CELL DEATH AND REGENERATION

TECHNISCHE UNIVERSITÄT DRESDEN

DFG

INVITED SPEAKERS

Junying Yuan	Harvard Medical School, Boston, USA
Pedro Friedmann-Angeli	University of Würzburg, Germany
Feng Shao	National Institute of Biological Science, Beijing, China
Tom Vanden Berghe	University of Antwerp and Ghent University, Belgium
Peter Vandenabeele	VIB-UGent Center for Inflammation Research, Ghent, Belgium
Mads Daugaard	University of British Columbia, Vancouver, Canada
Richard Kitsis	Albert Einstein College of Medicine Bronx, New York, USA
Peter Czabotar	The WEHI of Medical Research, Melbourne, Australia
Edward Mocarski	Emory University, Atlanta, USA
Bart Tummers	St. Jude Children's Research Hospital, Memphis, USA
Patricia Boya	Spanish National Research Council (CSIC), Madrid, Spain
Cristina Munoz Pinedo	IDIBELL Bellvitge Biomedical Research Institute, Barcelona, Spain
Sudan He	Chinese Academy of Medical Sciences, Suzhou, China
Carlo Croce	The Ohio State University, USA
Tania Watts	University of Toronto, Canada
Inna Lavrik	University of Magdeburg, Germany
Scott Lowe	Memorial Sloan Kettering Cancer Center, NY, USA
Mathieu Bertrand	VIB-UGent Center for Inflammation Research, Ghent, Belgium
Francis Chan	Duke University School of Medicine, UK
Judy Lieberman	Harvard Medical School, Boston, USA
Jochen Prehn	Royal College of Surgeons, Dublin, Ireland
Gerry Melino	MRC Toxicology Unit, Cambridge, UK
Seamus Martin	Trinity College Dublin, Ireland
Douglas Green	St. Jude Children's Research Hospital, Memphis, USA
Carol Prives	Columbia University, NY, USA
Mads Gyrd-Hansen	Ludwig Institute for Cancer Research, Nuffield Department of Medicine, University of Oxford, Oxford, UK
Brent Stockwell	Columbia University, NY, USA
Andreas Villunger	Medical University of Innsbruck, Austria
Alexei Degterev	Tufts University, Boston, USA
Stephanie Kreis	University of Luxembourg, Luxembourg
William Kaiser	UT Health San Antonio, USA
Kevin Ryan	Beatson Institute, Glasgow, UK
Shigekazu Nagata	Osaka University, Japan

CHAIR PERSONS

Markus Rehm

Amelio Ivano

Simone Fulda

Klaus-Michael Debatin

Boris Zhivotovsky

Patrizia Agostinis

WELCOME

Welcome to the ECDO 2019 Conference

It is our great pleasure to welcome you to the 27th conference of the European Cell Death Organization on the topic of: *"Cell Death and Regeneration"* from **September 25th-27th 2019** at the Hilton Conference Center, in the historical center of Dresden, Germany.

This years ECDO Conference will continue the long standing tradition of scientific excellence and lively debate. The format of the conference, with vivid poster sessions and many short oral communications selected from submitted abstracts, together with communal lunches, the conference dinner and social program, will create an ideal environment for stimulating discussions and contacts between participants at all levels.

Dagmar Kulms & Andreas Linkermann
Chairs of the 27th ECDO Conference on Cell Death

CONFERENCE VENUE

The conference will take place in the **Hilton Hotel Dresden.**

Addess conference hotel:

An der Frauenkirche 5
01067 Dresden
Germany
Phone: +49 351 86420

HOW TO GET THERE?

From Dresden Main Station

Pedestrian path along Prager Strasse to Altmarkt, cross Wilsdruffer Strasse and continue towards Neumarkt. In front of the Frauenkirche turn left and follow the signs to the Hilton Hotel (15 min walk)

Public transport

Tram (No. 3 dir. “Wilder Mann”; No. 7 dir. “Weixdorf”; No. 8 dir. “Hellerau”), exit at stop “Synagoge” and follow the signs to Frauenkirche /Hilton Hotel - 2,80 € per person/ single trip

From motorway

From north/east (Hamburg, Berlin, Bautzen) Across A13/14

Take exit No.81 Dresden Hellerau/city center. Follow B170/E55 towards city center / Prag. At the first set of traffic lights after the bridge Carolabruecke, turn left twice towards the river Elbe and follow the signs for the Hilton Dresden.

From west (Frankfurt/M, Munich, Leipzig) Across A4/A14

Take exit No.78 Dresden Old Town towards Dresden city center /B6 (approximately 4km) to the Semper Opera House / river Elbe. Follow the signs for the Hilton Dresden.

From south / Prag across E55

Take the B170 / E55 towards the city center. At the third set of traffic lights (just before the bridge) turn right towards the river Elbe and follow the signs for the Hilton Dresden.

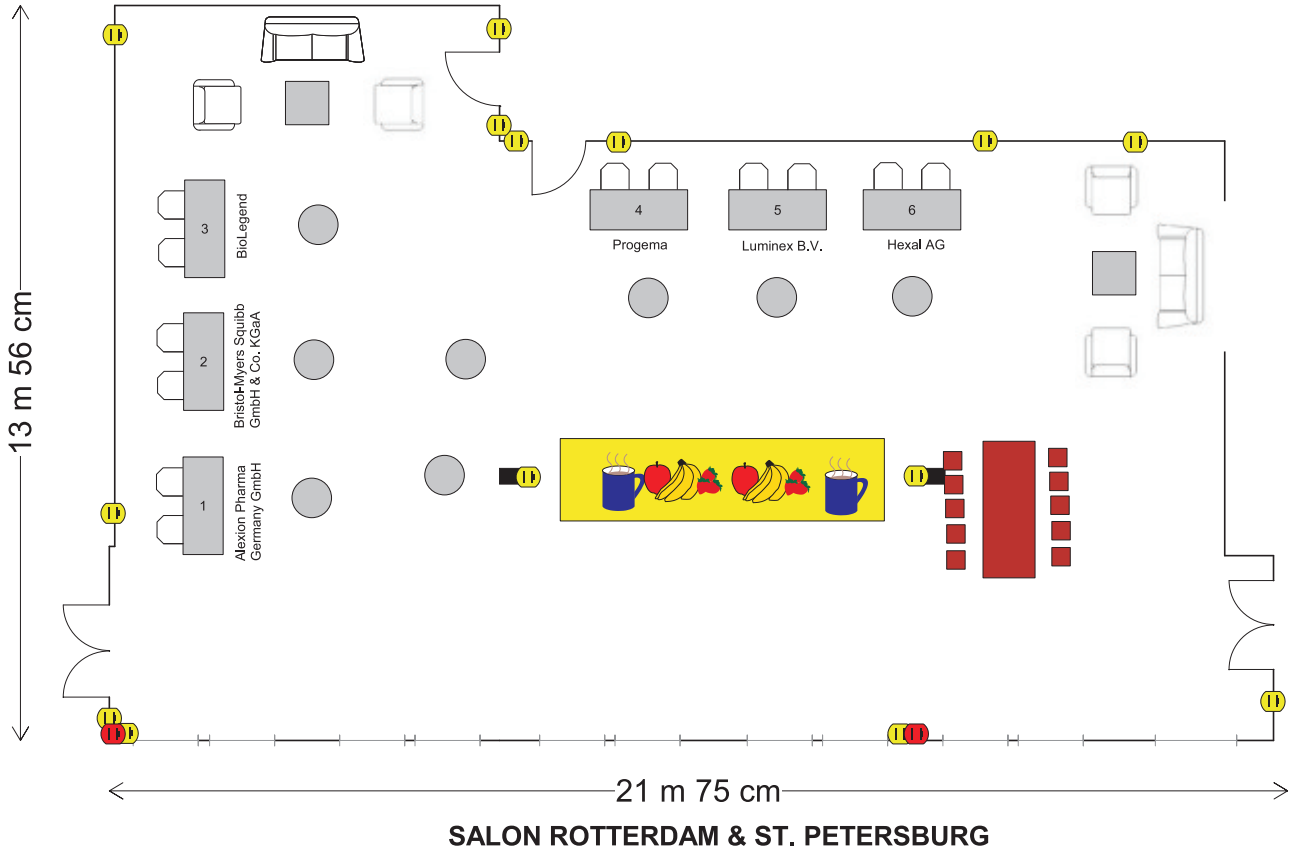
From the airport

Train No. S1 or S2 to Main Station, continue from there as stated above – 2,80 € per person/ single trip

Taxi about 25,00-30,00 € from Dresden airport to Hilton Hotel

By car follow airport road into town towards the city center. Keep straight and after the bridge “Carolabruecke” turn left, then left again towards the river Elbe and follow the signs for Hilton Dresden.

FLOOR PLAN



SALON ROTTERDAM & ST. PETERSBURG

EXHIBITORS

Alexion Pharma Germany GmbH

BioLegend GmbH

Bristol-Myers Squibb GmbH & Co. KGaA

Hexal AG

Luminex Munich GmbH

Progema GmbH

GENERAL INFORMATION

REGISTRATION DESK

The registration desk is located on the first floor.

Working hours:

24. September 2019	16:00 – 20:00
25. September 2019	08.00 – 18:00
26. September 2019	08:30 – 18:00
27. September 2019	08:30 – 18:00

INVITED SPEAKER AND ORAL PRESENTATIONS

We kindly ask you to provide your presentation on the USB stick to the technical specialist at the Presentation desk, at least two hours prier to the presention.

POSTER SESSIONS

Two poster sessions are scheduled during the meeting. Presenting authors are requested to be at their poster during their designated sessions.

Poster Session 1 (odd numbers):	Wednesday 20:00 – 22:00 (during the get together)
Poster Session 2 (even numbers):	Thursday 13:30 – 15:30 (during the lunch break)

Please mount your poster the day of presentation and remove it by the end of the session.

Delegates are neither allowed to take pictures from slides during oral presentations, nor from the posters without consent from the authors.

EXHIBITION

One important component of the event will be the exhibition of scientific companies, which will be located at Salon Petersburg & Rotterdam. Please visit our industrial partners there during the coffee breaks and any time you like.

GENERAL INFORMATION

MEALS

The get together on Wednesday evening (September 25) will be held in the piano bar at the **Conference Venue**. **All registered participants are kindly invited.**

Lunches on Wednesday, Thursday and Friday **and coffee breaks** are included in the registration fee.

Lunchtime

25. September 2019	13:00 – 14:30
26. September 2019	13:00 – 15:30
27. September 2019	13:30 – 15:30

Coffee breaks will be arranged at Salon Petersburg & Rotterdam

25. September 2019	17:15 – 17:45
26. September 2019	11:00 – 11:30
26. September 2019	17:00 – 17:30
27. September 2019	11:00 – 11:30
27. September 2019	17:00 – 17:30

The *Conference Dinner* will take place on Friday September 27 at 20:15 at the Restaurant “Sophienkeller”. The restaurant is in walking distance from the venue. Meeting point will be 20:00 at the Lobby of the Hilton Hotel.

CERTIFICATES OF ATTENDANCE

Certificates of Attendance will be provided in the conference bag.

QUESTIONS

If you have any questions, please do not hesitate to contact the registration desk.

CONFERENCE DINNER

The Conference Dinner will take place at the Restaurant “Sophienkeller” on Friday 27.09.2019, 20:15.

Situated in the historical centre of Dresden and close to most popular sights e.g. the famous Semper Opera and the Dresdner Zwinger you will find the restaurant “Sophienkeller”. It is furnished with tents, pavilions and a merry-go-round as the legendary festivity in a camp known as “Zeithainer Lager”. A variety of international dishes as well as German and Czech beer and a selection of wines will be served.

PROGRAM SCHEDULE

Wednesday | 25.09.2019

THE FUTURE OF CELL DEATH SCIENCE – THE YOUNG INVESTIGATOR PRESENTATIONS

Chair: Markus Rehm

08:30-10:30 **ECDO Board Meeting**

10:30-10:40 **Welcome and opening remarks by the organizers**

10:40-13:00 *(20 min each)*

Mathieu Bertrand - Ghent University, Belgium
Regulation of Ripk1 kinase-dependent cell death

Mads Daugaard - University of British Columbia, Vancouver, Canada
Function and regulation of the cancer glycocalyx

Christina Munoz Pinedo - Institut d'Investigació Biomèdica de Bellvitge, Bercelona, Spain
Starvation responses: beyond cell death

Alexei Degterev - Tufts University, Boston, USA
Therapeutic targeting of RIP kinases

Bart Tummers - St. Jude Children's Research Hospital, Memphis, USA
Mind Melt: FADD, caspase-8, MLKL, and inflammasome interactions in the control of lethal inflammation

Stephanie Kreis - University of Luxembourg
HNew ynergistic drug combinations effectively inhibit sensitive and drug-resistant melanoma cells

Jose Pedro Friedmann-Angeli - University of Würzburg, Germany
Characterization of a novel mechanism of ferroptosis suppression

13:00-14:30 **Lunch**

PROGRAM SCHEDULE

Wednesday | 25.09.2019

CELL DEATH AND IMMUNITY/INFLAMMATION

Chair: Ivano Amelio

14:30-15:15	**Keynote lecture 1** **Scott Lowe - Memorial Sloan Kettering Cancer Center, NY, USA** Restoring tumor suppression to advanced cancers
15:15-15:45	**Peter Vandenabeele - Ghent University, Belgium** Comparative analysis of immunogenic cell death (ICD) by apoptosis, necroptosis and ferroptosis
15:45-16:00	**Peter Krammer - German Cancer Research Center, Germany** The Annexin Check Point System
16:00-16:15	**Leonie Hartmann - Friedrich-Alexander-University, Germany** Mechanisms linking IFN-mediated mixed lineage kinase domain-like protein activation with intestinal inflammation
16:15-16:45	**Tania Watts - University of Toronto, Canda** TNFR–dependent survival signaling in lymphocytes during anti-viral immunity and cancer
16:45-17:15	**Mads-Gyrd-Hanssen - University of Oxford, UK** Regulation of innate immune signalling by the deubiquitinase CYLD
17:15-17:45	**Coffee break**
17:45-18:15	**Tom Vanden Berghe - University of Antwerb, Netherlands** Ferroptosis and inflammation research in critical illness
18:15- 18:30	**Daniel Frank - The WEHI of Medical Research, Melbourne, Australia** Post-translational regulation of RIPK3-induced cell death

PROGRAM SCHEDULE

Wednesday | 25.09.2019

18:30-18:50 *(2 min each)*

Divya Venkatesh - Columbia University, New York, USA
MDM2 and MDMX facilitate ferroptosis in the absence of p53

Stephanie Bleicken – Ruhr University, Germany
Towards structure determination of oligomeric Bcl-2 protein complexes using two-'color'-three-channel DEER

Ruoshi Peng - University of Oxford, UK
RIPK3 drives inflammatory signalling independently of cell death

Iris Stolzer - Friedrich-Alexander-University Erlangen, Germany
IFN-STAT1-MLKL axis drives necroptosis during gastrointestinal inflammation and infection

Stefan Küffer - University Medical Center, Göttingen, Germany
A Caspase 3 deletion with prognostic relevance in thymic epithelial tumors (TET)

Anja Krippner-Heidenreich - Princess Maxima Center for Pediatric Oncology, Utrecht, The Netherlands
Sensitisation of Smac-induced Cell Death by Tumour Necrosis Factor Receptor (TNFR) Type 2-mediated Signalling in T-cell Acute Lymphoblastic Leukaemia (T-ALL)

Nivetha Krishna Moorthy - University of Stuttgart, Germany
Development and testing of TRAIL variants intended for transcytotic delivery into the central nervous system

Graeme P. Sullivan - Trinity College Dublin, Ireland
The novel immune-regulatory cytokine Interleukin-37 exhibits pro-inflammatory activity upon N-terminal proteolytic processing

18:50-19:35

CDD honorary lecture
Carlo Croce - The Ohio State University, USA
MicroRNA dysregulation to identify targets for therapy in cancer

19:35-23:00

Poster session 1 (odd numbers) and get together

PROGRAM SCHEDULE

Thursday | 26.09.2019

CELL DEATH AND REGENERATION

Chair: Simone Fulda

09:00-09:30 **Kevin Ryan - Beatson Institute, Glasgow, UK**
Targeting metabolism to cause tumour cell death

09:30-10.00 **Sudan He – Chinese Academy of Medical Sciences, Suzhou, China**
Modulation of the tumor microenvironment by necroptosis regulators

10:00-10:15 **Elena I. Morgun - Moscow Institute of Physics and Technology, Russian Federation**
Caspase-3 Expression In The Model Of Chronic Skin Wound Regeneration

10:15-10:30 **Jinanbin Ruan - Harvard Medical School, Boston, USA**
Executioner of Pyroptosis: Revealing the Secrets of Gasdermins Driving Cell Death

10:30-11:00 **Edward Mocarski - Emory University, Atlanta, USA**
Inflammatory Consequences of Cell Death Pathways Suppressed by Herpesviruses

11:00-11:30 **Coffee break**

11:30-12:00 **Richard Kitsis - Albert Einstein College of Medicine, NY, USA**
Mechanistic connections between apoptosis and necrosis and combined small molecule therapeutic targeting of both

12:00-12:30 **Patricia Boya - Spanish National Resarch Consil, Spain**
New links between autophagy and cell death

12:30-12:45 **Motti Gerlic - Tel Aviv University, Israel**
Proteomic analysis of necroptotic extracellular vesicles

12:45-13:00 **Florian Bock - Beatson Institute, Glasgow, UK**
What doesn't kill you makes your neighbour stronger: paracrine resistance to chemotherapy

13:00-15:30 **Poster session 2 (even numbers) and lunch**

PROGRAM SCHEDULE

Thursday | 26.09.2019

CELL DEATH AND CANCER

Chair: Klaus-Michael Debatin

15:30-16:00 **Gerry Melino - MRC Toxicology Unit, Camebridge, UK**
ZNF281 contributes to the DNA damage response as well as to neuronal differentiation and is a prognostic maker for neuroblastoma

16:00-16:15 **Triona Ni Chonghaile - RCSI, Dublin, Ireland**
A small molecule screen in apoptosis resistant cancer cells identifies a new HDAC6 inhibitor

16:15-16:30 **Claudio Mauro - University of Birmingham, UK**
Metabolic control of immune-mediated inflammation

16:30-17:00 **Carol Prives - Columbia University, NY, USA**
Mdm2 and MdmX masters of life or death

17:00-17:30 **Coffee break**

17:30-18:00 **Andreas Villunger - Medical University of Innsbruck, Austria**
What doesn´t kill you makes you stronger - or resistant to cancer

18:00-18:15 **Lisa Bouchier-Hayes - Baylor College of Medicine, USA**
Caspase-2 regulates S-phase checkpoint activation to facilitate DNA repair

18:15-18:30 **Caitlin Brown - University of Massachusetts Medical School, USA**
Prominin2 Drives Ferroptosis Resistance by Stimulating Multivesicular Body/Exosome-Mediated Iron Export

18:30-19:15 **Keynote lecture 2**
Brent Stockwell - Columbia University, NY, USA
Ferroptosis: Mechanisms and Therapeutic Applications

20:00 **Dinner - Invited Speakers**

PROGRAM SCHEDULE

Friday | 27.09.2019

NON APOPTOTIC CELL DEATH MECHANISMS

Chair: Boris Zhivotovsky

09:30-10:00 **Francis Chan - Duke University School of Medicine, UK**
Necroptosis kinases in anti-viral immunity and tissue homeostasis

10:00-10:30 **William Kaiser - UT Health San Antonio, USA**
tba

10:30-10:45 **Anne Hamacher-Brady - Johns Hopkins University, Baltimore, USA**
Endolysosomal Interactions with Mitochondria Regulate BAX-Mediated Mitochondrial Permeabilization during Cell Death Signaling

10:45-11:00 **Benjamin Demarco - University of Lausanne, Switzerland**
Physiological function of caspase-3/-7-dependent inactivation of Gasdermin-D

11:00-11:30 **Coffee break**

11:30-12:00 **Junying Yuan - Harvard Medical School, Boston, USA**
Regulation of RIPK1 activation in neurodegenerative diseases associated with aging

12:00-12:30 **Seamus Martin - Trinity College Dublin, Ireland**
Cell Stress and Inflammation

12:30-13:00 **Feng Shao - National Institute of Biological Science, Beijing, China**
Innate immunity to cytosolic LPS: pyroptosis and beyond

13:00-13:30 **Douglas Green - St. Jude Children's Research Hospital, Memphis, USA**
LC3-associated endocytosis (LANDO): A noncanonical function of autophagy proteins regulates microglial activation and neurodegenerative disease

13:30-15:30 **Lunch**

PROGRAM SCHEDULE

Friday | 27.09.2019

STRUCTURAL AND SYSTEMS BIOLOGY IN CELL DEATH

Chair: Patrizia Agostinis

15:30-16:00 **Peter Czabotar - The WEHI of Medical Research, Melbourne, Australia**
Creating a Killer: Conversion of Bax and Bak into Membrane Permeabilising Executioners

16:00-16:15 **Marion MacFarlane - MRC Toxicology Unit, University of Cambridge, UK**
Molecular Architecture of FADD:Caspase-8 Signalling Complexes - co-ordinated Control of Life/Death Decisions

16:15-16:30 **Nadine Pollak – University of Stuttgart, Germany**
Mcl-1 governs sublethal caspase-8 activation and transmitotic resistance to extrinsic apoptosis

16:30-17:00 **Jochen Prehn - Royal College of Surgeons, Dublin, Ireland**
Combination of molecular subtyping and systems analysis of apoptotic and proliferative pathways predicts Cetuximab responses in patient-derived xenografts models of metastatic colorectal cancer

17:00-17:30 **Coffee break**

17:30-18:00 **Inna Lavrik - University of Magdeburg, Germany**
Targeting death receptor network using rationally designed chemical probes

18:00-18:30 **Judy Lieberman - Harvard Medical School, Boston, USA**
Microptosis: Programmed Cell Death in Microbes Activated by Killer Lymphocytes

18:30-19:15 **ECDO honorary lecture**
Shigekazu Nagata - Osaka University, Japan
Phosphatidylserine-dependent efferocytosis and entosis

19:15-19:45 **ECDO general assembly, meeting announcement, poster prizes, concluding remarks**

20:15- 00:00 **Conference Dinner**

Regulation of Ripk1 kinase-dependent cell death

M. Bertrand

VIB-UGent Center for Inflammation Research

The serine/threonine protein kinase RIPK1 has emerged as an important component of the immune response activated downstream of PRRs/TNFRs. As a scaffold, RIPK1 inhibits caspase-8-dependent apoptosis and RIPK3/MLKL-dependent necroptosis. As a kinase, RIPK1 paradoxically induces these cell death modalities. The molecular mechanism regulating the switch between RIPK1 pro-survival and pro-death functions has remained enigmatic. We identified ubiquitination-dependent phosphorylation of RIPK1 on Ser25 by IKKa/b as a key mechanism directly inhibiting RIPK1 kinase activity and protecting cells from TNF cytotoxicity. We demonstrate the importance of this regulatory mechanism in models of infection and inflammation.

Function and regulation of the cancer glycocalyx

M. Daugaard

University of British Columbia, Vancouver, BC, V5Z 1M9, Canada

The tumor glycocalyx consists of bulky glycoproteins and highly sulfated glycosaminoglycans (GAGs) associated with malignancy and therapy resistance. During disease progression, tumors reconfigure the glycocalyx, but the function and regulation of individual components remain obscure. Here we show that sulfated GAGs are regulated by hormones and required for progression of prostate cancer. Upon androgen deprivation or loss of hormone signaling activity, prostate cancer cells become dependent on sulfated GAGs to drive cell proliferation and motility. Inhibition of GAG sulfation leads to loss of cell-cell and cell-matrix anchorage and cell death. The androgen receptor (AR) represses the expression of sulfotransferase genes and when AR activity is lost, sulfotransferase expression is unleashed, shifting GAG composition into a highly sulfated state. Indeed, elevated sulfotransferase expression correlates with high Gleason grade, stage, risk, metastasis formation and predicts poor overall survival of prostate cancer patients. Our work functionally links GAG alterations with AR activity and prostate cancer progression.

Starvation responses: beyond cell death

F. Püschel[1], J. Redondo-Pedraza[1], E. Lucendo[1], R. Iurlaro[1], S. Marchetti[2], E. Nadal[1], J. Ricci[2], E. Chevet[3], C. Muñoz-Pinedo[1]

1 Cell Death Regulation Group, Oncobell Program, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet, 08908, Spain

2 Université Côte d'Azur, INSERM, C3M, Nice, France

3 Inserm U1242 «Chemistry, Oncogenesis, Stress, Signaling», Université Rennes 1, 35042 Rennes, France

Cells react to nutrient deprivation by adapting their metabolism, or if starvation is severe, they undergo cell death. Starvation of individual cells promotes adaptive, cell-cell responses to reorganize the tissue like angiogenesis. In order to explore other adaptive responses to nutrient deprivation, we analyzed secretomes from glucose deprived cancer cells before they die. We show that cancer cells from different origins upregulate multiple immune cytokines and chemokines while downregulating others. The upregulated cytokines include pro-inflammatory and pro-tumorigenic factors like Interleukin 6 and the chemokine and pro-angiogenic cytokine Interleukin 8 (CXCL8). These proteins are induced and secreted in a manner dependent on NF-κB and the transcription factor of the Integrated Stress Response ATF4. mTORC1 inhibition was not sufficient to induce this response, and IRE1/XBP1 did not participate. Glutamine deprivation and the anti-metabolic drugs 2-deoxyglucose and metformin also promoted inflammatory cytokine release. Conditioned medium from starved cells attracted macrophages and neutrophils, suggesting that starvation in the tumor may promote pro-tumorigenic inflammation and regulate the immune response in a paracrine manner.

Funding

This project has received funding from the BFU2016-78154-R grant from the Ministry of Economy and Competitiveness-MINECO, Spain (cofunded by FEDER funds/European Regional Development Fund – "a way to build Europe"), and European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreements No 675448 (TRAINERS) and No 766214 (META-CAN).

Therapeutic targeting of RIP kinases

B. Dai[1], L. Schlicher[2], M. Hrdinka[2], C. Suebsuwong[3], . Daniel M Pinkas[4], A. N Bullock[4], G. D Cuny[3], M. Gyrd-hansen[2], A. Degterev[1]

1 Department of Developmental, Molecular & Chemical Biology, Tufts University School of Medicine, Boston, United States

2 Nuffield Department of Clinical Medicine, Ludwig Institute for Cancer Research, University of Oxford, Oxford, United Kingdom

3 Department of Chemistry, University of Houston, Houston, United States

4 Nuffield Department of Clinical Medicine, Structural Genomics Consortium, University of Oxford, Oxford, United Kingdom

RIPK1, RIPK2, and RIPK3 kinases are homologous protein kinases that play distinct roles in the activation of inflammatory signaling and, in case of RIPK1 and RIPK3, regulated necroptotic and apoptotic cell death in response to ligands of different (TLR and NOD) families of innate immune Pattern Recognition Receptors. These kinases attracted significant interest due to their involvement in a broad range of inflammatory and necrotizing pathologies. Our recent efforts have focused on the development of inhibitors for the three RIP kinases and characterization of their non-trivial modes-of-action. Our presentation will summarize our data from these projects.

Mind Melt: FADD, caspase-8, MLKL, and inflammasome interactions in the control of lethal inflammation

D. R. Green, B. Tummers

St. Jude Children's Research Hospital, Memphis, TN 38139

Here's what you need to know. Mice lacking caspase-8 or FADD do not survive embryogenesis. Mice lacking MLKL or both caspase-1 and caspase-11 (C1/11) develop normally. Mice lacking MLKL and either caspase-8 or FADD (or both) develop normally, but eventually succumb to autoimmune lymphoproliferative syndrome (ALPS), characterized by expansion of $B220^{+}CD3^{+}$ T cells, which is also seen in mice or humans with defective CD95 or CD95L. We have known all this for some time and think we understand it all.

Here's another thing. Mice with a homozygous mutant caspase-8, F122G,L123G (FGLG), develop normally, and are resistant to agonistic anti-CD95 in vivo. These animals do not develop ALPS. Nor do animals that are caspase-8^{FGLG}, $MLKL^{-/-}$. Therefore, ALPS does not appear to be caused by a defect in apoptosis induced by CD95 signaling. This is supported by mice with a homozygous mutant allele of caspase-8, D387A, (caspase-$8^{DA/DA}$), which also develop normally, as do caspase-$8^{DA/DA}$, $MLKL^{-/-}$ mice, but again, neither displays ALPS. Caspase-$8^{DA/DA}$ mice are similarly resistant to agonistic anti-CD95 in vivo. In contrast, caspase-$8^{DA/DA}$, $MLKL^{-/-}$ mice are sensitive to the lethal effects of agonistic anti-CD95, but without ensuing apoptosis in the liver (unlike WT mice, which show extensive hepatocyte apoptosis). This inflammatory syndrome is not prevented by deletion of C1/11.

Caspase-8 participates in signaling by the so-called FADDosome, a complex including FADD and RIPK1, in response to death receptor signaling. Indeed, caspase-$8^{DA/DA}$, $MLKL^{-/-}$ mice have elevated cytokine expression not seen in WT or caspase-$8^{-/-}$, $MLKL^{-/-}$ mice. In line with this idea, removing one allele of either FADD or RIPK1 prevents the inflammatory disease in caspase-$8^{DA/DA}$, $MLKL^{-/-}$ mice.

But now it gets very strange. Caspase-$8^{DA/DA}$, $MLKL^{-/-}$ mice that also lack FADD all die by postnatal day 14 (P14), with massive macrophage ($F4/80^{+}$) inflammation of skin, lung, and gut (without bacteremia). This lethal effect is not seen in caspase-$8^{wt/wt}$, $FADD^{-/-}$, $MLKL^{-/-}$ mice (or in caspase-$8^{-/-}$, $FADD^{-/-}$, $MLKL^{-/-}$ mice).

This is all very confusing. But here is the "mind melt:" Postnatal death in the caspase-$8^{DA/DA}$, $FADD^{-/-}$, $MLKL^{-/-}$ mice depends on C1/11; caspase-$8^{DA/DA}$, $MLKL^{-/-}$, $FADD^{-/-}$, $C1/11^{-/-}$ mice are fine for several weeks. The early, lethal inflammatory disease in caspase-$8^{DA/DA}$, $FADD^{-/-}$, $MLKL^{-/-}$ mice is dependent on caspase-1 or caspase-11. Then they get ALPS.

Our results unambiguously show that caspase-8 generates signals, regulated by its cleavage, that are independent of FADD. We observed no defects in ASC oligomerization or association with caspase-$8^{DA/DA}$ in response to LPS/nigericin in caspase-$8^{DA/DA}$, $MLKL^{-/-}$, $FADD^{-/-}$, $C1/11^{-/-}$ macrophages. What is going on? We invite you to explore the possibilities with us.

New synergistic drug combinations effectively inhibit sensitive and drug-resistant melanoma cells.

C. Margue, D. Philippidou, I. Kozar, G. Cesi,[1], D. Kulms[2], D. Nashan[3], E. Letellier, C. Haan, S. Kreis.[1]

1 Life Sciences Research Unit, University of Luxembourg

2 Experimental Dermatology, Department of Dermatology, Technical University Dresden, Germany

3 Klinikum Dortmund, Dortmund, Germany

Melanoma is the most aggressive and deadly form of skin cancer with increasing case numbers worldwide. The development of inhibitors targeting mutated BRAF (present in 50-60% of melanoma patients) has markedly improved overall survival of patients with late-stage tumors, even more so when combined with MEK inhibitors targeting the same signaling pathway. However, invariably patients become resistant to this targeted therapy resulting in rapid progression with treatment-refractory disease.

We have recently found a new mechanism of acquired drug resistance involving the activation of a truncated form of ALK transported in extracellular vesicles (EVs). Knock down or inhibition of ALK re-sensitised resistant cells to BRAF inhibition and induced apoptosis while combined inhibition of ALK and BRAF dramatically reduced tumour growth in vivo.

