, MPST, and CTH are investigated using genetic, dietary, and pharmacological approaches in mouse models of renal IRI. Genetic loss-of-function models are combined with CR or SR preconditioning and pharmacological modulation of CDO1, MPST, and CTH to dissect their contribution to renal stress resistance and evaluate their potential as therapeutic targets. Transcriptomic, proteomic, persulfidome, and targeted metabolomic analyses, together with H₂S measurements and quantitative MALDI-MSI-based isotope tracing, are integrated to characterize cysteine metabolism and downstream mechanisms. To assess translational relevance, human kidney biopsies, perirenal adipose tissue, serum, and urine from a randomized-control clinical trial in the setting of living kidney donation (NCT05709600) are analyzed following CR by a fasting-mimicking diet (FMD), SR, or a ketonic diet compared to control [3].

Results: Previous analyses showed that CR and SR induce overlapping changes in cysteine catabolism in the AKI-susceptible proximal tubule. CDO1, CTH, and MPST were consistently induced at transcript and protein level and accompanied by changes in sulfur metabolites [2]. These findings provide the basis for the current functional studies addressing whether individual enzymes are required for diet-induced nephroprotection and whether pharmacological modulation can reproduce protective metabolic adaptations. The translational approach will determine whether diet-induced changes are conserved in humans.

Discussion: The coordinated regulation of oxidative and H₂S-producing branches of cysteine catabolism suggests that metabolic adaptation may integrate redox control, sulfur signaling, and cellular stress responses to promote renal resilience. Dissecting the individual roles of CDO1, MPST, and CTH will clarify whether these enzymes represent shared of pathway-specific mediators of diet-induced nephroprotection.

Perspectives: Combining mechanistic mouse studies with human samples from a clinical trial establishes a translational framework from metabolic adaptation to therapeutic target identification. Defining conserved mechanisms of cysteine-dependent renal stress resistance may ultimately enable pharmacological strategies that mimic beneficial dietary preconditioning for the prevention of AKI.

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Adeno Associated Virus (AAV) Based Gene Therapy Approach for the Treatment of L-2-Hydroxyglutaric Aciduria

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Background:

L-2-hydroxyglutaric aciduria (L-2-HGA) is a rare autosomal recessive neurometabolic disorder characterized by developmental delay, epilepsy, cerebellar dysfunction, and progressive cognitive and motor impairment. It is caused by pathogenic variants in L2HGDH, encoding the mitochondrial enzyme L-2-hydroxyglutarate dehydrogenase. Loss of L2HGDH activity leads to pathological accumulation of L-2-hydroxyglutarate (L2HG), which is markedly elevated in blood, cerebrospinal fluid, and urine. Currently, treatment is limited to supportive care, and no disease-modifying therapy is available.

Objective:

We aimed to establish an adeno-associated virus (AAV)-mediated L2HGDH gene replacement strategy and evaluate its therapeutic potential for L-2-HGA. We hypothesize that restoring functional L2HGDH expression, particularly in the brain, can compensate for the enzymatic deficiency, normalize L-2-HG metabolism and mitochondrial homeostasis, and thereby slow progressive neurodegeneration.

Methods and Results:

Plasmids encoding human L2HGDH were generated and used for in-house production of recombinant AAV9 and AAV PHP.eB vectors. The vectors were validated in HEK293 cells and human induced pluripotent stem cell (iPSC)-derived neurons. Western blotting confirmed robust L2HGDH expression, while immunofluorescence demonstrated correct mitochondrial localization. A dichlorophenolindophenol (DCIP)-based enzymatic assay showed increased L2HGDH activity, confirming preservation of catalytic function following AAV-mediated gene transfer. For in vivo validation, AAV9 and AAV PHP.eB were administered to wildtype C57BL/6N mice by intravenous (IV) or intracerebroventricular (ICV) injection. Immunofluorescence confirmed transgene expression and mitochondrial localization in the brain following both administration routes and with both AAV serotypes. qPCR and Western blot analyses suggested higher brain transduction following ICV compared with IV administration. Transgene expression was also detected to varying degrees in peripheral organs, including the heart and kidney, indicating additional systemic biodistribution.

Therapeutic studies in L2hgdh knockout mice are currently ongoing, comparing IV and neonatal ICV delivery to determine how route of administration influences brain and peripheral tissue transduction. Tissue-specific L2HGDH expression and L-2-HG levels will be assessed in the brain and peripheral organs, together with histological and biochemical analyses, to evaluate therapeutic efficacy and treatment response.

Conclusion:

We successfully established and validated an AAV-mediated L2HGDH gene delivery system. In vitro and in vivo studies demonstrated robust transgene expression, correct mitochondrial localization, and preserved enzymatic activity. These findings provide a foundation for further preclinical evaluation of AAV-mediated L2HGDH gene replacement as a potential therapeutic strategy for L-2-HGA.

Keywords:

AAV; gene therapy; L-2-hydroxyglutaric aciduria; L2HGDH; mitochondrial metabolism

Investigation of the Molecular Mechanisms of Scar Free Cutaneous Wound-Healing in Adult Zebrafish

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Adult Zebrafish are able to fully regenerate full thickness dermal wounds without the formation of a scar. While they, like mammals, initially form a collagen-rich granulation tissue, it later gets resolved and doesn't lead to fibrotic tissue [1]. Even though the early phases of wound healing like initial wound closure and early inflammation are already pretty well investigated [2], the later phases of tissue resolution and remodeling are still not well understood. We therefore use this model to further investigate scar-free cutaneous wound healing and compare the observed mechanisms to fibrotic mammalian models, thereby trying to elucidate difference-making factors involved in scar resolution and whole tissue remodeling.

To understand the complex cellular and molecular interactions during granulation tissue resolution we conducted single-cell RNA sequencing analysis of four major relevant timepoints of the wound healing process and analyzed the spatial distribution of interesting genes found in the transcriptomics via Hybridization Chain Reaction (HCR) [3].

Using this combination of methods, we were able to show that macrophages and neutrophils seem to directly cooperate in the resolution of wound collagen fibers by expressing *mmp13a*, which is a collagenase, in neutrophils and *mrc1b*, which is a receptor for collagen phagocytosis, in macrophages. Finding these two specific cell-types in pairs strongly suggests a functional role in collagen resolution. Additionally, we find exactly these *mrc1b* positive macrophages located in the margins of cell-free lacunae that will later be occupied by regenerated adipocytes indicating an even broader function in whole-tissue remodeling.

Myofibroblasts are pro-fibrotic cells that produce excessive collagen and play a role in wound contraction in mammalian wounds. It was not very clear if these kinds of cells also exist in zebrafish regeneration. We could demonstrate the existence of myofibroblast-like cells located at the wound-muscle junction of adult zebrafish wounds that strikingly do not produce collagen. These cells could therefore be a “net-positive” myofibroblast population that is only responsible for wound contraction and muscle relocation, but not fibrotic collagen deposition.

Interestingly we did not only find differences in cell function and composition between mammals and zebrafish, but we also found indicators of differing granulation tissue ECM composition. Zebrafish fibroblasts express *col2a1b*, *col10a1b* and *clip* which are all rather known from cartilage and not from skin [3].

Based on these observations we want to conduct functional studies to investigate the different mechanism of tissue remodeling in more detail to find factors that could make the difference between scarring and scar-free wound healing.

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Mitochondrial Metabolism Controls Macrophage Transcriptional and Epigenetic Reprogramming during Tissue Repair

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Successful wound healing requires macrophage transition from inflammatory to pro-resolving and reparative states [1,2], a process tightly coupled to metabolic reprogramming. Mitochondrial metabolism regulates stage-specific macrophage functions during skin wound healing, with IL-4R α -mediated mitochondrial respiration characterizing late-stage pro-resolving macrophages [3]. Beyond bioenergetics, mitochondrial metabolites can regulate chromatin-modifying enzymes and link metabolism to transcription. α -Ketoglutarate (α KG), an intermediate of glutamine metabolism and the tricarboxylic acid cycle, promotes alternative macrophage activation through metabolic and epigenetic mechanisms [4]. However, how mitochondrial respiration shapes chromatin regulation during wound macrophage activation remains unclear. We therefore investigated how disruption of mitochondrial complex IV affects IL-4/IL-13-induced macrophage reprogramming.

Bone marrow-derived macrophages from control and macrophage-specific Cox10-deficient (Cox10^{MKO}) mice, characterized by impaired mitochondrial complex IV-dependent respiration, were stimulated with IL-4/IL-13. Activation-associated gene expression was analyzed under complete and glutamine-free conditions and following α KG supplementation. Chromatin accessibility was profiled by ATAC-seq. Because STAT6 is the central transcription factor downstream of IL-4/IL-13 signaling, genome-wide pSTAT6 occupancy and regulatory histone modifications were characterized using DynaTag [5].

IL-4/IL-13 induced transcriptional responses in both control and Cox10^{MKO} macrophages, but the magnitude of several alternative activation-associated genes differed between genotypes. Glutamine deprivation altered this response, while α KG supplementation modulated activation-associated gene expression, supporting a contribution of mitochondrial metabolite availability to macrophage reprogramming. ATAC-seq is being used to assess whether impaired mitochondrial respiration alters chromatin accessibility, while DynaTag compares genome-wide pSTAT6 occupancy and associated histone states between control and Cox10^{MKO} macrophages under basal and IL-4/IL-13-stimulated conditions.

Together, these findings suggest that mitochondrial metabolism contributes to alternative macrophage activation through a metabolic-epigenetic axis. Integrating ATAC-seq and DynaTag will test whether Cox10 deficiency alters chromatin accessibility, pSTAT6 occupancy and regulatory histone states underlying macrophage gene regulation.

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The Role of Nope in Hepatocellular Carcinogenesis under Western Diet Conditions

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Background:

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and remains a leading cause of cancer-related mortality worldwide. Its increasing association with metabolic dysfunction-associated steatotic liver disease (MASLD) highlights the need to better understand the molecular mechanisms driving hepatocarcinogenesis in a metabolically challenged liver. Neighbor of Punc E11 (Nope/IGDCC4) is an oncofetal cell surface protein that is highly expressed in the fetal liver but largely absent in healthy adult hepatocytes. Re-expression of Nope has been observed in HCC, particularly in non-cirrhotic tumors, as well as during hepatic progenitor cell activation and loss of hepatocyte polarity. These observations suggest an association between Nope expression and hepatocellular dedifferentiation. However, whether Nope merely represents a marker of hepatocellular transformation or functionally contributes to HCC initiation and progression remains unknown.

Methods:

This project investigates the functional significance of Nope in hepatocarcinogenesis using a liver-specific conditional Nope knockout (NKO) mouse model. We hypothesize that loss of hepatic Nope alters tumor development and that its functional relevance may depend on the underlying metabolic environment. NKO and control mice are therefore studied in two complementary experimental settings: chemically induced hepatocarcinogenesis using diethylnitrosamine (DEN) under standard diet and a combined DEN/Western diet model to investigate tumor development under metabolic stress. Animals are analyzed at six and nine months to assess different stages of hepatocarcinogenesis.

Tumor development is characterized by macroscopic assessment of tumor number and size and comprehensive histopathological evaluation. Immunohistochemical analyses are used to characterize proliferation, hepatic differentiation, fibrosis, and immune cell infiltration. These analyses are complemented by quantitative gene expression profiling, proteomic approaches, and serum biomarkers reflecting liver injury, inflammation, and tumor progression. Pulmonary tissue is additionally examined for metastatic lesions.

Expected Impact:

This study will provide the first functional characterization of Nope in an in vivo model of hepatocarcinogenesis. By combining a liver-specific knockout model with chemical and metabolic drivers of HCC, we aim to distinguish whether Nope is primarily a marker of hepatocellular transformation or actively contributes to tumor initiation and progression. Furthermore, comparison of standard and Western diet conditions may reveal whether the role of Nope is influenced by metabolic liver injury. Understanding the biological consequences of hepatic Nope deficiency may provide new insights into HCC pathogenesis and help evaluate the potential of Nope as a diagnostic biomarker and therapeutic target.

Development of a Biomarker-Based Therapeutic Approach for Pediatric Rhabdomyosarcomas

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Introduction:

Pediatric rhabdomyosarcomas (RMS), comprising embryonal and alveolar subtypes, account for approximately 3% of all childhood and adolescent malignancies. Although current multimodal treatment regimens achieve substantial response rates, they are associated with considerable acute and long-term toxicities, including growth impairment, organ damage, infertility, and secondary malignancies. There is therefore a critical need for targeted and less toxic therapeutic approaches. In contrast to many adult cancers, pediatric RMS are often characterized by a pronounced dependency on developmental transcription factors rather than a high mutational burden. The embryonic transcription factor SIX1 plays a central role in maintaining proliferative features, suppressing apoptotic signaling, and promoting tumor stem cell-associated features.

Material and Method:

Here, we evaluate a biomarker-driven strategy based on targeted degradation of SIX1 using a novel PROTAC (ELX19) that recruits the E3 ubiquitin ligase VHL to induce proteasomal degradation.

Results and Discussion:

In the alveolar RMS cell line Rh30, treatment with ELX19 (2 μ M) for 24 hours resulted in marked reduction of SIX1 protein levels and led to a near-complete loss of proliferative capacity, followed by substantial tumor cell elimination within 48 hours. These functional effects were accompanied by downregulation of pro-proliferative genes (including CDK4, CCNE1, and PCNA) and induction of pro-apoptotic pathways. Pharmacological rescue experiments confirmed the VHL- and proteasome-dependent mechanism of action. Notably, addition of cisplatin did not enhance the anti-tumor effect of ELX19 under the tested conditions. Pharmacokinetic analyses demonstrated in vivo detectability of ELX19 for up to 48 hours after intraperitoneal administration without immediate signs of toxicity. In parallel, we established a sequential multiplex immunofluorescence workflow to quantitatively assess SIX1 expression and its spatial relationship to the tumor microenvironment in archived patient specimens, aiming to identify predictive biomarkers of response.

Conclusion:

In conclusion, these data provide a translational proof-of-concept for targeting a lineage-defining transcription factor in pediatric RMS and establish the foundation for biomarker-guided clinical development of SIX1 degradation strategies.

Consequences of Age-Related G-Quadruplex Formation for the Fidelity of Mammalian Transcription

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DNA can adopt alternative conformations in living cells, including G-quadruplex (G4) structures¹. G4s are stable, four-stranded DNA configurations that can stall replication forks and impede transcription, resulting in replication stress and double-strand breaks that drive genomic instability. DNA helicases such as BRIP-1/FANCI, WRN, and BLM efficiently resolve G4s in vitro, thus preventing such instability. High-throughput sequencing has revealed that G4 structures are enriched in nucleosome-depleted regions (NDRs) controlled by transcription factors (TFs), where they not only shape transcriptional programs but also serve as epigenomic biomarkers for genome instability and gene activity^{2,3}. Moreover, many TFs directly bind to G4s to regulate transcription, indicating that G4 landscapes may predict TF activity.

Epigenetic alterations and genomic instability are hallmarks of aging and cancer, yet how these factors affect transcriptional fidelity remains unclear. Sirtuins, proteins associated with aging, decline in activity with age and are known to regulate helicases that resolve G4s and R-loops. Dysfunction in sirtuin activity may therefore lead to helicase failure, resulting in the accumulation of G4s and R-loops. This accumulation can stall polymerases, introduce transcriptional errors, and ultimately disrupt protein homeostasis.

We hypothesize that sirtuin dysfunction disrupts helicase activity, leading to increased G4 and R-loop levels, which in turn compromise transcription fidelity and proteostasis. To test this hypothesis, we will perturb sirtuin activity, identify the interacting helicases, and map genome-wide changes in G4 and R-loop structures. We will then assess the impact on transcriptional accuracy and protein folding in a replicative senescence model. This project aims to provide mechanistic insight into how age-related decline in sirtuin function contribute to genome instability and proteostasis defects.

