The 30th European Cell Death Organization Conference

Cell death at the crossroads of neurodegeneration and cancer

9 - 11 October 2024
University of Luxembourg

ECDO
European
Cell Death
Organization

Luxembourg 2024

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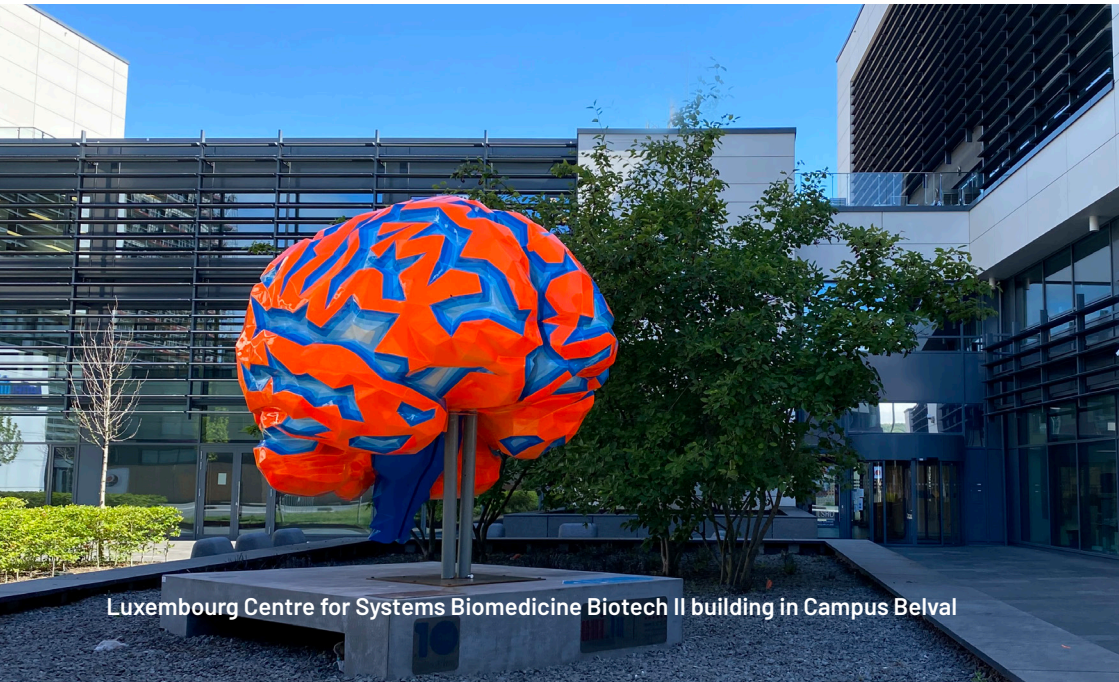

Luxembourg Centre for Systems Biomedicine Biotech II building in Campus Belval

INTRODUCTION

The European Cell Death Organization (ECDO) conference is one of the leading conferences on cell death control attracting outstanding scientists from Europe, USA, Australia and Japan.
Over the years, ECDO conferences have become a major forum for the presentation and discussion of central paradigms in cell death research, giving an overview of all latest developments in the field, initiating cooperation projects between international scientists and driving scientific progress in the field of biomedical research.

This year the conference is organised by the Luxembourg Centre for Systems Biomedicine (LCSB), a special emphasis will be put on diverse aspects of cell death, addressing molecular mechanisms such as apoptosis, necroptosis and autophagy, exploring its implications in neurodegenerative diseases like Alzheimer's and Parkinson's, examining the crossroads of metabolism and cell death, delving into fundamental aspects of cell death in cancer and related clinical applications, as well as unraveling the complex relationship between cell death and inflammation.

ABOUT THE ORGANISER

The Luxembourg Centre for Systems Biomedicine (LCSB) is an Interdisciplinary Research Centre of the University of Luxembourg. It combines experimental and computational approaches to analyse complex biological systems and disease processes with a strong focus on neurodegeneration. The overarching strategy of the LCSB is to systematically combine interdisciplinary approaches to contribute to the prevention, diagnosis and cure of neurological diseases.

CO-FUNDING

Supported by the Luxembourg National Research Fund (18808287)

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PROGRAMME

Wednesday 9 October

8:45 **Welcome** – **Simone Niclou**, Vice-rector for research, University of Luxembourg; **Dirk Brenner**, Luxembourg Centre for Systems Biomedicine, University of Luxembourg

Session 1. Cell death in neurodegeneration: Alzheimer's & Parkinson's disease

Chair: Inna Lavrik, Otto von Guericke University Magdeburg and ECDO President

9:00 **Keynote | Luc Buée**, Neuroscience and Cognition Research Centre, Lille
Tau protein: a double etched sword

9:45 **Short talk: Guy Brown,** University of Cambridge

10:00 **Flash talk: Ege Solel,** University of Bergen

10:07 **Flash talk: Svenja Lorenz,** Helmoltz Center Munich

10:15 Coffee break with posters & industry exhibition

10:45 **David Rubinsztein**, UK Dementia Research Institute
Autophagy and Neurodegeneration

11:15 **Darren Baker**, Mayo Clinic
Senescent glial cells promote neurodegeneration

11:45 **Aaron Gitler**, University of Stanford
Expanding mechanisms and therapeutic targets for neurodegenerative disease

12:15 Lunch with posters & industry exhibition (ECDO Board meeting)

13:30 **Poster session, industry exhibition & "Meet the editors" event (reception area)**

Session 2. Molecular mechanisms of apoptosis, necroptosis and autophagy

Chair: Eleonora Candi, University of Roma "Tor Vergata"

15:00 **Keynote | Katja Simon**, Max Delbrück Center for Molecular Medicine Berlin
Autophagy's impact on aging, differentiation and metabolism in the immune system

15:45 **Short talk: Nakano Hiroyasu,** Toho University School of Medicine

16:00 **Flash talk: Anna Saorin,** University of Zurich, Kinderspital Zurich

16:07 **Flash talk: Iratxe Uranga Murillo,** Health Research Institute of Aragon

16:15 **Sabrina Sofia Burgener**, Institute for Molecular Bioscience, University of Queensland
Novel insights into the molecular mechanism of inflammasome-driven disease

16:45 Coffee break with posters & industry exhibition

17:15 **Manolis Pasparakis**, Institute for Genetics, University of Cologne
Regulation of necroptosis and its role in inflammation

17:45 **Mathieu Bertrand**, VIB-UGent Center for Inflammation Research & Ghent University
Regulation of TNF cytotoxicity by unconventional autophagy

18:15 **Ceremony and talk for CDD prize** *(introduced by Gerry Melino)*:
Brent Stockwell, Columbia University
Metabolism, drugs, and diet: ferroptosis as a new therapeutic paradigm

19:00 **Boris Zhivotovsky,** General Secretary of the European Cell Death Organization and Karolinska Institute
The 30th Anniversary of the European Cell Death Organization

19:10 Opening reception with poster session at the conference venue
Invited speakers dinner (Nyx Restaurant on Belval Campus)

Thursday 10 October

Session 3. Metabolism & cell death

Chair: Patrizia Agostinis, VIB-Center for Cancer Biology and ECDO board member

9:00 **Keynote | Dirk Brenner,** Luxembourg Centre for Systems Biomedicine, University of Luxembourg and Luxembourg Institute of Health
cROSsing barriers: Metabolism meets T cell function

9:45 **Short talk: Kamyar Hadian,** Helmoltz Center Munich

10:00 **Flash talk: Melanie Grusdat-Pozdeev,** University of Luxembourg

10:07 **Flash talk: Francesca Rizzollo,** VIB-KU Leuven Center for Cancer Biology

10:15 **Guido Kroemer**, Université Paris Cité
Autophagy checkpoint inhibition – A novel strategy for therapeutic autophagy enhancement

10:45 Coffee break with posters & industry exhibition

11:15 **Maria Mittelbrunn**, Severo Ochoa Centre for Molecular Biology
Decoding the contribution of the immune system to aging

11:45 **Ceremony and talk for ECDO prize** *(introduced by Peter Vandenabeele)*:
Marcus Conrad, Institute of Metabolism and Cell Death, Helmholtz Munich
Ferroptosis as the underlying mechanism in neurodegenerative disease

12:30 Lunch with posters & industry exhibition (CDD meeting)

13:30 **Poster session, industry exhibition (reception area)**

14:45 **Sean Sapcariu,** Luxembourg National Research Fund
Funding opportunities

Session 4. Cancer: fundamental mechisms & clinical applications

Chair: Boris Zhivotovsky, Karolinska Institute and ECDO General Secretary

15:00 **Keynote | Henning Walczak,** University of Cologne & UCL Cancer Institute
Death receptors and ubiquitin in cell death, inflammation and immunity

15:45 **Short talk: David Andrews**, Sunnybrook Research Institute

16:00 **Flash talk: Ilaria Deidda**, University Medical Center Göttingen, Georg-August University

16:07 **Flash talk: Roberto Fernández Acosta,** University of Antwerpen

16:15 **Stephen Tait,** Cancer Research UK Scotland Institute and University of Glasgow
Mitochondria and Anti-Tumour Immunity – A Case of Bacterial Mimicry

16:45 Coffee break with posters

17:15 **Judy Lieberman,** Boston Children's Hospital & Harvard Medical School
Turning up the heat on cancer

17:45 **Ping Chih Ho,** Ludwig Institute for Cancer Research & University of Lausanne
Disturbed mitochondrial dynamics instruct T cell exhaustion and beyond cell death

18:15 **Andreas Linkermann**, Heidelberg University
Genomic and non-Genomic Anti-Ferroptotic Functions of Estradiol Define Cell Death Propagation and Sex Differences in Acute Kidney Injury

18:45 **Inna Lavrik,** President of the European Cell Death Organization
Presentation of ECDO conference 2025

18:50 *ECDO General Assembly (moderated by Inna Lavrik)*

19:05 Wrap up

19:30 Gala dinner (Halle des Poches à Fonte)

Friday 11 October

Session 5. Cell death & inflammation

Chair: Ivano Amelio, University of Konstanz and President elect of ECDO & Peter H. Krammer, German Cancer Research Center and ECDO board member

9:00 **Keynote | Andreas Strasser**, Walter and Eliza Hall Institute
How does the tumour suppressor p53 instruct cell fate – apoptosis vs cell survival and cell cycle arrest ?

9:45 **Short talk: Sjoerd van Wijk,** Goethe University Hospital

10:00 **Flash talk**: **Simon Verdonck,** VIB-UGent University of Gent

10:07 **Flash talk**: **Kirsten Kenney,** University of Queensland

10:15 **Lynn Wong,** Department of Molecular Sciences, University of Zurich
New tricks for XIAP in cell death and inflammation

10:45 Coffee break with posters & industry exhibition

11:15 **Ceremony and talk for Juerg Tschopp prize** *(introduced by Mauro Piacentini)*: **Junying Yuan,** Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences
RIPK1-mediated cell death and inflammation in aging and human diseases

12:00 **Short talk: Esther Hoste,** Ghent University

12:15 **Flash talk**: **Georgia Atkin-Smith,** Walter and Eliza Hall Institute

12:22 **Flash talk**: **Wei Xie,** VIB - UGent - Center for Inflammation Research

12:30 **Patricia Maciel**, Life and Health Sciences Research Institute and University of Minho School of Medicine
Serotonin signaling protects neurons against protein aggregation-induced dysfunction and degeneration

13:00 Lunch & Industry exhibition

13:45 **Short talk: Walker Jackson**, Linköping University

14:00 **Short talk: Ana Janic,** Universidad Pompeu Fabra

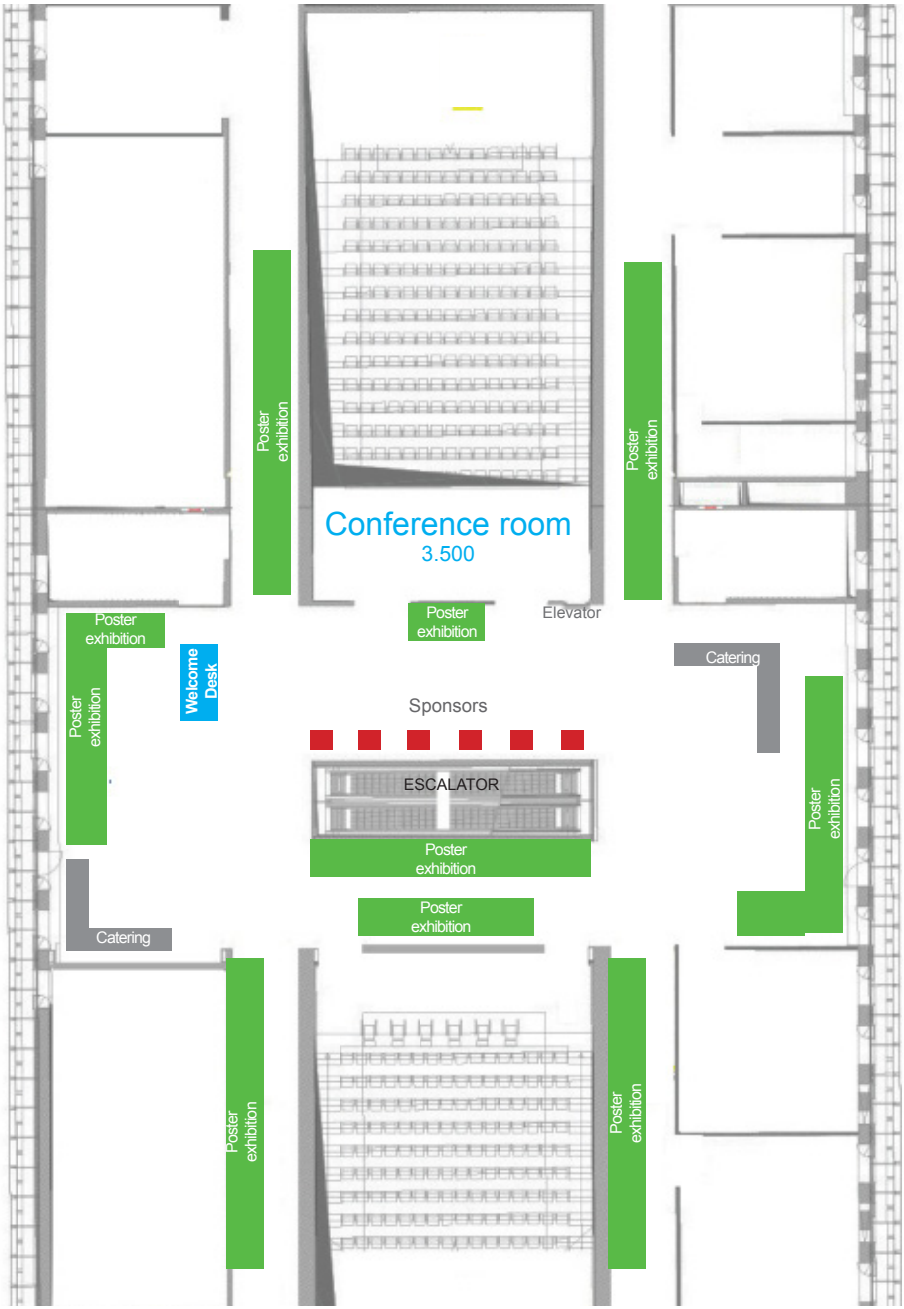
14:15 **Short talk: Frank Essmann,** Robert Bosch Center for Tumor Diseases

14:30 **Michael Heneka**, Luxembourg Centre for Systems Biomedicine, University of Luxembourg
Microglial pyroptosis as driving force of amyloid pathology in Alzheimer disease

15:00 *Poster prizes ceremony & concluding remarks*

15:10 Wrap up and end

VENUE


Poster exhibition
Conference room
3.500
Poster exhibition
Elevator
Poster exhibition
Poster exhibition
Welcome Desk
Poster exhibition
Catering
Sponsors
ESCALATOR
Poster exhibition
Poster exhibition
Poster exhibition
Catering
Poster exhibition
Poster exhibition

DARREN BAKER

SENESCENT CELLS OFFER A SUPPORTIVE TUMOR MICROENVIRONMENT

Professor and Principal Investigator, Mayo Clinic, MN, USA

Link to his research

Darren Baker, PhD, is a Professor of Biochemistry and Molecular Biology and Pediatrics at the Mayo Clinic in Rochester, Minnesota. He received his bachelor's degree from the University of Minnesota in Biology and Chemistry, his master's degree from Mayo Clinic College of Medicine in Tumor Biology and his Ph.D. in Biomedical Sciences at the University of Nijmegen in the Netherlands. His laboratory focuses on the involvement of senescent cells to aging and age-related diseases, with particular focus on neoplastic transformation and neurodegenerative disease. Using novel genetically engineered mouse models that allow for the elimination of senescent cells, he was one of the first to demonstrate that both premature and normal aging are driven by increased incidences of senescence. Furthermore, utilisation of this model has been instrumental in demonstrating that lung tumors in mice can be attenuated through removal of these damaging cells. Studies from his lab have been identified as one of the top 10 breakthroughs of the year by Science in both 2011 and 2016. In 2019, he was recognised with the Rising Star Award from the Mahoney Institute for Neurosciences from the University of Pennsylvania for his work in neurodegenerative disease. He is funded by the NIH (NCI and NIA), Cure Alzheimer's Fund, Ellison Medical Foundation, Mayo Clinic Children's Research Center, and the Alzheimer's Disease Research Center of Mayo Clinic. Additionally, he is a co-director of the Glenn Laboratories of Mayo Clinic for Senescence Research sponsored by the Glenn Foundation for Medical Research and the director of the Transgenic and Gene Knockout facility of Mayo Clinic.

MATHIEU JM BERTRAND

REGULATION OF TNF CYTOTOXICITY BY UNCONVENTIONAL AUTOPHAGY

Principal Investigator, VIB-UGent Center for Inflammation Research & Ghent University, Belgium

Link to the lab

Mathieu JM Bertrand studied bioengeneering at the University of Louvain (UCL), and did his PhD on the functional characterisation of the MAGE genes in the lab of Prof. De Backer at the Ludwig Institute for Cancer Research (LICR) and University of Namur. He then moved to the lab of Prof. Barker at McGill University for a first postdoctoral training during which he studied regulation of innate immune signalling by ubiquitylation. He later joined the lab of Prof. Vandenabeele at the Vlaams Instituut voor Biotechnologie (VIB) and Ghent University for a second postdoc on the regulation of cell death signalling by postranslational modifications. He was appointed with a professor position at Ghent University in 2011, and since then leads a research team at the VIB-UGent Center for Inflammation Research (IRC). Research in his lab aims at characterising the interplay between cell death and inflammation, and at defining the role of cell death in the context of infection and inflammatory pathologies. The lab has expertise in the elucidation and characterisation of the molecular mechanisms regulating cell survival/death and inflammatory responses downstream of TNF receptor superfamily members and pattern recognition receptors. The lab recently got very interested in the role of autophagy in the various aspects of the inflammatory response.

DIRK BRENNER

cROSSING BARRIERS: METABOLISM MEETS T CELL FUNCTION

Principal Investigator, Immunology & Genetics group, Luxembourg Centre for Systems Biomedicine
Principal Investigator, 'Experimental & Molecular Immunology lab, Luxembourg Institute of Health.

Link to the lab (LCSB)
Link to the lab (LIH)

Dirk Brenner is since 2020 Full Professor of Immunology and Genetics, Principal Investigator of the Immunology & Genetics group at the Luxembourg Centre for Systems Biomedicine (LCSB) and heads the 'Experimental & Molecular Immunology' lab (EMI) at the Luxembourg Institute of Health (LIH).

He studied biochemistry at the Universities of Bonn (Germany), Witten/Herdecke (Germany), Stanford University (USA), and Harvard University (USA). He completed his PhD and early postdoctoral studies in Heidelberg (Germany). At the German Cancer Research Center (DKFZ, Germany), he was a member of the research group of Prof Dr Peter H. Krammer, who pioneered research on cell death. As an 'Alexander von Humboldt' fellow, he joined the laboratory of Prof Dr Tak W. Mak at the Ontario Cancer Institute in Toronto (Canada), known for the discovery of the T cell receptor, the first physiological description of CTLA-4 and for reversed genetics. During that time, Prof Brenner was awarded with four postdoctoral fellowships including the 'Alexander von Humboldt foundation' (Germany) and the 'Cancer Research Institute' (USA).

Dirk Brenner is an immunologist who connects molecular and metabolic principles with the regulation of inflammatory diseases and cancer. He has a long-standing publication record as first, as well as senior author, in the fields of immunology, metabolism and cancer. He and his lab are specialised in in vivo applications and have, in the recent past, contributed to our understanding of how metabolism controls immunity. His lab investigates the metabolic regulation of the immune system and how this ensures a coordinated immune response and homeostasis. They seek to define the molecular, metabolic and cellular processes of inflammation and integrate in vitro with in vivo studies to gain a comprehensive picture of inflammation and cancer.

LUC BUÉE

TAU PROTEIN: A DOUBLE ETCHED SWORD

Research Director at the CNRS, Lille Neuroscience & Cognition Research Centre, Inserm, Université de Lille, CHU-Lille, France

Link to the lab

Luc Buée, CNRS Research Professor, is Director of the Lille Neuroscience & Cognition Research Centre and Head of the Inserm laboratory «Alzheimer & Tauopathies» at the University of Lille, France. Located on the Lille hospital campus, his laboratory belongs to the Lille Centre of Excellence in Neurodegenerative disorders (LiCEND) and is also part of the LabEx DISTALZ (National consortium on Alzheimer's disease).

Luc Buée has been working on Alzheimer's disease and related disorders for more than thirty years (>300 articles in Pubmed). He began his work on the role of proteoglycans in Alzheimer's disease during his doctoral training at Mount Sinai Medical Center, New York. He made some of the pioneering neuropathological observations on microvasculature abnormalities in neurodegenerative disorders. He was involved in the initial biochemical characterisation of tau aggregates in certain neurodegenerative disorders (barcode tauopathies). He then developed experimental models to better understand the role of post-translational modifications in tau aggregation and secretion. These experimental models are now widely used to evaluate therapeutic strategies for tauopathies (immunotherapy, intrabodies, small molecules, non-drug therapy…). With his team, he has also discovered numerous non-microtubular functions of the tau protein. He is currently working on the pathophysiological consequences of neurofibrillary degeneration and their links with amyloid pathology and inflammation in Alzheimer's disease.

Luc Buée is also involved in different scientific advisory boards, scientific programmes and operating committees. He is a foreign member of the Royal Academy of Medicine of Belgium and the organiser of the Eurotau meetings. Moreover, he has received different awards.

SABRINA SOFIA BURGENER

NOVEL INSIGHTS INTO THE MOLECULAR MECHANISM OF INFLAMMASOME-DRIVEN DISEASE

Deputy Lab Head of the Inflammasome Group, Centre for Inflammation and Disease Research, Institute for Molecular Bioscience, University of Queensland

Dr. Sabrina Sofia Burgener is a Novartis Foundation for Medical-Biological Research Fellow and Deputy Lab Head of the Inflammasome Group at the Institute for Molecular Bioscience (IMB) leading the pre-clinical disease models team. In 2019, Dr. Burgener joined the Inflammasome Group as a Senior Research Fellow with back-to-back fellowships from the Swiss National Science Foundation and Novartis Foundation for Medical-Biological Research.

In the Inflammasome lab, her research programme focus is on pre-clinical disease models to study inflammation with a focus on I) how the innate immune system drives disease, II) identifying novel therapeutic targets in these diseases, and III) defining potential trade-offs in preventing inflammation, to harness the development of new diagnostics and anti-inflammatory therapeutics.

Her research is internationally recognised for its contributions, having received several international awards, and is supported by prestigious Fellowships, including the Janggen-Poehn Fellowship (2017), Swiss National Science Foundation Postdoc Mobility Fellowship (2020-2022) and the Novartis Foundation for Medical and Biological Research Fellowship (2022-2023), along with competitive project funding: Research Excellence Award from the UQ Australian Infectious Disease Research Centre (2020) and the MetroSouth Co-funded Collaboration Project (2023). Dr. Burgener is the vice president of the Australian Cell Death Society (ACDS) with more than 350 members, and organiser of the 3rd Japanese and Australian Cell Death Meeting (JAM) in 2023. She is committed to guiding the next generation of rising scientists, focusing on mentoring and providing impactful research projects. Her outstanding leadership capabilities have been awarded with Leading Edge Leadership Scholarship from Women in Leadership Australia, two Commendations for IMB Leadership Impact Award and short-listed as UQ Excellence in PhD supervision.

MARCUS CONRAD

FERROPTOSIS AS THE UNDERLYING MECHANISM IN NEURODEGENERATIVE DISEASE

Director, Institute of Metabolism and Cell Death, Helmholtz Zentrum München

Link to the lab

Marcus Conrad is Director of the Institute of Metabolism and Cell Death at Helmholtz Zentrum München. Marcus Conrad studied biology at the University of Konstanz, Germany, and received his doctorate with distinction from Ludwig-Maximilians-University Munich in 2001. After a short postdoctoral period, he was an independent group leader until 2009 at Helmholtz Zentrum München. From 2009 – 2010 he was employed as Laboratory Head at Bayer-Schering AG, Berlin. After two years at the newly founded German Center for Neurodegenerative Diseases, he returned to Helmholtz Zentrum München as research group leader in 2012. In 2020, he was appointed Director of the Institute of Metabolism and Cell Death.

His laboratory has pioneered the field of ferroptosis and made a number of groundbreaking contributions to our understanding of oxidative cell death both in (neuro)degenerative diseases and cancer. He discovered the first in vivo active ferroptosis inhibitors, the so-called liproxstatins, whose next generation variants have already entered preclinical development to be used for the treatment of (neuro)degenerative disease. Marcus Conrad has published more than 160 articles, holds several patents and is co-founder and shareholder of ROSCUE Therapeutics GmbH. He was recently appointed as a new member of the Hinterzarten Circle of the Deutsche Forschungsgemeinschaft (DFG) on cancer research.

AARON GITLER

EXPANDING MECHANISMS AND THERAPEUTIC TARGETS FOR NEURODEGENERATIVE DISEASE

Professor of Genetics, Stanford University

Link to the lab

Prof. Aaron Gitler, Ph.D., is the Stanford Medicine Basic Science Professor in the Department of Genetics at Stanford University. He received his B.S. degree from Penn State University and did his PhD studies on cardiovascular development in the laboratory of Dr. Jonathan Epstein at the University of Pennsylvania. Then he performed his postdoctoral training with Dr. Susan Lindquist at the Whitehead Institute for Biomedical Research and MIT. In 2007, he established his laboratory at the University of Pennsylvania and moved to Stanford in 2012.

His laboratory has been using a combination of yeast and human genetics approaches to investigate pathogenic mechanisms of ALS. His laboratory has made several fundamental discoveries into neurodegenerative disease mechanisms. These discoveries include discovery of modifiers of aggregation and cytotoxicity of the FTD/ALS disease protein TDP-43, a mechanism to explain how FTD/ALS-linked TDP-43 mutations affect the protein and contribute to disease, and the discovery of novel genetic contributors to human FTD and ALS, including mutations in the ataxin-2 gene as one of the most common genetic risk factors for ALS and a role of cryptic splicing of UNC13A and other synaptic protein encoding genes as a mechanism in ALS and FTD. Gitler's work has helped to uncover unexpected and novel therapeutic targets for ALS, including preclinical studies of ataxin-2 as a therapeutic target for sporadic ALS, demonstrating that reduction of ataxin-2 levels markedly extends lifespan in TDP-43 transgenic mice. These efforts have provided the foundation for an ongoing clinical trial in human ALS patients to test ataxin-2 targeting antisense oligonucleotides as a therapy.Leadership Scholarship from Women in Leadership Australia, two Commendations for IMB Leadership Impact Award and short-listed as UQ Excellence in PhD supervision.

MICHAEL T. HENEKA

MICROGLIAL PYROPTOSIS AS DRIVING FORCE OF AMYLOID PATHOLOGY IN ALZHEIMER DISEASE

Director, Luxembourg Centre for Systems Biomedicine, University of Luxembourg and Principal Investigator of the Neuroinflammation group

Link to the lab

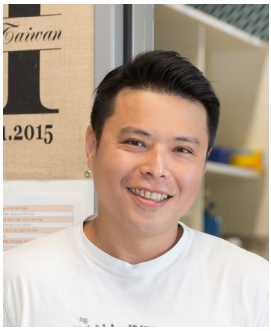
Michael Heneka is the Director of the Luxembourg Centre for Systems Biomedicine at the University of Luxembourg and the head of the Neuroinflammation group.

He studied medicine in Tübingen, Lausanne and London from 1990-1996. He obtained his medical degree at the Institute of Pharmacology for which he received the 1998 Attempto Award of the University of Tübingen. He started his clinical residency in Neurology at the Dept. of Neurology of the Univ. of Tübingen in 1996 and joined the Dept. of Neurology at the University of Bonn in 1999. After his clinical board examination (2002) and habilitation (2003) he took the chair as Professor for Molecular Neurology at the University of Münster in 2004. In 2008 he was appointed Professor for Clinical Neurosciences at the University of Bonn heading the DFG Clinical Research Unit 177. At the clinical level he has established a neurodegenerative outpatient unit at the University of Münster and thereafter at the University of Bonn from 2008-2016. The latter has been the basis for the foundation of the Dept. of Neurodegenerative Disease and Geriatric Psychiatry in 2016, which he was heading until his move to the Luxembourg Centre for Systems Biomedicine (LCSB) in January 2022.

He is an editorial board member of various neuroscience journals and serves as scientific advisory board member of the Paris Brain Institute, the Dementia Research Institute UK and the Dementia Discovery Fund. He is the organiser of the biannual meeting "Venusberg Meeting on Neuroinflammation" since 2009 as well as co-organiser of various international meetings and symposia.

Michael Heneka's research focus is on neurodegenerative diseases with a special emphasis on immune mechanisms in the central nervous system with the goal of developing new biomarkers and medical intervention programs. The group works e.g. on elucidating the role of tunnelling nanotubes for cell-to-cell communication and on exploring how immune cells regulate neuronal functions.

PING-CHIH HO



DISTURBED MITOCHONDRIAL DYNAMICS INSTRUCT T CELL EXHAUSTION AND BEYOND CELL DEATH

Professor, Ludwig Institute for Cancer Research, University of Lausanne

Link to the lab

Ping-Chih Ho grew up in Taiwan and obtained his basic biomedical training, including bachelor degree (Life Science) and master degree (Biochemical Science), at National Taiwan University. He then obtained his PhD in Department of Pharmacology at University of Minnesota. Ping-Chih then did postdoctoral training with Susan Kaech at Yale University, where he demonstrated how cancer cells evade T cell immunosurveillance by depriving infiltrating T cells of glucose, which is consumed in large amounts by malignant cells. In September 2015, he relocated to Switzerland as a tenure-track assistant professor in the Department of Oncology at the University of Lausanne and act as an adjunct scientist at the Ludwig Institute for Cancer Research. Ping-Chih was promoted as a tenured associate professor at the University of Lausanne in August 2019 and promoted to Associate Member at the Ludwig Institute for Cancer Research in January 2020. Since Jan. 2023, Ping-Chih is appointed as a Full Professor at University of Lausanne and a Full member at Ludwig Institute for Cancer Research. Ping-Chih's research is also supported by University of Lausanne, the European Research Council, the Cancer Research Institute, the Swiss National Science Foundation, the Melanoma Research Alliance, the Swiss Cancer League, Harry J. Lloyd Fund, Anna Fuller Fund, the ISREC foundation. His research is also acknowledged by several international awards, including the CRI Lloyd J. Old STAR Award, EMBO Young Investigator award, MRA-SITC Young Investigator award, MRA Established Investigator Award, CRI CLIP-investigator award, and the Swiss Bridge Award.

GUIDO KROEMER

AUTOPHAGY CHECKPOINT INHIBITION - A NOVEL STRATEGY FOR THERAPEUTIC AUTOPHAGY ENHANCEMENT

Professor at Université Paris Cité

Link to the lab

Guido Kroemer is currently Professor at the Faculty of Medicine of the University of Paris, Director of the research team "Metabolism, Cancer and Immunity" of the French Medical Research Council (INSERM), Director of the Metabolomics and Cell Biology platforms of the Gustave Roussy Comprehensive Cancer Center, and Hospital Practitioner at the Hôpital Européen George Pompidou, Paris, France. Dr. Kroemer's work focuses on the pathophysiological implications of cell stress and death in the context of aging, cancer and inflammation. With over 1600 articles including 67 papers in the 'CNS' Journals Cell (15 papers), Nature (6), Nature Medicine (21), Science (18) and Science Translational Medicine (7) and an h-index of 284, he is Europe's most cited researcher in biomedical research. His contributions have been recognised with multiple awards including the most prestigious cancer research and immunology prizes from Belgium (Baillet-Latour Health Prize), France (Prix Duquesne, Prix Léopold Griffuel, Grand Prix Ruban Rose), Spain (International Prize for Oncology Ramiro Carregal, Boulle-SEI Award) and Switzerland (Brupbacher Prize), the European Union-sponsored Descartes Prize, as well as the most important Italian science prize (Lombardia & Ricerca Prize). He also received two European Research Council (ERC) Advanced Investigator Awards. Kroemer is the founding Editor-in-Chief of six journals: Cell Death & Disease, Cell Stress, OncoImmunology, MedComm Cancer, Microbial Cell, and Molecular & Cellular Oncology. He is member of the Austrian Academy of Sciences, Academia Europaea, European Academy of Sciences (EAS), Chinese Academy of Engineering (CAE), European Academy of Sciences and Arts (EASA), European Academy of Cancer Sciences (EACS), European Molecular Biology Organization (EMBO), German Academy of Sciences (Leopoldina), Institut Universitaire de France (IUF) and Spanish Royal Academy of Sciences. He is the Founding President of the European Academy of Tumor Immunology (EATI), as well as the President-Elect of the European Network for Cancer Immunotherapy (ENCI). American Association of Immunologists-Thermo Fisher Meritorious Career Award. By continually advancing basic and translational research, she remains at the forefront of her field.

JUDY LIEBERMAN

TURNING UP THE HEAT ON CANCER

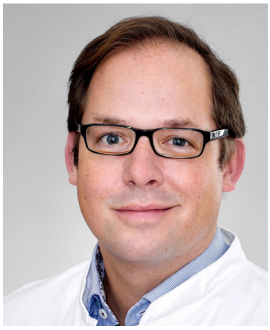
Endowed Chair in Cellular and Molecular Medicine, Program in Cellular and Molecular Medicine, Boston Children's Hospital

Link to the lab

Judy Lieberman (MD, PhD) holds an Endowed Chair in Cellular and Molecular Medicine at Boston Children's Hospital and is Professor of Pediatrics at Harvard Medical School. Her lab studies the innate and adaptive immune response to infection and cancer, especially the molecular pathways used by killer lymphocytes to induce programmed cell death of both mammalian cells and microbes and the mechanisms responsible for inflammatory death (pyroptosis) triggered by innate immune recognition of invasive pathogens and danger signals. Recent work identified roles for pyroptosis in SARS-CoV-2, Yersinia, and Group A streptococcal infections, in immune control of cancer and in neurodegeneration. Her lab was also in the forefront of developing RNAi-based therapeutics, using RNAi for genome-wide screening and studying the role of microRNAs in cancer.

She graduated from Radcliffe College, Harvard, received a PhD in theoretical physics at Rockefeller and an MD in the joint Harvard MIT Program in Health Sciences and Technology. She worked as a theoretical high energy physicist at the Institute for Advanced Study in Princeton and at Fermilab. She was a postdoc with Herman Eisen in immunology at MIT and worked as a hematologist/oncologist at New England Medical Center. She is a member of the American Academy of Arts and Sciences, the National Academy of Sciences and the National Academy of Medicine.

ANDREAS LINKERMANN



GENOMIC AND NON-GENOMIC ANTI-FERROPTOTIC FUNCTIONS OF ESTRADIOL DEFINE CELL DEATH PROPAGATION AND SEX DIFFERENCES IN ACUTE KIDNEY INJURY

Chair of the Clinic for Internal Medicine V, UMM Mannheim of the Heidelberg University

Andreas Linkermann is the Chair of the Clinic for Internal Medicine V, UMM Mannheim of the Heidelberg University, Germany. He was trained to specialize in internal medicine, nephrology and transplantation at the Division of Nephrology at the Christian-Albrechts-University Kiel, Germany until 2016 where he became a Fellow of the American Society of Nephrology (FASN) with a clinical focus on kidney transplantation and acute kidney injury. Until September 2024, he served as a Heisenbergprofessor at the Carl Gustav Carus Hospital of the Technical University of Dresden.

As a clinician scientist, research in his laboratory includes work on the basic understanding of regulated cell death and the mechanisms of necroinflammation. The immunogenicity of regulated necrosis was identified as a potential therapeutic target not only during transplantation and acute kidney injury, but also in myocardial infarction, stroke and intoxications. Recently, the laboratory focused on cell death propagation in the renal tubules and two specific signalling pathways of necroptosis and ferroptosis. In collaboration with medicinal chemists, small molecule inhibitors of these pathways have been developed. Since 2018, the lab has focused on sex differences in cell death and acute kidney injury. Another recent but exciting project investigates kidney xenotransplantation.

Work in the Linkermann laboratory is funded by the German Research Foundation including the collaborative research centers SFB/TRR205 (Adrenal) SFB/TRR127 (Xenotranplantation), the SPP2036 on ferroptosis, the international research training group IRTG2251, the TransCampus project with King´s college London and several private foundations. For the identification of the mechanistic details of signalling pathways of regulated necrosis and their role in pathophysiologic settings, Linkermann received the Carl-Ludwig-Award and the Franz-Volhard-Award of the German Society of Nephrology (DGfN), the Rudolf-Pichlmayr-Award of the German Society of Transplantation, the young investigator award of the American Transplantation Society, the Scientific excellence prize by the European Renal Association and was entitled honorary member of the European Academy of Tumor Immunology. Since 2021, he is a Clarivate Highly Cited researcher.

PATRICIA MACIEL

SEROTONIN SIGNALING PROTECTS NEURONS AGAINST PROTEIN AGGREGATION-INDUCED DYSFUNCTION AND DEGENERATION

Associate Professor, University of Minho School of Medicine;
Director, Life and Health Sciences Research Institute (ICVS)

Patrícia Maciel obtained a B.Sc. in Biochemistry (1993) and a Ph.D. in Biomedical Sciences – Genetics (1998) at the University of Porto, having developed her thesis work at Hôpital Necker-Enfants Malades, France, and at the Centre for Research in Neuroscience, McGill University, Canada.
Dr. Maciel is currently an Associate Professor at the University of Minho School of Medicine, Director of the MSc program in Health Sciences and Director of the Life and Health Sciences Research Institute (ICVS), where she also leads the Translational Neurogenetics research team. Her work, focusing on the discovery of molecular mechanisms and therapeutics for neurological diseases, of a neurodegenerative and neurodevelopmental nature, has led to over 150 publications in peer-reviewed scientific journals, with an H-index of 41 (Researcher ID)/50 (Google Scholar). She has supervised 20 PhD students and 20 MSc students to thesis completion. She is an inventor in two patents and has multiple collaborations with pharmaceutical companies for drug discovery and development.
In addition to serving as reviewer for over 30 scientific journals and for national and international funding entities, Dr. Maciel integrates the Inter-Ministerial Committee for an Integrated Strategy for Rare Diseases, the Policy Board of the European Joint Programme on Rare Diseases, and served as Expert and Rapporteur for the National Agenda for Research and Development promoted by the Portuguese Foundation for Science and Technology (FCT). She is an elected member of the General Council of the University of Minho since July 2020.

MARÍA MITTELBRUNN

DECODING THE CONTRIBUTION OF THE IMMUNE SYSTEM TO AGING

Head of Immunometabolism and Inflammation Lab, Severo Ochoa Centre for Molecular Biology

Link to the lab

María Mittelbrunn received the degree of Doctor in Biomedicine, Biochemistry and Molecular Biology from the School of Medicine at the Autonomous University of Madrid in 2006, and performed postdoctoral work at Spanish National Center for Cardiovascular Research (CNIC) (Madrid, Spain) from 2007 to 2016. Since 2017, she is Group Leader of the Immunometabolism & Inflammation Laboratory at the Molecular Biology Center (Madrid). Since 2021, she is Research Scientist of the Spanish Research Council (CSIC) .

Among her original contributions as PI are the demonstration that the deterioration of immune system function with aging not only compromises the response to infection, cancer, vaccination, or predisposes to autoimmunity but also increases the risk for cardiovascular, metabolic, and cognitive decline, thereby placing the immune system as a controller of healthy aging. Dr Mittelbrunn has contributed to decoding the molecular mechanisms by which aged T cells contribute to inflammaging and age-related diseases. Additionally, she has proposed new therapeutic targets to delay age-related multimorbidity and to reverse aortic aneurysms, thus preventing sudden death due to aortic dissections.

She has obtained funding from the major European and Spanish funding organisations, including an European Research Council Starting Grant in 2016, and Consolidator Grant in 2022.

For her scientific achievements, she has been awarded with Doctoral thesis Extraordinary Prize (2006), LÓREAL UNESCO for Women in Science (2015), BANCO SABADELL AWARD for Biomedical Research (2022), and Royal Spanish Academia of Science for young researchers among others. Since 2024, she is Visiting Professor at CCTI, Columbia

MANOLIS PASPARAKIS

REGULATION OF NECROPTOSIS AND ITS ROLE IN INFLAMMATION

Professor, CECAD Research Center, Institute for Genetics, University of Cologne

Link to the lab

Manolis Pasparakis studied Biology at the University of Athens and did his PhD at the Hellenic Pasteur Institute in Athens, Greece. After postdoctoral training in the Institute for Genetics of the University of Cologne he joined the Mouse Biology Programme of EMBL in Monterotondo, Italy as a group leader. Since 2005 he is a professor at the Institute for Genetics of the University of Cologne.

His research aims to understand the mechanisms regulating inflammation and the pathogenesis of inflammatory diseases and cancer. Topics of particular interest in his lab include IKK/NF-kB signalling and its function in physiology and pathology, RIP kinases and their role in health and disease, as well as ZBP1 as a sensor of Z-nucleic acids that mediates cell death, inflammation and interferon responses.