Furthermore, a larger screening of a 274-compound kinase inhibitor library in 3 BRAF mutant melanoma cell lines (each one sensitive or made resistant to 2 distinct BRAFi) identified eight inhibitors targeting Wee1, Checkpoint kinase 1/2, Aurora kinase, MEK, Polo-like kinase, PI3K and Focal adhesion kinase, which killed melanoma cells synergistically when combined with a BRAFi. Combination of a Wee1 and Chk inhibitor showed synergistic killing effects not only on sensitive cell lines, but also in intrinsically BRAFi- and treatment induced-resistant melanoma cells. Interestingly, continuous treatment with several of these drugs, alone or in combination, did not lead to emergence of resistance.

Taken together, we have identified a novel mechanism of drug resistance transfer between cells and have found promising synergistic drug combinations, which together with the new immunotherapies could be an important step towards improved 1st and 2nd line treatments for late-stage melanoma patients.

Characterization of novel mechanism of ferroptosis suppression

F. Porto Freitas[1], S. Doll[2], B. Proneth[2], A. Schulze[3], A. Kurz[4], M. Sauer[4], A. Goya Grocin[5], E. William Tate[5], R. Shah[6], D. Pratt[6], M. Conrad[2]

1 Rudolf Virchow Center for Experimental Biomedicine - Julius Maximilian University of Würzburg

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Ferroptosis is an iron-dependent form of necrotic cell death marked by oxidative damage to phospholipids. To date, ferroptosis was believed to be restrained only by the phospholipid hydroperoxide-reducing enzyme glutathione peroxidase 4 (GPX4). The factors which underlie a given cell type's sensitivity to ferroptosis is critical to understand the pathophysiological role of ferroptosis and/or how it may be exploited for cancer treatment. Although metabolic constraints and phospholipid composition have been identified to contribute to ferroptosis sensitivity, no cell-autonomous mechanisms have yet been identified that account for ferroptosis resistance. Hence, we undertook an expression cloning approach to identify genes able to complement GPX4 loss. These efforts revealed a novel oxido-reductase as a previously unrecognized anti-ferroptotic gene. These gene, hereafter renamed ferroptosis-suppressor-protein 1" (FSP1), confers an unprecedented protection against ferroptosis elicited by the loss of GPX4. We further demonstrate that ferroptosis suppression by FSP1 is mediated via recycling of an endogenous antioxidant that is able to trap lipid peroxyl radicals which propagates the process of phospholipid peroxidation. Moreover, we demonstrate that pharmacological targeting of FSP1 strongly synergizes with GPX4 inhibitors and trigger ferroptosis in a number of cancer entities. Conclusively, FSP1 exists as a stand-alone parallel system, which co-operates with GPX4 and GSH to suppress phospholipid peroxidation and ferroptosis

Comparative analysis of immunogenic cell death (ICD) by apoptosis, necroptosis and ferroptosis

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Immunogenic cell death (ICD) is the combined result of adjuvanticity caused by the release of cytokines, chemokines and damage-associated molecular patterns (DAMPs) as well as antigenicity of dying tumor cells, which induce an anti-tumor immune response. Most stable cancer cell lines that are used for ICD studies express virally derived peptides that are recognized by the immune system as tumor-associated antigens (TAAs). Yet the impact of different cell death modalities on the response towards endogenous TAA remains largely understudied. Using a colon carcinoma cell line, CT26, transduced with different Tet-On inducible cell death systems, we found that both in the case of necroptosis, as of apoptosis, the in vivo immunogenicity of dead CT26 cells was independent of NF-κB activity. Interestingly, we found that the endogenous AH1 retroviral TAA expression in the CT26 cell line accounted entirely for the immunogenicity of apoptotic cancer cells. However, necroptotic AH1KO CT26 cells elicited no anti-AH1 immunity, as expected, but still revealed an anti-tumor immune response specific for a mixture of CD8- and CD4-specific neo-epitopes. Our results demonstrate that necroptotic, but not apoptotic, colon carcinoma cells are immunogenic even in the absence of the otherwise immunodominant AH1 tumor antigen expression. This underlines the larger potency of necroptosis as compared to apoptosis in the same cellular context in eliciting a host response to neo-epitopes and makes this cell death modality a strong candidate for anti-tumor immunotherapy. Ferroptosis is another necrotic form of cell death that depends on redox-active iron that mediates the formation of phospholipid peroxyl radicals through Fenton-type reactions and activation of lipoxygenases. Ferroptosis is proposed as a very effective way of killing drug-resistant cancer cells, even metastatic cells. We examined the immunogenic potential of ferroptosis in a tumor vaccination model and will report on the results obtained.

The Annexin Check Point System

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After about 15 years of work, we succeeded in developing a new immunological checkpoint system, the Annexin Checkpoint System. This system was developed in my laboratory and is unique worldwide. The Annexin Checkpoint System allows regulating the activity of the immune system in an antigen specific fashion on the molecular, biochemical and cellular level. Our results also comprise data from disease relevant animal experiments. The system makes it possible to downregulate highly increased immune activity in allergy and autoimmunity, and can reestablish peripheral immune tolerance. It also enables a high increase of anti-tumor immune responses.

In view of the efficiency of the checkpoint system and our years of work with this system, the Annexin Checkpoint System should be transferred into the clinic as soon as possible for the benefit of patients.

Papers describing the Annexin Checkpoint system are presently being prepared.

Mechanisms linking IFN-mediated mixed lineage kinase domain-like protein activation with intestinal inflammation

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Interferons (IFNs) are immune-modulatory cytokines expressed by epithelial and mucosal cells in response to viral and bacterial infection. Just recently, we discovered a correlation between IFN-λ expression and disease activity in human Crohn's disease (CD) patients. In addition, we have identified a new type of regulated necrosis that is IFN dependent and was executed by a mixed lineage kinase domain-like protein (MLKL)-dependent pathway but independent of RIPK3 and controlled by Caspase-8. Furthermore, IFN-λ-dependent non-apoptotic epithelial cell death was associated with strong MLKL up-regulation. This background leads us to the following hypothesis: IFN-λ induces MLKL-dependent programmed necrosis in the gut and this pathway drives intestinal inflammation in mice and in human IBD patients. Our central question of the current project is if and by which pathways interferon-regulated necrosis of epithelial cells contributes to intestinal inflammation and how these mechanisms could be targeted for future therapeutic intervention.

We here uncovered that gene expression of the cell death mediators Mlkl and Caspase-8 is dependent on IFN-λ-mediated JAK-STAT1 signaling. Therefore, pharmacological inhibition of this signaling pathway by different JAK-inhibitors appears to be a promising strategy to interfere with cell death pathways in the pathogenesis of intestinal inflammation. The non-specific pan JAK-inhibitor Tofacitinib is able to attenuate gene expression of Mlkl and Caspase-8 in vitro as well as in vivo. It prevents non-apoptotic as well as apoptotic cell death of small intestinal organoids stimulated with IFN-λ and is sufficient to prevent small intestinal tissue destruction in Casp8ΔIEC mice challenged with IFN-λ. Additionally, we use the selective JAK1-inhibitor Filgotinib to limit the targeted JAK-STAT signaling pathways to only JAK1-STAT1 signaling and thus reduce side effects of the inhibitor on other signaling pathways. This had a similar effect as Tofacitinib suggesting that IFN controls MLKL mediated cell death via JAK1.

In summary our results indicate that targeting IFN-λ-mediated JAK-STAT1 signaling by the small molecules Tofacitinib and Filgotinib impedes induction of Mlkl and Caspase-8 mediated cell death pathways. Therefore, it prevents the pathogenesis of small intestinal inflammation and thus maintains the barrier integrity.

TNFR–dependent survival signaling in lymphocytes during anti-viral immunity and cancer

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During an immune response, lymphocytes require signals through their antigen specific receptors as well as through additional co-stimulatory receptors in order to proliferate and survive. TNFR family members play key roles in providing NF-kappa B-mediated survival signals to lymphocytes. In this talk I will first discuss the role of the TNFR family member GITR in normal T cells during viral infection, and then I will turn to the role of TNFR signaling in chronic lymphocytic leukemia (CLL). The initial activation of T cells involves the recognition of antigen bound to MHC (signal 1), a costimulatory signal through CD28 (signal 2) as well as cytokines (signal 3), provided by dendritic cells in the draining lymph node. Recently, using the TNFR superfamily member 18 (GITR) and its ligand, TNFSF18 (GITRL), as proof of concept, we showed that TNF family ligands expressed on monocyte-derived APC, rather than on classical dendritic cells provide an important post-priming checkpoint, signal 4, for T cell survival during chronic viral infection (Chang et al., Immunity, 2017). More recently, we demonstrated that GITR/GITRL (signal 4), in the lung tissue is important in allowing effector T cells to survive, accumulate and give rise to tissue resident memory T cells in the lung during acute influenza virus infection (Chu et al. Mucosal Immunology, 2019). While TNFR family members are critical in promoting protective immunity, they can also contribute to leukemia cell survival. TNFR family members play important roles in driving NF-kappa B activation in B-CLL, however little has been done to target this signaling axis. Here we show that the protein kinase C related kinase PKN1 is required to protect the pro-survival signaling adaptor TRAF1 from degradation during constitutive CD40 signaling. Accordingly, treatment of patient B-CLL cells with a PKN1 inhibitor lowered TRAF1, Mcl-1 and Bcl-xL protein levels with concomitant increases in levels of activated caspase 3 and cell death. B-CLL cells that survive treatment with the Bcl-2 antagonist venetoclax are enriched for TRAF1hi cells. We further show that combined treatment with venetoclax and a PKN1 inhibitor results in increased cell death, correlating with loss of TRAF1, Mcl-1, and Bcl-xL. These findings identify TRAF1 as a mediator of venetoclax resistance and PKN1 as a new target for B-CLL.

Regulation of innate immune signalling by the deubiquitinase CYLD

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Innate immune receptors facilitate activation of NF-kappaB-mediated inflammation and other host-defence processes designed to control infections, but these processes also contribute to chronic inflammatory diseases and cancer. Signalling by innate immune receptors rely on the generation of non-degradative polyubiquitin chains linked via the N-terminal methionine (Met1-linked ubiquitin chains, also termed linear ubiquitin) and Lys63-linked ubiquitin chains. Met1-linked ubiquitin chains are generated by the ubiquitin ligase LUBAC (linear ubiquitin chain assembly complex) and are disassembled by two deubiquitinases; OTULIN and the cylindromatosis tumour suppressor CYLD. We recently discovered that CYLD forms a stable complex with LUBAC via the adaptor protein SPATA2, and found that this complex is recruited to receptor signalling complexes where it regulates ubiquitination and receptor signalling. I will discuss our on-going investigations of the regulation of innate immune signalling by the LUBAC-SPATA2-CYLD complex.

Ferroptosis and inflammation research in critical illness

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The complexity of critical illness in intensive care units essentially requires a precision approach. Organ failure is one of the key detrimental factors in critical illness, and fundamentally driven by an auto-amplifying loop of cell death and inflammation. This process feeds dynamic disease fluctuations and heterogeneity in critical care, which might partially explain inconsistent translatability. Patients with similar clinical presentations typically have different cellular and molecular responses due to individual genetic differences and co-morbidities. To deal with this form of heterogeneity, a precision approach is needed to allow patient stratification. There is a growing list of detrimental circulating biomolecules related to cell death and inflammation, which potentially could be novel biomarkers for stratification of critically ill patients. To pave the way for precision medicine v2.0, we are currently dynamically monitoring ferroptosis and inflammation in plasma to stratify critically ill patients for treatment with new ferroptosis and/or inflammation intervention strategies. This interdisciplinary approach aims at answering some key questions: Is there a molecular and/or cellular signature in biofluids that reflects organ or systemic dysfunction? Which circulating biomolecules and/or immune cell profiles have prognostic value for disease progression, sepsis, multi-organ failure and/or mortality? Is there therapeutic value in targeting ferroptosis and/or inflammation in context of critical illness?

Post-translational regulation of RIPK3-induced cell death

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Receptor Interacting Serine/threonine Kinase 3 (RIPK3) signalling is important for the protection against infections, but can also be pathologically activated in various inflammatory disorders. Although RIPK3 is mostly studied for its critical role in activating the terminal effector of necroptotic cell death, Mixed Lineage Kinase Domain-Like Protein (MLKL), recent research has revealed that RIPK3 can also signal apoptosis, or activate the inflammasome protein complexes, to cause disease. Despite these roles in animal disease models, the post-translational control of RIPK3 signalling is not fully understood. Using mass-spectrometry, we have identified a novel ubiquitylation site on a lysine residue beyond the RHIM (BTR) domain of murine RIPK3. The complementation of RIPK3-deficient cells with wildtype RIPK3, or a RIPK3 BTR mutant unable to be targeted for ubiquitination (lysine to arginine substitution; RIPK3 BTRKtoR), demonstrated that ubiquitination of RIPK3 BTR is required to limit phosphorylation of RIPK3, subsequent MLKL activation, and hence necroptotic cell death. The enhanced cell death resulting from the loss of RIPK3 BTR ubiquitination occurred in the absence of any upstream signals, such as TNF, and was spontaneously triggered by expression of RIPK3 BTRKtoR alone. Loss of ubiquitination in RIPK3 BTR increased RIPK3 stability, suggesting that RIPK3 BTR ubiquitination suppresses RIPK3 activity, at least in part, by targeting RIPK3 for degradation. Surprisingly however, the total ubiquitylation of the activated RIPK3 BTRKtoR mutant was enhanced, as was traditionally activated endogenous RIPK3, which correlated with enhanced migration of activated RIPK3 into Triton X-insoluble membrane fractions prior to cell death. Therefore, ubiquitylation of RIPK3 BTR may also act to prevent RIPK3 from being modified with activating ubiquitin chains on alternate lysine residues. Current studies are examining the in vivo consequences of RIPK3 ubiquitination BTR via analysis of CRISPR knock-in RIPK3 BTRKtoR mice.

MicroRNA dysregulation to identify targets for therapy in cancer

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We have shown that loss of miR-15/16 genes on chromosome 13q14 is the most common alteration in CLL and then shown that miR-15/16 target BCL2, a gene overexpressed in most CLLs. We have also shown that miR-15/16 also target RORI, and that venetoclax, the anti Bcl2 drug, and monoclonal antibodies against RORI are synergistic in killing CLL cells. An additional miR-15/16 locus exists on chromosome 3q26. We have knock out this additional locus in the mouse and the KO mice developed predominantly CLL.

Recently we have knock out both miR-15/16 loci and observed that 77 % of the double KO mice developed AML.

Then we discovered that a large fraction of AMLs lose the expression of both mirR-15/16 loci leading to AML. Thus AMLs with low miR-15/16 expression should be treated with venetoclax and monoclonal antibodies against RORI.

Targeting metabolism to cause tumour cell death

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It is now well established that tumours alter their cellular metabolism in order to meet their energetic and metabolic needs. This can range from changes in the metabolism of external nutrients to changes in the availability and utilization of intracellular constituents through processes like autophagy. One common feature of many cancer is their enhanced uptake and increased dependency on glucose. This facet of tumours is so common that it is frequently used as diagnostic tool in which labelled forms of glucose are used to detect tumours with the aid of positron emission tomography. Due to this elevated demand and need for glucose many strategies have been developed to interfere with the uptake and metabolism of this important sugar, and these strategies have also been shown to regulate cell death. Recently, we have shown that the simple natural sugar mannose also interferes with the metabolism of glucose, causing a reduction in tumour cell growth both in vitro and in vivo. Moreover, we have found that mannose can enhance tumour cell death in combination with conventional forms of chemotherapy. This effects involves enhancement of apoptosis via modulation of members of the Bcl2 family. Recent insights into the effects of mannose in cancer and how it can be utilized to promote cell death will be discussed.

Modulation of the tumor microenvironment by necroptosis regulators

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Necroptosis is a tightly regulated form of necrosis that requires the activation of receptor-interacting protein (RIP) kinases RIP1 and RIP3, as well as the RIP3 substrate MLKL (mixed lineage kinase domain-like protein). Necroptotic cells release cellular contents including damage-associated molecular patterns (DAMPs) that evoke immune responses. The RIP1/RIP3 necrosome has been reported to promote pancreatic oncogenesis. Here, we demonstrate that RIP3 deficiency but not inhibition of RIP1 kinase activity suppresses tumor growth in syngeneic mouse tumor models. Loss of RIP3 promotes the infiltration of immune cells into tumor tissues. Compared to wild type mice, RIP3 deficient mice exhibit increased frequencies of cytotoxic CD8+ T cell and enhanced cytotoxicity of effector T cells in tumor microenvironment. Therefore, inhibition of RIP3 enhances the anti-tumor immune response and contributes to tumor suppression. The mechanism underlying RIP3-mediated modulation of tumor microenvironment will be discussed.

Caspase-3 Expression In The Model Of Chronic Skin Wound Regeneration

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Objective: To study expression of apoptosis marker caspase-3 during chronic skin wound regeneration.

The aim of the work is to evaluate caspase-3 expression in chronic wound regeneration under the influence of living skin equivalent (LSE) transplantation and in untreated controls.

Materials and methods: Balb/c mice were used in this study. Manipulations with mice were done under anesthetics and with acceptance of the Ethic committee. LSE was created by cocultivating of donor mouse keratinocytes with mesenchymal stem cells on the matrix, containing hyaluronic acid and collagen type I. Animals were divided into three groups: «control», «matrix», and «LSE». The H-shaped 10×30 mm skin flap with full-thickness circular wound in the middle was inflicted onto the back of an animal. The wound healing phases were studied on day 5, 7, 14 and 21 by methods of histology; expression of apoptosis marker was revealed by immunohistochemical detection of caspase-3.

Results: Histological analysis showed the inflammatory phase on day 5; proliferation and maturation of granulation tissue were observed on days 7-14; epithelization and mature scar formation indicated the complete wound healing on day 21. Immunohistochemical analysis showed a few caspase-3+ cells in the wounds of all animal groups on the days 5-7, and the high expression of caspase-3 at the wound edges on days 14-21. However, in the wound bed, the caspase-3 expressing cells were still few. We suppose that apoptosis of the wound bed cells during the wound healing terminates due to the other effector caspase.

Conclusions: Apoptosis marker caspase-3 is expressed in the wound edges during proliferative and remodeling phases of chronic wound regeneration.

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Executioner of Pyroptosis: Revealing the Secrets of Gasdermins Driving Cell Death

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Gasdermin D (GSDMD) is a recently identified downstream effector of inflammasomes. It contains a functionally important N-terminal domain (GSDMD-NT) that is shared in the gasdermin family. Inflammatory caspases specifically cleave the linker between GSDMDNT and GSDMD C-terminal (GSDMD-CT) domains. Upon cleavage, the GSDMD-NT specifically binds to phosphatidylinositol phosphates (PIPs), phosphatidylserine (PS) and cardiolipin, and exhibits strong membrane-disrupting cytotoxicity in mammalian cells. However, the molecular mechanism of GSDMD-NT pores assembly remains to be understood. Here we present the cryo-electron microscopy structures of the 27-fold and 28-fold single-ring pores formed by the N-terminal fragment of mouse GSDMA3 (GSDMA3-NT) at 3.8 and 4.2

Inflammatory Consequences of Cell Death Pathways Suppressed by Herpesviruses

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Herpesviruses encode cell death suppressors to sustain infection and restrict inflammation in their mammalian hosts. Virus-induced apoptosis and necroptosis have been revealed through studies of cell death suppressor mutant viruses showing the critical contribution of cell-autonomous death signaling in the elimination of infected cells and as drivers of inflammation. During cytomegalovirus infection, programmed cell death triggered either via mitochondrial stress, resulting in activation of serine protease Htra2 (also called Omi) is suppressed by the viral mitochondrial inhibitor of apoptosis (vMIA), which targets Bax and Bak. Apoptosis that results from autoactivation of caspase-8 is suppressed by the viral inhibitor of caspase-8 apoptosis (vICA). Necroptosis is an alternate, lytic pathway triggered via RHIM-dependent activation of receptor interacting protein kinase (RIPK)3, resulting in RIPK3 phosphorylation of MLKL, which leads to membrane rupture after allosteric activation by highly phosphorylated forms of inositol phosphate (IP5/IP6). Necroptosis has emerged as a highly potent means to eliminate infected cells when pro-apoptotic caspase-8 activity has been compromised. Necroptosis is triggered by the pathogen sensor Z-nucleic acid binding protein (ZBP)1 (also called DAI and DLM1) in the course of infection with cytomegaloviruses and herpes simplex viruses, as well as poxviruses and some RNA viruses. The resulting virus-induced ZBP1-RIPK3-MLKL pathway is naturally blocked by RHIM-signaling competition through the action of the herpesvirus-common homolog of ribonucleotide reductase, MCMV M45, encoding the viral inhibitor of RIP activation (vIRA) or HSV UL39, encoding ICP6. Either of these prevents recruitment and activation of RIPK3 by ZBP1 in the natural host species. In contrast, vaccinia E3L encodes a competitor of nucleic acid recognition by ZBP1 that is separate from the domain that inhibits protein kinase R (PKR). Necroptosis-sensitive mutants of MCMV or vaccinia cannot infect mice due to the uniform death of cells at sites of inoculation. Inflammatory outcomes are influenced by the pattern of cell death in experimental settings, as well as the cell types that are infected. Cell death impacts subsequent patterns of adaptive immunity. Necroptosis represents an important, but underappreciated innate host defense mechanism capable of preventing virus infection and dissemination in mammals. Death pathways contribute to inflammatory disease outcomes and influence both levels and duration of adaptive immunity.

Mechanistic connections between apoptosis and necrosis and combined small molecule therapeutic targeting of both

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Cell death is mediated by multiple programs. While each is usually studied in isolation, these programs arose over evolution in a presumably coordinated manner. In addition, they share common molecular components and mechanisms. An important challenge in the field is to understand how the various programs are coordinated and why a particular program predominates in the case of cellular stresses that have the potential to activate multiple routines. We are interested in connections between apoptosis and necrosis at the mitochondria. One unifying component is the BCL-2 protein BAX, which mediates apoptosis through permeabilization of the outer mitochondrial membrane and necrosis through potentiating Ca2+-induced opening of the permeability transition pore in the inner mitochondrial membrane. I will discuss mechanistic connections between these programs and the design of selective small molecule BAX inhibitors that abrogate both apoptotic and necrotic cell death. I will also discuss the application of these inhibitors to prevent a common disease paradigm that involves both apoptotic and necrotic cell death: heart failure from the anthracycline chemotherapeutic doxorubicin.

New links between autophagy and cell death

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The retina provides an excellent model system to study autophagy in the context of neural development, and to establish its relationship with proliferation, differentiation and cell death. Autophagy is a lysosomal degradative pathway that allows the recycling of cell constituents, and plays important roles during neurodevelopment. We have demonstrated that the elimination of mitochondria by autophagy is essential for retinal ganglion cell differentiation by regulating a metabolic change towards glycolisis. At earlier embryonic stages we also have shown that autophagy contributes to retinal neurogenesis providing energy for cell corpse removal after physiological cell death, a process associated with retinal neurogenesis. We now extend these findings to mouse models with autophagy deficiency, and demonstrate that autophagy-deficient microglia engulf dead cells but they are not capable of degrading them and thus the apoptotic bodies accumulate inside activated microglia. In addition, we show that autophagy proteins regulate a LC3-associated phagocytosis, a non-canonical autophagy pathway that recruits LC3 to single membranes during phagocytosis, know as LAP (LC3-associated phagocytosis). A mouse model that shows just deficiency in LAP, but not in general autophagy displays accumulation of apoptotic bodies inside microglial cells and defects in their degradation. Thus, a non-canonical autophagy pathway is essential for cell corpse removal during physiological cell death associated with retinal neurogenesis.

Proteomic analysis of necroptotic extracellular vesicles

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Necroptosis is a regulated but inflammatory form of cell death. As with necrosis, necroptosis is characterized by cell swelling and membrane permeabilization, resulting in the release of cell content containing pro-inflammatory molecules. This was believed to be the major driving force of necroptosis-mediated inflammation. We and others have previously reported that necroptotic cells release extracellular vesicles (EVs). This process is initiated during the early stage of necroptosis, while the plasma membrane is still intact. We found that necroptotic EVs are 0.1-0.4 um in size, and are loaded with proteins, including the key necroptosis executor factor, phosphorylated mixed lineage kinase domain-like (MLKL). However, the exact necroptotic EVs proteins composition and impact have not been delineated yet. Here, we show that necroptotic EVs expose phosphatidylserine, and can be phagocytosed by peritoneal macrophages. This, in turn, led to modulation of chemokine and cytokine secretion. To characterize their content, EVs from necroptotic and untreated U937 cells were isolated by ultracentrifugation and analyzed by mass spectrometry. A total of 3648 proteins were identified, sharing ~95 % with the exosome proteome database, Vesiclepedia, and ~70 % with the Exocarta data base, including 25 of 30 the most frequently identified proteins. Principal component analysis revealed that the necroptotic and untreated EVs samples were clustered separately. 304 proteins were significantly abundant in the necroptotic EVs. Among these, are MLKL (q-value 0.003) and caspase-8 (q-value 0.00001). Noticeably, MLKL abundance confirmed our previous reports, and was validated by western blot. Gene Ontology (GO) analysis of the differentially abundant proteins demonstrated the significant enrichment of terms related to the endosomal sorting complexes required for transport (ESCRT) machinery (Cellular Component, q-value 0.0000004). This supports the reported role of ESCRT components in the budding of MLKL-damaged plasma membrane, while also suggesting the involvement of new unstudied components of this machinery, as well as other vesicular trafficking proteins. The GO enrichment term: Immune Effector Process was also found to be enriched (Biological Process, q-value 0.00004), with candidates suggestive to facilitate cell-cell communication during necroptosis. Among these are interleukin-32 (q-value 0.0006), a pro-inflammatory cytokine with an unknown release mechanism, and the calcium-dependent phospholipid scramblase, TMEM16F (q-value 0.008), with an inconclusive involvement in necroptosis. In summary, our study reveals a new layer of regulation during the early stage of necroptosis by the secretion of specific EVs that may influence their microenvironment. Our future investigation aims to shed light on new players in the necroptosis signaling and its related EVs, as well as to uncover the functional tasks accomplished by the cargo of these necroptotic EVs.

What doesn't kill you makes your neighbour stronger: paracrine resistance to chemotherapy

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Conventional and targeted anti-cancer therapies, such as BCL-2 inactivating BH3-mimetics, work by engaging tumour cell apoptosis. However, resistance to cell killing therapies represents a key hurdle to effective cancer treatment. My aim is to understand cancer cell resistance to cell death using BH3-mimetics as a paradigm. A common form of resistance is the upregulation of BCL-2 family members to inactivate BH3 mimetics, mediated by selection pressure on the cancer cell population. This eventually leads to the outgrowth of tumours unresponsive to BH3 mimetics and potentially worse prognosis. However, I find that treatment with BH3 mimetics, as well as overexpression of BH3-only proteins, can promote transient and reversible upregulation of BCL-2 proteins (BCL-2, MCL1 and BCL-Xl), arguing against selection as the underlying factor. Furthermore, experiments using conditioned medium showed that upregulation can be transferred to neighbouring cells, pointing towards a secreted ligand to promote activation of MEK/ERK and subsequent transcriptional upregulation of BCL-2 family proteins. Accordingly, the transfer of conditioned medium can promote resistance to chemotherapeutic drugs in a MEK/ERK dependent manner. These data suggest that stressed cells can signal to their microenvironment to promote the survival of neighbouring cells - this may have important implications for physiological engagement of apoptosis. Similarly, chemotherapeutic treatment can increase paracrine resistance against BH3-mimetics or other apoptotic drugs. Inhibiting this effect may represent a promising strategy to prevent the emergence of chemoresistance.

ZNF281 contributes to the DNA damage response as well as to neuronal differentiation and is a prognostic maker for neuroblastoma

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ZNF281 is a zinc-finger factor affecting cell death, which expression increases after genotoxic stress caused by DNA-damaging drugs. ZNF281 silencing significantly increases cell death upon chemotherapy in vitro. Here, it regulates XRCC2 and XRCC4, two genes that take part in homologous recombination and non-homologous end joining, respectively. These are transcriptionally activated by ZNF281 through a DNA-binding-dependent mechanism, involving an interaction with c-Myc. Conversely, ZNF281 decreases during neuronal differentiation of embryonic stem cells and in retinoic acid-induced differentiation of neuroblastoma cells (NB). Silencing of ZNF281 induces neuronal differentiation of NB cells and its ectopic expression causes the opposite effect. Furthermore, the expression of ZNF281 is inhibited by TAp73 through miR34a. Contrariwise, MYCN promotes the expression of ZNF281 at least in part by inhibiting the expression of miR34a. These findings imply a functional network that includes p73, MYCN and ZNF281 in NB cells where ZNF281 acts by inhibiting neuronal differentiation. Array analysis of NB cells silenced for ZNF281 expression identified GDNF and NRP2 as two transcriptional targets inhibited by ZNF281. Bioinformatic analysis of NB datasets indicates that ZNF281 is a prognostic marker of aggressive, undifferentiated NB. These observations suggest that ZNF281 is a regulator of cell death during DNA Damage Response as well as of neuronal differentiation, with relevant implication in cancer. Finally, we identify a novel role for the zinc-finger protein ZNF281 in regulating the ordered recruitment of the NHEJ repair factor, XRCC4, at damage sites. ZNF281 is recruited to DNA lesions within seconds after DNA damage through a mechanism dependent on poly-ADP ribose polymerase (PARP) activity and on its DNA binding domain.

A small molecule screen in apoptosis resistant cancer cells identifies a new HDAC6 inhibitor

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Multi-drug resistance remains one of the greatest challenges in oncology. One of the identified major contributors to drug resistance is resistance to cell death signaling. We set up a screen to identify novel targets in apoptotic resistant cancer cells. We performed a high-throughput screen to identify small molecules that are cancer selective but can kill apoptosis-resistant TNBC cells. We screened a total of 30,000 compounds in duplicate across the cell line in which the pro-death proteins BAX/BAK were knocked down, and a non-transformed cell line. There was a hit rate of 0.3% in the screen and 85 compounds were retested in the validation cherry pick screen. From this screen 18 compounds were further validated with low-throughput assessment for mitochondrial independent killing and selectivity for cancer cells.

To identify the mechanism of action of the lead compound we used a genetic approach to generate an RNAi signature for the compound, which indicated the lead compound was most likely that of histone deacetylase inhibitors (HDAC). From a series of novel unbiased screening approaches, we identified a new histone deacetylase 6 inhibitor (HDAC6i), BAS-2, which in vitro only inhibited HDAC6. Using an in vitro HDAC inhibitor screen, we identified that the compound specifically inhibited HDAC6.

Current known substrates of HDAC6 include proteins involved in cell motility and protein homeostasis. Using the specificity of BAS-2 we compared the acetylome following both HDAC6 inhibition and HDAC6 knockdown in breast cancer cells and found remarkable overlap. We also measured the interactome and found for the first time that HDAC6 bound to and altered the acetylation of four key glycolytic enzymes. Both knockdown and inhibition of HDAC6 confirmed the new role for HDAC6 in altering glycolytic metabolism in cells in vitro. HDAC6 inhibition as a single agent reduced tumor volume in both breast and lung cancer models and remarkably also reduced glycolytic metabolites in vivo. Using unbiased screening approaches we have identified a new HDAC6i that kills apoptotic resistant cells. Targeting vulnerabilities of the deregulated metabolic pathways through inhibition of HDAC6 opens up potential new therapeutic regimens for the treatment of both breast and lung tumors.

Metabolic control of immune-mediated inflammation

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The research in my group focusses on the interconnections between metabolic and inflammatory pathways and how systemic and cellular metabolic alterations in diseases with an inflammatory component lead to aberrant immune cell responses, which favour both the establishment and the propagation of inflammation. In particular, we investigate the mechanisms of metabolic control of T cell-mediated immune responses, including migration, differentiation and cytokine production in physiology and under metabolic stress.

One key area of our research is how small metabolites, like lactate, which accumulates locally in the inflamed tissue or systemically during acute and chronic inflammation, can impact the fate of the immune-inflammatory response via induction of intracellular metabolic rewiring with immediate effect on broad range human diseases. I will present our latest data, which are at a final stage of revision.