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Roles and Mechanisms of Specific Tau Isoforms in Neuronal Development and A β -Induced Pathology in MAPT-KO Human 2D/3D Neurons and Mapt-KO Mice

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Background: Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and is characterized by the accumulation of neurofibrillary tangles composed primarily of hyperphosphorylated and misfolded TAU. TAU is a microtubule-associated protein encoded by MAPT that, under physiological conditions, contributes to microtubule stability, neuronal polarity and axonal transport. In AD and related tauopathies, pathological tau modifications cause its detachment from microtubules, somatodendritic redistribution, aggregation and neuronal dysfunction. The human brain expresses six major tau isoforms generated by alternative MAPT splicing, defined by three or four microtubule-binding repeats (3R/4R) and zero, one, or two N-terminal inserts (0N/1N/2N). Increasing evidence suggests that tau isoforms in addition to different biochemical properties have distinct, context-dependent functions in neurodevelopment and neurodegeneration. In AD A β oligomers induce tau hyperphosphorylation, mislocalization, and aggregation, while tau mediates A β -mediated synaptic dysfunction and neuronal vulnerability. Our previous studies showed that re-expression of 1N4R tau in tau knockout human neurons restores susceptibility to A β oligomer-induced neuronal dysfunction.

Objective: This project aims to systematically characterize the functional roles of distinct tau isoforms in neuronal development and AD related stress conditions. Specifically, we seek to determine how individual tau isoforms regulate neuronal vulnerability, A β mediated toxicity, tau localization, and disease associated neuronal dysfunction. Understanding isoform specific mechanisms may reveal novel therapeutic targets for tau related neurodegenerative disorders.

Methods & Preliminary Results: Human iPSC-derived glutamatergic neurons have been established from MAPT knockout (TAU KO) and wild type backgrounds. Individual human TAU isoforms will be reintroduced into TAU KO neurons using lentiviral vectors. Neuronal function, microtubule dynamics, axonal transport and APP-induced vulnerability will be assessed using electrophysiological recordings, live-cell imaging, immunofluorescence analysis and photoconversion. Three-dimensional cerebral organoids will be generated from iPSCs. TAU KO organoids will be complemented with individual TAU isoforms through lentiviral re-expression. Organoid maturation, neuronal development, microtubule organization, axonal transport and responses to A β /APP-induced pathology will be analyzed by immunostaining, live imaging, calcium imaging, and single-cell RNA sequencing. For in vivo validation, TAU knockout mouse models will be generated with isoform-specific TAU re-expression using adeno-associated viral (AAV) vectors. The effects of different TAU isoforms on neuronal development, A β -associated pathology and cognitive function will be evaluated through immunofluorescence, immunohistochemical staining and behavioral tests. Stable neuronal models expressing individual TAU isoforms have been established. Photoconversion based live-cell imaging was performed to visualize and quantify microtubule dynamics and TAU transport behavior.

Conclusion: This study will provide a comprehensive understanding of the distinct roles of tau isoforms in neuronal development and Alzheimer's disease pathogenesis. By combining human cellular models, brain organoids, and animal models, this project aims to uncover how individual tau isoforms influence A β -induced neurotoxicity and tau-related neuronal dysfunction. The findings may advance our understanding of tau biology and contribute to the identification of isoform specific therapeutic strategies for Alzheimer's disease and related tauopathies.

Macrophage Necroptosis Disrupts Tissue Architecture during Skin Repair

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How a cell dies shapes the tissue around it. Apoptosis is largely immunologically silent and drives early inflammatory macrophage clearance via FADD-mediated apoptosis during skin repair. Necroptosis is lytic: the plasma membrane ruptures and intracellular contents spill into tissue, giving it a strong immunogenic potential. Macrophages are recruited into the injured skin and carry the machinery for both death modes, yet how macrophage necroptosis affects tissue architecture and repair remains unknown.

We used full-thickness excisional skin injury in mice, in which monocyte-derived macrophages are recruited to the wounds. Using an inducible Cx3cr1^{CreER}-mediated deletion strategy, we drove a fraction of wound macrophages into necroptosis (*Fadd* deletion; FADD^{IMKO}) or enhanced apoptosis (*Birc2/Birc3* deletion; cIAP1^{IMKO}cIAP2^{-/-}). Outcomes were assessed by histomorphometry, flow cytometry, single-cell RNA sequencing (scRNA-seq), and ligand-receptor analysis (LIANA+).

Macrophage necroptosis disrupted structural integrity at mid-stage healing, producing hemorrhagic granulation tissue, a reduced reparative Ly6C^{low} macrophage compartment, and impaired myofibroblast differentiation. Enhanced apoptosis lowered macrophage numbers comparably without these defects, dissociating the phenotype from cell loss. ScRNA-seq linked the structural deficit to aberrant cell-cell communication. At 2 days post-injury (dpi), necroptosis reprogrammed neutrophils toward inflammatory, matrix-producing states and rewired neutrophil-to-fibroblast signaling via cytokine/chemokine axes. At 7 dpi, neutrophils failed to resolve and stayed elevated, while surviving macrophages signaled aberrantly to fibroblasts, shifting from SPP1- to retinoic acid-dominated signaling. Loss of SPP1 (osteopontin), a known driver of myofibroblast differentiation, was consistent with the reduced myofibroblast population detected by scRNA-seq. Concurrently, a Lyve1⁺ macrophage population with a tissue-resident, pro-regenerative signature expanded in the perilesional dermis.

Together, the mode of macrophage death, not the extent of macrophage loss, governs the structural repair outcome. By reprogramming neutrophils and surviving macrophages, necroptosis corrupts their instructive signaling to fibroblasts and limits the fibrotic response needed to maintain skin architecture. We hypothesize the expanded Lyve1⁺ regenerative macrophages are recruited to contain damage and support tissue resolution seen by 14 dpi.

The consequences of cell death depend not only on which cells are lost but on how they die, identifying macrophage death mode and its paracrine environment as a determinant of repair outcome, with implications for therapies targeting cell death in fibrosis and inflammation.

GNAS Dysfunction Alters ECM Secretion and Deposition, Causing Ectopic Skin Mineralization

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Introduction: The GNAS c.565_568del GACT variant causes dermal fibrosis and ossification by impairing Gsα-mediated cAMP signaling¹. However, the mechanisms linking reduced cAMP to pathological extracellular matrix (ECM) remodeling and osteogenic conversion of dermal fibroblasts remain unclear. We hypothesized that Gsα deficiency promotes heterotopic ossification by enhancing profibrotic signaling and dysregulating ECM secretion and deposition.

Methods: Dermal fibroblasts from patients and controls underwent proteomic and secretomic profiling, including site-specific hydroxylation analysis, with candidate targets validated by Western blotting and ELISA. Intracellular cAMP and alkaline phosphatase activity were measured, and ER-Golgi ultrastructure was assessed by transmission electron microscopy (TEM). The effects of ciprofloxacin were evaluated by TEM, proteomics, and hydroxylation analysis. GNAS knockdown in BJ-5ta fibroblasts was used to further investigate the underlying mechanisms.

Results: Patient fibroblasts showed reduced cAMP, increased TGFβ secretion and activity, and elevated ALP activity. Proteomic and secretomic profiling revealed enhanced ECM production, including collagen I, fibrillin-2, and periostin. TANGO1 was upregulated in patient fibroblasts, and pharmacological inhibition of cAMP production similarly increased TANGO1 expression². TEM revealed dilated Golgi and ER cisternae, while COL1A1 showed increased hydroxylation. Ciprofloxacin normalized ER-Golgi morphology, ECM secretion, and COL1A1 hydroxylation³. GNAS knockdown reproduced reduced cAMP and elevated COL1A1 secretion.

Discussion: Increased ALP activity indicates osteogenic commitment of patient-derived fibroblasts. Gsα deficiency disrupts TANGO1 mediated secretion, promoting profibrotic ECM deposition and ER-Golgi abnormalities. Ciprofloxacin reversed these defects, highlighting its potential for drug repurposing in rare fibrotic and heterotopic ossification disorders.

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Targeting SARS-CoV-2 Papain-Like Protease: Structure-Based Discovery of Paraherquamide E as a Selective Inhibitor

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Rationale and Objectives:

Papain-like protease (PLpro) is a crucial enzyme involved in the proteolytic processing required for SARS-CoV-2 replication. Therefore, PLpro represents a promising therapeutic target. This study aimed to identify potential PLpro inhibitors through virtual screening and evaluate their pharmacokinetic and drug-likeness properties.

Methodology:

A set of 112 molecules targeting PLpro was retrieved from the ChEMBL database based on structural features of reported SARS-CoV-2 PLpro inhibitors. The compounds were subjected to molecular docking against SARS-CoV-2 PLpro (PDB ID: 6WX4), followed by pharmacokinetic, drug-likeness, ADMET, and toxicity assessments.

Results and Conclusion:

Ten compounds demonstrated favourable binding energies compared with reported inhibitors. Among them, Paraherquamide E (ZINC000000603253) showed the most favourable docking score (–9.1 kcal/mol) and interacted strongly with key residues GLN174, SER170, PRO173, and MET206. Pharmacokinetic profiling based on Lipinski's rule of five revealed no violations for Paraherquamide E. The compound also demonstrated favourable drug-likeness, pharmacokinetic, ADMET, and toxicity profiles. Overall, Paraherquamide E emerged as a promising candidate for selective SARS-CoV-2 PLpro inhibition, warranting further preclinical experimental evaluation.

Keywords:

SARS-CoV-2, Proteases, Molecular Docking Simulation, Drug Discovery, Infectious disease

The Impact of Obesity and GLP-1 Agonists on T Cell Phenotype and Function

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Introduction:

The prevalence of obesity is continuously rising with tremendous implications for personal and public health. Obesity is associated with increased cancer risk and severe viral infections; however, underlying mechanisms remain poorly understood limiting our ability to design therapies to protect this highly vulnerable patient cohort. Glucagon-like-peptide-1 agonists (GLP-1A) have revolutionised weight loss therapy but their effect on the immune system is still unknown.

Based on findings from murine models and cancer, we hypothesize that obesity impacts antiviral T cells by inducing senescence and exhaustion due to altered lipid metabolism. We further hypothesize that GLP-1A have immunomodulatory potential irrespective of alterations in body weight.

Methods:

PBMC from obese and non-obese participants were analysed with high-dimensional multicolour flow cytometry to assess T cell phenotype and function.

Results:

While obesity did not alter T cell frequency, T cell effector function was limited after polyclonal stimulation. Obesity particularly impacts SARS-CoV-2-specific CD8⁺ T cell cytokine production while CD4⁺ T cells were not affected. Surprisingly, we also show an immunoinhibitory effect of *in vitro* GLP-1A resulting in limited proliferation, cytokine production and cytotoxicity.

Discussion:

Obesity limits T cell effector function and our future work aims to determine underlying mechanisms as well as the reversibility with weight loss. *In vitro* GLP-1A have an immune dampening effect. Considering the sudden rise in prescriptions, it will be critical to further investigate these.

Perspective:

We plan to pin down molecular mechanisms leading to the immune failure in obesity, particularly focusing on alterations in lipid metabolism and further T cell subsets such as T_{REG}. Utilizing a well-characterised cohort of participants undergoing weight-loss therapies, we will be able to assess the reversibility of these findings and pin down the *in vivo* effect of GLP-1A. Determining mechanisms leading to immune failure is critical to identify at risk populations and to suggest new immunotherapeutic targets.

Age-Dependent Metabolic Programming is Associated with Regenerative Capacity in Airway Epithelial Cells

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The respiratory tract is continuously exposed to environmental and intrinsic challenges that damage epithelial tissue, requiring efficient regenerative responses. This need is particularly critical in neonates, who must rapidly establish tissue homeostasis immediately after birth. Age-dependent metabolic programming may contribute to differences in cellular function, as previously demonstrated in neonatal monocytes, in which enhanced oxidative phosphorylation (OXPHOS) was linked to myeloid differentiation.

To investigate age-dependent differences in the regenerative capacity of airway epithelia and their association with the cellular metabolic programming, we established air-liquid interface cultures of primary nasal airway epithelial cells (AECs) collected from neonatal and adult donors. AECs were cultured using distinct medium systems providing either high or low support for epithelial differentiation. Epithelia were characterized by assessing differentiation success and cell viability, bulk RNA sequencing, profiling of single-cell energy metabolism (SCENITH), and *in vitro* wound-healing assays including live-cell imaging.

Upon transition to *in vitro* culture, neonatal AECs generated epithelial tissues with higher cellular yield and viability, despite similar culture failure rates compared with adult AECs. Transcriptomic analyses revealed distinct age-dependent epithelial profiles. Neonatal AECs showed increased expression of genes associated with mitosis and epithelial morphogenesis, together with enhanced ribosome biogenesis and OXPHOS-related pathways. In contrast, adult AECs displayed increased expression of genes associated with epithelial remodeling, ciliary function, and immune-related signaling. Consistent with the transcriptomic profile, SCENITH demonstrated higher overall metabolic activity and greater mitochondrial dependence in neonatal AECs, accompanied by lower glucose dependence compared with adult AECs. Functionally, under conditions providing lower support for epithelial differentiation, wound-healing capacity was significantly greater in neonatal than in adult airway epithelia.

Together, these findings identify age-dependent differences in the metabolic and regenerative programs of human airway epithelial cells. Neonatal AECs exhibit enhanced regenerative capacity associated with an OXPHOS-biased metabolic program and the maintenance of proliferative and morphogenetic features, particularly under conditions of limited differentiation support. These findings suggest that metabolic programming may contribute to age-dependent differences in airway epithelial regeneration.

Age-Associated G-Quadruplexes Affect DNA Stability in Regulatory Regions

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Genome instability is a hallmark of aging, yet why DNA breaks recur at regulatory DNA remains unclear. Understanding molecular vulnerabilities that facilitate DNA breakage is essential to mitigate age-related frailty and diseases in humans. DNA secondary structures such as G-quadruplex (G4) DNA structures can pose significant challenges for genome integrity, especially at transcriptionally active genes.

We have found that G4 DNA structures accumulate in promoter regions in human and murine models of Hutchinson-Gilford progeria syndrome (HGPS) and in physiologically aged *Caenorhabditis elegans*. Human and murine HGPS cells accumulate unrepaired DNA double-strand breaks (DSBs) in their genome. To directly link chromatin context to DNA breakage, we developed Cleavage Under Targets, tagmentation and DNA double-strand break capture (CUT&Break), which enables genome-wide detection of chromatin features adjacent to DSBs. Applying G4-CUT&Break in mouse liver nuclei reveals elevated G4-DSBs in HGPS vs. WT, which are associated with promoters of aberrant transcriptional hepatocyte states. This indicates a specific susceptibility of G4-forming regulatory DNA to form unrepaired DSBs which affect transcriptional regulation. Investigating the causes of stabilized G4 DNA structure formation, we identified a mechanism in which reduced protein levels of the deacetylase SIRT7 in HGPS leads to hyperacetylation of the helicase DDX21, and increased occupancy of inactive DDX21 at fragile G4 promoters. Overexpressing SIRT7 or DDX21 in HGPS cells is sufficient to reduce G4 and DSB levels.

We propose a model of aging in which a decrease in nuclear sirtuin levels leads to accumulation of compromised helicases that fail to maintain levels of secondary structures, thus promoting genome instability.

Microtubule Surface Regulation by Polyglutamylating TTLL Enzymes In Human Tauopathy Models: Implications for Neurodegeneration and Healthy Brain Aging

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Background: Neuronal maintenance critically depends on the organization and dynamic regulation of the microtubule cytoskeleton, which supports intracellular transport and neuronal connectivity. In age-associated neurodegenerative diseases such as Alzheimer's disease, microtubule dysfunction contributes to impaired axonal transport, synaptic dysfunction, and progressive neuronal degeneration. Microtubule function is dynamically regulated by tubulin post-translational modifications. Polyglutamylation, mediated by tubulin tyrosine ligase-like (TTLL) enzymes, alters the molecular properties of the microtubule surface and may thereby influence the binding and function of microtubule-associated proteins such as TAU. Dysregulation of TAU-microtubule interactions is a central feature of tauopathies and can contribute to cytoskeletal dysfunction and impaired neuronal integrity. However, how individual TTLL enzymes modulate the microtubule surface and TAU-dependent neuronal phenotypes in human neurons remains poorly understood.