DAVID RUBINSZTEIN

AUTOPHAGY AND NEURODEGENERATION

Professor of Molecular Neurogenetics and UK Dementia Research Institute Group Leader, University of Cambridge

Link to the lab

David Rubinsztein is Professor of Molecular Neurogenetics and a UK Dementia Research Institute Group Leader at the University of Cambridge. He is Deputy Director of the Cambridge Institute for Medical Research. Dr. Rubinsztein earned his MB ChB, BSc(Med) Hons, and PhD degrees from University of Cape Town. He came to Cambridge in 1993 as a Senior Registrar in genetic pathology and was the first person to complete formal training in this field in the UK.

His research is focused in the field of autophagy, particularly in the context of neurodegenerative diseases. His laboratory pioneered the strategy of autophagy upregulation as a possible therapeutic approach in various neurodegenerative diseases, and has identified drugs and novel pathways that may be exploited for this objective. He has made contributions that reveal the relevance of autophagy defects as a disease mechanism and to the basic cell biology of this important catabolic process.

Rubinsztein was elected Fellow of the Academy of Medical Sciences (2004), EMBO member (2011), Fellow of the Royal Society (2017) and membership of Academia Europaea (2022). He was awarded the Graham Bull Prize (2007), Thudichum Medal (2017), Roger de Spoelberch prize (2017) and the Goudie Medal (2020).

KATJA SIMON

AUTOPHAGY AND NEURODEGENERATION

Group Leader, Cell Biology of Immunity, Max Delbrück Center for Molecular Medicine Berlin

Link to the lab

Prof. Katja Simon trained as an Immunologist at the Deutsche Rheumaforschungszentrum, Berlin and during her PhD showed that TH1 cytokines are found in excess in human rheumatoid arthritis joints. For this, she was awarded the European League Against Rheumatism EULAR award.

After postdocs at the Centre d'Immunologie Marseille Luminy and at the Weatherall Institute in Oxford, where she focused on cell death pathways in the immune system, she became a principal investigator. With her team, she pioneered the field of autophagy in the immune system. Her group discovered that autophagy, the main cellular bulk degradation pathway, promotes differentiation of healthy red blood cells and neutrophils, and maintains long-lived cells such as stem and memory T cells. She also showed that it prevents ageing of immune cells and can be used to reverse immune senescence.

She has been a Wellcome investigator since 2015. In 2016 she moved to the Kennedy Institute of Rheumatology Oxford and became a full professor. In 2022 she started a new group at the Max Delbrück Center for Molecular Medicine in Berlin with funding from the Helmholtz Society for distinguished professors. She received the 2018 Ita Askonas prize for outstanding achievements as a female European group leader in Immunology and became a fellow of the UK Academy of Medical Sciences.

BRENT STOCKWELL

METABOLISM, DRUGS, AND DIET: FERROPTOSIS AS A NEW THERAPEUTIC PARADIGM

Chair of the Department of Biological Sciences and a Professor at Columbia University in the Departments of Biological Science and Chemistry

Link to the lab

Brent R. Stockwell, PhD, is the William R. Kenan Jr. Professor of Biological Sciences and Chair of the Department of Biological Sciences, and Professor of Chemistry in Arts & Sciences, Columbia University, and Professor of Pathology and Cell Biology, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center. His research involves the discovery of small molecules that can be used to understand and treat cancer and neurodegeneration, with a focus on biochemical mechanisms governing cell death. In a series of papers from 2003-2012, Dr Stockwell discovered compounds that activate a previously unrecognised form of cell death that he termed ferroptosis. His lab defined key mechanisms governing ferroptosis, its therapeutic implications, and key reagents for studying this new form of cell death.

Dr Stockwell has received numerous awards, including being elected to the US National Academy of Medicine, a Burroughs Wellcome Fund Career Award at the Scientific Interface, a Beckman Young Investigator Award, a Howard Hughes Medical Institute Early Career Scientist Award, the BioAccelerate NYC Prize, the Lenfest Distinguished Columbia Faculty Award, the Great Teacher of Columbia College Award from the Society of Columbia Graduates, the Dean Peter Awn Commitment to the LGBTQ community Faculty Award, and an NCI R35 Outstanding Investigator Award. He has been in the top one percent of highly cited researchers the last four years and was named as one of the 50 most influential life science individuals in New York. He has published >190 scientific articles, been awarded 23 US patents, and received >50 research grants for >$40 million.

ANDREAS STRASSER

HOW DOES THE TUMOUR SUPPRESSOR P53 INSTRUCT CELL FATE – APOPTOSIS VS CELL SURVIVAL AND CELL CYCLE ARREST?

Head of the Blood Cells and Blood Cancer Division at The Walter and Eliza Hall Institute

Link to the lab

Andreas Strasser is Head of the Blood Cells and Blood Cancer Division at the Walter and Eliza Hall Institute. He is a world leader in cancer and immunology, with a particular focus on the role of programmed cell death (apoptosis). By exploiting mouse genetics, he was the first to demonstrate that abnormalities in the control of apoptosis can cause autoimmune disease and amongst the first to show that this can cause cancer and render tumor cells refractory to anti-cancer therapy. These discoveries have major biological implications and suggest novel therapeutic strategies for cancer, autoimmunity and degenerative diseases. Current research interests include identification of the signalling pathways that mediate developmentally programmed cell death in mammals and those that are responsible for chemotherapy-induced killing of cancer cells, with the goal to develop improved strategies for treatment of cancer and autoimmune diseases.

Andreas Strasser earned his PhD at Basel Institute for Immunology under Fritz Meichers in 1988 and moved for postdoctoral studies under Suzanne Cory to the Walter and Eliza Hall Institute in 1989 where he has remained ever since. He has been a Senior, Principal, then Senior Principal Research Fellow, then Australia Fellow of the National Health and Medical Research Council. Since 2006 he has been the Joint Division Head of the Molecular Genetics of Cancer Division at Walter and Eliza Hall Institute. He has published more than 240 primary research papers as well as a further 100+ review articles, and has received more than 35,000 citations overall. He has been recognised with the Burnet Prize, the Josef Steiner Cancer Research Prize, the Friedrich Miescher Prize from the Swiss Society for Biochemistry, shared the Glaxo Wellcome Australia Prize with Professor David Vaux, and the Victoria Prize in 2011. In 2003 he was elected to the Australian Academy of Science. In 2009, became a foreign associate member of the European Molecular Biology Organisation, and in 2023 he was elected a Fellow of the American Association of Cancer Research (AACR).

STEPHEN TAIT

MITOCHONDRIA AND ANTI-TUMOUR IMMUNITY – A CASE OF BACTERIAL MIMICRY?

Deputy Head of School of Cancer Sciences, University of Glasgow; Professor of Mitochondrial Cancer Biology, University of Glasgow; Senior Group Leader, Cancer Research UK Scotland Institute

Link to the lab

Prof. Stephen Tait investigates mitochondrial regulation of cell death and inflammation in the context of cancer. His lab is based at the Cancer Research UK Scotland Institute in Glasgow, UK.

His lab's major contributions include:

1. The discovery that sub-lethal engagement of mitochondrial apoptotic signaling can be oncogenic
2. Killing cancer cells under caspase inhibited conditions engages anti-tumor immunity
3. Discovery that mitochondria trigger various inflammatory pathways during cell death
4. Mitochondrial permeabilisation directly connects cell death and senescence

His lab is supported by funding from Cancer Research UK, BBSRC and Prostate Cancer UK.

HENNING WALCZAK

DEATH RECEPTORS AND UBIQUITIN IN CELL DEATH, INFLAMMATION AND IMMUNITY

Professor of Biochemistry, Institute of Biochemistry I, University of Cologne.
Centre for Cell Death, Cancer and Inflammation, UCL Cancer Institute, University College London

Link to the lab (ULC)
Link to the lab (University Cologne)

Professor Henning Walczak holds a dual position as Alexander-von-Humboldt Professor of Biochemistry at the Centre of Biochemistry & CECAD at the University of Cologne and Chair of the Centre for Cell Death, Cancer and Inflammation (CCCI) in the Cancer Institute, University College of London.

After completing his PhD in 1995 at the German Cancer Research Centre (DKFZ, Heidelberg, Germany), he worked as postdoctoral researcher at Immunex Corporation in Seattle (WA, USA). After returning to Europe in 1998, he became group leader at the DKFZ in 2000 following receipt of a BioFuture Prize awarded by the German Ministry for Science and Education. In October 2007, he was appointed as Chair of Tumour Immunology at Imperial College London. He joined UCL in January 2013. Research by the Walczak group has been funded through Programme grants from Cancer Research UK, the Wellcome Trust, MRC and ERC since 2008. In 2019, he was appointed as Alexander-von-Humboldt Professor of Biochemistry and Chair of the Institute of Biochemistry I of the Medical Faculty of the University of Cologne.

The Research group headed by Prof Walczak investigates cell death and ubiquitin in inflammation, immunity to infection, auto-immunity and cancer. A particular emphasis of the group's work is on harnessing newly gained insight on the functional interplay between cell death, ubiquitin and inflammation regarding their role in tumour immunity.

WENDY WEI-LYNN WONG

NEW TRICKS FOR XIAP IN CELL DEATH AND INFLAMMATION

Group leader, Institute of Experimental Immunology,
Department of Molecular Life Sciences, University of Zurich

Link to the lab

Wei-Lynn Wong (Lynn) hails from Canada. She pursued her undergraduate education at the University of Guelph, participating in the co-operative program, earning a Bachelor of Science degree. Continuing her academic journey, she obtained her PhD from the University of Toronto, conducting research at the Princess Margaret (PM) Cancer Centre, formerly recognized as the Ontario Cancer Institute.

Her postdoctoral years led her to Melbourne, Australia, where she was mentored by John Silke and David Vaux at the Walter & Eliza Hall Institute of Medical Research and LaTrobe University. In 2011, she transitioned to Switzerland, assuming the role of a group leader at theInstitute of Experimental Immunology, University of Zurich . After receiving the Clöetta Medical Research fellow, she became Assistant Professor within the same institute in 2016. In 2022, her group moved to the Department of Molecular Life Sciences at the University of Zurich.

The research of her lab explores the vital function of cell demise in maintaining healthy tissue homeostasis. The manner in which a cell meets its end profoundly influences the neighbouring tissue and the immune system's reaction. Their emphasis lies in pinpointing the regulatory mechanisms governing cell demise signaling and its implications in detecting danger cues and/or generating inflammation, such as activation of the inflammasome or an interferon stimulated gene signature. The lab strives to uncover whether these responses vary across different primary cell types or share commonalities. This has unveiled how multifaceted cell death signaling pathways can often blur the line in regulating cell demise or cellular function.

JUNYING YUAN

TALK TITLE

Director of Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences

Link to the lab

Junying Yuan received her undergraduate degree from Fudan University, Shanghai, China, in 1982 and her Ph.D. in Neuroscience from Harvard University in 1989. Dr. Yuan carried out her Ph.D. thesis work at the Massachusetts Institute of Technology in the laboratory of H. R. Horvitz. She was first appointed as Assistant Professor at Harvard Medical School in 1992, when she became a Principal Investigator of the Cardiovascular Research Center at Massachusetts General Hospital. She joined the Department of Cell Biology in 1996 and was appointed a Professor of Cell Biology at Harvard Medical School in 2000. In 2014, Dr. Yuan was appointed as Elizabeth D. Hay Professor of Cell Biology, a Professorship that honours the late Professor Elizabeth D. Hay, the first female full professor in the history of Harvard Medical School. Dr. Yuan returned to China in 2020 and joined Shanghai Institute of Organic Chemistry as the Director of Interdisciplinary Research Center on Biology and Chemistry.

Dr. Yuan is a pioneer in the cell death field. Her 256 published papers have been highly cited with collective citations of more than 120,000 times (H index 136). Dr. Yuan made transformative discoveries on apoptosis and necroptosis in mammalian cells. Her discovery of mammalian caspases led to a molecular era in apoptosis research. She used chemical biology to demonstrate the existence and significance of a regulated necrosis mechanism, termed necroptosis, in human inflammatory and neurodegenerative diseases and the role of RIPK1 as a key mediator of necroptosis. RIPK1 inhibitors, first discovered and described by Dr. Yuan, have been advanced into human clinical trials for the treatment of human inflammatory and neurodegenerative diseases worldwide. She is an elected member of the National Academy of Sciences (US) and the American Academy of Arts and Sciences, and a foreign member of the Chinese Academy of Sciences.

SHORT TALKS SELECTED FROM ABSTRACTS

DAVID ANDREWS

Sunnybrook Research Institute, Canada

DIRECT BINDING INTERACTIONS OF C-TERMINAL SEQUENCES OF BCL-2 FAMILY PROTEINS REGULATE CELL DEATH

GUY BROWN

University of Cambridge, United Kingdom

CELL DEATH BY PHAGOCYTOSIS CONTRIBUTES TO NEURODEGENERATION

FRANK ESSMANN

Robert Bosch Center for Tumor Diseases, Germany

PREFERENTIAL HOMOTYPIC INTERACTION OF C-TERMINAL DOMAINS FROM PRO-APOPTOTIC EFFECTOR PROTEINS BAX, BAK AND BOK

SHORT TALKS SELECTED FROM ABSTRACTS

HADIAN KAMYAR

Helmholtz Centre Munich, Germany

SUPPRESSION OF FERROPTOSIS BY VITAMIN A OR RADICAL-TRAPPING ANTIOXIDANTS IS ESSENTIAL FOR NEURONAL DEVELOPMENT

ESTHER HOSTE

Ghent University, Belgium

STABILIZATION OF BETA-CATENIN AVERTS DERMATITIS CAUSED BY OTULIN DEFICIENCY

WALKER JACKSON

Linköping University, Sweden

MICROGLIA DO SOMETHING STRANGE TO THEIR RIBOSOMES DURING NEUROINFLAMMATION

SHORT TALKS SELECTED FROM ABSTRACTS

ANA JANIC

Universidad Pompeu Fabra, Spain

COMBINED ABSENCE OF TRP53 TARGET GENES ZMAT3, PUMA AND P21 CAUSE A HIGH INCIDENCE IN MICE

HIROYASU NAKANO

Toho University School of Medicine, Japan

LIVE CELL IMAGING OF NECROPTOSIS AND THE RELEASE OF DAMPS IN VITRO AND IN VIVO

SJOERD VAN WIJK

Institute for Experimental Cancer Research in Pediatrics, Goethe University Hospital Germany

LINEAR UBIQUITINATION AT DAMAGED LYSOSOMES INDUCES LOCAL NF-κB ACTIVATION AND CONTROLS CELL SURVIVAL

FLASH TALKS SELECTED FROM ABSTRACTS

GEORGIA ATKIN-SMITH

Walter and Eliza Hall Institute, Australia

IDENTIFICATION OF ENDOTHELIAL CELL EXTRACELLULAR VESICLES AS A BIOMARKER OF VASCULAR DEGRADATION DURING BLOOD MALIGNANCIES

ILARIA DEIDDA

University Medical Center Göttingen, Georg-August University, Germany

RUNX1 MODULATES APOPTOSIS VIA EPIGENETIC REPROGRAMMING IN PANCREATIC CANCER

ROBERTO FERNÁNDEZ ACOSTA

University of Antwerpen, Belgium

EPIGENETIC RECONDITIONING OF FERROPTOSIS-RESISTANT HIGH-RISK NEUROBLASTOMA: CTRL+ALT+DEL FOR TUMOR DEFENSE MACHINERY

MELANIE GRUSDAT-POZDEEV

Luxembourg Centre for Systems Biomedicine, University of Luxembourg

GLUTATHIONE REGULATES NATURAL KILLER CELL IMMUNITY AND MEDIATES TUMOR CONTROL

FLASH TALKS SELECTED FROM ABSTRACTS

KIRSTEN KENNEY

University of Queensland, Australia

CORTICOSTERONE DYSREGULATES NLRP3 INFLAMMASOME SIGNALLING

SVENJA LORENZ

Helmholtz Centre Munich, Germany

NEURONAL FERROPTOSIS IN MICE CAUSES SIGNATURES REMINISCENT OF NEURODEGENERATIVE DISEASES IN MAN

FRANCESCA RIZZOLLO

VIB - KU Leuven Center for Cancer Biology, Germany

IRON TRAFFICKING AND FERROPTOSIS VULNERABILITY IN MELANOMA IS CONTROLLED BY MITOCHONDRIA-LYSOSOME CONTACT TETHERING

ANNA SAORIN

University of Zurich, Kinderspital Zurich Switzerland

TRANSCRIPTIONAL REMODELING SHAPES THERAPEUTIC VULNERABILITY TO NECROPTOSIS IN ACUTE LYMPHOBLASTIC LEUKEMIA

FLASH TALKS SELECTED FROM ABSTRACTS

EGE SOLEL

University of Bergen, Norway

IMPACT OF GSDME ON PYROPTOSIS IN GLIOBLASTOMA

IRATXE URANGA MURILLO

Health Research Institute of Aragon, Spain

FASL, A CRUCIAL HOST FACTOR DRIVING COVID-19 PATHOLOGY AND LETHALITY

SIMON VERDONCK

VIB-UGent University of Gent, Belgium

RIPK1 PREVENTS STING-DEPENDENT SKIN INFLAMMATION

WEI XIE

VIB - UGent - Center for Inflammation Research

ROLE OF APOPTOTIC EXECUTIONER CASPASE-3/7 IN INTESTINAL INFLAMMATION AND COLORECTAL CANCER

ABSTRACTS OF SHORT / FLASH TALKS

Author	Affiliation	Title	Talk, Slot & Poster no.
David Andrews	Sunnybrook Research Institute	Direct binding interactions of C-terminal sequences of BCL-2 family proteins regulate cell death	Short talk Session 4 (10.10, 15:45) Poster no. 1
Georgia Atkin-Smith	WEHI	Identification of endothelial cell extracellular vesicles as a biomarker of vascular degradation during blood malignancies	Flash talk Session 5 (11.10, 12:15) Poster no. 2
Guy Brown	University of Cambridge, United Kingdom	Cell death by phagocytosis contributes to neurodegeneration	Short talk Session 1 (09.10, 09:45)
Ilaria Deidda	Universitätsmedizin Göttingen, Georg-August Universität	RUNX1 Modulates Apoptosis via Epigenetic Reprogramming in Pancreatic Cancer	Flash talk Session 4 (10.10, 16:00) Poster no. 3
Frank Essmann	Robert Bosch Center for Tumor Diseases	Preferential homotypic interaction of C-terminal domains from pro-apoptotic effector proteins BAX, BAK and BOK	Short Talk Session 5 (11.10, 14:15) Poster no. 4
Roberto Fernández Acosta	University of Antwerpen	Epigenetic reconditioning of ferroptosis-resistant high-risk neuroblastoma: Ctrl+Alt+Del for tumor defense machinery	Flash talk Session 4 (10.10, 16:07) Poster no. 5

ABSTRACTS OF SHORT / FLASH TALKS

Author	Affiliation	Title	Talk, Slot & Poster no.
Melanie Grusdat-Pozdeev	Luxembourg Centre for Systems Biomedicine	Glutathione regulates natural killer cell immunity and mediates tumor control	Flash talk Session 3 (10.10, 10:00) Poster no. 6
Kamyar Hadian	Helmholtz Zentrum München	Suppression of ferroptosis by vitamin A or radical-trapping antioxidants is essential for neuronal development	Short talk Session 3 (10.10, 09:45)
Esther Hoste	Ghent University	Stabilization of beta-catenin averts dermatitis caused by OTULIN deficiency	Short Talk Session 5 (11.10, 12:00) Poster no. 7
Walker Jackson	Linköping University	Microglia do something strange to their ribosomes during neuroinflammation	Short Talk Session 5 (11.10, 13:45)
Ana Janic	Universidad Pompeu Fabra	Combined absence of TRP53 target genes ZMAT3, PUMA and p21 cause a high incidence in mice	Short Talk Session 5 (11.10, 14:00)
Kirsten Kenney	University of Queensland	Corticosterone Dysregulates NLRP3 Inflammasome Signalling	Flash talk Session 5 (11.10, 10:07) Poster no. 8

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Author	Affiliation	Title	Talk, Slot & Poster no.
Svenja Lorenz	Helmholtz Zentrum München	Neuronal ferroptosis in mice causes signatures reminiscent of neurodegenerative diseases in man	Flash talk Session 1 (9.10, 10:07) Poster no. 9
Hiroyasu Nakano	Toho University School of Medicine	Live cell imaging of necroptosis and the release of DAMPs in vitro and in vivo	Short talk Session 2 (9.10, 15:45)
Francesca Rizzollo	VIB - KU Leuven	Iron trafficking and ferroptosis vulnerability in melanoma is controlled by mitochondria-lysosome contact tethering	Flash talk Session 3 (10.10, 10:07) Poster no. 10
Anna Saorin	UZH, Kinderspital Zurich	Transcriptional remodeling shapes therapeutic vulnerability to necroptosis in acute lymphoblastic leukemia	Flash talk Session 2 (9.10, 16:00)
Ege Solel	University of Bergen	Impact of GSDME on Pyroptosis in Glioblastoma	Flash talk Session 1 (9.10, 10:00) Poster no. 11
Iratxe Uranga Murillo	Health Research Institute of Aragon	FasL, a crucial host factor driving COVID-19 pathology and lethality	Flash talk Session 2 (9.10, 16:07) Poster no. 12

ABSTRACTS OF SHORT / FLASH TALKS

Author	Affiliation	Title	Talk, Slot & Poster no.
Sjoerd van Wijk	Institute for Experimental Cancer Research in Pediatrics, Goethe University Hospital	Linear ubiquitination at damaged lysosomes induces local NF-κB activation and controls cell survival	Short talk Session 5 (11.10, 09:45)
Simon Verdonck	VIB-UGent	RIPK1 prevents STING-dependent skin inflammation	Flash talk Session 5 (11.10, 10:00)
Wei Xie	VIB - UGent - Center for Inflammation Research	Role of apoptotic executioner caspase-3/7 in intestinal inflammation and colorectal cancer	Flash talk Session 5 (11.10, 12:22) Poster 96

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DIRECT BINDING INTERACTIONS OF C-TERMINAL SEQUENCES OF BCL-2 FAMILY PROTEINS REGULATE CELL DEATH

Andrews D.W. (1,2), Nguyen D. (2), Trebinjac S. (1)

1) Odette Cancer Program, Sunnybrook Research Institute, Sunnybrook Hospital, Toronto, Ontario, Canada; 2) Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

Programmed cell death via the both intrinsic and extrinsic pathways is regulated by interactions of the Bcl-2 family protein members that determine whether the cell commits to apoptosis via mitochondrial outer membrane permeabilization (MOMP). Recently the conserved C-terminal sequences (CTSs) that mediate localization of Bcl-2 family proteins to intracellular membranes, have been shown to have additional protein-protein binding functions that contribute to the functions of these proteins in regulating MOMP. We have discovered pivotal roles of CTSs in Bcl-2 family interactions including: (1) homotypic interactions between anti-apoptotic and pro-apoptotic proteins (2) heterotypic interactions between pro-apoptotic and anti-apoptotic proteins that prevent MOMP, and (3) heterotypic interactions between the pro-apoptotic executioner proteins and the pro-apoptotic direct activator proteins that promote MOMP.

We use fluorescence spectroscopy, fluorescence lifetime imaging and automated fluorescence microscopy to study protein-protein and protein-membrane interactions in live cells and the effects of these interactions on cell death1-3. Examination of these interactions using purified mitochondria and liposomes with full-length recombinant proteins revealed that Bcl-XL inhibits apoptosis as dimers that bind multiple BH3 proteins. Bcl-XL dimerization mediated by its C-terminal sequence (CTS) enables a unique form of allosteric regulation in which the BH3 sensitizer Bad confers switch-like activity to the activation of Bax by BH3-proteins4. Similarly, Bax and Bak form homodimers in membranes that are stabilized by interactions between the CTS sequences. Dimerization via the CTS may provide an interface distinct from the BH3-binding site resulting in the formation of Bax and Bak multimers that permeabilize membranes.

What makes a BH3 protein and activator of Bax and Bak? Our analyses of the pro-apoptotic BH3 proteins Bim and Puma revealed a double-bolt lock mechanism in which Bim and Puma bind their targets via both the well-known BH3 interaction and by direct binding of their respective CTS sequences2,3. For both Bim and Puma it is this additional CTS mediated interaction that enables efficient activation of Bax and Bak. Furthermore, this double bolt lock mechanism prevents BH3 mimetics from displacing Bim or Puma from anti-apoptotic proteins in live cells1.

These interactions have direct implications for the use of BH3-mimetics as anti-cancer agents. The underlying hypothesis has been that treatment with targeted chemotherapy agents can be used to induce dimer formation between pro- and anti-apoptotic proteins such as activated Bax and Bcl-XL that can then be released by drugs such as the Bcl-2/Bcl-XL inhibitor Navitoclax resulting in selective kill of cancer cells. We are testing this premise directly in pre-clinical studies using patient derived CLL and AML cells and for other cancers using organoids. We are also testing the hypothesis in patients with high grade serous ovarian cancer in an ongoing Phase I/II clinical trial combining the PARP inhibitor Olaparib with the Bcl-2/Bcl-XL inhibitor Navitoclax.

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IDENTIFICATION OF ENDOTHELIAL CELL EXTRACELLULAR VESICLES AS A BIOMARKER OF VASCULAR DEGRADATION DURING BLOOD MALIGNANCIES

Georgia K Atkin-Smith (1,2,3,4), Jascinta Santavanond (3,4), Amanda Light (1,2), Joel S Rimes (1,2), Andre Samson (1,2), Jeremy Er (1,5), Joy Liu (1,2), Darryl N Johnson (6), Mélanie Le Page (7), Pradeep Rajasekhar (1,2), Raymond Yip (1,2), Niall Geoghegan (1,2), Kelly L Rogers (1,2), Catherine Chang (1), Vanessa L Bryant (1,2,8), Mai Margetts (1), M Cristina Keightley (3,9), Trevor Kilpatrick (10,11), Michele D Binder (10), Sharon Tran (12,13), Erinna F Lee (3,12,13), W Douglas Fairlie (3,12,13), Dilara C Ozkocak (3,4), Andrew Wei (1,2,14), Edwin D Hawkins (1,2,Y,*) & Ivan KH Poon (3,4,Y,*)

1) WEHI, Melbourne, VIC, AUS. 2) Department of Medical Biology, The University of Melbourne, Melbourne, VIC, AUS. 3) Department of Biochemistry and Chemistry, La Trobe University, Melbourne, VIC, AUS. 4) Research Centre for Extracellular Vesicles, La Trobe University, VIC, AUS. 5) Clinical Haematology Department, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, VIC, AUS. 6) Materials Characterisation and Fabrication Platform, University of Melbourne, VIC, AUS. 7) Alfred Research Alliance, Monash University, Melbourne, VIC, AUS. 8) Department of Clinical Immunology and Allergy, Royal Melbourne Hospital, Melbourne, VIC, AUS. 9) Department of Rural Clinical Sciences, La Trobe Rural Health School, Bendigo, VIC AUS. 10) Florey Institute of Neuroscience and Mental Health, Melbourne, VIC, AUS. 11) Florey Department of Neuroscience and Mental Health, University of Melbourne, Melbourne, VIC, AUS. 12) Olivia Newton-John Cancer Research Institute, Heidelberg, VIC, AUS. 13) School of Cancer Medicine, La Trobe University, Bundoora, VIC, AUS; 14) Peter Mac Callum Cancer Centre, Melbourne, VIC, AUS.

Extracellular vesicles (EVs) are subcellular membrane cargo generated by both live and dying cells, such as when cells undergo apoptosis or autophagy. The formation of large EVs has recently emerged as a key mechanism to aid the disposal of cellular waste, as well as aid intercellular communication. However, the in vivo formation and clearance of endothelial cell-derived EVs remains to be fully defined.

We established a 4D intravital imaging approach to track the cellular dynamics of endothelial cells by using the endothelial reporter mouse stain, Flk1-GFP. Extensive intravital microscopy of the bone marrow calvarium identified a population of large EVs generated by endothelial cells under steady state conditions. These EVs contained polarized mitochondria, active caspase 3/7, exposed the 'eat me' signal, phosphatidylserine (PtdSer), and were also identified in zebrafish and human samples. Through extensive immune cell panelling and Image Flow Cytometry, we identified a number of immune cell populations including monocytes and neutrophils that could interact with and engulf these EVs. Notably, EV clearance was in part driven through a PtdSer dependent mechanism as EVs accumulated in the spleen of mice lacking the engulfment machinery, MerTK.

Next, we adopted three different blood cancer models to explore the impact of EV formation during malignant haematopoiesis. This included acute myeloid leukemia (AML), T cell acute lymphoblastic leukemia (T-ALL) and EμMyc-driven B cell lymphoma. Strikingly, live intravital imaging of the bone marrow calvarium and dual confocal/multiphoton microscopy of cleared long bones revealed that both AML and T-ALL resulted in the degradation of the bone marrow vasculature at late-stage of disease. This correlated with elevated numbers of endothelial cell-derived EVs found in circulation. Excitingly, we captured the complete fragmentation of endothelial cells during AML by extensive time-lapse intravital microscopy, and quantified EV biogenesis. Moreover, injection of Annexin V prior to im-

aging enabled detection of PtdSer positive endothelial cells and EVs in the bone marrow microenvironment. In comparison to the extensive vasculature degradation observed during AML and T-ALL, expansion of EμMyc lymphoma resulted in drastic remodelling of the bone marrow blood vessels but not endothelial cell death. As such, circulating EV levels remained unchanged.
Together, this study identified a new EV population that is generated and cleared under steady state and malignant conditions. Moreover, EV numbers correlate with endothelial cell degradation and thus, can provide a snapshot into the health status of the endothelium at distal sites during disease.

RUNX1 MODULATES APOPTOSIS VIA EPIGENETIC REPROGRAMMING IN PANCREATIC CANCER

Leonie Witte (1), Ilaria Deidda (1), Christian Schneeweis (2), Selen Selcen (1), Judith Achler-Galitzki (1), Michael Ghadimi (1), Günter Schneider (1), Matthias Wirth (1,3,4)

1) University Medical Center Göttingen, Department of General, Visceral and Pediatric Surgery, Göttingen, Germany, 2) Technical University Munich, Institute for Translational Cancer Research and Experimental Cancer Therapy, Munich, Germany, 3) Charité Universitätsmedizin Berlin, Department of Hematology, Oncology and Cancer Immunology, Campus Benjamin Franklin, Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany, 4) Max-Delbrück-Center for Molecular Medicine, Berlin, Germany

Pancreatic ductal adenocarcinoma (PDAC) displays high adaptability towards therapeutic interventions, leading to the development of resistance towards cell death and, ultimately, therapy failure. Starting from an unbiased screening approach, we identified RUNX1 as a potential therapeutic target in PDAC. We show that small molecule inhibition of RUNX1 is associated with the induction of the BH3-only protein NOXA and enriched H3K27 acetylation, an active chromatin mark (Doffo et al. 2022).

During our study, we conducted an unbiased compound screen with 1842 pharmacological compounds including experimental preclinical anti-cancer drugs as well as FDA-approved drugs. This screening was carried out in isogenic human and murine NOXA knockout cell lines of the aggressive basal-like PDAC subtype. To explore the regulation of potential cell death modulators genetically, we employed ATACseq, ChIPseq, and RNAseq, and performed extensive database analyses. We analyzed single and combination treatments using live cell imaging and quantitative endpoint measurements to assess viability (ATP quantification, metabolic activity), cell cycle progression (flow cytometry), and cell death (Annexin V/PI). Additionally, immunoblotting and qPCR assays were used to validate the mode of cell death and specific targets. We tested these pharmacological interventions in a variety of murine and human cell line models, predictive patient-derived organoid (PDO) models, and cell line-derived xenograft (CDX) models.

We find that a RUNX1/HDAC2 complex specifically controls the special epigenetic architecture of NOXA, suggesting an overarching role of NOXA in the maintenance of the apoptotic balance in PDAC. The combined inhibition of RUNX1 and BET proteins such as BRD4 further shifts the apoptotic balance towards cell death by simultaneously releasing the expression of pro-apoptotic BCL2 family members and repressing anti-apoptotic BCL2 family members transcriptionally. The simultaneous inhibition of RUNX1 and BRD4/BET proteins both genetically and pharmacologically therefore induced massive apoptosis in PDAC cell lines, patient-derived organoids, and xenograft models and effectively suppressed PDAC tumor growth. Likewise, we find a significantly increased response to BET inhibitor treatment in the absence of RUNX1.

Together, our data suggest RUNX1 as a potential biomarker for the prediction of BET inhibitor treatment response and suggests a potential epigenetic-based therapeutic strategy for PDAC patients.

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CELL DEATH BY PHAGOCYTOSIS CONTRIBUTES TO NEURODEGENERATION

Guy C. Brown

Department of Biochemistry, University of Cambridge, U.K.

Cell death by phagocytosis (phagoptosis) is the death of a cell as a result of a cell being engulfed, killed and digested by another cell. It is one of the commonest forms of cell death in mammals, contributing to development, immunity, tissue turnover, cancer and neurodegeneration [1]. Neurodegeneration can result from microglia (brain macrophages) phagocytosing neurons or synapses. Many of the genes associated with Alzheimer's disease (AD) risk affect microglial phagocytosis, including TREM2, PLCG2, ABI3, CD33, PILRA, SIGLEC11, ABCA1, ABCA7, CR1, GRN, CLU and APOE. However, it is unclear whether this effect on AD is via phagocytosis of amyloid and debris, or live synapses and neurons. We found that the R47H variant of TREM2 (which increases AD risk 4-fold) increased microglial phagocytosis of synapses and neurons, suggesting that microglial phagocytosis is detrimental in AD [2]. Extracellular TAU accumulates and spreads through the brain in AD, and we found that extracellular TAU induced microglial phagocytosis of live neurons via TLR4-NLRP3 inflammasome signaling [3]. We have previously shown that this TAU-induced neuronal loss can be prevented by blocking the microglial phagocytic receptor P2Y6 in culture and in vivo [4]. We also found that blocking the microglial P2Y6 receptor can prevent microglial phagocytosis of synapses, contributing to loss of neurons and memory [5].

Blood and brain levels of lipopolysaccharide (LPS) are elevated in AD, and potentially contribute to disease progression by activating microglial phagocytosis [6]. Screening for drugs that prevent LPS-induced neuronal death in glial-neuronal cultures identified multiple pathways, including the tyrosine kinase SYK [7]. TREM2 and other phagocytic receptors induce microglial phagocytosis via SYK, and we found that SYK inhibitors could prevent LPS-induced neuronal loss by blocking microglial phagocytosis of neurons [7,8]. Overall, the above results, and unpublished results to be presented, indicate that microglial phagocytosis of neurons and synapses contributes to neurodegeneration, and this may be prevented by blocking TREM2, SYK or P2Y6.

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PREFERENTIAL HOMOTYPIC INTERACTION OF C-TERMINAL DOMAINS FROM PRO-APOPTOTIC EFFECTOR PROTEINS BAX, BAK AND BOK

Beigl TB (1), Paul A (1), Fellmeth TP (2), Nguyen D (3,4), Barber L (1), Weller S (1), Schäfer B (1), Gillissen B (5), Aulitzky We (6), Kopp H-G (1,6), Rehm M (7,8), Andrews DW (3,4,9), Pluhackova K (2), Essmann F (1,10)

1) Robert Bosch Center For Tumor Diseases, Stuttgart, Germany; 2) Cluster Of Excellence Simtech, University Of Stuttgart, Germany; 3) Department Of Medical Biophysics, Faculty Of Medicine, University Of Toronto, Canada; 4) Biological Sciences Platform, Sunnybrook Research Institute, Canada 5) Department Of Hematology, Oncology, And Tumorimmunology, Charité University Medicine, Berlin; Germany; 6) Robert-bosch-hospital, Stuttgart, Germany; 7) Institute Of Cell Biology And Immunology, University Of Stuttgart, Germany; 8) Stuttgart Research Center Systems Biology, University Of Stuttgart, Germany; 9) Department Of Biochemistry, Faculty Of Medicine, University Of Toronto, Canada; 10) Department Of Molecular Medicine, Interfaculty Institute For Biochemistry, Eberhard Karls University Tübingen, Germany

Mitochondrial apoptosis signaling is critically regulated by the Bcl-2 protein family. Oligomerization of the pro-apoptotic downstream effector proteins BAX, BAK or BOK and subsequent release of cytochrome c initiate the caspase cascade and proteolysis of regulatory and structural downstream substrates. The pro- and anti-apoptotic members of the Bcl-2 family constitute an intricate network of specific interaction. Interaction is mediated by binding of the Bcl-2 Homology Domain 3 (BH3) into the hydrophobic groove of anti-apoptotic proteins. Thus, the BH3 has been identified as lead structure for small molecule drugs that block anti-apoptotic Bcl-2 proteins. These BH3-mimetic drugs are effective in anti-cancer therapy.

While the role of the C-terminal α9 helix, also coined transmembrane domain (TMD), for the subcellular localization of Bcl-2 proteins is widely accepted, studies on the direct interaction of Bcl-2 TMDs are rare. We developed and utilized a split-luciferase assay to investigate the interaction of TMDs from Bcl-2 proteins. We identify preferential homotypic interaction of the transmembrane domains from pro-apoptotic effector proteins BAX, BAK and BOK. These results are supported by data from molecular modeling of effector-TMD interaction in mimics of subcellular membranes. Exchange of TMDs in pro-apoptotic effector proteins verifies the significant part of TMD sequence in mediating subcellular localization, protein-protein interaction and, consequently, cell death regulation.

The results i) underline the critical role of Bcl-2 TMDs in mediating localization to intracellular membranes, ii) support their function in Bcl-2 protein interaction, and iii) indicate the impact of TMDs on regulation of cell death. Further investigation will characterize Bcl-2-TMD interactions and their role in fine tuning Bcl-2 regulated apoptosis. Analysis of tumor-specific TMD mutations might establish TMDs as marker for anti-cancer therapy.

EPIGENETIC RECONDITIONING OF FERROPTOSIS-RESISTANT HIGH-RISK NEUROBLASTOMA: CTRL+ALT+DEL FOR TUMOR DEFENSE MACHINERY

Roberto Fernández-Acosta (1), Ine Koeken (1), Iuliana Vintea (1, 2), Behrouz Hassannia (1, 3), Gerben Menschaert (2), Wim Vanden Berghe (1), Tom Vanden Berghe (1, 3)

1) Cell Death Signaling Lab, Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium; 2) Biobix, Lab of Bioinformatics and Computational Genomics, Department of Mathematical Modelling, Statistics and Bioinformatics, Ghent University, Ghent, Belgium; 3) Ferroptosis and Inflammation Research, VIB-UGent Center for Inflammation Research, Ghent, Belgium

Neuroblastoma (NB) is the most common extracranial solid tumor in children and is a leading cause of pediatric cancer-related mortality (1, 2). Patients with high-risk forms of neuroblastoma often exhibit poor response to therapy and frequent relapse post-treatment (3, 4). In previous studies, we demonstrated that inducing ferroptosis, an iron-dependent cell death driven by excessive lipid peroxidation, effectively eradicates therapy-resistant, aggressive NB in mice (5). However, high-risk NB tumors adapt to develop ferroptosis resistance mechanisms, which include decreased levels of polyunsaturated fatty acids (PUFAs) and iron availability, alongside the upregulation of antioxidant pathways (6-8). Epigenetic modifications are key contributors to drug resistance (9), prompting us to explore epigenetic modulation to sensitize high-risk NB to ferroptosis.

NB exhibits significant clinical heterogeneity (10), leading us to stratify NB cell lines based on their sensitivity to ferroptosis. Our analysis revealed SH-SY5Y cell line as particularly resistant. Leveraging this model, highly refractory to ferroptosis, we screened a library of 1,210 epigenetic drugs, identifying 4 potent sensitizing compounds (ferroptosis induced mostly in combination with a ferroptosis trigger), while 16 induce ferroptosis independently. Notably, a majority of these compounds are inhibitors of BET proteins, histone deacetylases (HDACs), or kinases, underscoring the pivotal role of these epigenetic regulators in sensitizing NB to ferroptosis. Additionally, novel targets unrelated to ferroptosis, such as tankyrase proteins, were also identified, presenting exciting avenues for further investigation. Furthermore, we discovered and validated in vitro, for the first time, two FDA-approved drugs and two drugs currently in phase 2 clinical trials as potent ferroptosis triggers (with EC50 values ranging from 3 to 120 nM), which could expedite clinical translation.

Building upon these findings, we selected a BET inhibitor (BETi) for additional analysis of its sensitizing effect in vitro. We found that the combination of RSL3 or ML162 with the BETi synergistically induced a robust, nearly unstoppable wave of ferroptotic cell death. Transcriptomic and DNA methylation (WGBS and ONT) analysis of SH-SY5Y cells treated with 200 nM of the BETi revealed substantial upregulation/activation of histone-related genes and pathways, accompanied by a decrease in transcript levels of SLC7A11 and its regulators (DKK1 and GDF15). Moreover, drug connectivity analysis correlated the BETi transcriptional signature with the profile of two HDACs inhibitors (one FDA-approved and one under clinical trial) from our screening, suggesting reduced ferroptosis defense mechanisms through histone expression (post-translational) changes.

Collectively, our study furnishes valuable insights into harnessing epigenetic modulation to reset ("Ctrl+Alt+Del") the antiferroptotic mechanisms of NB. Additionally, it represents the first seminal endeavour in delineating a ferroptotic epifingerprint reflective of therapy responsiveness.

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GLUTATHIONE REGULATES NATURAL KILLER CELL IMMUNITY AND MEDIATES TUMOR CONTROL

Melanie Grusdat (1,2), Luana Guerra (1,2), Takumi Kobayashi (1,2), Carole Binsfeld (1,2), Dirk Brenner (1,2,3)

1) Experimental and Molecular Immunology, Dept. of Infection and Immunity (DII), Luxembourg Institute of Health, Luxembourg; 2) Immunology & Genetics, Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Luxembour; 3) Odense Research Center for Anaphylaxis (ORCA), Department of Dermatology and Allergy Center, Odense University Hospital, University of Southern Denmark, Denmark.