Another important aspect of the research is how fatty acids can impact the outcomes of an immune response, with repercussions on obesity-related diseases. I will present our latest data on the impact of omega-3 fatty acids on T cell responses in the adipose tissue, again data at a final stage of revision prior to publication.

Our published studies so far indicate that interfering with metabolic pathways (i.e., lipid, glucose and oxidative metabolism) alters immune cell effector functions and can be exploited for therapy (PLoS Biol 2015, Cell Metab 2017, Nat Comm 2017).

Mdm2 and MdmX masters of life or death

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Mdm2 and MdmX, negative regulators of the p53 tumor suppressor protein, can work separately and as a heteromeric complex to restrain p53 functions. Mdm2 also has pro-oncogenic roles in cells, tissues, and animals that are independent of p53. There is less information available about p53-independent roles of MdmX or the Mdm2-MdmX complex.

We have discovered that several human cancer cell lines that do not express p53 require Mdm2 and MdmX for growth and survival. Specifically, upon siRNA or pharmacological ablation of Mdm2 or MdmX, cells undergo arrest and in some settings cell death. The presence of cancer-related mutant forms of p53 does not rescue the impact of Mdm2/MdmX ablation. In some cases loss of Mdm2 is accompanied by loss of p73. Remarkably, in these cells, siRNA ablation of p73 also leads to cell cycle arrest suggesting that maintaining p73 levels is one mode by which Mdm2 promotes cell cycle progression. Identification of the isoform(s) of p73 required for maintaining cell cycle is under investigation.

In another setting we have discovered that Mdm2 and MdmX can facilitate the form of cell death known as ferroptosis by which cells die as a result of iron-dependent lipid peroxidation. Small molecules antagonists of the Mdm2-X complex or RNA interference reagents that ablate Mdm2 or MdmX counteract the ability of ferroptosis inducers to promote cell death. On the other hand, MdmX over-expression can increase ferroptotic death. Relevantly, we found that Mdm2 and MdmX alter the lipid profile of cells to favor ferroptosis. Additionally Mdm2 or MdmX antagonists increase levels of the antioxidant coenzyme Q10 suggesting that Mdm2 and MdmX play roles in cellular defense against lipid peroxidation. Mechanistically, the activity of PPARα, a master transcriptional regulator of lipid synthesis, is needed for Mdm2 and MdmX to promote ferroptosis. Our data suggest that the Mdm2-MdmX complex regulates lipids through altering PPARα activity rather than PPARα levels. These findings reveal the complexity of cellular responses to Mdm2 and MdmX in cancer cells and present the possibility that these two proteins may also have roles in degenerative diseases in which ferroptosis has been implicated.

What doesn´t kill you makes you stronger - or resistant to cancer

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The pro-apoptotic BCL2 family member BIM plays critical roles in immune cell development and acts as a barrier against autoimmunity and malignant disease. These functions have first been revealed in loss-of-function studies using gene-modified mice and their relevance for human disease has been confirmed. Gain-of-function studies, however, have so far not been deemed possible based on experiences made when overexpressing BH3-only proteins, such as BIM, that proved to be highly toxic. Using a genetic trick to increase endogenous BIM levels by less than 50% over endogenous we generated SUPER-BIM mice. This was achieved by deleting a set of miRNA binding sites targeted by the miR17-92 cluster in the exon encoding the 3´UTR of Bim mRNA. Analysis of SUPER-BIM mice revealed that miR17-92 mediated regulation of BIM expression levels is disepensable for normal B cell development, constrasting earlier results, but highly relevant for B cell lymphomagenesis. Animals heterozygous for the mutated version of the BIM 3´UTR were highly refractory to MYC-driven transformation. In vitro analysis of cells derived from pre-malignant and diseased mice suggest that even a mild increase in BIM protein levels that can be tolerated in steady state sufficiently primes B cells to undergo apoptosis at higher rates when facing oncogenic stress. Moreover, increasing BIM levels in established tumors by conditional replacement of the wild type with the mutated 3´UTR suffices to spontaneously eradicate MYC lymphoma cells suggesting that tumor initiation as well as tumor maintenance depends on miR17-92-mediated BIM repression. We propose that measures that increase apoptotic priming, e.g. by increasing endogenous levels of BIM, e.g. by antagomirs, can be exploited to prevent or treat cancer.

Caspase-2 regulates S-phase checkpoint activation to facilitate DNA repair

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Despite being the most evolutionarily conserved caspase, the role of caspase-2 in apoptosis has been difficult to unravel. In fact, accumulating evidence suggests that caspase-2 has non-apoptotic roles and may even regulate cell division. Loss of caspase-2 is known to increase proliferation rates but how caspase-2 is regulating this process is currently unclear. We show that caspase-2 is activated in dividing cells. In the absence of caspase-2, cells exhibit numerous S-phase defects including delayed exit from S-phase and impaired ATR and ATM activation, leading to increased aneuploidy and DNA damage following S-phase arrest. We also have noted that caspase-2 deficient cells have a much higher frequency of stalled replication forks and S-phase associated chromosomal aberrations. These functions appear to be independent of the pro-apoptotic function of caspase-2 because blocking caspase-2-induced cell death had no effect on cell division. We propose that these functions of apoptosis and cell division are regulated by different caspase-2 signaling complexes. We recently reported that DNA damage induces the assembly of two distinct activation platforms for caspase-2: a cytoplasmic platform that is RAIDD-dependent but PIDD-independent, and a nucleolar platform that requires both PIDD and RAIDD as well as the nucleolar resident protein, nucleophosmin (NPM1). Inhibition of NPM1 impaired caspase-2-dependent inhibition of cell growth suggesting that it is the nucleolar PIDDosome that is responsible for the cell cycle effects of caspase-2. Thus, our data supports a model where the cytoplasmic complex induces apoptosis and the nucleolar PIDDosome is required to regulate cell cycle events to facilitate DNA repair.

Prominin2 Drives Ferroptosis Resistance by Stimulating Multivesicular Body/Exosome-Mediated Iron Export

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Ferroptosis, regulated cell death characterized by the iron-dependent accumulation of lethal lipid reactive oxygen species, contributes to tissue homeostasis and numerous pathologies, and it may be exploited for therapy. Cells differ in their sensitivity to ferroptosis, however, and a key challenge is to understand mechanisms that contribute to resistance. Using RNA-Seq to identify genes that contribute to ferroptosis resistance, we discovered that pro-ferroptotic stimuli including inhibition of the lipid hydroperoxidase GPX4 and detachment from the extracellular matrix induce expression of prominin2, a pentaspanin protein implicated in regulation of lipid dynamics. Prominin2 facilitates ferroptosis resistance in mammary epithelial and breast carcinoma cells. Mechanistically, prominin2 promotes the formation of ferritin-containing multi-vesicular bodies (MVBs) and exosomes that transport iron out of the cell, inhibiting ferroptosis. These findings reveal that ferroptosis resistance can be driven by a prominin2-MVB/exosome-ferritin pathway and have broad implications for iron homeostasis, intracellular trafficking, and cancer.

Ferroptosis: Mechanisms and Therapeutic Applications

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Ferroptosis is an emerging form of regulated, non-apoptotic cell death that is driven by peroxidation of phospholipids containing polyunsaturated fatty acyl tails. Ferroptosis is tightly integrated with metabolism of iron, amino acids, lipids, and ROS, and is implicated in diverse diseases. Recent data obtained in my laboratory evaluated the potential utility of inducing ferroptosis in cancers that are addicted to cystine import through SLC7A11/system xc-. We developed an optimized inhibitor of system xc-, termed imidazole ketone erastin (IKE), and found that it has anti-tumor activity in a mouse cancer models. We demonstrated that IKE can induce pharmacodynamic markers of ferroptosis in these models, as well as inhibiting tumor growth. We also found effective combination regimens for IKE, and developed new methods of detecting ferroptosis in animal and patient samples. These data suggest that system xc- inhibitors such as IKE may be suitable for advancing to clinical studies.

Necroptosis kinases in anti-viral immunity and tissue homeostasis

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Necroptosis is an inflammatory form of cell death with key roles in anti-microbial defense. Receptor Interacting Protein Kinase 1 (RIPK1) and RIPK3 are two key regulators of necroptosis. In addition to induction of cell death to limit the microbial replication factory inside cells, RIPK1 and RIPK3 may also facilitate immune response against pathogens through stimulating cell death-independent cytokine expression. Many herpesviruses encode active inhibitory mechanisms to inhibit necroptosis, highlighting the role for this cell death pathway in anti-viral immunity. In contrast to herpesviruses, we previously showed that vaccinia virus infection sensitizes cells to necroptosis. Moreover, RIPK1/RIPK3-dependent necroptosis has a critical role in innate immune defense against vaccinia virus infection. However, the consequence of impaired necroptosis during innate immune response on adaptive immunity has not been examined. Here, we will discuss how necroptosis-associated inflammation during innate immune response impact subsequent adaptive immunity. We will also discuss the role of necroptosis in controlling infection by other poxviruses.

Endolysosomal Interactions with Mitochondria Regulate BAX-Mediated Mitochondrial Permeabilization during Cell Death Signaling

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Inter-organelle communication is emerging as a key component of organelle function and cell signaling. We recently reported that in response to pro-apoptotic BH3-only protein signaling, XIAP E3 ligase activity leads to mitochondrial ubiquitylation, associated with recruitment of endolysosomal machinery to and into mitochondria. These events require the presence of BAX/BAK, suggesting a functional connection to mitochondrial outer membrane permeabilization (MOMP). We employed live cell fluorescence imaging to record the dynamics of organelle interactions, mitochondrial ubiquitylation and MOMP-related events. Quantitative extraction of spatio-temporal maps revealed frequent transient contacts between endolysosomes and mitochondria under physiological conditions. In response to mitochondrial bioenergetic or apoptotic stresses, these interactions increase and culminate in a largescale, switch-like targeting of endolysosomes to the entire mitochondrial compartment. XIAP E3 ubiquitin ligase and USP15 deubiquitinase orchestrate the mobilization of endoplasmic reticulum-anchored endolysosomes and their routing to mitochondria. Specificity of endolysosomal vesicle identities, and interactions with mitochondria, are determined by engagement of ubiquitin-binding endocytic adapters, and endocytic effector and Rab GDP/GTP exchange factor (GEF) activities. Strikingly, we show that during apoptosis endolysosomal targeting of mitochondria is temporally and functionally integrated with MOMP, and that these inter-organelle interactions are required for full in situ apoptotic pore formation.

Physiological function of caspase-3/-7-dependent inactivation of Gasdermin-D

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Inflammasomes are signalling complexes assembled by cytosolic pattern-recognition receptors upon detection of microbial or damage-associated stimuli. The main function of inflammasomes is to facilitate the recruitment, dimerization and auto-proteolytic activation of the inflammatory caspases. Canonical inflammasomes activate caspase-1 in humans and mice, while the non-canonical inflammasome controls activation of caspase-4, -5 in humans and caspase-11 in mice. These caspases process pro-interleukin (IL)-1b and pro-IL-18 into their mature active forms, and also cleave the pore-forming protein gasdermin D (GSDMD) to drive a form of lytic cell death known as pyroptosis.

We recently demonstrate that apoptotic caspases-3 and -7 inactivate GSDMD by cleaving at position aspartate 88 (mouse, 87 in human), suppressing caspase-8-driven GSDMD activation and lytic cell death during extrinsic apoptosis1. However, it is still unknown if caspase-3/-7 negatively regulate GSDMD-dependent pyroptosis upon canonical or non-canonical inflammasome activation, and whether this has a physiological relevance during infection or inflammation in vivo.

In this study, we investigated the impact of caspase-3/-7-dependent GSDMD inactivation upon engagement of the canonical NLRP3, NAIP-NLRC4 and AIM2 inflammasomes, as well as the non-canonical pathway, in bone marrow-derived macrophages and neutrophils. In addition, we explored the physiological impact of GSDMD inactivation in vivo, by challenging a novel GsdmdD88A knock-in mouse line, in which GSDMD resist caspase-3/-7 cleavage, with the pathogen Salmonella enterica serovar Typhimurium, tumor necrosis factor (TNFa) or lipopolysaccharide (LPS). In conclusion, our study contributes to deepen the understanding of how GSDMD-dependent cell lysis is regulated in cultured cells and in vivo in mice.

Regulation of RIPK1 activation in neurodegenerative diseases associated with aging

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Upregulation of death receptor family ligands, such as TNFα can sensitize cells in the CNS to apoptosis and a form of regulated necrotic cell death known as necroptosis that is mediated by RIPK1, RIPK3 and MLKL. Necroptosis promotes cell death and neuroinflammation to mediate pathogenesis in multiple neurodegenerative diseases, including multiple sclerosis, amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease. RIPK1 kinase activity is suppressed by inhibitory phosphorylations mediated directly by TAK1 and by the kinases activated by TAK1, including MK2 and IKKs, and TBK1.

Aging is a major risk factor for both genetic and sporadic neurodegenerative disorders. However, it is unclear how aging interacts with genetic predispositions to promote neurodegeneration. We investigated how partial loss-of-function of TBK1, a major genetic cause for amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) comorbidity, leads to age-dependent neurodegeneration. We show that TBK1 is an endogenous inhibitor of RIPK1 and the embryonic lethality of Tbk1-/- mice is dependent on RIPK1 kinase activity. In aging human brains, another endogenous RIPK1 inhibitor, TAK1, exhibits a marked decrease in expression. We show that in Tbk1+/- mice, the reduced myeloid TAK1 expression promotes all the key hallmarks of ALS/FTD, including neuroinflammation, TDP-43 aggregation, axonal degeneration, neuronal loss and behavior deficits, which are blocked upon inhibition of RIPK1. Thus, aging facilitates RIPK1 activation by reducing TAK1 expression, which cooperates with genetic risk factors to promote the onset of ALS/FTD.

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Cell Stress and Inflammation

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Inflammation is initiated in response to Infection, Injury or Cell Stress, that all share the ability to elicit the production of an array of soluble factors that recruit and activate cells of the immune system and initiate wound repair. While it is very well established that conserved components of infectious agents, called PAMPs (pathogen-associated molecular patterns), and molecules released by necrotic cells, called DAMPs (damage-associated molecular patterns), promote inflammatory responses, it is less well appreciated that conditions which provoke Cell Stress (such as misfolded protein-induced ER stress, mitochondrial depolarization, heat shock, DNA damage) can also instigate inflammatory cytokine production in a manner that is poorly understood at present. Stress-induced inflammation is suspected to underlie the slow buildup of inflammation that is seen in multiple chronic conditions such as cancer, obesity and neurodegeneration that contributes to disease progression. However, although we now know a great deal about how infection and tissue necrosis promote inflammation, how inflammation is initiated in response to Cell Stress is obscure. I will discuss recent data from our laboratory suggesting that cell stress leads to inflammation via death receptor upregulation and autoactivation.

Innate immunity to cytosolic LPS: pyroptosis and beyond

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Lipopolysaccharide (LPS), the major cell-wall component of Gram-negative bacteria, is long known to be sensed by the plasma membrane-bound TLR4 receptor. Ligation of TLR4 by the lipid A of LPS stimulates NF-kB and IRF-mediated inflammatory cytokine production. Recently, we showed that caspase-11, 4 and 5 are cytosolic immune receptors for LPS and activated by direct binding to its lipid A part. Like caspase-1 activation by the canonical inflammasome, caspase-11/4/5 activation induces pyroptosis, both of which are critical for antibacterial defense and development of immunological diseases. Caspase-11/4/5 and caspase-1 cleave Gasdermin D (GSDMD) to release the autoinhibition of its N-terminal domain that bears an intrinsic pore-forming activity for executing pyroptotic cell death. Gsdmd-/- mice are susceptible to various bacterial infections and also resist LPS-induced septic shock. GSDMD belongs to a large Gasdermin family sharing the pore-forming domain. Another family member GSDME whose expression is silenced in most cancer cells, is activated by caspase-3 cleavage; inflammatory damages caused by GSDME-mediated pyroptosis is the major determinant for the toxicity of those DNA-damaging chemotherapy drugs. Lastly, we discover that ADP-heptose, the precursor for LPS inner core oligosaccharide, is recognized in host cytosol by a novel kinase receptor ALPK1. Like lipid A-activated TLR4, ADP-heptose-activated ALPK1 potently stimulates NF-kB-dependent inflammatory responses both in cells and mice. These findings shift the paradigm of immune sensing of LPS and antibacterial defense, and open several new areas of research on innate immunity and pyroptosis-mediated inflammation. The cell-entry property of ADP-heptose also suggests a new way of modulating immune responses in mammals.

LC3-associated endocytosis (LANDO): A noncanonical function of autophagy proteins regulates microglial activation and neurodegenerative disease

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Two ancient metabolic processes, phagocytosis and macroautophagy, arose as mechanisms of meeting the energy demands of the cell. Both also evolved into mechanisms of host defense. We have described a process we term "LC3-Associated Phagocytosis" (LAP), in which signals that are generated upon engulfment of particles by phagocytic cells induce components of the autophagy machinery to associate with the phagosome, promoting its fusion to lysosomes (phagosome maturation). Like macroautophagy, this LC3 association depends on Beclin1, PI3P generation, ATG5, and ATG7, but unlike autophagy, LC3 associates with the single phagosome membrane to promote phagosome maturation and degradation of the contents. Further, unlike macroautophagy, LAP proceeds in the absence of elements of the autophagic pre-initiation complex, ULK1, ATG13, and FIP200, and involves a novel PI3K complex. This raises an intriguing possibility: It is now well established that defects in some components of the autophagy machinery promote inflammatory disease and compromise host defense to intracellular infections. While such effects are generally interpreted as consequences of defective macroautophagy, the existence of LAP as a discrete phenomenon suggests that at least *some* such effects may specifically relate to LAP. In studying the role of LAP in the clearance of Beta-amyloid in an Alzheimer's Disease (AD) model, we found that autophagy components associated with LAP profoundly inhibit disease, but that a) this is related to endocytosis, not phagocytosis of Abeta oligomers and b) maturation of the endosome and degradation of Beta-amyloid are unaffected by these LAP components. We have instead uncovered a novel process of LC3-associated endocytosis (LANDO) which functions in the recycling of Abeta receptors from cytosolic endosomes to the plasma membrane. This regulates the inflammatory response to Abeta oligomers and inhibits neurodegeneration.

Creating a Killer: Conversion of Bax and Bak into Membrane Permeabilising Executioners

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Conversion of Bax and Bak from inert monomers into membrane perforating oligomers is a key event within the intrinsic apoptotic pathway. Recent structural studies have provided molecular details of the activation events initiating these transformations and the ensuing conformational changes that lead to formation of key intermediate dimer units. However, questions remain as to how these dimers assemble into large oligomers, how these assemblies interact with lipids from the mitochondrial outer membrane and how they permeabilise this barrier. Here we present unpublished crystal structures of Bak dimers interacting with lipids and lipid like molecules. These are the first structures of any Bcl-2 family member associated with lipids. They reveal novel lipid binding sites not predicted from previous Bak structures, provide clues as to how Bak and Bax permeabilise membranes and suggest a new model for Bak and Bax oligomerisation, with oligomers building through lipid-protein interactions to create the complexes that permeabilise the outer mitochondrial membrane.

Molecular Architecture of FADD:Caspase-8 Signalling Complexes - co-ordinated Control of Life/Death Decisions

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Caspase-8, a key regulator of cell fate, is recruited/activated within large multiprotein signalling platforms including the Death-Inducing Signalling Complex (DISC), Necrosome and Ripoptosome. The best characterized of these, the DISC, is critical for initiation of death receptor-induced apoptosis; consequently, its formation and activation must be tightly regulated. Recruitment of Procaspase-8 to the adaptor molecule FADD is mediated via Death Effector Domain (DED) interactions. Subsequently, multiple Procaspase-8 molecules are required to interact via their tandem DEDs to form a helical Caspase-8-activating chain (Dickens et al. 2012 Mol. Cell; Schleich et al. 2012 Mol. Cell). c-FLIP isoforms, which are key regulators of procaspase-8 activation, are recruited to the complex via a co-operative/hierarchical binding mechanism involving Procaspase-8 (Hughes et al. 2016 Mol. Cell). The ratio of c-FLIPL/S to Procaspase-8 is a critical regulator of Caspase-8 dimerization/activation within tDED oligomers and crucially defines how c-FLIP isoforms differentially control cell fate, predicting that c-FLIPS blocks Caspase-8 activation by somehow disrupting tDED triple helix assembly. Now, using negative-stain Electron Microscopy (EM) and Cryo-EM, we have visualized for the first time the 3D structural architecture of FADD:Caspase-8 complexes. Further investigation, including nano-gold immunolabelling, has confirmed the LC-MS/MS-determined stoichiometry of the FADD:Caspase-8 Complex and provided new information on the domain architecture of the active Caspase-8 catalytic dimer, as well as its regulation by key tDED regulators (e.g. c-FLIP). Our data provide important novel insights into the co-ordinated control of life/death decisions by FADD:Caspase-8 signalling platforms including the DISC, Necrosome and Ripoptosome.

Mcl-1 governs sublethal caspase-8 activation and transmitotic resistance to extrinsic apoptosis

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Extrinsic apoptosis relies on the activation of TNF-family receptors, such as TRAIL receptors, by immune cells or receptor-activating biologics. Here, we monitored cell cycle progression at minutes resolution to relate apoptosis kinetics and death decisions to cell cycle phases, and found that cells in mid to late S phase delay TRAIL-induced death in favour for mitosis, thereby passing on an apoptosis-primed state to their offspring. This translates into two distinct fates, apoptosis execution post mitosis or cell survival from inefficient apoptosis. Transmitotic resistance is controlled upstream of mitochondrial outer membrane permeabilisation (MOMP) and is linked to the upregulation of Mcl-1 from mid S phase onwards, which allows cells to pass through mitosis with activated caspase-8. Worryingly, cells escaping apoptosis execution after mitosis sustain sublethal DNA damage. Antagonizing Mcl-1 by BH3-mimetics efficiently suppresses cell cycle-dependent delays in apoptosis, prevents apoptosis-resistant progression through mitosis and averts unwanted survival from apoptosis induction. Overall, cell cycle progression therefore modulates signal transduction during extrinsic apoptosis, with Mcl-1 modulating cellular decision making between death, proliferation and survival from inefficient apoptosis.

Combination of molecular subtyping and systems analysis of apoptotic and proliferative pathways predicts Cetuximab responses in patient-derived xenografts models of metastatic colorectal cancer

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Antibodies targeting the human epidermal growth factor receptor (EGFR) are used for the treatment of Ras wild-type metastatic colorectal cancer (mCRC). A significant fraction of patients remain unresponsive to this therapy. Molecular subtyping of CRC tumors has provided important insights into the molecular heterogeneity of CRC, and demonstrated utility as prognostic/predictive tool in the clinical setting. In this talk, I will outline that the prognostic/predictive power of molecular subtyping is greatly enhanced by a subtype-specific systems analysis of two tumour hallmarks: apoptosis and proliferation. Transcriptome and reverse phase protein array (RPPA) analysis was performed on 93 RAS wild-type mCRC tumours from patient-derived xenografts (PDX) treated with Cetuximab. Levels of BCL-2, BCL-XL, BAX, BAK and MCL-1 were used as inputs for a dynamic systems model to determine apoptosis capacity, and transcripts of panel of proliferative genes to determine proliferative capacity of individual tumours. CRC intrinsic subtype (CRIS) analysis grouped responders and non-responders across all molecular subtypes. Interestingly, responders in CRIS B and D, but not in other subtypes, showed high apoptosis capacity compared to non-responders. Conversely, responders in CRIS A, C and E showed low proliferative capacity. Unsupervised hierarchical (phospho)proteome clustering identified 3 protein clusters. Protein cluster 3 partially overlapped with CRIS C but was exclusively composed of responders. Protein clusters 1 and 2 composed of both responders and non-responders, with responders in cluster 1 characterised by low proliferative, and in cluster 2 with high apoptosis capacity. Collectively, our data highlights the importance of combining molecular subtyping with systems-based signalling network analyses to accurately identify responders to anti-EGFR therapy.

Targeting death receptor network using rationally designed chemical probes

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Pharmacological targeting via rationally designed chemical probes has recently acquired an emerging importance as a valuable tool to delineate molecular mechanisms. The formation of death inducing signaling complex (DISC) and death effector domain (DED) filaments is critical for regulation of death receptor networks. DISC comprises CD95, FADD, procaspase-8/10, and c-FLIP, serving as a key platform for initiator procaspase-8 activation leading to induction of apoptotic and non-apoptotic pathways. To get more insight into the detailed molecular mechanisms of this network regulation we have rationally designed chemical probes targeting the core components of the DISC. In particular, we focused on c-FLIP and caspase-8. Specifically, we rationally designed a small molecule that binds to c-FLIPL at the interface of the heterodimer caspase-8/c-FLIPL, aiming to stabilize the active center of caspase-8 in the caspase-8/c-FLIPL heterodimer after processing at Asp374. The optimized chemical compound enhanced caspase-8 activity at the DISC and promoted cell death. Moreover, a generated systems biology model of the DISC further supported the suggested mechanism on the quantitative level. Furthermore, we have generated peptides specifically targeting c-FLIP interactome and tested their action experimentally. These studies led to the novel understanding of the network regulation and provided fascinating insights into the mechanisms of death receptor control. Our most recent findings will be presented.

Microptosis: Programmed Cell Death in Microbes Activated by Killer Lymphocytes

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When killer lymphocytes recognize infected cells, perforin delivers cytotoxic proteases (granzymes) into the target cell to trigger caspase-independent programmed cell death. What happens to intracellular microbes during this process has been unclear. Granulysin, an antimicrobial peptide, is also a component of human, but not rodent, cytotoxic granules. Mice expressing transgenic granulysin in killer cells are better able than wild-type mice to clear Listeria monocytogenes and survive lethal Toxoplasma gondii and Trypanosoma cruzi challenges. Killer lymphocytes kill intracellular microbes when they recognize and kill infected cells, which requires the concerted action of granzymes, perforin and granulysin. Granulysin and perforin deliver granzymes into bacteria and parasites to induce programmed microbial cell death, which we term "microptosis". In bacteria, granzymes cleave electron transport chain complex I (as they do in mammalian mitochondria) and oxidative stress defense proteins, generating ROS that rapidly kill bacteria. ROS scavengers and bacterial antioxidant protein overexpression inhibit bacterial death. Anerobic bacteria still die, but more slowly, suggesting that granzymes disrupt multiple bacterial pathways. We used differential proteomics to identify granzyme B substrates in three unrelated bacteria, Escherichia coli, Listeria monocytogenes and Mycobacteria tuberculosis. In all 3 bacteria, granzyme B cleaves a highly conserved set of proteins, which function in vital biosynthetic and metabolic pathways that are critical for bacterial survival under diverse environmental conditions. Key proteins required for protein synthesis, folding and degradation are also substrates, including multiple aminoacyl tRNA synthetases, ribosomal proteins, protein chaperones and the Clp system. Parasite death has features of granzyme-mediated mammalian cell death, including ROS generation, mitochondrial swelling, transmembrane potential dissipation, membrane blebbing, phosphatidyl serine exposure, DNA damage and chromatin condensation. The pathways targeted by granzyme B in T. cruzi overlap strongly with the essential pathways targeted in bacteria. Thus, killer lymphocytes use a multi-pronged strategy to target vital microbial pathways. Because multiple critical pathways are inactivated, microbes may not easily develop resistance to killer cell attack.

Phosphatidylserine-dependent efferocytosis and entosis

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Phosphatidylserine (PtdSer) is confined to the inner leaflet by the action of flippases that translocate PtdSer from the outer to inner leaflet. When cells undergo apoptosis, PtdSer is exposed to the cell surface, and is recognized as an “eat me” signal by macrophages for “efferocytosis” or "engulfment of apoptotic cells". Two P4-type ATPases (ATP11A and 11C) function as the flippase, and are inactivated by the caspase-cleavage during apoptosis. This flippase-inactivation is necessary but not sufficient for the apoptotic PtdSer exposure. To quickly expose PtdSer to the surface, scramblases of the XKR family containing 10 transmembrane regions must be activated by caspase to non-specifically and bi-directionally translocate phospholipids between the inner and outer leaflets. Among the 9 XKR members, XKR8 is ubiquitously expressed, and the apoptotic PtdSer exposure and efferocytosis was strongly delayed in Xkr8-/- hematopoietic cells. The two plasma membrane flippases (ATP11A and 11C) were ubiquitously expressed except for B cell progenitors, in which only ATP11C was present. ATP11C-/- mice suffered from B cell lymphopenia, which could be rescued by double deficiency of Axl and MerTK genes coding for tyrosine-kinase receptors involving PtdSer-dependent efferocytosis. The ATP11C-/- B cell progenitors in Axl-/- MerTK-/- background constitutively exposed PtdSer, and were efficiently engulfed by resident macrophages in vitro. These results indicate that PtdSer is sufficient for cells to be engulfed, and plays an important role in "entosis" or "engulfment of living cells". We recently found that PtdSer-exposure is regulated not only by caspase-mediated cleavage of flippase and scramblase, but also kinase-mediated their phosphorylation. I will discuss the PtdSer-exposure in various cell death processes, and other biological processes.

(References)

Nagata S (2018) Apoptosis and the clearance of apoptotic cells. Annu. Rev. Immunol. 36:489-517.

Nagata S, and Tanaka M (2017) Programmed cell death and the immune system. Nat. Rev. Immunol. 17:333-340.