Methods: We use human iPSC-derived cortical neurons to investigate the functional consequences of manipulating individual polyglutamylating TTLL enzymes during neuronal maturation and in TAU-dependent disease models. TTLL expression is modulated by lentiviral overexpression and shRNA-mediated knockdown. Neuronal morphology, dendritic architecture, tubulin post-translational modifications, and TAU localization are quantified by immunocytochemistry, including comparisons with TAU-knockout neurons to assess TAU dependence. Live-cell EB3 imaging is used to characterize microtubule growth dynamics, while multielectrode array (MEA) recordings assess neuronal network activity and functional connectivity. To investigate the relevance of TTLL-dependent mechanisms *in vivo*, AAV-mediated TTLL1 knockdown is currently being evaluated in hTAU transgenic mice.

Results: Modulation of individual TTLL enzymes produces distinct, stage-dependent effects on neuronal cytoskeletal organization and microtubule dynamics. TTLL overexpression alters the tubulin modification landscape in mature neurons, including changes in acetylation and tyrosination. TTLL1 and TTLL6 differentially affect axonal and dendritic architecture, with several phenotypes being attenuated in TAU-deficient neurons, indicating TAU-dependent effects. Live-cell imaging further revealed enzyme-specific changes in microtubule growth behavior, including increased growth velocity and run length following TTLL6 overexpression. These findings suggest that changes in the microtubule surface can reshape neuronal structure and function in a context-dependent manner. TTLL1 was prioritized for *in vivo* investigation based on its pronounced effects on neuronal morphology and previous beneficial effects in an A β context.

Conclusion: Our findings identify TTLL enzymes as regulators of the neuronal microtubule surface with consequences for cytoskeletal organization, dynamics, and TAU-dependent phenotypes. Dysregulation of this pathway may contribute to neuronal vulnerability in neurodegenerative disease.

Diet-induced Kidney Protection is Mediated by a Cell-Type-Specific Modulation of Cysteine Metabolism

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Introduction:

Acute kidney injury has no targeted therapy. Preconditioning by caloric restriction (CR) or by restriction of sulfur-containing amino acids (SR) confers potent protection against renal ischemia-reperfusion injury (IRI), yet the molecular basis of this protection and its conservation in humans have remained undefined, stalling clinical translation.

Methods:

We combined transcriptomics, proteomics and persulfidomics in a murine model of renal IRI after 14 days of CR, SR or ad libitum feeding. Because susceptibility to ischemia differs between tubular cell types, we incorporated single-nucleus RNA sequencing (snRNAseq). Mechanism was probed by cell-culture redox assays and in vivo S-sulfocysteine treatment, and conservation by snRNAseq of human pre-implantation kidney biopsies from living donors randomized to CR or SR (DILKID trial).

Results:

Both diets converged on a cell-type-specific reprogramming of cysteine metabolism, most pronounced in the highly ischemia-susceptible third segment of the proximal tubule (PTS3), where 910 genes were shared with concordant fold changes ($R^2 \approx 0.77$) and recapitulated in the proteome. CR and SR coordinately reinforced hydrogen sulfide production and oxidative cysteine catabolism toward sulfite, increased persulfidation, namely of cysteine-metabolizing enzymes following injury, and limited lipid peroxidation and ferroptosis. Sulfite supported the regeneration of glutathione from its oxidized and persulfidated forms, and its reaction product with cystine, S-sulfocysteine, entered cells through system xc⁻ to replenish the cysteine and glutathione pools. Administration of S-sulfocysteine rendered proximal tubules ROS-resistant and significantly reduced histopathological damage after IRI. snRNAseq of the human biopsies confirmed a conserved, proximal-tubule-specific induction of the cysteine-metabolizing programme (1,817 shared genes, $R^2 = 0.85$).

Discussion and Perspectives:

These data define a conserved, sulfur-based mechanism of organ protection by dietary preconditioning and nominate sulfite and S-sulfocysteine as tractable and novel therapeutic approaches for acute kidney injury.

Chromatin Guided Metabolic Reprogramming in Metabolic Dysfunction Associated Steatotic Liver Disease

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Introduction: Metabolic dysfunction associated steatotic liver disease (MASLD) is a prevalent condition affecting approximately 30% of adults worldwide, it is driven by persistent hepatic lipid storage and inflammatory stress. Emerging evidence suggests that epigenetic alterations play a pivotal role in the development of MASLD, but the responsible regulatory mechanisms remain poorly understood. One potential mediator is lysine specific demethylase 1 (LSD1/KDM1A), a FAD dependent histone demethylase and transcriptional scaffold that may regulate hepatocyte metabolic adaptation. We, therefore investigated how loss of LSD1 affect diet induced steatosis.

Methods: To determine whether LSD1 contributes to diet-induced steatosis, male mice were fed a high fat diet and LSD1 function was disrupted using complementary pharmacological and genetic approaches. LSD1 was inhibited systematically with HCI-2509 or inactivated selectively in hepatocytes through AAV-mediated activation of a conditional catalytic mutant allele. The resulting effects on steatosis and liver injury were evaluated by histology, serum transaminases, hepatic triglyceride measurements, lipidomics and circulating cytokine profiling. To identify the underlying molecular changes, we performed bulk liver RNA sequencing, followed by differential expression and gene set enrichment analysis. These studies were complemented by H3K4me2 ChIP-seq in hepatoma cells. Fatty acid challenged primary murine hepatocytes were used to assess the cell autonomous effects of LSD1 inhibition in vitro, while single nuclei RNA sequencing was performed to characterize changes in hepatocyte gene expression and cellular states after LSD1 inactivation.

Results: Systemic inhibition of LSD1 with HCI-2509 markedly reduced hepatic steatosis, serum ALT and AST levels, and several triglyceride species. Hepatocyte specific genetic model was used to study whether hepatic phenotype was due to hepatocyte specific LSD1 inactivation or not. Hepatocyte specific LSD1 inactivation reduced steatosis and liver injury without affecting body weight and produced a similar histological phenotype. Consistent with these findings, bulk liver RNA sequencing showed that HCI-2509 altered genes and partially reversed the transcriptional programme induced by a high fat diet. This response was characterized by suppression of fatty acid metabolism, PPAR signaling and oxidative phosphorylation, together with reduced expression of genes involved in lipid uptake and storage. In hepatoma cells, LSD1 inhibition showed increased H3K4me2 levels at promoters of lipid metabolic genes showing epigenetic regulation by decreased LSD1 activity. snRNA seq data showed a redistribution from pericentral towards portal like hepatocyte states, with attenuation of the pericentral lipogenic programme.

Discussion: Concordant results from in vivo and in vitro models indicate that LSD1 helps maintain a steatosis associated programme within hepatocytes. The transcriptomic and H3K4me2 results link this phenotype to coordinated regulation of lipid metabolic pathways.

Perspectives: Further studies are needed to identify the direct genomic targets of LSD1 in primary hepatocytes and establish how this chromatin changes affect lipid handling.

Aging Compromises Cellular Plasticity and Autophagic Adaptation to Changes in Oxygen Levels via Hippo Pathway

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Cellular plasticity and the ability to adapt to environmental stress are crucial for preserving tissue homeostasis and regenerative capacity as we age. Autophagy plays a key role in this process by regulating the recycling and quality control of cells in response to environmental challenges.

We investigated age-dependent adaptation to changes in oxygen concentration in primary human dermal fibroblasts obtained from young and elderly female and male donors. The cells were either maintained under physioxia (3% O₂), which mimics the physiological oxygen environment of the skin, or exposed to atmospheric oxygen (21% O₂), which represents hyperoxia, a stressful condition¹. First, we confirmed the differences in our aging model by running a transcriptomic analysis (PCA) and verifying changes in specific autophagy genes² with age (heatmap). We then ran a proteomic analysis (western blot) to check for changes in LC3B levels analysing autophagy flux capacity changes with age or oxygen concentration. To better understand the mechanisms behind the cellular response differences between the samples, we then used small interfering RNA to silence a protein and check their implications in the autophagy flux.

Integrating transcriptomic profiling with measurements of autophagic flux revealed that basal autophagic activity remained largely unchanged across age and sex when the cells were exposed to physioxia. However, increased oxygen tension only induced a marked autophagic response in young fibroblasts, as measured using proteomic analysis. Gene expression and proteomic analyses identified the differential activation of the Hippo pathway regulators YAP-TAZ in young fibroblasts suggesting a potential link between environmental sensing and autophagy that is compromised during aging. When TAZ protein was silent, we have seen a significant decreased of Δ LC3B, a marker of the autophagy flux.

Overall, our findings suggest that aging reduces fibroblast plasticity, thereby limiting their ability to adapt to environmental stress through autophagy and potentially compromising cellular resilience and tissue homeostasis. Altered Hippo pathway signalling may contribute to this loss of adaptability by disrupting the integration of environmental and mechanical responses³.

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Defining how the Pentose Phosphate Pathway Supports Innate Immune Responses during Regeneration

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Regeneration in vertebrates requires a coordinated interplay between multiple cell and tissue types, many of which radically alter their behavior after injury. Work in several species has revealed that metabolic remodeling, especially of the fate of glucose, can be a critical contributor to regenerative success [1,2]. After tail amputation in *Xenopus tropicalis* tadpoles, a switch to the pentose phosphate pathway (PPP), a key metabolic route for redox balance and biosynthesis, as the primary fate of glucose is required for successful regenerative outcomes [3]. Analysis of scRNA-seq data from regenerating tail tissue reveals that PPP enzyme expression is partitioned into two populations: proliferating cells and the immune lineage. Innate immune cells play a critical conserved role in regeneration across all vertebrates, and while they execute a range of metabolically expensive functions, their metabolic responses to injury are largely unknown [4]. Elevated PPP gene expression in *X. tropicalis* coincides with secreted LY6/PLAUR domain containing 1-like (*slurp1l*), a marker of one of two wound-responsive myeloid cell types identified in the closely related species *Xenopus laevis* [5]. The specific functions of these two cell populations are not yet known but are essential for our understanding of mechanistic links between metabolic state and pre-regenerative cellular activity. Here we define the transcriptional and metabolic identities of primitive myeloid cells in *Xenopus* and test the central hypothesis that innate immune cells rely on PPP activity to support key regenerative processes including reactive oxygen species generation, phagocytosis, or recruitment. Comparative transcriptomics have elucidated both macrophage-like and neutrophil-like *Xenopus* myeloid cell populations in addition to a cell-type specific upregulation of PPP enzymes after injury. Orthogonal PPP inhibitors were used to assess global requirements for the PPP on innate immune cell functions during regeneration. Transplantation experiments will determine if the PPP is required cell-intrinsically for immune cell responses and successful tail regeneration. Findings from this project provide the opportunity to uncover fundamental principles of the immune response to injury that can be harnessed to enhance regenerative medicine strategies across species. This project is funded by NIH R01 GM148490 to AW and NSF-GRFP DGE-2140004 to BLM.

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uORF Regulates the Subcellular Localization and Expression of the NADase Prodegenerative Protein SARM1

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SARM1 is a key executor of the SARMoptosis that triggers cell death and axonal degeneration of neuronal cells. This member of the family of Toll-like receptor adaptor proteins consumes NAD(P)⁺ to produce cADPR or NAADP, and NAM after it is activated via an increase in [NMN]/[NAD⁺] or dsDNA binding. SARM1 activation induces a cascade of metabolic rewiring and signalling in the soma and neurons that leads cells to a grim end. Its activation has been related to multiple mitochondrial dysfunctions, induced via treatments or protein ablations (SPG7, MFN2). SARM1 has been described to have non-conventional MTS and ORF-SARM1 overexpression localized it to the mitochondria, despite the presence of two starting codons in its MTS. All of this makes SARM1 a high potential therapeutic target for neurodegeneration linked to mitochondrial dysfunction.

Our study discovered that, in endogenous conditions, SARM1 has a dual localization between cytosol and mitochondria, but the vast majority is cytosolic in contrary of what is described. This is explained by the presence of an uORF in the 5'UTR of SARM1 that regulates the translation coming from the first or the second starting codon present in SARM1's MTS and also its expression. Furthermore, it seems that the presence of the 5'UTR enables SARM1 to go inside the mitochondria, when studies report it is only present on the outer membrane. We tried to determine the precise localization of SARM1 in the mitochondria, and to establish whether the cytosolic and mitochondrial forms perform the same function.

Our findings open a new area of investigation in the regulation of SARM1 expression in different tissues, but also open the possibility of a different role and interactome due to this double localization.

Shared and Divergent Transcriptional and Metabolic Programs of Glioblastoma Invasion and 5-ALA Detectability

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Introduction: Complete surgical resection of glioblastoma (GBM) is limited by its ability to infiltrate beyond the contrast-enhancing tumour margin. Guided surgery using 5-aminolevulinic acid (5-ALA) improves the intraoperative visualisation; however, infiltrative tumour regions may remain weakly fluorescent. This may contribute to residual disease and subsequent recurrence. The exact molecular programs associated with reduced 5-ALA detectability remain incompletely understood.

Methods: A secondary bioinformatic analysis of publicly available transcriptomic datasets was performed. Paired transcriptomic profiles from 10 GBM patients were obtained from E-MTAB-8743 and analysed across core, rim and invasive tumour regions. The dataset also included matched FACS-isolated 5-ALA+ and 5-ALA- fractions. Differential expression and gene set enrichment analyses were performed using Hallmark and Reactome gene sets. Regional findings were assessed in independent spatial transcriptomic dataset GSE316917. An independent single-cell dataset GSE274987 was used to evaluate malignant cell states, cell-type-specific expression of PpIX-associated genes, and the cellular distribution of invasion-associated metabolic programs.

Results: Along the Core-to-Invasive transition, metabolic remodelling was clear, with enrichment of oxidative phosphorylation and respiratory electron transport in invasive tissue, together with phospholipid- and cholesterol-associated pathways. A 24-gene respiratory module increased in invasive tissue in all 10 patients ($p = 0.0059$). Independent spatial datasets provided support for selected regional signatures and showed inter-patient heterogeneity which is characteristic of GBM. In contrast, 5-ALA-negative fractions demonstrated a clear transcriptional phenotype dominated by cell-cycle and biosynthetic programs, including E2F targets, G2M checkpoint, DNA replication and mitotic pathways. Respiratory electron transport was also enriched in the 5-ALA-negative fraction. Across 1,226 Reactome pathways, 253 were invasion-specific, 15 were 5-ALA-specific and only three were concordantly significant. Respiratory electron transport represented the principal shared metabolic component; however, the magnitude of the respiratory shifts across the two axes did not correlate at the patient level (Spearman $\rho = -0.018$, $p = 0.960$). Single-cell analysis showed that the shared respiratory module decreased from tumour core to edge in malignant cells in all four paired patients. PpIX-associated genes also demonstrated marked cellular heterogeneity, with ABCG2 predominantly expressed in endothelial cells and HMOX1 enriched in myeloid cells.

Discussion: These findings indicate that GBM invasion and 5-ALA detectability represent largely distinct biological states rather than manifestations of a single transcriptional mechanism. Their limited molecular convergence and discordance between tissue-level and malignant-cell signals support the hypothesis that the tumour microenvironment may play an important role in shaping these phenotypes.

Perspectives: Future work will integrate spatially resolved transcriptomics with machine learning approaches to disentangle how both invasion and 5-ALA status are shaped by the composition of the microenvironment and malignant cell states. This may enable prediction of poorly fluorescent infiltrative niches and support more precise and data-driven strategies for fluorescence-guided GBM resection.

Establishing a Suitable Murine Model to Study Innate Immunological Aspect of Aortic Valve Stenosis (AVS)

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Aortic valve stenosis (AVS) is a progressive disease of the aortic valve characterized by leaflet thickening, fibrosis, and calcification, which lead to narrowing of the valve orifice and obstruction to blood flow from the left ventricle into the aorta (Lindman et al., 2016; Pawade et al., 2015). Over time, this obstruction results in left ventricular hypertrophy, impaired diastolic filling, heart failure, syncope, and, if untreated, premature death (Rajamannan et al., 2011). AVS has historically been considered a degenerative and passive calcification process, but recent studies demonstrate that it is an actively regulated pathobiological process involving inflammation, immune cell infiltration, and fibrosis (Coté et al., 2012) (Goody & Jansen, 2020). Other recognized risk factors include hypercholesterolemia (elevated levels of LDL cholesterol), hypertension, smoking, diabetes mellitus, and chronic kidney disease. These factors accelerate lipid infiltration, inflammation, and calcific processes in the valve leaflets (Kjeldsen et al., 2024). As aging significantly alters immune function by shifting macrophage polarization, reducing the effectiveness of regulatory mechanisms, and promoting pro-inflammatory cytokine production, understanding the intersection of immune aging and AVS pathology could identify novel biomarkers and therapeutic targets. The objective of this project is to establish and validate suitable murine models for the induction and progression of aortic valve stenosis (AVS) in order to provide the basis for subsequent immunological investigations. Using C57BL/6 wild type, ApoE knockout, LDL receptor knockout, and *Ercc1^Δ/Δ* mice of different ages and under chow versus high-fat diet conditions, we will determine how aging and metabolic stress contribute to AVS development. Disease progression will be monitored longitudinally by echocardiography, and terminal analyses of the heart, aorta, spleen, and liver will enable histological and immunological characterization. The ultimate goal is to identify a robust and reproducible model system that reflects the clinical risk factors of AVS and can be used to investigate innate immune mechanisms in valve disease.