Natural killer (NK) cells perform their regulatory and cytotoxic functions in highly oxidative environments, such as during viral infections and tumor growth. However, the antioxidant requirements of NK cells, particularly like glutathione (GSH), are not well understood. In this study, using a mouse model with conditional abrogation of GSH production in NK cells, we demonstrate that GSH is essential for IL-15-mediated activation of these innate lymphocytes. Without this antioxidant, in vitro activated NK cells accumulate reactive oxygen species (ROS) in the mitochondria, leading to reduced mitochondrial fitness and lower energy capacity. This results in impaired proliferation and cytokine production, along with diminished mTOR and STAT5 signaling. During acute viral infections, NK cells in mutant mice fail to control the anti-viral T cell response effectively. Additionally, the lack of GSH in NK cells significantly impairs their ability to control tumor spread. Therefore, GSH appears to be a critical checkpoint for NK cell activation and function.

SUPPRESSION OF FERROPTOSIS BY VITAMIN A OR RADICAL-TRAPPING ANTIOXIDANTS IS ESSENTIAL FOR NEURONAL DEVELOPMENT

Juliane Tschuck (1), Vidya Padmanabhan Nair (2), Ana Galhoz (3,4), Carole Zaratiegui (5), Hin-Man Tai (2), Gabriele Ciceri (6), Ina Rothenaigner (1), Jason Tchieu (6), Brent R. Stockwell (7), Lorenz Studer (6), Daphne S. Cabianca (5), Michael P. Menden (3,4), Michelle Vincendeau (2), and Kamyar Hadian (1)

1) Research Unit Signaling and Translation, Helmholtz Zentrum München, Neuherberg, Germany; 2) Endogenous Retrovirus Group, Institute of Virology, Helmholtz Zentrum München, Neuherberg, Germany; 3) Computational Health Center, Helmholtz Zentrum München, Neuherberg, Germany; 4) Department of Biology, Ludwig-Maximilians University Munich, Munich, Germany; 5) Institute of Functional Epigenetics, Helmholtz Zentrum München, Neuherberg, Germany; 6) Developmental Biology and Center for Stem Cell Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA; 7) Department of Biological Sciences, Department of Chemistry, Herbert Irving Comprehensive Cancer Center, Irving Institute for Cancer Dynamics, Columbia University, New York, NY, USA.

The development of functional neurons is a complex orchestration of multiple signaling pathways that control cell proliferation, differentiation, and homeostasis (1); the details of which are not fully understood. Because the balance of antioxidants and vitamins is important for neuronal survival and development, we hypothesized that ferroptosis — a cell death modality controlled by antioxidants (2, 3) — must be suppressed to gain neurons.

We found that removal of antioxidants diminishes neuronal development and laminar organization of cortical organoids. However, impaired neuronal development in conditions lacking antioxidants could be fully restored when ferroptosis was specifically inhibited by ferrostatin-1 or when neuronal differentiation occurred in the presence of vitamin A. Also using a C. elegans in vivo model, we could confirm that induction of ferroptosis leads to early developmental defect, which can be reduced by using vitamin A. We determined that all-trans retinoic acid (ATRA), the active metabolite of vitamin A, activates the heterodimeric nuclear receptor complex Retinoic Acid Receptor (RAR)/Retinoid X Receptor (RXR), which orchestrates the increased expression of the ferroptosis inhibitors GPX4, FSP1, GCH1, and ACSL3, amongst others. Surprisingly, retinal and retinol have a different mode of action by acting as radical-trapping antioxidants.

Together, our study reveals an unexpected function of vitamin A in coordinating the expression of essential cellular gatekeepers of ferroptosis (ATRA) or acting as radical-trapping antioxidants (retinal and retinol). We demonstrate that suppression of ferroptosis by radical-trapping antioxidants or by vitamin A is required to obtain mature neurons and proper laminar organization in cortical organoids.

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STABILIZATION OF β-CATENIN AVERTS DERMATITIS CAUSED BY OTULIN DEFICIENCY

Kim Lecomte (1,2), Maarten Ciers (1,2), Geert van Loo (1,2) and Esther Hoste (1,2)

(1) Department for Biomedical Molecular Biology, Ghent University, Belgium; (2) VIB Center for Inflammation Research, Ghent, Belgium

Mutations of the human OTULIN gene cause a severe autoinflammatory condition affecting different body sites, including the skin. OTULIN functions as a deubiquitinase with exclusive specificity for linear ubiquitin chains and is a crucial brake on TNFR1-mediated cell death in epithelial cells. Mice lacking OTULIN selectively in keratinocytes (Δ^{Ker}OTULIN mice) develop inflamed lesions that progress into squamous carcinomas, a phenotype driven by excessive cell death. Previous studies linked OTULIN to Wnt signalling, however the in vivo relevance of this link is unclear. We elucidated that depleting OTULIN from keratinocytes supresses Wnt target gene expression. OTULIN regulates Wnt activation in keratinocytes by directly interacting with β-catenin and removing its linear ubiquitin chains. Stabilization of β-catenin markedly improves the inflammatory pathology of Δ^{Ker}OTULIN mice in prophylactic and therapeutic settings. Activation of Wnt prevents the exaggerated cell death occurring in OTULIN-deficient keratinocytes. Notably, altered Wnt-dependent gene regulation is also observed in Sharpincpdm/cpdm mice, indicating that Wnt activation is a hallmark of linear ubiquitination-driven immune dysregulation.

MICROGLIA DO SOMETHING STRANGE TO THEIR RIBOSOMES DURING NEUROINFLAMMATION

Walker S. Jackson (1,2), Srivathsa Magadi (1,2), Maria Jonson (1,2)

1) Wallenberg Center for Molecular Medicine, Linköping University; 2) Department of Biomedical and Clinical Sciences, Linköping University

Neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, cause severe damage before clinical signs emerge. Therefore, early detection and treatments are urgently needed. However, our understanding of early mechanisms in neurodegeneration is incomplete. To generate new mechanistic insight, and possibly discover new biomarkers or therapeutic targets, we performed cell type-specific translatome analyses in mouse models of four neurodegenerative diseases (1-4). In follow-up work, we discovered that ribosomal protein S24 (abbreviated as Rps24 for the gene/mRNA and S24 for the protein) expresses a variant C-terminal tail that is induced during conditions that trigger neuroinflammation.

Although ribosomes are typically viewed as invariant protein-producing machines, we found that the expression of ribosome proteins is not uniform between brain regions and cell types. Furthermore, Rps24 is one of the most differentially alternatively spliced mRNAs across tissues and cell types in mice, humans, and non-human primates. The gene carries seven exons: exon 5 encodes a single lysine (K) followed by a stop codon (*), exon 6 carries only a stop codon, and exon 7 encodes proline-lysine-glutamate (PKE) followed by a stop codon and a 3' -untranslated region. RNA isoform Rps24c, the one that we find most fascinating, is formed by the exclusion of exons 5 and 6 and is the only isoform that encodes S24-PKE. Rps24c is normally abundant in certain organs, including the liver and spleen, but is in low amounts or absent in other tissues, including the brain and heart. Interestingly, Rps24 isoform ratios are regulated by mTOR and hypoxia.

To study the function of Rps24 isoforms we created a collection of customized tools, including an isotype-specific antibody, splice-switching oligonucleotides, transcript-specific droplet digital PCR assays, several mutant human cell lines, and a knock-in mouse line where we can force or prevent the expression of Rps24c/S24-PKE in specific cell types. Our novel antibodies do not bind to healthy brain sections, but clearly labeled human and mouse brain sections with neuroinflammation, such as that caused by neurodegeneration or cancer. Notably, forcing the expression of Rps24c/S24-PKE in excitatory neurons of mice causes embryonic lethality, indicating the isoforms are not redundant. Ongoing experiments aim to develop these findings as a biomarker and to test if controlling the isoforms impacts the brain's vulnerability to neuroinflammation caused by neurogenerative diseases or cancer. Importantly, since the Rps24 splice patterns and S24 sequences are identical between humans and mice, data generated from mouse studies may translate into humans.

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COMBINED ABSENCE OF TRP53 TARGET GENES ZMAT3, PUMA AND P21 CAUSE A HIGH INCIDENCE OF CANCER IN MICE

Margs S. Brennan (1,2,3), Kerstin Brinkmann (1,2), Gerard Romero Sola4, Geraldine Healey1,5, Leonie Gibson1, Lahiru Gangoda (1,2), Margaret A. Potts (1,2), Elizabeth Lieschke(1,2), Stephen Wilcox (1), Andreas Strasser (1,2), Marco J. Herold (1,2,5,6), Ana Janic (4)

1) The Walter and Eliza Hall Institute of Medical Research (WEHI), 1G Royal Parade, Parkville, Melbourne, Victoria 3052, Australia; 2) Department of Medical Biology, The University of Melbourne, Melbourne, VIC, Australia; 3) Department of Medicine Huddinge, Centre for Haematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden; 4) Department of Medicine and Life Sciences, Universidad Pompeu Fabra, Barcelona, Spain; 5) Genome Engineering and Cancer Modelling Program, Olivia Newton-John Cancer Research Institute, Melbourne, Victoria, Australia; 6) School of Cancer Medicine, La Trobe University, Melbourne, Victoria, Australia

Transcriptional activation of target genes is essential for TP53-mediated tumour suppression, though the roles of the diverse TP53-activated target genes in tumour suppression remains poorly understood. Knockdown of ZMAT3, an RNA-binding zinc-fi nger protein involved in regulating alternative splicing, in haematopoietic cells by shRNA caused leukaemia only with the concomitant absence of the PUMA and p21, the critical effectors of TRP53-mediated apoptosis and cell cycle arrest respectively. We were interested to further investigate the role of ZMAT3 in tumour suppression beyond the haematopoietic system. Therefore, we generated Zmat3 knockout and compound gene knockout mice, lacking Zmat3 and p21, Zmat3 and Puma or all three genes. Puma-/-p21-/-Zmat3-/- triple knockout mice developed tumours at a significantly higher frequency compared to wild-type, Puma-/- Zmat3-/- or p21-/-Zmat3-/-deficient mice. Interestingly, we observed that the triple knockout and Puma-/-Zmat3-/- double deficient animals succumbed to lymphoma, while p21-/- Zmat3-/- animals developed mainly solid cancers. This analysis suggests that in addition to ZMAT3 loss, additional TRP53-regulated processes must be disabled simultaneously for TRP53-mediated tumour suppression to fail. Our findings reveal that the absence of different TRP53 regulated tumour suppressive processes changes the tumour spectrum, indicating that different TRP53 tumour suppressive pathways are more critical in different tissues.

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CORTICOSTERONE DYSREGULATES NLRP3 INFLAMMASOME SIGNALLING

Kirsten Kenney (1), Sabrina Sofia Burgener (1) and Kate Schroder (1)

1. Institute for Molecular Bioscience (IMB), The University of Queensland, Brisbane, Queensland, Australia.

Inflammasomes are signalling hubs that assemble when specific innate immune receptors sense potential threats to homeostasis. The most studied signalling platform is the NLRP3 inflammasome that activates caspase-11. Activated caspase-1 cleaves and activates Gasdermin-D (GSDMD) to trigger lytic cell death (pyroptosis), and the pro-inflammatory cytokines pro-IL-1β and pro-IL-18 to induce immune signalling in neighbouring cells2,3. In metabolic dysfunction-associated steatotic liver disease (MASLD), NLRP3 inflammasome is linked to hepatocyte damage, hepatocyte death and fibrosis4. Yet, the specific contributions of downstream NLRP3 inflammasome signalling components, such as GSDMD, remain to be elucidated and whether co-morbidities, such as stress, influence MASLD disease outcome. In vitro, we found corticosterone dysregulates NLRP3 inflammasome signalling, exerting a time-dependent switch from pro-inflammatory to anti-inflammatory. Preliminary experiments indicate that glucocorticoid-induced leucine zipper (GILZ) is involved in the dual functionality of corticosterone; however, ongoing work will explore preferential receptor binding and the molecular interactions mediating this inflammatory response. We similarly investigated whether exogenous corticosterone exposure enhanced inflammation using an in vivo model of MASLD. We observed significant differences in hepatic inflammation, steatosis and damage between mice treated with and without corticosterone. These findings offer novel insights in the field of cell death and disease. Understanding the role of corticosterone in modulating NLRP3 inflammasome signalling and cell death, and its potential to exacerbate the inflammatory pathology of MASLD, is critical to combatting this condition and other related diseases.

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NEURONAL FERROPTOSIS IN MICE CAUSES SIGNATURES REMINISCENT OF NEURODEGENERATIVE DISEASES IN MAN

Svenja M. Lorenz (1), Mark Bostock (2), Adam Wahida (1), Tobias Seibt (1), Dietrich Trümbach (1), Marcel S. Woo (3), Jana K. Sonner (3), André Santos Dias Mourão (2), Sabine Hölter-Koch (4), Petra Steinacker (5), Ashok K. Jayavelu (6), Manuel Friese (3), Grzegorz M. Popowicz (4), Marcus Conrad (1)

1) Institute of Metabolism and Cell Death, Helmholtz Zentrum München, Neuherberg, Germany; 2) Institute of Structural Biology, Helmholtz Zentrum München, Neuherberg, Germany; 3) Institute of Neuroimmunology and Multiple Sclerosis, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 4) Institute of Developmental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany; 5) Department of Neurology, Martin-Luther-University of Halle-Wittenberg, Germany; 6) Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Martinsried, Germany

Neurodegenerative diseases (NDs), including Alzheimer's disease (AD), increasingly represent a global health concern, especially in aging societies. Apart from the serious health problems for patients suffering from a ND, there is as yet no curative treatment. Although the pathophysiological features described among different NDs can vary widely, the common denominator is neuronal cell death.
Emerging evidence suggests that ferroptosis, a recently described form of regulated necrotic cell death, may be the underlying cell death mechanisms accounting for neuronal cell death in several NDs. Selenium-containing glutathione peroxidase 4 (GPX4), the guardian of ferroptosis, plays a central role due to its unique ability to directly reduce peroxides in lipid bilayers, thereby preventing autoxidation and rupturing of cellular membranes. Here we show that spatiotemporal deletion of GPX4 in adult mouse brains leads to progressive loss of glutamatergic neurons, progressive cortical atrophy and extensive neuroinflammation. A neuroproteomic survey of cortical tissue of these mice revealed striking signatures strongly reminiscent of AD and other NDs. These findings corroborate the notion that ferroptosis could be the underlying cause of neuronal cell death, even in the absence of any known genetic susceptibilities in the GPX4 gene in AD. In addition, neuronal cell death could be prevented by pharmacological means using the ferroptosis inhibitor liproxstatin-1, making this mouse model a powerful tool for investigating new therapeutic approaches for previously untreatable NDs.

LIVE CELL IMAGING OF NECROPTOSIS AND THE RELEASE OF DAMPS IN VITRO AND IN VIVO

Hiroyasu Nakano (1), Shin Murai (1), Kenta Sumiyama (2), Yoshitaka Shirasaki (3), Mai Yamagishi (3), Kenta Terai (4), and Kenta Moriwaki (1).

1) Department of Biochemistry, Faculty of Medicine, Toho University, Tokyo, Japan; 2) Department of Animal Sciences, Graduate School of Bioagricultural Sciences, Nagoya University, Aichi, Japan; 3) Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan; 4) Department of Histology, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan.

Necroptosis is a regulated form of cell death implicated in various pathological conditions, including ischemic reperfusion injuries, viral infections, and drug-induced tissue injuries. Unlike apoptosis, necroptosis is characterized by early membrane rupture, leading to the release of danger-associated molecular patterns (DAMPs) and eliciting strong inflammatory responses. The precise spatiotemporal dynamics of necroptosis and the subsequent immune responses to released DAMPs in vivo remain unclear.

Previously, we developed a Forster resonance energy transfer (FRET) biosensor, termed SMART (Sensor for MLKL Activation by RIPK3 based on FRET), which tracks conformational changes in MLKL during necroptosis in human and murine cell lines in vitro. In this study, we generated transgenic (Tg) mice expressing the SMART biosensor across various tissues. Our findings demonstrated that the FRET ratio increased specifically during necroptosis, but not apoptosis or pyroptosis, in primary cells. Additionally, cisplatin-treated SMART Tg mice exhibited elevated FRET signals in renal tubular cells compared to untreated counterparts. These results indicate that SMART Tg mice serve as a valuable tool for monitoring necroptosis in diverse cell types both in vitro and in vivo.

To further elucidate the release of DAMPs, we generated transgenic mice expressing HMGB1 fused to the fluorescent protein mCherry. Analysis of DAMP release at single-cell resolution using cutting edge technology, live-cell imaging of secretion activity (LCI-S), revealed two distinct modes of HMGB1 release from primary macrophages, in contrast to a single release mode for IL-1β. These findings, combined with our previous results, suggest that the release mechanisms of HMGB1 are influenced by the intrinsic structure of HMGB1 as well as the size of the ruptured membrane.

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IRON TRAFFICKING AND FERROPTOSIS VULNERABILITY IN MELANOMA IS CONTROLLED BY MITOCHONDRIA-LYSOSOME CONTACT TETHERING

Francesca Rizzollo (1,2), Abril Escamilla-Ayala (3), Tito Calì (4), Jean Christophe Marine (5), Raphael Rodriguez (6), Julie Bonnereau (1,2), Patrizia Agostinis (1,2).

1) Cell Death Research and Therapy Laboratory, Center for Cancer Biology, VIB; 2) Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium; 3) VIB Centre for Brain Disease Research, Leuven, Belgium; 4) University of Padova, Department of Biomedical Sciences, Padova, Italy; 5) Laboratory for Molecular Cancer Biology, Center for Cancer Biology, VIB, Leuven, Belgium; 6) Chemical Biology of Cancer Laboratory, Institut Curie, Paris, France

Excessive iron uptake enhances cancer cell plasticity but increases their susceptibility to iron-dependent ferroptosis. Therefore, the distribution of redox-active Fe2+ among organelles must be carefully regulated to prevent unwanted cell death. This study identifies Bdh2, the mammalian homolog of the bacterial siderophore Enterobactin, as a mediator of inter-organelle iron transport in melanoma. Bdh2 localizes to mitochondria-lysosome contact sites (MLCs) to produce the siderophore 2,5-DHBA, promoting lysosomal acidity and Fe2+ transport to mitochondria. In invasive, drug-resistant melanoma cells, loss of MITF reduces Bdh2 expression, causing lysosomal iron retention and increased ferroptosis sensitivity. Restoring Bdh2 expression or providing 2,5-DHBA protects these cells from ferroptosis. Thus, Bdh2 is essential for transferring redox-active Fe2+ from lysosomes to mitochondria, enabling melanoma cells to sustain active mitochondria and avoid ferroptosis.

TRANSCRIPTIONAL REMODELING SHAPES THERAPEUTIC VULNERABILITY TO NECROPTOSIS IN ACUTE LYMPHOBLASTIC LEUKEMIA

Anna Saorin (1), Anna Dehler (1), Bartimée Galvan (1), Fabio Steffen (1), Dong Lu (2), Xin Yu (2), James Kim (1), Aneta Drakul (1), Samanta Kisele (1), Jin Wang (2,3), Jean-Pierre Bourquin (1) and Beat C. Bornhauser (1*)

1) Department of Oncology and Children's Research Centre, University Children's Hospital Zürich; 8032 Zürich, Switzerland; 2) The Verna and Marrs McLean Department of Biochemistry and Molecular Pharmacology, Baylor College of Medicine; Houston, Texas 77030, USA; 3) Department of Molecular and Cellular Biology, Baylor College of Medicine; Houston, Texas 77030, USA.

Insufficient eradication of cancer cells and survival of drug tolerant clones are major relapse driving forces. Underlying molecular mechanisms comprise activated pro-survival and anti-apoptotic signaling leading to inadequate, insufficient apoptosis and drug resistance. The identification of programmed cell death pathways alternative to apoptosis opens up for possibilities to antagonize apoptosis escape routes in drug resistant cancer (1,2). We have earlier shown that acute lymphoblastic leukemia (ALL) harbors a distinct propensity to undergo cell death by necroptosis, regulated by RIP1 Kinase (RIPK1) downstream of TNF receptor signaling (3). While depending on the presence of TNF receptors 1 and 2 (4), key regulatory events that drive RIPK1 dependent cell death remain elusive.

Here, we aimed to identify genetic determinants of necroptosis and the molecular mechanisms that drive necroptosis in ALL, in order to construct novel treatment strategies to eradicate resistant disease despite apoptosis incompetence.

We used our established ex vivo drug response profiling platform to screen for potential compounds that act as necroptosis modulators and identified, as expected, key apoptosis activators including venetoclax as potent combination partners for SM. Surprisingly, we further found synergistic drug activity of necroptosis induction by SM with histone deacetylase inhibitors (HDACIs), potent regulators of gene transcription, across B-cell precursor ALL, even sensitizing SM poor responder ALL to cell death in a RIPK1-dependent manner. Consequently, the combination of SM and HDAC inhibition effectively delayed leukemia progression in xenografted mice. Moreover, RNAseq analysis revealed RIPK1 dependent transcriptional regulation as an early response to SM, with a key regulatory role of HDACs and the transcription master regulator SP1. Corroborating these data, functional in vivo CRISPR sgRNA analysis indicated activity of SP1 and the histone acetyltransferase (HAT) p300 in sensitizing ALL towards necroptosis while HDAC2 was shown to have an inhibitory role. HATs such as p300 promote acetylation of the chromatin, facilitating gene expression required for necroptosis, while HDACs exert the opposite effect. Thus, transcriptional regulation downstream RIPK1 is a key driver of the necroptosis response, governed by mutual activity of SP1 and HDAC2 driven gene expression regulation, interference with which identifies a potent anti-leukemic therapy approach.

Altogether, our results pinpoint transcriptional dependency of RIPK1-driven necroptosis as key regulatory mechanism and identify novel targets for interference with and sensitization to necroptosis. This represents a thus far unexplored clinically relevant strategy which could be exploited to re-sensitize resistant leukemias towards cell death, to eradicate drug resistant clones that do not respond to apoptotic triggers.

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IMPACT OF GSDME ON PYROPTOSIS IN GLIOBLASTOMA

Ege Sole (1), Egil Brudvik (1), Rolf Bjerkvig (1), Jubayer Hossain (1), Hrvoje Miletic (1,2)

1) Department of Biomedicine, University of Bergen, Bergen, Norway; 2) Department of Pathology, Haukeland University Hospital, Bergen, Norway

Immunotherapy has seen limited success in treating glioblastoma (GBM), the most prevalent primary brain tumor. Despite extensive research on apoptosis and autophagy, our understanding of cell death mechanisms in GBM remains incomplete, regardless of their potential influence on the immune microenvironment. Pyroptosis, a distinct type of programmed cell death from apoptosis, is both immunogenic and lytic. In pyroptosis, gasdermin proteins create pores in the cell membrane, releasing cellular contents into the microenvironment. Gasdermin E (GSDME), frequently inactivated in cancers due to mutations or low expression, undergoes proteolytic cleavage by caspase 3 during apoptosis, leading to a shift towards pyroptosis (1,2). In this study, we investigated the relevance of GSDME-mediated pyroptosis in GBM. According to The Cancer Genome Atlas, GSDME is highly expressed in GBMs compared to many other cancers, with no observed mutations.

We utilized raptinal to induce apoptosis and then examined the expression of full-length and cleaved GSDME, as well as cleaved caspase 3, in syngeneic mouse and human patient-derived GBM stem-like cell lines using western blotting. To evaluate lytic cell death and viability, we employed lactate dehydrogenase (LDH) and WST-1 assays, respectively. Pyroptotic morphology was observed through a PI uptake assay. Additionally, we investigated the impact of inhibiting plasma membrane repair in human GBM cell lines using BAPTA-AM, a rapid Ca2+ chelator, to assess its effect on pyroptosis. The knockout (KO) of GSDME was achieved using CRISPR/Cas9 technology. Growth curve and cell cycle analysis were conducted to assess the effect of KO on growth and cell cycle. We further explored the potential impact of KOs on invasiveness through a collagen invasive assay. Finally, the in vivo effect of GSDME KO was evaluated using Kaplan-Meier survival curves and immunohistochemistry to assess immune system markers such as CD3, CD45, CD4, CD8, and F4/80.

Our findings confirmed that treatment with raptinal led to the cleavage of GSDME and caspase 3 in both mouse and human GBM cell lines, indicating that the molecular machinery for pyroptosis remained functional. Treatment with raptinal resulted in a significant decrease in cell viability, demonstrating successful execution of cell death. In mouse GBM cell lines, a significant LDH release was detected, which was attenuated by GSDME KO. Conversely, human GBM cells, despite exhibiting GSDME cleavage, showed less or no LDH release. Pyroptotic morphology was observed in mouse GBM cell lines after raptinal treatment, whereas it was delayed or inhibited in human GBM cells. BAPTA-AM treatment in human GBM cell lines enhanced pyroptotic morphology, suggesting a role for plasma membrane repair in inhibiting pyroptosis. Treatment of orthotopic CT2A tumors in mice with raptinal did surprisingly not increase survival compared to CT2A GSDME KO. Further, survival of untreated CT2A GSDME KO mice was increased compared to CT2A wild-type tumors. Immunohistochemical assessment of immune markers CD3, CD45, CD4, CD8, and F4/80 in untreated CT2A GSDME KO glioma revealed a distinct pattern when compared to the wild-type tumors.

The molecular machinery required for pyroptosis is active in both mouse and human GBM cells. Nevertheless, while mouse GBM cells clearly undergo pyroptosis upon apoptosis induction, human GBM cells exhibit a less efficient pyroptotic response, which can be par-

tially restored by inhibiting plasma membrane repair. In vivo, we did not observe an effect of pyroptosis on treatment effect/tumor immunity. On the contrary, GSDME by itself had a growth promoting effect and impact on the immune microenvironment. These results indicate a function of GSDME in GBM development, which is independent of pyroptosis.

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FASL, A CRUCIAL HOST FACTOR DRIVING COVID-19 PATHOLOGY AND LETHALITY

Iratxe Uranga-Murillo (1,2,3), Marie-Christine Albert (4,5), Maykel Arias (1,2,3), Michael Bergmann (6), Henning Walczak (4,5,7), Julián Pardo (1,2,3)

1) CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, 28029, Spain; 2) Aragón Health Research Institute (IIS Aragón), San Juan Bosco 13, 50009, Spain; 3) Department of Microbiology, Paediatrics, Radiology and Preventive Medicine and Public Health, University of Zaragoza, 50009, Spain; 4) Cell death, inflammation and immunity laboratory, CECAD Cluster of Excellence, University of Cologne, 50931, Germany; 5) Cell death, inflammation and immunity laboratory, Institute of Biochemistry I, Centre for Biochemistry, Faculty of Medicine, University of Cologne, 50931, Germany; 6) Div. of Visceral Surgery, Dept. of General Surgery, Comprehensive Cancer Centre, Medical University of Vienna, 1090, Austria; 7) Centre for Cell Death, Cancer, and Inflammation, UCL Cancer Institute, University College London, WC1E 6DD, UK

At the end of 2019, a novel coronavirus respiratory disease (COVID-19) emerged, caused by the SARS-CoV-2, leading to one of the deadliest pandemics of the last centuries, despite the unprecedentedly rapid development of effective vaccines and antiviral drugs.
While SARS-CoV-2 infection induces a wild range of symptoms, dismal disease outcome was due to severe lung failure, including acute respiratory distress syndrome (ARDS). SARS-CoV-2-induced lung failure is associated with an impaired initiation of the immune response against the virus, with a dysregulated interferon (IFN) response. This leads to excessive inflammation due to an increase in cytokines and chemokines, and the recruitment of neutrophils and inflammatory macrophages to the lower respiratory tract. The inflammation, paired with aberrant cell death and lymphopenia, eventually results in immunopathology and lung tissue damage.
However, the understanding of the mechanisms of the dysregulated immune response that are responsible of ARDS development and SARS-CoV-2-induced lung failure remains challenging, with current immunosuppressive treatments providing limited therapeutic benefit to COVID-19 patients. The death ligand members of the tumour necrosis factor (TNF) superfamily, including TNF and Fas ligand (FasL), which have been associated to inflammation, lymphocyte susceptibility to apoptosis and macrophage dysfunction, could act as determinant triggers of the damaging inflammation and cell death during SARS-CoV-2 infection. Accordingly, their modulation could provide therapeutic benefit against COVID-19.
Here, we report a novel mouse-adapted SARS-CoV-2 model (MA20), developed through serial passaging in mice, that acquired seven adaptive mutations, including, within the spike protein, the major contributor to mouse adaptation Q493R. Upon intranasal infection, SARS-CoV-2 MA20 induced mild respiratory signs in young C57BL/6 mice while severe disease and lethality was found in young BALB/c and mature C57BL/6 mice. Apart from age-dependant severity, SARS-CoV-2 MA20 infection recapitulated other key pathological features of COVID-19, such as neutrophil and inflammatory monocytic-macrophage (IMMs) recruitment and lymphopenia and the induction of acute lung injury. In addition, we found an enhanced expression of inflammatory pathways and increased cell death in the lungs of infected mice.
Using SARS-CoV-2 MA20, we identified the induction of cell death and inflammation and the expression of FasL to be highly associated with disease. During SARS-CoV-2 MA20 infection, Fasl mRNA expression was significantly upregulated in comparison to mock-infect-

ed controls, mainly expressed on the surface of IMMs and NK cells. Therapeutic inhibition of this death ligand significantly increased the survival of both young BALB/c and mature C57BL/6 mice from 40 % to 67 % and 90 % survival rates, respectively. This increase in the survival was characterised by a reduction in the cell death and inflammation in the lungs of the mice, including a decrease in cytokines that have been shown to correlate with increased mortality in COVID-19, including TNF, CXCL10 or IFNγ. Remarkably, FasL inhibition did not alter viral titres, affecting the immunopathology without altering pathogen control. Taken together, we identify a prominent role for FasL as a previously unrecognised key contributing factor to the inflammatory immunopathology characteristic of severe COVID-19 and propose, with the inhibition of FasL, a potential novel therapeutic opportunity for this disease.

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LINEAR UBIQUITINATION AT DAMAGED LYSOSOMES INDUCES LOCAL NF-KB ACTIVATION AND CONTROLS CELL SURVIVAL

Laura Zein (1, #), Marvin Dietrich (1, #), Denise Balta (2), Verian Bader (3), Christoph Scheuer (1), Suzanne Zellner (4), Nadine Weinelt (1), Julia Vandrey (2), Muriel C. Mari (5), Christian Behrends (4), Friederike Zunke (2), Konstanze F. Winklhofer (3, 6), Sjoerd J. L. van Wijk (1, 7, 8)

1) Institute for Experimental Pediatric Hematology and Oncology, Goethe University Frankfurt, Komturstrasse 3a, 60528 Frankfurt am Main, Germany; 2) Department of Molecular Neurology, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Schwabachanlage 6, 91054 Erlangen, Germany; 3) Department of Molecular Cell Biology, Institute of Biochemistry and Pathobiochemistry, Ruhr University Bochum, Universitätsstrasse 150, 44801 Bochum, Germany; 4) Munich Cluster for Systems Neurology (SyNergy), Faculty of Medicine, LMU Munich, Feodor-Lynen Strasse 17, 81377 München, Germany; 5) Department of Biomedicine, Aarhus University, Wilhelm Meyers Allé 3, Building 1234, 328, 8000 Aarhus, Denmark; 6) Cluster of Excellence RESOLV, 44801 Bochum, Germany; 7) German Cancer Consortium (DKTK) partner site Frankfurt/Mainz and German Cancer Research Center (DKFZ), Heidelberg, Germany; 8) University Cancer Centre Frankfurt (UCT), University Hospital Frankfurt, Goethe-University Frankfurt, Frankfurt, Germany

Lysosomes are the major cellular organelles responsible for nutrient recycling and degradation of cellular material. Maintenance of lysosomal integrity is essential for cellular homeostasis and lysosomal membrane permeabilization (LMP), induced by lysosomotropic agents, sensitizes towards cell death. Damaged lysosomes are repaired or degraded via lysophagy, during which glycans, exposed on ruptured lysosomal membranes, are recognized by galectins leading to K48- and K63-linked poly-ubiquitination (poly-Ub) of lysosomal proteins followed by recruitment of the autophagic machinery and degradation. Linear (M1) poly-Ub, catalyzed by the E3 ligase linear ubiquitin chain assembly complex (LUBAC) and removed by the OTU domain-containing deubiquitinase with linear linkage specificity (OTULIN) exerts important functions in immune signaling and cell survival, but the role of M1 poly-Ub in lysosomal homeostasis remains largely unexplored. Here, we demonstrate that L-leucyl-leucine methyl ester (LLOMe)-damaged lysosomes are decorated with M1 poly-Ub in a LUBAC-, OTULIN- and K63-dependent manner. LMP-induced M1 poly-Ub at damaged lysosomes contributes to lysosome degradation, recruits nuclear factor-κB (NF-κB) essential modulator (NEMO) and locally activates inhibitor of NF-κB kinase (IKK) to trigger NF-κB activation in a K63 poly-Ub-dependent manner. Inhibition of lysosomal degradation enhances LMP- and OTULIN-dependent cell death, indicating pro-survival functions of M1 poly-Ub during LMP and potentially lysophagy. Finally, we demonstrate that M1 poly-Ub also occurs at damaged lysosomes in primary mouse neurons and induced pluripotent stem cell-derived primary human dopaminergic neurons. Our results reveal novel functions of M1 poly-Ub during lysosomal homeostasis, LMP and degradation of damaged lysosomes, with important implications for NF-κB signaling, inflammation and cell death.

RIPK1 PREVENTS STING-DEPENDENT SKIN INFLAMMATION

Simon Verdonck (1.2), Evelien Dierick (1,2), Richard de Reuver (1.2), Katrien Staes (1.2), Eline Hessmann (1.2), Jonathan Maelfait (1.2)

1) VIB-UGent Center for Inflammation Research, Ghent, Belgium; 2) Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium

RIPK1 is a key signaling kinase involved in inflammation and regulated cell death. In mouse keratinocytes, loss of RIPK1 (Ripk1E-KO) leads to spontaneous activation of ZBP1/RIPK3/MLKL-mediated necroptosis, resulting in skin inflammation.1-3 Expression of ZBP1 is induced by interferons (IFNs) and expression levels are generally undetectable under steady-state conditions.4 Interestingly, the skin of Ripk1E-KO mice displays a clear antiviral immune response with elevated levels of ZBP1, while ex vivo cultured RIPK1-deficient keratinocytes show no spontaneous phenotype, suggesting the need for an additional (exogenous) trigger to initiate ZBP1-mediated necroptosis.2,3

Using germfree animals or mice lacking T cells, B cells and innate lymphoid cells, we show that RIPK1 expression in keratinocytes prevents a sterile autoinflammatory immune reaction. Transcriptome and RT-qPCR analysis of purified keratinocytes from Ripk1E-KO mice revealed an early IFN signature, before the onset of apparent inflammatory lesion development. We found that crossing Ripk1E-KO mice into a Stat1-deficient background significantly delays onset of inflammatory lesions, suggesting an IFN-driven pathology. To identify the pathway responsible for the pathological IFN production, we genetically deleted crucial adaptor proteins in the three primary pathways upstream of type I IFN production: the RIG-I-like receptor (RLR), Toll-like receptor (TLR) and cGAS-STING pathway. Deletion of Mavs or Ticam1 did not or only marginally, in the case of MyD88, rescue the mice from pathology, thereby excluding a role for RLRs and TLRs in pathogenesis. In contrast, we demonstrate that keratinocyte intrinsic and extrinsic activation of the STING-mediated dsDNA sensing pathway initiates disease.

Mechanistically, we propose a model whereby STING activation induces a STAT1-dependent interferon-response resulting in the expression of the interferon-stimulated gene Zbp1. Activated ZBP1 then induces MLKL-dependent necroptotic keratinocyte cell death. Together, this shows that dsDNA constitutes an in vivo damage-associated molecular pattern in the context of necroptosis-driven autoinflammation. Future work will show how loss of RIPK1 results in spontaneous activation of STING signaling and whether STING-signaling also causes necroptosis-mediated immunopathology in tissues other than the skin.

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METABOLIC REGULATORS OF MITOCHONDRIAL DNA RELEASE IN CELLULAR MODELS OF PRKN-LINKED PARKINSON'S DISEASE

Gideon Agyeah (1), Paul Antony (1), Jenny Ghelfi (1), Lucia Gallucci (1), Aleksandar Rakovic (2), Sandro L Pereira (1), Anne Grünewald (1,2)

1) Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg; 2) Institute of Neurogenetics, University of Lübeck, Lübeck, Germany

The mitochondrial DNA, which is located within mitochondria under physiological conditions, can be released into the cytosol or extra-cellular space in disorders such as PRKN-associated Parkinson's disease. This observation is of clinical relevance due to the implication of extramitochondrial mtDNA in inflammatory signalling in PRKN-PD. We previously demonstrated that a metabolic shift can induce the release of mtDNA in PRKN-PD patient-derived neurons and PRKN knockout neuroblastoma cell lines. Based on these findings, we investigated (i) the potential of NAD+ metabolism as a disease-modifying target in PRKN-PD. Moreover, we explored the (ii) mitochondrial mechanisms and (iii) structural changes contributing to the release of mtDNA in cellular models of PRKN-PD.
In order to achieve these aims, we pharmacologically induced various mitochondrial anomalies and explored how Parkin confers protection against mtDNA release in these scenarios. Using high-content imaging-based assays for the quantification of cytosolic mtDNA, mitochondrial mass, mtDNA abundance, and mitochondrial size, we aim to correlate mtDNA release with specific mitochondrial phenotypes.
First, we validated our previous finding of a link between mitochondrial metabolism and mtDNA leakage. This analysis revealed a significant increase in mtDNA release in both wild-type and PRKN knockout neuroblastoma lines after depleting NAD+ levels with cobalt chloride treatment. Conversely, we saw a significant rescue of the release phenotype, when NAD+ increasing drug quercetin was used. Quercetin increases NAD+ levels by activating NAMPT and inhibiting NAD+ catabolism. Increased mtDNA release was also mirrored in cells treated with (i) hydrogen peroxide to increase ROS signalling, (ii) Fumarate to induce cristae remodelling, or (iii) thapsigargin to interfere with calcium homeostasis. While the release phenotype was independent of the genotype, the effect size was generally larger in Parkin-deficient cells. Our results also suggest concordance between mitochondrial fragmentation and mtDNA release. Our data shows that mitochondrial size is inversely proportional to mtDNA release in over 90% of our drug treatments whereas mitochondrial mass sparingly predicted the release phenotype.
Taken together, our findings support the existence of a link between mitochondrial metabolism, mitochondrial size, mtDNA release, and, by extension, inflammation in PD. To further elucidate the mechanisms underlying Parkin-conferred protection against mtDNA release, we are currently performing transcriptomic and metabolomic analyses in cells exposed to the above-mentioned stress and rescue conditions. Ultimately, we hope that our work will uncover new entry points for metabolic therapies that can prevent or slow down the progression of PD.

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SEX-DEPENDENT ALTERATIONS OF APOPTOTIC PROCESSES IN ALZHEIMER'S DISEASE REVEALED BY LARGE-SCALE SINGLE-CELL TRANSCRIPTOMICS

Mohamed Soudy (1), Sophie Le Bars (1), Enrico Glaab (1)

1. *Biomedical Data Science Group, Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Esch-sur-Alzette, Luxembourg*

Previous epidemiological, clinical, and biological evidence indicates significant sex differences in Alzheimer's disease (AD); however, the molecular and cellular mechanisms underlying these differences in the pathophysiology of AD remain poorly understood. To help address these knowledge gaps, we conducted bioinformatics analyses of the largest public single-cell transcriptomic atlas of AD, derived from samples of the aged human prefrontal cortex from AD patients and controls (Mathys et al., 2023). This dataset comprises 2.3 million cells from postmortem brain samples of 427 individuals with varying degrees of AD pathology and cognitive impairment.

By building cell type-specific and sex-specific gene regulatory networks and simulating the modulation of upstream regulatory gene activity, we identified the gene YBX3 (Y-Box Binding Protein 3) as a primary regulator of male-specific network changes in astrocytes. YBX3 has previously already been identified as differentially expressed between male and female AD patients in the posterior cingulate cortex in an independent study (Sun et al., 2019). The gene is involved in the regulation of cell death pathways and immune responses, which are critical in the context of AD, as dysregulated cell death and chronic inflammation can accelerate brain tissue degeneration. In the single-cell data studied here, YBX3 has a highly significant ($p = 2.9E-20$) male-specific increased expression in AD, and its main target genes (B3GALNT2, and STIP1) also display consistent alterations. These findings suggest that YBX3 could serve as a target for further mechanistic studies to elucidate its potential role in sex-specific regulatory mechanisms in AD.

Overall, this study identified a potential sex-dependent cellular process alteration in astrocytes that may contribute to molecular sex differences in AD. The observation of significant male-specific alterations in YBX3 and its downstream network targets, as well as the known role of this gene in cell death and immune-related pathways with potential relevance for AD progression, suggest that YBX3 may merit further investigation in the context of sex-specific molecular mechanisms in AD.

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INDUCTION OF H2O2-MEDIATED APOPTOSIS AND FERROPTOSIS BY SANGUINARINE IN HUMAN CERVICAL CANCER

Ameer Alakkal (1,2), Faisal Thayyullathil (1), Siraj Pallichankandy (1), Karthikeyan Subburayan (1), Anees Rahman Cheratta (1), Sehamuddin Galadari (1)

1) Cell Death Signaling Laboratory, Division of Science (Biology), Experimental Research Building, New York University Abu Dhabi, Abu Dhabi P.O. Box 129188, United Arab Emirates; 2) Department of Biochemistry, College of Medicine and Health Sciences, UAE University, Al Ain P.O. Box 17666, United Arab Emirates

Sanguinarine (SNG), a quaternary benzophenanthridine alkaloid primarily extracted from Sanguinaria canadensis, Chelidonium majus, and Macleaya cordata, displays numerous clinical properties such as anticancer, anti-hypertensive, anti-inflammatory, and antimicrobial properties. SNG exhibits promising antineoplastic properties evidenced by its efficacy against diverse tumor cells in both in vitro and in vivo settings. Despite its recognized potential, the precise molecular pathways through which SNG operates to exert its therapeutic effects remain to be fully elucidated.

In this study, we demonstrate that SNG causes cell death in human cervical cancer (HeLa) cells through the activation of two distinct signaling pathways namely apoptosis and ferroptosis. SNG-induced apoptosis was characterized by caspase activation and cleavage of DNA damage repair enzyme Poly (ADP-ribose) polymerase (PARP). On the contrary, ferroptosis was characterized by the downregulation of solute carrier family 7 member 11 (SLC7A11) and glutathione (GSH), along with iron accumulation and lipid peroxidation (LPO).