POSTER

POSTER SESSIONS

Two poster sessions are scheduled during the meeting. Presenting authors are requested to be at their poster during their designated sessions

Poster Session 1 (odd numbers): Wednesday 20:00 – 22:00 (during the get together)
Poster Session 2 (even numbers): Thursday 13:30 – 15:30 (during the lunch break)

POSTER

1 **Dieter Adam**
TRAIL-induced necroptosis: analysis of potential protumoral side effects and efficacy against resting tumor cells

2 **So Ra Lee**
Accelerated degradation of cFLIPL and the sensitization of TRAIL DISC-mediated apoptotic cascade by A, a lignan isolated from plant B

3 **Divya Venkatesh**
MDM2 and MDMX facilitate ferroptosis in the absence of p53 Flash Talk 1

4 **Kaiwen Chen**
Extrinsic and intrinsic apoptosis activate pannexin-1 to drive NLRP3 inflammasome assembly

5 **Laura K.Hillert**
QUANTITATIVE ANALYSIS OF MACROMOLECULAR COMPLEXES OF THE CD95/FAS CELL DEATH PATHWAY

6 **Aleksandra Egorshina**
Interplay between necroptosis, autophagy and apoptosis as a consequence of mitotic catastrophe

7 **Johannes Espe**
Uncovering CD95-mediated activation of NF-?B

8 **Fabian Wohlfromm**
Recombinant lactaptin analogon RL2 inhibits TRAIL induced cell death in breast cancer cell lines

9 **Max Richter**
The recombinant fragment of human kappa-Casein induces cell death by targeting the proteins of mitochondrial import in breast cancer cells

10 **Viktorija Juric**
Interrogating Cyclin-Dependent-Kinase Inhibitors As A Novel Treatment Strategy For Glioblastoma Patients

11 **Richard de Reuver**
Study of the interplay between Z-RNA and the central regulator of the dsRNA response ADAR1

12 **Luciano Ferrada**
Ferroptosis-resistant human glioblastoma cells are sensitized to ferroptosis by co-treatment with dehydroascorbic acid

POSTER

13 **Stephanie Bleicken**
Towards structure determination of oligomeric Bcl-2 protein complexes using two-'color'-three-channel DEER

Flash Talk 2

14 **Gelina Kopeina**
Activation of caspase-2 upon genotoxic stress is regulated by phosphorylation and ubiquitination

15 **Corinna König**
Pharmacological targeting of c-FLIPL and Bcl-2 family members promotes apoptosis in cancer cells

16 **Nezka Kavcic**
CATHEPSIN C IS CRITICAL FOR THE RELEASE OF CATHEPSINS FROM LYSOSOMES IN LEUCYL-LEUCINE METHYL ESTER TRIGGERED APOPTOSIS

17 **Chiara Boccellato**
In vitro investigation of glioblastoma cells responsiveness to IZI1551 in combination with a brain-penetrant synergizer

18 **Inbar Shlomovitz**
Proteomic analysis of necroptotic extracellular vesicles

19 **ANNA LUGOVAYA**
THE COMPARATIVE EVALUATION OF THE DYNAMICS OF APOTOSIS AND AUTOTHAGY MARKERS IN THE ACUTE PERIOD OF ISHEMIC STROKE

20 **Ruoshi Peng**
RIPK3 drives inflammatory signalling independently of cell death

Flash Talk 3

21 **Christian Molnar**
Inhibition of PUMA-induced apoptosis preserves hematopoietic stem cell function and prevents bone marrow failure in a dyskeratosis congenita mouse model

22 **Ewelina Dobosz**
The role of MCPIP-1 in the regulation of neutrophil apoptosis

23 **Barbara Ruder**
Regulation of the host cell death machinery by a single viral protein

24 **Iris Stolzer**
IFN-STAT1-MLKL axis drives necroptosis during gastrointestinal inflammation and infection

Flash Talk 4

25 **Paolo Armando Gagliardi**
Apoptotic epithelial cells trigger an ERK/Akt activity wave in the neighbors to protect them from apoptosis

26 **Hadar Cohen**
Inflammasome activation by bacterial type VI secretion system

27 **Ziv Erlich**
Macrophages, rather than DCs, are responsible for inflammasome activity in the GM-CSF BMDC model

28 **Marie-Claire Fitzgerald**
Improving and Personalising Treatment Options for Paediatric Brain Tumour Patients

POSTER

29 **Seyed Mohammad Amin Moosavi**
Effects of metal oxide nanoparticles on regulated cell death pathways: apoptosis, autophagy, ferroptosis and necroptosis

30 **Uris Lianne Ros Quincoces**
Calcium signaling in necroptosis: one ion with many faces

31 **Gavin Fullstone**
The apoptosome molecular timer synergistically aids XIAP in shutting down caspase signalling during apoptosis execution

32 **Georgia Atkin-Smith**
The disassembly of apoptotic monocytes and Influenza infection

33 **Barbora Vesela**
Novel functions of caspase-9 in endochondral bone development

34 **Daniela Stöhr**
Stress-induced TRAILR2 expression overcomes TRAIL resistance in cancer cell spheroids

35 *cancelled by author*

36 **Nadezhda Mironova**
The search for RNA targets for exogenous ribonucleases associated with apoptosis failure in tumors

37 **Aleksandra Senkova**
Antitumor cell-free vaccines based on mannosylated liposomes and membrane vesicles derived from dendritic cells provide the formation of cytotoxic T-lymphocytes with enhanced killing properties

38 **Sanket More‘**
Therapeutic harnessing of iron-dependent cell death in melanoma; the Apolipoprotein E and ferroptosis link

39 **Jenny Sprooten**
Understanding the impact of necroptosis or apoptosis on myeloid inflammation in cancer.

40 **Anja Krippner-Heidenreich**
Sensitisation of Smac-induced Cell Death by Tumour Necrosis Factor Receptor (TNFR) Type 2-mediated Signalling in T-cell Acute Lymphoblastic Leukaemia (T-ALL)

Flash Talk 5

41 **Christian Holmgren**
A combination treatment of Smac mimetic LCL-161 and TRAIL induces apoptosis in sensitive breast cancer cells through RIP1 complex formation independent of RIP1 kinase function

42 **Katia Cosentino**
Real-time assembly mechanisms of Bax and Bak during apoptosis at the single molecule level

43 **Margs Brennan**
Humanised Mcl-1 mice enable accurate preclinical evaluation of MCL-1 inhibitors destined for clinical use

44 **Alexia Belavgeni**
Exceptional sensitivity to Ferroptosis in Adrenocortical Carcinomas

POSTER

45 **YizhouWang**
Creating structure-based RANKL mutants with low affinity for OPG to activate macrophage in fibrosis

46 **Vanessa Kraft**
An overexpression screen identifies new players in ferroptosis susceptibility

47 **Anna-Laura Kretz**
TRAILblazing: The role of CDK9 in pancreatic cancer

48 **Emma Morrish**
Targeting MDR1 sensitises leukaemia stem cells to IAP inhibitors to overcome treatment resistance in acute myeloid leukaemia

49 **Denise Müller**
The role of the anti-apoptotic factors Mcl-1 and Bcl-2 in the treatment resistance of malignant thymoma and thymic carcinoma

50 **Rodrigo M. Maza**
MIR-138: A NEUROPROTECTIVE TARGET FOR SPINAL CORD INJURY?

51 **Maria Livia Sassano**
Lipid trafficking at the ER-mitochondria contact sites and ferroptosis

52 **Stefan Küffer**
A Caspase 3 deletion with prognostic relevance in thymic epithelial tumors (TET)

Flash Talk 6

53 **Nivetha Krishna Moorthy**
Development and testing of TRAIL variants intended for transcytotic delivery into the central nervous system

Flash Talk 7

54 **Vincent Braun**
The PIDDosome in promoting p53 induced tumor suppression

55 **Sandra Muench**
ASTX660, a non-peptidomimetic antagonist of cIAP1/2 and XIAP, induces tumour cell death by direct and immune-mediated mechanisms

56 **Carla Bezjian**
Peroxidation of phospholipids containing two unsaturated fatty acyl tails is associated with execution of cell death during ferroptosis

57 **Vivek Venkataramani**
Amyloid Precursor Protein (APP) Supports Tumorigenesis by Counteracting Iron-mediated Oxidative Stress and p38MAPK-dependent DNA Damage

58 **Olga Koval**
Vaccinia virus in cell death induction

59 **Mar Orzaez**
Preclinical antitumor efficacy of combined senescence-inducing chemotherapy with targeted nanotherapeutic senolysis

POSTER

60 **Olga Troitskaya**
IDO inhibitors enhance antitumor vaccination effect of lactaptin-treated cancer cells

61 **Ekaterina Golubitskaya**
Selective cancer cell death induced by cold atmospheric plasma irradiation

62 **ESTEFANIA LUCENDO**
Unrevealing the roles of the MCL-1 transmembrane domain

63 **Claudia Meyer**
Analysis of the Anti-ferroptotic and Anti-Necroptotic Activities of Necrostatin-1 (Nec-1)

64 **Matthias Wirth**
Identification of NOXA dependent vulnerabilities by a high throughput drug screening in pancreatic cancer cells

65 **Jaewhan Song**
C-MYC functions as an anti-necroptotic factor by suppressing the formation of the necrosome complexflash

66 **Marie Oliver**
MetzigIn TNF-induced necroptosis NF?B/RelA dynamics control an incoherent feedforward loop to determine cell fate decisions

67 **Stephanie Liebig**
Serum Cell Death Biomarker Mirrors Liver Cancer Regression and Predicts Progression-Free Survival after Transarterial Chemoembolisation

68 **Marie Ehrlichová**
KRAS pathway expression changes in pancreatic cancer models caused by conventional and experimental taxanes

69 **Johannes Weiss**
Analyzing the impact of thePIDDosome on aneuploidy in Ph+ pre-B-ALL.

70 **Katrin Tagscherer**
Novel treatment approaches in prostate cancer: Targeting Cyclin K

71 **Juan Lin**
RIPK1 prevents ZBP1-mediated necroptosis to inhibit inflammation

72 **Anne-Kathrin Knuth**
The effect of necroptosis on peritoneal macrophages

73 **Victoria Granqvist**
TRAIL treatment results in induction of interferon signaling in MCF-7 breast cancer cells

74 **Marietta Zille**
Ferroptosis in neurons and cancer cells is similar but differentially regulated by histone deacetylase inhibitors

75 **Lohans Pedrera**
Plasma membrane damage and calcium fluxes activate ESCRT-III mediated repair mechanism upon ferroptosis

POSTER

76 **Kelley McQueeney**
Using ascites fluid and high throughput dynamic BH3 profiling to identify active cancer therapies

77 **Lorrie Kirshenbaum**
Novel Alternative Splicing Switches Bnip3 from Cell Death to Cell Survival.

78 **Bettina Proneth**
Ferroptosis and necroinflammation in tissue degeneration

79 **Patrick Connolly**
Development of a novel mitotic inhibitor targeting the NOXA-MCL1-BIM axis and producing a pro inflammatory response

80 **A. Nikolai von Krusenstiern**
Exploring cellular processing and localization of ferroptosis-inhibiting deuterated polyunsaturated fatty acids

81 **Graeme Sullivan**
The novel immune-regulatory cytokine Interleukin-37 exhibits pro-inflammatory activity upon N-terminal proteolytic processing

Flash Talk 8

82 **Pavel Davidovich**
FLIP suppresses Death Receptor-induced inflammation as well as cell death

83 **Bradlee L. Heckmann**
Crashing the computer: Apoptosis and Necroptosis in Neurodegeneration

84 **Baptiste Dumétier**
Analysis of the oncogenic properties of cIAP1

85 **Svenja Lorenz**
Molecular underpinnings of ferroptotic cell death in neurodegeneration

86 **Viacheslav V. Senichkin**
Bcl-xL and Bak serve as predictors of sensitivity of cancer cells to BH3-mimetic S63845

1 | TRAIL-induced necroptosis: analysis of potential protumoral side effects and efficacy against resting tumor cells

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TRAIL-based therapies to eliminate human cancer cells via enforced induction of apoptosis have been recently evaluated in clinical trials. Unfortunately, they have so far shown only limited efficacy, in part due to intrinsic or acquired apoptosis resistance of tumors. As an alternative, emerging strategy, necroptosis constitutes a mode of regulated cell death that is molecularly distinct from apoptosis. Employing homoharringtonine (HHT, a plant alkaloid approved by the U. S. Food and Drug Administration for the treatment of chronic myeloid lymphoma) as a patient-friendly replacement for the commonly used necroptosis sensitizer cycloheximide, we have previously confirmed the potential of TRAIL-induced necroptosis for the elimination of apoptosis-resistant cancer cells in vitro and validated the in vivo efficacy of TRAIL/HHT-induced necroptosis in a preclinical mouse model. However, this treatment may also elicit proinflammatory signals and unwanted protumoral and prometastatic side effects (as previously reported for apoptosis). To address this point in vitro, we have compared tumor cell lines with high, medium and low sensitivity for TRAIL/HHT-induced necroptosis for the production of proinflammatory markers and cytokines as well as for changes in their migratory, proliferatory and invasive properties. In addition, we have analyzed the efficacy of TRAIL/HHT-induced necroptosis against tumor cells with high stem cell potential (resting tumor cells) as well as in cell cycle-arrested tumor cells lines, and will present our results at the conference.

Friederike Dierks and Rieke Winkelmann contributed equally to this work.

2 | Accelerated degradation of cFLIPL and the sensitization of TRAIL DISC-mediated apoptotic cascade by A, a lignan isolated from plant B

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Plant-derived lignans have numerous biological effects including anti-tumor and anti-inflammatory activities. By screening purified constituents of plant B from TNF related apoptosis-inducing ligand (TRAIL)-resistant human gliobalstoma cells, a lignan A was found to be highly active TRAIL sensitizer. Here we demonstrate that treatment of nontoxic doses of A in combination with TRAIL induces rapid apoptosis and caspase activation in a number of glioblastoma cells, but not in normal astrocytes. Analysis of apoptotic signaling events revealed that A enhances TRAIL-mediated death-inducing signaling complex (DISC) formation and complete processing of procaspase-8 within DISC in gliobalstoma cells, in which caspase-8 is inactivated. Mechanistically, A down-regulates the expression of cellular FLICE-inhibitory protein (cFLIPL) and survivin through proteasome-mediated degradation without affecting death receptors or downstream intracellular apoptosis-related proteins. Furthermore, the sensitization of TRAIL-mediated apoptosis by A strictly depends on the expression levels of cFLIPL, which is regulated through the de novo protein synthesis, rather than NF-κB or p53 signaling. Taken together, our results indicate that A facilitates DISC-mediated caspase-8 activation by targeting cFLIPL in an early event in apoptotic signaling, and thus provide a potential therapeutic module for TRAIL-based chemotherapy.

3 | MDM2 and MDMX facilitate ferroptosis in the absence of p53

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The P53 tumor suppressor network plays a significant role in regulating cell survival and tumorigenesis. Two key proteins within this network, MDM2 and MDMX, most well studied as negative regulators of P53, can work either separately or as a heterodimeric complex to restrain P53 functions. Recent studies have revealed that MDM2 also has pro-oncogenic roles in cells, tissues, and animals that are independent of P53. There is less evidence for P53-independent roles for MDMX and the MDM2-MDMX complex. Here, we found that MDM2 and MDMX facilitate ferroptosis in cells with or without P53. Using small molecules, RNA interference reagents and mutant forms of MDMX, we found that MDM2 and MDMX, likely working as a complex, normally facilitate ferroptosis caused by all classes of ferroptosis inducers. We observed that MDM2 and MDMX alter the lipid profile of cells during ferroptosis to facilitate ferroptosis and that inhibition of MDM2 or MDMX led to increased levels of coenzyme Q10, an endogenous lipophilic antioxidant that restrains ferroptosis. This suggests that MDM2 and MDMX normally prevent cells from mounting an adequate defense against lipid peroxidation and thereby promote ferroptosis. We also propose that the activity of the transcription factor, PPAR alpha is essential for MDM2 and MDMX to promote ferroptosis in this manner. These results suggest that in some contexts MDM2/MDMX inhibition might be useful for preventing degenerative diseases involving ferroptosis.

4 | Extrinsic and intrinsic apoptosis activate pannexin-1 to drive NLRP3 inflammasome assembly

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Pyroptosis is a form of lytic inflammatory cell death driven by inflammatory caspase-1, caspase-4, caspase-5 and caspase-11. These caspases cleave and activate the pore-forming protein gasdermin D (GSDMD) to induce membrane damage. By contrast, apoptosis is driven by apoptotic caspase-8 or caspase-9 and has traditionally been classified as an immunologically silent form of cell death. Emerging evidence suggests that therapeutics designed for cancer chemotherapy or inflammatory disorders such as SMAC mimetics, TAK1 inhibitors and BH3 mimetics promote caspase-8 or caspase-9-dependent inflammatory cell death and NLRP3 inflammasome activation. However, the mechanism by which caspase-8 or caspase-9 triggers cell lysis and NLRP3 activation is still undefined. Here, we demonstrate that during extrinsic apoptosis, caspase-1 and caspase-8 cleave GSDMD to promote lytic cell death. By engineering a novel Gsdmd D88A knock-in mouse, we further demonstrate that this proinflammatory function of caspase-8 is counteracted by caspase-3-dependent cleavage and inactivation of GSDMD at aspartate 88, and is essential to suppress GSDMD-dependent cell lysis during caspase-8-dependent apoptosis. Lastly, we provide evidence that channel-forming glycoprotein pannexin-1, but not GSDMD or GSDME promotes NLRP3 inflammasome activation during caspase-8 or caspase-9-dependent apoptosis.

5 | QUANTITATIVE ANALYSIS OF MACROMOLECULAR COMPLEXES OF THE CD95/FAS CELL DEATH PATHWAY

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The formation of the death-inducing signaling complex (DISC) is the first crucial step to trigger extrinsic apoptosis. CD95/Fas DISC comprises CD95/Fas, FADD, c-FLIPs, procaspase-8 and procaspase-10. This is accompanied by the assembly of DED filaments that serve as a platform for procaspase-8 dimerization and further apoptosis induction. Regulation of

CD95/Fas-mediated caspase-8 activation by short and long c-FLIP isoforms is a central process in extrinsic apoptosis regulation.

To further investigate the role of c-FLIP proteins in DED filaments, we have analyzed the stoichiometry of CD95/Fas ligand-induced DISCs from HeLa cells stably overexpressing c-FLIP isoforms by quantitative AQUA mass spectrometry. This was supported by biochemical analysis and the cutting-edge technology of imaging flow cytometry. It could be shown that the increase of c-FLIP level directly influenced caspase-8 filament formation and changed the stoichiometry of the key components of the DISC. Strikingly, upon c-FLIP overexpression, we could also quantify an increase in the amount of DISC complexes. Furthermore, using the data from quantitative mass spectrometry, we have built a structural model of DED filaments that suggested the role of c-FLIP in the complex as a bridging motif between adjacent receptor complexes. Finally, the combination of a cutting-edge experimental setup and computational structural modelling allowed us to get more information about the dynamics of this macromolecular complex which offers new points of application in the discovery of novel medical drugs. Our latest findings will be presented.

6 | nterplay between necroptosis, autophagy and apoptosis as a consequence of mitotic catastrophe

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Mitotic catastrophe is a pathway resulted from incomplete mitosis in response to genotoxic stress, most often owing to gross chromosomal aberrations or mitotic spindle defects. Mitotic catastrophe represents an oncosuppression mechanism and seems to be a promising strategy in cancer treatment. The outcome of mitotic catastrophe depends on a balance between proteins regulating different cell death pathways. While mitotic catastrophe is a non-lethal process, it constitutes a crossroad that can drive cells into autophagy, apoptosis or necrosis. However, the question of what type of necrotic cell death is induced via mitotic catastrophe is still open.

In the present study, the mitotic catastrophe was induced in ovarian (Caov-4) and colorectal (HCT116 wt and HCT116 p53-/-) cancer cell lines using low doses of genotoxic agent doxorubicin (600nM) and microtubule poison colcemid (10mkg/ml). Western blot analysis demonstrated that phosphorylation of MLKL and RIP-1

7 | Uncovering CD95-mediated activation of NF-κB

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CD95/Fas stimulation is also known to induce NF-κB activation along with apoptosis pathway. However, mechanisms of CD95-induced NF-κB activation remain unclear. C-FLIPs (cellular FLICE-like inhibitory proteins) are reported to play a key role in these processes. Importantly, it was shown for v-FLIPs (viral FLIPs) that they directly bind to NEMO and subsequently induce the NF-κB pathway. Assuming a similar mechanism for c-FLIP, here we have created a homology model of the c-FLIP/NEMO complex using the reported structure of v-FLIP/NEMO complex. This was followed by a rational design of peptides targeting c-FLIP. The designed peptides were based on the structure of the HLX2 domain of NEMO. The experimental in vitro validation demonstrated that these peptides inhibit CD95-mediated NF-κB activation. Strikingly, the best inhibitory effects on CD95-mediated NF-κB activation are exhibited by the NEMO-derived peptides with the substitution D242Y of NEMO. This was followed by the analysis of NEMO/c-FLIP interactions in c-FLIPlong overexpressing cells and further delineation of the role of these interactions. Our latest data will be presented.

Taken together using a combination of bioinformatics and experimental approaches we obtained new insights into CD95-mediated NF-κB activation providing possibilities for targeting the death receptor network.

8 | Recombinant lactaptin analogon RL2 inhibits TRAIL induced cell death in breast cancer cell lines

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Lactaptin is a proteolytic fragment of κ-Casein, a protein of the human milk. The recombinant analogon of lactaptin (RL2) induces cell death of breast cancer cells. Despite well-established anti-cancer effects of RL2, the molecular mechanism of RL2 action in breast cancer cells remains unknown.

RL2 presents a promising therapeutic for treating breast cancer as a single drug or in a co-treatment with well-known therapeutics. In this study we have analyzed molecular mechanisms of RL2-mediated cell death. In particular, we have investigated the influence of RL2 on members of the Bcl-2 family and analyzed the effects of RL2 on cell death in a co-treatment with TRAIL in MDA-MB 231 and MCF-7 breast carcinoma cells. Strikingly, the inhibitory effects of RL2 on TRAIL-induced cell death have been observed upon short-term RL2/TRAIL incubation. These effects were observed using cell viability, metabolic and caspase-activity assays. We demonstrate that RL2 does not interact with components of TRAIL DISC, nor does RL2 block the TRAIL- induced DISC formation. Moreover, despite the fact that RL2 treatment alone induces Caspase-3/7 Activity, RL2 inhibits TRAIL induced Caspase-3/7 and Caspase-8 Activity in MDA-MB 231 cells Interestingly even though RL2 inhibits TRAIL-induced cell death for short time intervals, it sensitizes breast cancer cells to TRAIL upon long-term co-treatment. The inhibitory effects within short incubation periods give a hint about the unknown mechanisms of the cell death induction by RL2. The sensitization of breast cancer cells in long-term treatment to TRAIL stimulation point out that RL2 can act as a potential anti-cancer therapeutic.

9 | The recombinant fragment of human κ-Casein induces cell death by targeting the proteins of mitochondrial import in breast cancer cells

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Breast cancer is still one of the most occurring cancers for woman. Specified therapeutics are indispensable for optimal treatment. In the previous studies it has been shown that RL2, the recombinant fragment of human κ-Casein, induces cell death in breast cancer cells. However the molecular mechanisms of RL2-induced cell death remained largely unknown. Various non-breast cancer cell lines are unaffected by RL2 treatment. In this study mechanisms of RL2-induced cell death in breast cancer cells were systematically investigated. In particular, we demonstrate that RL2 induces cell death in breast cancer cells in caspase-independent and -dependent manners. The mass spectrometry-based screen for RL2 interaction partners identified mitochondrial import protein TOM70 as a target of RL2, which was subsequently validated. Further we show that RL2 is targeted to mitochondria after penetration into the cells. Finally, siRNA-induced downregulation of TOM70 reduced RL2-mediated cell viability loss. Taken together, this study demonstrates that RL2–TOM70 interaction plays a key role in RL2- mediated cell death and targeting this pathway may provide new therapeutic options for treating breast cancer.

10 | Interrogating Cyclin-Dependent-Kinase Inhibitors As A Novel Treatment Strategy For Glioblastoma Patients

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Glioblastoma (GBM) is the most common and aggressive primary brain tumour in adults. Despite intense effort to combat GBM with surgery, radiation and temozolomide (TMZ) chemotherapy, median survival for patients is 15 months and nearly all patients experience disease recurrence, usually within 6-8 months of treatment onset. Novel therapeutic approaches are urgently needed for both newly diagnosed and recurrent GBM patients.

CDKs are critical regulatory enzymes that drive cell cycle transitions and regulate transcription. Virtually all cancers, including GBM, harbour genomic alterations that lead to the constitutive activation of CDKs, resulting in the proliferation of cancer cells. Such observations have resulted in a widening avenue of research and clinical development for CDK inhibition as a novel anti-cancer therapeutic strategy, especially for those tumours that are particularly resistant to established treatments.

We now evaluate the potential of second-generation CDK inhibitors for the treatment of GBM. Second-generation CDK inhibitors have significantly improved potency and metabolic stability in comparison to first-generation inhibitors and offer greater hope for their future clinical utilization. We first established a clinically relevant model of the disease, eight patient-derived GBM cell lines, isolated from newly diagnosed and recurrent GBM tumours. We next demonstrated that two CDK inhibitors, CYC065 and THZ1, targeting CDK-9 and 7 respectively, significantly decreased viability of all cell lines, irrespective of whether they were grown in 2-D monolayers or 3-D sphere cultures. Cell cycle analysis revealed both inhibitors induced cell death-specific effects rather than cell cycle perturbations. Indeed, western blot analysis showed downregulation of the anti-apoptotic Mcl-1 protein in all cells treated with the CDK inhibitors. Critically CYC065 was well-tolerated in vivo, in an orthotopic PDX model of the disease. Cytostatic rather than cytotoxic effects were noted upon sole administration of CYC065 in vivo, however. We next investigated if combining the inhibitors with standard of care TMZ, would increase their therapeutic potential. No synergy was observed when either inhibitor was combined with TMZ in our panel of patient-derived cell lines.

These preclinical findings emphasize that second-generation cyclin-dependent kinase inhibitors can significantly impact the survival of newly diagnosed and recurrent GBM tumours in vitro. Such effects are most likely mediated by transcription-related events rather than direct targeting of the cell cycle. Combination strategies may be required to ensure maximum in vivo efficacy. Co-administering the CDK inhibitors with the death receptor ligand, TRAIL will next be examined.

11 | Study of the interplay between Z-RNA and the central regulator of the dsRNA response ADAR1

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Virus infection leads to an accumulation of double-stranded RNA (dsRNA) and mammalian cells respond to its presence by inducing a protective antiviral state. DsRNA helices typically adopt an A-form structure (A-RNA), however, A-RNA can transition into a different conformation called Z-RNA. Aberrant detection of both A-RNA and Z-RNA by antiviral dsRNA sensors from host-derived transcripts cause autoimmunity in the absence of virus infection.

The RNA editing enzyme ADAR is a central regulator of the dsRNA response. Mechanistically, ADAR1 directly binds to dsRNA and converts adenosine residues into inosines thereby disrupting base pairing of dsRNA structures, in a process called A-to-I editing. Although the molecular mechanisms by which ADAR1 prevents the accumulation of A-RNAs are well documented, its role in regulating Z-RNA metabolism has not been studied before. The identification of mutations that prevent the interaction of Z-RNA with ADAR1 in patients suffering from autoimmune disease suggest an important role in this process.

Here, we study the interplay between ADAR1 and Z-RNA at the molecular level and determine the consequences of disruption of Z-RNA-ADAR1 interaction in transgenic mice and human cells lines. I further wish to identify the putative antiviral nucleic acid sensors and cell death pathways that are activated by loss of Z-RNA binding to ADAR1. A better understanding of ADAR1 function is important to human health in context of viral infection and autoimmunity.

12 | Ferroptosis-resistant human glioblastoma cells are sensitized to ferroptosis by co-treatment with dehydroascorbic acid

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Introduction. Ferroptosis, an iron-dependent form of nonapoptotic cell death, has been identified as a type of death that could be activated in certain types of cancer. In addition, a popular hypothesis suggests that mega doses of the reduced form of vitamin C, ascorbic acid (AA), can act as an anti-cancer pro-drug. However, several cell types are resistant to mega doses of AA, such as glioblastoma. Interestingly, treatment with the oxidized form of vitamin C, dehydroascorbic acid (DHA), induces cell death by consumption of glutathione (GSH) and increasing reactive oxygen species (ROS) and lipid ROS in certain cell types. To date, it is unknown how to sensitize cells resistant to ferroptosis to this type of death. Here, we show that glioblastoma cells treated with erastin-DHA cotreatment are sensitized to death by ferroptosis.

Materials and methods. Human glioblastoma cells (U87) and normal primary rat astrocytes were treated with erastin, RSL-3, iron overload or glutamate, and were cotreated with DHA. Ferrostain and b-mercaptoethanol were used as ferroptosis inhibitors. Additionally, we evaluated the participation of autophagy in ferroptosis sensitization by DHA with bafilomycin A, chloroquine, and NH4Cl. Cell death was monitored by the incorporation of Sytox green with real-time live-cell microscopy using an IncuCyte S3 system. Total (CellRox), mitochondrial (mitosox) and lipid (Image-iT lipid perdoxidation) ROS levels were determined by FACS. We also evaluated mitochondrial fragmentation using superresolution microscopy and mitotracker.

Results. We found that human glioblastoma cells are resistant to conventional ferroptosis induction therapies (Erastin, RSL3, iron overload or glutamate treatment) despite expressing ACSL4. Interestingly, DHA-erastin co-treatment sensitizes glioblastoma cells to ferroptosis. While in primary astrocytes, treatment with DHA inhibits erastin-induced ferroptosis, suggesting that DHA-erastin would be specific for tumor cells.

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13 | Towards structure determination of oligomeric Bcl-2 protein complexes using two-‘color’-three-channel DEER

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Bax is mainly monomeric and cytosolic in healthy cells, while its activation leads to membrane-insertion into the mitochondrial outer membrane and pore formation. Structure determination of active Bax has proven to be difficult due to its membrane-embedded nature, its engagement in homo- or hetero-oligomers, its inhomogeneous oligomer size, and the complicated multistep transitions during its activation. However, we were able to propose a 3D model of active Bax based on double electron-electron resonance (DEER) spectroscopy in liposomes and isolated mitochondria. During those studies we faced the challenge that with even only one spin probe per monomer, Bax oligomerization created complex systems with multiple intra- and inter- monomeric spin distances that were difficult to disentangle. We reduced the complexity by using spectroscopic tricks to enrich and retrieve the intra-monomer distances, but our structural modeling would largely benefit from the assignment of intra- and inter-dimer distances. Towards this end and to further study hetero-complexes with other Bcl-2 proteins (determined by fluorescent techniques), we explore the use of spectroscopically distinguishable spin probes (or to simplify probes with two ‘colors’)) and a two-‘color’three-channel DEER strategy. In a nutshell, the experiment is similar to FRET, but DEER can probe not only the mean distance between “donor and acceptor” pairs but yields distributions of distances between “donor-acceptor”, “acceptor-acceptor” and “donor-donor” pairs. We succeed to produce active Bax homo- and hetero-complexes containing spin probes in two ‘colors’, to perform DEER experiments and to assign complex spin-systems that could not be disentangled before. We believe this success marks the beginning of an exciting journey to explore the structure of Bcl-2 protein complexes.

14 | Activation of caspase-2 upon genotoxic stress is regulated by phosphorylation and ubiquitination

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Caspase-2 was the first identified human caspase and still remains the least studied. Caspase-2, as a member of the caspase family, fulfills both pro-apoptotic and tumor-suppressive functions. The protein controls maintenance of genomic stability and triggers apoptosis in response to genotoxic stress. Recent studies have demonstrated its role in the elimination of potentially dangerous oncogenic aneuploid cells, resulting from a disruption of the cell division process. However, the detailed mechanisms for the activation and functioning of caspase-2 remain unclear and require further investigation. Post-translational modifications (PTMs), e.g. phosphorylation and ubiquitination, are known to play a crucial role in regulation of caspases activation. Although, multiple PTMs have been reported for initiator caspase-8 and -9, only three cases of phosphorylation have been described for caspase-2. Bioinformatic analysis allowed to predict potential phosphorylation sites of caspase-2 - S196, S220, S307, S384 and T380, which could regulate caspase-2 activation. The mutant variants of caspase-2 with substitutions S196D, S220A, S307A, S384A and T380A were generated. Wild-type caspase-2 and the four mutated variants of caspase-2 with substitution S196D, S220A, S307A, and T380A have been shown to undergo autocatalytic activation that led to accumulation of p32 and p19 active fragments. The mutation S384A was able to block caspase-2 processing. Moreover, genotoxic stress enhanced accumulation of caspase-2 active fragments; however, the substitution S384A fully inhibited cisplatin-induced generation of active subunits. Additionally, isolation of caspase-2 using affinity chromatography has shown that caspase-2S384A did not contain the p19active subunit. Consequently, these data confirmed that the mutation S384A in caspase-2 led to full inhibition of autocatalytic activation. Measurement of caspase-2 activity using fluorogenic peptide Ac-VDVAD-AMC corroborated that caspase-2S384A did not have catalytic activity under genotoxic stress. Taken together, the mutation of potential phosphorylation site S384 caused not only blockage of caspase-2 processing but also inhibition of its catalytic activity. Additionally, caspase-2 has been found to undergo ubiquitination. Under genotoxic stress caspase-2 was enriched with K48-linked polyubiquitin chains that suggested enhancement of its proteasomal degradation. MG132-mediated inhibition of proteasomal degradation led to accumulation of caspase-2 p19 fragment at 4 and 8 hours of incubation. The accumulation of p19 was enhanced under combined treatment with MG-132 and DNA-damaging agents. A decrease of procaspase-2 level was not detected, suggesting that the accumulation of p19 subunit was caused by inhibition of its proteasomal degradation but not through enchantment of procaspase-2 cleavage. Thus, under genotoxic stress caspase-2, in particular its active cleaved forms, undergoes ubiquitination that leads to its degradation.