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Restoring Mitochondrial Resilience: From Patient-Derived Preclinical Models to Therapeutic Intervention and Setup of Clinical Trials for POLG-associated Mitochondriopathies

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Introduction: Mitochondrial dysfunction contributes to the progressive decline in cellular and neuronal function observed across a broad range of metabolic and mitochondrial disorders. Maintenance of mitochondrial DNA (mtDNA) integrity is essential for preserving mitochondrial homeostasis, yet the impact of impaired mtDNA maintenance on neurodegenerative diseases remains poorly understood. POLG-spectrum disorders (POLG-SDs), caused by pathogenic variants in the mitochondrial DNA polymerase POLG, represent a genetically defined group of disorders, displaying chronic mitochondrial dysfunction, mtDNA depletion with progressive neurodegeneration and a significantly shortened lifespan. As such, POLG-related disorders provide a unique opportunity to investigate these defining effects of mitochondrial dysfunction in patients and to develop strategies to improve mitochondrial resilience and establish a pathway toward clinical trials.

Methods: To investigate these questions, we combine patient recruitment, patient-derived cellular models and therapeutic development within a translational research framework. This also has led to preparation and planning for implementation of investigator initiated clinical trials in adults and children.

Results: Fibroblasts obtained from individuals with POLG-SDs, which are reprogrammed into human induced pluripotent stem cells (hiPSCs) and differentiated into GABAergic and glutamatergic neurons, exhibit significant reductions in mtDNA copy number, ATP production, oxidative capacity and mitochondrial biogenesis (MB). Quantitative imaging with MitoTracker and antibodies against COI, TOMM20 and dsDNA confirmed decreased mitochondrial mass and mtDNA content. Using pharmacological interventions to stimulate MB, including AICAR, cilostazol and formoterol, we have been able to partially restore mtDNA levels and improve mitochondrial bioenergetics in fibroblasts, hiPSCs and differentiated GABAergic and glutamatergic neurons.

Discussion: Building on these findings, we have begun assessing gene-based strategies aimed at restoring mtDNA maintenance and MB, including lentiviral and adeno-associated viral (AAV) delivery of key mitochondrial regulators such as POLG, POLG2, TWINKLE and TFAM. Together, findings from our pharmacological and gene-based strategies are now guiding the development and implementation of clinical trials, including off-label case studies, with first indications towards positive treatment efficiency. These planned clinical trials, for which we have already begun recruiting patients, will evaluate mitochondrial targeted therapies in paediatric and adult patient cohorts with POLG-SDs and related mitochondrial disorders.

Perspectives: By integrating preclinical cellular models with the development of interventional clinical trials, these studies establish a translational pathway for therapeutic advancement in POLG-SDs and related mitochondrial disorders. By linking human disease modelling, therapeutic discovery and clinical translation, this work seeks to identify actionable strategies to improve mitochondrial function and resilience in mitochondrial disease. More broadly, these studies provide insight into mechanisms that support mitochondrial integrity during ageing and may inform therapeutic approaches for age associated neurodegenerative disorders characterised by mitochondrial dysfunction.

Redox-Modulating and Hepatoprotective Effects of *Calpurnia aurea* Seed Extract Against HAART-Induced Toxicity in a Wistar Rat Model

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Introduction: Highly active antiretroviral therapy (HAART) remains essential for HIV management but is frequently associated with hepatotoxicity and systemic oxidative stress, complicating long-term treatment. Increasing evidence indicates that HAART-induced toxicity is mediated by redox imbalance and impaired antioxidant defense systems. *Calpurnia aurea*, a medicinal plant widely used in Ethiopian traditional medicine, is rich in bioactive compounds with potential antioxidant properties. This study investigates the redox-modulating and hepatoprotective effects of *C. aurea* seed extract in a HAART-induced toxicity model.

Methods: Hydroethanolic (70%) extract of *C. aurea* seeds was prepared and subjected to phytochemical and in vitro antioxidant analysis. Male Wistar albino rats (n = 30) were divided into five groups and treated with HAART and graded doses of the extract (100, 200, and 300 mg/kg) for 35 days. Hepatic injury was assessed by measuring serum biomarkers (ALT, AST, ALP, total bilirubin), while oxidative stress and antioxidant defense were evaluated through superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA), and total antioxidant capacity (TAC). Histopathological analysis of liver tissue was performed to assess structural alterations.

Results: HAART administration significantly increased hepatic enzyme levels, lipid peroxidation (MDA), and disrupted antioxidant defense systems (decreased SOD, CAT, GSH, and TAC), indicating pronounced oxidative stress and hepatocellular injury. Treatment with *C. aurea* extract, particularly at 300 mg/kg, significantly ($p < 0.05$) restored antioxidant enzyme activity, reduced lipid peroxidation, and normalized liver function biomarkers. Histopathological findings confirmed attenuation of hepatic damage, with restoration of near-normal cellular architecture in extract-treated groups.

Discussion: HAART-induced hepatotoxicity was closely associated with oxidative stress and disruption of redox homeostasis, as shown by increased MDA and decreased SOD, CAT, and GSH, indicating excessive ROS generation and hepatocellular injury. *Calpurnia aurea* seed extract significantly reversed these alterations in a dose-dependent manner, restoring antioxidant defenses and improving liver histoarchitecture. This suggests strong redox-modulating and cytoprotective effects, likely through free radical scavenging and enhancement of endogenous antioxidant systems. These findings support the role of polyphenol-rich natural products in maintaining cellular redox balance and protecting against drug-induced oxidative damage.

Perspectives: Future studies should isolate and characterize the active phytochemicals responsible for the observed effects and investigate key redox signaling pathways such as Nrf2/ARE. Further work should evaluate pharmacokinetics, long-term safety, and optimal dosing in preclinical models. Translational studies are needed to assess clinical relevance in patients receiving long-term HAART. Overall, this work highlights *Calpurnia aurea* as a promising redox-active candidate and supports integration of ethnopharmacology into redox-targeted therapeutic strategies for managing drug-induced hepatic toxicity.

Ether Lipids Are Conserved Mediators of Diapause Across Species

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Introduction:

Diapause, a state of reversible organismal quiescence triggered by environmental stress, dramatically extends lifespan across species. Identifying molecular regulators of diapause-associated longevity could therefore reveal conserved strategies for healthy aging.

Methods:

Using untargeted lipidomics in *C. elegans* starvation-induced adult reproductive diapause (ARD), we profiled lipidome-wide changes upon diapause entry and exit. We characterized ether lipid biosynthesis mutants (*ads-1/AGPS*, *fard-1/FAR1*, *acl-7/GNPAT*) for survival, healthspan, biological aging (proteomics-based aging clock) during diapause, and somatic and germline stem cell resilience upon diapause exit. Cross-species conservation was assessed by pharmacological AGPS inhibition in killifish embryos and amino acid-starved cancer cells.

Results:

Ether lipids are strongly upregulated upon ARD entry. Ether lipid biosynthesis mutants exhibit reduced ARD survival, diminished healthspan, accelerated biological aging during diapause, and impaired somatic and germline stem cell resilience upon refeeding. Interestingly, the plasmalogen-deficient mutant (*tmem-189*) is unaffected, implicating non-plasmalogen alkyl-acyl ether lipid species as the active effectors. AGPS inhibition similarly prevents diapause entry in killifish embryos and sensitizes cancer cells to amino acid starvation, demonstrating evolutionary conservation. Mechanistically, ether lipid-deficient animals exhibit lysosomal dysfunction and mitochondrial fragmentation during ARD.

Discussion:

These findings establish ether lipids as essential and conserved mediators of diapause survival. The evolutionary conservation of this requirement, from nematode to killifish to cancer cell dormancy, suggests ether lipids are broad mediators of the diapause program rather than a species-specific adaptation. As ether lipid biosynthesis declines with age, its induction during diapause may represent an ancient mechanism for coupling nutrient sensing to longevity and opens new avenues for extending healthspan through ether lipid precursor supplementation.

Abundance Regulation of the Selective ECM Cargo Receptor TANGO1 via the mTORC1/ULK1 Axis

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The endoplasmic reticulum (ER) orchestrates the secretion of extracellular matrix (ECM) proteins, many of which exceed the size of conventional transport vesicles and therefore require specialized export machinery. TANGO1, encoded by *MIA3*, organizes ER exit sites (ERES) for bulky cargo export, yet the molecular basis of cargo recognition and its relationship to the collagen-specific chaperone HSP47 remain unclear.

Through quantitative secretome profiling of TANGO1- and HSP47-deficient fibroblasts, organelle immunoprecipitation, trafficking analysis, structural modeling, and binding assays, we show that TANGO1 directly and selectively recognizes a defined subset of ECM proteins, including specific collagen and fibrillin isoforms, through a conserved tyrosine residue in its luminal MOTH domain, independently of HSP47.

Novel *MIA3* variants identified in individuals with previously undescribed skeletal dysplasia disrupt this cargo-binding interface, resulting in selective intracellular retention of ECM proteins. These findings identify defective cargo recognition, rather than impaired ERES assembly, as a primary molecular defect underlying *MIA3*/TANGO1-associated skeletal dysplasia.

Furthermore, we identify a nutrient-sensitive mechanism controlling TANGO1 abundance at ERES membranes through the mTORC1/ULK1/RHBDL4 axis. Our data suggest that this pathway regulates collagen export from the ER in response to nutrient availability, thereby linking cellular nutrient sensing to ECM secretion.

Together, our findings establish TANGO1 as a selective ECM cargo receptor that functions independently of HSP47 and reveal that its abundance at ERES is actively regulated by the mTORC1 nutrient-sensing pathway. This provides a framework for understanding both selective bulky cargo export and the quantitative regulation of collagen secretion at the ER, and suggests that dysregulation of TANGO1 abundance may contribute to pathological ECM accumulation and fibrosis.

Uncovering SLUG's Role in Shaping Drug Tolerance in Non-Small Cell Lung Cancer (NSCLC)

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Targeted therapies have transformed lung cancer treatment by selectively eliminating oncogene-driven tumor cells. However, a cancer cell subset — known as drug-tolerant persister cells (DTPs)—survives initial therapy. These cells enter a reversible, slow-cycling state, serving as a reservoir for disease relapse and the eventual emergence of resistance. Although DTPs rely on distinct transcriptional programs to endure drug pressure, the regulatory mechanisms that support their survival remain poorly understood.

Through comparative analysis of DTPs from different models, we identified phenotypic adaption – particular mesenchymal transition – as a consistent hallmark. This transition is driven by cytokines such as therapy-induced TGFβ2, and transcriptionally orchestrated by core epithelial-to-mesenchymal transition (EMT)-transcription factors, among which SLUG is consistently upregulated.

Using integrated chromatin occupancy and transcriptomic analyses, we mapped SLUG's and MYC's genome-wide targets in DTPs and uncovered its involvement in cell cycle regulation. Notably, SLUG binds to genomic regions typically targeted by MYC, which is suppressed in the DTP state. CRISPR/Cas9-mediated knockout of SLUG, combined with reconstitution experiments, confirmed that DTPs rely on this compensatory mechanism for survival. Loss of SLUG led to the downregulation of proliferation-associated genes, limiting the proliferative capacity and ultimately resulting in their depletion – effectively preventing relapse.

Our study identifies SLUG as an essential survival factor in the persister state. While MYC protein levels are reduced in DTPs, SLUG binds to MYC target genes involved in cell cycle regulation. This compensatory occupancy reveals a novel mechanism of transcriptional plasticity in DTPs and suggests a potential therapeutic vulnerability to prevent resistance in NSCLC.

Impact of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) on Immune Control of Chronic Hepatitis B Virus Infection

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Chronic hepatitis B virus (CHB) infection is a major global health issue, with severe complications such as cirrhosis and hepatocellular carcinoma (HCC). Over the last few decades, the incidence of metabolic syndrome has increased rapidly, making metabolic dysfunction-associated steatotic liver disease (MASLD) the second-leading cause of liver disease. Comorbidity CHB and MASLD is therefore frequent and affects the clinical course and outcome of CHB. However, little is known about the underlying immunological mechanisms. We aim to address this knowledge gap.

We will collect peripheral blood mononuclear cells (PBMCs) to comprehensively analyze the repertoire, phenotype, and function of HBV-specific CD8⁺ T cells in overall 50 matched patients with or without concurrent MASLD. Rare populations of HBV-specific CD8⁺ T cells will be identified ex vivo by peptide/MHC-I tetramer enrichment and analyzed by multi-parametric flow cytometry. Repertoire and function will be determined by multi-parametric flow cytometry after peptide-specific in vitro expansion of HBV-specific CD8⁺ T cells. Moreover, the overall immune landscape will be analyzed in serum by cytokine profiling and in liver biopsy samples by single-cell RNA-sequencing.

A retrospective analysis of data from treatment-naïve, HBeAg-negative CHB patients with and without concurrent MASLD by Huang et al.¹ showed that MASLD facilitated loss of HBsAg (seroclearance), which is associated with a lower risk of HCC, when the cumulative incidence of seroclearance was analyzed after a 5-year follow-up. Patients with CHB only also showed a nearly four-fold higher viral load compared with patients with concurrent CHB and MASLD¹. However, they also observed that CHB and concurrent MASLD was associated with a higher risk of all-cause mortality compared with CHB and steatosis only².

We are currently establishing laboratory methods and are in the process of collecting data. Our results will contribute to the understanding of the underlying differences in the adaptive immune response in viral hepatitis, MASLD, and their comorbidity. The results will enhance understanding of immunopathological principles in chronic HBV infection. They will also have implications for the development of immunotherapeutic approaches for the cure of chronic HBV infection, which will most likely need to be adapted in the setting of chronic HBV/MASLD comorbidity.

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2. Huang SC, Su TH, Tseng TC, et al. All-cause and cause-specific mortality in patients with chronic hepatitis B and concurrent steatotic liver disease. *Journal of Hepatology*. 2025;83(1):43-51. doi:10.1016/j.jhep.2024.12.009

Assessing the Role of Olfactory Receptor 2 in a Diet-Induced Obesity Animal Model

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Adipose tissue macrophages (ATMs) are innate immune cells that play critical roles in regulating inflammation and metabolism. In obesity, the expansion and activation of ATM populations contribute to chronic low-grade inflammation, a key driver of metabolic dysfunction and associated systemic complications.

Recent studies have revealed substantial heterogeneity among ATMs and identified lipid-associated macrophages (LAMs) as a predominant population in obese adipose tissue. However, the mechanisms governing the functional state of LAMs and their contribution to obesity-induced inflammation remain poorly understood. Olfactory receptor 2 (Olf2), an odorant receptor activated by octanal, has emerged as a mediator of inflammatory signaling in macrophages.

We identified Olf2-expressing LAMs in epididymal adipose tissue, with their numbers increasing in obese mice. Notably, Olf2^{-/-} HFD-fed mice exhibited reduced body weight and adiposity compared with Olf2^{+/+} HFD-fed mice. This phenotype was accompanied by improved glucose tolerance and reduced expression of proinflammatory cytokine genes.

Single-cell transcriptomic analysis revealed enrichment of oxidative stress and redox-related pathways in Olf2^{-/-} LAMs, suggesting enhanced antioxidant responses to the metabolically stressed environment of obesity. Together, these findings identify Olf2 as a potential regulator of LAM functional state and suggest that Olf2-expressing LAMs represent a promising therapeutic target for mitigating obesity-induced inflammation.