Importantly, SNG elevated the generation of reactive oxygen species (ROS), particularly hydrogen peroxide (H2O2) during the cell death. Furthermore, pre-treatment with H2O2 scavengers effectively inhibited the initiation of both apoptosis and ferroptosis by revoking PARP cleavage, SLC7A11 downregulation, lipid peroxidation, and loss of cell viability, highlighting the pivotal involvement of H2O2 in the cytotoxic mechanism triggered by SNG. Interestingly, incubation with caspase inhibitor z-VAD-fmk not only abrogated the features of apoptosis but also negated markers of SNG-induced ferroptosis. Likewise, pretreatment with the ferroptosis inhibitor ferrostatin-1 (Fer-1), besides safeguarding cells from SNG-induced ferroptosis, also restrained the features of SNG-induced apoptosis.

Our discoveries propose a synergistic collaboration between apoptosis and ferroptosis in the context of SNG-induced tumor suppression in human cervical cancer cells. This study underscores the pivotal involvement of signaling pathways, notably apoptosis and ferroptosis, in orchestrating the antineoplastic efficacy of Sanguinarine. It highlights the significance of comprehending these molecular mechanisms for the advancement of targeted cancer therapies.

THE ROLE OF CERAMIDE ACTIVATED PROTEIN PHOSPHATASE (CAPP) IN FERROPTOSIS

Asma Al Nuaimi, Faisal Thayyullathil, Anees Rahman Cheratta, Siraj Pallichankandy, Karthikeyan Subburayan, Ameer Alakkal, and Sehamuddin Galadari

Cell Death Signaling Laboratory, Biology Program, Division of Science, Experimental Research Building, New York University Abu Dhabi, PO Box 129188, Saadiyat Island, Abu Dhabi, United Arab Emirates.

Ferroptosis is a regulated cell death modality characterized by the accumulation of reactive oxygen species (ROS) and extensive lipid peroxidation (LPO). Although the role of ceramide, a prime sphingolipid signaling molecule, in apoptosis has been well-documented, its involvement in ferroptosis remains unclear. This study elucidates a novel function of ceramide in the promotion of ferroptosis. Mechanistically, treatment of pancreatic cancer cells (BxPC3 cells) with RSL3 (a classical ferroptosis inducer) activates acid sphingomyelinase (ASM), leading to ceramide generation. This ASM-mediated ceramide production is essential for RSL3-induced dephosphorylation of Akt at Ser473 and Thr308, culminating in ferroptosis. Both pharmacological and genetic inhibition of ASM significantly attenuated RSL3-induced ceramide generation, Akt dephosphorylation, and ferroptosis, underscoring the critical role of ASM in these processes. Additionally, using a group of ceramide-activated protein phosphatases (CAPPs) inhibitors—calyculin A, okadaic acid, and cantharidin—demonstrated the involvement of protein phosphatase 2A (PP2A) in RSL3-induced Akt dephosphorylation and ferroptosis. Collectively, these findings reveal a previously unrecognized role of CAPP in the regulation of ferroptosis, providing potential therapeutic targets for conditions associated with ferroptosis.

PERSONALIZED FUNCTIONAL PROFILING FOR DRUG DISCOVERY IN MELANOMA

Cristian Angeli (1), Joanna Patrycja Wroblewska (1), Eliane Klein (1), Christiane Wurth-Margue (1), Demetra Philippidou (1), Stephanie Kreis (1)

1) Signal Transduction Laboratory Department of Life Sciences and Medicine, Faculty of Science, University of Luxemburg

Melanoma is an aggressive and highly metastatic cancer, associated with poor outcomes once advanced stages have been reached. Although efficient targeted therapies are available for patients carrying BRAF mutations (50%), the rapid emergence of resistance to BRAF inhibitors remains a serious clinical issue. Current efforts aim for an improved efficacy of the existing treatments and for delaying the onset of drug resistance. The clinical treatment options are even more limited for the remaining half of melanoma patients, who either carry an NRAS mutation (25%) or who have other or no well-defined mutations in key driver genes, underlying the urgent need for efficient therapeutical options. In the current project, we performed repurposing high-throughput drug screening using 3D melanoma models, which led to the identification of two novel compounds targeting topoisomerase II and CK1α, which show high efficacy in inhibiting NRAS mutated melanoma cell growth. Compounds will be further validated to serve as a potential targeted therapy option for this patient group. Moreover, we are establishing pre-clinical 3D in vitro models and patient-derived samples, directly extracted from fresh tumors, to be used for drug testing and functional studies.

PREFERENTIAL ENHANCEMENT OF THE ANTICANCER POTENTIAL OF BETULINIC ACID BY ITS CONVERSION INTO IONIC LIQUIDS. A STUDY WITH HORMONE-DEPENDENT (MCF-7) VERSUS HORMONE-INDEPENDENT (MDA-MB-231) BREAST CANCER CELL LINES

Sonia Apostolova (1), Irina Georgieva (1), Paula Ossowicz-Rupniewska (2), Joanna Klebeko (2), Maya Guncheva (3), Rumiana Tzoneva (1)

1) Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, BULGARIA; 2) Department of Chemical Organic Technology and Polymeric Materials, Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology, Szczecin, POLAND; 3) Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, BULGARIA

Betulinic acid (BA) is a pentacyclic triterpenoid, isolated mainly from the bark of white birch tree (Betula spp.), with well-studied biological activities, including anti-inflammatory, antipathogenic, antimalarial, antidiabetic, antioxidant, antitumor, etc [1, 2]. BA has been shown to exert potent antitumor activity against numerous human malignancies, inducing apoptosis, mainly through the activation of the intrinsic mitochondrial pathway [3]. However, due to its hydrophobic nature, BA has limited water solubility, resulting in poor absorption and low bioavailability, hindering its potential use as a therapeutic agent. The conversion of bioactive molecules into ionic liquids (ILs) is a promising strategy to modulate their physicochemical properties, particularly, enhancing the water solubility and activity, resulting in an improved therapeutic efficacy.

In this study, we synthesized fifteen novel ILs containing a cation ethyl ester of a polar, non-polar or charged amino acid [AAOEt] and an anion [BA]. We assessed the water solubility and cytotoxic potential of all [AAOEt][BA] formulations towards two human breast cancer cell lines MDA-MB231 and MCF-7, with different metastatic properties and hormonal status. MDA-MB-231 is highly metastatic and estrogen-independent breast cancer cell line, whereas MCF-7 represents an in vitro model for non-metastatic estrogen-dependent cancer. The non-tumorigenic, MCF10A cell line was used as a model of normal mammary epithelial cells.

Our results indicated that within the series of [AAOEt][BA] analyzed, the positively charged lysine-based BA [LysOEt][BA]$_2$ (ratio LysOEt:BA - 1:2), showed the highest cytotoxic effect on MCF-7 breast cancer cells with IC_{50} values 4.8 ± 1.3 μM compared to that of BA (11.5 ± 1.8 μM) 72h after treatment. In addition, the clonogenic survival was also significantly reduced showing ~20 % surviving fraction of MCF-7 cells after exposure to [LysOEt][BA]2 at a concentration near the IC50 of BA. Besides, morphological alterations associated with cellular shrinking and rounding, typical for apoptosis, were also detected in MCF-7 treated cells. Regarding metastatic and hormone-independent MDA-MB-231 breast cancer cells, [LysOEt][BA]$_2$, again was the most cytotoxic [AAOEt][BA] among the series tested. Interestingly, the IC50 values determined for MDA-MB-231 were significantly higher (31.9 ± 0.7 μM), compared to those for MCF-7, indicating selective cytotoxicity of [AAOEt][BA] towards non-metastatic and hormone-dependent breast cancer cells. The experiments performed on MCF10A, also showed that [LysOEt][BA]$_2$ was the most active IL-formulation suppressing the cell viability, with IC_{50} values (4.0 ± 0.2 μM) similar to those obtained for MCF-7 breast cancer cells.

In conclusion, our data clearly show that the charged AA-based BA - [LysOEt][BA]$_2$, one of the most soluble [AAOEt][BA], enhances BA's cytotoxic activity preferentially in the hormone-dependent, non-metastatic breast cancer cell line MCF-7, suggesting cancer-type specificity. Future research focused on the hormone receptor signalling and cell death pathways involved is needed to reveal the mechanisms of action of [AAOEt][BA] in estrogen-dependent breast cancer.

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SPHINGOLIPID RHEOSTAT DYNAMICS CONTROL APOPTOSIS AND LIVE APICAL CELL EXTRUSION IN ZEBRAFISH EPIDERMIS

Joy Armistead (1,2), Sebastain Höpfl (3), Pierre Goldhausen (1), Jan Kuom (1), Andrea Müller-Hartmann (1), Evelin Fahle (1), Julia Hatzold (1), Rainer Franzen (4), Susanne Brodesser (5), Nicole E. Radde (3), Matthias Hammerschmidt (1,2)

1) Institute of Zoology / Developmental Biology, University of Cologne, Cologne, Germany; 2) Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany; 3) Institute for Stochastics and Applications, University of Stuttgart, Stuttgart, Germany; 4) Max-Planck Institute for Plant Breeding Research, Cologne, Germany; 5) Lipidomics / Metabolomics Facility, Cluster of Excellence Cellular Stress Responses in Aging-associated Diseases (CECAD), University of Cologne, Cologne, Germany

The sphingolipid rheostat regulates levels of crucial bioactive lipids, and perturbations of rheostat homeostasis can dictate cell fate. We have previously shown that, in the epidermis of hypomorphic hai1a^{hi2217} mutant zebrafish embryos, Matriptase-dependent increased sphingosine kinase activity drives sphingosine-1-phosphate (S1P) – mediated live apical cell extrusion. This cell loss is sufficient to counter Matriptase-driven epidermal hyperplasia and the embryos heal spontaneously.

However, although amorphic hai1a^{fr26} embryos also display S1P-mediated apical cell extrusion, they are unable to heal and die within a few days. Our data show that this lethality is due to tp53-independent apoptosis primarily in outer peridermal cells, resulting in the loss of plasma membrane integrity owing to secondary necrosis and compromising the epidermal barrier. Our data pointed to perturbations in the sphingolipid rheostat as the cause of apoptosis. Indeed, lipidomics analyses both before and after the onset of apoptosis, revealed shifts in sphingolipid concentrations over time. Before the onset of apoptosis, pro-apoptotic C_{16} ceramides are significantly reduced in the hai1a^{fr26} mutants, likely consumed as the substrate for S1P synthesis. After the onset of apoptosis, a significant increase in C_{16} ceramides was found, accompanied by an upregulation of ceramide synthase (cers) gene expression.

Via mathematical modeling of sphingolipid rheostat dynamics, combined with in vivo manipulations of rheostat components, we uncovered a negative feedback loop sensing ceramide levels and controlling its de novo synthesis. Indeed, acute pharmacological depletion of de novo ceramide synthesis in wild-type fish followed by recovery, replicated several aspects of the hai1a^{fr26} mutant phenotype. Our results offer an in vivo perspective of the dynamics of sphingolipid homeostasis and its relevance for epithelial cell survival versus cell death, linking live apical cell extrusion and apoptosis.

INVESTIGATING CROSSTALK BETWEEN INTRACELLULAR CALCIUM SIGNALING AND MITOCHONDRIAL DYNAMICS

Sundas ARSHAD (1) and Alexander SKUPIN (1),

(1) Luxembourg Centre for Systems Biomedicine, Univeristy of Luxembourg

Parkinson's disease (PD) is the second most common neurodegenerative disease linked with loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Research over the last decades have identified several hallmarks of PD including mitochondrial dysfunction and impaired calcium homeostasis but the intricate mechanisms underlying these phenomena remain elusive. Lund human mesencephalic (LUHMES) cells, which are human embryonic neuronal precursor cells, have been utilized in several studies to investigate the PD and underlying mechanisms associated with it. In this study, we investigated the crosstalk between Ca^{2+} signaling and mitochondrial dynamics by combining live cell imaging and immunostaining techniques. The LUHMES cells were derived into dopaminergic neurons and live cell imaging was performed and Ca^{2+} activity was assessed at two time points of differentiation by addition of external ATP, Histamine which stimulate IP3 mediated Ca^{2+} signaling and FCCP to access the effect of morphological changes on Ca^{2+} signaling. After imaging acquisitions, the cells were analysed for inter-spike intervals, spike width and spike area mean for Ca^{2+} concentration in cytoplasm. To check the morphology of the mitochondria, the cells at day 5 were treated with ATP and histamine while FCCP was used as a positive control and stained with TOM20. Our analysis showed that relationship between calcium activity induced by ATP and histamine, and morphological changes in mitochondrial. Moreover, the results also suggested the higher activity of Ca^{2+} channels in post mitotic dopaminergic neurons as compared to the immature cells. Overall, our study on Ca^{2+} live cell imaging revealed novel aspects, which can be used as model to further understand Ca^{2+} signaling in dopaminergic neurons.

INHIBITING THE ACTIVITY OF THE AUTOPHAGY RELATED PROTEIN VPS34 ENHANCES THE RELEASE OF PRO-INFLAMMATORY CHEMOKINES CCL5 AND CXCL10 IN NEUROBLASTOMA CELLS.

Elisabetta Bartolini (1,2), Ruize Gao (1), Bassam Janji (1)

1) Tumor Immunotherapy and Microenvironment (TIME) group. Department of Cancer Research. Luxembourg Institute of Health, L-1210 Luxembourg City, Luxembourg; 2) Faculty of Science, Technology and Medicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg

Neuroblastoma (NB) represents the most prevalent extracranial solid tumor among pediatric cases, contributing to 15% of cancer-related deaths in children under 15 years old[1]. Characterized by various genetic alterations, MYC-N amplification occurs in approximately 20% of cases and correlates with high-risk disease and poor prognosis, representing a significant treatment challenge[2,3].

Despite the success of Immune Checkpoint Blockade (ICB)-based immunotherapy in several aggressive solid tumors, its efficacy in NB tumors is notably limited. NB tumors are classified as "cold" tumors, which rely on the immune landscape of the tumor microenvironment (TME). Unlike "hot" tumors, which exhibit immune cell infiltration into the TME, "cold" tumors have limited immune cell infiltration due to insufficient pro-inflammatory factors. Therefore, strategies aimed at inducing the release of pro-inflammatory chemokines hold promise in overcoming the non-responsiveness of NB to ICB therapy.

Building on our previous research[4], we investigated the impact of pharmacologically inhibiting the kinase activity of the autophagy gene *Vps34* on the cytokine and chemokine profile of MYC-N-amplified neuroblastoma (NB) cells using a Proteome Profiler array that can simultaneously detect 111 mouse cytokines and chemokines. Following treatment with *Vps34* inhibitors (*Vps34i*), we observed substantial release of two major pro-inflammatory chemokines, CCL5 and CXCL10, in NB cells. Moreover, our data indicated that the elevated levels of CCL5 and CXCL10 were associated with increased mRNA expression, suggesting that *Vps34i* induces the release of these chemokines through upregulation of their gene expression. Our in silico analysis highlights the significance of CCL5 and CXCL10 by demonstrating that their overexpression is associated with increased expression of cytotoxic immune cell markers in a cohort of neuroblastoma (NB) patients.

Taken together our results emphasize the potential of *Vps34* inhibitors (*Vps34i*) to induce the expression of pro-inflammatory chemokines in NB tumors, thereby attracting cytotoxic immune cells into the tumor microenvironment. This approach ultimately enhances the efficacy of NB tumors in response to immune checkpoint blockade (ICB) therapy. Upcoming in vivo experiments will evaluate the role of *Vps34* in improving NB tumor response to ICBs and provide the preclinical proof of concept for combining selective *Vps34* inhibitors with anti-PD-1 therapy in aggressive NB patients.

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HOW DO CELLS DIE UNDER GLUCOSE STARVATION?

Bas Z (1), Hopff A.-S. (1), Lingnau F (2), Rose C (2), Fendt S.-M. (3), Stangherlin A (4), Reifenberger G (1), Leprivier G (1)

1) Institute of Neuropathology, University Hospital Düsseldorf and Medical Faculty, Heinrich Heine University, Düsseldorf, Germany; 2) Institute of Neurobiology, Heinrich Heine University, Düsseldorf, Germany; 3) Laboratory of Cellular Metabolism and Metabolic Regulation, VIB-KU Leuven Center for Cancer Biology, Leuven, Belgium; 4) Institute for Mitochondrial Diseases and Ageing, CECAD, Cologne, Germany

Glucose is the main fuel of mammalian cells, such that its restriction represents a severe cellular stress that can eventually lead to cell death. Cancer represents a pathological condition of glucose deprivation due to increased glucose uptake and deficient tumor vasculature. It is surprisingly unclear what type of cell death is occurring under glucose deprivation, as it ranges from apoptosis, necrosis, entosis to disulfidptosis. We set out to characterize the form of cell death induced by glucose deprivation and investigated the underlying mechanisms. Using various cell models, our preliminary data indicate that none of the canonical forms of cell death, namely apoptosis, necroptosis nor ferroptosis were contributing to glucose starvation-induced cell death. We uncovered that cells dying under glucose deprivation exhibit specific morphological features, such as the presence of cytoplasmic vacuoles. By testing several pharmacological inhibitors, we found that inhibition of protein synthesis or of the glucose-sensing and mRNA translation regulator mTOR pathway, confers resistance to cell death under glucose deprivation. Investigation of the impact of glucose starvation on cellular metabolism revealed a decrease of glycolytic and TCA cycle intermediates, which was accompanied by a severe reduction of ATP levels. Supplementation of cells with methyl-pyruvate prevented glucose starvation-induced cell death, suggesting that ATP depletion is a trigger of this type of cell death. In addition to that, since glucose is essential for protein glycosylation, we analyzed levels of protein N-glycosylation and found those to be strongly reduced upon glucose removal. Addition of the protein glycosylation precursor N-acetyl-glucosamine inhibits cell death induced upon glucose deprivation, indicating that defects of protein glycosylation is another trigger of this cell death. By combining RNA-sequencing with genetic screening, we are currently searching for genetic mediators of this form of cell death. Our findings so far points to a model whereby glucose starvation induces a non-canonical form of cell death characterized by energy depletion and a defect of protein glycosylation downstream of the mTOR pathway.

GASDERMIN E DEPENDENT PYROPTOSIS DETERMINES DRUG SENSITIVITY IN LEUKEMIA

Uditi Bhatt (1), Anna Guinot (1), Mahya Eslami (1), Fabio D. Steffen (1), Anna Saorin (1), Caterina Mezzatesta (1), Malwine J. Barz (1), Alexandra Oertel (1), Liridon Abduli (1), Scott McComb (1,+), Denis Schewe (2), Martin Schrappe (2), Cornelia Eckert (3), Arend von Stackelberg (3), Martin Stanulla (4), Martin Zimmermann (4), Gunnar Cario (2), Jean-Pierre Bourquin (1), Beat C. Bornhauser (1)

1) Department of Oncology and Children's Research Centre, University Children's Hospital Zürich, 8032 Zürich, Switzerland; 2) Department of Pediatrics, University Medical Centre Schleswig-Holstein, Kiel, Germany; 3) Department of Pediatric Oncology/ Hematology, Charité Universitätsmedizin Berlin, Berlin, Germany; 4) Department of Pediatric Hematology and Oncology, Hannover Medical School, Hannover, Germany; 5) Human Health Therapeutics Research Centre, National Research Council Canada, Ottawa, ON K1A 0R6, Canada

Cancer relapse and refractory disease remains a significant challenge in the treatment of pediatric acute lymphoblastic leukemia (ALL), with notably poor clinical outcomes (1). Evasion of apoptosis due to dysregulation of cell death pathways is a primary mechanism by which drug-resistant clonal subpopulations persist and induce relapse (2). Thus, activation of apoptosis-independent cell death pathways is an appealing strategy to overcome drug resistance-induced relapse in ALL.

To mimic apoptosis incompetency, we generated primary ALL cells deficient in executioner caspase-3 and -7 through CRISPR-based genome editing. While these apoptosis-incompetent cells were refractory to BCL2 inhibition as expected, we detected notable sensitivity to various chemotherapeutic agents, including proteasome inhibitors, such as bortezomib (BTZ), and anthracyclines. An in vivo genome-wide sgRNA CRISPR screen identified the inflammatory caspase-4 and -14 to be crucial for BTZ-induced cell death in apoptosis-incompetent cells. Subsequent transcriptome analyses revealed the initiation of an early immune response upon treatment, with significant enrichment of genes associated with pyroptosis and the inflammasome.

BTZ-treated apoptosis-incompetent cells exhibited characteristic morphological features of pyroptosis as well as activation of gasdermin E (GSDME), a key regulator of chemotherapy-triggered pyroptosis (3). GSDME activation and subsequent cell death was found to be dependent on caspase-4. Substantiating the pivotal role of pyroptotic cell death in drug sensitivity, we observed a correlation between GSDME expression and the response to a variety of chemotherapeutics. GSDME expression was strongly associated with treatment response in patient samples of the international ALL-BFM trial, suggesting that GSDME-driven pyroptosis is required for clearance of leukemia blasts.

Consequently, GSDME-driven pyroptosis is deemed essential for a sustained cell death response and represents an avenue for the therapeutic exploitation of informed combinatorial strategies to overcome drug resistance arising from impaired apoptotis in cancer.

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OXIDATION FROM NEUROINFLAMMATION AND EFFECTS ON THE TOXICITY OF THE Aβ42 PEPTIDE - AN IN-SILICO INVESTIGATION

Daniel Bonhenry (1), Laurent Marichal (2), Nils Davoine (1), Michael Heneka (2) and Joshua T. Berryman (1)

1) Department of Physics and Materials Science, University of Luxembourg, Luxembourg City L-1511, Luxembourg; 2) Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Belvaux 4362, Luxembourg

Neurodegenerative diseases are associated with a spreading and progressive atrophy and cell death in the brain. These symptoms are linked to the presence of cytotoxic amyloid aggregates made from peptides secreted endogenously or coming from external pathogens1. Recent hypotheses bring oligomers, small agglomerates or inchoate fibrils, of Aβ peptides to the forefront in the aetiology of Alzheimer's disease (AD). In this picture, synaptic toxicity is mediated by soluble amyloid microaggregates targeting various receptors2 or compromising the integrity of cellular membranes via the formation of transient pores3. Aβ oligomers and fibrils are also immunogenic, leading to neuroinflammation. Large-scale activation of the cerebral immune system, connected to amyloid deposition, results in lasting metabolic and functional changes in cell behaviour which are central to the development of AD4. Highly oxidative endogenous molecules released by activated immune cells have a high affinity with amino acid sidechains therefore altering their chemical and physical properties.

In light of the importance of neuroinflammation in the development of AD, we explore the effect of oxidative modifications of the Aβ peptide on its properties related to cytotoxicity. We specifically focused on the tyrosine at position 10 as its oxidation has been shown to enhance Aβ aggregation and plaque formation5. To this end, molecular dynamics simulations are employed to explore the binding properties of oxidized Aβ42 oligomers to a model bilayer and their impact on the structural integrity of a membrane when inserted. Insertion of wild type Aβ oligomers in a bilayer induces the formation of transient water pores. We show that tyrosine nitration modulates the aggregation kinetics of Aβ, favouring low-weight oligomers as a result of the non-covalent chemistry of the nitrotyrosine moiety. We also show that nitration enhances the binding of these oligomers to membrane, without preventing the formation of water structures in the bilayer, thus offering an explanation for enhanced cell damage and death by amyloid due to neuroinflammatory modulation of the aggregate properties.

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DELVING INTO THE MECHANISM OF SELINEXOR-INDUCED DEATH IN MULTIPLE MYELOMA CELL LINES

Laura Cambronero-Arregui (1), Andrea Redrado-Domingo (1), Laura J. Fraga-Cabrejas (1), Eva Latorre (1), Javier Naval (1), Alberto Anel (1), Isabel Marzo (1)

1) Department of Biochemistry and Molecular and Cell Biology, University of Zaragoza –Zaragoza (Spain)

Exportin 1 (XPO1) is a protein involved in maintaining cellular homeostasis through the translocation of different RNAs and proteins from the nucleus to the cytoplasm - such as transcription factors, tumor suppressor proteins or cell cycle regulators. In several types of cancers, such as multiple myeloma (MM), the overexpression of this protein is related with poor prognosis. Selinexor is a selective inhibitor of nuclear export (SINE) approved in 2019 for the treatment of MM in combination with bortezomib and dexamethasone. It forces nuclear retention of XPO1 by blocking its cargoes' binding site, avoiding the translocation of key tumor suppressor proteins to the cytoplasm and favoring apoptosis of malignant cells. However, the mechanism by which this happens is not well understood. We have studied the activity of Selinexor in several MM cell lines, observing that death by apoptosis is induced. Besides, dose-response studies performed with seven wild-type MM cell lines demonstrated differences on the sensitivity of each cell line to Selinexor at different incubation times. On the other hand, the main apoptotic pathway activated by Selinexor is the intrinsic one, as we have observed a partial reduction in the sensitivity to this drug in a Bax/Bak knock-out (DKO) MM cell line. Additionally, extrinsic apoptosis might contribute to a lesser extent, since a pan-caspase inhibitor and a specific caspase-8 inhibitor reduced almost completely the apoptosis induced by Selinexor in the DKO MM cell line. The expression levels of some Bcl-2 family proteins after incubation with the drug for different times has been analyzed in each cell line, considering the importance of the intrinsic apoptotic pathway. In these studies, we have observed a correlation between the levels of Mcl-1 protein and the sensitivity to Selinexor; the most sensitive cell lines showed a reduction in Mcl-1 levels over time, whereas the most resistant ones exhibited an increase of this protein. These results suggest that Mcl-1 downregulation could be a critical event for Selinexor-induced cell death. Furthermore, a S63845 (a Mcl-1 mimetic)-resistant cell line generated in our laboratory, which expresses lower basal Mcl-1 levels than the parental cell line, is more sensitive to Selinexor, supporting our hypothesis. To elucidate whether these changes in Mcl-1 protein levels are due to differences in gene expression or protein stability we have performed real time PCR, which has shown little changes on Mcl-1 mRNA over time in the wild-type MM cell lines. These might indicate that Mcl-1 is not being degraded in those cell lines which are more resistant to Selinexor. The interaction of Mcl-1 with Bim and the role of this latter in Selinexor-induced cell death have also been studied.

MODELLING GLUCOSE NEUROTOXICITY IN A HUMAN IPSC-BASED MODEL UNRAVELS THE NEUROPROTECTIVE EFFECT OF P75NTR TARGETING

Konstantina Chanoumidou (1,2), Ioanna Zota (1,2), Marianna Papadopoulou (1,2), Chrystalla Konstantinou (2), Ioannis Charalampopoulos (1,2)

1) Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology-Hellas (IMBB-FORTH), Heraklion, Greece; 2) School of Medicine, University of Crete, Greece

Glucose concentration should be tightly regulated to maintain brain homeostasis since persistent episodes of high glucose, a common feature of Diabetes Mellitus (DM), cause complications including neuronal damage and inflammation. Considering that DM is a high-risk factor for the onset of Alzheimer's Disease (AD), the identification of the pathological mechanisms that are triggered by high glucose is of major importance to develop neuroprotective strategies in diabetic patients. $p75^{NTR}$ belongs to the TNF-receptor superfamily and signals apoptosis in different settings. $P75^{NTR}$ is upregulated in the plasma of diabetic patients and has been previously implicated in diabetic retinopathy and neuropathy (1). However, its role in the brain complications of Diabetes is unknown. To date, most studies address glucose neurotoxicity in animal diabetic models. Here, we aim to study the neurological manifestations of high glucose in a human iPSC – based model with emphasis on the role of p75 neurotrophin receptor ($p75^{NTR}$). We use mono- and co-cultures of human iPSC-derived neurons and astrocytes to investigate the involvement of $p75^{NTR}$ signaling in the direct and inflammation-mediated effects of hyperglycemia on neurodegeneration. Our results show that hyperglycemia triggers neuronal cell death in a dose-dependent manner accompanied by an up-regulation of $p75^{NTR}$ expression. Inhibition of $p75^{NTR}$ activity rescues neuronal cell death highlighting $p75^{NTR}$ as a mediator of glucose neurotoxicity. Additionally, high glucose makes neurons more susceptible to Amyloid-β induced toxicity indicating an interlink with AD. RNA-seq analysis of hyperglycemic neurons unraveled many affected processes including changes in cell cycle progression and deregulated synaptogenesis. High glucose does not show any effect on iPSC-derived astrocyte survival or activation in contrast to previous findings in rodents indicating species-specific differences. This finding suggests that microglia is possibly the primary source of inflammation, reported in rodents and human patients, in hyperglycemia. Collectively, our study provides insights into the brain deficits caused by hyperglycemia and suggests the neuroprotective effect of $p75^{NTR}$ targeting.

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PAR-4-MEDIATED REACTIVATION OF P53-MEDIATED TUMOR SUPPRESSION

Cheratta Anees, Thayyullathil Faisal, Pallichankandy Siraj, Subburayan Karthikeyan, Alakkal Ameer, Alnuaimi Asma, Galadari, Sehamuddin

New York University Abu Dhabi

Protein 53 (p53) is the single most frequently mutated tumor suppressor protein, with inactivation being present in more than 50% of all invasive human cancers. p53 has been implicated in a growing number of tumor suppressive processes, including cell cycle arrest, senescence, apoptosis, autophagy, and ferroptosis; hence it is known as the master regulator of tumor suppression. Considering its critical role in suppressing the cancer formation and progression, the concept of restoration of p53 for cancer therapy is very attractive. Normal p53 protein has an extremely short half-life of ~5–20 min, owing to its continuous degradation largely mediated by ubiquitin ligase known as MDM2.

In our investigations we have found that the tumor suppressor Par-4 is causing substantial induction of p53 in different cancers. Overexpression of par-4 alone was sufficient to induce p53, while knockdown of Par-4 reduced the p53 level. By regulating the p53 levels, we have confirmed that Par-4 is orchestrating various tumor suppressive mechanism(s) of p53 including apoptosis, autophagy, and senescence in response to different anticancer agents. We have confirmed that Par-4 increases the p53 protein by enhancing its stability. For instance, p53 half-life is significantly increased in Par-4 overexpressing cells, while transient knockdown decreases it. Mechanistically, Par-4 stabilizes the p53 protein by inhibiting its MDM-2 mediated ubiquitination and subsequent degradation. The Serine/threonine-specific protein kinase, Akt can phosphorylate MDM2 at ser166 and ser186, thereby promoting MDM2-mediated p53 ubiquitination and degradation. Clearly, Par-4 is shown to dephosphorylate Akt, thereby inhibiting the Akt-dependent activation of MDM2, leading to p53 stabilization and tumor suppression.

PT-112-INDUCED CELL DEATH MECHANISM IN HUMAN MULTIPLE MYELOMA CELL LINES

M. Pilar Corral-Sancho (1), Christina Y. Yim (2), Maria T. Congenie (2), Tyler D. Ames (2), Isabel Marzo (1), Eva Latorre (1), Alberto Anel (1)

1) Department of Biochemistry and Molecular and Cellular Biology, University of Zaragoza. Zaragoza. Spain; 2) Promontory Therapeutics Inc., New York, U.S.A.

The treatment of multiple myeloma (MM), one of the most common types of haematological malignancies, has evolved considerably in recent years. However, MM remains incurable due to the frequent development of therapy resistance and disease recurrence, highlighting the need for new therapeutic approaches. PT-112, a novel small molecule currently under Phase 2 development [1-4], inhibits ribosome biogenesis and causes nucleolar stress, leading to immunogenic cell death (ICD) in cancer cells [5-8]. In addition, PT-112's osteotropism and preclinical activity in MM mouse models led to a Phase I study of PT-112 in relapsed or refractory MM, which is now completed [9, 10]. Here, we investigate in vitro cytotoxicity of PT-112 in human MM cell lines to deepen our understanding of the drug's anticancer activity in this disease setting.

PT-112 sensitivity was evaluated in seven human MM cell lines (KMS-12-BM, KMS-34, MM.1S, NCI-H929, OPM-2, RPMI 8226, U266). In order to further characterize PT-112-induced cell death, we used specific caspase and necroptosis inhibitors and engineered cell lines lacking or overexpressing pro-apoptotic or anti-apoptotic proteins. We also analysed markers for nucleolar stress upon PT-112 treatment using NPM1 immunolabeling and confocal microscopy.

Our preliminary results demonstrate differential drug sensitivity in the MM cell lines, with RPMI-8226 and KMS-12-BM cells being the most sensitive to PT-112 in the panel. In RPMI-8226 cells, PT-112-induced cell death was inhibited by co-treatment with the pan-caspase inhibitor Z-VAD-fmk. Additionally, cells overexpressing the anti-apoptotic protein Mcl-1 were less sensitive to PT-112 compared to control cells, suggesting that the mechanism of cell death involves the mitochondrial apoptotic pathway. In contrast, cell death was not affected by Z-VAD-fmk in KMS-12-BM cells, suggesting cell line-specific effects of PT-112. We also observed potential changes in nucleolar morphology via NPM1 staining upon PT-112 treatment in RPMI-8226 and KMS-12-BM cells, possibly indicative of nucleolar stress. Future work will analyse markers of ICD in response to PT-112 in this panel of MM cell lines.

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MICROBIOME-DRIVEN CHROMATIN STATE CHANGES IN COLON CANCER CELLS

Sophia Croce (1), Aurélien Ginolhac (1), Thomas Sauter (1), Elisabeth Letellier (1), Lasse Sinkkonen (1)

1) Department of Life Sciences and Medicine, University of Luxembourg, Belvaux, Luxembourg

Colorectal cancer (CRC) poses a significant global health concern, ranking as the third most common and second most lethal cancer in 2022[1]. While extensive research has been done on the role of the microbiome in CRC initiation, progression, and resistance to chemotherapy, the intricate regulatory and epigenetic mechanisms through which microbiome-associated factors mediate these effects in CRC cells remain poorly understood.

Throughout CRC progression, various bacterial strains and microbiome-associated metabolites are implicated in a complex, multistep process that influences disease advancement[2]. Recent research has highlighted the importance of the synergistic relationships within the microbial community, shifting the focus in the microbiome field towards a deeper understanding of these interactions and their impact on cancer biology. Specifically, the role of bacterial metabolites in modulating key cancer-related processes, has garnered significant attention.

In this context, our research aims to screen a library of human microbiome-derived metabolites to identify those that activate the epithelial-mesenchymal transition (EMT) phenotype in CRC cells. To comprehensively investigate the gene activation and epigenetic changes associated with this process, we will integrate various techniques, including RNA-seq, ATAC-seq, and CUT&Tag. By identifying specific metabolites that drive this phenotypic shift and their epigenetic profile, we aim to uncover the molecular basis of microbiome-mediated CRC progression.

Furthermore, we intend to identify the bacterial sources of these metabolites and evaluate their prevalence in CRC patients using publicly accessible datasets. This comprehensive analysis will correlate specific bacterial species and their metabolites with clinical outcomes and molecular subtypes of CRC, offering new insights into the microbial contributions to cancer heterogeneity.

Another key part of our investigation will involve exploring the co-occurrence and cooperative interactions of bacterial species frequently associated with CRC. Based on our prior research on EMT process, we will focus on a selected community of bacteria and metabolites. We will microinject these into colorectal microorganoids to model their influence on the tumor microenvironment and explore their cooperative effects. Single-cell analysis techniques will be employed to examine the molecular interactions between these components and CRC cells, providing a high-resolution understanding of the epigenetic processes involved.

Overall, this research will offer significant contributions to our understanding of the microbiome's role in CRC, highlighting the complex interplay between bacterial metabolites, epigenetic regulation, and cancer progression.

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INVESTIGATING THE REGULATION OF THE NLRP3 INFLAMMASOME IN ALZHEIMER'S DISEASE

Shilauni DADWAL (1), Bora TASTAN (1), Michael HENEKA (1,2)

1) Luxembourg Centre for Systems Biomedicine, University of Luxembourg, 6 avenue du Swing, 4367 Belval, Luxembourg; 2) Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA 01605, USA.

Inflammasomes are complex protein assemblies that activate caspases, leading to inflammation in response to various stimuli, including pathogens, protein aggregates such as amyloid plaques, and hyper-phosphorylated tau. Among the numerous inflammasomes studied, such as NLRP1, NLRP3, AIM2, NLRP4, and others, NLRP3 has been extensively investigated due to its wide range of recognition mechanisms that streamline immune activation and eliminate pathogens. To develop targeted therapies for neuroinflammatory diseases, it is crucial to fully understand how various molecules and signaling pathways regulate the assembly and activation of NLRP3 inflammasome. Therefore, the regulation of the NLRP3 inflammasome is a pivotal component of the innate immune system and is marked by complexity and dynamism in the literature. We aim to identify new regulatory elements by elucidating the regulatory network among the NLRP3 inflammasome complex and the kinases.

Accordingly, we employed lipopolysaccharide (LPS) for priming and nigericin/amyloid-ß for activation to induce NLRP3 inflammasome activation in primary microglia, and secondary cell lines. Subsequently, we conducted immunocytochemistry and Western blot techniques to analyze the outcomes further. Thus far, our investigations have demonstrated different expressions of NLRP3, ASC, caspase-1, NEK7, MARK4, and other kinases in samples subjected to LPS and nigericin treatments. For further experiments, the most well-known NLRP3 inflammasome regulators like NEK7 and MARK4, will be silenced for functional studies.

Overall, this project aims to provide a deep understanding of NLRP3 inflammasome regulation in the canonical pathway. By unraveling the complex interactions among these regulatory factors, we seek to enhance our knowledge of inflammasome biology, offering valuable insights for therapeutic approaches in neuroinflammatory diseases.

JNK REGULATES EFFICIENT ONCOLYSIS BY ONCOLYTIC-VIRUS/ MICROTUBULE-TARGETING-AGENT COMBINATION

Sucheta De and Marcelo Ehrlich

Shmunis School of Biomedicine and Cancer Research, Faculty of Life Sciences, Tel Aviv University

Background. Oncolytic viruses (OVs) are immunotherapy agents that specifically infect and kill cancer cells and stimulate anti-tumour immunity. In accordance with their overall heterogeneity, cancer cells differ in susceptibility to OVs, and a subset of tumor types exhibits resistance to OVs. Development of therapeutic strategies to overcome such resistance is critical for broadening the breadth and potency of clinical OV applications. In this context, combination of OVs with distinct anti-cancer compounds may overcome resistance, increase infection and oncolysis and enhance OV efficacy. Microtubule targeting agents (MTAs) perturb microtubule dynamics, arrest cells at the spindle-assembly checkpoint and induce different modes of mitotic cell death. However, the functional interactions between MTAs and OVs has not been fully characterized. Results. Here, we target OV-resistant human bladder cancer cells by combining 2-methoxyestradiol (2ME2), a natural estrogen metabolite and MTA, with EHDV-TAU (Epizootic Hemorrhagic Disease Virus – Tel Aviv University)[1, 2, 3] a double stranded RNA oncolytic orbivirus. While 2ME2 or EHDV-TAU showed limited efficacy as single therapy agents, the 2ME2-EHDV-TAU combination increased infection, potently induced caspase activity and the expression of pro-apoptotic factor NOXA, and stimulated the plasma-membrane exposure of calreticulin, an indicator of immunogenic cell death. Notably, the 2ME2+EHDV-TAU combination resulted in marked activation of c-Jun N-terminal kinase (JNK). Inhibition of JNK with SP600125 abrogated the increased oncolytic efficacy of the 2ME2-EHDV-TAU combination, resulting in significant reductions in infection and cell-death-related parameters. Conclusion. Together, our data point to oncolysis-enhancing potential of OV/MTA combinations, and identify JNK as a regulator of OV-induced immunogenic cell death.

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TAB2 PREVENTS TNF CYTOTOXICITY BY DIRECTLY MODULATING COMPLEX II

Tom Delanghe (1,2), Lisa Wijns (1,2), Inge Bruggeman (1,2), Mike Vadi (1,2), Peter Vandenabeele (1,2) and Mathieu JM Bertrand (1,2)

1) VIB Center for Inflammation Research, 9052 Ghent, Belgium; 2) Department of Biomedical Molecular Biology, Ghent University, 9052 Ghent, Belgium.

Tumor Necrosis Factor (TNF) is a pivotal pro-inflammatory cytokine and a key pharmacological target in various inflammatory diseases. TNF sensing by TNFR1 promotes inflammation either directly by activating the MAPK and NF-κB signaling pathways or indirectly by triggering cell death, in the form of apoptosis or necroptosis. The outcome of TNF sensing depends on the successive assembly of two protein complexes, the receptor-bound signaling complex I and the cytosolic lethal complex II. Usually, molecular brakes, known as cell death checkpoints, repress TNF-induced cytotoxicity by counteracting complex II assembly or activity1. However, genetic predisposition and environmental factors can disrupt these checkpoints, causing aberrant cell death and disease.

TAB2 (TAK1-binding protein 2) is widely reported as a critical component of TNFR1 complex I, essential for both TAK1 recruitment and activation. Consequently, TAB2 is assumedly required for TNF-mediated TAK1-dependent MAPK and NF-κB signaling and cell death inhibition2. Surprisingly, our study found that TAB2 deficiency did not affect TNF-dependent signalling to MAPK and NF-κB but shifted the TNF response from survival to apoptosis. Moreover, the induced death in TAB2 deficient cells was not caused by a defect in the currently known cell death checkpoints, including those regulated by TAK1. We show that while the pro-survival role of TAB2 depends on its ability to bind K63-linked ubiquitin chains, it is independent of its recruitment to TNFR1 complex I. Instead, we report that TAB2 associates with K63-linked ubiquitin chains in cell death-inducing complex II, limiting the amount of complex that is formed.

These findings collectively reveal a novel role for TAB2 in the TNF signaling pathway, independent of its known function to activate signaling via TNFR1 complex I, underscoring its importance in regulating cell death-inducing complex II. Many patients have been described with haplo-insufficient mutations in TAB2, which lead to non-syndromic congenital heart disease (CHD)3. Our study suggests that aberrations in TAB2 function could lead to faulty complex II regulation upon TNF sensing which induces cell death, potentially contributing to CHD progression. Consistently, mouse models of CHD due to TAB2-deficiency in cardiomyocytes underline a TNF- and cell death-dependent pathology4.