15 | Pharmacological targeting of c-FLIPL and Bcl-2 family members promotes apoptosis in cancer cells

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The development of efficient combinational treatments is one of the key tasks in modern anti-cancer therapies. Cancer cells are characterized by deregulation of both extrinsic and intrinsic pathways. Procaspase-8 activation in death receptor (DR)-mediated apoptotic pathway is controlled by c-FLIP proteins. We have recently developed small molecule FLIPinB targeting c-FLIPL in the caspase-8/c-FLIPL heterodimer. These small molecules enhanced caspase-8 activity in the death inducing signaling complex (DISC), CD95L/TRAIL-induced caspase activation and subsequent apoptosis. To further increase the pro-apoptotic effects of FLIPinB and enhance its therapeutic potential in this study we investigated costimulatory effects of FLIPinB in combination with the pharmacological inhibitors of the anti-apoptotic Bcl-2 family members, in particular, ABT-263, ABT-199 and S63845. This analysis has been carried out in several cancer cell lines such as cervical cancer, pancreatic cancer and T-Lymphoma cells. The administration of these inhibitors together with FLIPinB increased CD95-induced caspase activation and the number of the apoptotic cells. Taken together, our study suggests new approaches for the development of novel cancer therapies specifically targeting apoptosis pathways both at mitochondria and at the DR levels.

16 | CATHEPSIN C IS CRITICAL FOR THE RELEASE OF CATHEPSINS FROM LYSOSOMES IN LEUCYL-LEUCINE METHYL ESTER TRIGGERED APOPTOSIS

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The lysosomotropic detergent LLOMe (leucyl-leucine methyl ester) was used in clinical trials to efficiently kill immune cells. It was also suggested to be used as an anticancer drug because it triggered apoptosis in cancer cells. We showed that LLOMe perturbed lysosomes of promonocytes (U-937 and THP-1) and cancer cells (U-87-MG and HeLa) because its polymerization by the lysosomal enzyme cathepsin C. LMP (lysosomal membrane permeabilization) was followed by the release of other cysteine cathepsins into the cytosol resulting in apoptosis. However, immune cells were found to be far more sensitive to LLOMe in comparison to cancer cells. We, therefore, suggest that the intracellular level of cathepsin C, as an upstream molecule, determines the cell sensitivity to the LLOMe treatment. Additionally, the results showed that cysteine cathepsins B, L and S are involved in downstream apoptotic signaling by using two gene ablation models. Stefin B deletion in primary mouse cancer cells resulted in sensitizing to cell death without affecting the release of cathepsins. On the other hand, simultaneous deletion of cathepsins B and L protected primary mouse embryonic fibroblasts against cell death. Therefore, we propose that due to the extreme sensitivity of immune cells to LLOMe and the risk of systemic toxicity, the drug is not suitable for anticancer therapy.

17 | In vitro investigation of glioblastoma cells responsiveness to IZI1551 in combination with a brain-penetrant synergizer

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Glioblastoma is the most aggressive primary malignancy of the central nervous system (CNS). Surgical resection is still the first step in the management of glioblastoma and despite the addition of chemotherapy (TMZ:temozolomide) or radiation, the outcome of glioblastoma patients remains poor with a median life expectancy of 15-17 months. Moreover, a proportion of patients gain no survival advantage to TMZ therapy at all. Additional therapeutic strategies that can activate cell death pathways are therefore required to face the challenge of treating glioblastoma patients.

IZI1551 is a 2nd generation TRAIL (tumor necrosis factor related apoptosis inducing ligand)-variant with an increased valency and half-life compared to previous TRAIL variants.

We aim to study cell death induction by IZI1551, both as a single agent and in combination with the blood-brain barrier (BBB) permeable proteasome inhibitor Marizomib. The potential synergistic effects of such treatments will be assessed.

For our in vitro model, we adopted short term cultivation of a panel of primary and recurrent glioblastoma patient derived cell lines (PDCLs), maintained in cancer stem-like cells preserving conditions.

Initial viability assays in which we screen combinations of IZI1551/Marizomib in applicant-relevant concentrations revealed that while glioblastoma PDCLs are only moderately sensitive to single agent treatments, the combination of both drugs can induce synergistic responses, as calculated by Webb's fractional product.

Subsequent Annexin V/PI based flow cytometry indicates that the combination of IZI1551 with Marizomib can induce cell death, and that apoptosis seems to be the main mechanism of death. Treatment responsiveness will also be assessed in 3D models of glioblastoma PDCLs.

Finally, we will aim to study if heterogeneity in protein expression patterns in GBM PDCLs prior to treatment will allow us to predict the efficacy IZI1551/Marizomib-based treatments.

18 | Proteomic analysis of necroptotic extracellular vesicles

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Necroptosis is a regulated but inflammatory form of cell death. As with necrosis, necroptosis is characterized by cell swelling and membrane permeabilization, resulting in the release of cell content containing pro-inflammatory molecules. This was believed to be the major driving force of necroptosis-mediated inflammation. We and others have previously reported that necroptotic cells release extracellular vesicles (EVs). This process is initiated during the early stage of necroptosis, while the plasma membrane is still intact. We found that necroptotic EVs are 0.1-0.4 um in size, and are loaded with proteins, including the key necroptosis executor factor, phosphorylated mixed lineage kinase domain-like (MLKL). However, the exact necroptotic EVs proteins composition and impact have not been delineated yet. Here, we show that necroptotic EVs expose phosphatidylserine, and can be phagocytosed by peritoneal macrophages. This, in turn, led to modulation of chemokine and cytokine secretion. To characterize their content, EVs from necroptotic and untreated U937 cells were isolated by ultracentrifugation and analyzed by mass spectrometry. A total of 3648 proteins were identified, sharing ~95 % with the exosome proteome database, Vesiclepedia, and ~70 % with the Exocarta data base, including 25 of 30 the most frequently identified proteins. Principal component analysis revealed that the necroptotic and untreated EVs samples were clustered separately. 304 proteins were significantly abundant in the necroptotic EVs. Among these, are MLKL (q-value 0.003) and caspase-8 (q-value 0.00001). Noticeably, MLKL abundance confirmed our previous reports, and was validated by western blot. Gene Ontology (GO) analysis of the differentially abundant proteins demonstrated the significant enrichment of terms related to the endosomal sorting complexes required for transport (ESCRT) machinery (Cellular Component, q-value 0.0000004). This supports the reported role of ESCRT components in the budding of MLKL-damaged plasma membrane, while also suggesting the involvement of new unstudied components of this machinery, as well as other vesicular trafficking proteins. The GO enrichment term: Immune Effector Process was also found to be enriched (Biological Process, q-value 0.00004), with candidates suggestive to facilitate cell-cell communication during necroptosis. Among these are interleukin-32 (q-value 0.0006), a pro-inflammatory cytokine with an unknown release mechanism, and the calcium-dependent phospholipid scramblase, TMEM16F (q-value 0.008), with an inconclusive involvement in necroptosis. In summary, our study reveals a new layer of regulation during the early stage of necroptosis by the secretion of specific EVs that may influence their microenvironment. Our future investigation aims to shed light on new players in the necroptosis signaling and its related EVs, as well as to uncover the functional tasks accomplished by the cargo of these necroptotic EVs.

19 | THE COMPARATIVE EVALUATION OF THE DYNAMICS OF APOTOSIS AND AUTOTHAGY MARKERS IN THE ACUTE PERIOD OF ISHEMIC STROKE

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Apoptosis, autophagy and necrosis are the main mechanisms of neuronal death after acute ischemic stroke (IS). However, it is not completely clear which of these processes prevails at a particular stage of the ischemic cascade and affects the outcome of the disease. Impact on certain stages of enhanced autophagic flux and apoptosis can contribute to neuronal survival. We proposed that a comparative assessment of the dynamics of apoptosis and autophagy markers will lead to better understanding of the cross-interactions or, on the contrary, the competitive relationships between these processes at different stages of the acute period of IS.

Objective. Comparative evaluation of the dynamics of the concentration of apoptosis and autophagy markers in the blood of patients in the acute period of IS compared with the dynamics of the severity of neurological deficit and the volume of brain damage.

Methods. All studies were agreed with the University ethical committee. The dynamics of apoptosis and autophagy markers in serum of 35 patients in the acute period of newly diagnosed IS in comparison to dynamics of the severity of the neurological condition and the volume of brain damage were studied. Clinical and neurological dynamic examinations with the assessment of neurological deficit using NIHSS (National Institutes of Health Stroke Scale) and determination of the brain infarct volume by MRI scan (Magnetic resonance imaging) were carried out. The serum levels of p53, Bcl-2, Beclin 1 and LC3 (ELISA kits; Abcam, UK) were investigated. The comparison group consisted of 29 healthy donors. Blood sampling was carried out within 12 hours after the ischemic attack and daily for the next 14 days.

Results. Statistically significant elevated levels of serum proteins p53, Beclin 1 and LC3 were detected already in the first 24 hours after acute IS. The increase in p53 protein content positively correlated with the severity of neurological deficit (NIHSS>10) and the amount of brain damage according to MRI data already on the 1st day after IS and for the next 10 days. Increased Beclin 1 and LC3 levels positively correlated with the severity of neurological deficit and the extent of brain damage from the 1st through the 3rd and 1st through the 5th days, respectively, which probably indicates a joint involvement of apoptosis and autophagy in neuronal death at the early stages of the acute period of IS. A strong direct correlation between the elevated level of Bcl-2 and the large volume of brain damage was observed only from the 11th to the 14th day. Perhaps this is due to the time required to activate compensatory antiapoptotic processes.

Conclusions. The data obtained indicate the involvement of p53, Bcl-2, Beclin 1 and LC3 proteins in ischemic brain damage at various stages of the acute period of IS. Our results confirm the active participation of autophagy, pro- and antiapoptotic processes in the formation of delayed neuronal death after acute IS.

20 | RIPK3 drives inflammatory signalling independently of cell death

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Inflammatory signalling and programmed cell death are important aspects of the innate immune defence. The nucleotide-sensing receptor Z-NA–Binding Protein 1 (ZBP1) is essential for restriction of proliferation of certain viruses like murine cytomegalovirus. Upon activation, ZBP1 induces necroptosis and inflammatory responses via Receptor Interacting Protein Kinase 3 (RIPK3). However, the signalling mechanism after ZBP1-induced RIPK3 activation remains poorly explored.

We show that ZBP1-induced RIPK3 activation leads to TAK1- and IKK–mediated production of inflammatory cytokines independently of cell death. To investigate how RIPK3 mediates inflammatory signalling, we employed a ligand-induced RIPK3 oligomerisation model. Similar to ZBP1, RIPK3 activation induced by oligomerisation leads to MAPK- and NF-κB¬–mediated inflammatory signalling without cell death. We further show RIPK3-mediated inflammatory signalling is regulated by the E3 ligases cellular Inhibitor of APoptosis proteins (cIAPs) and the Linear UBiquitin chain Assembly Complex (LUBAC) as well as Caspase-8. Finally, Receptor Interacting Protein Kinase 1 (RIPK1) is dispensable for RIPK3 to induce inflammatory signalling. However, RIPK1 is required to recruit cIAPs and Caspase-8 to activated RIPK3 to exert their regulatory functions.

Taken together, the data suggests that the ZBP1-RIPK3 receptor-kinase module, like TNFR1-RIPK1 and NOD2-RIPK2, mediates inflammatory signalling in a process regulated by non-degradative ubiquitin chains generated by IAP and LUBAC.

Alternative perspectives:

Taken together, the data suggests that the ZBP1-RIPK3 receptor-kinase module mediates inflammatory signalling independently of cell death.

Taken together, the data suggests that the ZBP1-RIPK3 receptor-kinase module, like TNFR1-RIPK1, mediates inflammatory signalling independently of cell death and RIPK3's kinase activity, in a process regulated by non-degradative ubiquitin chains generated by cIAPs and LUBAC.

21 | Inhibition of PUMA-induced apoptosis preserves hematopoietic stem cell function and prevents bone marrow failure in a dyskeratosis congenita mouse model

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Dyskeratosis congenita (DC) is one of the most frequent inherited bone marrow failure syndromes and characterized by severe hematological complications including risk of secondary myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). The disorder is caused by premature telomere shortening due to mutations in the telomere machinery. Critically short telomeres cause a DNA damage response with p53-mediated cell cycle arrest, senescence and/or apoptosis; the latter primarily mediated via transcriptional activation of pro-apoptotic PUMA. p53 pathway activation is regarded pivotal for the exhaustion of hematopoietic stem and progenitor cells (HSPCs) in DC pathogenesis. Inactivating this checkpoint could possibly mitigate the hematopoietic phenotype but would increase risk of genomic instability and leukemia. Based on our earlier mouse model of secondary leukemia (Genes Dev, 24(15):1602-7), we hypothesized that exclusive inhibition of p53-mediated apoptosis - while all other p53-induced pathways remain active - could delay hematopoietic failure and prevent malignant transformation.

We created a DC model by serially transplanting HSPCs from generation 3 mTerc-/- (G3mTerc-/-) mice with dysfunctional telomerase, into lethally irradiated wildtype recipients. After secondary transplantation 45% of the recipients died of bone marrow failure. Surviving recipients displayed severely reduced HSPC viability. With the aim to inhibit apoptosis of hematopoietic cells, we deleted Puma in G3mTerc-/- mice. Puma deficiency significantly improved bone marrow cellularity, HSPC viability (72% vs. 50% viable HSPCs, $p<0.01$), and hematopoietic output. Most importantly, only 21% of secondary recipients died in the absence of Puma. Telomere length in G3mTerc-/-Puma-/- HSPCs was significantly longer compared to their Puma proficient counterparts indicating reduced cell turn-over when PUMA-mediated apoptosis was inhibited. Importantly, no signs of leukemia, genetic instability or karyotypic abnormalities were observed.

Our data indicates that inhibition of intrinsic apoptosis is sufficient to prevent loss of cells with critically short telomeres, leading to reduced proliferation pressure in the HSPC compartment. Exhaustion of stem cells is prevented, while their functional and genetic integrity remains intact. We anticipate that this is also sufficient to impede outgrowth of malignant clones and transformation to secondary MDS and AML.

22 | The role of MCPIP-1 in the regulation of neutrophil apoptosis

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Neutrophils (PMNs, *polymorphonuclear cells*) are a dominating population of human blood leukocytes. Their lifespan has to be strictly controlled as abnormalities in this process can lead to the release of toxic bactericidal factors and damage of host tissues, what play a role in the pathogenesis of many inflammatory diseases. As the regulation of spontaneous apoptosis of neutrophils still remains unsolved phenomenon, thus we decided to analyze the role of MCPIP-1 (*monocyte chemoattractant protein – induced protein -1*), also called Regnase-1, in this process. Our idea comes from the study showing that MCPIP-1 can act as RNase for anti-apoptotic protein in tumor cells leading to the induction of apoptosis. Moreover, we observed elevated expression of MCPIP-1 in neutrophils shortly post isolation from the blood of healthy donors followed by the induction of programmed cell death. Therefore we performed large-scale analysis using neutrophils obtained from a cohort of healthy blood donors and found a strong positive correlation between the MCPIP-1 expression and intensity of spontaneous apoptosis. The role of MCPIP-1 was confirmed in PMNs isolated from transgenic mice devoid of MCPIP-1 expression selectively in phagocytic cells (MCPIP1-Flox-M-lys-CRE). We documented that apoptosis of murine MCPIP-1-deficient neutrophils is delayed compared to cells isolated from WT animals. Finally, we noted that MCPIP-1 expression is inhibited by broadly described anti-apoptotic cytokine GM-CSF (*granulocyte-macrophage colony - stimulating factor*), further confirming the role of MCPIP-1 in the control of the PMNs life-span. Taken together, the results of our experiments shed light on the regulation of physiology of neutrophils.

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23 | Regulation of the host cell death machinery by a single viral protein

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A plethora of viruses is generally present in the intestinal microflora therefore viruses are currently discussed as a potential causative trigger for the development of inflammatory bowel disease. A number of viruses, belonging to the family of Herpesviridae or Poxviridae, express proteins, denoted as viral FLIPs (vFLIPs), which share structural similarities with cellular FLIPs (cFLIPs). These proteins are important regulators of the host cell death machinery in intestinal epithelial cells (IECs). In our study, we analysed the consequences of vFlip expression in IECs in mice on intestinal homeostasis and cell death and the ability of vFLIP to compensate for cFLIP. Our results show that mice constitutively expressing vFlip in IECs were characterized by chronic intestinal inflammation accompanied by loss of Paneth cells, increased cell death and altered NFkB signalling. Additional blocking of the alternative NFkB pathway attenuated mucosal inflammation and Paneth cell loss induced by vFLIP. Moreover, in an inducible model, transient expression of vFlip in IECs reduced apoptotic cell death and counteracted loss of barrier integrity induced by cFlip deletion. Interestingly, our data indicate that a single viral protein is able to disrupt epithelial homeostasis and to initiate chronic intestinal inflammation in mice. Moreover, vFLIP alters NFkB signalling and is partially able to acquire functions from the cellular regulator cFLIP, suggesting that viruses are able to deregulate host cell death pathways by interfering with cellular proteins. Regulating the host cell death machinery might be a potential mechanism of viruses to gain time for replication and determine the right time point for further propagation. Since the general impact of viruses on intestinal homeostasis and disease development is not fully understood, this study might help to improve our understanding of the viral influence on intestinal homeostasis, cell death, barrier function and disease pathogenesis in humans.

24 | IFN-STAT1-MLKL axis drives necroptosis during gastrointestinal inflammation and infection

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Interferons (IFNs) are potent immune-modularity cytokines that are strongly expressed by mucosal immune cells and by intestinal epithelial cells (IECs) in response to inflammation and infection. It has just recently been reported that expression of the Mixed lineage kinase domain-like protein (Mlkl) can be induced by interferons via STAT1 and that this IFN-dependent pathway potentially influences the pathogenesis of several inflammatory diseases by mediating regulated necrosis. Intestinal homeostasis and maintenance of the intestinal epithelial barrier integrity are essential components of host defense during gastrointestinal infection. Maintaining a functional barrier requires a strict regulation of cell death. However, molecular mechanisms of cell death regulation as well as novel forms of cell death are not fully understood. Thus, we elucidated the contribution of different forms of regulated cell death and upstream regulatory components during gastrointestinal inflammation and infection. In vitro and in vivo, we revealed that MLKL-mediated necroptosis is impaired as a result of deletion of STAT1 during inflammation and infection. Mice lacking Caspase8 (Casp8ΔIEC mice), a negative regulator of necroptosis, are highly sensitive towards gastrointestinal infection and inflammation associated with lethality of these mice. Our results demonstrate that epithelial STAT1 signaling controls Mlkl gene expression in intestinal epithelial cells during Salmonella Typhimurium infection and that lethality of Casp8ΔIEC mice is mediated by epithelial STAT1 signaling. A deletion of STAT1 ensures the survival of Casp8ΔIEC mice during the early phase of a Salmonella infection with a milder course of disease based on decreased epithelial cell death and increased epithelial integrity. This protective effect appears to be linked to interferon signaling rather than an altered number of Paneth Cells and impaired antimicrobial peptide expression. Furthermore our data uncovers that STAT1 is particularly important during disease initiation in the early phase of infection but not during disease progression by controlling cell death. Additionally, this pathway seems to be pathogen specific since STAT1 has only a minor contribution to coordinate cell death during Citrobacter rodentium infection. Collectively, our study reveals that STAT1-signaling maintains homeostasis of the intestinal epithelial barrier by altering Mlkl expression. Thus our data suggest that interferons and epithelial STAT1-signaling maintains intestinal tissue homeostasis during Salmonella infection by controlling host cell death.

25 | Apoptotic epithelial cells trigger an ERK/Akt activity wave in the neighbors to protect them from apoptosis

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Homeostasis between proliferation and cell death is crucial to maintain epithelial barrier function. Whereas the control of proliferation events on the base of cell density has been well characterized, less is known how epithelial cells coordinate cell death events.

By using an in vitro human epithelial model, fluorescent biosensors for ERK and Akt activity and computer-vision image analysis, we report that apoptotic cells induce a wave of ERK and Akt kinase activity in the neighbors. This wave propagates from the first layer of cells, directly in contact with the apoptotic one, to the other layers in a radial and unidirectional fashion. We describe the properties and spatiotemporal organization of this phenomenon. Mechanistically, the propagation of ERK/Akt wave requires the activity of the epidermal growth factor receptor (EGFR). Functionally, such ERK and Akt activity wave protects neighboring cells from further apoptosis for a period of about 4 hours. We provide direct demonstration of this phenomenon by observing several apoptotic events. In addition, we confirmed the ability of ERK/Akt activity pulses to induce death resistance by generating such pulses with an optogenetic actuator of the two pathways.

In sum, we found that epithelial cells can coordinate apoptotic events by communicating pulses of ERK and Akt activity. To our knowledge, this is the first demonstration of this phenomenon in mammalian cells.

26 | Inflammasome activation by bacterial type VI secretion system

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The type VI secretion system (T6SS) is a bacterial pertinacious machinery by which toxic proteins, called effectors, are delivered into a neighboring cell or to the external environment. Most characterized T6SSs mediate anti-bacterial activities, whereas few T6SSs were shown to target eukaryotic cells. We hypothesis, that T6SS may act directly or as a system to deliver virulence factors to eukaryotic host cells. To test this hypothesis we used the marine bacterium Vibrio proteolyticus, as it lack T3SS. Here, we show that one of the three T6SSs encoded by the marine bacterium Vibrio proteolyticus (i.e., T6SS-3) induces death in eukaryotic host cells that is independent of a previously described cytotoxic pore-forming hemolysin, VPRH. Specifically, we found that T6SS-3, but not T6SS-1 or T6SS-2, is responsible for the induced cell death. We further demonstrate that T6SS-3 activate the NLRP3 inflammasome leading to caspase 1 activation and GSDMD cleavage that results in IL-1b secretion and pyroptotic cell death. Future studies will reveal the specific inflammasome pathway and identify the new bacterial T6SS effectors that target and activated this pathway. Finally, this study set the foundation for understanding the immunological consequences of inflammasome activation by T6SSs.

27 | Macrophages, rather than DCs, are responsible for inflammasome activity in the GM-CSF BMDC model

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Since the discovery of the inflammasome by Jurg Tschopp's laboratory in 2002, thousands of studieshave investigated the molecular and cellular mechanismsrequired for inflammasome activation. In thepast, the vast majority ofinflammasome studies have used monocyte and macrophage cell lines and/orL-con BM-derived macrophages (BMDMs). As research into the regulation and effects of inflammasomes in disease has rapidly expanded, a variety of cell types, including dendritic cells (DCs), have been suggested to be inflammasome competent. However, while most of the inflammasome studies in DCs relies on the granulocyte-macrophage colony-stimulating factor (GM-CSF) derived bone marrow–derived dendritic cells (BMDCs) protocol, this protocol was recently found to comprise a heterogeneous population of conventional DCs and monocytes.Here we describe a major fault in the widely used DC–inflammasome model of BMDCs generated with the cytokine GM-CSF. We found that within GM-CSF bone marrow–derived cell populations, monocyte-derived macrophages, rather than BMDCs, were responsible for inflammasome activation and IL-1βsecretion. Therefore, GM-CSF BMDCs should not be used to draw conclusions about DC-dependent inflammasome biology, although they remain a useful tool for analysis of inflammasome responses in monocyte–macrophage.

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28 | Improving and Personalising Treatment Options for Paediatric Brain Tumour Patients

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Medulloblastoma (MB) is the most commonly occurring brain tumour in children, and while the survival rate is approximately 70-80%, aggressive multimodal treatment - surgery, chemotherapy and craniospinal radiation therapy (RT) - is associated with severe side effects and significant radiation-induced brain injury. This highlights an urgent need for personalised approaches to select the most appropriate drug for each patient to (1) reduce/eliminate the need for RT and (2) reduce side effects associated with inefficient therapy.

Using a panel of cell lines representing the main MB subgroups, we highlight heterogeneity in both their response to standard, clinically relevant chemotherapeutic agents, and their expression profiles of proteins involved in cell death signalling. We hypothesise that the chemoresistance characterising MB is due to dominance of anti-apoptotic signals or defects in key components of the apoptotic machinery, whereby identifying these signals could lead to development of more rational individualised therapeutic strategies.

Initial studies using an innovative, systems-based strategy (APOPTO-CELL), developed by our lab, showed that expression of apoptotic proteins downstream of mitochondrial activation is not predictive of chemotherapy response, and hence we hypothesise that causes of chemoresistance are more likely upstream of mitochondrial activation.

We aimed to assess the contribution of the Bcl-2 family of proteins to chemoresponsiveness, and first confirmed expression of the anti-apoptotic proteins Bcl-2, Bcl-xL and Mcl-1 via western blotting. Using BH3 profiling, we identified a dependence on Bcl-xL in particular in one subset of cell lines (SHH subgroup). Combination treatment of cisplatin and ABT-263 was synergistic in these cell lines, while the addition of an Mcl-1 inhibitor (S-63845) and Bcl-2 inhibitor (ABT-199) to standard treatment yielded no additional effect. We are currently exploring the use of additional targeted agents including WEHI-539 in these cell lines, and further expanding this work to include other subgroups of MB.

In the long term, this work has potential to create a clinical decision-making tool to facilitate delivery of personalised, effective chemotherapy in specific patient cohorts, allowing the reduction/elimination of RT, and ultimately reducing long-term sequelae.

29 | Effects of metal oxide nanoparticles on regulated cell death pathways: apoptosis, autophagy, ferroptosis and necroptosis

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The most predominantly investigated feature of nanoparticles (NPs), in particular metal oxide (MO) NPs, is the ability to induce oxidative stress in the biological environment. Reactive oxygen species (ROS) produced by these NPs can damage cell membrane, as well as intracellular DNA, proteins and lipids, leading to the eventual induction of regulated cell death (RCD). A deep understanding of the mechanistic basis of nanoparticle (NP) interactions with the RCD machinery greatly help in defining their eventual adverse and/or positive effects in human cells, as well as their potential for the development of anti-cancer therapies. Here we describe the effects of several MO NPs, including Fe3O4, ZnO, and TiO2 NPs, on RCD pathways in breast (MCF7) and melanoma (A375 and B16-F10) cancer cell lines. Although low-to-moderate concentrations of these NPs (

30 | Calcium signaling in necroptosis: one ion with many faces

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Necroptosis is an inflammatory type of regulated cell death that depends on the activation of the mixed-lineage kinase domain-like (MLKL) protein. MLKL mediates plasma membrane permeabilization that ultimately kills the cell. Calcium has a dual role in necroptosis, as it is required both for cell death and for the activation of ESCRT-III mediated plasma membrane repair mechanisms. However, it is not clear yet how MLKL induces the activation of calcium fluxes and, in a more general sense, how necroptotic cell death and cell survival, as two opposing mechanisms, are regulated by calcium. Here, we performed a comprehensive analysis of the effect of calcium signaling in necroptosis, by combining live-cell confocal microscopy and classic cell biology. We found that calcium fluxes require MLKL and are activated quickly after the formation of MLKL clusters in the cells. This process anticipates cell rounding and plasma membrane breakdown, two typical hallmarks of necroptotic cell death that are related to osmotic forces. The ESCRT-III component CHMP4B forms intracellular clusters after cytosolic calcium influx. Chelation of both extracellular and intracellular calcium attenuates the level of observed cell death and CHMP4B cluster formation. We also demonstrate that inhibitors of endogenous calcium channels from the endoplasmic reticulum protect cells, pointing to a probable role of calcium stores during necroptosis. This work provides new insights into the complex role of calcium fluxes in necroptosis.

31 | The apoptosome molecular timer synergistically aids XIAP in shutting down caspase signalling during apoptosis execution

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The execution phase of apoptosis is a critical process in programmed cell death in response to a multitude of cellular stresses. A crucial component of this pathway is the apoptosome, a platform for the recruitment, activation and cleavage of pro-caspase 9 (PC9). Recent findings have shown that autocleavage of PC9 to C9(p35/p12), a process dependent on PC9 homodimerisation on the apoptosome, not only permits XIAP-mediated C9 inhibition but also temporally shuts down the activity of the apoptosome due to the low affinity of C9(p35/p12) to the apoptosome, forming a molecular timer. Interestingly, C3-mediated feedback cleavage of C9(p35/p12) to the C9(p35/p10) isoform relieves XIAP-inhibition and restores C9 affinity to the apoptosome. In order to broadly analyse and delineate the combined contributions of XIAP and the apoptosome molecular timer to apoptosis execution we utilised a systems biological modelling approach. We demonstrate that a cooperative binding model for PC9 recruitment to the apoptosome, based on existing PC9-apoptosome interaction data, is important for efficient formation of PC9 homodimers, subsequent autocatalytic cleavage and thus dual regulation by XIAP and the molecular timer across a wide-range of biologically relevant PC9 and APAF1 concentrations. We next screened physiologically relevant concentration ranges of APAF1, PC9, PC3, XIAP and SMAC for cellular scenarios where the molecular timer can actively prevent apoptosis. We discovered that the molecular timer can prevent apoptosis execution in specific scenarios after both complete or partial mitochondrial outer membrane permeabilisation (MOMP). Furthermore, its ability to prevent apoptosis is intricately tied to a synergistic combination with XIAP. We therefore postulate that the physiological function of the molecular timer is to aid XIAP in the shutdown of caspase-mediated signalling. This shutdown potentially facilitates a switch to pro-inflammatory caspase-independent responses subsequent to Bax/Bak pore formation.

32 | The disassembly of apoptotic monocytes and Influenza infection

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Apoptosis is a fundamental biological process responsible for the turn-over of billions of cells within the human body every day. Although the induction of apoptosis and the removal of apoptotic cells has been thoroughly studied, the intermediate step (a process called apoptotic cell disassembly) has been largely overlooked. Nevertheless, we recently demonstrated that the disassembly of apoptotic monocytes is a highly regulated process, controlled by a series of proteins and cellular processes. Moreover, we discovered a previously undefined membrane protrusion coined beaded-apoptopodia that fragments to generate an abundance of small membrane-bound vesicles known as apoptotic bodies (ApoBDs).

As Influenza A virus (IAV) infection commonly induces immune cell apoptosis, we next investigated whether IAV could induce monocyte ApoBD formation, and the functional consequence of this process. We first developed a series of flow cytometry methodologies to accurately detect, quantify and isolate ApoBDs, and validated that IAV can induce monocyte apoptosis and apoptotic cell disassembly in vitro and in vivo. Next, we demonstrated that IAV proteins, genomic material and lethal virions are distributed into the beaded-apoptopodia and ApoBDs of infected monocytes. Consequentially, the ApoBDs derived from IAV-infected monocytes could propagate IAV infection and induce cell death in vitro, and elicit an innate and adaptive immune response in vivo. Next, through a high-throughput drug screen we identified a commonly used anti-psychotic as a potent inhibitor of apoptotic cell disassembly. Treatment of infected monocytes with this inhibitor could significantly impair ApoBD-mediated viral propagation in vitro and reduce disease severity in vivo. Taken together, these results demonstrate a novel role of apoptotic cell disassembly in aiding IAV propagation, unveiling new therapeutic strategies to combat viral infection.

33 | Novel functions of caspase-9 in endochondral bone development

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Caspase-9, as an initiator of the mitochondrial apoptotic pathway, triggers cell death by activation of the effector caspases -3, -6 and -7. Currently, the action of caspases has been re-evaluated because of their non-apoptotic roles in many systems. We have previously shown non-apoptotic activation of pro-apoptotic caspases during bone development. Here we have focused on caspase-9, which becomes activated in non-apoptotic chondrocytes of the growth plate of endochondral (long) bones. The growth plate is an important part of endochondral bone as it allows for elongation.

To further clarify the role of caspase-9 in these chondrogenic cells we utilised an in vitro micromass system. Caspase-9 was inhibited by the specific pharmacological inhibitor Z-LEHD-FMK. After one week of cultivation, cells were lysed, RNA isolated, cDNA prepared and a panel of 84 osteogenesis-related genes analysed by PCR Array.

In the treated cultures, expression of 8 genes was significantly altered. The most prominent change was observed in expression of Mmp-9, which decreased 53-times in treated cultures. Mmp-9 has the ability to cleave a variety of extracellular matrix components including collagens and has been described in the degradation and damage of articular cartilage. To determine the dynamics of Mmp-9 expression in micromass cultures, we detected RNA levels of Mmp-9 during the course of caspase-9 inhibition. These results showed that decrease of Mmp9 expression during cultivation positively correlated with the time of caspase-9 inhibition. Further, co-localization of caspase-9 and Mmp-9 was observed in the prenatal growth plate during long bone development in vivo.