Keywords: Adipose tissue macrophages, olfactory receptor 2, inflammation

Epigenetic Reprogramming of FOXA1-MYC Networks Underlying Acquired Chemoresistance in Small Cell Lung Cancer

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Small cell lung cancer (SCLC) is a highly aggressive cancer characterized by the almost universal acquired resistance to chemotherapy treatment. Epigenetic mechanisms, including transcription factor (TF) occupancy and chromatin remodeling are increasingly recognized as central determinants of SCLC tumor heterogeneity, subtype identity and also acquired resistance to chemotherapy. However, the molecular mechanisms by which specific TF programs drive chemotherapy resistance remain a major unmet challenge.

Our results from integrative analysis of multiomics data, including single-nuclei (sn)RNA-seq, snATAC-seq and snDynaTag, revealed a notable relationship between FOXA1 and MYC occupancies and their regulated gene networks. In SCLC PDX tumors, FOXA1 and MYC showed extensive co-occupancy at the same promoter regions. By contrast, in cisplatin-resistant SCLC cells, this co-occupancy was replaced by a redistribution of MYC away from FOXA1-bound targets toward genes associated with pluripotency and cellular reprogramming, consistent with MYC's established role as one of the Yamanaka factors. Of note, in SCLC-resistant cells, gained FOXA1 occupancy is associated with a neuroendocrine identity program, whereas MYC gains occupancy at stemness related genes, thus suggesting an epigenetic switch from co-occupied FOXA1-MYC-driven neuroendocrine identity toward a stem-like chemo-therapy-resistant state. These findings establish FOXA1 and MYC as relevant epigenetic regulators following chemotherapy treatment of SCLC and reveal their interplay as a candidate therapy escape mechanism.

As follow-up, we will further comprehensively define whether FOXA1 is causally required to establish MYC occupancy-driven resistance programs and ultimately reveal the causal epigenetic mechanisms underlying SCLC chemotherapy sensitivity and acquired resistance. Altogether, the ultimate goal of this project will be the translation of these findings into novel diagnostic and therapeutic strategies for this aggressive malignancy.

Tuning Helicase Activity to Counteract G4 Structures and Interconnected DNA Damage in Premature Aging

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Guanine quadruplexes (G4s) are secondary, four-stranded structures formed by guanine-rich nucleic acid sequences and stabilized by Hoogsteen hydrogen bonding¹. They are enriched in regulatory genomic regions, where they influence gene expression and genome stability. Notably, G4 structures can induce DNA double-strand breaks (DSBs)^{2,3}, making them important contributors to genomic instability and potentially aging. G4 helicases, such as DEAD-box helicase 21 (DDX21), bind and resolve G4 structures⁴, thereby limiting their accumulation. DDX21 activity is upregulated by post-translational modifications, including deacetylation by sirtuin 7 (SIRT7) at lysine residues K18, K137, and K600⁵.

Hutchinson-Gilford progeria syndrome (HGPS) is a premature aging disorder characterized by elevated levels of progerin, which destabilizes SIRT7 and promotes its proteasomal degradation⁶. We hypothesize that SIRT7 loss results in increased levels of acetylated, less active DDX21, leading to G4 accumulation, DNA damage, and accelerated aging phenotypes.

To test this, we used CRISPR activation (CRISPRa) to upregulate endogenous DDX21 or SIRT7 in HGPS-derived induced mesenchymal stem cells (HGPS-iMSCs). Overexpression of either factor significantly reduced G4 structures and DNA damage (γH2AX), as shown by immunofluorescence staining. Gene expression analysis revealed decreased expression of the senescence marker p21 and increased levels of the proliferation marker Ki67. Consistently, β-galactosidase staining uncovered a reduced proportion of senescent cells, suggesting partial rejuvenation *in vitro*.

To further investigate the role of DDX21 acetylation, we expressed wild-type, hyperacetylation-mimic (DDX21^{3KQ}), and hypoacetylation-mimic (DDX21^{3KR}) variants in HEK293T cells. Following SIRT7 knockdown, DDX21^{3KQ} increased G4 accumulation and γH2AX levels, whereas DDX21^{3KR} had the opposite effect. These findings demonstrate that DDX21 acetylation critically regulates G4 resolution and genome stability. Overall, our results suggest that restoring DDX21 and SIRT7 activity mitigates G4-associated genomic instability and rescues aging-related phenotypes, highlighting a potential therapeutic strategy for HGPS and age-associated pathologies.

¹ Hänsel-Hertsch et al., 2016. G-quadruplex structures mark human regulatory chromatin. *Nat Genet*, 48(10), 1267-1272

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³ Lensing et al., 2016. DSBcapture: in situ capture and sequencing of DNA breaks. *Nat Methods*, 13(10), 855-857.

⁴ McRae et al., 2017. Human DDX21 binds and unwinds RNA guanine quadruplexes. *Nucleic Acids Res*, 45(11), 6656-6668.

⁵ Song et al., 2017. SIRT7 and the DEAD-box helicase DDX21 cooperate to resolve genomic R-loops and safeguard genome stability. *Genes Dev*, 31(13), 1370-1381.

⁶ Sun et al., 2020. Vascular endothelium-targeted Sirt7 gene therapy rejuvenates blood vessels and extends life span in a Hutchinson-Gilford progeria model. *Sci Adv*, 6(8), eaay5556.

Characterizing B-Cell Lymphoma-Induced T-Cell Dysfunction to Design CRISPR-Engineered CAR T Cells

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Chimeric antigen receptor (CAR) T cell therapy induces durable remissions in B cell non-Hodgkin lymphoma (B-NHL), yet a substantial fraction of patients exhibits primary resistance. The tumor cell intrinsic mechanisms underlying non-response prior to therapy remain poorly defined. Here, we investigate whether pre-existing T cell dysfunction within the lymphoma microenvironment can instruct the design of novel CRISPR engineered CAR T cells.

We analyzed single-cell RNA sequencing (scRNA-seq) data from our previously published cohort comprising lymphoma biopsies from B-NHL patients collected prior to CAR T cell therapy. Application of multiple curated T cell dysfunction gene signatures revealed elevated dysfunction scores in CD8⁺ T cells from patients exhibiting a non-durable response (NDR). RNA velocity analysis indicated that these cells occupy stable transcriptional states consistent with terminal exhaustion. To identify lymphoma cell signals associated with this phenotype, we performed cell–cell interaction analysis using CellPhoneDB. Comparative analysis of ligand–receptor interactions revealed a specific signaling axis ligand Y – receptor X to be enriched in NDR samples. To functionally interrogate this interaction, we successfully leveraged our non-viral platform to simultaneously knock-in an anti-CD19 CAR into the TRAC locus and knock-out receptor X. Ongoing studies aim to define the impact of disrupting this inhibitory axis on CAR T cell activation, proliferation, dysfunction, and antitumor efficacy.

Collectively, our work identifies tumor–T cell inhibitory interactions present prior to therapy as potential drivers of CAR T cell resistance, and provides a rationale for engineering innovative CAR T cell products with CRISPR/Cas9.

PEAR1 Signaling Regulates Fibroblast Phenotype and Is Necessary for Spinal Cord Regeneration in Zebrafish

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After spinal cord injury (SCI) in mammals, fibroblasts can transition to a myofibroblast phenotype, characterized by increased contractility, extracellular matrix (ECM) production, and proliferation. Myofibroblasts contribute to a fibrotic scar that impairs axonal regrowth in mammals (1).

In contrast, in larval zebrafish, fibroblasts support spinal cord regeneration, which leads to return of swimming function within 48 hours post-lesion (2). The Platelet Endothelial Aggregation Receptor 1 (PEAR1) has been shown to suppress the myofibroblast state (3) and preliminary analysis of our single-cell RNA-seq data during spinal cord regeneration in zebrafish larvae identified *pear1* expression in lesion-associated *pdgfrb*⁺ fibroblasts. We hypothesized that Pear1 controls the myofibroblast state to promote spinal cord regeneration. *In situ* hybridization confirmed enriched expression of *pear1* in lesion site fibroblasts. Somatic mutation of *pear1* completely abrogated spinal cord reconnection.

Additionally, spinal cord-associated fibroblasts were decreased in volume and showed a more elongated morphology, as well as increased proliferation, consistent with a myofibroblast state. Nevertheless, ECM deposition was not detectably altered.

Currently, we are testing the role of potential Pear1 ligands in these processes. Our preliminary findings suggest that Pear1 signaling regulates pro-regenerative fibroblast behavior after SCI in zebrafish.

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This work is support by the Alexander von Humboldt Professorship Award (to CGB) and by the Technische Universität Dresden (Core Funding to CGB).

Dendritic Cell Plasticity: How the Cytokine Environment Shapes Phenotype and Tolerogenic Responsiveness

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Introduction:

Dendritic cells (DCs) are central to immune regulation at the ocular surface and represent potential cellular vehicles for antigen-specific tolerance. For allergic conjunctivitis, however, the suitability of individual DC populations for tolerogenic conditioning remains poorly defined. We investigated how DC developmental origin, differentiation environment, and timing of tolerogenic exposure influence DC phenotype and plasticity.

Methods:

Bone marrow-derived DCs were generated with Fms-like tyrosine kinase 3 ligand (FLT3L) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Classical DC type 1, classical DC type 2, plasmacytoid DC, and monocyte-derived DC populations were resolved by multiparameter flow cytometry. Rapamycin, interleukin-10, or dexamethasone was administered either throughout differentiation (days 1–8) or after differentiation (days 6–8). DC phenotypes from spleen, lymph node, and conjunctiva were characterized in parallel, and spleen- and lymph node-derived DCs were assessed following short-term tolerogenic stimulation.

Results:

FLT3L and GM-CSF generated markedly different DC landscapes: FLT3L favored classical DC populations, whereas GM-CSF produced a heterogeneous inflammatory compartment enriched in monocyte-derived DCs and CD11b⁺ classical DC type 2 cells. Tolerogenic conditioning during differentiation altered both subset distribution and activation phenotypes, while treatment after differentiation produced comparatively limited changes. GM-CSF-derived DCs were particularly susceptible to phenotypic modulation, including reduced co-stimulatory marker expression and increased monocytic features, whereas FLT3L-derived populations remained more stable. Conjunctival DCs were characterized by a prominent monocyte-enriched profile. Spleen- and lymph node-derived DCs showed distinct responses to short-term tolerogenic stimulation, highlighting differences between tissue-derived and bone marrow-derived DCs.

Discussion:

Our findings demonstrate that tolerogenic DC programming is not a uniform process but depends on the developmental context in which DCs arise and when conditioning is applied. The contrasting behavior of FLT3L- and GM-CSF-derived populations indicates that commonly used DC generation systems may yield substantially different substrates for tolerance induction. Tissue-specific phenotypes further emphasize the importance of considering physiological DC origin when translating in vitro tolerogenic strategies toward ocular immunity.

Perspectives:

Defining the relationship between DC origin, subset identity, and conditioning response may enable rational selection of cellular populations for tolerogenic approaches. These findings support further development of subtype- and timing-optimized DC-based vaccination strategies for allergic conjunctivitis and other ocular inflammatory diseases.

Exploring Macrophage-Fibroblast Communication as Driver of Regenerative versus Fibrogenic Wound Initiation

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In most organs mammalian tissue damage results in fibrotic repair leaving a scar behind. Scar tissue imposes an immense burden on the organism, as it perturbs tissue architecture and function and ultimately leads to complete organ failure. In contrast, there are regenerative organisms such as the axolotl, the naked mole rat and the zebrafish, which are capable of replacing injured tissue, e.g., skin wounds or even entire organs without scar formation. The mechanisms by which some organisms are able to regenerate, while others are not, remains unresolved. As the primary producers of scar tissue, but also as main players in true regeneration, fibroblasts represent a central target for understanding mechanisms of different healing outcomes. Immune signals, in particular macrophages, are emerging as critical sensors of tissue damage and orchestrators of fibroblast differentiation in regenerative vs. fibrogenic wound healing.

We hypothesize that macrophage-fibroblast communication is a critical regulatory axis directing wounds toward fibrosis or regeneration. To investigate cellular communication over the time course of wound healing we generated scRNA-seq datasets of full thickness dorsal mouse skin wounds covering the spectrum from early- towards late-stage healing phases (naïve skin, 4, 7, 14 days post-injury). We focused our investigation on the annotation of wound macrophages and fibroblasts and their communication.

scRNA-seq data was pre-processed and clustered according to standard protocols. Broad cell types were annotated based on cluster-specific expression of known canonical markers from the single-cell literature. Macrophages and fibroblasts were further divided into subpopulations. Macrophages were annotated according to the framework published by Sanin et al. (2022)¹. Fibroblasts were annotated based on the model developed by Gallegos et al. (2023), which describes a wound induced stepwise transition of fibroblasts towards a fibrotic myofibroblast state during mouse skin wound healing².

Macrophages showed a distinct shift in subpopulation composition from early towards late stages of healing. Fibroblast subpopulations also changed over the course of repair and largely reverted towards the naïve skin state during late phase of wound resolution. Interestingly three wound-induced fibroblast subpopulations were identified that were specific to the injury and disappeared at the late healing stage. Macrophage-fibroblast communication analysis showed specific interactions between macrophage subpopulations and these wound-induced fibroblast populations. Investigation of interaction networks indicated that macrophages direct wound-induced fibroblast transitions.

We anticipate that insights gained from these studies will elucidate mechanistic underpinnings of opposing healing outcomes and ultimately identify druggable targets to prevent scar formation and to promote regeneration.

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Sci. Immunol. 2022, 7,eabl7482

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Unlocking the Temporal Sequence of Sensory Dysfunction Diabetic Neuropathy

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Diabetes mellitus is a group of diseases characterized by persistent hyperglycemia. Type-II diabetes (T2D) accounts for many diabetes patients worldwide and it is caused by relative insulin deficiency resulting from insulin resistance in target cells¹. Diabetic neuropathy (DN) is the most prevalent complication of diabetes², leading to sensory dysfunctions and chronic pain. As DN progresses, some peripheral sensory neurons residing in the dorsal root ganglia (DRGs) degenerate, while others become hyperactive or hypertrophic, thereby altering sensory processing^{3,4}. The heterogeneity of sensory neurons has hindered our understanding of the selective vulnerability of individual DRG neuron types to diabetic stress. Here, we aim to define the early anatomical and functional changes in DRG neurons to better understand the pathophysiology of DN.

We established a mouse model recapitulating the temporal progression and metabolic characteristics of T2D-associated DN, and we developed an integrative framework to define how behavioral and anatomical changes correlate with sensorimotor dysfunctions. Mice of both sexes were randomly selected and placed on chow diet (control), 60% kcal high-fat diet (HFD; pre-diabetes), or HFD combined with streptozotocin (HFD+STZ; diabetes) to induce persistent hyperglycemia through pancreatic β -cells depletion. We identified three distinct phases of DN progression: pre-diabetes, during which mice displayed transient hyperglycemia and reduced sensitivity to touch; early diabetes, characterized by persistent hyperglycemia and a further reduction in tactile sensitivity, and late diabetes, when mice developed motor coordination deficits, became insensitive to touch, while also exhibiting hyperalgesia.

Next, we assessed changes in peripheral sensory axons and sensory end organs, including muscle spindles and Meissner corpuscles, at the experimental endpoint across control, HFD, and HFD+STZ mice. Surprisingly, muscle spindles, which are proprioceptive sensory organs located within skeletal muscles, did not show anatomical signs of degeneration. Instead, they appeared to be more numerous in both HFD and HFD+STZ mice compared with controls, suggesting that mechanisms other than structural loss of muscle spindles may contribute to the observed motor deficits. In contrast, Meissner corpuscles, mechanoreceptive sensory organs located in glabrous skin, were hypertrophic in pre-diabetic HFD mice but showed partial degeneration and structural remodeling in diabetic HFD+STZ mice.

Our findings are consistent with clinical observations that patients with DN can simultaneously experience sensory loss and hyperalgesia, while also being at increased risk of falls due to impaired motor coordination². Future experiments focusing on the anatomical characterization at intermediate disease stages will help pinpoint the selective loss or hypertrophy of specific DRG neuron types underlying the onset and progression of DN. This work may ultimately enable the development of targeted therapeutic strategies for DN, with the goal of preventing or delaying the further progression of sensory dysfunction.