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NSS PROTEIN OF TOSCANA VIRUS MODULATES CELL DEATH AFTER INFECTION

Maëva Duboeuf (1), Felix Streicher (2), Céline Garnier (1), Qilin Xin (1), Shawon Gupta (2), Pierre-Yves Lozach (1,2), Carine Maisse (1)

1) Infections Virales et Pathologie Comparée (IVPC, UMR754), INRAE, Université Claude Bernard Lyon 1, EPHE. 50 Avenue Tony Garnier, 69366 Lyon Cedex 07, France; 2) Center for Integrative Infectious Diseases Research (CIID), Department of Virology, University Hospital Heidelberg. Im Neuenheimer Feld 344, 69120 Heidelberg, Germany.

Toscana virus (TOSV) is a neurotropic virus transmitted to humans by sandflies. TOSV is present all around the Mediterranean basin, and millions of people are exposed. The virus has recently been associated to numerous cases of unexplained encephalitis in Spain, indicating that it could be one of the major causes of encephalitis and meningoencephalitis during the summer season in South Europe. However, TOSV surveillance, prevention, and research remain largely neglected.

TOSV belongs to the Phenuiviridae family, like the Rift Valley Fever Virus (RVFV), another neurotropic virus transmitted by mosquitoes and considered by the WHO to present a significant risk of causing large-scale zoonotic epidemics. TOSV and RVFV are both single-stranded RNA-negative viruses, encoding just half a dozen proteins, among which the non-structural protein NSs. This protein is notoriously known for its strong antagonist effect on the antiviral cell response.

During viral infections, regulated cell death is generally described as a defensive mechanism to limit virus replication, thus preventing the infection of nearby cells and participating in immune response induction. Interestingly, while the RVFV NSs protein has been extensively studied and described as a pro-apoptotic protein, we have observed that the TOSV NSs is able to delay cell death after infection.

Typically, the wild-type (wt) TOSV induces a late Caspase 8 (Casp8) activation which leads to cell death in a mitochondria-independent pathway. In stark contrast, TOSV genetically modified to no longer express NSs (TOSV dNSs), induces a rapid cell death through Casp8 activation and mitochondria depolarization. Altogether, our results suggest that NSs prevents cell death through its inhibitory action on PAMPs receptors and proteins of the Type-I Interferon pathway.

A better knowledge of how TOSV modulates cell death will provide insights into how this virus manipulates the immune system. This information is crucial for understanding the propagation of the virus from the initial infection site, i.e., the skin, to the brain, ultimately leading to inflammatory disease.

CANCER-ASSOCIATED FIBROBLASTS ALTER CATHEPSIN EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA SPHEROIDS

Ida Eriksson (1), Emilia Wiechec (1,2), Karin Roberg (1,2)

1) Division of Cell Biology, Department of Biomedical and Clinical Sciences, Linköping University, Linköping; 2) Department of Otorhinolaryngology, Region Östergötland, Linköping, Sweden

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, accounting for approximately 900,000 new cases and over 400,000 deaths annually. Despite progress in treatment, advanced HNSCC is associated with poor prognosis and high mortality rates, due in part to its complex and supportive tumor microenvironment. Cancer-associated fibroblasts (CAFs) are critical components of the tumor microenvironment and play pivotal roles in therapy resistance. Lysosomes, degradative organelles essential for cellular homeostasis, have been increasingly recognized for their role in cancer progression and metastasis. Alterations in lysosomal function can affect the tumor microenvironment and contribute to therapy resistance by modulating the secretion of enzymes such as cathepsins.

Here, we demonstrate that including patient-correlated CAFs in HNSCC spheroids reduces the intracellular expression of lysosomal cathepsins in the tumor cells, while enhancing their secretion to the extracellular environment. Cathepsin D is equally expressed in both cell types in the cocultures, whereas cathepsin B is predominantly found in the CAFs. Moreover, exposure to radiation increases cathepsin expression in the tumor cells. Interestingly, including CAFs in radiation-resistant spheroids further enhances cathepsin B, but not cathepsin D expression. Conversely, in radiosensitive cells, inclusion of CAFs reduces cathepsin B and D levels instead.

To conclude, various cathepsins are differently modulated by CAFs in response to radiation therapy. Understanding and targeting the role of lysosomal cathepsins in the interactions between the tumor stroma and cancer cells presents a promising therapeutic strategy to modulate the tumor microenvironment and improve outcomes for HNSCC patients.

PROGRAMMED CELL DEATH AND HOST-IMMUNE DEFENSE.

Picard M (1), André S (1), Kundura L (2), Mammano F (1), Silvestre R (3), Corbeau P (3,4), Estaquier J (1,5)

1) INSERM-U1124, Université Paris, Paris, France; 2) Institut de Génétique Humaine UMR9002 CNRS-Université de Montpellier, Montpellier, France; 3) Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal; 4) Laboratoire d'Immunologie, CHU de Nîmes, Nîmes, France; 5) Université Laval Research Center, Québec City, Québec, Canada. estaquier@yahoo.fr.

During the last decades, the importance of programmed cell death (PCD) involving both the extrinsic (death receptors) and the intrinsic pathways (members of the Bcl-2 family) associated with mitochondrial membrane permeabilization and the release of apoptogenic factors, as well as the activation of cysteine proteases (caspases) that contribute to the executionary phase of both PCD and inflammation (inflammasome, pyroptosis, and necroptosis) has been extensively studied in the context of immune defenses. Thus, subversion of cell death mechanisms is of central importance in the establishment and dissemination of pathogens leading, like a cancer, to altered environments and potentially to death or disability. The recent pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has underlined once again the importance of PCD. We will present a series of results on the role of PCD in SARS-CoV-2 pathogenicity at the acute phase and for long COVID form in which pain, inflammation and neurological symptoms have been described. Strategies aiming to modulate PCD could be of interest for people living with COVID-19.

STUDY OF FERROPTOSIS AND THE JAK/STAT PATHWAY AS A POSSIBLE THERAPEUTIC TARGET IN GLIOBLASTOMA AND ALZHEIMER'S DISEASE

Fuentes-Baile M (1), García-Ayllon MS (2, 3), García-Morales P (1), Fuster-Picher S (3), Martínez N (1), Chihaia E (1), Pérez-Valenciano E (1), de Juan Romero C (1,2), Saceda M (1,2)

1) Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universidad Miguel Hernández, Elche (Alicante), Spain; 2) Unidad de Investigación, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO), Hospital General Universitario de Elche, Elche (Alicante), Spain; 3) Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, Sant Joan d'Alacant, Spain.

Recent studies suggest a relationship between genes involved in the genesis and maintenance of glial tumors, especially glioblastomas, and genes related to neurodegenerative diseases (1, 2). Several works have shown that WNT, ERK-AKT-p21, and angiogenesis control signaling pathways are inversely regulated in glioblastoma and Alzheimer's disease (AD) (3, 4). In fact, there is an inverse correlation in the levels of different miRNAs between both pathologies (2). It is necessary to deepen our understanding of this interconnection to elucidate the molecular mechanisms involved in the development and evolution of these diseases and, more importantly, to identify more effective therapeutic strategies for both types of pathologies.

Our initial aim was to determine whether ferroptosis and the JAK/STAT/SOCS3 pathway could be therapeutic targets in both pathologies. We have used Erastin, Sorafenib and MMRi62 as inducers of ferroptosis, as well as JAK/STAT inhibitors in several glioblastoma cell lines and the neuroblastoma cell line SH-SY5Y, which is accepted as an in vitro model for the study of Alzheimer's disease.

Interestingly, we found that all three ferroptosis inducers produced cell death in the glioblastoma cell lines, but this cell death was not blocked by ferrostatin. When analyzing by RT-qPCR the expression of three genes involved in ferroptosis: KEAP1, TFRC and GPX4, we observed the opposite of what was expected in the ferroptotic cell death. Glutathione (GSH) levels and the presence of lipid peroxidation were also analyzed after the different treatments. In this case, a decrease in the percentage of glutathione was observed after treatment with Erastin and MMRi62, which did not occur with Sorafenib. In contrast, lipid peroxidation levels increased with all three inducers. In the case of the SH-SY5Y cell line, there was a differential effect when comparing the effect of the ferroptosis inducers between the undifferentiated cell line and the differentiated cell line, which is considered a model of Alzheimer's disease. In the differentiated cell line, cell death is inhibited by ferrostatin, whereas in the undifferentiated cell line, cell death cell also occurs but is not affected by ferrostatin.

The differential effects of JAK/STAT pathway inhibitors in glioblastoma cell lines and in the undifferentiated and differentiated SH-SY5Y cell lines have also been analyzed, showing an antiproliferative effect. Finally, we performed an analysis of the expression of 25 genes related to ferroptosis and the JAK/STAT pathway in a cohort of glial tumors (including glioblastomas), non-tumor brains and brains with different degrees of Alzheimer's disease.

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UNRAVELING THE ROLE OF LC3-INDEPENDENT AUTOPHAGY IN INTESTINAL INFLAMMATION

Sergio Garcia Sanchez (1,2), Dario Priem (1,2), Maude Jans (1,2), Jon Huyghe (1,2), Mathieu J M Bertrand (1,2) and Geert van Loo (1,2)

1) VIB Center for Inflammation Research, B-9052, Ghent Belgium; 2) Department of Biomedical Molecular Biology, Ghent University, B-9052 Ghent, Belgium

The intestinal epithelium maintains a physical barrier established by the tight contact of intestinal epithelial cells (IECs) preventing bacterial infiltration. Maintenance of this intestinal epithelial barrier is of pivotal importance to prevent intestinal inflammation, and defects in barrier integrity is generally observed in inflammatory bowel disease (IBD). IECs are highly sensitive to TNF-mediated cell death that may trigger tissue damage and intestinal barrier breakdown under conditions of infection and damage. We recently identified an LC3-independent form of ATG9A-mediated autophagy as an essential mechanism to prevent TNF cytotoxicity. In this study, we aim to determine the role of LC3-independent autophagy in intestinal homeostasis and inflammation.

To do so, we generated and compared the phenotypes of mice specifically lacking Atg9a or Atg16L1 in the intestinal epithelial cells (IECs), by crossing mice with floxed Atg9a or Atg16L1 alleles with transgenic mice expressing Cre recombinase under control of the Villin promotor. IEC-specific Atg9a knockout (Atg9aFL/FL Villin-Cre, Atg9aIEC-KO) mice develop normally but are significantly smaller compared to WT controls. Histologic analysis of tissue sections of Atg9aIEC-KO mice shows normal morphology of both the small intestine and the colon, but a reduction in the number of goblet cells and Paneth cells. However, a significant number of IECs stained positive for cleaved caspase-3, reminiscent of epithelial cell death in the small intestine of Atg9aIEC-KO mice. IEC-specific Atg16L1 knockout (Atg16L1FL/FL Villin-Cre, Atg16L1IEC-KO) mice did not develop such a phenotype, demonstrating that the anti-inflammatory role of Atg9A in the small intestine is not mediated by LC3-dependent canonical autophagy. Moreover, Atg9aIEC-KO mice are hypersensitive to TNF that upon injection induces massive epithelial cell apoptosis and intestinal barrier breakdown leading to lethality of the mice. Together, these data identify LC3-independent autophagy as a major protective pathway in the intestinal epithelium and suggest that defects in the Atg9a-dependent unconventional autophagy pathway may be involved in inflammatory bowel disease in humans.

IL-4-INDUCED MICROGLIAL EXTRACELLULAR VESICLES EXERT NEUROPROTECTIVE EFFECTS AGAINST ROTENONE-INDUCED NEURONAL INJURY THROUGH MIR-191-5P

Burak Ibrahim Arıoz (1,2), Aysen Cotuk (1,2), Bilgesu Genc (1,2), Cagla Kiser (1,2), Tutku Yaras (1 ,2), Alper Bagriyanik (1,2,3), Gokhan Karakulah (1,2), Pembe Keskinoglu (4), Sermin Genc (1,2,5), Kemal Ugur Tufekci (1,6,7)

1) Izmir Biomedicine and Genome Center, Izmir, Turkey; 2) Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Turkey; 3) Department of Histology and Embryology, Faculty of Medicine, Dokuz Eylül University, Izmir, Turkey; 4) Department of Biostatistics and Medical Informatics, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey; 5) Department of Neuroscience, Institute of Health Sciences, Dokuz Eylul University, Izmir, Turkey; 6) Department of Health Care Services, Vocational School of Health Services, Izmir Democracy University, Izmir, Turkey; 7) Center for Brain and Neuroscience Research, Izmir Democracy University, Izmir, Turkey

Background-Aim: Microglia, innate cells of the central nervous system (CNS), are involved in important physiological functions such as phagocytosis, neuronal survival, cell death and synaptogenesis. They respond to pathological conditions with different phenotypes. For example, microglia responding to Interleukin-4 (IL-4) are known to suppress inflammatory responses and promote neuronal survival. The aim of this study was to determine the expression profile of miRNAs enriched in IL-4-induced microglial extracellular vesicles (IL-4 EVs) and to evaluate neuroprotective effects of miRNA on rotenone-induced neuronal injury model in SH-SY5Y cells.

Methods: In this study, human microglia HMC3 cells were induced with IL-4. Microglial extracellular vesicles were isolated by ultracentrifugation. They were characterized by Western blot, qPCR, electron microscopy and Nanoparticle tracking analysis. Next generation sequencing was used to select miRNAs significantly expressed in IL-4 EVs. Neuronal damage was induced by rotenone in SHSY-5Y human neuroblastoma cell line. To look at neuroprotective effect, IL-4 EVs and control EVs were administered to SHSY-5Y cells. IL-4 EVs were transfected with miR-191-5p inhibitor to look for neuroprotective effect mediated by miR-191-5p. Cell death was examined by PI staining.

Results: According to sequencing result, miR-191-5p expression was most significantly increased in IL-4 EVs and was therefore selected for further experiments. IL-4 EVs prevented rotenone induced neuronal damage at statistical significance (19.03%) when compared to control EVs (34.28%). To confirm the neuroprotective effect of miR-191-5p in IL-4 EVs, we performed functional study by using miR-191-5p antogomir. This protective effect of IL4 EVs against Rotenone induced neurotoxicity was not observed in miR-191 antagomir transfected. This finding confirmed that the IL-4 EV induced neuroprotective effect is partially mediated through miR-191-5p.

Conclusion: Our results show IL-4-induced microglial extracellular vesicles have protective effect against rotenone-induced neuronal damage. This protective effect is partially mediated by miR-191-5p. The results of our study suggest miRNAs carried by microglial EVs may have potential therapeutic effects on neurological diseases.

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CONTEXT-DEPENDENT MODULATION OF EXTRACELLULAR VESICLE SECRETION BY ATM INHIBITION AND TEMOZOLOMIDE IN CANCER CELLS

Irina Georgieva (1*), Aleksander Aleksandrov (2*), Radoslav Aleksandrov (2), Dimitar Iliev (2)

*1) Institute of Biophysics and Biomedical Engineering – Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 21, Sofia, 1113, Bulgaria; 2) Institute of Molecular Biology "Rumen Tsanev" – Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 21, Sofia, 1113, Bulgaria; *) equal contribution*

ATM kinase plays a key role in the DNA damage response. This has prompted the investigation of ATM as a potential target for cancer treatment, as its inhibition aims to sensitize cancer cells to chemotherapy or radiation. ATM inhibitors (ATMi) have already been used in clinical trials (1). Little is known, however, about how ATMi might affect the release and content of extracellular vesicles (EVs) secreted by cancer cells in response to DNA-damaging agents, despite the prominent roles of EVs in the tumor microenvironment and disease progression.

Our current data indicate that treatment with the ATMi KU-55933 in combination with the alkylating agent Temozolomide (TMZ)(2,3) has context-dependent effects on EV secretion, varying based on cell line and TMZ resistance.

In THP-1 monocytes, concurrent treatment led to a synergistic increase in the secretion of both large and small EVs, bearing markers that signify an autophagosomal origin (LC3-II). Exosome markers, such as CD63 and Syntenin, were also synergistically upregulated in small EVs. In contrast, U-87MG glioblastoma cells exhibited a more varied response, particularly in the small EVs fraction, with discrepancies between the secretion patterns of the observed markers, suggesting the presence of distinct populations of EVs.

Furthermore, TMZ resistance did not significantly alter the release of most markers, with the notable exception of caveolin-1, whose non-caveolar localization is associated with increased tumor aggressiveness (4). Regardless of TMZ sensitivity, KU-55933 universally induced the secretion of caveolin-1-laden small EVs. In non-resistant cells, the addition of TMZ potentiated this effect synergistically. However, in resistant cells treated with the combination, less caveolin-1 was secreted compared to cells treated with KU-55933 alone. Taken together, these results highlight the lack of a universal response mechanism of cancer cells to treatment with KU-55933, as well as its potentially complex interaction with DNA damaging agents.

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TARGETING NR1D2 TO REVERSE IMMUNOSUPPRESSION IN GLIOBLASTOMA

Melike Gezen (1), Andrea Scafidi (1,2), Frida Lind-Holm (1,2), Eleonora Campus (1), Tony Kaoma (3), Arnaud Muller (3), Anna Golebiewska (4), Petr V. Nazarov (3), Alessandro Michelucci (1), and Aurélie Poli (1)

1) Neuro-Immunology Group, Department of Cancer Research, Luxembourg Institute of Health, L-1210 Luxembourg, Luxembourg; 2) Doctoral School of Science and Technology, University of Luxembourg, Esch-sur-Alzette, Luxembourg; 3) Bioinformatics Platform, Department of Medical Informatics, Luxembourg Institute of Health, L-1445 Strassen, Luxembourg; 4) NORLUX Neuro-Oncology Laboratory, Department of Cancer Research, Luxembourg Institute of Health, Luxembourg, Luxembourg

Glioblastoma (GBM), the most aggressive brain tumor, remains incurable and has a poor response to immunotherapy due to its immunosuppressive nature. GBM immunosuppression is marked by a reduced adaptive immunity and an increase in immunosuppressive myeloid cells, where tumor associated macrophages (TAMs) recruited from the periphery play a central role1.

Patient studies highlight a protective role of allergic diseases on GBM susceptibility and prognosis2. We modeled this in mice, showing that allergic airway inflammation (AAI) prolongs survival following intracranial implantation of the GL261 GBM cell line, enhancing microglial and adaptive anti-tumoral immunity3. AAI also induces transcriptomic reprogramming of microglia, with nuclear receptor subfamily-1 group-D member-2 (NR1D2) being the most significantly upregulated gene. Bioinformatic explorations of publicly available transcriptomic data from patient biopsies reveal NR1D2 as a marker interconnecting GBM patient survival with tumor malignancy and reduced local immunosuppression. Furthermore, single-cell RNA sequencing analysis of peripheral blood immune cells from our cohort of GBM patients shows classical monocytes with a decreased NR1D2 expression and dampened immune response profile compared to healthy individuals.

We hypothesize that targeting NR1D2 can promote an anti-GBM phenotype by modulating TAMs, reversing GBM-related immunosuppression. This project aims to characterize NR1D2 expression patterns and its effects on TAMs' anti-tumoral features. We have established in vitro procedures, including transcriptomic, proteomic and molecular techniques, to characterize the NR1D2 functions in TAMs. To assess NR1D2's impact on TAMs, we will use drugs targeting NR1D2 and CRISPR/Cas9 genome editing for gain- and loss- of-function approaches.

Our results show a correlation between increased NR1D2 expression and limited immunosuppression in GBM. This research offers new insights into the connection between allergy and GBM, enhancing our understanding of NR1D2's role in myeloid cells within GBM.

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DEVELOPMENT OF PDE6D CELL-PENETRATING INHIBITOR PEPTIDES TO TARGET KRAS MUTANT CANCERS

Atanasio Gómez-Mulas (1), Rohan Chippalkatti (1), Daniel Abankwa (1)

1) Cancer Cell Biology and Drug Discovery, Department of Life Sciences and Medicine, University of Luxembourg

RAS genes are mutated in 19% of all human cancer cases and, among them, 70% are caused by KRAS mutations (1). Downstream of Ras, the Ras-MAPK pathway regulates vital cellular processes, including proliferation and differentiation. Its overactivation is associated with cancer and multi-organ congenital disorders collectively called RASopathies.
The KRAS-encoded isoform K-Ras4B (hereafter K-Ras) is activated by extracellular signalling cascades at the plasma membrane. The intracellular redistribution of prenylated K-Ras necessary for its plasma membrane localization and activity is facilitated by the prenyl-binding trafficking chaperone PDE6D. K-Ras has proven difficult to target directly, for that reason PDE6D is considered a surrogate target of K-Ras-driven pathologies.
Previous studies have discovered inhibitory molecules that can bind to the hydrophobic pocket of PDE6D. However, it has been shown that compounds displaying higher affinity for the PDE6D hydrophobic pocket also suffer from increasingly poor solubility and off-target effects (2). Although solubility and efficacy of small-molecule PDE6D inhibitors could be increased in our previous work (2), novel PDE6D-targeting strategies must be devised.
Based on the sequence of natural high-affinity PDE6D cargo, the affinity of K-Ras towards PDE6D can be increased by introducing point mutations in the four residue-stretch upstream of the prenylated C-terminal cysteine. These amino acid substitutions increase the affinity of the mutant K-Ras towards PDE6D several thousand-fold, leading to the sequestration of K-Ras in the nucleo-cytoplasm (3).
Based on this high-affinity binding mutant K-Ras and using a rational inhibitor development pipeline, we have designed small genetically encoded peptides that interact with PDE6D and plug its prenyl-binding pocket. Using these peptides, we aim to block the trafficking activity of PDE6D, preventing K-Ras from reaching the PM, and thus, from activating downstream effectors. In a next step, these genetically encoded peptides will be functionalized with a cell-penetrating moiety. Finally, further optimization of peptidomimetics or small molecule analogues will be performed to establish novel types of PDE6D inhibitors.

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SPECIFIC METABOLIC REGULATIONS OF P53 MUTANT VARIANTS IN PANCREATIC CANCER

Sabrina Caporali (1,*), Alessio Butera (1,*), Alessia Ruzza (1,*), Carlotta Zampieri (2), Marina Bantula Fontes (3), Sandra Scharsich (1), Anna-Katerina Ückert (4), Ivana Celardo (4), Ian U. Kouzel (5), Luigi Leanza (6), Andreas Gruber (5), Joan Montero (3), Angelo D'Alessandro (7), Thomas Brunner (8), Marcel Leist (4) and Ivano Amelio (1,$)

*1) Chair for Systems Toxicology, Department of Biology, University of Konstanz, Germany; 2) Department of Pathology, University of California San Diego School of Medicine, La Jolla, California, USA; 3) Department of Biomedical Sciences, Universitat de Barcelona, Casanova 143, Barcelona, 08036; 4) Chair for in vitro Toxicology and Biomedicine, University of Konstanz, Konstanz, Germany; 5) Applied Bioinformatic Group, University of Konstanz, Konstanz, Germany; 6) Department of Biology, University of Padua, Padua, Italy; 7) University of Colorado Anschutz Medical Campus, Aurora, CO 80045, US; 8) Chair for in Biochemical Pharmacology, University of Konstanz, Konstanz, Germany; * These authors have equally contributed to this article.; $ Correspondence to: Ivano Amelio, ivano.amelio@uni-konstanz.de*

Approximately half of all human cancers harbour mutations in the p53 gene, leading to the generation of neomorphic p53 mutant proteins. These mutants can exert gain-of-function (GOF) effects, potentially promoting tumor progression by altering the chromatin landscape and dysregulating genomic integrity and gene expression. However, the clinical significance of p53 GOF mutations, as well as the specificity of individual variants, remains unclear.

To elucidate the metabolic regulations and molecular underpinnings associated with the specific p53R270H and p53R172H mutant variants (the mouse equivalents of human p53R273H and p53R175H, respectively), we employed a comprehensive approach, including integrating global metabolomic analysis with epigenomic and transcriptomic profiling in mouse pancreatic cancer cells. Our investigation revealed that the p53R270H variant, but not p53R172H, sustains mitochondrial function and energy production while also influencing cellular antioxidant capacity. Conversely, p53R172H, while not affecting mitochondrial metabolism, attenuates the activation of pro-tumorigenic metabolic pathways such as the urea cycle. Thus, the two variants selectively control different metabolic pathways in pancreatic cancer cells. Mechanistically, p53R270H induces alterations in the expression of Lamin B1 and Slc3a2 genes, accompanied by changes in chromatin accessibility. Dysregulation of Lamin B1 and Slc3a2 is associated with oxidative stress and disruptions in mitochondrial respiration. In contrast, p53R172H specifically impacts the expression levels of arginase 2 (Arg2) and creatine kinase B (CKB), pivotal enzymes in urea metabolism. However, our analysis of cell proliferation, cell competition, mitochondrial apoptotic priming suggested that the expression of either p53R270H or p53R172H does influence the tumorigenic properties of cancer cells nor confer any selective advantage. Importantly, however correlation between the expression of the identified downstream regulators and p53 mutational status, together with a clear prognostic impact, provides a potential clinical significance to the observed regulations.

Our study elucidates the mutant-specific impact of p53R270H and p53R172H on metabolism of PDAC cancer cells, highlighting the need to shift from viewing p53 mutant variants as common entities to a systematic assessment of each specific p53 mutant protein. Furthermore, our analysis of cancer cell properties underscores the importance of evaluating the impact of specific microenvironmental conditions on mutant p53 GOF, using experimental systems that accurately replicate the biology of real tumour ecosystems.

TARGETING CK1 AND RSK: A STRATEGIC APPROACH TO MITIGATE NECROPTOSIS

Eden Haseeb (1), Benjamin Pfeuty (1) and Franck B. Riquet (1)

1) Université de Lille, CNRS, UMR 8523-PhLAM-Physique des Lasers Atomes et Molécules, 59000 Lille, France

Necroptosis, a programmed form of cell death, plays a crucial role in inflammation due to the release of cytokines, chemokines, and damage-associated molecular patterns. This mechanism is implicated in pathological conditions, including neurodegenerative and cardiovascular diseases [for review [1]]. The receptor-interacting serine/threonine-protein kinase 1 (RIPK1) is a crucial mediator of death receptor-induced necroptosis. Despite numerous clinical trials to inhibit RIPK1, no effective necroptosis inhibitors have been successfully translated into clinical practice. Therefore, we reasoned that additional molecular targets might exist and drug repurposing may provide alternative therapeutic strategies for necroptosis management.

We conducted a high-throughput screening of 2800 FDA-approved compounds to evaluate their modulatory effects on TNF-induced necroptosis in L929sAhFas cells. Secondary screening validated 151 compounds that modulated TNF-induced necroptosis significantly, of which [2] compounds spiked an interest. The first one is a Casein Kinase 1 (CK1) activator that enhanced necroptosis significantly as compared to the mTNF treated condition which led us to concentrate on the role of CK1 in necroptosis. We found that prior studies have indicated that CK1 inhibition can attenuate necroptosis in murine and human cellular models by inhibiting the phosphorylation of RIPK3 at Serine 227 which blocks necroptosis [2]. The second compound family is Epidermal Growth Factor Receptor (EGFR) inhibitors that reduced TNF-induced necroptosis by 50-70% in confirmation screening. Interestingly, components of the Extracellular Regulated Kinase (ERK) pathway such as p90 ribosomal S6 kinase (RSK) that is downstream of the EGFR have shown efficacy in attenuating necroptosis[3,4]. RSK phosphorylation of pro-Caspase 8 (Casp8) at Thr 265 contributes to passing the Casp8 checkpoint of necroptosis[4]. Since RSK and CK1 are important components involved in the regulation of the necrosome, we reasoned that their combined inhibition could robustly suppress TNF-induced necroptosis. With inhibitors of both kinases already in clinical trials, our project presents potential therapeutic applications for managing necroptosis.

We are now developing experimental strategies to: i) study the effect of combined inhibition of CK1 and RSK in murine and human cell lines, ii) validate results using genetic approaches, iii) repurpose RSK and CK1 inhibitors as inhibitors of TNF-induced necroptosis.

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UNVEILING THE ROLE OF INTER-ORGANELLAR COMMUNICATION IN PINK1-MEDIATED MITOCHONDRIAL QUALITY CONTROL AND NEURONAL HEALTH

Marlena Helms (1, 2, 3), Simone Wanderoy (1, 4), Tabitha Hees (1, 2, 4), Angelika Harbauer (1, 3, 5)

1) Max Planck Institute for Biological Intelligence; 2) International Max Planck Research School – Biological Intelligence; 3) Integrated Research Training Group – TRR353, 4) Graduate Center of Medicine and Health, Technical University of Munich; 5) Institute for Neuronal Cell Biology, Technical University of Munich

Functional mitochondria are especially important for energy-demanding cells such as neurons. Thus, quality control of mitochondria plays a crucial role in neuronal health[1]. The PTEN induced kinase 1 (PINK1), a protein that, when mutated, is associated with Parkinson's disease, acts as a sensor for mitochondrial damage and can trigger a mitophagy cascade culminating in the removal of defective mitochondria via lysosomes[2–4].

Our lab has shown that a hitchhiking complex – consisting of the RNA-binding protein Synaptojanin 2a (SYNJ2a) and its mitochondrial binding protein (SYNJ2BP) – allows for transport of Pink1 mRNA into the axons of neurons[5]. AMP-activated protein kinase (AMPK), a master regulator of energy metabolism, regulates this transport by ensuring SYNJ2a-SYNJ2BP interaction through phosphorylation of SYNJ2BP[6]. In combination with local translation in distal parts of the neuron, Pink1 mRNA transport thereby ensures a constant supply of the short-lived PINK1 protein.

Unsurprisingly, the interaction and communication of mitochondria with other organelles are not only crucial for mitochondrial quality control. Lysosomes additionally serve as an important signaling hub in metabolism and many other cellular processes including calcium signaling[7]. In order to buffer cellular calcium, mitochondria also work closely together with the endoplasmic reticulum (ER)[8].

Interestingly, our data show that knockdown as well as overexpression of SYNJ2BP result in a loss of neuronal viability. This cell death is characterized by phenotypic changes in cellular calcium regulation and can partially be rescued by overexpression of SYNJ2a, highlighting the importance of modulating SYNJ2BP phosphorylation to ensure the presence of SYNJ2a. However, a phosphatase that can regulate SYNJ2a SYNJ2BP interaction has not been identified yet. The precise mechanisms by which inter organellar calcium signaling is involved in this process and downstream PINK1-dependent mitochondrial quality control also remain unclear.

Using advanced live cell imaging tools such as the MS2/PP7-split Venus system for Pink1 mRNA visualization, the SunTag reporter for active PINK1 translation monitoring, calcium imaging, and FLIM FRET, we assess the role of mitochondrial communication in SYNJ2BP modulation and PINK1 biology. Overall, we emphasize the importance of inter-organellar signaling in maintaining mitochondrial quality control and subsequently in promoting neuronal health.

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FUNCTIONAL DIVERGENCE OF SUCLG2 ISOFORMS IN CANCER

Hrysiuk M (1,2,3), Dvorakova S (1), Cecchini G (3), Maklashina E (3), Boukalova S (1,2), Neuzil J (1,2,4,5)

1 Institute of Biotechnology, Academy of Sciences of the Czech Republic, Prague-West, Czech Republic; 2) Faculty of Science, Charles University, Prague, Czech Republic; 3) San Francisco VA Medical Center & UCSF, San Francisco, USA; 4) School of Pharmacy and Medical Sciences, Griffith University, Southport, Qld, Australia; 5) First Faculty of Medicine, Charles University, Prague, Czech Republic

Succinate-CoA ligase (SUCL) is an enzyme with heterodimeric structure comprising the α-subunit SUCLG1 and a substrate-specific β-subunit SUCLA2 or SUCLG2 that generate either ATP or GTP, respectively. SUCL holds a central position at the crossroads of numerous metabolic pathways, being a vital component of the tricarboxylic acid (TCA) cycle, catalyzing the conversion of succinyl-CoA and ADP/GDP into succinate, CoA-SH, and ATP/GTP. We have recently identified mutations in SUCLG2 among patients with paraganglioma and pheochromocytoma (PPGL), linking these mutations to defects in the assembly of electron transport chain complex II (CII), a known tumor suppressor in PPGL [1]. Our research reveals that the assembly of CII is specifically regulated by one of the two primary splice isoforms of SUCLG2, termed isoform 204 (SUCLG2204). Interestingly, SUCLG2204 does not form a complex with SUCLG1 and is not involved in the enzymatic activity of SUCL. Conversely, the 201 splice isoform of SUCLG2 (SUCLG2201) supports the GTP-dependent activity of SUCL but does not facilitate CII assembly. Our findings highlight the distinct functional divergence of SUCLG2204. This isoform has lost its role in succinate production via the SUCL complex but enhances succinate consumption within the TCA cycle by promoting CII assembly. We propose that, through this unique function, SUCLG2204 acts as a tumor suppressor in PPGL.

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NON-PROTEOLYTIC UBIQUITINATION OF RIPK3 VIA SMURF1 AND USP5 CONTROLS NECROPTOSIS

Chi Hyun Hwang (1), Jaewhan Song (1)

1) College of Life Science & Biotechnology, Yonsei University, Seoul, Republic of Korea

Receptor-interacting protein kinase 3 (RIPK3) is a central regulator protein of necroptosis that programmed cell death causing immune responses. There are several E3 Ligases that can regulate necroptosis by determining RIPK3 protein stability. However, little is known about the mechanisms underlying the regulation of different types poly ubiquitination of RIPK3. Here, we identify that SMAD-specific E3 ubiquitin protein ligase 1 (SMURF1) and Ubiquitin-specific peptidase 5 (USP5) are associated with the necrosome complex, which can be assembled during necroptosis. SMURF1 deficiency accelerates necroptosis, and the acceleration can be reversed by reconstituting only SMURF1 and not SMURF1 C699A, suggesting that E3 ligase activity is required for this activity. On the contrary, USP5 deficiency suppresses necroptosis, and the suppression can be reversed by reconstituting only USP5 and not USP5 C335A, suggesting that deubiquitinase activity is required for this activity. Mechanistically, SMURF1 prevents the formation of the Necrosome complex by promoting K63 poly ubiquitination of RIPK3 during necroptosis. Vice versa, USP5 acts de-ubiquitination of K63 poly ubiquitination of RIPK3, which already regulated by SMURF1. Finally, down-regulation of SMURF1 enhances necroptosis in leukemia cells and suppresses tumor growth in a xenograft model upon treatment with birinapant and emricasan. These findings demonstrate that SMURF1 and USP5 regulate necroptosis through antagonistic regulation of K63 poly ubiquitination of RIPK3 and necrosome complex formation.

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PTGS2 PROMOTES FERROPTOSIS AND MODULATES LIPID METABOLISM IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

Benedetta Imperiali (1), Francesco Rugolo (1), Livia Concetti (1), Eleonora Candi (1), Gerry Melino (1) and Massimiliano Agostini (1)

1) Department of Experimental Medicine, TOR, University of Rome "Tor Vergata", 00133 Rome, Italy.

Squamous cell carcinomas including head and neck (HNSCC), are highly malignant treatment-refractory tumours and represent a major cause of death worldwide. Although surgical resection and other therapies including radiation, chemotherapy and immunotherapy have improved the life quality of the patients, resistance to targeted therapy remains a largely insurmountable challenge. Here, we explore the possibility that HNSCC cancer cells which had escaped cell death may be selectively killed by ferroptosis (1). We have identified that Prostaglandin-Endoperoxide Synthase 2 (PTGS2 also known as COX-2) expression positively correlates with the sensitivity of the HNSCC cell lines to ferroptosis induced by Erastin. Indeed, genetic and pharmacological inhibition of PTGS2 resulted in a significant reduction of both cell death and lipid peroxidation after Erastin treatment.

PTGS2 is mainly located in the lumen of the endoplasmic reticulum (2). Nevertheless, several observations indicate that in cancer cells PTGS2 may localizes in the mitochondria and/or lipid droplets (LDs) (3), two key organelles involved in regulating ferroptosis. Hence, we monitored PTGS2 intracellular localization both in unstimulated and erastin-treated cells. Immunofluorescence analysis shows that in untreated HNSCC cell lines PTGS2 partially localize on LDs. Importantly, following induction of ferroptosis PTGS2 migrates and accumulates on LDs, suggesting a possible cooperation between PTGS2 and LDs. Then, we explored if LDs dynamic is affected by ferroptosis induction and whether PTGS2 may participate in the regulation of the process. Erastin treatment caused a significant increase in LDs number per cell in both A253 and HN30 cell lines. On the contrary, the size (LD area) and fluorescence intensity of LDs was significantly reduced. However, upon inhibition of PTGS2 expression the effect of erastin on LD number, area and intensity was significantly reduced, indicating that PTGS2 participates to the regulation of LDs dynamics during ferroptosis.

Then, we investigated whether our results may have a clinical relevance. Patients which are under a platinum-based treatment have a better prognosis when the expression of PTGS2 is low. Interestingly, HNSCC patients that are characterized by high levels of PTGS2 do not respond to the Cisplatin treatment suggesting that PTGS2 may regulate chemosensitivity to Cisplatin therapy.

Overall, our findings indicate that the expression of PTGS2 discriminates susceptibility to ferroptosis in HNSCC, and therefore we would like to speculate that PTGS2 may act as biomarker for stratification of HNSCC patient that may potentially respond to an alternative cell death pathway such as ferroptosis.

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THE CASPASE-2 SUBSTRATE P54NRB EXERTS ITS TUMORIGENIC FUNCTION VIA EXPRESSIONAL REGULATION

Gergely Imre

South Dakota State University, Brookings, United States

Caspase-2 is an evolutionarily conserved enzyme that plays a crucial role in genotoxic stress-induced apoptosis, metabolic changes associated with aging, and the elimination of aneuploid cells in tumors. The genetic deletion of caspase-2 has been linked to increased tumor susceptibility in vivo, highlighting its importance as a possible tumor suppressor. However, the precise downstream signaling mechanisms through which caspase-2 exerts its tumor suppressive effects remain elusive. Unique among caspases, caspase-2 is localized in the nucleus and other cellular compartments.

In this study, we identify p54nrb as a nuclear-specific substrate of caspase-2. Caspase-2 selectively cleaves p54nrb at D422, disrupting its C-terminal site, which is the putative DNA binding region of the protein. P54nrb is an RNA and DNA binding protein involved in RNA editing, transport, and the transcriptional regulation of genes. Notably, we demonstrate that p54nrb is overexpressed in several human tumor types, including cervical adenocarcinoma, melanoma, and colon carcinoma. Conversely, the loss of p54nrb in tumor cell lines increases susceptibility to cell death and significantly reduces tumorigenic potential.

Using high-resolution quantitative proteomics, we demonstrate that the loss or cleavage of p54nrb leads to altered expression of oncogenic genes. Among these, the downregulation of the tumorigenic protease cathepsin-Z and the anti-apoptotic protein gelsolin is consistently observed across three tumor cell types: adenocarcinoma, melanoma, and colon carcinoma. Furthermore, we show that p54nrb interacts with the DNA of cathepsin-Z and gelsolin, but not their RNA.

This study reveals a previously unrecognized mechanism by which caspase-2 exerts its tumor suppressor functions in human tumor cells. Our findings suggest that the cleavage of p54nrb by caspase-2 is a critical event that modulates the expression of key oncogenes, thereby influencing tumorigenesis and cell death susceptibility. These insights provide a deeper understanding of the molecular pathways involved in caspase-2 mediated tumor suppression and highlight potential targets for therapeutic intervention in cancer.

METABOLIC VULNERABILITIES OF AGGRESSIVE LUNG TUMOURS WITH CONCOMITANT MUTATIONS IN TP53 AND KRAS

Júlia Urgel i Solas (1,2) Etna Abad (1), Laura Ortet (1)Savvas Kourtis (2), Ilir Sheraj (2), Sara Sdelci (2) , Ana Janic (1)

1) Department of Medicine and Life Sciences, Universidad Pompeu Fabra, Barcelona,Spain; 2) Centre of Regulation of Genomics (CRG), Barcelona, Spain

Tumours with concurrent mutations in KRAS and TP53 represent 15% of all non-small cell lung cancers (NSCLC), and it has been shown that this genetic asset confers a particularly aggressive phenotype. TP53 and KRAS are known to be widely involved in carcinogenesis, being two of the most mutated genes in tumours. Upon activation, TP53 can trigger a range of downstream pathways, including metabolic regulation and cell homeostasis. On the other hand, when KRAS is mutated, it acquires oncogenic properties, promoting the disregulation of many processes, including the reprogramming of the tumor metabolism. Despite the involvement of mutated TP53 or KRAS signalling in cancer metabolism, the knowledge regarding the metabolic rewiring occurring in KRAS/TP53 double-mutant tumours is limited. To identify novel interconnections between KRAS/TP53 double-mutant driven NSCLC proliferation and metabolism rewiring we conducted metabolic CRISPR/Cas9 drop-out functional genomics screens within the whole mouse and 2D human cell lines. We revealed purine synthesis as a metabolic dependency of KRAS/TP53 mutant driven lung cancer cells. Interestingly, we interrogated essentialities in publicly available Cancer Cell Line Encyclopedias (CCLE) datasets and our results indicate that our hits corresponding to purine synthesis appeared to be more essential in cancer cells with TP53 low activity, resembling TP53 KO cancer cells. Interestingly we have shown that KRAS/TP53-mutant cells are more sensitive to purine synthesis inhibitor than control KRASmut/TP53-wt. Next, we have shown that TP53 loss could promote purine synthesis metabolic pathway, in particular adenosine and adenosine derivates. The results of this project has lead to the identification of novel therapeutic approaches for lung adenocarcinomas harbouring mutations in TP53 and KRAS, which exhibit a critically aggressive phenotype and cause globally approximately 200,000 deaths per year.