Our results point to caspase-9 as having a significant impact on Mmp-9 regulation. Since caspases mostly operate through substrate cleavage, an indirect effect on Mmp-9 expression would be expected. Therefore, our ongoing research quests for the mechanism behind the impact on MMPs.

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34 | Stress-induced TRAILR2 expression overcomes TRAIL resistance in cancer cell spheroids

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Tumor cell populations can show considerable heterogeneity in their susceptibility to apoptosis, the primary cell death modality induced by anti-cancer therapies. Here, we studied the susceptibility of cancer cell spheroids, grown to the size of micro-metastases, to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Interestingly, we observed that pronounced, spatially coordinated response heterogeneities manifest within spheroidal microenvironments: In spheroids grown from genetically identical cells, TRAIL-resistant subpopulations develop and form layers that enclose and protect TRAIL-hypersensitive cells, thereby leading to an overall increased treatment resistance. TRAIL-resistant cell layers form at the interface of proliferating and quiescent cells and are characterized by the loss of both TRAILR1 and TRAILR2 protein expression. In contrast, oxygen and nutrient deprivation promote high amounts of TRAILR2 expression in TRAIL-hypersensitive cells in inner spheroid layers. COX-II inhibitor celecoxib further enhanced TRAILR2 expression in spheroids and thereby re-sensitized TRAIL-resistant cell layers to treatment, likely as a consequence of increased endoplasmic reticulum (ER) stress. Our analyses provide novel insight into how TRAIL response heterogeneities manifest within well-defined multicellular environments, and how cells within these environments can be manipulated to minimize or eliminate barriers of TRAIL resistance.

36 | The search for RNA targets for exogenous ribonucleases associated with apoptosis failure in tumors

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Exogenous ribonucleases, namely ribonuclease binase from Bacillus pumilus and bovine pancreatic RNase A were shown to display pronounced antitumor activity which is likely mediated via their ability to degrade intracellular and extracellular coding and non-coding RNAs. However the spectrum of their targets depending on the tumor type is not well evaluated yet.

Here, the miRNA profiles of several tumor models of mice were assessed in vitro and in vivo during cells/tumors treatment with binase or RNase A – enzymes decreasing their invasive and metastatic potential. In connection with the known ability of binase to induce apoptosis of tumor cells, special attention was paid to miRNAs involved in the regulation of apoptosis events.

In vitro upon B-16 melanoma cells treatment with binase or RNase A the downregulation of oncomirs miR-155 and miR-10b mediating negative regulation of apoptosis and the upregulation of tumor suppressor miR-145 promoting TNF-α-induced apoptosis were found. It was shown that alteration of miRNA in B16 melanoma and lymphosarcoma RLS40 cells under the action of binase resulted in a drop of mitochondrial potential, increase in calcium concentration and inhibition of cell respiration that indicated on the activation of intrinsic apoptotic pathway. The subsequent binase-mediated synthesis of TNF-alpha by the cells triggers apoptosis via extrinsic pathway.

In vivo RNase A in LLC/C57Bl model as well as binase in lymphosarcoma RLS40/CBA model caused the decrease of the levels of major part of miRNA in bloodstream of tumor-bearing animals including oncomirs and tumor-suppressor miRNAs. Using LLC/C57Bl model it was proved that such alterations of miRNA profile in blood provide the rearrangement of miRNA profile of tumor tissue that lead to the increase of the expression of genes encoding proteins that function as positive inducers of apoptosis, both caspase-dependent and mitochondrial (Pcpb4, Faim, Pycard, Plekhf1 and Dapk1), and the reduction of expression of the genes Hipk3 and Bcl2l2 involved in negative regulation of apoptosis.

Cumulative observations allow us to conclude that ribonucleases cause a change in the miRNA profile associated with apoptosis failure in tumors at the level of the cell and organism, which mediates the tumor death.

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37 | Antitumor cell-free vaccines based on mannosylated liposomes and membrane vesicles derived from dendritic cells provide the formation of cytotoxic T-lymphocytes with enhanced killing properties

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Antitumor vaccines based on dendritic cells (DCs) are considered as promising tools for the tumor treatment. However, the preparation of cell-based vaccines is associated with many difficulties, such as long-term storage of cells, the problem of standardization and instability of cell vaccines. To solve these problems, new cell-free antitumor vaccines targeted to antigen-presenting cells or cytotoxic lymphocytes (CTL) are currently being developed.

In this study, we investigated two types of cell-free antitumor vaccines based on (1) tumor RNA-loaded mannosylated liposomes, addressed to lectin receptors of antigen-presenting cells (in particular, DCs) (ML) and (2) cytochalasin B-induced membrane vesicles, obtained from murine DCs transfected with complexes of ML/tumor RNA (MV).

ML consisted of the mannosylated lipid conjugate with diethylsquarate linker, the polycationic lipid 2X3, and the lipid-helper DOPE at a molar ratio of 1 : 3 : 6 [1]. ML vaccines were obtained by the loading of ML with total RNA of murine melanoma B16 or lymphosarcoma RLS40. MV vaccines were obtained from murine bone marrow-derived DCs and tsDCs transfected with ML/tumor RNA complexes by treatment with cytochalasin B followed by mechanical disruption of cells.

The level of antitumor CTL priming in vivo under the action of cell-free vaccines was much higher for cytochalasin B-induced MVs in comparison with ML-based vaccines and similar to the level of CTL priming by classical DC vaccines. A study of the antitumor immune response in lymphosarcoma RLS40/CBA model after treatment with cell-free vaccines showed that double immunization with MV-based vaccines resulted in 50-fold inhibition of tumor growth, and their efficiency was 20-fold much higher in comparison with ML-based and classical DC vaccines. ML- or MV-based vaccines inhibit primary tumor grows and metastasis, stimulate lymphocyte migration to the tumor node and induce apoptotic and necrotic changes in the tumor tissue. Histological study of spleen and thymus demonstrated that cell-free vaccines cause the increase of the white pulp and diameter of lymphoid follicles in the spleen and increase of the thymus cortex reflecting an effective activation of immune system. Evaluation of biochemical parameters of blood serum and liver morphology showed that ML- and MV-based vaccines have no significant liver injury.

Thus, the obtained data provide the basis for the development of new effective immunotherapeutic approaches that allow the transition from antitumor vaccines based on dendritic cells to cell-free vaccines based on targeted liposomes or extracellular vesicles of dendritic cell origin.

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38 | Therapeutic harnessing of iron-dependent cell death in melanoma; the Apolipoprotein E and ferroptosis link

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Dedifferentiation is a hallmark of cancer progression, and in highly plastic melanoma cells it is a source of cross-resistance to both targeted and immune therapies. Recent studies, across diverse cancer lineages have provided evidence that the drug-resistant dedifferentiated cancer cell state hallmarked by increased activity of enzymes, promoting the synthesis of polyunsaturated fatty acids (PUFA) facilitating the high mesenchymal and motile cancer phenotype. In a recent study, using insilico based approach on wide range of melanoma cell lines and validating through patient sample database melanoma was classified in four progressive differentiation states; Melanocytic (M), Transitory (T) Neural Crest like (NC) and Undifferentiated (U), further refining the Hoek's main classification in proliferative/melanocytic (MITFhighSOX9lowAXLlow) and invasive/undifferentiated (MITFlowSOX9highAXLhigh). Pharmacogenomics drug sensitivity profile revealed that the undifferentiated/invasive cluster of the melanoma cell lines was selectively sensitive to agents inducing ferroptosis; necrotic cell death executed by iron-dependent peroxidation of PUFAs(1). However, the mechanistic underpinning of ferroptosis susceptibility or resistance mechanisms in melanoma cells remains largely undefined.

Using a panel of different melanoma cells lines belonging to the four main melanoma subtypes, we found that cells with U subtype promptly undergo Ferrostatin-1 and Deferoxamine inhibitable cell death after treatment with the ferroptosis inducer RSL3, which is accompanied by high level of lipid peroxidation. Lipid profiling of these melanoma subtypes, show that melanoma cells with U subtype have high level of PUFAs followed by the NC, T and M subtypes. Hierarchical clustering using a list of lipid metabolism genes on a RNAseq dataset consisting of 53 melanoma cell lines identified two main clusters; cluster 1 (M and T subtypes) and cluster 2 (NC and U subtypes) with Apolipoprotein E (APOE) being top differentially expressed gene between the clusters. In line with this, protein expression levels of APOE are high in M and T melanoma subtypes while the NC and U melanoma subtypes harbour low levels of APOE. Notably, culturing the U subtype of melanoma cells in presence of recombinant APOE significantly reduced induction of ferroptosis. These observations thus far suggest APOE as a candidate molecule regulating differential sensitivity towards ferroptosis in melanoma. Ongoing research aims to unravel the molecular and in vivo mechanisms through which melanoma-associated APOE confers resistance to ferroptosis and track at the single-cell level the pathway that APOE coordinates in ferroptosis-resistant melanoma cells.

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39 | Understanding the impact of necroptosis or apoptosis on myeloid inflammation in cancer.

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Cell death pathways have important implications for cancer-associated inflammation and immunity. Two major programmed cancer cell death pathways, i.e. apoptosis or necroptosis, strongly differ in their inflammatory potential. While apoptosis is largely tolerogenic or immunosuppressive (especially extrinsic apoptosis) in a physiological cancer setting yet, necroptosis is regarded as pro-inflammatory. This necroptosis associated inflammation may be vital for eliciting anticancer immunity, especially in absence of apoptosis. Unfortunately, research addressing this has produced contradictory observations wherein necroptosis was found to elicit anticancer immunity, immunosuppressive effects or even pro-tumourigenic inflammation depending on the context. Studying the immunological effects of apoptosis vs. necroptosis in cancer has been complicated by the lack of murine tumor models thereby making in vivo immunological scrutiny difficult. This needs to be addressed urgently since immunotherapies partly operate via extrinsic cancer cell death stimuli (e.g. death receptor ligands), which positions necroptosis and extrinsic apoptosis at the core of cancer immunotherapy

To this end, we decided to exploit the commonly used murine tumour model of TC1 lung carcinoma cells to address this avenue, since it was recently shown to have necroptosis susceptibility. We used a system, consisting of WT TC1 cells and TC1 cells knocked-out for the pro-necroptotic gene, Mlkl via CRISPR/Cas9 method By testing various cell death parameters and markers we established that WT TC1 cells indeed underwent bona fide necroptosis, which could be switched to apoptosis in Mlkl ablated TC1 cells. However the overall kinetics were different depending on the cell death stimulus i.e. TNF or TRAIL. Interestingly, our preliminary analyses found both necroptosis and apoptosis to cause suppression (rather than activation) of (type I) interferon immunogenic responses in interacting J774 myeloid (macrophage-like) cells. Conversely, TC1 cells basely elicited significant NFkBbased signaling from J774 myeloid cells wherein, contrary to expectations, necroptosis or apoptosis didn't alter NFkB signaling further. We are currently investigating the factors mediating these responses and hope to unravel their implications for anticancer immunity and immunotherapy.

40 | Sensitisation of Smac-induced Cell Death by Tumour Necrosis Factor Receptor (TNFR) Type 2-mediated Signalling in T-cell Acute Lymphoblastic Leukaemia (T-ALL)

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Increased TNF expression is associated with poor prognosis in leukaemia. In line with this, autocrine TNF can promote cell survival and leukaemia progression by exhibiting anti-apoptotic and pro-proliferative functions. Critical factors regulating these functions are IAP proteins found to be upregulated in leukaemia, thereby, promoting pro-survival effects of TNF. Inhibition of IAPs by Smac-mimetics can sensitise some but not all leukaemic cells to TNF-induced cell death. Here, we aim to elucidate the mechanism of SM-sensitivity in T-ALL by investigating individual components of the TNF-system.

In this study, we evaluated the efficacy of TNFR-signalling to induce cell death in combination with the Smac-mimetic (SM) Birinapant against a diverse set of T-ALL cell lines and patient-derived xenograft (PDX) T-ALL cells.

We found constitutive low level expression of cell surface TNFR1, whereas TNF and cell surface TNFR2 was detected at various expression levels in approximately 30% of both T-ALL cell lines and PDX T-ALLs. SM Birinapant promotes TNF-induced cell death in various T-ALL cell lines and PDX T-ALLs. As expected, SM Birinapant-induced cell death is dependent on TNF production as it could be abolished by anti-TNF treatment. Interestingly, correlation analyses revealed a very good correlation between SM-sensitivity and cell surface TNFR2 suggesting that surface TNFR2 primes PDX T-ALLs towards SM Birinapant-induced cell death. In line with this, (i) efficient activation of TNFR2 by a TNF-variant mimicking membrane-bound TNF augments SM Birinapant induced cytotoxicity, (ii) shRNA-mediated knockdown of TNFR2 is sufficient to significantly diminish TNF/SM-induced cell death in T-ALL cell lines and (iii) ectopic expression of TNFR2 primed TNFR2-negative T-ALL cell lines for TNF/SM-induced cell death.

Together, our data demonstrate large differences in the expression pattern of TNFR2 on T-ALL cells. TNFR2 expression seems to sensitise T-ALL cells for SM Birinapant-induced killing, thus, could potentially be used as biomarker for SM sensitivity in T-ALL.

41 | A combination treatment of Smac mimetic LCL-161 and TRAIL induces apoptosis in sensitive breast cancer cells through RIP1 complex formation independent of RIP1 kinase function

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Smac mimetics are a class of compounds designed to mimic the inhibitor of apoptosis protein (IAP)-binding functions of the Smac protein and are currently investigated as potential anti-cancer agents. They act by disinhibiting caspases and facilitating cell death. However, most cancer cell lines are resistant to Smac mimetics as single agents and for this reason, they are mainly studied in combination with other agents to induce death of cancer cells.

One potential strategy of using Smac mimetics in a cancer setting is to combine them with death receptor agonists. We are currently investigating if Smac mimetics can be used to increase sensitivity to TNF-related apoptosis-inducing ligand (TRAIL) in breast cancer cells and how cell death is mediated by the combination treatment. In our studies, we found that the breast cancer cell lines MDA-MB-468 and CAMA-1 are resistant to the Smac mimetic LCL-161 and display a moderate sensitivity to TRAIL in a WST-1 assay. When LCL-161 and TRAIL is combined, the TRAIL sensitivity of the cell lines are highly increased. The cell viability decrease is correlated with increased cleavage of caspase-8 and caspase-3 and the cell death measured with an Annexin-V/propidium iodide flow cytometry assay can be completely blocked by pre-treatment with the caspase inhibitor z-VAD-fmk, indicating that the cells die through apoptosis. Downregulation of caspase-8 using siRNA almost completely blocked cell death induced by LCL-161 and TRAIL, suggesting that apoptosis is primarily mediated by this caspase. In a third breast cancer cell line, MCF-7, the combination treatment did not result in decreased cell viability nor in increased caspase-8 cleavage. In the sensitive CAMA-1 cells, apoptosis was preceded by RIP1 association with caspase-8, an interaction that could not be seen in MCF-7 cells. The interaction was correlated with subsequent RIP1 cleavage and decreases in FLIP-L protein levels. Downregulation of FLIP in MCF-7 cells showed a tendency towards increased sensitivity to the combination treatment. Furthermore, downregulation of RIP1 in CAMA-1 cells significantly decreased Annexin-V positivity induced by LCL-161 and TRAIL, suggesting that RIP1 has an important role in apoptosis induced by the combination treatment. In contrast, inhibition of RIP1 kinase activity using necrostatin-1 did not increase cell death induced by the combination treatment, suggesting that the pro-apoptotic role of RIP1 in this setting is primarily mediated by a non-catalytic function.

In conclusion, our study shows that a combination therapy of LCL-161 and TRAIL can be used to induce apoptosis in breast cancer cells and identifies an important, non-catalytic function of RIP1 in mediating apoptosis under these settings. This function is preceded by an association of RIP1 with caspase-8 that does not occur in resistant MCF-7 cells.

42 | Real-time assembly mechanisms of Bax and Bak during apoptosis at the single molecule level

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The Bcl-2 family members Bax and Bak drive apoptotic cell death by assembling into oligomers that permeabilize the mitochondrial outer membrane (MOM).

Despite Bax and Bak have largely overlapping functions, whether they follow similar or different mechanisms of action during apoptosis is yet not known.

Here we use single particle imaging to quantify the real time dynamics of assembly of Bax and Bak in the mitochondria of apoptotic cells. Our data reveal divergent kinetics of oligomerization for these two proteins: while Bak oligomers assemble faster than Bax and reach an equilibrium, Bax oligomers continuously grow overtime. Interestingly, by using a Bax mutant, which retains full Bax activity, but localizes at the mitochondria as Bak, we have proved that the different behavior for Bax and Bak is independent of protein concentration and localization at the mitochondria, but is due to a different mechanism of assembly.

By complementary stoichiometry analysis in model membranes we have dissected the mechanism of assembly of Bak oligomers and revealed a concentration-dependent behavior for Bax, but not for Bak, oligomer formation. In line with this finding, Bak forms smaller pore structures than Bax, as revealed by atomic force microscopy.

Overall, our study shed new light on the molecular mechanisms by which Bax and Bak proteins mediate MOM permeabilization and strongly question their apparently redundant functional role in apoptosis and in its poorly understood, emerging inflammatory outcome.

43 | Humanised Mcl-1 mice enable accurate preclinical evaluation of MCL-1 inhibitors destined for clinical use

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MCL-1 is a pro-survival BCL-2 family member essential for the sustained growth of many cancers, making it an attractive therapeutic target. Recently a highly specific MCL-1 inhibitor, S63845, showing 6-fold higher affinity to human MCL-1 compared to mouse MCL-1 was described. To accurately test the efficacy and tolerability of S63845 in preclinical models of disease, we developed a humanised Mcl-1 (huMcl-1) mouse strain in which the genomic region of the murine Mcl-1 locus was replaced with its human homolog. HuMcl-1 mice are phenotypically indistinguishable from wild-type mice, and the intrinsic apoptotic pathway remains intact in their cells with no compensation by other BCL-2 family proteins observed. Lymphoid cells from the huMcl-1 mice died in a comparable manner to wild-type counterparts in response to diverse cytotoxic stimuli, however, they were considerably more sensitive to S63845. As anticipated, the maximum tolerated dose of S63845 was lower in huMcl-1 mice than wild-type mice. To test whether malignant cells from the humanised MCL-1 mice also show higher sensitivity to the MCL-1 inhibitor, we tested Eμ-Myc lymphoma cells from huMcl-1;Eμ-Myc mice. As predicted, these lymphoma cells were ~6 times more sensitive to S63845 in vitro compared to Eμ-Myc lymphoma cells expressing mouse MCL-1. When huMcl 1;Eμ Myc lymphoma cells were transplanted into huMcl-1 mice, treatment with S63845 led to tumour-free survival in >60% of mice. Furthermore, treatment with very low doses of S63845 in combination with sub-optimal doses of cyclophosphamide led to almost 100% regression of huMcl-1;Eμ-Myc lymphoma cells growing in huMcl-1 mice. This demonstrates that a therapeutic window for MCL-1 inhibitors in the clinic could be reached either as single agents or in combination with conventional chemotherapeutic drugs. These results demonstrate that our huMcl-1 mouse model represents an ideal preclinical tool to test current and future available MCL-1 inhibitors alone or in combinatorial treatment regimens for a broad range of cancers, allowing accurate prediction of efficacy against tumour cells and on-target toxicity to normal tissues.

44 | Exceptional sensitivity to Ferroptosis in Adrenocortical Carcinomas

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Adrenocortical carcinomas (ACCs) are rare and highly malignant cancers associated with poor survival of patients. Currently, mitotane, a non-specific derivative of the pesticide DDT (1,1-(dichlorobiphenyl)-2,2-dichloroethane), is used as the standard treatment, but its mechanism of action in ACCs remains elusive. Here, we demonstrate that the human ACC NCI-H295R cell line is remarkably sensitive to induction of ferroptosis, while mitotane does not induce this iron dependent mode of regulated necrosis. Supplementation with insulin, transferrin and selenium (ITS) is commonly used to keep NCI-H295R cells in cell culture. We show that this supplementation prevents spontaneous ferroptosis, especially when it contains polyunsaturated fatty acids (PUFAs), such as linoleic acid. Inhibitors of apoptosis (zVAD, emricasan) do not prevent the mitotane-induced cell death, but morphologically prevent membrane blebbing. The expression of glutathione peroxidase 4 (GPX4) in H295R cells, however, is significantly higher when compared to HT1080 fibrosarcoma cells, suggesting a role for ferroptosis. Direct inhibition of GPX4 in H295R cells led to high necrotic populations compared to control, while co-treatment with ferrostatin-1 (Fer-1) completely reverted ferroptosis. Interestingly, the analysis of public databases revealed that several key players of the ferroptosis pathway are hypermethylated and/or mutated in human ACCs (1, 2). Finally, we also detected that growth hormone-releasing hormone (GHRH) antagonists, such as MIA602, kill H295R cells in a non-apoptotic manner. In summary, we found elevated expression of GPX4 and higher sensitivity to ferroptosis in ACCs. We hypothesize that instead of treatment with mitotane, human adrenocortical carcinomas may be much more sensitive to induction of ferroptosis.

45 | Creating structure-based RANKL mutants with low affinity for OPG to activate macrophage in fibrosis

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Fibrosis is a chronic disease characterized by progressive alteration of the tissue structure due to the excessive production of extracellular matrix (ECM). As the end result of chronic inflammation, fibrosis progresses to an irreversible process and ultimately lead to tissue failure. Macrophages, which are abundantly present in fibrotic tissue, can show antifibrotic properties by producing secreted matrix metalloproteinases (MMPs) and cathepsins to degrade fibrotic ECM. The cytokine system Receptor activator of nuclear factor NF-κB ligand (RANKL)/RANK/Osteoprotegerin (OPG) was discovered to play an important role in the regulation of bone ECM formation and degradation, while the importance of this pathway in liver or pulmonary ECM degradation is currently unclear. Interestingly, increased decoy receptor OPG levels are found in fibrosis process. We hypothesized that RANKL could stimulate RANK on macrophages and initiate the process of ECM degradation, which may be inhibited by highly expressed OPG in fibrosis. A structure-based RANKL mutants library is built and the Q236 residue of RANKL is found to be important for OPG binding. RANKL_Q236D was found to maintain activating RAW macrophage and to escape from the obstruction by exogenous OPG. The generation of RANKL mutants with low affinity for OPG is a promising strategy for the exploration of new therapeutics against fibrosis.

46 | An overexpression screen identifies new players in ferroptosis susceptibility

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Ferroptosis is an iron-dependent form of programmed non-apoptotic cell death connecting iron, lipids and glutathione levels to degenerative processes and tumor suppression. To date, ferroptosis has been mapped to molecular mechanisms that affect the biochemistry or stoichiometry of redox substrates. Here, we performed an overexpression screen in ferroptosis-sensitive cells to identify new players in ferroptosis. Gene overexpression screens, as opposed to knockout screens, are not restricted by functional redundancy and can uncover previously unknown mechanisms in cell death.

We used three different ferroptosis inducers RSL3, imidazole ketone erastin (IKE) and genetic ablation of Gpx4 and identified a cohort of genes with overlapping protection against each stimulus. Strikingly, one gene was universally protective against all three. Several known genes featured among the set, however, the majority of protective genes make their debut for the first time as ferroptosis regulators. We generated individual overexpressing cell lines and validated specific anti-ferroptotic activity in viability assays as well as diminished lipid peroxidation, a hallmark of ferroptosis, for several of them including the top hit.

Expression levels in tumors of the top overlapping hit correlate to aggressive cancer types that are known to be more resistant to ferroptosis like breast, lung and colon cancers. This property signifies potential to serve as a biomarker for susceptibility to ferroptosis induction in certain cancers. The cumulative results are expected to substantially widen the scope of ferroptosis-related mechanisms.

47 | TRAILblazing: The role of CDK9 in pancreatic cancer

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Background: Even there was a distinct progress in diagnosis and therapy of pancreatic-ductal-adenocarcinoma (PDAC) over the last decade, prognosis for the most patients still remains very poor. Therefore, new efficient therapy approaches are urgently needed.

The family of cyclin-dependent kinases (CDK) comprises 20 kinases which contribute to malignancy of tumour cells due to regulation of proliferation, invasion, and apoptotic resistance. Based on that knowledge CDK-inhibitors with different inhibitory profiles have been developed. In this work we analyse the role of CDK9 in pancreatic cancer. Furthermore, the therapeutic potential of CDK9 inhibition by Dinaciclib (SCH-727965), a CDK inhibitor in clinical Phase III, was tested as new tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL/Apo2L)-sensitizing therapy approach. The “silver bullet” in cancer therapy – the ability to selectively kill tumour cells while sparing healthy cells - seemed close at hand in 1995 when TRAIL was introduced. After much testing, this selectivity holds to this day, however, its efficacy to induce significant levels of apoptosis in some resistant cancers like PDAC will have to be helped through additional means.

Methods and Results: Tumour and normal tissue of PDAC patients was immunohistochemically analysed on CDK9 expression and correlated with patients' survival. In comparison to normal tissue, CDK9 is overexpressed in pancreatic cancer tissue. In addition, high CDK9 expression in tumour tissue is associated with significantly shortened survival. In this connection the therapeutic efficiency of Dinaciclib on human PDAC cells was analysed. Various cancer hallmarks are simultaneously targeted and for instance viability, proliferation, clonogenic survival, and migration suppressed. Moreover, CDK9 inhibition is capable to intensify the therapeutic effect of TRAIL by the suppression of short-lived antiapoptotic proteins such as Mcl-1 and c-FLIP.

Conclusion: CDK9 is a negative prognostic marker for pancreatic cancer. Pharmacological CDK9-inhibition provides a new, TRAILblazing therapeutic approach for pancreatic cancer.

48 | Targeting MDR1 sensitises leukaemia stem cells to IAP inhibitors to overcome treatment resistance in acute myeloid leukaemia

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Background: Overexpression of inhibitor of apoptosis (IAP) proteins, responsible for the regulation of TNF-mediated apoptosis, have been correlated with cancer progression and treatment resistance. Natural IAP antagonists exist, leading to the pharmaceutical development of Smac-mimetics (SMs). The clinical SM birinapant is currently in clinical trials for a range of cancers, including acute myeloid leukaemia (AML). AML is an aggressive disease with a current 5-year survival rate of only ~30%. Although, birinapant has shown promising anti-cancer effects, boosting its efficacy and overcoming resistance are still major challenges.

Methods: Using a library of >5,700 bioactive compounds we examined the emergence of resistance to birinapant in AML and identified multidrug resistance protein 1 (MDR1/ABCB1) inhibitors as a class of clinical drugs that can overcome SM resistance in cancer.

Results: We show that 3rd generation clinical MDR1 inhibitors synergise with birinapant and other SMs to kill AMLs. Inhibition of MDR1 in SM resistant leukaemia cells increased intracellular levels of birinapant, and sensitised AML and cancer patient cells to birinapant killing. Genetic studies confirmed MDR1 specificity of this combination therapy and that expression/activity of MDR1 is a predictor of response to birinapant-based therapies in cancer cells. Moreover, co-inhibition of MDR1 and IAPs is well-tolerated in vivo and more effective against leukaemic cells, compared to normal haematopoietic progenitors. Importantly, birinapant combined with MDR1 inhibitors effectively killed leukaemic stem cells (LSCs) and prolonged survival in vivo, suggesting a therapeutic opportunity for AML.

Significance: This study identifies MDR1 as a biomarker of SM therapy and reveals a new combination therapy that can potentiate SM treatment. Notably, this therapy can suppress LSCs, reduce the chance of disease relapse and improve AML patient outcomes.

49 | The role of the anti-apoptotic factors Mcl-1 and Bcl-2 in the treatment resistance of malignant thymoma and thymic carcinoma

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Aim:

Thymic epithelial tumors (TET) are the most common neoplasms of the anterior mediastinum. According to the WHO classification of TET, thymomas are sub classified into the clinically indolent types A, AB, B1 and the aggressive types B2 and B3. In general, complete surgical removal is the most important prognostic factor and only curative treatment of malignant TET. Advanced and recurrent TET that cannot be completely resected require neoadjuvant or adjuvant systemic treatment, often in combination with radiotherapy. Cisplatin-based regimens such as the PAC scheme (cisplatin, adriamycin, cyclophosphamide) are most often used. TET have been described as the tumors with the lowest mutational burden among adult cancers. However, next to the enrichment of non-targetable HRAS, NRAS, TP53, and GTF2I mutations, copy number gain (18q21.33) and overexpression of Bcl-2 go along with decreased survival in TET. Therefore, targeting Bcl-2 has been suggested as a combinatorial treatment of malignant TET. We investigated the cell death mechanism of Bcl-2 inhibition in the TP53 mutated thymic carcinoma cell line 1889c.

Methods:

1889c was treated with different Bcl-2 and Mcl-1 inhibitors in combination with Cisplatin, Etoposide, Sorafinib and Sunitinib. Cell viability was analyzed 48h and 72h after treatment with MTS. Cytochrome C release was measured using a panel of BH3 domain peptides. Apoptotic and anti-apoptotic signaling factors including Casp3, -7, -8, -9, XIAP, PARP, γH2AX, Bcl-2, Mcl-1 and AIF were monitored by western blot. Tissue microarrays (TMAs) with TH and TC samples were immunohistochemically stained for Bcl-2, Mcl-1 and TP53.

Results:

The inhibition of Bcl-2 reduced cell viability of 1889c up to 80%. Cell death was induced without activation of any effector caspases. In addition, BH3 profiling resulted in almost no cytochrome C release. However, the combination of common chemotherapeutics with low concentrations of Bcl-2 inhibitors showed additional cell lethality effects.

Conclusion:

The specific inhibition of Bcl-2 alone leads to a strong reduction of cell viability in 1889c suggesting a Bcl-2 dependency of Bcl-2 amplified TETs. Even though the mechanism laying behind this caspase-independent cell death pathway is not clear, we propose a combined treatment with specific Bcl-2 inhibitors in addition to regular chemotherapy in TP53 mutated TETs containing a Bcl-2 amplification.

50 | MIR-138: A NEUROPROTECTIVE TARGET FOR SPINAL CORD INJURY?

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Injury to the spinal cord alters the local expression of multiple microRNAs. These are short RNA sequences that inhibit the expression of hundreds of genes to regulate cell state and fate. The therapeutic potential of dysregulated microRNAs is currently under study because evidence indicates that the gene expression changes they induce contribute to the onset of key processes of the spinal cord injury such as astrogliosis.

MiR-138 is a microRNA highly enriched in the central nervous system. In situ hybridizations of spinal cord samples reveal that miR-138 is highly expressed in neurons, and, to a lesser extent, in oligodendrocytes. Real-time PCR data confirm previously described miR-138 downregulation during the first week after contusive spinal cord injury. Histology reveals that downregulation is particularly evident among neurons in the perilesional regions.

Potential targets of miR-138 include components of cell death pathways upregulated in the damaged spinal cord. We employed luciferase reporter assays plus expression analyses to validate that mir-138 targets three key components of the apoptosis pathway –caspase 3, caspase 7 and Bak-1–. Gene expression data reveals that the downregulation of miR-138 during the first week after injury coincides with the upregulation of the three apoptotic targets. In accordance, transfection of miR-138 mimetics in neural cell line C6 reduces the expression of caspase 3, caspase 7, and Bak-1, and is cytoprotective against apoptosis induced by etoposide.

Validated miR-138 targets are involved in the secondary damage of the spinal cord injury. Caspases and Bak-1 together with previously validated targets such as Mst-1 and Bim contribute to the onset and execution of apoptosis which underlies oligodendrocyte dismissal during the first days after injury. CDK6, EZH2, E2F2, and other targets promote cell cycle activation and induce neuronal death in different neurological pathologies, including spinal cord injury.