¹ Tuomi et al., 2014² Feldmann et al., 2019³ Fukami et al., 2017⁴ Yang et al., 2025

Identification and analysis of differentially expressed genes during wound healing

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Introduction: Re-epithelialization is one of the fundamental stages for wound healing. The disruption of this process inevitably leads to chronic non-healing wounds. Understanding the transcriptional networks during skin regeneration is critical for uncovering reliable markers for diagnostics and targeted treatment.

Methods: Secondary analysis of the public genome-wide GSE28914 dataset was conducted using R. Preprocessed expression data for approximately 54,000 probes across 25 biopsy samples were extracted from the Series Matrix file. Alongside metadata detailing patient identifiers and timepoints (Intact, Acute, Day 3, and Day 7) was structured. Following this, statistically significant candidate genes were imported into JASP for descriptive statistics, linear mixed-effects models, estimated marginal means, contrasts, and correlation profiling.

Results: Comprehensive genome analysis revealed extensive stage-dependent transcriptional remodeling during epidermal wound healing. A total of 26,638 probes showed a significant overall temporal effect after Benjamini-Hochberg correction ($FDR < 0.05$). Distinct expression patterns were observed across Acute, Day 3, and Day 7 compared with intact skin. Among the top-ranked genes were IL37, KRT2, MMP1, PI3, SOX6, SERPINB4 and TIMP1, reflecting changes in extracellular matrix remodeling, epithelial differentiation, and inflammatory or defense-associated pathways.

In response to epithelial barrier disruption, PI3 and SERPINB4 are significantly upregulated to defend local tissue from immune cell proteases ($r = 0.99$, $p < 0.001$). Subsequent matrix remodelling is characterized by a strong positive correlation between MMP1 and its inhibitor TIMP1 ($r = 0.97$, $p < 0.001$). This is highly likely due to the organism's self-regulation of the extracellular matrix disassembly and organization. In addition, MMP1 is thought to aid in keratinocyte migration across the collagen matrix during re-epithelialization. However, this proteolytic process temporarily slows the cell differentiation, and that explains the inverse correlation with KRT2 ($r = -0.64$, $p < 0.001$ with MMP1) ($r = -0.73$, $p < 0.001$ with TIMP1). KRT2 expression restores during later stages of wound healing, as it is known to be responsible for terminal cornification and epidermal barrier restoration. SOX6 was also found to be upregulated at later time points. Given that it stimulates keratinocyte proliferation and inhibits epithelial-mesenchymal transition, this indicates that both of the genes play critical roles in the completion of re-epithelialization ($r = 0.95$, $p < 0.001$ with KRT2). Moreover, the co-expression of IL37, an anti-inflammatory cytokine, suggests that skin regeneration and inflammation suppression occur simultaneously ($r = 0.97$, $p < 0.001$ with KRT2) ($r = 0.94$, $p < 0.001$ with SOX6).

Discussion: These findings demonstrate a tightly regulated molecular mechanisms during cutaneous wound healing, shifting from antiprotease defense (PI3, SERPINB4) to matrix remodelling (MMP1, TIMP1) and late barrier formation (KRT2, SOX6). Disruption of these key biological checkpoints may contribute to formation of non-healing chronic wounds.

Perspectives: The genes analyzed in this study represent promising biomarkers for monitoring wound healing and detecting possible tissue repair dysfunctions. However, further research is needed for the functional validation in 3D skin models and evaluation of these potential prognostic indicators for non-healing wounds.

Interferon- γ -Signaling Regulates Local Tissue Perfusion, Tissue Oxygenation and Antimicrobial Control

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Low levels of oxygen (O₂) prevail in infected or inflamed tissues. Low lesional O₂ levels promote the survival of the intracellular parasite *Leishmania (L.) major*, whose control depends critically on the ability of macrophages to produce high levels of NO through the O₂-dependent antimicrobial enzyme type 2 nitric oxide synthase. However, the mechanism leading to local O₂ deficiency in the infected lesion is unknown. The focus of this study is to understand the regulation of oxygenation and perfusion in *L. major*-infected tissues.

Using non-invasive optical imaging of O₂ and LASER speckle perfusion measurements in a C57BL/6 (healer) / BALB/c (non-healer) mouse model, we observed a substantial increase in tissue perfusion in C57BL/6 mice, but not in BALB/c mice, which was paralleled by normalization of O₂ levels and clinical resolution of disease. Global transcriptomic analysis revealed an enrichment of interferon- γ -dependent signaling in lesional endothelial cells, fibroblasts, T cells and myeloid cells. Therefore, we tested the contribution of adaptive immune system in mice lacking T and B cell (Rag2-KO). Rag2-KO animals on C57BL/6 background showed a non-healing course without an increase in perfusion and no normalization of tissue O₂, which could be rescued by adaptive T cell transfer. In contrast, adaptive T cell transfer from interferon- γ knockout animals was not able to improve tissue perfusion and displayed non-resolving course of infection.

We show that tissue perfusion serves as a driver for O₂ normalization and lesion healing. In addition to their antimicrobial role, T cell-dependent interferon- γ signaling contribute to an appropriate immunovascular tissue response, which is critical for infection control and resolution.

DNA-Methylation Profiling of CLL Patients with Complex and Non-Complex Karyotypes

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Background:

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of malignant B cells with clinically heterogeneous outcomes. The TP53 status is a potent predictor for disease progression with patients harbouring WT TP53 exhibiting an overall superior prognosis. Nonetheless, a subset of patients with no detectable TP53 alterations experience an adverse disease course. These patients often share a higher karyotype complexity, defined as three or more chromosomal abnormalities, a known predictor for adverse outcomes. Epigenetic alterations play a critical role during CLL pathogenesis through aberrant global DNA hypomethylation and promoter specific hypermethylation at tumor suppressor sites. Moreover, DNA methylation signatures can classify CLL into subtypes with prognostic implications, highlighting the complexity of CLL disease progression beyond TP53. As part of CLL-CLUE we aim to elucidate the genetic and epigenetic background of the phenotypes related to complex and simple karyotypes during disease progression to uncover the mechanisms that drive karyotype high-risk CLL subgroup and identifying novel diagnostic and therapeutic approaches.

Early Clonal Divergence Shapes Evolutionary Trajectories of Neuroblastoma

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Introduction: Neuroblastoma, the most common extracranial solid tumor in childhood, follows a strikingly heterogeneous clinical course, ranging from spontaneous regression to fatal outcome. A central challenge in high-risk neuroblastoma is treatment-refractory relapse: Patients initially respond well to first-line therapy, yet metastatic relapse carries an overall survival below 10%. How these tumors evolve under and in the absence of treatment pressure remains poorly understood, as most genomic studies rest on the investigation of single timepoint biopsies. Resolving this evolutionary trajectory is key to understanding mechanisms of resistance and to identifying treatment options. We reconstructed the evolutionary history of 61 neuroblastoma patients across multiple timepoints of the disease course.

Methods: Patients were eligible if tumor material was available from at least two distinct timepoints. Between two and maximal seven timepoints were analyzed per case. Whole-exome and/or -genome sequencing was performed for 153 samples, and bulk RNA-sequencing was performed for all samples with sufficient RNA quality, yielding 105 transcriptomes. To reconstruct tumor evolution, mutations were assigned a cancer cell fraction using ScIust, and phylogenetic trees were inferred from SNVs. Refphase was used to resolve haplotype-specific copy-number states, and copy-number-based phylogenies were reconstructed using MEDICC2. We further investigated mutational signatures and determined tumor mutational burden.

Results: We identified three evolutionary patterns across the cohort. A subset of tumors evolved linearly from the most recent common ancestor, whereas most diverged early and underwent repeated branching, resulting in pronounced intratumoral heterogeneity. In several cases, mutations dominating the relapse were traceable to minor subclones at diagnosis, present at cancer cell fractions that would have escaped detection without the subsequent timepoint. Third, we observed parallel evolution on both the copy-number and the SNV level: at least one mirrored subclonal allelic imbalance (MSAI) event was detected in 26/61 patients, indicating that independent clones acquire the same imbalance - most frequently at 17q - through distinct routes. Somatic *ALK*-mutations occurred at different hotspots in different clones of the same tumor. At the transcriptomic level, diagnostic and relapse samples showed largely concordant expression profiles, whereas biopsies obtained under therapy diverged substantially.

Discussion and Perspectives: Our data indicate that relapse-driving clones are frequently present already at diagnosis and persist through first-line therapy to seed relapse years later. We next aim to integrate clinical annotations to test whether the presence of such clones at diagnosis is associated with outcome, and to characterize their transcriptional state. Identifying these clones early and defining resistance-associated trajectories could inform risk stratification and provide a rationale for clone-directed therapeutic strategies in neuroblastoma.

Hedgehog – Gli2 Signalling Determines Lineage fFte and Metabolic Programming of Progenitor Cells during Skin Morphogenesis

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The formation of mammalian skin appendages, like hair follicles (HFs) and sebaceous glands (SGs), requires Hedgehog (Hh) signalling and the activation of Gli transcription factors (TFs), particularly Gli2. Although the Hh-Gli2 signalling controls both, HF and SG epithelial progenitor cells, the exact mechanisms remain unclear.

To define the precise role of epidermal Hh signalling, our lab generated a mouse model with epidermal knockout of Gli2 (Gli2^{EKO}). Gli2^{EKO} mice exhibit delayed HF development and reduced HF number, but enlarged SGs compared to their littermates, and thus suggesting a dual role of Gli2: supporting HF growth while restricting SG expansion.

This project aims to understand the underlying mechanism of the specific role of Gli2 in regulating distinct HF progenitor cell types during skin morphogenesis. Our data show that Gli2-mediated HF and SG defects initiate early during embryonic morphogenesis.

Single-cell RNA sequencing and GeneTrajectory analyses revealed that Hh-Gli2 signalling controls not only SG progenitor identity but also their metabolic state. SG progenitors from Gli2^{EKO} mice show increased expression of lipid-metabolic pathways relative to littermate controls. In addition, we mapped the genetic programme governing SG formation and examined how Hh-Gli2 signalling regulates this complex cellular process.

Together, these novel results identify how Hh-Gli2 signalling controls both progenitor-cell behaviour and metabolic activity during skin appendage development.

Constitutive B Cell-Specific Overexpression of the Akt Kinase Initiates Immunometabolic Epigenetic Alterations Leading to Macrophage-Like CD11b⁺CD68⁺ B Cells

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Our group established a mouse model, of B cell-specific AKT overexpression in C57BL/6 mice (CD19^{cre/+}Akt1^{fl/STOP-IRES-GFP}), demonstrating that enhanced Akt signalling promotes differentiation toward the CD21⁺CD1d⁺ marginal zone B cell lineage and facilitates Richter transformation in TCL1-driven chronic lymphocytic leukaemia ^{1,2}. More recently, we observed complete loss of the B cell receptor in 20-30% of total B cells and upregulation of macrophage-associated markers, CD11b and CD68, coinciding with downregulation of PAX5. Based on these findings, we hypothesized that AKT-driven metabolic reprogramming induces epigenetic alterations that suppress the B cell-commitment transcriptional programs and by extension B cell identity, effectively leading to lineage trans-differentiation.

These PAX5^{low} cells were enriched for IgKappa-IgLambda⁺ CD19⁺ B cells and displayed decreased histone H3K9 acetylation, as measured with flow cytometry. Autofluorescence-based metabolic measurement of NADH/FAD ³ revealed a suppressed optical redox ratio across all B cell subsets, consistent with diminished OXPHOS activity, and subsequently reduced acetyl-CoA production. Notably, the IgKappa-IgLambda⁺ subset appeared to be most metabolically compromised, consistent with histone hypoacetylation. Mitochondrial mass and membrane potential were also reduced, as measured by MitoTracker and TMRE staining. Fate-mapping of GFP⁺ AKT-overexpressing B cells further identified a small 2-10% population of GFP⁺ cells within the CD19⁺CD90⁺ compartment, potentially corresponding to ex-B cells.

In summary, AKT overexpression induces mitochondrial perturbations and epigenetic remodelling that destabilize B cell identity, through downregulation of PAX5. Reduced OXPHOS activity likely limits acetyl-CoA availability, contributing to histone hypoacetylation and epigenetic silencing of PAX5. This enables the emergence of CD11b⁺CD68⁺ macrophage-like B cells, consistent with a failure to maintain the PAX5-dependent B cell lineage-commitment programs. To contextualize this finding, we looked at publicly available scRNA-seq patient datasets of splenic marginal zone lymphoma (SMZL), in which we detected expansion of CD11b⁺CD68⁺ B cells, hereby showing that our mouse model phenocopies elements of SMZL. Finally, we attempted a B cell-specific knock-out the ACC1 enzyme (CD19^{+/cre}AKT1^{fl/stop}ACC1^{fl/fl}), to boost intracellular acetyl-CoA levels ⁴, which may rescue the phenotype described.

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Ratiometric Fluorescent Citrate Biosensors Reveal Citrate Dynamics and Cellular Heterogeneity in Cancer and Mouse Embryonic Stem Cells

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Metabolism is subject to the control of a myriad of metabolite interconnections and plays a critical role in maintaining cellular function. Mass spectrometry-based techniques are widely used to detect metabolite levels in biological contexts, and are beginning to reveal the complexity of metabolism in mammalian tissue. However, the spatiotemporal dynamics of cellular metabolites, particularly at the subcellular and single-cell level, remain largely unclear. Citrate is a key metabolite that links the tricarboxylic acid cycle in the mitochondria and lipid biosynthesis in the cytosol. Tools for monitoring spatiotemporal citrate dynamics in the mitochondria and cytosol are essential to improving our understanding of cellular metabolism. Here, we have developed genetically encoded ratiometric biosensors for citrate based on our previous intensimetric green fluorescence protein-based citrate biosensor, Citron1.

The new ratiometric citrate biosensor, ratioCitron1, enables quantitative analysis due to its ratiometric response. When expressed in mammalian cells, these biosensors enable citrate dynamics to be monitored in both the cytosol and the mitochondria. Comparative analysis across multiple human breast cancer cell lines reveals differences in citrate levels and heterogeneity specific to each cell line, which may be linked to malignancy. Furthermore, flow cytometry-based measurements in mouse embryonic stem cells demonstrate the proteomics signatures associated with population-level variability in citrate concentrations and citrate rewiring during stem cell differentiation. Together, these results demonstrate that these ratiometric citrate biosensors enable the quantitative, compartment-resolved and population-scale analysis of cellular metabolism.

Intracellular Persistence Factors in Urinary Tract Infections

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Recurrent urinary tract infections (UTIs) are a major health concern due to increasing antibiotic resistance, mortality and comorbidities^{1,2}. Approximately 10% of UTIs are caused by *Klebsiella pneumoniae*, a uropathogen that is most frequently associated with carbapenem antibiotics which are used as a last resort to treat bacterial infections^{3,4}.

K. pneumoniae invades bladder epithelial cells via receptor-mediated endocytosis and is able to persist intracellularly, enabling immune evasion, and resistance to urinary clearance and antibiotics. Its ability to form quiescent intracellular reservoirs (QIRs) within vacuoles plays a key role in recurrent UTIs, complicating patient management^{3,5}. However, the mechanisms enabling *K. pneumoniae* to survive within bladder epithelial cells are unknown.

In this doctoral project the molecular mechanisms that enable *K. pneumoniae* to persist in LAMP1 positive vacuoles in bladder epithelial cells will be characterized. To achieve this, an arrayed transposon mutant library in luminescent *K. pneumoniae* strain will be generated and screened for mutants impaired in intracellular survival via direct measurement of luminescence followed by whole genome sequencing to identify the location of the transposon insertion. Mutants displaying a strong defect in intracellular survival will be validated with clean knockout mutants and investigated via confocal microscopy to characterize the *Klebsiella*-containing vacuole (KCV), KCV exocytosis, and lysosome fusion. Co-immunoprecipitation experiments will identify possible protein-protein interactions of bacterial and host cell factors that are involved in intracellular bacterial survival. Taken together, this study will provide a foundation to gain a better understanding of *K. pneumoniae*'s ability to cause recurrent infections.