THE FUNCTION OF TRIM28 AS AN RNA BINDING PROTEIN AND ITS ROLE IN CONTROLLING BOK MRNA EXPRESSION

Ali Jazaeri Jouneghani (1), Daniel Bachmann (1), Yuniel Fernandez Marrero (1), Thomas Kaufmann (1)

1) Institute of Pharmacology, University of Bern, Bern, Switzerland

BOK (BCL-2 related ovarian killer) is a member of the BCL-2 family killer proteins. It structurally resembles the pro-apoptotic "killers" BAX and BAK. Studies have shown that BOK expression is frequently repressed by various means in cancer, pointing towards possible tumor-suppressor-like function of this protein (1). Moreover, a contribution of BOK in mouse development was revealed, as BAX/BAK/BOK triple knockout mice display a more severe phenotype than BAX/BAK double knockout mice (2). Besides this, it seems that BOK is involved in some other cellular processes like cell multiplication, unfolded protein response, autophagy and uridine metabolism (3).
Recent studies have shown TRIM28 (Tripartite motif containing 28) as a novel RNA-binding protein that binds to AU-rich elements in BOK mRNA, causing its degradation (1). Of note, high TRIM28 typically correlates with poor patient survival in cancer. Since TRIM28 does not have a known nuclease activity, it is conceivable that it recruits and cooperates with other proteins to degrade BOK mRNA. We are using proximity dependent biotin labeling approaches (TurboID), followed by mass spectrometric analysis, to identify interaction partners of TRIM28 at the level of mRNA complexes. We further plan to identify other mRNAs affected by TRIM28 using RNA-immunoprecipitation, followed by RNA sequencing. Furthermore, we have generated transgenic mice in which the ARE-site in BOK has been deleted and we will investigate the physiological relevance of this regulatory element in development and in the adult mouse.

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NATURAL FLAVONOIDS TARGETING G2/M CELL CYCLE-RELATED GENES AND SYNERGIZE WITH SMAC MIMETIC LCL-161 TO INDUCE NECROPTOSIS AND IMMUNOGENIC CELL DEATH IN CHOLANGIOCARCINOMA CELLS

Siriporn Jitkaew (1,2), Thanpisit Lomphithak (3), Hardyanti Eka Putri (1,2), Patthorn Jaikla (3), Apiwit Sae-Fung (3), Sasiprapa Sonkaew (3)

1) Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand; 2) Center of Excellence for Cancer and Inflammation, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand; 3) Graduate Program in Clinical Biochemistry and Molecular Medicine, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand.

Cholangiocarcinoma (CCA) is an aggressive cancer associated with a very poor prognosis and low survival rates, primarily due to late-stage diagnosis and low response rates to conventional chemotherapy. Therefore, there is an urgent need to identify effective therapeutic strategies that can improve patient outcomes. Flavonoids, such as quercetin and kaempferol, are naturally occurring compounds that have attracted significant attention for their potential in cancer therapy by targeting multiple genes. In this study, we employed network pharmacology and bioinformatic analysis to identify potential targets of quercetin and kaempferol. The results revealed that the target genes of these flavonoids were enriched in G2/M-related genes, and higher expression of G2/M signature genes was significantly associated with shorter survival in CCA patients. Furthermore, in vitro experiments using CCA cells demonstrated that quercetin or kaempferol induced cell-cycle arrest in the G2/M phase. Additionally, when combined with a Smac mimetic LCL-161, an IAP antagonist, quercetin or kaempferol synergistically induced RIPK1/RIPK3/MLKL-mediated necroptosis and immunogenic cell death in CCA cells while sparing non-tumor cholangiocyte cells. These findings shed light on an innovative therapeutic combination of flavonoids, particularly quercetin and kaempferol, with Smac mimetics, suggesting great promise as a necroptosis-based approach for treating CCA and potentially other types of cancer.

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IDENTIFYING NOVEL REGULATORS OF EXPRESSION OF THE BH3-ONLY PROTEIN PUMA USING WHOLE GENOME CRISPR/CAS9 KO SCREENS TO ENHANCE THE EFFICACY OF CANCER TREATMENT

Deeksha Kaloni (1,2), Elizabeth Lieschke (1,2), Andrew Kueh (1,3), Lin Tai (1,3), Catherine Chang (1), Marco Herold (1,3), Andreas Strasser (1,2), Sarah Diepstraten (1,2) and Gemma Kelly (1,2)

1) Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia; 2) Department of Medical Biology, University of Melbourne, Parkville, Victoria 3052, Australia; 3) Olivia Newton-John Cancer Research Institute, Heidelberg, Victoria, Australia

The mitochondrial-mediated apoptotic pathway, also known as the intrinsic apoptotic pathway, is governed by the BCL-2 (B-cell lymphoma 2) protein family, which includes both pro-survival and pro-apoptotic members. BH3 (BCL-2 homolog region [BH] 3)-only proteins (e.g., PUMA, BIM, BID, NOXA) are pro-apoptotic members of the BCL-2 protein family that are required for the initiation of apoptosis, and they play crucial roles in the killing of malignant cells by diverse anti-cancer agents. PUMA is an important pro-apoptotic BH3-only protein that plays a significant role in triggering apoptosis through both p53-dependent and p53-independent pathways. The gene encoding PUMA is a direct transcriptional target of p53. Our research focuses on identifying novel genes that can regulate Puma expression in both p53-dependent and p53-independent manners in blood cancer cells.

To do this we have engineered and validated a novel reporter mouse strain, replacing the Puma coding region (Bbc3 gene) with the tdTomato gene while retaining the endogenous regulatory regions. Using the Puma-tdTomato reporter mouse model, we have established mouse models of Acute Myeloid Leukemia (AML) by introducing retroviruses expression known AML-driving oncogenes into haematopoietic stem and progenitor cells from Puma-tdTomato reporter mice. The injection of these HSPCs into irradiated recipient mice led to AML-like tumours within a month and cell lines could be derived from the tumours. Additionally, we have crossed Puma-tdTomato mice with the Eμ-Myc transgenic mouse model of lymphoma and derived lymphoma cell lines derived from the resulting tumours. Subsequently, we monitored Puma gene induction in response to death stimuli in malignant cells derived from these tumours using FACS and microscopy to track tdTomato expression. Furthermore, to identify novel regulators of Puma expression, we have performed CRISPR/Cas9 whole genome knock-out screens in the Puma reporter-expressing AML cell lines. We selected the top and bottom 3% of tdTomato expressing cells and carried out Next Generation Sequencing to identify the enriched single guide RNAs. Validation of hits was carried out in AML cancer cell lines expressing p53 and in AML cell lines engineered by CRISPR/Cas9 to be p53 deficient.

Our study, employing a Puma-tdTomato reporter mouse model in pre-clinical models of AML, has uncovered novel regulators of Puma expression. Such hits could be targets for existing or novel anti-cancer drugs that could increase PUMA expression and thereby enhance cancer cell death. Importantly, targeting hits that validate in p53-deficient cancer cells will enable induction of Puma expression and subsequently cell death in cancer cells that are mutated for p53.

GENOME-WIDE INVESTIGATION REVEALS THE GENETIC UNDERPINNINGS OF GRANULOVACUOLAR DEGENERATION BODIES OBSERVED IN NEURODEGENERATIVE BRAIN DISEASES

Celeste Laureyssen (1,2), Fahri Küçükali (1,2), Jasper Van Dongen (1,2), Klara Gawor (3), Sandra O. Tomé (3), Alicja Ronisz (3), Markus Otto (4), Christine A.F. von Arnim (5), Philip Van Damme (6,7), Rik Vandenberghe (7,8), Dietmar Rudolf Thal (3,9), Kristel Sleegers (1,2)

1. Complex Genetics of Alzheimer's Disease group, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium; 2. Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium; 3. KU Leuven, Laboratory for Neuropathology, Department of Imaging and Pathology, and Leuven Brain Institute, Leuven, Belgium; 4. Martin-Luther-University Halle-Wittenberg, Department of Neurology, Halle (Saale), Germany; 5. University Medical Center Göttingen, Department of Geriatrics, Göttingen, Germany; 6. VIB-KU Leuven, Laboratory for Neurobiology, Leuven, Belgium; 7. UZ Leuven, Department of Neurology, Leuven, Belgium; 8. KU Leuven, Laboratory for Cognitive Neurology, Department of Neurosciences; Leuven Brain Institute, Leuven, Belgium; 9. University Hospital Leuven, Department of Pathology, Leuven, Belgium

Introduction: Neurodegenerative brain diseases (NBDs) are very complex, heterogenous proteinopathies with a detrimental outcome of memory loss, accompanied by a wide range of additional debilitating symptoms. Despite clear differences amongst these diseases, some overlap can be observed on a pathological level. The most prevalent NBD, Alzheimer's disease, presents itself in the majority of cases with comorbid lesions additional to the AD hallmark features. One lesion that is frequently co-observed alongside specific NBD hallmark pathology is granulovacuolar degeneration, characterized by vacuoles in the neuronal cytoplasm termed granulovacuolar degeneration bodies. Interestingly, these vacuoles contain aggregations of pMLKL, RIPK1 and RIPK2. These proteins are essential markers of the necroptotic cell death pathway, implicating this pathway as a commonality between NBDs. In this study, a genome-wide investigation of granulovacuolar degeneration bodies in a neuropathological cohort was performed, offering an elucidated view of genetic factors that might play a role in the development of these disease-overarching lesions.
Methods: A neuropathological cohort of 367 individuals suffering from a variety of NBDs was interrogated by staining granulovacuolar lesions and assessing the stage of GVD pathology based on the spread throughout the brain (stage 0 – stage V). DNA was extracted from cerebellar tissue and low-coverage whole-genome sequencing was performed using Element Biosciences AVITI sequencing, followed by read alignment, extensive QC, base-calling and imputation with GLIMPSE (genotype-likelihood imputation and phasing method). The resulting dataset was employed to investigate possible association between genetic single-nucleotide polymorphisms (SNPs) and GVD stage. Association was tested by constructing linear regression models correcting for age, sex and the first 3 principal components. Linear regression was performed using the PLINK glm function.
Results: One locus on chromosome 3 passed Bonferroni correction for multiple testing with an index SNP p-value of 1.7*10-8. Downstream mapping of the locus revealed that the index SNP was located in an intergenic region, neighboring two genes: VHL (Von Hippel Lindau) and IRAK2 (Interleukin-receptor associated kinase 2). The former being a tumor suppressor gene and the latter having a strong implication in IL-1 and TLR signaling, resulting in NF-kB release. Expression quantitative trait locus (eQTL) investigation revealed however that the index SNP itself is strongly influencing expression of IL-17 which is locat-

ed upstream of the risk locus.
Conclusion: This work offers novel insights in the potentially relevant genetic predisposition for developing GVD pathology, reflecting underlying necroptosis. The importance of this form of cell death in NBDs and especially Alzheimer's disease has recently been highlighted, making it a pivotal area of investigation. Genetic studies can also be a valuable tool in uncovering potentially interesting novel pathway interactors and biological mechanisms involved in necroptotic cell death.

CROSS-DISEASE STUDY OF APOPTOTIC SIGNALING CHANGES IN ALZHEIMER'S AND PARKINSON'S DISEASE USING SINGLE-CELL ANALYSES OF BRAIN ORGANOIDS

Sophie Le Bars (1), Mohamed Soudy (1), Sarah Nickels (1), Jens Christian Schwamborn (1), and Enrico Glaab (1)

1) Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Esch-sur-Alzette, Luxembourg

Alterations in apoptotic pathways are a common feature of neurodegenerative diseases, yet the underlying mechanisms and their cell type-specificity remain incompletely understood, and it is unclear to which these mechanisms are consistent across different degenerative disorders. This study aims to elucidate relevant shared mechanisms of apoptosis in neurodegenerative diseases at single-cell resolution, focusing on potential common patterns between Alzheimer's (AD) and Parkinson's disease (PD).

Using brain organoid samples to mimic pathological conditions in key affected brain regions in AD (2 samples for AD and 2 samples for healthy controls (HC)) and PD (7 samples for PD and 3 samples for HC), we conducted a comprehensive analysis of single-cell RNA-sequencing data encompassing differential expression, pathway analysis, differential gene regulatory net-work analysis, and cell-cell communication analysis. Our results revealed significant shared biological process alterations in astrocytes between AD and PD, with pronounced changes af-fecting pathways associated with apoptotic signaling and oxidative stress response.

A cell type-specific gene regulatory network construction and network perturbation analysis identified the gene SFPQ (Splicing Factor Proline and Glutamine Rich), a DNA and RNA binding protein, as a key regulator of the sub-network of shared differentially expressed genes between both diseases for astrocytes. SFPQ is implicated in the positive regulation of oxidative stress-induced intrinsic apoptotic pathways. Additionally, CDKN1A, which encodes the cyclin-dependent kinase inhibitor 1A (also known as p21), was identified as a further important regu-lator of common downstream network changes. CDKN1A is both a mediator of oxidative stress signaling and a downstream effector of the P53 pathway, a major apoptotic signaling pathway.

In summary, we have identified shared regulatory network alterations in apoptotic pathways between AD and PD and identified SFPQ and CDKN1A as common upstream regulators of these pathways. These findings pave the way for follow-up mechanistic studies to further elucidate the roles of SFPQ and CDKN1A in the regulation of apoptosis and oxidative stress in neurodegenerative diseases.

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ANTI-CANCER DRUG SALINOMICYN REVERSES ENDOTHELIAL CELL ANERGY IMPAIRING THE FORMATION OF MELANOMA METASTASIS

Valentina Brillo (1), Daniele Bonesso (1), Margherita Zamberlan (1), Diego de Stefani (1), Ildiko Szabo (1), Luca Scorrano (1), Giovanna Pontarin (1), Anna Rita Cantelmo (2), Luigi Leanza (1)

1) Department of Biology, University of Padova, Padova, Italy; 2) Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, Lille, France

Endothelial cells are pivotal in the formation and function of blood and lymph vessels, and dysregulation of their proliferation leads to pathological angiogenesis or vascular malfunctions. Tumor angiogenesis results in abnormal branching and vessel leakiness that led to an uneven distribution of anticancer drugs within the tumor and an inadequate elicitation of the immune response. At a cellular level, tumor endothelial cells become unresponsive to inflammatory stimuli, a phenomenon called "endothelial anergy", by the downregulation of specific endothelial adhesion molecules. This, together with the peculiar secretion of factors and the exhibition of specific receptors impedes the recruitment and extravasation of specific immune cell subtypes, establishing an immune-privileged microenvironment that promotes immune escape and facilitates tumor progression. In recent years, various strategies, such as anti-angiogenic therapy and vessel normalization, have been implemented to overcome endothelial anergy. The goal is to restore proper vascularization and establish an immune-activated microenvironment within the tumor. Achieving this objective involves the synergistic use of anti-angiogenic agents and immune checkpoint inhibitors. Therefore, in this work we studied the effects of anticancer drugs on endothelial cells signaling and metabolism, both in vitro and in vivo. We observed that specific anticancer drugs, such as Salinomycin and Nigericin, targeting K+ homeostasis, exerted at sublethal concentrations an impact on endothelial functions such as migration and tubule formation in vitro and in vivo. Hence, we elucidated a new mitophagy-induced NF-κB/JNK signaling axis, that in turn upregulated endothelial adhesion molecules expression upon the treatment, inducing vessel normalization and decreasing lung metastases formation in vivo in a mouse melanoma model. Together, these findings provide a better comprehension of the interplay between cancer cells, endothelium, and anticancer drugs, and ultimately could be innovative therapeutic strategies that account for their influence also on the tumor microenvironment, by establishing a connection between anticancer drugs and the reversal of endothelial anergy within a tumor context.

PARK7/DJ-1 DEFICIENCY IMPAIRS MICROGLIAL ACTIVATION IN RESPONSE TO LPS-INDUCED INFLAMMATION

F. Lind-Holm Mogensen (1,2), C. Sousa (2,3), Corrado Ameli (2), Katja Badanjak (2), Arnaud Muller (1,4) , Tony Kaoma (1), Djalil Coowar (2), Andrea Scafidi (1,2), Suresh K Poovathingal (2,5), Natalie Nicot (4), Aurélien Ginolhac (2), Daniela M Vogt Weisenhorn (6,7), Wolfgang Wurst (6,7,8,9,10), Aurélie Poli (1), Petr V Nazarov (1), Alexander Skupin (2,11), Anne Grünewald (2,12), Alessandro Michelucci (1)

1) Luxembourg Institute of Health, Luxembourg, Luxembourg; 2) University of Luxembourg, Esch-sur-Alzette, Luxembourg; 3) Current address: International Iberian Nanotechnology Laboratory, Braga, Portugal; 4) LuxGen Genome Center, LIH & Laboratoire National de Santé, Dudelange, Luxembourg 5) Single Cell Analytics & Microfluidics Core, Vlaams Instituut voor Biotechnologie-KU Leuven, Leuven, Belgium; 6) Helmholtz Zentrum München-German Research Center for Environmental Health, Institute of Developmental Genetics, Neuherberg, Germany; 7) Technische Universität München-Weihenstephan, Freising, Germany; 8) German Center for Neurodegenerative Diseases (DZNE), Munich, Germany; 9) Munich Cluster for Systems Neurology (SyNergy), Munich, Germany; 10) Deutsche Zentrum für Psychische Gesundheit (DZPG), Munich, Germany; 11) Department of Neuroscience, University of California San Diego, La Jolla, USA; 12) Institute of Neurogenetics, University of Lübeck, Lübeck, Germany

The development of Parkinson's disease (PD) involves oxidative stress and microglia activation. Genetic factors, including PARK7 mutations leading to DJ-1 deficiency, contribute to about 10% of PD cases. DJ-1 has diverse roles, notably in protecting against reactive oxygen species. However, the precise mechanisms through which DJ-1 deficiency leads to early-onset PD require further investigations. Hence, our study aims to investigate the role of DJ-1 deficiency in microglia. To achieve our goals, we conducted a comparative analysis of microglial cell phenotypic characteristics between PARK7/DJ-1 knock-out (KO) and wildtype (WT) littermate mice, both at baseline and following an intraperitoneal injection of lipopolysaccharide (LPS) for 6 and 24 hours. We combined single-cell and bulk RNA-sequencing with multicolour flow cytometry and immunofluorescence analyses to discern differential phenotypic acquisition.

Our findings reveal that microglia isolated from PARK7/DJ-1 KO mice exhibit a distinct phenotype, particularly evident in type II interferon and DNA damage response signalling, in response to LPS, when compared with wildtype mice. Furthermore, we detected discrete transcriptional signatures in human PARK7/DJ-1 mutant iPSC-derived microglia, highlighting the translational prospects of our findings. These transcriptional signatures were also reflected morphologically, with microglia from LPS-treated PARK7/DJ-1 KO mice displaying a less amoeboid cell shape compared with wildtype mice. These findings suggest that the underlying oxidative stress associated with the lack of PARK7/DJ-1 affects microglial activation states. As balanced immune responses are crucial for CNS homeostasis, compromised microglial responses under DJ-1 deficiency could contribute to PD development and progression.

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STATUS AND FUNCTIONS OF THE DEUBIQUITINATING ENZYME CYLD DURING PROGRAMMED CELL DEATH

Yanis Macé (1,2), Cara J. Ellison (3), Paul R. Elliott (3), Julie Gavard (1,2,4), Nicolas Bidère (1,2)

1) Team SOAP, CRCI2NA, Nantes Université, INSERM, CNRS, Université d'Angers, 44000 Nantes, France; 2) Équipe Labellisée Ligue Nationale Contre le Cancer, 75013 Paris, France; 3) Department of Biochemistry, University of Oxford, Oxford, OX1 3QU, United Kingdom; 4) Institut de Cancérologie de l'Ouest (ICO), 44800 Saint-Herblain, France

The deubiquitinating enzyme CYLD, a protease that hydrolyses ubiquitin chains from substrates (1, 2), plays a critical role in balancing cell survival, inflammation, and programmed cell death. For instance, CYLD participates in necroptosis, a lytic and inflammatory form of cell death. During apoptosis induced by death receptors, CYLD is cleaved and inactivated by caspase (CASP) 8 to prevent necroptosis and its associated inflammation (3). Additionally, CYLD restricts the activity of the NLRP3 inflammasome (4), a multiprotein complex that forms in response to cellular perturbations or microbes, leading to the activation of CASP1 and initiating a pro-inflammatory cell death called pyroptosis. However, the regulation and activity of CYLD during pyroptosis or in response to CASP8-independent apoptotic signals remain unclear.

Using CRISPR/Cas9 genetic knockout in the human monocytic THP-1 cell line, we first confirmed that CYLD limits cell death by pyroptosis. In contrast, CYLD knockout cells treated with BH3 mimetics to induce mitochondrial apoptosis, which partly relies on CASP3, display reduced cell death. Moreover, we observed that CYLD is differentially and directly cleaved by CASP1 and CASP3 in vitro and in cellulo at previously unreported sites. By combining molecular biology and biochemistry approaches, we identified that CASP3 cleaves CYLD at aspartic acid in position 364 and that this proteolysis is dispensable for its catalytic activity. Importantly, cells engineered by CRISPR/Cas9 to express a CASP3-resistant version of CYLD display delayed apoptotic cell death.

Collectively, these results suggest that CYLD cleavage by CASP3 finely tunes apoptosis and may participate in regulating the equilibrium between inflammation and programmed cell death.

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TYROSINE NITRATION ON AMYLOID β PEPTIDES INDUCES THE FORMATION OF TOXIC OLIGOMERS

Laurent Marichal (1), Daniel Bonhenry (2), Pablo Botella Lucena (1), Frances Evans (1), Joshua T. Berryman (2), and Michael Heneka (1)

1) Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Belvaux 4362, Luxembourg; 2) Department of Physics and Materials Science, University of Luxembourg, Luxembourg City L-1511, Luxembourg

One of the pathophysiological trademarks of Alzheimer's disease (AD) is the formation of amyloid β (Aβ) plaques in the brain extracellular matrix. Aβ plaques are made primarily of Aβ fibrils, that possess a distinctive cross-β structure. The existence of a causative link between the Aβ fibril accumulation and the onset of AD has long been thought to be the key to understand the fundamental mechanism behind AD (amyloid cascade hypothesis). A large body of evidence challenged this theory and another type of Aβ aggregates is now thought to play a crucial role in the AD pathology: Aβ oligomers. The more recent oligomer hypothesis (1) is based on the fact that Aβ oligomers tend to be more toxic than Aβ fibrils, at the cellular level.

Concomitantly, the AD onset is strongly correlated with chronic neuroinflammation. This inflammation state has many consequences such as inflammasome activation, phagocytosis, or even neuronal cell death. Another effect of neuroinflammation is the occurrence of nitro-oxidative stress in the brain. This stress can, in particular, induce the post-translational modification called nitration. Aβs that are parts of the amyloid plaques were shown to be partly nitrated on the tyrosine 10 moiety (2). Moreover, injection of nitrated Aβ into mice brains lead to plaque formation in vivo.

Recently we realized that Aβ nitration induces the formation of oligomers as opposed to fibrils for non-nitrated Aβs, in physiological conditions. The structure of these oligomers possesses subtle differences compared to typical Aβ oligomers (differences in secondary and quaternary structure). Thus, we are investigating the biological effect of these oligomers. In particular, we are combining cell toxicity assays on neurons and immune response assays on microglia. The goal of this study is to have a complete picture on potential specificities regarding these nitrated Aβ oligomers.

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INTERROGATING THE ROLE OF THE BCL-2 PROTEIN FAMILY IN ADVANCED PROSTATE CANCER

Laura Martínez-Escardó (1), Xiuhe Chong (1), Laura Galbraith (1), Jayanthi Anand (1), Leah Officer-Jones (1), Ian R Powley (1), Asma Ahmed (1,2), Stephen Tait (1,2), John Le Quesne (1,2), Imran Ahmad (1,2), Hing Leung (1,2), Karen Blyth (1,2) and Kirsteen Campbell (1,2)

1) CRUK Scotland Institute, Glasgow, G61 1BD, UK; 2) School of Cancer Sciences, University of Glasgow, Glasgow G61 1QH, UK

Prostate cancer, the second most common cancer in men worldwide, has a very good prognosis when detected at early stages, but is an incurable disease once it has spread from the prostate to other parts of the body. Unfortunately, 35% of men diagnosed with prostate cancer in Scotland already have metastatic disease and despite advances in their treatment, including hormone and chemotherapy, prostate cancer continues to rank among one of the leading causes of cancer death in Scotland, and worldwide. Elevated expression of pro-survival BCL-2 members (BCL-2, BCL-XL and MCL-1) has been shown in prostate cancer tissue and cell lines, where associations have been made with treatment resistance, and high expression levels of pro-survival BCL-2 family proteins are particularly prominent in advanced stages of the disease [1].

Here, we demonstrate, both genetically and pharmacologically with BH3 mimetic drugs, a co-dependency on BCL-XL and MCL-1 for metastatic prostate cancer cell survival. With this work, we are interrogating strategies to harness this dependency on pro-survival BCL-2 proteins using both in vitro and in vivo models of prostate cancer as a vulnerability that can be exploited in combination with the standard of care for prostate cancer patients to improve treatment responses. Furthermore, we are optimizing multiplex immunocytochemistry for a spatial mapping of BCL-2 family proteins in a prostate tumour tissue microarray (TMA) to better understand the interplay between different members of the BCL-2 family and their expression at different stages of the disease.

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MICROGLIA, ASTROCYTES AND PERIVASCULAR MACROPHAGES CONTRIBUTE TO THE PERI-Aβ PLAQUES NEURODEGENERATION IN ALZHEIMER'S DISEASE

Mónica Miranda de la Maza (1, 2, 3, 4), Sonja Fixemer (1, 2), Gaël Paul Hammer (2, 3), Félicia Jeannelle (2, 3), Sophie Schreiner (2, 3) , Jean-Jacques Gérardy (2, 3), Naguib Mechawar (5), Michel Mittelbronn (1, 2, 3, 4, 6, 7), David S. Bouvier (1, 2, 3)

1) Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Belval, Luxembourg; 2) Luxembourg Centre of Neuropathology (LCNP), Dudelange, Luxembour; 3) Laboratoire national de santé (LNS), National Center of Pathology (NCP), Dudelange, Luxembourg; 4) Department of Cancer Research (DOCR), Luxembourg Institute of Health (LIH), Luxembourg; 5) Douglas Mental Health University Institute, Department of Psychiatry, McGill University, Montreal, Quebec, Canada; 6) Department of Life Sciences and Medicine (DLSM), University of Luxembourg, Esch-sur-Alzette, Luxembourg; 7) Faculty of Science, Technology and Medicine (FSTM), University of Luxembourg, Esch-sur-Alzette, Luxembourg

Alzheimer's disease (AD) displays multifaceted neuropathological features, including β amyloid plaques (Aβ), neurofibrillary tangles (NFTs) and neuroimmunological alterations. The contribution of glial and immune cells to AD progression is still under debate. Microglia, the resident immune cells of the central nervous system (CNS), are highly responsive to pathological changes. A common observation in AD is the accumulation of microglia in a rosette-like conformation around Aβ plaques. These plaque-associated microglia (PaM) have been particularly studied, yet their role remains controversial, with evidence suggesting both protective and pathological contributions to AD progression. Astrocytes also play a crucial role in maintaining neuronal health and homeostasis. In the context of AD, reactive astrocytes contribute to the formation of a glial net around Aβ plaques. Peripheral immune cells (e.g. T-cells and perivascular macrophages) can infiltrate the parenchyma in neurodegenerative areas, but their impact remains poorly understood. To further understand the role of the glial-immune component in the amyloid pathology, we characterised the distribution of microglia, astrocytes and infiltrating immune cells in hippocampal subfields (Dentate Gyrus (DG), Cornu Ammonis (CA) 4, CA3, CA1)) and their relationship to Aβ plaques in human post-mortem samples from age-matched controls (CTL) and AD patients using multiplex chromogenic immunohistochemistry (IHC), confocal microscopy, digital pathology analysis and spatial profiling. Briefly, we measured the number of iba1+ and GFAP+ cells around Aβ plaques (labelled with 4G8) using chromogenic multiplex and found a positive correlation between the number of PaM and astrocytes with Aβ plaque area, across all subfields but a higher proportion of plaques bearing both PaM and astrocyte-nets in CA1 and CA4, 26% and 42% of them, respectively. We then investigated CD8+ T cells infiltration. In CTL hippocampus, CD8+ cells were mainly found in blood vessels (BV) with occasional presence in the brain parenchyma. In AD patients, we measured a slight, non-significant increase in number of cells in both BV and parenchyma. Our multiplex IHC showed that infiltrated CD8+ cells weren't associated with Aβ plaques vicinity. However, infiltrated CD163+ cells, representing the perivascular macrophages, were often associated with the core of Aβ plaques and co-distributed with PaM. Furthermore, our spatial profiling analysis with GeoMx nanostring further unveiled molecular characteristics of the peri-plaque neurodegenerative microenvironment, such as the high expression of the complement components C1q, C3 and C4d by PaM and astrocytes. We also observed a clear enrichment of phosphorylated mixed lineage kinase domain-like protein (pMLKL), part of the necrosome

complex, and severe tau pathology encapsulated by the glial-immune peri-plaque. Our research provides crucial insights into the complex interplay between glial cells, infiltrated immune cells, neuroinflammatory processes and their association with Aβ pathology in AD.

STUDYING ER-MITOCHONDRIAL CONTACT SITES IN FERROPTOSIS USING THE SPLITFAST PROBE

Teresa Mitchell-Garcia (1), Jasper Kuipers (1), Michela Rossini (2), Paola Pizzo (2), Riccardo Filadi (2), Arjan Kortholt (3), Amalia Dolga (1)

1) Department of Molecular Pharmacology, Faculty of Science and Engineering, Groningen Research Institute of Pharmacy (GRIP), University of Groningen, Groningen, The Netherlands; 2) Department of Biomedical Sciences, University of Padua and Institute of Neuroscience, National Research Council (CNR), Padua, Italy; 3) Department of Cell Biochemistry, Faculty of Science and Engineering, University of Groningen, Groningen, The Netherlands

Ferroptosis is a form of regulated cell death characterized by iron-dependent lipid peroxidation and imbalances in Ca2+ homeostasis. Ferroptosis cell death is one of the mechanisms associated with neurodegenerative diseases1, that are affecting millions of people worldwide. Interactions between the ER and mitochondria play an important role in ferroptosis facilitating processes including Ca2+ transfer. Dysfunction of these processes at ER-mitochondrial contact sites can support ferroptotic cell death, making these contact sites important to understand the underlying mechanisms of ferroptosis and neurodegenerative diseases. Recently, a split fluorescence-activation and absorption shifting tag (splitFAST) has been developed to characterize the dynamic and reversible ER-mitochondrial contact sites in live cells2. We aimed to optimize the transfection of the novel splitFAST system in the mouse hippocampal neuronal HT-22 cell line to visualize the ER-mitochondrial contact sites. Using ferroptotic inducer RSL3, we analyzed the effect of ferroptosis induction on ER-mitochondrial contact sites and we tested the effects of ferroptotic inhibitor, Ferrostatin-1 and mitochondrial calcium uptake inhibitor, MCU-i43. We successfully established the splitFAST system in HT22 cells and quantified the amount of the ER-mitochondrial contact sites in different ferroptotic conditions. Our data shows that Ferrostatin-1 or MCU-i4 elicit changes in the ER-mitochondrial contact sites, alongside protective effects on cell survival of ferroptotic HT22 cells. Overall, our data show that splitFAST system can be used to gain a deeper understanding of the interactions between the ER and mitochondria in conditions of cell death.

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DEFECTIVE CELL DEATH MECHANISMS AT THE ROOT OF ANGIOIMMUNOBLASTIC T CELL LYMPHOMA APPEARANCE

Barbara Chalhoub (1), Lucía Filotrani (1), Laia Rivas (1), Laura Mondragón (1)

1) T cell lymphoma group, Josep Carreras Leukaemia Research Institute (IJC), Badalona, Spain. e-mail: lmondragon@carrerasresearch.org

Angioimmunoblastic T-cell lymphoma (AITL) is a rare and aggressive subtype of peripheral T-cell lymphoma (PTCL), constituting 15-30% of PTCL cases and 1-2% of all non-Hodgkin lymphomas. Affecting primarily elderly individuals, AITL originates from the malignant transformation of T-follicular helper (Tfh) cells, crucial for germinal center B (GC B) cell survival. Malignant Tfh cells drive excessive proliferation of GC B cells, fostering an inflammatory tumor microenvironment1. Despite therapeutic efforts, AITL carries a poor prognosis with a five-year survival rate of about 32%. Recent identification of mutations in epigenetic regulators associated with AITL has prompted the development of animal models aimed at mimicking the disease; however, existing models often fail to fully replicate the clinical and pathological features of human AITL, particularly its late onset.

In this regard, the host lab contributed to the characterization of a new preclinical mouse model for AITL: the plck-GAPDH. This model overexpresses the glycolytic protein GAPDH in the T cell lineage. Considering, GAPDH overexpression also plays a cytoprotective role in caspase-independent cell death (CICD), we hypothesize that modulation of other proteins implicated in cell death would lead to the identification of new preclinical models for AITL2. To prove our hypothesis, we bred and aged the mouse model Apaf-1+/-, which presents a reduced expression in the pro-apoptotic protein apoptotic protease activating factor 1 (Apaf-1). Apaf-1 is implicated in cell death induction after mitochondria outer membrane permeabilization. It is a key regulator of the mitochondrial apoptotic cell death pathway, being the central element of the multimeric complex called apoptosome, also formed by procaspase-9 and cytochrome c. Apoptosome is assembled just after cytochrome c mitochondrial release leading to the final activation of caspases, a group of proteases responsible for the final dismantle of the cell in the final stages of apoptosis3.

Despite presenting no detectable phenotype at early stages of life, aged Apaf-1+/- mice replicate all symptoms of human AITL, such as hepatomegaly, splenomegaly, and enlarged lymph nodes, with a disease onset around 18 months. Besides, it displays key immunophenotypic characteristics of AITL, including increased Tfh cells, an activated memory phenotype and increased GC B cells, among others. Finally, this model also shows an unusual increase in TCR-γδ Tfh cells, which could serve as an early biomarker for the disease, potentially improving early diagnosis and patient outcomes.

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ASSESSMENT OF PROGRAMMED CELL DEATH PROTEINS IN ORAL SQUAMOUS CELL CARCINOMA

Ndukaife Augustine Chidera

Oral Pathology Department, College of Medicine, University of Ibadan, Nigeria

Oral squamous cell carcinoma (OSCC) is a significant health concern in Nigeria and although the prevalence is relatively low compared to other populations, late patient presentation, yet to be clearly defined etiology and inadequate facilities for management result in high mortality rates. Chronic inflammation, which borders on immunological concepts of cancer biology have been proposed as contributory. Seeing that immunology is a 'double-edged sword' that can be manipulated for therapy, it is needful to explore this model in OSCC found in Nigerian patients. We aim to investigate the expression of and relationship between PD-1, PD-L1 and PD-L2 in OSCC. This is important because there are now immunotherapies that target the cell programmed death pathway. Twenty FFPE blocks of OSCC were prepared for immunohistochemistry to Abcam Mouse monoclonal Anti-PD1 antibody, Rabbit monoclonal Anti-PD-L1 antibody and Rabbit Polyclonal Anti-PD-L2. Cytoplasmic/membrane staining was taken as positive for the antibodies. The Sinicrope scoring method was used to evaluate staining intensity and proportion. We found that tumor associated macrophages and neoplastic cells expressed PD1, PD-L1 and PD-L2 in differing proportions, but most of the cases were negative for these antibodies. Our results have shown that immunotherapy may be relevant when considering the management of OSCC patients in Nigeria.

miRNA PROFILE IN HUMAN PANCREATIC β-CELLS DURING FATTY ACID-INDUCED AND REGULATED APOPTOSIS

Němcová V (1), Bouškova V (2), Šrámek J (1), Kovář J (1), Souček P (2)

1) Charles University – Third Faculty of Medicine, Department of Biochemistry, Cell and Molecular Biology & Center for Research of Diabetes, Metabolism and Nutrition, Prague, Czech Republic; 2) The National Institute of Public Health, Toxicogenomics Unit, Prague, Czech Republic

Elevation in circulating fatty acids (FAs) contributes to the gradual worsening of β-cell function that eventually culminates in β-cell death. Research indicates that the harmful effects of FAs depend on their saturation levels. Saturated FAs like stearic acid (SA) exert a toxic effect on pancreatic β-cells. In contrast, unsaturated FAs such as oleic acid (OA) are less toxic and can even mitigate the cell death induced by saturated FAs. Molecular mechanisms of apoptosis induction and its regulation by FAs in β-cells are not fully elucidated. However, it was shown that specific microRNAs (miRNAs) may be involved at least in the first process. This study aimed to expand upon previous findings to identify other miRNAs possibly involved in molecular mechanism of saturated SA-induced apoptosis in the human pancreatic β-cell line NES2Y. In addition, we also wanted to identify miRNAs possibly involved in mechanisms of the regulation of this process by unsaturated OA.
In experiments, a defined serum-free medium supplemented with 1 mM SA, a combination of SA with 0.2 mM OA, or 0.2 mM OA alone bound to a 2% FA-free bovine serum albumin was used. miRNAs transcriptome analysis was processed with SurePrint G3 Human miRNA Microarrays. The expression data were extracted from the scanned image using Feature Extraction software (Agilent). Differences in the expression of miRNAs between the treated (SA, OA, SA+OA) and control (CTRL, SA) groups were analyzed using Genespring GX program (Agilent Technologies).
Among three sets of samples 337 miRNAs were identified as expressed over a specific detection limit. Principal component analysis of these miRNAs as well as cluster analysis showed that OA treatment clustered together with controls while SA treatment clustered closely with treatment using a combination of SA and OA. 99 miRNAs were significantly differentially expressed (fold change ≥ 2) between four sample groups. Comparisons of interest were: (1) SA-treated cells vs. control cells, (2) OA-treated cells vs. control cells, (3) cells treated with a combination of SA and OA vs. control cells, and (4) cells treated with a combination of SA and OA vs. SA-treated cells. Among 99 miRNAs differentially expressed only 8 miRNAs, i. e. hsa-miR-6126, hsa-miR-4763-3p, hsa-miR-3656, hsa-miR-3960, hsa-miR-4739, hsa-miR-6724-5p, hsa-miR-3665 and hsa-miR-24-1-5p, may be possibly involved in mechanisms of SA-induced apoptosis of pancreatic β-cells; and 7 miRNAs, i.e. hsa-miR-4651, hsa-miR-6789-5p, hsa-miR-629-3p, hsa-miR-150-3p, hsa-miR-8072, hsa-miR-6727-5p and hsa-miR-6850-5p may be involved in mechanisms of inhibition of SA-induced apoptosis of pancreatic β-cells by unsaturated OA.
To conclude, 15 miRNAs were identified to be possibly involved in mechanisms of apoptosis induction and its regulation by FAs in β-cells

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IMPACT OF UV-INDUCED EXPRESSION OF MMP1 AND COX-2 ON TUMOR PROGRESSION IN TUMOR SPHEROIDS OF MALIGNANT MELANOMA

Linda Vainikka, Linda Rößler, Fahima Akter, Karin Öllinger

Division of Cell and Neurobiology, Department of Biomedical and Clinical Sciences, Linköping University, Linköping Sweden

Malignant melanoma is the deadliest form of skin cancer, and UV radiation is a well-established risk factor for melanoma initiation. UVB radiation induces direct DNA damage, while UVA causes indirect damage by production of reactive oxygen species. The precise role of UV in cancer progression and metastasis remains, however, a subject of ongoing investigation and is the focus on this study. The impact of UV irradiation on tumor growth and metastatic potential was analyzed by exposing malignant melanoma tumor spheroids to sublethal doses of UVA and UVB for three consecutive days, followed by embedding in Matrigel and microscopic evaluation to follow the metastatic spread. Both UVA and UVB promote tumor growth into the Matrigel, and interestingly UVA appear to have a higher stimulatory effect than UVB. A microarray analysis of UV radiated tumor spheroids revealed higher number of genes upregulated after UVA exposure than UVB, and identified several candidate genes, including COX-2 (cyclooxygenase-2) and MMP1 (matrix metalloprotease-1) to promote UVA-induced signaling. UVA stimulated MMP1 secretion to the extracellular space, and MMP1 inhibition reduced tumor spheroid growth. Interestingly, inhibition of COX-2 potentiated UVA-induced protein expression of MMP1 and promoted tumor spreading into the Matrigel. These findings indicate activation of a reaction axis between COX-2-MMP1 upon UV-irradiation and support that UVA radiation might stimulate the progression of malignant melanoma in the skin.

UNDERSTANDING THE SPRED/ RAF INTERACTION AND ITS IMPACT ON RAS INACTIVATION

Göksu Oral (1), Elisabeth Schaffner-Reckinger (1), Matthew J Smith (2,3), Daniel Kwaku Abankwa (1,*)

1) Cancer Cell Biology and Drug Discovery Group, Department of Life Sciences and Medicine, University of Luxembourg, 4365 Esch-sur-Alzette, Luxembourg; 2) Institute for Research in Immunology and Cancer, Université de Montréal, Montréal, Québec, Canada; 3) Department of Pathology and Cell Biology, Faculty of Medicine, Université de Montréal, Montréal, Québec, Canada; * Correspondence: daniel.abankwa@uni.lu

Mutational activation of RAS proto-oncogenes frequently induces hyperactivation of the MAPK pathway, which plays a major role in RAS-dependent tumorigenesis and rare diseases referred to as RASopathies (1). Within the MAPK pathway, three RAF paralogs ARAF, BRAF and CRAF act as Ras effector proteins and share a similar domain structure with an N-terminal regulatory and a C-terminal kinase domain. Numerous signals and interactions tightly regulate the MAPK pathway. Negative regulation involves SPRED, whose C-terminal Sprouty domain is required for its plasma membrane localization, and the Ras GTPase-activating protein NF1, which is recruited to the plasma membrane by SPRED. This recruitment relies on the interaction between the SPRED EVH1 domain and the NF1 GRD domain (1).
We previously showed that SPRED1 recruitment to the plasma membrane involves BRAF (2). Additionally, NF1 expression increases during cell differentiation and its overexpression enhances and downregulation inhibits differentiation, respectively (unpublished data). While BRAF mutations frequently lead to a constitutively active, oncogenic protein, SPRED and NF1 mutations impair their tumor suppressor function (1). These alterations disrupt the proper regulation of RAS signaling, leading to uncontrolled cell proliferation and impaired differentiation. Based on these findings, we propose to explore the structural and functional details of SPRED/RAF interaction.
We employed Bioluminescence Resonance Energy Transfer (BRET) to quantitatively assess the interaction between different RAF and SPRED paralogs. Our initial findings reveal distinct binding activities between different paralog pairs suggesting that small variations in domain structures can influence their interaction. In future studies, we will identify the interacting domains, evaluate their binding affinity using isothermal titration calorimetry (ITC), map them with protein NMR spectroscopy and determine their structures by crystallography. Additionally, we will investigate how cancer- or RASopathy-associated mutations at the SPRED/RAF interface affect their interaction using BRET. The effect of these mutations on protein localization will be visualized by confocal microscopy and their impact on differentiation will be analyzed using our well-established flow cytometry-based differentiation assay in C2C12 cells (3). Ultimately, this combined approach will contribute to understanding the mechanism and spatio-temporal regulation of Ras inactivation during differentiation.