51 | Lipid trafficking at the ER-mitochondria contact sites and ferroptosis

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The largest membranous network of the cell is the endoplasmic reticulum (ER), which represents the primary cellular site for protein quality control, lipid biosynthesis and calcium homeostasis and signaling. In order to conduct its various functions, the ER' dynamic membranous structures form specific sub-domains or sites of close apposition between organelles, which allow the communication between the ER and other organelles or cellular structures. Chief among them are the mitochondria-associated membranes (MAMs) which represent a tight interface where mitochondria and ER communicate and exchange signals. Emerging evidence demonstrated the fundamental role of this tight cross-talk in regulating several physiological processes, such as calcium homeostasis, mitochondria bioenergetics, lipid-mediated signaling, metabolism and cell death.

Several proteins have been identified enriched at the ER-mitochondria interface, whose function is still not known. In particular, we are focusing the interest on the ER pancreatic kinase PERK, known for being a key mediator of the unfolded protein response (UPR), but also identified by our lab at MAMs. Our previous studies show that the ER stress sensor PERK is an integral member of the MAMs and has a tethering role at the ER-mitochondria contact sites, independently of its UPR activity. PERK deficient cells display weakened ER-mitochondria contact sites and thereby increased apoptosis resistance against agents that simultaneously mobilize Ca2+ and induce ER stress through ROS. In line with this, here we found that PERK deficiency counteracts the mitochondrial calcium uptake, following depletion of the ER Ca2+ stores by IP3 mobilizing agents.

Analysis of the PERK-interactome and co-IP assays uncovered that PERK physically interacts with the Ca2+-regulated phospholipid-binding protein Extended Synaptotagmin-1 (E-Syt1), which tethers the ER to the PM upon ER store Ca2+ depletion thus exchanging phospholipids at this interface. We reveal that PERK and E-Syt1 interact within the MAMs subdomain and we mapped the specific domains of E-Syt1 responsible for this interaction. Furthermore, we found that PERK is required for the recruitment of E-Syt1 at MAMs.

Lipidomics analysis of MAM- and mitochondria fractions show that the presence of PERK, as well as E-Syt1, modulate the distribution of several phospholipids, within these compartments. Additionally, our ongoing studies reveal that the cell's sensitivity to lipid peroxidation and thereby to ferroptosis in response to the ferroptosis inducer RSL3 is significantly reduced in PERK deficient cells. PERK knock out cells displayed reduced overall and mitochondrial-associated lipid peroxidation and maintained mitochondria morphology in response to cell death induced by RSL3. Ongoing research is addressing whether the role of PERK in promoting ferroptosis is caused by the modulation of the lipid distribution at MAMs through the interaction with E-Syt1.

52 | A Caspase 3 deletion with prognostic relevance in thymic epithelial tumors (TET)

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Thymic epithelial tumors (TET) are the most common neoplasms of the anterior mediastinum. According to the WHO classification of TET, thymomas are sub classified into the clinically indolent types A, AB, B1 and the aggressive types B2 and B3. In general, complete surgical removal is the most important prognostic factor and only curative treatment of malignant TET. Advanced and recurrent TET that cannot be completely resected require neoadjuvant or adjuvant systemic treatment, often in combination with radiotherapy. Cisplatin-based regimens such as the PAC scheme (cisplatin, adriamycin, cyclophosphamide) are most often used. TET have been described as the tumors with the lowest mutational burden among adult cancers. However, next to the enrichment of non-targetable HRAS, NRAS, TP53, and GTF2I mutations, copy number gain (18q21.33) and overexpression of Bcl-2 go along with decreased survival in TET. Therefore, targeting Bcl-2 has been suggested as a combinatorial treatment of malignant TET. We investigated the cell death mechanism of Bcl-2 inhibition in the TP53 mutated thymic carcinoma cell line 1889c.

1889c was treated with different Bcl-2 and Mcl-1 inhibitors in combination with Cisplatin, Etoposide, Sorafinib and Sunitinib. Cell viability was analyzed 48h and 72h after treatment with MTS. Cytochrome C release was measured using a panel of BH3 domain peptides. Apoptotic and anti-apoptotic signaling factors including Casp3, -7, -8, -9, XIAP, PARP, γH2AX, Bcl-2, Mcl-1 and AIF were monitored by western blot. Tissue microarrays (TMAs) with TH and TC samples were immunohistochemically stained for Bcl-2, Mcl-1 and TP53.

The inhibition of Bcl-2 reduced cell viability of 1889c up to 80%. Cell death was induced without activation of any effector caspases. In addition, BH3 profiling resulted in almost no cytochrome C release. However, the combination of common chemotherapeutics with low concentrations of Bcl-2 inhibitors showed additional cell lethality effects.

The specific inhibition of Bcl-2 alone leads to a strong reduction of cell viability in 1889c suggesting a Bcl-2 dependency of Bcl-2 amplified TETs. Even though the mechanism laying behind this caspase-independent cell death pathway is not clear, we propose a combined treatment with specific Bcl-2 inhibitors in addition to regular chemotherapy in TP53 mutated TETs containing a Bcl-2 amplification.

53 | Development and testing of TRAIL variants intended for transcytotic delivery into the central nervous system

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Glioblastoma (GBM) is a grade IV glioma, which is the most malignant and aggressive form of glioma. It accounts for 80% of the primary malignant brain tumours and is associated with a poor survival rate. Tumour Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL)-based therapeutics potently induce apoptosis in cancer cells, including GBM cells, by binding to death receptors (DR4 and DR5). However, the blood-brain barrier (BBB) is a major obstacle for these biologics to enter the central nervous system. Receptor mediated transcytosis (RMT) is a mode of transport capable of carrying large proteins and lipoproteins across the BBB. For example, the angiopep-2 peptide crosses the BBB through LRP1-mediated transcytosis. We, therefore, study if antibody-based fusion proteins that combine the apoptosis-inducing TRAIL with angiopep-2 based transcytosis-inducing targeting moieties can be developed. So far, we designed two different constructs with the aim of achieving transcytosis and apoptosis rendered by angiopep-2 and TRAIL, respectively, and these are now being expressed, purified and tested for on-target bioactivity in GBM cells. Initial testing has demonstrated that our constructs retain apoptotic potency against GBM cell line A172. In parallel, we now begin testing a BBB model to determine if such constructs can be transported efficiently across the barrier in vitro.

54 | The PIDDosome in promoting p53 induced tumor suppression

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Aneuploidy is a common feature of cancer cells. One cause of this unequal distribution of chromosomes can be the accumulation of supernumerary centrosomes. During mitosis this can result in the formation of multipolar spindles, which increase the chance of chromosome miss segregation, a first step towards aneuploidy. The PIDDosome, a protein complex containing PIDD1, RAIDD and Caspase 2, has been described to be activated in response to extra centrosomes. This limits genomic instability by inducing a p53-mediated cell cycle arrest or, potentially, apoptosis. Hence, in consequence PIDDosome activation should protect cells from malignant transformation. Yet, it is still unclear whether the PIDDosome acts as a first barrier against cancer in response to supernumerary centrosomes in vivo.

To address this question, extra centrosomes will be generated in mice carrying a transgene encoding for Polo-like kinase 4, fused to a fluorescent marker, EYFP (PLK4-EYFP). This transgene is placed under control of a CRE-excisable STOP cassette to allow tissue-specific overexpression of PLK4, the master regulator of centriole biogenesis. Using CRE recombinase under control of the villin promotor, I will study the effects of extra centrosome on colorectal cancer development. Intercrossing PLK4-transgenic mice with mice deficient for components of the PIDDosome, will allow me to dissect the role of the PIDDosome in promoting p53-induced tumor suppression downstream of extra centrosomes. First experiments and preliminary results will be discussed.

55 | ASTX660, a non-peptidomimetic antagonist of cIAP1/2 and XIAP, induces tumour cell death by direct and immune-mediated mechanisms

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Inhibitor-of-apoptosis proteins (IAPs) are important mediators of apoptosis resistance in normal and transformed cells. Members of the protein family include cellular IAPs (cIAP1 and cIAP2) and X-linked IAP (XIAP) which have been shown to directly bind and inhibit caspase activity. Besides their part in regulation of apoptosis, IAPs function as important regulators of NF-κB signaling and therefore play an important role in inflammation and immunity. Upregulation of IAPs has been demonstrated in a variety of cancers and antagonists of this protein family have been developed as anti-cancer therapies. These compounds induce cell death in cancer cells in vitro and in vivo. In addition, antagonism of IAPs has been shown to enhance stimulation of T cells via NF-κB activation.

ASTX660 is a potent, non-peptidomimetic antagonist of cIAP1/2 and XIAP, which is currently being tested in a first in human phase I-II study in patients with advanced solid tumors and lymphomas (NCT02503423). Preliminary efficacy and safety data from the relapsed/refractory (r/r) peripheral T-cell lymphoma (PTCL) and cutaneous T-cell lymphoma (CTCL) Phase 2 cohorts have been reported recently (Mehta, Hollebecque et al. 2019). In the work presented here we aim to explore KRAS mutant non-small cell lung cancer (NSCLC) as a potential patient population for IAP antagonism. KRAS mutant NSCLC cell lines treated with ASTX660 show induction of apoptosis in vitro and in vivo in a subset of cell lines but this effect relies on either autocrine or exogenous TNFα. Interestingly, the A549 cell line, which is resistant to apoptosis induction by ASTX660 in standard in vitro assays, exhibits a robust cell death response when treated with ASTX660 in co-culture with activated peripheral blood mononuclear cells (PBMCs). These co-culture experiments demonstrate that ASTX660 potentiates tumor killing by PBMCs.

In summary, ASTX660 can achieve anti-tumour activity not only by releasing a brake on apoptosis, but also by enhancing immune cell-mediated mechanisms in the tumour microenvironment. Ongoing work will investigate the mechanisms, by which ASTX660 controls tumour progression.

Mehta, A., A. Hollebecque, F. Foss, J. Lister, M. Mita, N. Wagner-Johnston, M. J. S. Dyer, B. You, A. Prica, F. Hernandez-Llizaliturri, R. Ferraldeschi, D. Chan, J. Zhang and F. Samaniego (2019). PS1073: Preliminary results of ASTX660, a novel non-peptidomimetic cIAP1/2 and XIAP antagonist, in relapsed/refractory peripheral T-cell lymphoma and cutaneous T-cell lymphoma. 24th EHA Annual Congress. Amsterdam.

56 | Peroxidation of phospholipids containing two unsaturated fatty acyl tails is associated with execution of cell death during ferroptosis

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Ferroptosis is a form of regulated, non-apoptotic cell death driven by peroxidation of phospholipids containing polyunsaturated fatty acyl tails. Here, a genome-wide, overexpression screen was used to identify an anti-ferroptotic pathway. We describe how the upregulation of this pathway led to changes in the lipid profile cells, and resulted in protection of specific lipids from depletion through peroxidation. Ferroptosis-specific lipidomic signatures were observed in both protected cells and cells undergoing cell death; however, the protected cells had a small set of lipids that were protected from degradation, suggesting that execution of ferroptosis requires the peroxidation of only a small set of specific lipids. In particular, we found that the oxidative degradation of phospholipids with two polyunsaturated fatty acid (PUFA) tails was associated with cell death, but not degradation of phospholipids with only one PUFA tail and one saturated lipid tail. These findings suggest a new model for the mechanism by which lipid peroxidation drives cell death during ferroptosis.

57 | Amyloid Precursor Protein (APP) Supports Tumorigenesis by Counteracting Iron-mediated Oxidative Stress and p38MAPK-dependent DNA Damage

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The amyloid precursor protein (APP) plays a pathophysiological key role in Alzheimer disease (AD) due to its neurotoxic proteolytic amyloid-beta peptide fragments. However, full-length APP as well as its large N-terminal soluble sAPPalpha processing product exert neuroprotective features that are intricately involved in iron metabolism. Yet, the functional attributes of APP outside the nervous system and downstream pathways remain unclear. Here, we report that APP functions as iron and redox homeostatic protein that promotes tumorigenesis. We confirmed previous findings that APP interacts with heme-degrading enzyme hemeoxygenase-1 (HO-1). Loss-of-APP using APP-knockout MEFs and stable APP-depleted prostate cancer cell lines results in the translocation of HO-1 from the membrane fraction of the plasmamembrane and endoplasmic reticulum to mitochondria, resulting in an overactivity of HO-1 releasing free unchecked redox-active iron (Fe2+). Similar to (Lipid-)ROS blocker, pharmacological inhibition of HO-1 activity using QC-308 restored the impaired clonogenic growth of APP-knockout MEFs. On the molecular level, we discovered a previously unrecognized mechanisms by which APP suppresses pro-oxidative Fe2+ accumulation that results in deleterious oxidative stress-induced DNA damage in cancer cells, with p38MAPK stress signaling as a crucial mediator of this process. Consonant with these findings, in human data set analyses of prostate cancers, high levels of APP are significantly associated with a poor event-free survival rate and negatively correlated with tumor suppressive p38MAPK activation. As proof of concept, pharmacological disruption of APP increased levels of redox-active Fe2+ and p38MAPK-dependent DNA damage and significantly reduced tumor cell clonogenicity in vitro and tumor growth in a xenograft model. Taken together, our results uncover that APP depletion represents a potential novel approach to disrupt iron homeostasis, releasing unchecked pro-oxidative Fe2+ that hinders survival and propagation of oncogenic transformed cells and cancer cells as well as increasing the responsiveness to ferroptosis-inducing therapies.

58 | Vaccinia virus in cell death induction

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Cell death of virus-infected cells is crucial for organism survival therefore these mechanisms are evolutional developed. Vaccinia virus (VV) predominantly causes lysis of infected tumor cells but the nature of cell death phenomenon is not well understood. Non-modified VV usually activates programmed necrosis. We have previously constructed therapeutic recombinant VV coding human granulocyte-macrophage colony-stimulating factor and apoptosis-inducing protein lactaptin (VV-GMCSF-Lact). VV-GMCSF-Lact activated a set of critical apoptosis markers in infected cells: phosphatidylserine externalization, caspase -3, -7 activation, DNA fragmentation, up-regulation of pro-apoptotic protein BAX and efficiently decreased mitochondrial membrane potential of infected cancer cell.

Here, the effects of combinatorial treatment of VV with a set of inhibitors on cell death modality were analyzed: necrostatin, z-VAD and tyrphostin (AG1478, an EGF receptor inhibitor). Combinatorial treatment of cancer cells with VV-GMCSF-Lact and necrostatin didn't suppress the efficiency of VV-induced death. The combination of VV-GMCSF-Lact and z-VAD decreased rate of cell death in comparison to single VV-GMCSF-Lact treatment. These results suggest that VV-GMCSF-Lact activates caspase-dependent death of infected cells.

We showed that combinatorial treatment of tumor cells with VV-GMCSF-Lact and tyrphostin decreased the efficiency of cell death only in EGFR-high cells (MDA-MB-231, BT549 and A431 cells), but not EGFR-low cells (MCF-7). These findings have proved the importance of EGF signaling pathway in VV-dependent cell death.

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59 | Preclinical antitumor efficacy of combined senescence-inducing chemotherapy with targeted nanotherapeutic senolysis

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Senescence induction produces a stable cell cycle arrest of cancer cells, arresting tumour growth and promoting immune clearance. However, incomplete clearance of senescent cells may favour tumour dormancy and recurrence, plausibly limiting the positive effects of these drugs in the long term. An approach to overcome this situation is the combination of senescence induction with the subsequent elimination of senescent cells. In this work, we explore the antitumor efficacy of a combination therapy involving senogenesis and targeted senolysis, in an orthotopic immunocompetent model of the highly aggressive 4T1 triple negative breast cancer. Senogenesis is induced with palbociclib, whereas senolysis is achieved with nano-encapsulated navitoclax. We show that treatment with palbociclib and further elimination of senescent cells with encapsulated navitoclax delays or even prevents remission, reduces metastases and has the additional benefit of diminishing the systemic toxicity of navitoclax. Conceivably, this principle could be applied to other senescence-inducing chemotherapies and tumor types.

60 | IDO inhibitors enhance antitumor vaccination effect of lactaptin-treated cancer cells

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Nowadays immunotherapy is one of the most promising approaches to the treatment of malignant tumors. Dual strategy, when antitumor drug directly induces the death of cancer cells and simultaneously activates the immune system by the induction of immunogenic cell death (ICD) to form a sustained antitumor immune response is highly desirable. However, to avoid immune system attack, tumors can involve various mechanisms of immune suppression and tolerance. Tumor microenvironment is a key structure which protects tumors from the host immune system. Immunosuppressive microenvironment secrets anti-inflammatory cytokines and metabolic enzymes which stimulates the growth of tumor cells and promotes the immune escape. Metabolic enzyme indoleamine-2,3-dioxigenase (IDO) degrades amino acid tryptophan and facilitates tumor immunotolerance. In this context we suggested that IDO inhibitors can enhance tumor infiltration with cytotoxic T-lymphocytes and increase anticancer vaccination effects of ICD inducers.

In this study we investigated whether IDO inhibitors enhance antitumor vaccination effect of lactaptin-treated cancer cells. Two IDO inhibitors - ethyl pyruvate and 1-methyltryptophan were used in this work.

We have shown previously that recombinant analog of pro-apoptotic protein lactaptin (RL2) induced immunogenic cell death in vitro with a whole set of ICD markers: calreticulin and HSP70 exposure on outer plasma membrane as well as ATP and HMGB1 release into the extracellular space. To study mouse antitumor vaccination with RL2-treated tumor cells, MX-7 mouse rhabdomyosarcoma cells we used. RL2-treated MX-7 cells were transplanted on C3H/He syngeneic mice and next, one week after, live MX-7 cells were also inoculated into mice. Vaccination effect was evaluated by animal survival and tumor nodes appearance. Several schemes of treatment with IDO inhibitors were realized. We showed that ethyl pyruvate increased the survival rate of vaccinated animals from 67% to 83% and percent of tumor-free mice – from 33% to 50%. Peritoneal macrophages of vaccinated mice showed high capacity to engulf intact MX-7 cells. Overall, we showed that indoleamine 2,3-dioxygenase inhibitors can enhance antitumor vaccination effects in vivo with RL2-treated cancer cells.

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61 | Selective cancer cell death induced by cold atmospheric plasma irradiation

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Over the past decade biophysical methods for cancer treatment have undergone a renaissance. Cold atmospheric plasma (CAP) showed promising therapeutic activity against cancer cells in vitro. CAP is an ionized gas consisting of charged particles, active uncharged particles, an electric field and UV radiation. An attractive feature of a cold plasma jet for antitumor research is the low temperature in the area of contact of cold plasma with a biological object. For the introduction to the clinical practice the selectivity of the action of CAP on tumor cells have to be demonstrated.

The biological effect of plasma-activated medium on the epidermoid carcinoma cells A431 and normal human embryonic kidney cells HEK-293 has been investigated. The culture medium was exposed to CAP irradiation generated in argon gas or helium for 2–8 min at a voltage of 4.9 kV and then added to the cells. The proliferation of the treated cells in real time mode was measured using iCELLigence device. The level of intracellular reactive oxygen species (ROS), the activation of caspases -3 and -7 and the proportion of apoptotic and necrotic cells were analyzed by the flow cytometry. These experiments revealed CAP irradiation conditions leading to the death of A431 tumor cells, but not healthy HEK-293 cells. Under these selective conditions the operation characteristics of plasma device generating CAP jet were studied in numerical simulations.

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62 | Unrevealing the roles of the MCL-1 transmembrane domain

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The proteins from the Bcl-2 family control cell fate modulating the permeabilization of the mitochondrial outer membrane. Most of the members of this family have a C-terminal domain with membrane targeting functions. Recent evidences show that some Bcl-2 transmembrane segments have also a role in the modulation of apoptosis.

This work deeply analyzes the pro-apoptotic role of the Mcl-1 transmembrane domain (Mcl-1 TMD). Mcl-1 TMD forms homo-oligomers and hetero-oligomerices with Bok TMD in cellular membranes. Overexpression of this TMD induces apoptosis in a Bax/Bak-independent, Bok-dependent manner. Our results suggest a model in which Mcl-1 TMD competes with full-length protein and exerts antagonistic functions.

To study the contribution of Mcl-1 TMDs to pathological situations we have investigated the appearance of mutations in cancer patients. Analysis of COSMIC data reveals the existence of high pathological score somatic mutations that affect the transmembrane region. Introduction of these mutations in the Mcl-1 TMD disturbs the oligomerization network and alters its apoptotic induction capability.

These results support a Bcl-2 protein interaction model in which lateral interactions among TMDs contribute, along with the rest of protein domains, to apoptosis regulation and highlight the relevance of understanding and modulate the Mcl-1 TMD interactome in cancer studies.

63 | Analysis of the Anti-ferroptotic and Anti-Necroptotic Activities of Necrostatin-1 (Nec-1)

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Although ferroptosis and necroptosis are considered two independent regulated cell death pathways, the receptor interacting kinase 1 (RIPK1) inhibitor necrostatin-1 (Nec-1) intrinsically prevents ferroptosis as induced by erastin or RSL3. This is in contrast to the more potent necrostatin-1s that does not affect ferroptosis. However, the underlying mechanism of this protective effect remains unclear. We therefore generated a biotinylated Nec-1 employing a tryptophan ester binding site that had been previously used to add the 7-O-chloride in Nec-1s, and additionally generated an intermediate product that contains a linker but no biotin. The murine cell line NIH 3T3 was subjected to type I and type II ferroptosis inducers (FINs, erastin and RSL3), as well as induction of necroptosis via TNFα treatment combined with caspase inhibition. By altering the structure of the indole backbone we were able to identify the critical group involved in ferroptotic protection. As expected from the previously published structure of the RIP1 kinase pocked bound to Nec-1s, both the intermediate and the biotinylated version of Nec-1 cannot prevent necroptosis. In contrast, however, the intermediate version potently inhibits ferroptosis induced by any FIN, confirming the hydantoin to be responsible for this effect. In addition, we will employ the biotinylated Nec-1 for pull down experiments and mass spectrometry to identify proteins binding to the ferroptosis-inhibiting portion of Nec-1.

64 | Identification of NOXA dependent vulnerabilities by a high throughput drug screening in pancreatic cancer cells

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Background:

Pancreatic ductal adenocarcinoma (PDAC) is the most frequent type of pancreatic cancer and one of the most lethal malignancies. Genetic heterogeneity is a crucial contributor towards the failure of current clinical treatments. Recent studies have shown distinct molecular subtypes of PDAC associated with different prognosis and therapy response. Interestingly, the most aggressive subtype shows a high expression of the pro-apoptotic BH3-only NOXA, which correlates with worse overall survival. In this study we aim to identify unknown NOXA dependent vulnerabilities in this subtype by an unbiased pharmacological screening.

Methods:

Eight different PDAC cell lines including CRISPR/Cas9-mediated NOXA knock out were used for a drug screening of 1842 compounds (FDA approved and experimental drugs) at a single dose concentration of 600 nM. Multivariate analysis of variance (MANOVA) was performed with the software Infostat using a confidence interval of 95% (α=0,05).

Results:

Comparison of NOXAhigh/wildtype and NOXAlow/knockout showed an increased sensitivity towards 2% of all substances in the NOXAhigh/wildtype group. Of those, we found hits involved in DNA-synthesis (20%), Cytoskeleton dynamics (17,5%), PLK1 (10%), Epigenetic modulators (7,5%), Proteasome inhibition (5%), mTOR/PI3K (2,5%) among other pathways. In the latter, two hits were exclusively present: a glucocorticoid steroid and Raltitrexed, an inhibitor of thymidylate synthase. Identified hits have been validated in multi-dose experiments in a larger set of cell lines and in patient derived PDAC organoids, which have been molecularily subtyped by RNAseq. Additionally, we reanalyzed transcriptome profiles of different public available datasets, which corroborates our findings.

Conclusion:

Our data supports the notion that high-throughput drug screenings represent a powerful tool to elucidate molecular pathways to find novel strategies for personalized treatment approaches.

65 | C-MYC functions as an anti-necroptotic factor by suppressing the formation of the necrosome complex

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c-MYC is a potent oncogene that modulates various cellular processes including proliferation, differentiation, inflammation and metabolism. We observed that c-MYC functions as an anti-necroptotic factor. Upon TNF-α-dependent necroptotic stimulation, the c-MYC pathway was drastically downregulated, this was detected by mRNA sequence analyses and western blotting. The suppression of c-MYC-associated pathways was due to the decrease in c-MYC levels. The down-regulation of c-MYC levels required RIPK3 activation upon necroptotic stimulation, and was mediated via ubiquitination and proteasome-dependent degradation. Under normal conditions, the N- and C-termini of c-MYC bind to the RHIM and kinase domains of RIPK3, respectively. This binding prevents the formation of the RIPK3 and RIPK1 pre-necrosome under normal conditions by preventing the binding of RIPK3 to RIPK1. Overexpression of c-MYC induces the dissociation between RIPK3 and RIPK1, which leads to the destabilization of RIPK3 and RIPK1 by CHIP E3 ligase. On the other hand, depletion of c-MYC induces the stabilization of RIPK3 and RIPK1 via formation of the complex between RIPK3 and RIPK1, which might be resistant to E3 ligase dependent degradation and facilitates necroptosis. As c-MYC is a potent oncogene, we further tested the effect of necroptosis on the suppression of tumorigenesis by employing the leukemia cell line, Molm13, which is susceptible to necroptotic stress induced by birinapant, an inhibitor of cIAP E3 ligase, and emricasan, a caspase inhibitor. The addition of CPI-203, which inhibits the expression of c-MYC, led to a drastic increase in necroptotic cell death. The growth of orthotopic and skin xenografts of the Molm13 cell line was severely suppressed by the injection of birinapant, emricasan and CPI-203 (BEC), implicating that necroptotic stimuli could suppress cancer cell growth. Furthermore, the lifespan of mice with the orthotopic xenograft of Molm13 increased by one hundred percent upon BEC treatment indicating that CPI-203 (BEC) could serve as a prospective therapeutic strategy against leukemia.

66 | In TNF-induced necroptosis NFκB/RelA dynamics control an incoherent feedforward loop to determine cell fate decisions

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Balancing life-death decisions is crucial to maintain tissue homeostasis and prevent disease. Tumor necrosis factor (TNF) does not only control inflammatory responses via nuclear factor κB (NFκB), but triggers signaling mechanisms that can lead to necroptotic cell death. However, whether NFκB-responsive expression of pro-survival factors provides protection from necroptosis is unknown.

Here, we present a systems biology approach that provides quantitative and dynamic understanding of NFκB-mediated necroptosis control. Live-cell microscopy, motivated by conceptual mathematical modeling, suggests that death rate kinetics of TNF-treated L929 fibrosarcoma cells are indicative of a stimulus-induced, rather than pre-existent survival mechanism. CRISPR-mediated knockout identifies the NFκB/RelA-dependent target gene that mediates transient protection in a fraction of cells. In contrast, perturbing the NFκB-regulatory system reveals that hyper-activated RelA is able to provide long-term protection from necroptosis. Mathematical modeling of NFκB-responsive regulators explains the timing of death and fractional survival in response to transient TNF exposure in the relevant contexts of hypo-, normo- and hyper-RelA activity.

In sum, our work reveals an incoherent feedforward loop of pro- and anti-necroptotic signaling as a circuit design principle that distinguishes the two physiological functions of TNF; it ensures cellular survival for the NFκB/RelA-mediated inflammatory control functions of transient TNF stimuli, but preserves sensitivity to the cytotoxic functions of long-term TNF exposure.

67 | Serum Cell Death Biomarker Mirrors Liver Cancer Regression and Predicts Progression-Free Survival after Transarterial Chemoembolisation

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Hepatocellular carcinoma (HCC) represents an increasing health problem with limited therapeutic options. In patients with intermediate disease stage, transarterial chemoembolization (TACE) is widely applied. Treatment response is routinely assessed by imaging techniques according to the international response evaluation criteria in solid tumors, which consider tumor regression (RECIST) or additionally tumor necrosis (mRECIST). Evaluation of treatment response, however, by these methods is time- and cost-intensive and usually performed at earliest several months following TACE. We therefore investigated the suitability of novel non-invasive cell death biomarkers for an earlier prediction of TACE response. For this purpose we analyzed activation of pro-apoptotic caspases and the proteolytic cleavage of the caspase substrate CK-18 in liver tissues and sera from HCC patients by immunohistochemistry, a luminometric substrate assay and ELISA.

Both caspase activity and caspase-cleaved CK-18 fragments were elevated in HCC patients compared to healthy controls. CK-18 serum levels significantly increased during the first three days and peaked at day two following TACE. Interestingly, we found significant differences in CK-18 levels between patients with and without tumor regression. Detection of CK-18 fragments revealed a promising performance for the early prediction of TACE response with an area under the curve value of 0.76. Patients who reached the identified CK-18 cut-off for prediction of treatment response showed a significantly higher progression-free survival rate compared to those with CK-18 levels below this value.

In conclusion, caspase-cleaved CK-18 levels mirror liver cancer regression and allow an earlier prediction of TACE response and progression-free survival. The concordance with mRECIST suggests that detection of CK-18 levels immediately after TACE might be used as short-term decision guide to continue or change HCC therapy.

68 | KRAS pathway expression changes in pancreatic cancer models caused by conventional and experimental taxanes

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Pancreatic ductal adenocarcinoma (PDAC, OMIM: 260350) represents the fourth leading cause of cancer-related death worldwide. Systemic chemotherapy is administered to more than 80% of PDAC patients, but the mortality, paralleling the incidence indicates failure of either conventional chemotherapy or any so far tested targeted therapeutics. In case of the use of classical anticancer drugs taxanes, inherited or acquired resistance of tumor cells presents one of the major obstacles in successful therapy. Our previous work has shown that novel taxanes (taxanes of second-generation called “Stony Brook Taxanes”; SB-T) possess extremely high potency against drug-resistant cancer cells expressing the multidrug resistance (MDR) phenotype. This study aimed to explore interactions of conventional paclitaxel and experimental taxane SB-T-1216 with the KRAS signaling pathway expression in in vitro and in vivo PDAC models, because this pathway is pivotal for pancreatic ductal adenocarcinoma (PDAC) development.

KRAS wild type (BxPc-3) and mutated (MiaPaCa-2 and PaCa-44) cancer cell line models were treated with paclitaxel to verify a dysregulation of the KRAS signaling pathway gene expression profile in vitro and investigate role of KRAS mutation status. The cellular models used to study PDAC were different in basal transcriptional level according to the state of the KRAS mutation, but the mechanism of action of taxanes (paclitaxel) was found to be independent on these differences. Furthermore, no difference in cytotoxicity on the action of taxanes was observed in the KRAS mutant cell lines (Paca-44, MIAPaCa-2) and the BcPC-3 (wild type) cell line. Subsequently, mouse PDAC PaCa-44 xenograft model was used for evaluation of changes in transcription and protein levels of the KRAS signaling pathway caused by administration of experimental taxane SB-T-1216 in vivo. The taxane administration caused a downregulation of the KRAS signaling pathway both in vitro and in vivo, but this effect was not dependent on the KRAS mutation status.

In conclusion, putative biomarkers for prediction of taxane activity or targets for stimulation of taxane anticancer effects were not discovered by the KRAS signaling pathway profiling in various PDAC models.

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69 | Analyzing the impact of thePIDDosome on aneuploidy in Ph+ pre-B-ALL.

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The PIDDosome, a protein complex consisting of PIDD1, RAIDD and Caspase 2, was recently described to recognize extra centrosomes and subsequently trigger a p53-dependent cell cycle arrest. Every cell usually harbours one centrosome that duplicates exactly once per cell cycle to ensure proper segregation of chromosomes in mitosis. However, a cell can acquire multiple centrosomes due to various reasons ranging from a malfunctioning centrosome-duplication to failed cytokinesis. Cells with extra centrosomes are a threat to the organism as in such cells the risk of multipolar spindle assembly, chromosome missegregation, chromosomal instability and thus the risk for aneuploidisation is elevated. By activating a p53-dependent cell cycle arrest in response to supernumerary centrosomes, the PIDDosome might therefore have a yet unidentified but important barrier function in cancer development. It has been described that aneuploidy is frequent in pre-B acute lymphoblastic leukaemia (ALL) and that the oncogene BCR-ABL, which causes pre-B ALL, can lead to aneuploidy in these malignant cells. Surprisingly, ALL patients with multiple centrosomes seem to have a better clinical outcome than those with normal centrosome numbers. This would indicate that the PIDDosome protein complex is active in such cells to, at least slow down proliferation of leukemic cells and therefore enabling a better outcome for the patients. To investigate this question, we set-up a mouse model for pre-B ALL in PIDDosome-deficient mice and investigate the role of the PIDDosome in human pre-B ALL . Deficiency of PIDD1, RAIDD or Caspase-2 should favour the survival and proliferation of cells with supernumerary centrosomes, driven by the BCR-ABL oncogene and thus potentially alter tumour onset or progression.