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Establishing an Inducible HEK293 Suspension Cell Platform for Controlled AAV Vector Production

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Adeno-associated viral (AAV) vectors are widely used for gene replacement in neurogenetic diseases and hold promise for the treatment of age-associated diseases. However, current AAV production approaches largely rely on transient multi-plasmid transfection, which can complicate process standardization and contribute to batch-to-batch variability. We are currently establishing a viral core facility to support (pre)clinical translation and aim to develop a robust and reproducible platform for AAV vector production.

Here, we aim to establish an inducible stable HEK293 suspension producer cell platform for controlled AAV vector manufacturing. Our approach is based on transposon-mediated genomic integration of AAV production cassettes, allowing stable maintenance of the genetic elements required for vector production. In combination with inducible ON/OFF switches, this system is designed to enable controlled, on-demand activation of AAV production while cells are expanded under non-induced conditions. This approach is intended to separate cell expansion from vector production and thereby provide greater control over the production process.

By establishing a versatile platform for controlled AAV manufacturing using alternative induction strategies, we aim to simplify the production workflow and improve process reproducibility. The resulting engineered producer cell line is expected to facilitate scalable bioprocessing and provide a flexible basis for the development of more consistent AAV production processes. Ultimately, this platform may support the implementation of reproducible AAV manufacturing workflows for future gene therapy applications in an academic setting.

Co-Translational Quality Control of Transmembrane Proteins

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The ribosome-associated protein quality control (RQC) pathway recognizes prolonged translational arrests and targets incomplete proteins for degradation^{1,2}. Multipass transmembrane proteins are particularly susceptible to RQC-mediated degradation, yet the molecular basis of their translational stalling and how these stalls are sensed remain unclear^{3,4}. Here, we investigated the sequence determinants of terminal ribosomal stalling of model multipass transmembrane proteins, such as the cystine/glutamate antiporter SLC7A11 and the cystic fibrosis transmembrane conductance regulator CFTR. Using dual fluorescence reporter assays, we observed that deletion of all 12 transmembrane segments (TMs) of SLC7A11 completely abolished ribosome stalling, indicating that the translational problem is caused by TM synthesis. Systematic deletion of TM pairs did not significantly change the rate of stalling, indicating that the translational problem cannot be attributed to specific TMs. In agreement, isolated expression of individual TM segments demonstrated that all SLC7A11 TMs can independently induce stalling, but to different extents. Together, these results support a model in which nascent TM segments directly interfere with ribosome elongation, most likely due to inappropriate interactions with the ribosome and/or the membrane insertion machinery⁵. Our results reveal a new type of translational problem contributing to the essential function of the RQC in proteome homeostasis

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Investigation of the Importance of Metabolic Rewiring in a Neural Tumor Model in *Drosophila melanogaster*

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A common alteration during tumor progression is the metabolic rewiring in favor of aerobic glycolysis to fulfill energy demands [1]. Glioblastoma is a very aggressive human cancer of CNS, that arises from astrocytes or neural stem cells and shows cellular heterogeneity and metabolic reprogramming [2]. We have developed a neural stem cell-like tumor in *D. melanogaster*, where primary hyperplasias are formed in the brain of developing larvae using a constitutively active form of Notch. In the process, asymmetric cell divisions are interrupted and aberrant neural stem cells (neuroblasts) are formed, eventually causing hyperplasias. Following transplantation into the abdomen of healthy flies (T0), the tumor spreads throughout the body cavities, leading to lifespan reduction [3].

Allograft tumors were collected, and sc RNA seq was performed. It revealed that two neighboring clusters are characterized by cellular stress and metabolic reprogramming in favor of glycolysis, as Gadd45 and Ldh are upregulated respectively. Gadd45 is expressed after DNA damage [4] and Ldh catalyzes the conversion of pyruvate to lactate, maintaining the NADH/NAD⁺ balance. Ldh is frequently upregulated in cancer [5], and it is identified as a Myc, Sima/HIF1a, dERR and Crc/ATF4 responsive gene [6]. What is the role of Gadd45⁺ and Ldh⁺ subpopulations in the physiology and spread in the hosts?

While Ldh is not expressed in primary hyperplasias, ~22% of the T0 allograft cells acquire a glycolytic state, displaying a dispersed distribution in the tumor. Ldh upregulation, however, is not correlated with hypoxia, as Ldh⁺ cells are present for several days in O₂-rich cell culture medium. Ldh⁺ cells are mostly apparently healthy stem-cell-like tumor cells (dnp⁺), as they do not express apoptotic factors like Dcp1 or accumulate lipofuscin [7]. They do not accumulate DNA damage either (H₂Ax). In contrast, they are pro-differentiative and proliferative, as they express the pro-differentiation factors Elav and Pros and can be labeled with EdU.

To investigate the roles of Gadd45 and Ldh in tumor progression, RNAi-mediated knockdown was performed. After Gadd45^{KD}, the lifespan of injected flies is reduced significantly, while there is no change in Ldh^{KD}. In Ldh^{KD} tumors, however, the cellular composition changes in favor of differentiation, as there are more Elav⁺ cells in the tumor mass. Applying the opposite setup, RNAi against Elav, protein crucial for neural differentiation, reduces the Ldh population, suggesting a potential connection between aerobic glycolysis and the differentiation ability of the aberrant NBs, which is under further investigation.

Moving on, Seahorse analysis will be performed after specific knockdowns of crucial enzymes for glycolysis and respiration. ECAR of the TME will be measured after Ldh^{KD} or incubation with the Ldh inhibitor oxamate [8]. Secondly, to investigate if Ldh⁺ aberrant NBs are a clonal lineage, live imaging will take place in explanted tumor cells. In addition, the lactate sensor CanlonicSF will be studied before and after incubation with L-lactate. Lastly, as lifespan in Ldh^{KD} tumors is not altered but the cellular composition is changed, tumors with Ldh^{KD} and w^{KD} (control) will be transplanted in Ldh^{KD} hosts, to investigate potential metabolic compensation between the host environment and tumor. For the stress related cluster, the cellular composition of Gadd45^{KD} tumors will be investigated for signs of DNA damage, apoptosis and cellular differentiation.

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Targeting Serine Metabolism Rescues Contractile Dysfunction across Genetic and Acquired Heart Failure

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Dilated cardiomyopathy (DCM) is a leading cause of heart failure and cardiac transplantation, yet the metabolic mechanisms coupling genetic sarcomeric defects to impaired contractility remain poorly understood. Building on our discovery that ATF4-driven serine biosynthesis regulates cardiomyocyte function¹, we now identify the PHGDH-serine axis as both a determinant of normal contractility and a disease-selective, druggable vulnerability in heart failure.

Using isogenic iPSC-derived cardiomyocytes (iPSC-CMs) carrying the TNNT2 R183W DCM variant alongside corrected controls, integrated with pixel-based contractility tracking, engineered heart tissue (EHT) force measurements as a functional model of myocardial contraction, untargeted metabolomics, and ¹³C-glucose stable isotope tracing, we show that PHGDH is essential for cardiomyocyte function: its knockdown impairs contractility in healthy iPSC-CMs and reduces EHT force, while its overexpression improves contractility and enhances force generation in DCM iPSC-CMs. Metabolomic profiling reveals broad depletion of glycolytic and TCA cycle intermediates in DCM cardiomyocytes; ¹³C-glucose tracing confirms increased serine availability upon PHGDH induction. Mitochondrial respiratory capacity is unaffected, pointing to a contractile rather than bioenergetic mechanism. Strikingly, exogenous L-serine selectively restores contractility in our 2D and 3D DCM models but does not affect healthy cardiomyocytes. This represents a disease-specific metabolic dependency. We further confirmed this in a second DCM genotype using an independent phospholamban R14del iPSC-CM model. In vivo, AAV9-mediated cardiac PHGDH overexpression stabilizes ejection fraction (a measure of cardiac pump function) in genetic DCM mice over the 10-week study period in contrast to progressive decline in untreated controls². In our catecholamine-induced model of acquired heart failure, both dietary serine supplementation and PHGDH induction halted or reduced further ejection fraction decline, demonstrating therapeutic efficacy across etiologically distinct forms of cardiac dysfunction.

These findings establish serine metabolism as a shared metabolic vulnerability across genetic and acquired heart failure and position the PHGDH-serine axis as a novel therapeutic target. Ongoing ¹³C-serine tracing and phosphoproteomics studies aim to define the downstream signaling and lipid remodeling networks mediating cardioprotection - a critical step toward clinical translation.

¹ Perea-Gil et al. Eur Heart J. 2022;43:3477-3489.

² Kay M et al. Metabolism. 2025;156395.

Metabolism Dictates Effector Fate in Human MAIT Cells

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Mucosal-associated invariant T (MAIT) cells are innate-like T lymphocytes abundant in human blood and tissues. Their semi-invariant T cell receptor (TCR), MR1 restriction, and limited alloreactivity make MAIT cells attractive candidates for off-the-shelf cellular therapies. MAIT cells rapidly respond to microbial metabolites through TCR-dependent activation but can also be activated independently of the TCR by inflammatory cytokines, positioning them at the interface of innate and adaptive immunity. However, how these distinct modes of activation shape human MAIT cell metabolism and functional fitness remains incompletely understood. Here, we investigated how distinct stimulation contexts shape the metabolic and functional programs of human MAIT cells. TCR-dependent activation preferentially induced IL-17A and TNF production and was associated with enhanced oxidative phosphorylation. In contrast, TCR-independent stimulation with IL-12 and IL-18 promoted IFN γ production and was linked to increased glycolytic activity. Targeted metabolic modulation of glycolysis selectively reduced IFN γ production, while modulation of oxidative phosphorylation affected TCR-driven effector functions. Together, our findings identify the activation context as a key determinant of metabolic programming and effector function in human MAIT cells. TCR- and cytokine-driven activation engage distinct metabolic pathways that support specific functional outputs, demonstrating that human MAIT cell effector responses are metabolically constrained. Defining the metabolic requirements of distinct MAIT cell effector programs may enable targeted metabolic engineering for therapeutic application, generating MAIT cell-based therapies with tailored effector functions and improved fitness.

Metabolic Control of Macrophage Immunity

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Type 2 inflammation is a core driver of tissue repair, however, if sustained it also contributes to the development of pathological tissue fibrosis. Hence, pharmacological blocking of type 2 cytokine-signaling provides a promising strategy to prevent fibrosis. Yet, despite the relevance, molecular features driving type 2-mediated repair and fibrosis remain largely unknown. Macrophages (MFs) play a pivotal role in wound healing and their dysfunction abrogates repair and promotes tissue fibrosis. The mechanistic basis of type 2-activated MFs in repair and tissue fibrosis is unclear. Recently, we showed that oxidative metabolism in late-stage wound macrophages is regulated by IL-4R α signaling. Here we addressed the question whether type 2-elicited oxidative metabolism (OXPHOS) in wound MFs contributes to their pro-fibrotic function.

In this project, we leveraged novel genetic resources (including conditional mouse models, multiomic datasets) to tackle the function of OXPHOS in wound MFs. By generating mice with myeloid cell-specific *Cox10* deficiency (deletion of complex IV of the electron transport chain) in wound MFs (*Cox10*^{MKO} mice), we demonstrated that OXPHOS is required to mediate IL-4R α -elicited pro-fibrotic repair programs in wound MFs.

Wound healing in *Cox10*^{MKO} mice was significantly impaired, as revealed by significantly lower numbers of alpha-smooth muscle actin-positive myofibroblasts. Interestingly, OXPHOS-defective wound MFs also failed to downregulate inflammatory gene expression (*Il1b*, *Nos2*). scRNA-seq analyses of total wound cell suspensions isolated from control and *Cox10*^{MKO} mice showed that *Cox10*-deficient wound macrophages were impaired in their ability to properly progress through the phagocytic activation pathway. At the same time, fibroblasts were stuck in an inflammatory and proto-myofibroblast state and were unable to differentiate efficiently into myofibroblasts.

CellChat analysis revealed perturbed communication pathways between *Cox10*-deficient wound macrophages and fibroblasts. Mechanistically, we found that in contrast to controls, *Cox10*-deficient macrophages could not adjust the ratio of the tricarboxylic acid-cycle metabolites α -ketoglutarate and succinate in response to type 1- and type 2-cytokines. Proper macrophage activation in *Cox10*-deficient macrophages could be partially restored by treatment with α -ketoglutarate *in vitro*.

Collectively, these findings identified a novel role of IL-4R α -induced OXPHOS in wound MFs that is required to balance both downregulation of potent early-phase pro-inflammatory mediators and induction of pro-fibrotic late-phase repair programs. Prospectively, our findings provide a mechanistic basis for repurposing type 2 blockers to target OXPHOS in wound MFs and to prevent tissue fibrosis.

Episomal Reprogramming of Patient-Derived Keratinocytes and Fibroblasts for Modeling POLG-Associated Mitochondrial Disease

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Introduction: Mitochondrial disorders comprise a heterogeneous group of genetic diseases characterized by impaired mitochondrial function and disrupted cellular energy metabolism. The mitochondrial DNA polymerase γ , encoded by POLG, plays a central role in mitochondrial DNA (mtDNA) replication and maintenance. Pathogenic variants in POLG can result in mtDNA depletion and/or deletions, leading to mitochondrial dysfunction compromising mtDNA integrity and oxidative phosphorylation. Patient-derived human induced pluripotent stem cells (hiPSCs) provide a powerful cellular platform to investigate disease mechanisms and allow the generation of different cell types to investigate the effect on cellular function and metabolism. However, efficient reprogramming of primary somatic cells remains technically challenging. Hair follicle-derived keratinocytes provide an accessible and minimally invasive alternative to fibroblasts obtained from skin biopsies, which is particularly advantageous for pediatric studies. Here, we describe a feeder-free, episomal reprogramming approach for the generation of hiPSCs from keratinocytes and fibroblasts using lipofection.

Methods: Primary keratinocytes isolated from plucked hair follicles and fibroblasts obtained from skin biopsies were cultured and expanded prior to reprogramming. Cells were transfected twice by lipofection with non-integrating episomal vectors encoding OCT3/4, Sox2 and KLF4 and L-MYC and LIN28, together with shRNA mediated suppression of p53. Following transfection, cells were transferred to vitronectin coated plates and maintained under feeder-free conditions using TeSR-E7 medium and monitored for the emergence of colonies with hiPSC-like morphology. Individual colonies were manually selected, expanded and maintained for further characterization.

Results: Episomal reprogramming by lipofection successfully generated multiple hiPSC colonies from primary keratinocytes under feeder-free conditions. Two sequential transfections supported efficient colony formation, whereas a single transfection was insufficient to induce reprogramming in fibroblasts, indicating that repeated transfection may also be required for this cell type using these vectors. Selected colonies displayed typical hiPSC-like morphology and could be manually picked and expanded. Further characterization of established clones, including confirmation of pluripotency and genomic stability, is ongoing.

Discussion: Here, we establish a non-integrating approach for reprogramming primary somatic cells using episomal plasmids while avoiding electroporation and feeder-dependent cultivation. Successful reprogramming of hair follicle-derived keratinocytes is particularly relevant for studies involving pediatric patients, where minimally invasive sampling is advantageous. Our findings also indicate that repeated transfection may be important for successful reprogramming using this approach.

Perspectives: Reprogramming of patient-derived keratinocytes and fibroblasts carrying pathogenic POLG variants is ongoing. Following characterization and validation, established hiPSC lines will be differentiated into disease-relevant cell types, including glutamatergic and GABAergic neurons to investigate the consequences of POLG variants on mtDNA maintenance, mitochondrial function and cellular metabolism. These models will also provide a basis for future interventional studies aimed at modifying disease-associated cellular phenotypes.

The Role of Fumarate Hydratase 1 (FH1) in Atherosclerosis

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Introduction: Atherosclerosis is a lipid-driven vascular inflammation in which macrophages play a central role in plaque initiation and progression. Excessive cholesterol uptake drives metabolic dysregulation in macrophages, promoting foam cell formation and disease progression. The mitochondrial TCA cycle enzyme fumarate hydratase (FH1) has been implicated in macrophage metabolic regulation and pro-atherosclerotic cytokine release, but its role in foam cell formation and atherosclerosis remains entirely unknown.

Aim: To investigate the role of FH1 in foam cell formation and atherosclerotic plaque progression, and to explore its potential clinical relevance in humans.