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TUMOR SUPPRESSOR PROTEIN PROSTATE APOPTOSIS RESPONSE-4 (PAR-4) REGULATES OXEIPTOSIS MEDIATED CELL DEATH

Siraj Pallichankandy (1), Faisal Thayyullathil (1), Anees Rahman Cheratta (1), Karthikeyan Subburayan (1), Ameer Alakkal (1), Asma Albadi Alnuaimi (1), Vivek M Rangnekar (2), Sehamuddin Galadari (1)

1) Cell Death Signaling Laboratory, Biology Program, Division of Science, Experimental Research Building, New York University Abu Dhabi, PO Box 129188, Saadiyat Island, Abu Dhabi, United Arab Emirates. 2Department of Radiation Medicine and Markey Cancer Center, University of Kentucky, Lexington, KY 40536, USA.

Prostate apoptosis response-4 (Par-4) is a tumor suppressor protein with a unique ability to selectively induce cancer cell death without affecting normal cells. This study investigates the role of Par-4 in mediating reactive oxygen species (ROS) generation and oxeiptosis, a non-apoptotic form of cell death driven by oxidative stress, specifically H_2O_2 Using a combination of molecular biology techniques, ROS assays, and viability assays, we explored how Par-4 regulates ROS levels and execute oxeiptosis in cancer cells. Our results demonstrate that Par-4 activation leads to a significant increase in intracellular ROS generation and cell death. Both knockdown and knockout of Par-4 significantly inhibited intracellular ROS generation and oxeiptosis, characterized by loss of viability and AIFM1 dephosphorylation at Ser116, a key event during the oxeiptosis induction. Furthermore, the role of Par-4 in oxeiptosis was confirmed by re-introducing wild-type Par-4 into CRISPR knockout Par-4 cells. The results showed that reintroduced Par-4 significantly induces oxeiptosis compared to CRISPR-Par-4 knockout cells. These findings elucidate the critical function of Par-4 in promoting oxidative stress-induced cell death and suggest its potential as a therapeutic target for selectively eliminating cancer cells. This study advances our understanding of Par-4-dependent ROS signaling and its involvement in oxeiptosis, highlighting its significance in the development of novel cancer therapies.

RIPK1 PRESERVES THE SUPPRESSIVE TRANSCRIPTIONAL PROFILE OF REGULATORY T CELLS PREVENTING THE RISE OF SPONTANEOUS AUTOIMMUNITY

Carlos Plaza-Sirvent (1), Hannah Sturm (2), Maximilian K. Nocke (2,3), Fatemeh Ghorbani (1), Clara Bessen (1), Marina C. Greweling-Pilsc (4), Stefan Floess (5), Jana Niemz (5), Jochen Huehn (5), Robert Geffers (6), Daniel Todt (2,3), Peter Vandenabeele (7,8) and Ingo Schmitz (1)

1) Department of Molecular Immunology, Ruhr University Bochum, Bochum, Germany, 44801; 2) Department of Molecular and Medical Virology, Ruhr-University Bochum, Bochum, Germany, 44801; 3) European Virus Bioinformatics Center (EVBC), Jena, Germany, 07743; 4) Mouse Pathology Platform, Helmholtz Centre for Infection Research, Braunschweig, Germany, 44869; 5) Experimental Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany, 44896; 6) Genome Analytics, Helmholtz Centre for Infection Research, Braunschweig, Germany, 44869; 7) Cell death and Inflammation Unit, VIB-UGent Center for Inflammation Research, Ghent, Belgium, 9052; 8) Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium, 9000

Regulatory T (Treg) cells are essential to maintain immune homeostasis. Thanks to their suppressive ability, Treg cells preserve self-tolerance in the organism avoiding autoimmune disorders. Receptor-interacting protein kinase 1 (Ripk1) is a key regulator of cell death and inflammation. Recruited by death receptors, Ripk1 participates in the initiation of two interconnected cell death types, namely apoptosis or necroptosis. In addition, Ripk1 regulates inflammatory-related signaling pathways via the NF-κB pathway, which is important for the development, survival and function of many cell types of the immune system. Several studies have shown that Ripk1 is implicated in the maintenance of the T cell and Treg cell homeostasis (1-3). However, it is not clear whether the reduction of these cell populations is a direct consequence of the Ripk1 absence or it us caused by inflammatory cues presumably produced by a defect in the suppressive capacity of the Treg cells. In this work, we took advantage of the lox-cre system to generate chimeric mice that contain Ripk1-proficient and Ripk1-deficient Treg cells, allowing the study of these cells in the absence of inflammation.

In agreement with previous studies, our work show that Treg-specific ablation of Ripk1 leads to a reduction of Treg cells and spontaneous systemic autoimmunity in conditional knock out mice. Interestingly, using chimeric mice that allowed us to study Treg cells in the absence of inflammatory conditions, we observed that key Treg molecules, such as CTLA-4, TIGIT, KLRG1, Gpr15 and CD103 were downregulated. Importantly, although the Ripk1-deficient population displayed a clear homeostatic disadvantage, we did not observe impaired viability of the Ripk1-deficient Treg cells. Furthermore, bulk and single-cell RNA sequencing revealed that Ripk1 is required for the maintenance of the Treg cell transcriptional signature, which is essential for the suppressive function of Treg cells. In conclusion, our findings highlight the fundamental role of Ripk1 in maintaining immune homeostasis by preserving the suppressive phenotype of Treg cells.

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UNCONVENTIONAL AUTOPHAGY AS A NEW AND BROAD ANTI-INFLAMMATORY MECHANISM IN THE SKIN

Dario Priem (1,2), Jon Huyghe (1,2), Barbara Gilbert (1,2), Esther Hoste (1,2), Jonathan Maelfait (1,2), Geert van Loo (1,2) and Mathieu JM Bertrand (1,2)

1) VIB Center for Inflammation Research, 9052 Ghent, Belgium; 2) Department of Biomedical Molecular Biology, Ghent University, 9052 Ghent, Belgium.

Autophagy is a cellular degradation mechanism that ensures nutrient availability, controls protein turnover and promotes the clearance of damaged organelles. Defective autophagy has been linked to various inflammatory diseases. Previous studies have reported on the existence of an LC3-independent unconventional selective autophagy pathway that is mechanistically different from classical autophagy, however, its (patho)physiological importance has remained unclear. Recently, we have demonstrated that the unconventional autophagic pathway is crucial to counteract TNF-mediated cytotoxicity (1). We showed that blocking unconventional autophagy in mice (by deleting the core autophagy protein ATG9A) leads to early embryonic lethality, which is completely driven by excessive TNF-mediated death. In order to study the pathophysiological functions of this process in adult mice, we specifically blocked unconventional autophagy in mouse keratinocytes. We found that the skin-specific deletion of ATG9A leads to the spontaneous development of a severe inflammatory skin disease, which was caused by aberrant TNF-mediated death. Remarkably, we found that unconventional autophagy also counteracts type I IFN production in the skin. We will present data demonstrating that unconventional autophagy prevents nucleic acid release, limits nucleic acid sensing and inhibits nucleic acid-driven ZBP1-mediated keratinocyte cell death. In summary, we show that unconventional autophagy emerges as a new and broad anti-inflammatory mechanism that is crucial to prevent skin pathology.

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THE RIPK1 INHIBITOR PRIMIDONE REDUCES PSORIASIS-LIKE SKIN INFLAMMATION

Theresa Riebeling (1), Benedikt Kolbrink (1), Yao Zhang (1), Rebecca Philippsen (2), Eileen Dahlke (3), Franziska Theilig (3), Agatha Schwarz (2), Ulrich Kunzendorf (1), Roland Schmitt (1), and Stefan Krautwald (1)

1) Department of Nephrology and Hypertension, University Hospital Schleswig-Holstein, 24105 Kiel, Germany; 2) Department of Dermatology and Allergology, University Hospital Schleswig-Holstein, 24105 Kiel, Germany; 3) Institute of Anatomy, Christian-Albrechts-University Kiel, 24118 Kiel, Germany

Blocking the signaling mediated by receptor-interacting protein kinase (RIPK)1 represents a promising therapeutic target for a plethora of inflammatory and autoimmune diseases. Despite the clinical testing of newly developed RIPK1 inhibitors in various pathologies, none of these agents have yet been approved for clinical use [1]. In a recent study, we identified for the first time the FDA-approved drug primidone, in addition to its conventional use as an anticonvulsant, as a selective inhibitor of the kinase activity of RIPK1 [2].

Psoriasis is one of the most common inflammatory skin diseases and is characterized, among others, by dysregulated systemic inflammation. Therefore, in the following study, we investigated the potentially protective effect of primidone in this clinically highly relevant disease. We induced a psoriasis-like skin inflammation in mice through the topical application of the TLR7 agonist imiquimod [3]. To assess the general efficacy of primidone in this disease model, we added the drug to the drinking water before the initiation of imiquimod application. In another cohort, primidone was administered therapeutically through the drinking water at the onset of the first symptoms, which more closely resembles the clinical setting.

In both, the preventive and therapeutic scenarios, we found that mice receiving primidone in the psoriasis disease model exhibited milder symptoms, with less redness, thickening, and scaling of the skin, lower PASI scores, and less pronounced splenomegaly. These findings demonstrate the drug's effectiveness in mitigating the disease course.

Our results underscore once more the therapeutic potential of RIPK1 inhibition in inflammatory diseases. The significant benefit observed with its therapeutic application suggests that primidone is a promising candidate to improve psoriasis treatment. Therefore, its repurposing should be tested in a clinical setting in humans.

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IMPACT OF TRANSGLUTAMINASE TYPE 2 (TG2) ON CANCER CELL METABOLISM

Ruzza A. (1), Vianello F. (1), Zaltron E. (1), Brillo V. (1), Scavezzon M. (1), Celotti I. (1), Palazzo A. (1), Severin F. (1), Leanza L. (1)

1) Department of Biology, University of Padova, Padova, Italy

Transglutaminase type 2 (TG2) is the most ubiquitously expressed and well characterized member of the transglutaminase family. It is a multifunctional enzyme implicated in the regulation of several cellular processes, including cell growth and differentiation, cell death, autophagy, inflammation, ECM assembly and remodeling. Due to its multiple localizations both inside and outside the cell, TG2 participates in the regulation of many crucial intracellular signaling cascades in a tissue- and cell-specific manner, making this enzyme an important player in disease development and progression. However, TG2's role in cancer is still controversial and highly debated; it has been described both as an oncogenic and tumor-suppressive factor and is linked to all the processes of tumorigenesis. On this scenario, we are characterizing the role of TG2 in tuning intracellular metabolism, by impacting on mitochondrial function. The obtained results in two different cancer types, breast cancer and melanoma, demonstrated that TG2 ablation can impair respiratory chain complexes expression and activity, thus impacting on mitochondrial respiration. These data will suggest a role for TG2 as a possible target to regulate cancer cells metabolism, unveiling novel vulnerabilities of therapeutic interest.

CELL DEATH MECHANISMS INDUCED BY CLYTA-DAAO CHIMERIC ENZYME IN HUMAN CANCER CELL LINES

Fuentes-Baile M (1), Pérez-Valenciano E (1), García-Morales P (1), de Juan Romero C (2), Lloret E (2), Berna JM (2), Saceda M (1,2)

1) *Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universidad Miguel Hernández, Avda. Universidad s/n, Ed. Torregaitán, 03202 Elche (Alicante), Spain; 2) Unidad de Investigación, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO), Hospital General Universitario de Elche, Camí de l'Almazara, 11, 03203 Elche (Alicante), Spain*

Suicide enzyme therapy consists of targeting an enzyme to the tumor and then administering systemically a harmless prodrug that must be a substrate for the enzyme, which will become an active anticancer drug after reacting with it (1). In this work, treatment efficacy with the enzyme D-amino acid oxidase (DAAO) from Rhodotorula gracilis together with D-Alanine against pancreatic carcinoma, colorectal carcinoma and glioblastoma cell lines has been evaluated. DAAO catalyzes the oxidation of D-amino acids, converting them into alpha-ketoacids and ammonia, and generating H2O2, which induces oxidative stress (2). Specifically, a chimera has been used, which presents DAAO bound to the choline-binding domain of the N-acetylmuramoyl-L-alanine amidase (CLytA) from Streptococcus pneumoniae, allowing the enzyme immobilization on supports containing choline or derivatives, such as diethylaminoethanol (DEAE) (3).

Our results demonstrate that the combination of CLytA-DAAO and D-Alanine induces cell death in several pancreatic and colorectal carcinoma and glioblastoma cell lines (4). Analysis of the cell death mechanisms showed that treatment with CLytA-DAAO and D-Ala triggers different types of cell death (5).

In glioblastoma cell lines, CLytA-DAAO-induced cell death was inhibited by a pan-caspase inhibitor, suggesting a classical apoptotic cell death. Meanwhile, the cell death induced in pancreatic and colon carcinoma cell lines is a type of programmed necrosis, but the mechanisms that trigger that cell death are context dependent. We acquire a further insight into the necrotic cell death induced in pancreatic and colorectal carcinoma cell lines. We analyzed the intracellular calcium mobilization, mitochondrial membrane potential, PARP-1 participation and AIF translocation. In the RWP-1 exocrine pancreatic carcinoma cell line, the induced cell death is parthanatos. Death in IMIM-PC-2 is independent of PARP-1 and intracellular calcium mobilization, and in the case of colorectal cancer cell lines, SW-480 and SW-620, death is PARP-1 and calcium mobilization dependent but independent of AIF. Overall, our results provide a better understanding of the cell death mechanism induced by CLytA-DAAO, a promising therapy against cancer.

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ACSL3 IS AN UNFAVORABLE PROGNOSTIC MARKER IN CHOLANGIOCARCINOMA PATIENTS AND CONFERS FERROPTOSIS RESISTANCE IN CHOLANGIOCARCINOMA CELLS

Apiwit Sae-Fung (1,2), Nawaporn Vinayavekhin (3), Bengt Fadeel (2), and Siriporn Jitkaew (4,5,*)

1) Graduate Program in Clinical Biochemistry and Molecular Medicine, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand; 2) Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 3) Center of Excellence in Natural Products Chemistry, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand; 4) Center of Excellence for Cancer and Inflammation, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand; 5) Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand.

Cholangiocarcinoma (CCA) constitutes a heterogenous group of malignancies arising from the bile duct. In a previous comprehensive analysis of ferroptosis-related genes, we found that ferroptosis-related gene expression could be applied for the stratification of CCA patients into low-risk and high-risk groups based on survival time. Here, we explored the underlying mechanism and role of ferroptosis in CCA. Based on the mRNA expression profile and clinical information of CCA patients retrieved from public databases, we identified acyl-CoA synthetase long chain family member 3 (ACSL3) as a potential ferroptosis suppressor in high-risk CCA patients. Using a panel of cell lines, we confirmed that ACSL3 expression was upregulated in cell lines corresponding to high-risk CCA, and this was, in turn, correlated with resistance to the ferroptosis-inducing agent, RSL3. Mass spectrometry-based lipidomic analysis demonstrated that the ferroptosis-resistant CCA cell lines expressed higher levels of monounsaturated fatty acid (MUFA)-containing phospholipids when compared to ferroptosis-sensitive cell lines. Silencing of ACSL3 by shRNA technology sensitized the ferroptosis-resistant CCA cell lines to RSL3. Moreover, the suppression of ferroptosis was found to be dependent on exogenous MUFAs. We also found that the resistance to RSL3 was enhanced upon inhibition of lipid droplet biogenesis. These findings highlight ACSL3 as a promising target for therapeutic strategies aimed at overcoming ferroptosis resistance in patients with CCA.

NON-CANONICAL NECROPTOTIC-TO-APOPTOTIC SIGNALING IS A REFRACTORY FEATURE OF INFLAMMATORY BOWEL DISEASE

Jiyi Pang# (1,2), Aysha Al-Ani# (1,2,3), Komal Patel (1,2), Isabella Kong (4), Samuel Young (1,2), Siqi Chen (1,2), Marilou Barrios (1,2), Imadh Azeez (1,2), Siavash Foroughi (1,2), James Rickard (3), Wayne Cawthorne (1,2), Sebastian Hughes (1,2), Lisa Giulino-Roth (4), Rory Bowden (1,2), Andre Samson* (1,2), Britt Christensen* (1,2,3), James Vince* (1,2), James Murphy* (1,2,5).

*1) Walter and Eliza Hall Institute of Medical Research, Parkville, Australia; 2) University of Melbourne, Parkville, Australia; 3) Royal Melbourne Hospital, Parkville, Australia; 4) Pediatric Hematology/Oncology, Weill Cornell Medical College, New York, USA; 5) Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Australia; *these authors contributed equally; #these authors jointly supervised the work*

Inflammatory bowel disease (IBD) is a major global health challenge. IBD is characterized by areas of gastrointestinal inflammation due to imbalanced cytokine signaling, immunity, microbial sensing and gut barrier function. Patients with IBD are placed on therapies that correct these imbalances to achieve remission. However, in most cases, patients will eventually relapse or become refractory to therapy. Thus, IBD remains a chronic condition where the drivers of disease persist during remission and in the face of therapy. Identifying these underlying drivers of IBD is of immense interest.

Programmed cell death is inextricably linked to inflammation, although which subroutines of cell death occur in IBD remains unclear. To address this, we have defined the prevalence and mechanisms of intestinal cell death in adults with IBD (n=32), relative to individuals without IBD (n=28). Most of the patients recruited to this study were on maintenance therapy, and most were in remission or had mild disease. To gain insight into the chronology of IBD, we collected biopsies from non-inflamed, marginally inflamed and fully inflamed intestinal tissues from each patient. The accuracy of biopsy collection was confirmed by blinded histopathology scoring, then biopsies were analyzed in parallel by 1) immunoblotting, 2) RNA sequencing, 3) microbial sequencing, and 4) functional epithelial organoid assays.

Our data suggest that necroptotic signaling arises during nascent inflammation, which then transitions to apoptotic signaling during the later stages of inflammation in IBD. We find that necroptotic signaling does not correlate with RIPK1 activation, which may explain why RIPK1 inhibitors were found to been ineffective in IBD patients. Rather, an alternative interferon-inducible form of necroptosis prevails in IBD. Similarly, interferon-induced gene upregulations are critical for two distinct mechanisms of mitochondrial apoptosis in IBD – one that promotes intestinal epithelial stem cell death and one that governs the death of absorptive enterocytes.

Collectively, we have uncovered three inflammation-induced forms of cell death that arise in IBD. As these mechanisms persist in patients with well-controlled IBD and in patients on various classes of treatment (including anti-TNF therapy), we propose that they are a new IBD-associated pathway that could be targeted to achieve deeper remission.

AN IMPROVED PDE6D INHIBITOR COMBINES WITH SILDENAFIL TO SYNERGISTICALLY PROMOTE KRAS-MUTANT CANCER CELL DEATH

Pelin Kaya (1), Elisabeth Schaffner-Reckinger (1), Ganesh babu Manoharan (1), Vladimir Vukic (2), Alexandros Kiriazis (3,#), Mirko Ledda (4), Maria Burgos (1), Karolina Pavic (1), Anthoula Gaigneaux (5), Enrico Glaab (4), Daniel Kwaku Abankwa (1,3,*)

*1) Cancer Cell Biology and Drug Discovery Group, Department of Life Sciences and Medicine, University of Luxembourg, 4365 Esch-sur-Alzette, Luxembourg; 2) University of Novi Sad, Faculty of Technology, 21000 Novi Sad, Serbia; 3) Turku Bioscience Centre, University of Turku and Åbo Akademi University, 20520 Turku, Finland; 4) Luxembourg Center for Systems Biomedicine, University of Luxembourg, 4365 Esch-sur-Alzette, Luxembourg; 5) Bioinformatics Core, Department of Life Sciences and Medicine, University of Luxembourg, 4365 Esch-sur-Alzette, Luxembourg; # current address: Orion Corporation, Orion Pharma, Espoo, Finland; * Correspondence: daniel.abankwa@uni.lu*

Significant progress has been made in the past decade in directly targeting oncogenic Ras; nevertheless, the need for alternative approaches remains of utmost importance. PDE6D (or PDEδ) is a trafficking chaperone for prenylated proteins. By binding the farnesyl-moiety of K-Ras, PDE6D promotes K-Ras diffusion in the cytoplasm and helps maintain K-Ras plasma membrane localization and activity. This has led to the nomination of PDE6D as a surrogate target for K-Ras and to the development of several high-affinity PDE6D inhibitors (PDE6Di) that block its prenyl-binding pocket (1). However, their cellular efficacy remained below expectation, preventing their clinical development.

In the past, our group has developed PDE6Di with a hexamethylene-amide backbone, which had low micromolar affinities for PDE6D (2). Here, an in silico library of compounds was established by cross-hybridizing moieties of previously established high-affinity PDE6Di with our hexamethylene-amide backbone. Following computational docking experiments, 16 novel PDE6Di were synthesized and characterized. Among them, the best PDE6Di was identified and named Deltaflexin3 (3).

Deltaflexin3 is a highly soluble, low nanomolar PDE6Di, which has the lowest off-target activity as compared to three prominent reference compounds (1). Deltaflexin3 reduces K-Ras-signaling and selectively decreases the growth of KRAS-mutant and PDE6D-dependent cancer cells. We further exploit that PKG2-mediated phosphorylation of the K-Ras residue Ser181 lowers K-Ras binding to PDE6D. Thus, Deltaflexin3 combines with the clinically approved PKG2-activator Sildenafil to synergistically inhibit PDE6D/ K-Ras binding, cancer cell viability, K-Ras signaling and microtumor growth. As observed previously, inhibition of K-Ras-dependent signaling and cancer cell viability remained overall modest. Our results suggest reevaluating PDE6D as a K-Ras surrogate target in cancer.

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INVESTIGATING MOLECULAR DIFFERENCES IN THE HIPPOCAMPUS IN ALZHEIMER'S DISEASE

S. Schreiner (1,2), F. Jeannelle (1,2), M. Miranda De la Maza (2,4), M. Mittelbronn (1,2,3,4), D. Bouvier (1,2,3)

1) Laboratoire National de Santé (LNS), National Center of Pathology (NCP), Dudelange; 2) Luxembourg Center of Neuropathology (LCNP), Dudelange; 3) Luxembourg Center of Systems Biomedicine (LCSB), University of Luxembourg (UL), Belval; 4) Department of Cancer Research (DoCR), Luxembourg Institute of Health (LIH), Strassen

Age-related neurodegenerative disorders like Alzheimer's disease (AD) cause progressive neuronal death, leading to significant cognitive and motor impairments in affected patients1. In AD, pyramidal neurons in the hippocampal CA1/CA2 (cornu Ammonis) areas are particularly vulnerable2, and their loss is linked to memory decline3. Beside the prominent Tau pathology in this region4, neuroinflammation contributes to neurodegeneration in AD5. Investigating the relationship between Tau pathology and the complement immune system can provide deeper insights into the molecular processes underlying CA1/CA2 neurons vulnerability and may open up new therapeutic strategies. To characterize how the severity and the distribution of the tau pathology affects the expression of complement immune system components, we used single and multiplex chromogenic immunohistochemistry (IHC) with quantitative digital pathology, as well as immunofluorescence combined with 3D confocal imaging on a collection of human post-mortem hippocampal tissue from age-matched control (CTL) and AD patients (AD). To distinguish the stages of the neurofibrillary tangle maturation, we used AT8 (Ser202 and Ser205) and ps396 (phospho-Tau Ser396) antibodies and quantified C1q staining, a prominent complement component, in AD in the hippocampal subfields dentate gyrus (DG), CA4, CA3 and CA1/CA2. As expected, we found a global 58-fold increase in AT8 and a 13-fold increase in ps396 relative surface coverage in AD compared to CTL, with ps396 staining being 4-fold less widespread than AT8 in AD. Specifically, in AD patients, the CA1/CA2 region was the most affected by Tau pathology, with between 5-fold and 20-fold increase compared to respectively DG, CA4 and CA3. Multiplex IHC for AT8 and ps396 revealed neurons that were positive for either AT8 or ps396, or double-positive. When C1q distribution was examined, an inverse trend was observed, with a specific decrease of C1q-positive stained neurons in the CA1/CA2 area of AD compared to CTL. Confocal imaging showed neurons in the AD hippocampus, most of which were only positive for C1q or AT8 and a few were double positive for both markers. Our data highlight the heterogeneity of neuronal disease phenotypes across hippocampal subfields, the potential association between pTau accumulation and the classical complement activation, and its vulnerability of the CA1/CA2 hippocampal in AD. To elucidate the mechanisms underlying these observations and their implications for the pathogenesis, we will extend our investigation to other Tau pathology markers and design in-vitro experiments.

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EFFECTS OF MIR-155-5P ON MIGRATION AND AUTOPHAGY IN LUNG CANCER CELLS

Elif APAYDIN (1), Gamze YILMAZ (1), Zerrin SELLER (1)

1) Biochemistry Department, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey

Lung cancer is one of the most common causes of cancer-related death worldwide (1). Despite advances in early detection and development of new targeted agents, the prognosis of lung cancer remains poor. Therefore, the development of more accurate methods for lung cancer diagnosis is urgently required.

miRNAs are modulators that govern the translation of protein-coding mRNAs and regulate gene expression after transcription (2). miR-155-5p is an oncogenic miRNA whose expression is altered in many cancers. In particular, miR-155-5p is overexpressed in lung cancer, leading to shorter survival and poor prognosis in patients (3).

In this study, the effects of miR-155-5p on autophagy and migration, and its relationship with the p53 gene, when miR-155-5p expression was increased and suppressed in A549 cells. To determine whether miR-155-5p exerts its effects on autophagy and migration through p53, p53 was silenced using siRNA in A549 cells. The effects of miR-155-5p on autophagy were determined by fluorescent labeling of autophagosomes using a fluorescence microscope, and its effects on migration were determined using a real-time cell analyzer (Xcelligence). The effects of miR-155 on autophagy and migration and the mRNA expression of p53, SIRT1, RHEB, Snail, and vimentin, the key molecules of these pathways, were determined by PCR analysis.

After miR-155-5p (50 nm) mimic transfection in A549 cells, p53 mRNA expression level decreased 0.38-fold, SIRT-1 expression level increased 1.6-fold, and RHEB expression level, which suppresses autophagy via mTOR, decreased to 0.4. After miR-155-5p mimic transfection, the fluorescence intensity of autophagosomes increased slightly compared with that of the mimic negative control. After miR-155-5p inhibitor (10 nm) transfection of A549 cells, p53 mRNA expression increased 1.8-fold, SIRT-1 expression increased 1.4-fold, and RHEB expression decreased to 0.45. After the suppression of miR-155-5p by inhibitor transfection, the autophagosome fluorescence intensity increased compared to inhibitor-negative control and mimic transfection. Suppression of miR155-5p expression in A549 cells increased autophagy mediated by p53, RHEB, and SIRT.

The effects of miR-155-5p on migration were evaluated by Xcelligence for 120 hours. When miR-155 was suppressed by inhibitor transfection, migration decreased to 77.8% at 24 hours and to 74.6% at 120 hours. The snail mRNA expression level increased 2.3-fold 48 hours after mimic transfection. Vimentin expression level decreased by 0.71 after mimic transfection and by 0.30 after inhibitor transfection. miR-155-5p inhibitor suppressed migration by decreasing vimentin mRNA expression.

To determine the effects of p53 on miR-155-5p, 0.1-fold p53 mRNA expression was suppressed by siRNA transfection (10pg/ul). After p53 suppression, the miR-155-5p mimic and inhibitor were transfected for a second time. In p53-suppressed cells, p53, RHEB, SIRT1, Snail, and vimentin mRNAs were significantly suppressed. Fluorescence microscopy showed that the rate of autophagy decreased when miR-155-5p was increased or suppressed when p53 was suppressed. According to migration studies, increased miR-155-5p expression due to p53 suppression increased migration by 213% at 24 h and 265% at 96 h. The suppression of miR-155-5p expression was 88%.

As a result of these studies, it was determined that miR-155-5p works in a p53-mediated manner in A549 cells, and when p53 is suppressed, autophagy is suppressed and migration is increased.

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THE MODULATION OF THE ER-MITOCHONDRIA TETHER GRP75 REGULATES MELANOMA CANCER CELL AGGRESSIVENESS

Filippo Severin, Elisabetta Zaltron, Ilaria Celotti, Alessia Ruzza, Federica Vianello, Matteo Scavezzon, Alberta Palazzo, Valentina Brillo and Luigi Leanza

Department of Biology, University of Padua, Padua, Italy

Mitochondria are organelles not only involved in cellular respiration but also in several other pathways important for cell life and death. They are not isolated within the cells but are closely interconnected with other organelles, among which the Endoplasmic Reticulum (ER). Defective ER-mitochondria crosstalk and ER stress impacts on several cellular functions as well as on important intracellular pathways that promote the cancer development. Modulation of ER-mitochondria contacts have a role in cancer development and resistance to pharmacological therapy by impacting on cellular bioenergetics and metabolism. More recently, we showed that a reduction of mitochondria-ER contacts sites, by the down-regulation of the tether GRP75, can tune cancer cells intracellular signaling (e.g. Wnt signaling) both in vitro and in vivo, ultimately impacting on cancer cells proliferation.

We demonstrated that organelle contacts are mutually regulated in response to metabolism rewiring so affecting cancer formation/progression and cancer cells sensitivity to drugs. In details, we showed that a fine tuning of mitochondrial metabolism can modulate Wnt signaling and that the down-modulation of GRP75 reduces ER-Mito contacts and impair Mito-Wnt axis. Moreover, GRP75 knock-down impacts on melanoma cell proliferation, migration and invasion both in vitro and in vivo. Finally, we pointed out how various nutrients impact differently on melanoma progression in wild-type cells with respect to GRP75 knock-down clones.

These findings reveal that affecting mitochondria-ER tethering may be beneficial against cancer by altering the cellular signaling, and in turn sensitizing tumor cells to chemotherapeutic treatment.

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HYPOXIA POTENTIATES PRO-APOPTOTIC EFFECT OF STEARIC ACID AND INHIBITS PROTECTIVE EFFECT OF OLEIC ACID IN PANCREATIC β-CELLS

Šrámek J (1), Němcová V (1), Polák J (2), Kovář J (1)

1) Charles University – Third Faculty of Medicine, Department of Biochemistry, Cell and Molecular Biology & Center for Research of Diabetes, Metabolism and Nutrition, Prague, Czech Republic; 2) Charles University – Third Faculty of Medicine, Department of Pathophysiology, Prague, Czech Republic

Increased levels of fatty acids (FAs) in the blood are factors contributing to the failure and death of pancreatic β-cells. Therefore, it can contribute to the development of type 2 diabetes mellitus (T2DM). Saturated FAs, e.g. stearic acid (SA), induce apoptosis in pancreatic β-cells while unsaturated FAs, e.g. oleic acid (OA) have nearly no detrimental effect. Moreover, unsaturated FAs are capable of inhibiting the pro-apoptotic effect of saturated FAs. Hypoxia is also known to have deleterious effects on β-cell function and viability. These factors influencing β-cell viability are usually studied separately in in vitro studies. We have tested the combined effect of FAs (i.e. saturated SA, unsaturated OA and combination of these FAs) and hypoxia (1% O2) on the growth and viability of human (NES2Y, 1.1B4) and rodent (MIN6, INS1E) pancreatic β-cells in an attempt to mimic more closely the milieu present in the body of obese individuals suffering from fat-rich diet and hypoxia, e.g. due to sleep apnea syndrome.

In experiments, depending on cell line a defined serum-free medium supplemented with 0.75 or 1 mM SA, a combination of SA with 0.2/0.4/0.5 mM OA, or 0.2/0.4/0.5 mM OA alone bound to a 2% FA-free bovine serum albumin was used. We used 1% O2 concentration to generate strong hypoxia. 13% O2 concentration was applied as normoxia.

We found that hypoxia increased the pro-apoptotic effect of saturated SA. Endoplasmic reticulum stress signaling, autophagy and some kinase signaling pathways seemed to be involved in the mechanism of this effect. Hypoxia also decreased the protective effect of unsaturated OA against the pro-apoptotic effect of SA. Thus, in the presence of hypoxia, OA was unable to save SA-treated β-cells from apoptosis induction.

These pioneering data suggest that hypoxia, although solely seems to have rather only weak detrimental effect on β-cell viability, in combination with other pro-apoptotic factor(s) could represent a decisive factor in pancreatic β-cell death and survival and thus in the development of T2DM. Data also insinuate the necessity to study the effects of individual T2DM contributing factors in the context of the other factors to enable a comprehensive understanding of their role in T2DM development.

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ONCOGENIC RAS SIGNALING CO-OPTS UDP-GALACTOSE-4`-EPIMERASE FUNCTION TO PROMOTE MALIGNANT TRANSFORMATION

Frauke Stölting (1), Gabriel Leprivier (1), Ulla Gerling-Driessen (2), Jonathan Lim (1),

1) Institute of Neuropathology, University Hospital Düsseldorf and Medical Faculty, Heinrich Heine University, Düsseldorf, Germany; 2) Institute for Macromolecular Chemistry, University of Freiburg, Germany

The RAS family of genes are among the most mutated genes in human cancer, being found in more than 25% of all human tumors. Mutations in one particular isoform, KRAS, characteristically appear in pancreatic, colorectal and lung cancer. To date, drugs that target RAS directly lead to resistance or relapse. This drives the impetus to generate novel understanding of oncogenic RAS function towards the design of more efficacious therapies. Elucidating novel mechanisms of oncogenic RAS-mediated transformation may lead to the discovery of new therapeutic targets downstream of RAS. Using an integrated proteomics and transcriptomics approach in engineered murine cell lines expressing mutant KRAS, we discovered that UDP-galactose-4`-epimerase (GALE) is upregulated by oncogenic RAS signaling.

GALE plays an important role in the biosynthesis of glycoproteins and glycolipids, by balancing the nucleotide sugars UDP-Glucose/UDP-Galactose and their corresponding N-acetylhexosamines (UDPGlcNAc/UDPGalNAc). Notably, the contribution of GALE to tumorigenicity is poorly explored, and the molecular pathways and mechanisms by which it is regulated in tumors is not clearly described. In support of our finding from the transcriptomic and proteomic approach, knockdown of RAS in human lung cancer cells with shRNA, as well as targeting KRASG12C-mutant human pancreatic cells with a FDA-approved RAS inhibitor led to significant downregulation of GALE. Using pharmacological inhibitors against components of MEK-ERK or PI3K-AKT, we show that GALE is regulated by RAS downstream of the MEK-ERK signaling cascade. Further, using siRNA targeting MYC, as well as pharmacological inhibition of MYC, we found that GALE is transcriptionally regulated via MYC.

To investigate the effects of GALE on metabolic flexibility of transformed cells, knockdown cell lines were established and GALE-deficient cells seem to be more sensitive to glucose-starvation induced cell death. To further explore the involvement of GALE on oncogenic transformation, soft agar colony formation assays were performed. Our GALE knockdown cell lines showed decreased colony formation capability under high as well as physiological glucose concentrations. As an outlook, we plan to elucidate the metabolic flexibility that GALE renders by tracing 13C-labeled sugar hexoses in 3T3 KRASG12V GALE control vs. knockdown cells under glucose starvation induced cell death.

Notably, we found that GALE expression exhibits strong clinical relevance as a prognostic biomarker. Namely, gene expression data in tumor types with activating mutations in RAS, pancreatic adenocarcinoma (PAAD), colon adenocarcinoma (COAD) and lung adenocarcinoma (LUAD) tissues revealed overexpression of GALE. Further, GALE is overexpressed in glioblastoma, a tumor type known to harbor RAS pathway activation even in the absence of activating mutations in RAS, and associated with poor patient prognosis. In relation to this, genes that are co-expressed with GALE in glioma are enriched for oncogenic RAS pathway signatures further suggesting that oncogenic RAS signaling may adapt GALE function to promote malignant transformation.

In conclusion, our data suggests that oncogenic RAS signaling regulates GALE via MEK-

ERK-Myc signaling. GALE contributes to transformation, possibly via flexible regulation of shifting metabolism, e.g. towards the hexosamine biosynthesis pathway. GALE is clinically relevant and could serve as biomarker and new target to specifically treat RAS transformed cancers.

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TUMOR SUPPRESSOR PAR-4 PLAYS A KEY ROLE IN PROMOTING AUTOPHAGY-MEDIATED FERROPTOSIS

Karthikeyan Subburayan (1), Faisal Thayyullathil (1), Siraj Pallichankandy (1), Anees Rahman Cheratta (1), Ameer Alakkal (1), Mehar Sultana (2), Nizar Drou (3), Muhammad Arshad (3), Palanikumar Loganathan (4), Mazin Magzoub (4), Vivek M Rangnekar (5), and Sehamuddin Galadari (1)

1) Cell Death Signaling Laboratory, Biology Program, Division of Science, Experimental Research Building, New York University Abu Dhabi, PO Box 129188, Saadiyat Island, Abu Dhabi, United Arab Emirates; 2) Center for Genomics and Systems Biology (CGSB), and 3) CGSB Core Bioinformatics, Experimental Research Building, New York University Abu Dhabi, PO Box 129188, Saadiyat Island, Abu Dhabi, United Arab Emirates; 4) Biology Program, Division of Science, Experimental Research Building, New York University Abu Dhabi, PO Box 129188, Saadiyat Island, Abu Dhabi, United Arab Emirates; 5) Department of Radiation Medicine and Markey Cancer Center, University of Kentucky, Lexington, KY 40536, USA.

Ferroptosis is a specific iron-dependent form of cell death characterized by extensive lipid peroxidation. While there is growing evidence of its link to autophagy-mediated cell death, the detailed molecular mechanisms governing ferroptosis are not fully understood. In this study, we used an unbiased genome-wide screening approach to reveal the involvement of the versatile tumor-suppressor protein Par-4/PAWR in ferroptosis. Functional analyses demonstrate that the genetic removal of Par-4 hinders ferroptotic processes, while its overexpression makes cells more susceptible to ferroptosis. Importantly, we found that Par-4-mediated ferroptosis operates through the autophagy machinery. Increased levels of Par-4 stimulate ferritinophagy, the autophagic breakdown of ferritin, through the nuclear receptor co-activator 4 (NCOA4), leading to the release of labile iron and increased lipid peroxidation, thus intensifying ferroptosis. Significantly, inhibiting Par-4 disrupts the NCOA4-mediated ferritinophagy axis. Our data also show a direct link between Par-4 activation and the generation of reactive oxygen species (ROS), which are crucial for ferritinophagy-mediated ferroptosis. Furthermore, the depletion of Par-4 effectively prevents ferroptosis-mediated tumor suppression in murine xenograft models. Overall, these findings highlight the important and novel role of Par-4 in ferroptosis, providing promising opportunities for leveraging this pathway in cancer therapy.

TBK1 AND IKKε PROTECT TARGET CELLS FROM IFNγ-MEDIATED T CELL KILLING VIA AN INFLAMMATORY APOPTOTIC MECHANISM

Nicholas Sun (1), Allison Carr (1), Erica Krogman (1), Yogesh Chawla (1), Jun Zhong (2), Matthew Guttormson (1), Mark Chan (1), Michelle Hsu (1), Haidong Dong (1,3), Dusan Bogunovic (4), Akilesh Pandey (2,5), Laura Rogers (1), Adrian Ting (1)

1) Department of Immunology, Mayo Clinic, Rochester, MN 55905, USA; 2) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905, USA; 3) Department of Urology, Mayo Clinic, Rochester, MN 55905, USA; 4) Columbia Center for Genetic Errors of Immunity, Department of Pediatrics, Columbia University Irving Medical Center, New York, NY 10032, USA; 5) Center for Individualized Medicine, Mayo Clinic, Rochester, MN 55905, USA

Cytotoxic T cells produce interferon gamma (IFNγ), which plays a critical role in anti-microbial and anti-tumor responses. However, it is not clear whether T cell-derived IFNγ directly kills infected and tumor target cells, and how this may be regulated. Here, we report that target cell expression of the kinases TBK1 and IKKε regulate IFNγ cytotoxicity by suppressing the ability of T cell-derived IFNγ to kill target cells. In tumor targets lacking TBK1 and IKKε, IFNγ induces expression of TNFR1 and the Z-nucleic acid sensor, ZBP1, to trigger RIPK1-dependent apoptosis, largely in a target cell-autonomous manner. Unexpectedly, IFNγ, which is not known to signal to NFκB, induces hyperactivation of NFκB in TBK1 and IKKε double-deficient cells. TBK1 and IKKε suppress IKKα/β activity and in their absence, IFNγ induces elevated NFκB-dependent expression of inflammatory chemokines and cytokines. Apoptosis is thought to be non-inflammatory, but our observations demonstrate that IFNγ can induce an inflammatory form of apoptosis, and this is suppressed by TBK1 and IKKε. The two kinases provide a critical connection between innate and adaptive immunological responses by regulating three key responses: (1) phosphorylation of IRF3/7 to induce type I IFN; (2) inhibition of RIPK1-dependent death; and (3) inhibition of NFκB-dependent inflammation. We propose that these kinases evolved these functions such that their inhibition by pathogens attempting to block type I IFN expression would enable IFNγ to trigger apoptosis accompanied by an alternative inflammatory response. Our findings show that loss of TBK1 and IKKε in target cells sensitizes them to inflammatory apoptosis induced by T cell-derived IFNγ.

INVESTIGATING MICROGLIAL MECHANORECEPTOR MEDIATED Aβ CLEARANCE IN AD

Hilmi Jaufer Thameemul Ansari (1), Michael T. Heneka (1, 2)

1) Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, 4362, Belvaux, Luxembourg; 2) Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, Massachusetts, USA

Mechanoreceptors can respond to any change in mechanical pressure or distortion in a biological environment. Piezo1 is a mechanoreceptor expressed in microglia and plays a role in clearing amyloid beta (Aβ) from the brain (Hu et al., 2023; Jantti et al., 2022). We investigate the mechanism of Piezo1-mediated clearance of Aβ in Alzheimer's disease model. Paralally, we are also exploring the expression of other possible mechanoreceptors expressed in microglia.