70 | Novel treatment approaches in prostate cancer: Targeting Cyclin K

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Resistance towards therapeutic drugs and a lack of clinically relevant biomarkers are key challenges in the treatment of prostate cancer and novel therapeutic approaches are urgently needed. Cyclin K (CycK) regulates proliferation and survival of cancer cells as well as transcription of DNA damage repair genes in association with CDK12. However, its functional role in prostate cancer is unknown. We found that silencing of CycK by siRNAs caused apoptotic cell death and inhibited proliferation accompanied by an accumulation of cells in the G2/M phase in prostate cancer cells. Moreover, immunofluorescence staining of microtubules upon CycK depletion revealed an induction of mitotic catastrophe as underlying mechanism. To investigate the clinical relevance of CycK, we analyzed CycK expression in tissue from 91 prostate cancer patients and identified CycK as putative predictive biomarker for therapy response. In addition, we examined the newly synthesized covalent CDK12/CDK13 inhibitor THZ531 in prostate cancer cells and fresh tumor tissue of prostate cancer patients. Incubation of tumor cells with THZ531 demonstrated anti-tumorigenic effects comparable to CycK reduction. Interestingly, we found a reduced expression of several DNA damage repair genes accompanied by an elevation of DNA double strand breaks. Moreover, treatment of fresh prostate cancer tissue with THZ531 caused considerable DNA damage. These results provide for the first time a rationale for the clinical relevance of CycK/CDK12 inhibition and warrant the further investigation of CycK as therapeutic target for the treatment of prostate cancer.

71 | RIPK1 prevents ZBP1-mediated necroptosis to inhibit inflammation

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RIPK1 exhibits kinase activity-dependent and -independent functions that are important for the regulation of cell death and inflammation. RIPK1 kinase activity induces FADD-caspase-8-mediated apoptosis and RIPK3-MLKL-dependent necroptosis. RIPK1 was also shown to inhibit caspase 8-dependent apoptosis and RIPK3-dependent necroptosis via kinase independent scaffolding functions. Specifically in the epidermis, loss of RIPK1 caused RIPK3-MLKL-dependent keratinocyte necroptosis

and skin inflammation. In order to study if RIP homotypic interaction motif (RHIM)-dependent RIPK1 functions to inhibit necroptosis in keratinocytes, we generated knock-in mice expressing RIPK1 with mutated RHIM (RIPK1mRHIM). RIPK1mRHIM mice die perinatally and displayed epidermal hyperplasia, increased immune cell infiltration and cell death in the dermis. Perinatal lethality and skin hyperplasia in RIPK1mRHIM mice were rescued by RIPK3, MLKL or ZBP1 deficiency, indicating ZBP1-dependent necroptosis causes this phenotype. Consistent with the identified inhibitory function of RHIM of RIPK1 during late embryonic life, mice with skin epidermal specific RIPK1 RHIM mutant protein expression (RIPK1mRHIM/E-KO) developed skin inflammation that was prevented by loss of RIPK3, MLKL or ZBP1. Immunoprecipitation experiments showed that ZBP1 interacted strongly with and activated RIPK3 in cells expressing RIPK1 with mutated RHIM, suggesting that RIPK1 acts via its RHIM to inhibit ZBP1-dependent activation of RIPK3. Collectively, our results showed that RIPK1 prevents perinatal lethality as well as skin inflammation by inhibiting ZBP1-RIPK3-MLKL-mediated necroptosis.

72 | The effect of necroptosis on peritoneal macrophages

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Objective:

Apoptosis and necroptosis, two different types of regulated cell death, affect the immune system in different ways. Whereas apoptosis is a weak inducer of inflammation and actively contributes to its resolution, necroptosis releases danger associated molecular patterns (DAMPs) and is considered a strong inducer of inflammation. The role of necroptosis in the resolution of inflammation is so far unclear. Here, we investigate the effect of necroptotic and apoptotic cells on the immune system by analyzing the impact of dying cells on the polarization of co-cultured macrophages.

Material and Methods:

We use a murine fibroblast cell line (NIH 3T3) expressing human caspase 8 or murine receptor-interacting serine/threonine-protein kinase 3 (RIPK3), which are able to dimerize or oligomerize upon treatment with B/B homodimerizer to induce apoptosis or necroptosis, respectively. Cell death was analyzed by PI/Hoechst staining using ImageXpress Micro XLS or PI/Annexin staining using flow cytometry. Peritoneal macrophages were isolated by peritoneal lavage of C57 BL/6 mice. Macrophages were co-cultured with NIH 3T3 cells for 48h using a transwell system. Levels of anti- and pro-inflammatory cytokines and chemokines in the supernatant of co-cultures were analyzed using Cytometric bead array, ELISA or Legendplex- kits. Marker gene expression at the mRNA levels in NIH 3T3 cells or peritoneal macrophages was determined with real-time PCR.

Results:

Cell death of NIH 3T3 cells expressing caspase 8 or RIPK3 upon inducible dimerization /oligomerization was confirmed using specific necroptosis and apoptosis inhibitors. mRNA and protein levels of the pro-inflammatory cytokine IL-6 were elevated in the supernatant of necroptotic cells and significantly downregulated in the supernatant of apoptotic cells. Supernatants of peritoneal macrophages 48h co-cultured with necroptotic cells contained higher levels of IL-6, TNF-α, CCL17 and CCL22 protein in comparison to macrophages co-cultured with apoptotic cells. Furthermore, peritoneal macrophages showed decreased TNF-α and IL-23 mRNA levels after 24h and CD206 and LOX15 mRNA levels after 48 h when co-cultured with necroptotic cells. RNA sequencing was used to determine global changes in macrophage gene expression upon co-culture with necroptotic versus apoptotic cells.

Conclusion:

Our results provide new insights into the effect of necroptotic cells on macrophages. Necroptotic cells lead to the release of pro-inflammatory mediators, but also chemokines able to recruit regulatory T cells and Th2 cells. Analyzing gene expression profiles in detail will identify macrophage activation markers and signaling pathways triggered upon contact with necroptotic cells

73 | TRAIL treatment results in induction of interferon signaling in MCF-7 breast cancer cells

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Background

The prognosis for breast cancer, which is the most common cancer affecting women, is generally good. However, there is still a need of novel treatment since there are a substantial number of patients that suffer from relapse. TNF-related apoptosis-inducing ligand (TRAIL) could potentially be used to treat cancers as it initiates the apoptotic machinery in cancer cells, which ultimately results in cancer cell death. Previously, we have seen that TRAIL in combination with the small molecule Smac mimetic LCL-161 induces apoptosis in breast cancer cells. However, this combination of treatments does not induce cell death in MCF-7 breast cancer cells but rather an irreversible upregulation of various inflammatory-related genes.

Aim

Our aim is to study the molecular mechanisms of how TRAIL results in induction of IFN-induced genes in breast cancer cells.

Methods

Gene expression was analyzed with microarray, RNA-sequencing and RT-qPCR. Western blot was used to determine activation of cell signaling pathways. Knock-down experiments were performed with a siRNA approach.

Results

Results from global mRNA expression analysis after 24 h of treatment with Smac mimetic LCL-161 and TRAIL showed that genes related to NF-κB- and IFN-signaling were upregulated. The same genes were also upregulated, to a larger extent, after long-term treatment and re-seeding of cells in regular medium without treatments for three days, indicating that the effects on gene expression are sustained and irreversible. Both non-canonical NF-κB- and IFN-signaling pathways were confirmed to be active as the combination of LCL-161 and TRAIL resulted in elevation of p52 protein levels and phosphorylation of STAT1. TRAIL alone was found to be sufficient for the phosphorylation of STAT1 as well as induction of IFN target genes. This could be inhibited upon knock-down of interferon alpha receptor 1 (IFNAR1), as well as with pre-treatment with a JAK inhibitor. Knock-down of caspase-8 prevented STAT1 phosphorylation and inhibited downstream STAT1 signaling, whereas a caspase inhibitor had no suppressive effect. Analysis of the roles of TRAIL receptors DR4 and DR5 on IFN signaling showed that primarily a siRNA targeting of DR5 had a suppressive effect on STAT1 phosphorylation.

Conclusions

Our results from global gene expression analysis demonstrate that treatment with Smac mimetic LCL-161 and TRAIL induces an inflammatory response in luminal MCF-7 breast cancer cells. TRAIL is responsible for induction of IFN signaling, which conceivably goes through the IFN-receptor. There is dependence on caspase-8, but not on its catalytic activity for activation of the IFN signaling pathway. In addition, the TRAIL-receptor DR5 seems to be more important for the downstream signaling resulting in IFN signaling as compared to DR4.

74 | Ferroptosis in neurons and cancer cells is similar but differentially regulated by histone deacetylase inhibitors

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Ferroptotic death is a mechanism for tumor suppression by pharmacological inhibitors that target the Xc- transporter (cystine/glutamate antiporter) in a host of non-CNS and CNS tumors. Inhibition of this transporter leads to reduction of cystine uptake, cyst(e)ine deprivation, subsequent depletion of the versatile antioxidant glutathione, and reactive lipid species dependent death. Accordingly, pharmacological inhibitors of the Xc- transporter can also induce neuronal cell death raising concerns about toxicity in the CNS and PNS if these agents are used for chemotherapy. Here, we show that ferroptotic death induced by the canonical ferroptosis inducer erastin is similar in HT1080 fibrosarcoma cells and primary cortical neurons although cell death is mediated more potently in cancer cells. Reducing the toxicity of ferroptosis inducers will require, among other things, the identification of agents that protect neurons from ferroptosis but exacerbate it in tumor cells. While we show that a number of agents known to block ferroptosis in primary mouse neurons also inhibit ferroptosis in fibrosarcoma cells, class I histone deacetylase (HDAC) inhibitors selectively protect neurons while augmenting ferroptosis in cancer cells. Our results further suggest that cell death pathways induced by erastin in these two cell types is statistically identical to each other and identical to oxidative glutamate toxicity in neurons, where death is also mediated via inhibition of Xc- cystine transport. Together, these studies identify HDACs inhibitors as a novel class of agents to augment tumor suppression by ferroptosis induction and to minimize neuronal toxicity that could manifest as peripheral neuropathy or chemo brain.

75 | Plasma membrane damage and calcium fluxes activate ESCRT-III mediated repair mechanism upon ferroptosis

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Ferroptosis is an iron-dependent form of regulated necrosis associated with lipid peroxidation that results in the release of damage-associated molecular patterns (DAMPs) following plasma membrane permeabilization. Ferroptosis has attracted considerable attention due to its implication in several pathophysiological processes such as: cancer, neurodegeneration, acute renal failure, hepatic and heart ischemia-reperfusion injury, as well as antiviral immunity. A key step in ferroptosis execution is the final disruption of the plasma membrane integrity, which mediates the release of intracellular factors. However, little is known about the molecular events leading to the disruption of the plasma membrane during this type of cell death. Here we show that increase in cytosolic Ca2+ is a hallmark of ferroptosis that precedes complete bursting of the cell. Common to necroptosis and pyroptosis, our findings with ferroptosis reveal that sustained high levels of cytosolic Ca2+ prior to plasma membrane disruption is a common feature in lytic forms of regulated cell death. We identified the formation of pores of around 2.7 nm radius as a core mechanism that triggers plasma membrane permeabilization and the increase in cytosolic calcium in ferroptosis, and correlated these processes with the activation of ESCRT-III-mediated membrane repair mechanism. Furthermore, our results position Ca2+-dependent membrane repair mechanisms as a central machinery to protect cells from inflammatory forms of regulated cell death.

76 | Using ascites fluid and high throughput dynamic BH3 profiling to identify active cancer therapies

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The use of imperfect models and ex vivo culture systems to try to predict patient drug response represents an enormous bottle neck in cancer treatment. Determining how effective an approved drug will be for a given cancer patient, as well as identifying novel compounds that may be beneficial to a specific population requires the use of primary tumor cells. High-throughput dynamic BH3 profiling (HT-DBP) provides an assay platform that allows us to maximize the amount of information obtained from a limited number of cells and requires only 24 hours of culture ex vivo to more accurately reflect the behavior and vulnerabilities of the primary tumor. HT-DBP is a microscopy-based assay performed on adherent cells that visualizes and quantifies mitochondrial outer membrane permeabilization to identify compounds that induce mitochondrial apoptotic signaling.

Ovarian cancer is a devastating disease desperately in need of novel therapeutic interventions. The standard-of-care cytotoxic agent regimen remains largely unchanged over the last 15 years. Although there has been some success using molecular targeted agents and maintenance therapies, the genetic complexity and lack of common molecular drivers in the disease have made the selection of patient populations most likely to benefit from therapies difficult. Ovarian cancer, however, frequently has a unique pathology wherein fluid, known as ascites fluid, accumulates in the peritoneum of patients. This ascites fluid frequently contains tumor cells and is routinely aspirated from patients throughout treatment. Therefore, ascites fluid presents a potentially abundant and easily accessible source of tumor cells from ovarian cancer patients.

We have optimized the use of tumor cells from primary ascites samples in the adherent HT-DBP assay. To evaluate how susceptible the tumor cells from individual patients will be to currently available chemotherapeutic agents, we have treated the cells with a range of concentrations of approved compounds. We observed varying sensitivities to these frequently used drugs. In an effort to identify new drugs, or drug targets, that may benefit patients, we have also performed an 880 compound screen at 3 concentrations per compound on primary ascites samples. This screen identified novel chemical vulnerabilities to investigate in the future. In total, we have demonstrated the potential utility of tumor cells isolated from ascites samples in the HT-DBP assay to both predict patient drug response and identify new therapies.

77 | Novel Alternative Splicing Switches Bnip3 from Cell Death to Cell Survival.

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Programmed cell death is recognized as a central feature of myocardial infarction and tumoriogenesis in cancer.

Previously, we established the hypoxia-inducible factor Bnip3 as an integral component of the intrinsic apoptosis death pathway that can also signal autophagy but the underlying mechanisms that regulate these divergent properties remain cryptic. Alternative mRNA splicing provides a versatile mechanism by which cells can generate proteins with different or even antagonistic properties. Herein, we provide novel evidence that inclusion or skipping of Bnip3 exon3 (Bnip3ex3) generated by alternative splicing of Bnip3 mRNA provides a molecular switch that functionally determines whether Bnip3 triggers mitochondrial perturbations and apoptosis of cardiac myocytes and cancer cells under physiological conditions. Here we show that during hypoxia in vitro and in vivo two distinct Bnip3 mRNA isoforms are synthesized, one mRNA encodes a Bnip3 protein of 26kDa and previously unrecognized truncated form missing exon3 that encodes a Bnip3 protein of 8.2kDa. Notably, whereas the mitochondrial associated Bnip3 isoform provokes apoptosis and autopahgy, gain of function of Bnip3ex3 by promoting exon3 skipping abrogated the effects of Bnip3. Furthermore, genetic inhibition of Bnip3ex3 inhibited cell proliferation and sensitized hypoxia and doxorubicin resistant Glioblastoma, Pancreatic ductal carcinoma and breast cancer cells to necrotic cell death. Cellular stress induced by nutrient deprivation induced the same changes in Bnip3 mRNA alternative splicing as hypoxia in cardiac myocytes and cancer. Given the otherwise lethal consequences of de-regulated Bnip3 expression, alternative splicing of Bnip3 mRNA highlights a novel unrecognized survival mechanism that opposes mitochondrial defects and cell death in tumors induced by Bnip3 during hypoxic stress. Hence, our novel findings fundamentally explain how cancer cells avert cell death during tumoriogenesis imposed by hypoxic and nutrient stress.

78 | Ferroptosis and necroinflammation in tissue degeneration

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Ferroptotic cell death is considered as a major root cause for early cell loss and tissue demise in numerous diseases including neurodegeneration, organ failure and tissue ischemia/reperfusion injury (IRI). Preliminary studies suggest that bursting ferroptotic cells trigger immune cell recruitment, as exemplified by reactive astrogliosis and macrophage infiltration in diseased tissues of brain and kidney, respectively. By exploiting conditional mouse knockout models of the ferroptosis key regulators glutathione peroxidase 4 (GPX4) and acyl-CoA synthetase long chain family member 4 (ACSL4) we now show, that expression of these enzymes not only mediates tissue damage upon IRI in the liver but also regulates the early infiltration of effector cells. In addition, treatment of mice subjected to hepatic IRI using an optimized and metabolically more stable version of the first in vivo active ferroptosis inhibitor liproxstatin-1 (2nd generation liproxstatin) resulted in significant tissue protection along with reduced effector cell recruitment. In summary, our data further corroborate a crosslink between ferroptosis and immune cell activation and stress the need for translating new therapeutic concepts based on the prevention of ferroptosis and associated inflammatory events into the clinic for the treatment of IRI-related conditions and other degenerative diseases.

79 | Development of a novel mitotic inhibitor targeting the NOXA-MCL1-BIM axis and producing a pro inflammatory response

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Antimitotic chemotherapy is a major treatment modality for both solid and hematological neoplasms. Cells which are arrested in mitosis can undergo two major fate; mitotic cell death and mitotic slippage. Caspase-2 is activated in response to extended mitotic arrest and is an essential mediator of both mitotic cell death and the response to slippage. We took advantage of this property to identify novel mitosis targeting agents. Using a bimolecular fluorescence-based genetic reporter we screened a library of 2050 druglike compounds for caspase-2 activation. We identified 372 as a potent mitotic inhibitor. Through its binding and depolymerization of microtubules, 372 prevents cells from progressing through prometaphase, thereby inducing an extended mitotic arrest, accompanied by proteasomal MCL1 degradation. In cell types which are prone to undergo apoptotic cell death upon mitotic arrest, this death is mediated through the pro-apoptotic BCL-2 family proteins PUMA, NOXA, BAX and BAK. In cells prone to slippage, 372 promotes mitotic exit into a highly micronucleated state. These micronuclei contain destabilized chromosomes with high levels of DNA damage and a strong p53 response. The primary nucleus harbors minimal DNA damage or activated p53. The result of this micronucleation is a potent inflammatory response characterized by phospho-IκBα-driven NF-kB activation, leading to expression of a range of pro-inflammatory cytokines. 372 is active against a range of tumor cell lines as well as primary leukemia bone marrow progenitors. A back-screen against 133 other antitumor agents revealed that 372 displays synergy with BCL-ABL inhibitors, and is able to deplete both with-type and p53-mutant BCR-ABL positive cells. 372 likely binds to the colchicine-type site on microtubules and is able to overcome paclitaxel and vinblastine resistance.

80 | Exploring cellular processing and localization of ferroptosis-inhibiting deuterated polyunsaturated fatty acids

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Ferroptosis is a non-apoptotic form of iron-dependent cell death via lipid peroxidation that has been implicated in several disease pathologies, including neurodegenerative disorders, renal tubular necrosis, and myocardial ischemia. Thus, there is great interest in elucidating the mechanisms of induction and inhibition of ferroptotic cell death. Peroxidation of polyunsaturated fatty acids (PUFAs) by lipoxygenase enzymes and free iron has been identified as a key driver of ferroptosis. Treatment of cells with PUFAs that are deuterated at the bisallylic positions (D-PUFAs) potently rescues from ferroptotic death in vitro. This inhibition of ferroptosis is likely due to the kinetic isotope effect, as hydrogen abstraction is the first step of PUFA peroxidation. D-PUFAs have also been shown to abate neurodegeneration in mouse models of Parkinson's and Alzheimer's diseases. It was previously unknown where within cells D-PUFAs accumulate to exert their protective effect. We identified the subcellular destination of D-PUFAs using stimulated Raman spectroscopy imaging, an imaging technique that allows localization in live cells without a fluorescent label due to the unique C-D vibrational frequency in these deuterated compounds. We then evaluated the role these sites of accumulation play in ferroptosis inhibition using chemical and genetic techniques, and assessed incorporation of D-PUFAs using mass spectrometry-based lipidomics. The exploration of subcellular processing and sites of accumulation of D-PUFAs not only provides a deeper understanding of their protective mechanism, but also contributes to the elucidation of key subcellular sites of lipid peroxidation in ferroptotic death.

81 | The novel immune-regulatory cytokine Interleukin-37 exhibits pro-inflammatory activity upon N-terminal proteolytic processing

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IL-37 is an enigmatic member of the extended IL-1 cytokine family that is thought to be the sole anti-inflammatory member of this family. IL-37 has been suggested to function through engaging the IL-18 receptor and SIGGR upon processing of this cytokine by caspase-1. However, contrary to reports that IL-37 is an anti-inflammatory cytokine, here we show that IL-37 exhibits robust pro-inflammatory activity upon processing by neutrophil elastase, skin-associated cathepsins or proteases derived from multiple allergens. Of note, the latter proteases processed IL-37 at physiologically relevant concentrations, whereas caspase-1 failed to do so. Mutation of critical residues within the N-terminus of IL-37 ablated the activation of this cytokine by elastase or cathepsin S, whereas generation of an N-terminal truncation produced a constitutively active IL-37 that induced robust inflammation in vitro and in vivo. Consistent with the structural homology between IL-37 and IL-36, elastase- or cathepsin S-processed IL-37 exhibited highly similar inflammatory signatures by RNASeq analysis. Collectively, these data challenge the idea that IL-37 is an exclusively anti-inflammatory cytokine and argue that IL-37 can exert pro-inflammatory effects similar to other IL-1 family cytokines.

82 | FLIP suppresses Death Receptor-induced inflammation as well as cell death

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TRAIL and FasL are potent inducers of apoptosis that can also promote inflammation through assembly of FADDosome (FADD/caspase-8/RIPK1) NF-κB-activating complexes. FLIP isoforms are also recruited to the latter complexes to suppress apoptosis, however the role of FLIP in regulating NFB activation has been controversial. Here we show that silencing of FLIPL or FLIPs, or both, led to dramatically enhanced Fas- or TRAIL-induced inflammatory cytokine production, as well as cell death, suggesting that FLIP acts to suppress both death receptor-associated apoptosis, as well as NF-κB activation. Here, we will discuss the mechanistic basis for these effects.

83 | Crashing the computer: Apoptosis and Necroptosis in Neurodegeneration

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Microglial cells are the resident innate immune cells of the central nervous system (CNS) and are responsible for regulating inflammatory activation in response to both physiological and pathological immune perturbations and ultimately neuron health and survival. The mechanisms that fully control and dictate the nature of an inflammatory response in the CNS are still poorly understood. We have recently identified a novel pathway where components of the canonical autophagy machinery function to conjugate the microtubule-associated protein LC3 to endosomal membranes, a process we have termed LC3-associated endocytosis (LANDO). We further identified that abrogation of LANDO in microglial cells promotes pro-inflammatory cytokine production *in vitro.* To characterize LANDO and its role in immune regulation in the CNS, we utilized a well-established model of neuroinflammation induced by β-amyloid deposition. We found that mice lacking LANDO but not canonical autophagy in either the myeloid compartment or specifically in microglia, have a robust increase in pro-inflammatory cytokine production in the hippocampus and have increased levels of neurotoxic β-amyloid accumulation. This inflammation and β-amyloid deposition led to reactive microgliosis and hyperphosphorylation of tau, a protein that is vital to neuronal structure and function. As a consequence, LANDO-deficient AD mice have massive neuronal cell death, resulting in impaired neuronal signaling and consequential behavioral and memory deficits. The causative mechanism of cell death in neurodegeneration his currently a highly controversial topic and unresolved question. Herein, we use our unique LANDO-deficient AD model of neurodegeneration to investigate the contribution of both apoptosis and necroptosis to the observed neuronal loss. In brief, we will show that inhibition of necroptosis through genetic abrogation of MLKL has an impact on neurodegeneration as does pharmacological inhibition using Necrostatin-1s. We further compare and contrast the differences between apoptotic and necroptotic death in our model and provide substantiating evidence for a role of necroptosis and necroinflammation *in vivo*. Our results further delineate a putative physiological role for necroptosis in neuronal death, long thought to be dominated by apoptosis alone, and opens the possibility for the development of novel therapeutics for AD and other neurodegenerative conditions.

84 | Analysis of the oncogenic properties of cIAP1

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Cellular inhibitor of apoptosis 1 (cIAP1) is a major regulator of the innate immunity, inflammatory response, cell differentiation and cell death. It is a member of IAP family harboring an E3-ubiquitine ligase activity. Thanks to its ability to modify a large number of protein substrates it regulates several intracellular signaling pathways such as the tumor necrosis factor receptor (TNFR)-, NF-κB (Nuclear Factor-Kappa B), and MAPK (Mitogen-Activating Protein Kinase) signaling pathways. We and other also identified a function for cIAP1 in the regulation of actin cytoskeleton reorganization and transcription factor activity. cIAP1 displays oncogenic properties in mouse models, and its expression has been found to be altered in several human cancer samples.

Our project aims to analyze the mechanisms involved in the cIAP1 oncogenic properties. Mouse Embryonic Fibroblasts (MEFs) WT, devoid of cIAP1 (SKO) or devoid of cIAP1 and cIAP2 (DKO) were transformed by H-Ras-V12 oncogene. Deletion of cIAPs considerably altered the ability of cells to grow in a mice xenograft model and to colonize lung in metastatic experimental model in mice. Expressing cIAP1 in DKO cells restored the growth and invasive abilities of cells. The deletion of the BIR1 domain of cIAP1 (ΔBIR1 mutant) is sufficient to inhibit oncogenic properties of cIAP1. The BIR1 is dedicated to the interaction with the signaling intermediate TRAF2 (Tumor necrosis factor Receptor Associated Factor). Mutation of Leucine 47 residue that abolished the cIAP1-TRAF2 interaction inhibited the oncogenic properties of cIAP1. Immunofluorescence analysis of TRAF2 in cIAP1-restored DKO MEFs showed the presence of TRAF2 clusters revealing activation. Such TRAF2 clusters were not observed in DKO MEFs or in DKO MEFs expressing the ΔBIR1 mutant. Isolated BIR1 domain was demonstrated to trimerize TRAF2 (Zheng and al. *Mol Cell* 2010). We then expressed the isolated BIR1 domain in DKO MEFs. We observed the presence of TRAF2 clusters. The isolated BIR1 was sufficient to stimulate tumor cell growth in soft agar medium and in the xenograft mouse model. The analysis of tumor samples demonstrated a significant correlation between cIAP1 expression and tumor growth, and between cIAP1 expression and IL-6 expression. The kinases JAK2 and STAT3 are phosphorylated in cIAP1-expressing cells revealing the activation of the IL-6 signaling pathway. Deletion of the BIR1 decreased JAK2 and STAT3 phosphorylation. Altogether, our data demonstrated the importance of TRAF2 in oncogenic properties of cIAP1. Activation of TRAF2 is sufficient to stimulate tumor growth. The IAP antagonists currently under investigations target the cIAP1-BIR2 and BIR3 domains, mimicking SMAC activity. Targeting the BIR1 in order to block cIAP1-TRAF2 interaction could be an alternative strategy to counteract cIAP1 oncogenic activity.

85 | Molecular underpinnings of ferroptotic cell death in neurodegeneration

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Amyotrophic lateral sclerosis (ALS) is characterized by the selective loss of motor neurons (MN) eventually culminating in paralysis and early death of affected patients. Oxidative damage and deregulated iron handling are both common features of ALS, yet it still remains elusive how this damage culminates in MN death. Besides ALS, dementia, the most prevalent age-associated neurodegenerative disease, still has no clear genetic pattern with a still poorly understood molecular pathogenesis. In this context, ferroptosis, a regulated necrotic form of cell death, is of particular interest as it is triggered by iron-dependent lipid peroxidation (Dixon et al., Cell, 2012). Glutathione peroxidase 4 (GPX4) act as the key regulator of ferroptosis due to its unique ability to efficiently reduce peroxidized lipids in lipid bilayers thus preventing further lipid peroxidation chain reaction (Angeli et al., Nat Cell Biol, 2014; Yang et al., Cell, 2014). *In vivo* studies using cell-type specific GPX4 knockout mice demonstrated its importance in the brain, especially for cortical neuron survival and MN homeostasis (Seiler et al., Cell Metab, 2008; Hambright et al., JBC 2015). To study the consequences of increased phospholipid oxidation in different neuronal subtypes and associated neuroinflammation, relevant mouse models have been generated. Inducible cortical neuron-specific GPX4 KO mice show massive neuronal loss, progressive cortical atrophy and neuroinflammation, hallmarks of dementia. MN-specific GPX4 KO mice are viable at birth and are indistinguishable from their wildtype littermates, but at the age of 4-6 months develop progressive ALS-like symptoms. These include kinky tail, kyphosis, motor dysfunction and muscle weakness. To prevent ferroptotic cell death and associated neuronal loss in those novel mice models, it is planned to pharmacologically treat these mice with 3rd generation liproxstatins, highly efficient and blood brain barrier-permeable ferroptosis inhibitors. Hence, all these studies aim to understand the role of ferroptosis in neuronal loss, knowledge to be translated in patients suffering from neurodegenerative disease such as ALS and dementia.

86 | Bcl-xL and Bak serve as predictors of sensitivity of cancer cells to BH3-mimetic S63845

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One of the most commonly exploited strategies in anticancer treatment is based on the induction of apoptotic cancer cell death. There are numerous agents which cause various types of cellular stresses and eventually lead to the mitochondrial outer membrane permeabilization (MOMP) and activation of intrinsic pathway of apoptosis. MOMP is tightly controlled by proteins of the Bcl-2 family, which consists of more than 20 members, which are either proapoptotic or antiapoptotic. Antiapoptotic proteins of the Bcl-2 family (such as Bcl-2, Bcl-xL, Mcl-1) represent attractive targets for anticancer therapy and, consistently, the so-called BH3-mimetics – inhibitors of antiapoptotic Bcl-2 proteins – are thoroughly investigated in both preclinical and clinical studies. Recently, the inhibitor of Bcl-2 Venetoclax was approved by FDA for clinical use, underlining the great potential of BH3-mimetics. Nowadays, at least three BH3-mimetics specifically targeting Mcl-1 are evaluated in clinical trials. In general, these compounds represent a powerful tool for induction of apoptosis. As BH3-mimetics act as direct regulators of MOMP, they are much more specific in triggering apoptosis in selected cell types compared to other agents, such as genotoxic drugs or microtubule poisons. At the same time, sensitivity to one or other BH3-mimetic varies between different tumor cells, which highlights the importance of biomarkers that will correlate to response to such drugs. Our studies have shown that among two BH3-mimetics targeting Mcl-1, A1210477 and S63845, the first one demonstrates lower efficacy compared to MCL1 knockdown, while S63845 is as potent as downregulation of Mcl-1 using siRNA. Apparently, differences in efficacy of these two compounds are due to diverse impact on activation of proapoptotic Bcl-2 family member Bak. Indeed, whereas A1210477 results in a moderate level of apoptosis in a Bak-independent fashion, S63845 induces higher level of apoptosis in a Bak-dependent manner. Next, using siRNA-mediated knockdown it was shown on different tumor cell lines (such as HeLa and H23) that S63845-induced apoptosis depends on level of Bak but not on Bax or Bim (other proapoptotic members of the Bcl-2 family). We have also generated cell lines, characterized by higher resistance to S63845 compared to parental cell lines. It was shown that acquired resistance to S63845 is accompanied by higher expression of prosurvival member of Bcl-2 family Bcl-xL. Finally, it was shown on a panel of cancer cell lines that lower levels of Bcl-xL correlate with better response to S63845. Taken together, these data demonstrate that Bak and Bcl-xL serve as biomarkers for sensitivity of cancer cells to BH3-mimetics targeting Mcl-1.