Methods/Results: In silico analysis of single-cell RNA sequencing of leukocytes from murine atherosclerotic aortas revealed decreased Fh1 expression in Trem2⁺ macrophages. Immunofluorescent imaging of atherosclerotic plaques in low-density lipoprotein receptor knock-out (LDLr^{-/-}) mice fed western-type diet for 12 weeks confirmed reduced Fh1 abundance in CD68^{pos} BODIPY^{pos} foamy macrophages compared to CD68^{pos} BODIPY^{neg} macrophages (n=3; P<0.001). FACS confirmed decreased FH1 abundance in aortic foamy macrophages in LDLr^{-/-} mice (n=4/group; P=0.029). In silico CRISPR screen-analysis of BV2 myeloid cells undergoing foam cell formation ranked Fh1 as the TCA enzyme most associated with oxLDL uptake (n=5/group; P<0.001). Interestingly, in vitro, Fh1 expression was unchanged in oxLDL-treated bone marrow-derived macrophages (BMDMs), but co-stimulation with LPS, mimicking plaque inflammation, significantly reduced FH1 (n=3/group; P=0.001). Compared to vehicle, pre-stimulation with the FH1 inhibitor (FHIN1) reduced DIL-labeled oxLDL uptake in BMDMs (n=3/group; P=0.015). Genetic Fh1-deficiency in BMDMs reduced expression of foam cell associated genes including Trem2 (n=3/group; P=0.001), Cd36 (P=0.034) and Plin2 (P=0.044). In vivo, macrophage-specific Fh1 deletion (Cx3cr1-CreERT2 x Fh1-flox-flox (Cx3cr1^{Fh1}) mice) showed reduced Fh1 expression in peritoneal Cx3cr1⁺ leukocytes after 1 week of tamoxifen-containing HFD ((TAM-HFD) n=3/group; P=0.034). In comparison to controls, transplantation of Cx3cr1^{Fh1} bone marrow into lethally-irradiated LDLr^{-/-} mice increased atherosclerotic burden after 12 weeks of HFD followed by 4 weeks TAM-HFD (n=7/group; P=0.021). Ongoing work is investigating phenotypic and inflammatory changes in the plaque microenvironment. In 1071 patients undergoing carotid endarterectomy, higher FH1 expression in plaques was associated with lower occurrence of stroke/TIA at the time of surgery (OR 0.84; 95% CI 0.73–0.96; P=0.009), independent of risk factors and lipid biomarkers.

Conclusion: Fh1 is downregulated in foam cells both in vitro and in vivo, and higher Fh1 expression in human plaques is associated with reduced cerebrovascular events, suggesting potential predictive relevance. FH1 deficiency decreased oxLDL uptake, altered foam cell gene expression, and increased atherosclerotic burden in mice. These findings highlight FH1 as a potential target for atherosclerosis prevention and therapy.

Domain-Specific Tau Mechanisms and Tau Suppression as Therapeutic Strategies Supported by PET-Based Microtubule Imaging in Alzheimer's Disease

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Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and neuronal loss. Increasing evidence identifies TAU pathology as a central driver of neurodegeneration. Under physiological conditions, TAU stabilizes microtubules and supports axonal transport through distinct functional domains that regulate microtubule binding, intracellular localization, and protein interactions. In AD, however, TAU becomes hyperphosphorylated and mislocalized, resulting in microtubule destabilization and impaired neuronal function. However, the contribution of individual TAU domains to microtubule stability remains incompletely understood. As cytoskeletal alterations are thought to occur early in disease progression, there is a need for imaging approaches capable of detecting microtubule dysfunction before the appearance of advanced pathological hallmarks.

Methods: To investigate the role of TAU and its individual domains in regulating microtubule stability, human induced pluripotent stem cell (iPSC)-derived neurons were combined with a MAPT knockout (MAPT-KO) model lacking endogenous TAU expression. This approach enabled the controlled re-expression of selective human TAU deletion constructs and the assessment of domain-specific functions independent of endogenous TAU. Lentiviral transduction was used to express TAU variants lacking the proline-rich region 2 (PRR2), N-terminal, or C-terminal domains. Neuronal morphology was assessed by immunocytochemistry, while microtubule dynamics were quantified using live-cell imaging of EB3 comets. In parallel, a fluorine-18-labeled PET tracer targeting destabilized microtubules was developed and evaluated in in vitro models with pharmacologically modulated microtubule stability. Tracer uptake kinetics and binding characteristics were analyzed to assess sensitivity to cytoskeletal alterations.

Results: Re-expression of selective TAU deletion constructs in MAPT-KO neurons revealed domain-specific effects on neuronal morphology. Sholl analysis demonstrated increased dendritic complexity compared with transduction controls, with the strongest effect observed following expression of the N-terminal TAU construct. These findings indicate that distinct TAU domains differentially contribute to the maintenance of neuronal architecture and cytoskeletal organization. In vitro characterization of the fluorine-18-labeled PET tracer in HEK293 cells demonstrated time-dependent uptake, reaching a plateau after approximately one hour of incubation. Pharmacological modulation of microtubule stability revealed increased tracer accumulation under conditions of microtubule destabilization, whereas microtubule stabilization reduced tracer uptake, indicating preferential binding to destabilized microtubules. Preliminary in vivo PET studies further demonstrated brain uptake of the tracer. Interestingly, pharmacological blocking did not reduce tracer signal but instead resulted in increased uptake, suggesting that the treatment generated additional binding sites through enhanced microtubule destabilization. Together, these findings support the proposed binding mechanism and provide initial evidence that microtubule alterations can be detected non-invasively using PET imaging.

Conclusion & Perspective: This work combines mechanistic investigations of TAU biology with the development of a novel molecular imaging strategy targeting microtubule dysfunction. The in vitro findings demonstrate TAU- and domain-specific contributions to microtubule regulation and provide evidence that microtubule destabilization can be detected using a fluorine-18-labeled PET tracer. Ongoing in vivo studies in TgF344-AD rats and rTg4510 mice will evaluate the translational potential of this approach as a biomarker for early neurodegenerative changes, disease progression, and therapeutic response.

Mitochondrial Control of TAU-Based Neurodegeneration and Gene-Therapy Approaches

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Introduction: Aging is the major risk factor for Alzheimer's disease (AD) and related tauopathies, with increasing evidence linking age-associated mitochondrial dysfunction to neurodegeneration. TAU, a microtubule-associated protein predominantly localized to axons, undergoes aberrant missorting, an early sign of NDD-typical TAU pathology, and aggregation in tauopathies, including sporadic AD and inherited dementias. As key regulators of neuronal energy metabolism and calcium homeostasis, mitochondria are essential for neuronal function, and their dysfunction is closely associated with synaptic impairment and neurodegeneration. Recently, we found that both global and axon initial segment-specific mitochondrial dysfunction are sufficient to induce TAU missorting. We therefore hypothesize that mitochondrial dysfunction acts upstream of TAU pathology and may represent a potential therapeutic target for AD and tauopathies.

Aim: This study aims to determine whether mitochondrial dysfunction induces TAU pathology and to assess the effects of mitochondria-targeted therapeutic strategies on TAU-related alterations.

Methods and Results: Human iPSC-derived neurons are used as the primary disease-relevant model. Mitochondrial dysfunction was induced pharmacologically (IMT1B, Actinonin, Antimycin A, Rotenone, and ddC) or genetically by disrupting mtDNA maintenance, including POLG knockdown with shRNA. POLG knockdown was confirmed by qPCR, showing reduced POLG mRNA levels, while IMT1B and ddC treatment similarly reduced mtDNA content, indicating impaired mitochondrial genome maintenance. Importantly, TAU missorting was consistently observed following Actinonin, Antimycin A, Rotenone, and ddC treatment, as well as POLG knockdown, whereas IMT1B-induced mtDNA depletion alone did not produce detectable TAU alterations. Viral gene delivery platforms, including lentiviral POLG shRNA and AAV production systems, have been established to enable bidirectional modulation of mitochondrial function in vitro and in vivo. Expression constructs targeting mitochondrial function, including POLG, TFAM, TWINKLE, PGC-1 α , POLG2 (Pol- γ B), and SSBP1, have also been generated. Future studies will extend these findings to murine K320E-Twinkle and murine POLG shRNA models, further characterize neuronal and mitochondrial structural alterations, and investigate the molecular mechanisms underlying mitochondrial dysfunction-induced TAU pathology using proteomic approaches.

Conclusion: These findings implicate mitochondrial dysfunction in early TAU pathology and TAU missorting, supporting mitochondria as a potential therapeutic target for neurodegenerative and mitochondrial diseases.

Pharmacokinetic Evaluation of Ademethionine-Loaded Red Blood Cell-Based Drug Delivery Systems

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Introduction: Sepsis-associated liver dysfunction is an important component of abdominal sepsis and is associated with impairment of the metabolic, detoxification, and immune functions of the liver. Ademethionine (S-adenosyl-L-methionine, SAMe) is a hepatoprotective and metabolically active compound. However, its relatively rapid systemic clearance and nonspecific distribution may limit drug exposure at sites of hepatic inflammation, potentially reducing therapeutic exposure and necessitating repeated dosing. Erythrocyte carriers represent a biocompatible drug-delivery platform that may modify the pharmacokinetic profile of loaded drugs and provide a more gradual release into the circulation. Therefore, this study aimed to compare the pharmacokinetics of free ademethionine and ademethionine-loaded erythrocyte carriers in rats as a potential approach to prolonged drug delivery for future application in sepsis-associated liver dysfunction.

Methods: Both formulations were administered intravenously at an equivalent dose of 100 mg/kg. Blood samples were collected at 10 and 30 min and at 1, 1.5, 2, 3, 6, 9, and 24 h after administration. Plasma ademethionine was quantified by liquid chromatography-mass spectrometry (LC-MS). The analytical signal was monitored for S-adenosyl-L-methionine at *m/z* 399.145 with a retention time of 0.85 min. Pharmacokinetic indices were estimated from the plasma concentration-time profiles.

Results: Free ADM showed a high early plasma concentration, reaching an observed C_{max} of 95.20 ppm at 10 min, followed by a decrease to 32.04 ppm at 30 min and 16.80 ppm at 1 h; the drug was not detected from 1.5 h onward. RBC-ADM showed a lower observed C_{max} of 17.82 ppm at 30 min. Mean detectable concentrations were 6.20 ppm at 1 h, 6.30 ppm at 1.5 h, 5.98 ppm at 2 h, and 2.60 ppm at 3 h; no signal was detected at 6, 9, or 24 h. Estimated MRT was 0.68-0.81 h for Free ADM and 1.12-1.40 h for RBC-ADM.

Discussion: Compared with free ademethionine, RBC-ADM reduced the early plasma peak by approximately 5.3-fold, shifted the observed T_{max} from 10 to 30 min, and prolonged the presence of detectable ademethionine in plasma from 1 to 3 h. The increase in estimated MRT further indicates a slower pharmacokinetic profile of the erythrocyte-loaded formulation. These findings demonstrate that incorporation of ademethionine into erythrocyte carriers substantially modifies its plasma concentration-time profile and provides a more gradual decline in plasma concentrations.

Perspectives: These findings suggest that erythrocyte-based delivery may offer a promising strategy for improving the therapeutic management of sepsis-associated liver dysfunction. Further studies are warranted to establish whether the observed pharmacokinetic modifications result in enhanced hepatic accumulation and improved therapeutic efficacy of ademethionine in experimental models of sepsis-associated liver dysfunction.

Funding: This research is funded by Nazarbayev University under Collaborative Research Program Grant № 211123CRP1614, A.G.

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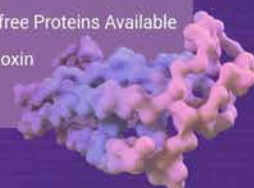
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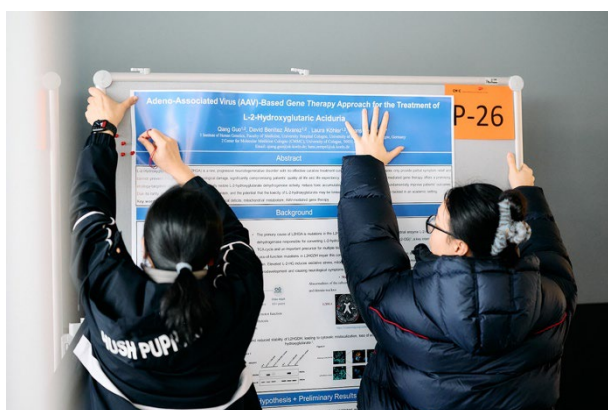
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CMMC Symposium 2026

Event Management

Poster, Program, Abstract Book and Symposium Website: Design, Layout, Preparation in house by the CMMC Office Team

- Dr. Debora Grosskopf-Kroiher - Scientific Coordinator / Managing Director
- Dr. Nadja Bochers - Dr. Claudia Herr - Kerstin Heber - Bettina Kranz - CMMC Office Team

Audiovisual and Technical Support:

- Peter Fey - Sebastian Zetsche

Acknowledgement

- We gratefully acknowledge the support of all CMMC members who contributed to the symposium.

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CMMC SYMPOSIUM 2026

Metabolic Control of Tissue Regeneration and Degeneration – Sept. 28 - 30, 2026

Short Time Table

Venue: Main Lecture Hall, Faculty of Medicine, University of Cologne

Monday - Sept. 28, 2026

12.45 p.m.	Welcome Address and Opening the CMMC Symposium 2026 C Niessen – S Eming – G Schwarz – J Brüning
Session 1	Regeneration and Metabolism
Chairs	Elly Tanaka – Matthias Hammerschmidt
1.00 - 2.30 p.m.	Almudena Chaves Perez – Mónica M Sousa – Ahmed I Mahmoud
2.30 - 3.00 p.m.	Coffee Break
Session 2	Carbohydrate Metabolism and Diabetes
Chairs	Gerald Shulman – Carien Niessen
3.00 - 4.30 p.m.	Jens Brüning – Arvid Sandforth – Roy Taylor
4.30 - 5.00 p.m.	Coffee Break with Biotec Exhibition
5.00 - 6.00 p.m.	Keynote Lecture I Elly Tanaka
Poster Session	with Welcome Get-Together
6.00 - 9.00 p.m.	Poster Presenters of Poster 01 – P 71 moderated by Nathalie Schmidt – Sebastian Theobald

Tuesday - Sept. 29, 2026

Session 3	Redox Metabolism and Regeneration
Chairs	Gunter Schwarz – Jan Riemer
9.00 - 10.30 a.m.	Ruma Banerjee – Hozumi Motohashi – Can Aztekin
10.30 - 10.40 a.m.	Conference Photo
10.40 - 11.00 a.m.	Coffee Break
Session 4	Immunity and Metabolism
Chairs	Hamid Kashkar – Esther von Stebut
11.00 - 12.30 p.m.	Jane Reznick – Kenji Kabashima – David E Sanin
12.30 - 2.30 p.m.	Lunch Break with Poster Presentation at the venue
Session 5	Nucleic Acid Metabolism
Chairs	Axel Roers – Henning Walczak
2.30 - 4.00 p.m.	Ana Martin-Villalba – Min Ae Lee-Kirsch – Hendrik Poeck
4.00 - 4.05 p.m.	Announcement of the three Poster Prize Winners – CMMC Symposium 2026
4.05 - 4.30 p.m.	Coffee Break
4.30 - 5.30 p.m.	Keynote Lecture 2 Axel Roers
6.30 p.m.	Conference Dinner

Wednesday - Sept. 30, 2026

9.00 - 9.30 a.m.	Short talks of the poster prize winners – CMMC Symposium 2026
Chairs	Nathalie Schmidt – Sebastian Theobald; Chairs Poster Evaluation Committee
Session 6	Lipid and Fatty Acid Metabolism
Chairs	Thomas Benzing – Michal-Ruth Schweiger
9.30 - 11.00 a.m.	Triantafyllos Chavakis – Gregory Steinberg – Kitt Petersen
11.00 - 11.30 a.m.	Coffee Break
11.30 - 12.30 p.m.	Keynote Lecture 3 Gerald Shulman
12.30 - 12.45 p.m.	Summary, open questions, closing remarks and leave-taking Sabine Eming – Gunter Schwarz