We have shortlisted a few mechanoreceptors based on literature and our existing scRNAseq data from microglia. From the initial qPCR data, we understood that the expression of Piezo1 is abundant among other mechanoreceptors in microglia. Other mechanoreceptors are also expressed but at a very low level, and they are Trpov4, Kcnk4, and Asic3. We are yet to study whether Piezo1 is the sole mechanoreceptor responsible for Aβ phagocytosis by microglia. However, upon Piezo1 activation with an agonist, Yoda1, there is an enhanced phagocytosis of (fluorescent-tagged) TAMRA-Aβ. The mechanism is not yet defined, and a report indicated Piezo1 acts via NF-κB mediated inflammatory signaling upon LPS activation (Malko et al., 2023). We will study the immune response triggered by microglial Piezo1 and its effect on cellular health.

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METABOLIC STRESS TRIGGERS DISULFIDPTOSIS-LINKED NECROPTOSIS VIA ACTIVATING KEAP1-NRF2-SLC7A11 AXIS

Faisal Thayyullathil, Anees Rahman Cheratta, Siraj Pallichankandy, Karthikeyan Subburayan, Ameer Alakkal, Asma Al Nuaimi, and Sehamuddin Galadari

Cell Death Signaling Laboratory, Biology Program, Division of Science, Experimental Research Building, New York University Abu Dhabi, PO Box 129188, Saadiyat Island, Abu Dhabi, United Arab Emirates.

Necroptosis is a form of regulated cell death dependent on receptor-interacting serine/threonine kinase 1 (RIP1), RIP3, and its substrate, mixed lineage kinase domain-like pseudokinase (MLKL). It is implicated in various human diseases, including ischemic organ damage and cancer. Disulfidptosis, a newly identified form of cell death triggered by disulfide stress, is characterized by the collapse of cytoskeleton proteins and F-actin due to intracellular accumulation of disulfides. Insufficient nutrient disrupts physiological homeostasis, leading to diseases and potentially death. Given the significant physiological and pathological consequences of metabolic stress, understanding the adaptive responses cells employ under these conditions is of great interest.

We show that under the condition of glucose starvation, cells initiate and execute disulfidptosis-linked necroptosis through Kelch-like ECH-associated protein 1 (KEAP1)-nuclear factor erythroid-2-related factor 2 (NRF2)-solute carrier family 7 member 11 (SLC7A11) signaling pathway, ultimately leading to accumulation of cystine, which is crucial for disulfidptosis. Mechanistically, metabolic stress leads to the proteasomal degradation of KEAP1, resulting in the activation of NRF2-mediated SLC7A11 expression and cystine accumulation. Consequently, RIPK1, RIPK3, and MLKL are activated, leading to necroptotic cell death. Inhibition of disulfidptosis, either pharmacologically or through genetic inhibition of SLC7A11 or its upstream regulator NRF2, abrogated the accumulation of disulfidptosis-associated markers and prevented necroptotic cell death. Together, metabolic stress activates KEAP1-NRF2-SLC7A11 axis, leading to cystine accumulation and disulfidptosis-linked necroptotic cell death.

USING FLUORESCENT REPORTER MICE AND INTRAVITAL IMAGING TO INVESTIGATE THE P53 RESPONSE TO ONCOGENE ACTIVATION DRIVING TUMOUR DEVELOPMENT

Annabella F Thomas (1,2), Elizabeth Lieschke (1), Lin Tai (1), Andrew Kueh (1), Edwin Hawkins (1,2), Marco J Herold (1,2), Georgia Atkin-Smith (1,2), Gemma L Kelly (1,2), Andreas Strasser (1,2)

1) The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; 2) The Department of Medical Biology, The University of Melbourne, Melbourne, Victoria, Australia

The p53 gene is mutated in ~50% of human cancers and these mutations often contribute to poor responses to cancer therapy. The p53 protein is a master regulator of several cellular responses to diverse stresses, such as activation of oncogenes or DNA damage. It functions as a homo-tetrameric transcription factor that directly transcriptionally regulates ~500 genes, some of which suppress tumorigenesis. This includes p21, which is required for p53-mediated induction of cell cycle arrest/cell senescence, and Puma, which is critical for p53-mediated apoptosis induction. Deregulated over-expression of the transcription factor c-MYC promotes tumorigenesis by causing aberrant growth and proliferation of cells. As a safeguard against tumorigenesis, deregulated MYC over-expression can trigger apoptosis through the activation of p19Arf which then activates p53, leading to the expression of pro-apoptotic PUMA.

To investigate the p53-mediated cellular responses promoting tumour suppression, we created two reporter mouse lines in which GFP is knocked into the p21 locus behind an IRES, or in which the Puma coding region was replaced with tdTomato. We also inter-crossed these p21 and Puma reporter mice to explore why certain cells undergo cell cycle arrest/senescence after p53 activation, while others undergo apoptotic cell death. We validated these novel reporter mice through comprehensive flow cytometry analysis and intravital imaging of live mice, revealing reporter activity in diverse immune cell types in various organs, including the bone marrow calvarium.

To investigate the p53-response in a tumorigenic setting, reporter mice were crossed to the Eμ-Myc transgenic mouse model, where c-MYC is over-expressed in B lymphoid cells. Flow cytometry analysis of B cell subsets revealed an increase in Puma but not p21 reporter expression, compared to control B lymphoid cells that do not over-express c-MYC. Furthermore, intravital imaging of these mice also revealed a significant increase in Puma but not p21 reporter activity in cells within the bone marrow. Ongoing studies aim to elucidate which p53 target genes, in addition to Puma, are induced in response to MYC oncogene expression in B lymphoid cells to effect tumour suppression. This will contribute to the understanding of tumour initiation and tumour suppressive responses and may reveal new targets for cancer therapy.

SERATRODAST INHIBITS FERROPTOSIS BY SUPPRESSING LIPID PEROXIDATION

Juliane Tschuck (1), Wulf Tonnus (2), Ina Rothenaigner (1), Andrea Kolak (1), Derek Pratt (3), Andreas Linkermann (2,4), and Kamyar Hadian (1)

1) Research Unit Signaling and Translation, Helmholtz Zentrum München, Neuherberg, Germany; 2) Division of Nephrology, Department of Internal Medicine 3, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Germany; 3) Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada; 4) Division of Nephrology, Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, USA

Ferroptosis is a regulated and non-apoptotic form of cell death that is mediated by iron-dependent peroxidation of polyunsaturated fatty acyl tails in lipids. Research of the past years has shed light on the occurrence of ferroptosis in organ injury and degenerative diseases of the brain, kidney, heart, and others. Hence, ferroptosis inhibition may prove therapeutically beneficial to treat the aforementioned diseases. In this study, we explored the ferroptosis-modulating activity of seratrodast, an inhibitor of thromboxane A2 (TXA2) receptor, which is approved in some countries for the treatment of asthma. Surprisingly, seratrodast suppressed ferroptosis, but not apoptosis and necroptosis; thus, demonstrating selective anti-ferroptotic activity. Mechanistically, seratrodast counteracted lipid peroxidation through its quinone moiety exhibiting radical-trapping activity. Importantly, seratrodast ameliorates the severity of renal ischemia-reperfusion injury in mice. Together, this study provides a drug repurposing case, where seratrodast – a marketed drug – may undergo fast-forward pre-clinical/clinical development for the inhibition of ferroptosis in distinct degenerative diseases.

COMBINED TREATMENT WITH MELATONIN AND FINGOLIMOD ENHANCES COGNITION AND SPHINGOLIPID METHABOLISM IN A RAT MODEL OF ALZHEIMER'S DISEASE

Rumiana Tzoneva (1), Irina Georgieva (1), Sonia Apostolova (1), Tzveta Dimitrova (2), Lidia Kortenska (2), Tsvetelina Oreshkova (3), Jana Tchekalarova (2)

1) Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria; 2) Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. Bl. 23, Sofia 1113, Bulgaria; 3) Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Tzarigradsko shose blvd. 73, Sofia 1113, Bulgaria

Alzheimer's disease (AD) is a neurodegenerative disorder that typically develops because of accumulation of amyloid plaques, leading to hyperphosphorylation of Tau, severe neuronal death, cognitive impairment, and memory loss. AD progression is associated with increased oxidative stress (OS), which contributes to the generation of Aβ and vice versa. Our previous studies demonstrated the positive effects of exogenous treatment with the antioxidant melatonin (MEL) on cognition in an intracerebroventricular (icv) Aβ-induced AD model in rats [1]. Moreover, AD is characterized by an imbalance between sphingosine-1-phosphate (S1P) and ceramide levels [2]. This imbalance, which results in decreased levels of S1P and increased levels of ceramide, has significant implications, as S1P promotes cell survival, while ceramide induces cell death. Therefore, we hypothesized that a combined treatment with MEL and fingolimod (FTY), an S1P analogue acting as an immunosuppressor, might cause a cross-talk between the pathways and exhibit a synergistic effect in neuron protection. FTY has been linked to OS [3], making the antioxidant properties of MEL particularly relevant in mitigating its undesirable effects. In this study, we administered FTY, MEL, or a combination of both intraperitoneally for two weeks after an icv infusion of Aβ fibrils to test the potential enhancement in memory performance in rats with AD. The combination of FTY and MEL significantly improved the rats' memory compared to the control and Aβ groups treated with or without FTY or MEL. Additionally, the combined treatment restored the rats' disturbed spontaneous alternation behavior, while FTY and MEL administered alone were ineffective. Gene expression data revealed a distinct pattern in the response between the hippocampus and frontal cortex. Specifically, Aβ in the hippocampus downregulated the expression of sphingosine kinase 1 (SPHK1), whereas FTY treatment reduced S1P receptor 1 expression in the frontal cortex. Moreover, FTY downregulated the expression of apoptosis-related genes, such as caspase 3, ceramide kinase, and SPHK2, only in the hippocampus; this effect was reversed by MEL in the combined treatment. The observed positive outcomes of combining FTY treatment with melatonin are encouraging, especially considering the potential of the combined treatment to improve the immune response in AD patients.

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IDENTIFICATION OF DIMETHYL FUMARATE (DMF) TARGETS IN TNF-INDUCED APOPTOSIS AND NECROPTOSIS

Mike Vadi (1,2), Wei Xie (1,2), Laura Wyckaert (1,2), Peter Vandenabeele (1,2)

1) Cell Death and Inflammation Unit, VIB-UGent Center for Inflammation Research (IRC), Ghent, Belgium, BE; 2) Department of Biomedical Molecular Biology (DBMB), Ghent University, Ghent, Belgium, BE

Dimethyl fumarate (DMF) is an ester from the Krebs cycle intermediate fumarate. It is currently used as an FDA/EMA-approved drug for the treatment of relapsing-remitting form of multiple sclerosis and plaque psoriasis. Although the therapeutic mechanism of DMF remains largely unknown, it was demonstrated in many studies that DMF has great impact on inflammatory signaling pathway (Bresciani et al., 2023). In this project, we aim to investigate the effect of DMF on TNF signaling with an emphasize on TNF-induced apoptosis and necroptosis. We recently demonstrated that DMF protects human HT-29 cells against TNF-induced RIPK1-dependent and -independent apoptosis and against necroptosis. Indeed, DMF prevents TNF-induced RIPK1 autophosphorylation at Ser166, the cleavage of caspase-8/-3/-7 and the phosphorylation of RIPK3 and MLKL. We also observed that DMF treatment leads to additional RIPK1 phosphorylation bands. Those are being identified and evaluated. We will now confirm the protective effect of DMF in in vivo inflammatory mouse models involving TNF and the role of RIPK1.

In an unbiased approach, we will try to understand how mechanistically DMF can protect cells against cell death. DMF, together with fumarate and other fumarate esters, can directly modify cysteines present on proteins, through a process named “succination” (Guberovic & Frezza, 2024). Many targets have been identified but for most of them, the impact of succination remains unexplored. We will identify DMF targets in TNF-induced apoptosis and necroptosis by combining click-chemistry with mass spectrometry and then evaluate the role of the succination of key targets in the protective effect of DMF against cell death. Our findings will not only greatly improve our understanding on the therapeutic mechanism of DMF, but also highlight the possible impact of the Krebs cycle in TNF-mediated inflammation and cell death.

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IDENTIFICATION OF ZFHX4 AS A NOVEL TRANSCRIPTION FACTOR CONTROLLING DOPAMINERGIC NEURON DIFFERENTIATION

Elena Valceschini (1), Borja Gomez Ramos (1,2), Jochen Ohnmacht (1,2), Anthoula Gaigneaux (1), Dimitrios Kyriakis (2), Kamil Grzyb (2), Anne Grünewald (2), Alexander Skupin (2), Thomas Sauter (1), Rejko Krüger (2), Lasse Sinkkonen (1)

1) Department of Life Sciences and Medicine, University of Luxembourg, Belvaux, Luxembourg; 2) Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Belvaux, Luxembourg

The selective degeneration of specific neuronal subtypes represents a pathological hallmark of several neurological diseases such as the degeneration of midbrain dopaminergic neurons (mDANs) in Parkinson's disease (PD) (1). Improving in vitro models of human neuronal subtypes that could recapitulate the heterogeneity of the human brain would have implications in the biomedical field as well as improve our ability to unravel the underlying molecular mechanisms of diseases. Transcription factors (TFs) and their co-regulators play a central role in the cell lineage commitment, acting in gene regulatory networks (GRNs) to control gene expression and define specific cellular identities (2). Nowadays, high-throughput sequencing methods have paved the way for data-driven multi-omics approaches that allow unbiased identification of cell-type specific transcriptional regulators. We have taken advantage of transcriptomic and epigenomic data from the International Human Epigenome Consortium (IHEC) to identify TFs with high and selective expression and regulation in human brain or during neurogenesis. Among the interesting TFs we identified ZFHX4. Single nuclei RNA-seq of the whole-brain transcriptomic atlas from Allen Brain Atlas confirmed a high expression of Zfhx4 in neurons from several areas of the mouse brain, including dopaminergic neurons.

We have previously generated time-series transcriptomic and epigenomic profiles of purified human induced pluripotent stem cell (hiPSC)-derived mDANs (3). From the integration of the generated high-throughput data, ZFHX4 was confirmed as one of the most prominent super-enhancer-controlled TFs in mDAN differentiation. We further investigated its role using RNA-seq analysis upon in vitro knockdown (KD) assays, as well as CUT&Tag analysis of ZFHX4 enrichment in mDANs. The depletion of ZFHX4 affected the mDAN differentiation while enrichment analyses on the differentially expressed genes resulted in an augmentation of cell-cycle related TFs and pathways. Flow-cytometry analysis of the cell-cycle state upon ZFHX4 depletion indicated an increase in the number of replicative cells suggesting a regulation of the G1 checkpoint. The RNA-binding protein LIN28A, involved in stem-cell maintenance, emerged among the most upregulated genes upon the ZFHX4 KD while the LIN28A regulatory locus was enriched for the ZFHX4 binding signal. Finally, the mDANs time-series analysis showed an anti-correlation of LIN28A and ZFHX4 induction. Our analysis indicates a pivotal role of ZFHX4 in the regulation of cell cycle, specifically in silencing pluripotency and proliferative programs while maintaining the mDANs in a post-mitotic state.

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MITOCHONDRIA REGULATION OF AGE-ASSOCIATED NEURODEGENERATIVE DISEASES VIA IRON-DEPENDENT SIGNALING PATHWAYS

Alfonso Schiavi (1), Silvia Maglioni (1,2), Kai Kremers (1), Lena Jentsch (1,2), Laura Schröter (1,2), Roberta Pensotti (3), Maria Elena Regonesi (3), Lois Armendariz (4), Natalia Kirienko (4), Leonie Thewes (5), Carsten Berndt (5), Natascia Ventura (1,2,5*)

*1) Leibniz Research Institute for Environmental Medicine (IUF), Düsseldorf, Germany; 2) Institute for Clinical Chemistry and Laboratory Diagnostic, Medical Faculty, Heinrich Heine University, Düsseldorf,Germany; 3) Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy 4) BioSciences Department, Rice University, 77005 Houston, Texas, USA; 5) Department of Neurology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany; *natascia.ventura@hhu.de*

Aging is one of the most important risk factors for the development of numerous disorders including neurodegenerative diseases and cancer, and is modulated by genetic, environmental and life-style factors.

I have pioneered the concept of "mitochondria hormesis" in the regulation of animals' health and lifespan. We found that finely tuning the activity of the mitochondrial respiratory chain (MRC) reprograms cellular metabolism and counteracts neuronal disease and aging through evolutionarily conserved iron-regulated mechanisms i.e., induction of mitophagy and inhibition of ferroptosis 1-2. We also identified dietary components (e.g., iron, lutein) and contaminants (nanoplastic particles) impacting C. elegans health and aging through mitohormetic paradigms 3-5.

In search of specific mitochondrial signaling pathways mediating the beneficial effects of iron depletion, we recently collected lifespan-resolved omics data (transcriptomic, proteomic, lipidomic) from C. elegans treated with pro-longevity mitochondrial stress (frataxin/frh-1 silencing), which we compared to changes in the gene expression profile induced by the iron chelator phenanthroline and siderophore pyoverdine, produced by Pseudomonas aeruginosa. Our preliminary analyses point towards common genes involved in cellular xenobiotic and immune response (e.g., cyps), redox and lipid homeostasis (e.g., glutathione peroxidase, methionine sulfoxide reductase), with TFEB/CeHLH-30 and NRF2/CeSKN-1as possible coordinating transcription factors. The expression of a selected number of genes has been validated by qPCR in response to frh-1 silencing and during animal aging and when available with C. elegans transgenic reporter lines. The implication of relevant genes in cell death pathways is currently being investigated in mammalian cells death assays. Moreover, we are using genetic epistatic analysis coupled to various behavioral tests to specifically address the involvement of these genes in C. elegans aging and age-associated neurodegenerative diseases. Results from our ongoing study will be presented at the conference.

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MULTI-OMICS ANALYSIS OF GUT MICROBIOTA UNVEILS MICROBIAL FUNCTIONS ALTERATIONS ASSOCIATED TO PARKINSON'S DISEASE.

Rémy Villette (1), Polina Novikova (1,) Julia Ortis-Sunyer (1), Velma Aho (1) , Viacheslav Petrov (1), Oskar Hickl (1), Charlotte De Rudder (1), Benoit Kunath (1), Susheel Bhanu Busi (1), Sebastian Schade (2), Annette Janzen (3), Wolfgang Oertel (3), Cédric Laczny (1), Patrick May (1), Brit Mollenhauer (2) and Paul Wilmes (1)

1) Luxembourg Center of Systems Biomedicine, University of Luxembourg, Belval, Luxembourg; 2) Movement disorders and Parkinson's Disease, Paracelsus-Kliniken Deutschland GmbH, Osnabruck, Germany; 3) Department of Neurology, Philipps-University Marburg, Marburg, Germany

Objective: Microbiome composition has been associated with Parkinson's disease (PD) at multiple stages. However, these associations are mainly studied using genomics technologies where the functional capacities of microbes are not fully investigated. In this study, we aim at describing the microbiome functions associated with PD using multi-omics technologies.
Methods: Using integrated multi-omics analysis, we performed deep phenotyping of the gut microbiome in a cross-sectional cohort of 49 healthy control (HC), 28 iRBD and 46 PD patients. Stool samples were phenotyped using shotgun metagenomics (MG), metatranscriptomics (MT), metaproteomics (MP) and metabolomics (MB).
Results: The gut microbiome showed no clear differences between groups both in alpha and beta-diversity for MG and MT taxonomic composition but differences in MT functions and MB. Roseburia, Blautia and Eubacterium were however reduced in PD for MT taxonomy. We observed a decrease in glycerol which correlated with Roseburia abundance and an increase beta-glutamate for PD that was correlated with Akkermansia and Methanobrevibacter abundance. We observed an increase and a decrease in diversity of gene expression in PD for Methanobrevibacter and Roseburia, respectively. Interestingly, we found important changes in gene expression that were related to glutamate transformation, chemotaxis-flagellin assembly and methane metabolism. Finally, we witnessed a decrease in functional diversity for Roseburia, Eubacterium and Blautia, while Methanobrevibacter gained functional diversity.
Conclusions: MT and MB represented the most powerful omics to differentiate the gut microbiome between HC and PD. Microbial functions appeared to be altered in PD context, especially for Roseburia and Methanobrevibacter that seemed to have contrary links to PD.

ZNF148 AS A REGULATOR OF CELL MIGRATION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

Alessia Violante, Rosalba Pecorari, Mara Mancini and Eleonora Candi

Department of Experimental Medicine, TOR, University of Rome "Tor Vergata", 00133 Rome, Italy

Head and neck squamous cell carcinomas (HNSCC) represents the sixth most common cancer worldwide1. Survival outcomes remain poor for most patients, highlighting the urgent need to identify novel potential therapeutic targets that could significantly improve quality of life2,3. In this context, over the past year, our laboratory has investigated the p63 interactome, identifying ZNf148 as an important co-factor with an oncogenic role in HNSCC (manuscript under revision)4. ZNF148 belongs to Krüppel-type zinc-finger family of transcription factors. We uncovered a p63-independent function of ZNF148 through ChIP-seq, RNA-seq, and ATAC-seq experiments. Specifically, ZNF148 regulates the transcription of genes involved in apoptosis, cell morphology, and the actin cytoskeleton. The latter two categories suggest its role in cancer cell migration and metastasis. Here, we report preliminary data confirming the role for ZNF148 in controlling the migration of FaDu cells. ChIP-seq analysis identified a potential novel direct target gene of ZNF148, PPFIA1 (also known as Liprin-α1), which is abnormally expressed in several types of malignancies, including HNSCC. PPFIA1 is involved in adhesion and cytoskeletal organization. It has also been recently reported that PPFIA1 plays a role in invasion and metastasis and could therefore be used to develop strategies to counteract tumor cell migration in aggressive cancers, as well as serve as a prognostic marker5. Bioinformatic analysis using publicly available datasets confirmed a direct correlation between the expression of ZNF148 and PPFIA1. ZNF148 appears to directly regulate PPFIA1 transcription by binding to the PPFIA1 promoter. We further demonstrated that ZNF148 knockdown, using siRNA, decreases PPFIA1 at both mRNA and protein levels, confirming it as a target gene. ZNF148 silencing appears to reduce the expression levels of tensillin, talin, vinculin, and paxillin, which are downstream targets of PPFIA1. All these findings suggest that ZNF148, through its direct target PPFIA1, is involved in modulating critical processes such as cell attachment, spreading, and migration. Further studies will be done to fully elucidate the contribution of ZNF148 in cancer metastasis and cell death.

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NANOPORE SEQUENCING OF PLASMA CELL-FREE DNA IN ICU PATIENTS FOR MONITORING INFECTION AND ORGAN INJURY

Cyril Willemart (1) , Mojca Strazisar (2, 3), Eric Hoste (4), Philippe Jorens (5, 6), Gerben Menschaert (7), and Tom Vanden Berghe (1, 8, 9)

1) Cell Death Signaling lab, Infla-Med Centre of Excellence, Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium; 2) Neuromics Support Facility, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium; 3) Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium 4) Intensive Care Unit, Department of Internal Medicine and Pediatrics, Ghent University Hospital, Ghent University, Ghent, Belgium; 5) Department of Intensive Care Medicine and Clinical Pharmacology, Antwerp University Hospital, Antwerp, Belgium; 6) Laboratory of Experimental Medicine and Pediatrics, Infla-Med Centre of Excellence, University of Antwerp, Antwerp, Belgium; 7) Department of Mathematical Modelling, Statistics and Bioinformatics, Ghent University, Ghent, Belgium; 8) VIB-UGent Center for Inflammation Research, Ghent, Belgium; 9) Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium.

In the intensive care unit (ICU), timely and correct diagnosis of organ injury and infections is crucial for treatment decisions, impacting patient outcomes and quality of life. Traditional diagnostic methods, while informative, often lack speed and specificity, leading to delayed critical interventions. This research aims to develop a rapid, integrative platform utilizing Oxford Nanopore Technologies (ONT) for real-time assessment of tissue injury and infections in ICU patients by sequencing plasma cell-free DNA (cfDNA). cfDNA refers to various extracellular nucleic acids found in body fluids, mainly originating from dying cells 1. Interestingly, cfDNA retains CpG methylation and it is thus possible to infer its tissue-of-origin through episequencing 2. Additionally, cfDNA sequences can be compared with microbial databases, thereby detecting infections and antibiotic-resistance genes, sensitively and faster than traditional culture methods 3.

In the last five years, numerous algorithms have emerged for tissue deconvolution based on cfDNA methylation markers, each varying in methodology, input types, sensitivity, and specificity. While certain methods are targeted, amplifying and analyzing a few sites for a specific tissue, we adopt the untargeted approach, to obtain an overview of all tissues. Our first objective is to evaluate these tissue deconvolution algorithms systematically by correlating their predictions with established clinical biomarkers for liver, kidney, heart, and lung injuries. For this, we sequenced samples from critically ill patients, selected based on gradients of biomarkers, to assess how accurately the algorithm(s) align with known indicators of organ dysfunction. We found that, for example, proportions of hepatocyte-derived cfDNA are highly correlated with ALT, a well-known biomarker for liver injury. Starting from simulations, we will optimize sample preparation to increase the sensitivity of the organ injury detection. To further empower the platform, microbial DNA detection will be added to the tissue injury pipeline. The sensitivity, specificity, and speed will be compared with those of classical clinical approaches. Besides strain determination, we aim to detect antibiotic-resistance genes to give appropriate antibiotics within hours after ICU admission. This study aims to improve ICU diagnostics by providing a faster and more accurate method for simultaneous diagnosing organ injuries, infections and antibiotic-resistance, ultimately improving patient outcomes, quality of life and reducing healthcare costs. Our platform's innovative integration of tissue-specific and microbial DNA detection represents a prom-

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TARGETING PROGRAMMED DEATH WITH SMALL MOLECULE INHIBITORS FOR NON-SMALL CELL LUNG CANCER

Liyang Wu (1), Anthony Marchand (2), Daniel Bachmann (1), Thomas Kaufmann (1)

1) Institute of Pharmacology, Faculty of Medicine, University of Bern, Bern, Switzerland; 2) Current address: School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Cisplatin-based and carboplatin-based doublets represent the standard-of-care of the first-line therapy for non-oncogene addicted advanced non-small cell lung cancer (NSCLC) patients (1). However, resistance is frequent also in patients who receive the full dose. Resistance to platinum drugs has been studied extensively in vitro, but the clinical relevance of each of the reported mechanisms is currently not entirely clear (2).
Inhibitors of apoptosis proteins (IAPs), in particular cIAP1, cIAP2, and XIAP, have major anti-apoptotic functions and have been identified for their potential to inhibit programmed cell death (PCD) (3). There are natural IAP antagonists which can promote the self-ubiquitination and proteasomal degradation of cIAP1/2 (4) and prevent XIAP from inhibiting caspase 3, 7, and 9 (5), but rarely induce its degradation (4). The observation that elevated expression of IAPs in some tumor types correlates with tumor survival and resistance to chemotherapy (6) has prompted the development of drugs that are small molecule peptidomimetics of Smac (7). These so-called Smac mimetics have shown promising effects on various cancer cells in vitro, with a mechanism involving autocrine TNF-mediated cell death (8), and TNF is an essential component for SM-mediated cell death (6). Several companies have already entered clinical trials with small-molecule IAP inhibitors. However, some clinical studies have shown that the tumor suppressive effects of single-use of Smac mimetics are not significant and easily tolerated (7). But Smac mimetics can also be used in combination with chemotherapeutic agents, other small molecule inhibitors or agonists, radiation, and immune stimulation to treat tumor patients more effectively (9).
Another important novel class of cancer drugs is the co-called BH3 mimetics, which are small-molecule inhibitors of anti-apoptotic BCL-2 proteins (10). Through protein-protein interactions, members of the Bcl-2 family control mitochondrial outer membrane permeability in the intrinsic apoptotic pathway (11). Recently, highly specific BH3 mimetics have been developed with the potential to inhibit individual anti-apoptotic BCL-2 family members (12). The first approved drug is the BCL-2 specific inhibitor Venetoclax (ABT-199) which has shown promising results in CLL (13) and AML (14). Multiple BH3 mimetics targeting BCL-XL or MCL-1, both of which are highly relevant in solid cancers, are in clinical trials (15,16,17). Navitoclax (ABT-263) is a bi-specific inhibitor of BCL-2 and BCL-xL and was the first specific BH3 mimetics to enter clinical trials of hematological malignancies and solid tumors; however, it did not reach the clinics yet due to thrombocytopenia (18). BH3 mimetics can show great anti-cancer potential when used with classical and conventional anti-cancer drugs and are among the candidates for the treatment of a range of cancers.
Targeting different tumor cells as well as resistance to therapy in individual patients using ideally personalized combination regimens, has or will become key to successful therapy (6).
So far, I have obtained preliminary data demonstrating the anti-proliferative and pro-apoptotic properties of BH3 mimetic and Smac mimetic in NSCLC cell lines when combined with cisplatin, respectively. I will next focus on specific inhibitor targets and delve into the

effects of drug combinations on NSCLC cell apoptosis and necroptosis. We expect to identify better-dosing regimens and new targets through this project.

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INVESTIGATING THE IMMUNOREGULATORY PROPERTIES OF LIPID DROPLETS AND METABOLITES FOLLOWING FERROPTOSIS INDUCTION IN CANCER

Laura Wyckaert (1), Wei Xie (1), Mike Vadi (1), Peter Vandenabeele (1,2)

1) Unit of Molecular Signaling and Cell Death, VIB Center for Inflammation Research, Ghent, Belgium; 2) Department of Biomedical Molecular Biology, Cancer Research Institute Ghent (CRIG), Ghent University, Ghent, Belgium

Cancer cell resistance against drugs that induce apoptotic cell death remains a major hurdle. Ferroptosis, a caspase-independent cell death modality, could offer an alternative treatment to eradicate these apoptosis-resistant cancer cells (Hassania et al. 2018). Although ferroptosis seems a compelling strategy for novel treatment, its immunogenic potential and inherent adjuvanticity are incompletely understood. Ferroptosis induction is associated with lipid droplet formation, COX2 activation, and PGE2 production, all factors that could possibly interfere with the cross-presentation ability of DCs and as such stimulate tumor evasion. The detrimental effects of PGE2 on immunosurveillance and survival have been well established. Moreover, lipid droplet accumulation has been associated with compromised functionality of DCs, particularly in terms of their ability to cross-present antigens.

The working hypothesis is based on recently obtained data that exposure of BMDC to ferroptotic cells despite abundant release of DAMPs, cytokines, and interferons, induce a tolerance state of DCs by reducing their capacity to support antigen presentation to antigen-specific T-cells in vitro. An established in vivo model of 'prophylactic cancer cell vaccination' shows that ferroptotic cells do not induce immunological protection against cancer cells, in contrast to other known 'immunogenic' cell death modalities (Wiernicki et al. 2022). Transcriptomics analyses revealed that ferroptosis modifies gene programs in dendritic cells (DCs) toward dampening of T cell immunity. This advances the concept that ferroptosis is not associated with a strong adaptive immunity response.

In summary, we will report on results that will illustrate the impact of lipid peroxidation, lipid droplets formation, and related metabolites and how these processes could be modulated to improve the anticancer immunogenic response during ferroptosis induction in cancer.

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ROLE OF APOPTOTIC EXECUTIONER CASPASE-3/7 IN INTESTINAL INFLAMMATION

Wei Xie (1,2), Laura Wyckaer (1,2), Mike Vadi (1,2), Tatyana Divert (1,2), Bruno Verstraeten (1,2) , Peter Vandenabeele (1,2)

1) Molecular Signaling and Cell Death Unit, VIB-UGent Center for Inflammation Research, Flanders Institute for Biotechnology, Ghent, Belgium; 2) Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium

An appropriate gut barrier is crucial during infection, for nutrition absorption and immunoregulation. Previously we reported that the apoptotic caspase-3/7 are dispensable in these processes at steady-state conditions, while the role of caspase-3/7 in stressed conditions remains unknown.

Following challenge with dextran sulphate sodium (DSS), increased inflammation, lethality and retarded regeneration were observed in mice that lack executioner caspase-3/7 in intestinal epithelial cells(IECs), arguing for a protective role of caspase-3/7 in control of inflammation and tissue regeneration.

Transcriptome analysis of IECs reveals an aberrant inflammatory response and cell cycle regulation in the absence of epithelial caspase-3/7, which may mediated by microbiota dysbiosis as broad antibiotics pretreatment completely eliminated difference between two genotypes. Moreover, full length 16S rRNA sequencing revealed a dramatic depletion of probiotic, including Lactobacillus group, when apoptotic IECs were not presented during colitis.

Our research identified caspase-3/7 as important regulators for intestinal inflammation and explored the crosstalk between IECs and gut microbiota, which further increased our fundamental understanding of the (pre)clinical application of caspase-inhibitors.

PHOTO-OXIDATIVE DAMAGE INDUCES GASDERMIN-E-MEDIATED PYROPTOSIS IN PRIMARY AND RECURRENT GLIOBLASTOMA

Yihan Yang (1,2,3), Frederik De Smet (4,5), Steven De Vleeschouwer (3), Patrizia Agostinis (1,3)

1) Research Group Experimental Neurosurgery and Neuroanatomy, Department of Neurosciences, KU Leuven, Leuven, Belgium; 2) Laboratory of Cell Death Research & Therapy, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium; 3) VIB Center for Cancer Biology Research, Leuven, Belgium; 4) Department of Imaging and Pathology, KU Leuven, Leuven, Belgium; 5) Leuven Institute for single-cell omics (LISCO), Leuven, Belgium; 6) Department of Neurosurgery, University Hospitals Leuven, Belgium; 7) Leuven Brain Institute (LBI), KU Leuven, Belgium

Hypericin-based photodynamic therapy (Hyp-PDT) is a potent inducer of photo-oxidative stress-mediated immunogenic apoptosis in different cancer types1–4. In an orthotopic murine model of glioblastoma (GBM) Hyp-PDT based DC vaccines robustly induced anti-tumor immunity and synergized with temozolomide (TMZ) in curative settings, leading to tumour regression5. However, the heterogeneous nature of human GBM raises inquiries regarding the effectiveness of Hyp-PDT and its potential to drive inflammatory and immunogenic responses.

Here, by employing several patient-derived cell lines (PDCLs) from primary GBM and their matched recurrence, we found that Hyp-PDT triggers caspase-dependent cell death characterized by necrotic-like features reminiscent of pyroptosis. In response to photo-oxidative stress, we observed a prompt caspase-mediated cleavage of GSDME generating the N-terminal fragment (NT-GSDME) pyroptosis mediator. Under the photodamage, caspase 3-mediated NT-GSDME and necrotic cell death were more pronounced in PDCLs of recurrent GBM as compared with their matched primary tumours. Particularly, the recurrent GBM of the mesenchymal phenotype exhibited exacerbated NT-GSDME formation and enhanced release of key damage-associated molecular patterns (DAMPs) such as ATP and HMGB1. This suggests the ability of Hyp-PDT to induce pro-inflammatory and immunogenic cell death mediators in GBM with acquired TMZ resistance through a GSDME-mediated mechanism. Analysis of in-house GBM single-cell RNA sequencing data and publicly available transcriptomic and proteomic datasets indicated that GSDME expression is particularly upregulated in GBM, in contrast with the methylation of GSDME frequently observed in other cancer types, suggesting its biomarker and therapeutic potential in GBM. Spatial omic studies, using patient tissues of the corresponding PDCLs, are ongoing to validate the expression of apoptotic and pyroptosis mediators in GBM subtypes and their associated immune profiles. Mechanistically, we found that silencing GSDME in PDCLs did not prevent overall cell killing but shifted the cell death mechanism from necrotic cellular demise to apoptosis, suggesting that the presence of GSDME is decisive for the ultimate inflammatory profile of photo-oxidative stress induced caspase signaling. We are currently evaluating DC maturation to characterize the immunogenic potential of Hyp-PDT treatment in GBM under these cell death settings.

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INSULIN SIGNALLING ROLE IN DOPAMINERGIC NEURON SURVIVAL

Alise Zagare (1), Ahmed Hemedan (1), Paul Antony (1), Enrico Glaab (1), Gemma Gomez-Giro (1), Rejko Krüger (1,2), Marek Ostaszewski (1), Giuseppe Arena (1), Jens C Schwamborn (1)

1) Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, 2) Transversal Translational Medicine, Luxembourg Institute of Health (LIH),

Parkinson's disease (PD) is not only one of the most common but also the fastest-growing neurodegenerative disorder, with its causes still unclear for most of the cases. Identifying risk factors for PD pathology is crucial for developing effective preventive or therapeutic strategies. Increasing evidence suggests that central insulin resistance plays an important role in PD pathology. However, it is unclear whether insulin resistance stems from external factors and lifestyle, comorbidities such as Type 2 diabetes, or occurs in the brain of PD patients independently from peripheral insulin resistance.

In this study, we investigate insulin resistance and its role in the pathogenesis and severity of Parkinson's disease associated with the GBA-N370S mutation. We used midbrain organoids generated from iPSCs of PD patients with the GBA-N307S heterozygous mutation (GBA-PD) and healthy donors, exposing them to varying insulin concentrations to alter insulin signalling activity. Additionally, we performed transcriptomics analysis to examine insulin signalling gene expression in GBA-PD and identify potential targets for rescuing GBA-PD-associated phenotypes.

Our results show that genes associated with the insulin signalling pathway are dysregulated in GBA-PD. Notably, we demonstrate that FOXO transcription factors are among significantly differentially expressed genes between GBA-PD and healthy controls. We also show that a knockdown of FOXO1 reduces the loss of dopaminergic neurons and cell death in GBA-PD midbrain organoids. Furthermore, our findings suggest that the anti-diabetic drug Pioglitazone shows therapeutic potential in decreasing dopaminergic neuron loss in GBA-PD. Overall, this study highlights that local insulin signaling dysfunction plays a substantial role in GBA-PD pathogenesis, exacerbating dopaminergic neuron death.

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INVESTIGATING α-SYNUCLEIN AGGREGATION IN PATIENT-SPECIFIC IMMUNOCOMPETENT MIDBRAIN ORGANOIDS IN PARKINSON'S DISEASE.

Elisa Zuccoli (1), Henry Kurniawan (1), Alise Zagare (1), and Jens C. Schwamborn (1)

1) Luxembourg Centre for Systems Biomedicine, University of Luxembourg

Parkinson's disease (PD) is, after Alzheimer's disease, the second most prevalent neurodegenerative disorder. The main pathological characteristic of PD is the degeneration of dopaminergic neurons in the substantia nigra pars compacta of the midbrain and the aggregation of a key protein, α-synuclein, with the appearance of the so-called Lewy pathology. Microglia, the innate immune cells of the brain, have been previously described to be critical for PD pathogenesis. They play a key role as the first line of defense of the CNS in case of infections, but they also play physiological role in synaptic homeostasis, maintenance, and functioning. We hypothesize that deregulated autophagy in PD patient-specific microglia contributes to their deleterious activity during disease progression. Here, we developed an in vitro assembloid model with the integration of patient-specific microglia in the midbrain organoid, derived from human induced pluripotent stem cells (hiPSCs). With this integration, we investigate the effect of microglia on dopaminergic neuron degeneration and disease-associated protein aggregation. For the phenotyping of the diseased model, we investigated the expression of microglia, midbrain specific markers, and α-synuclein-related markers via immunofluorescence staining, Western blot and FACS. Our findings revealed that the diseased microglia indeed exert an influence on the aggregation of disease-associated proteins in the assembloid model. Additionally, the results indicate that α-synuclein dysfunction adversely affects multiple pathways, including mitochondrial activity and oxidative stress. Furthermore, iPSC-derived microglia were CRISPR/Cas9 engineered with a fluorescent autophagy reporter, which is suitable for high content imaging and screening. Eventually, we will use this system to screen the available natural compound library for small molecule compounds targeting altered autophagy in PD-specific microglia.

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ADDITIVE STING ACTIVATION SHIFTS CISPLATIN TOXICITY FROM KIDNEY INJURY TO LIVER FAILURE BY GSDMD-DEPENDENT NETOSIS

Wulf Tonnus (1), Karolin Flade (1), Anne Brucker (1), Jorunn Becker (1), Francesca Maremonti (1), Shubhangi Gavali (1), and Andreas Linkermann (1)

1) Medizinische Klinik und Poliklinik 3, University Hospital CGC at the Technische Universität Dresden, Germany

Background: Activation of cGAS/STING signaling leads to both type I interferon production and NFkB-dependent transcription. During acute kidney injury (AKI), we recently described a population of innate immune cells expressing gasdermin D to be recruited to the surroundings of areas of acute tubular necrosis. Interestingly, these GSDMD+ cells seemed to feed back on the ongoing cell death, a concept termed necroinflammation. As recent work demonstrated a crucial role for free DNA and RNA fragments in the pathogenesis of AKI, we hypothesized that these might contribute to innate immune activation via the cGAS/STING pathway.
Methods: We treated both STING-deficient mice and C57Bl/6J controls with 20 mg/kg cisplatin +/- 3 mg/kg STING agonist diABZI. Survival and underlying organ injury were assessed for up to 48h. Different mouse strains deficient for cell death effectors were utilized for mechanistic insights, supported by studies in BMDMs.
Results: STING-deficient mice were indistinguishable from wild type controls upon cisplatin treatment. However, forced STING activation in this model led to rapid deterioration in wild type mice, which we found to be caused by acute liver failure. We found this to be dependent on regulated cell death, which was neither apoptosis, necroptosis, nor ferroptosis. Interestingly, GSDMD-deficient mice could not be further sensitized by STING-activation. Western Blot analyzes from primary tissue confirmed a correlation between type 1-IFN signaling, and GSDMD expression. Liver tissue demonstrated increased levels of proteolytically activated GSDMD, which correlated to aberrant NETosis leading to secondary hepatocyte necrosis. Studies in BMDMs yielded further insights into IFN-modulated activation of GSDMD in myeloid cells.
Discussion: We found STING to be critically involved to prime neutrophils for aberrant NETosis. In this second-hit model utilizing cisplatin-induced organ injury, NETosis led to secondary liver failure due to microthrombosis. Thus, this study provides further insight into the pathogenesis of septic organ injury.