
Age-Related Human Diseases
Special Focus: Autophagy

# Poster Abstracts

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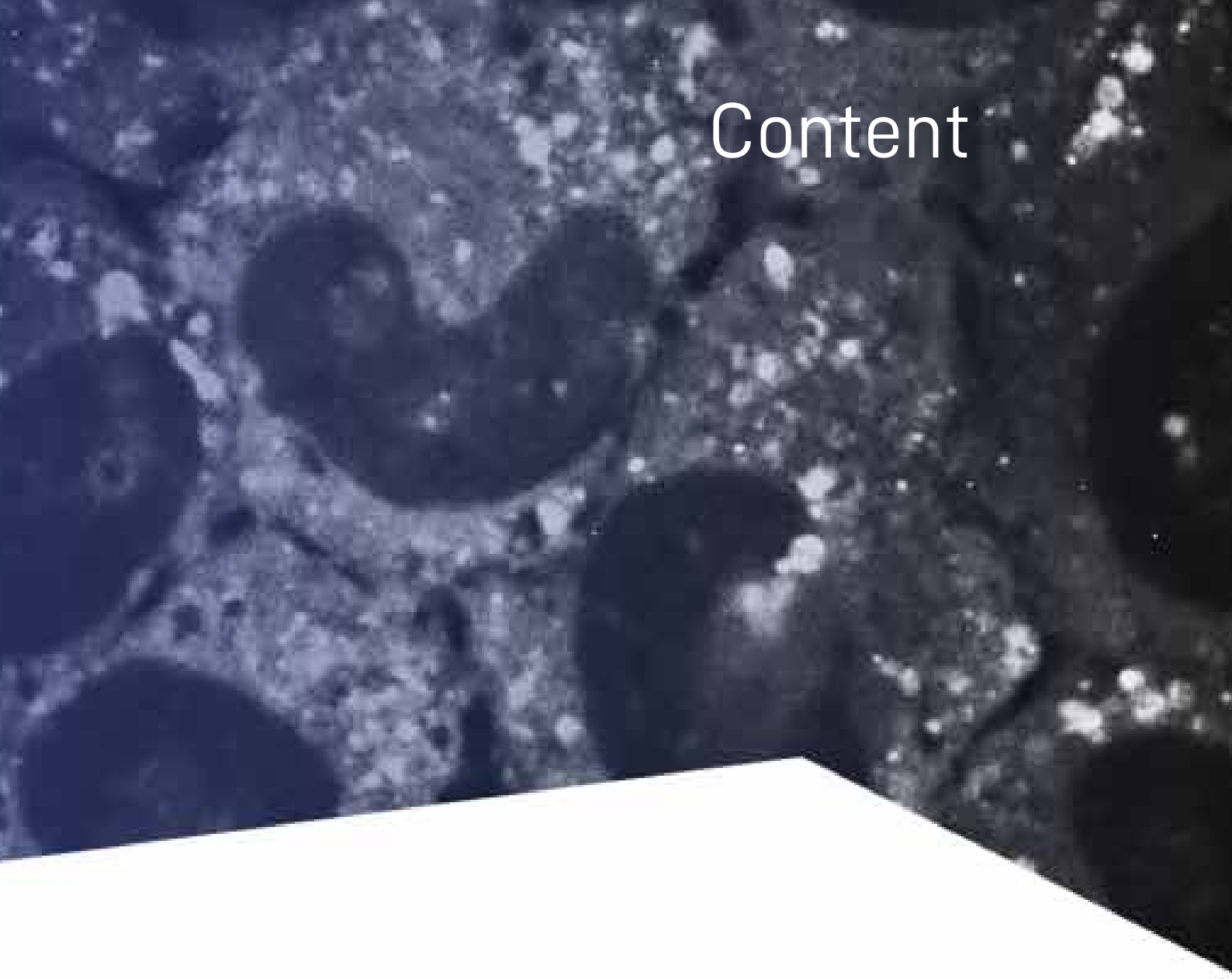

# Content

# Abstracts - Poster

**Identification of monensin A as a novel inhibitor of the leukemia protein MYB**

Authors:
**L. Abdel Ghani[1], M. Yusenko[1], A. Trentmann[1], K. - H. Klempnauer[1]**

[1] Westfälische-Wilhelms Universität, Institut für Biochemie - , Münster, North Rhine-Westphalia, Germany
Email: l_abde01@uni-muenster.de

Recent work has shown that deregulation of the transcription factor MYB contributes to the development of leukemia, making MYB an attractive target for drug development. By employing a MYB-reporter cell line we have identified the polyether ionophores monensin A, salinomycin and nigericin as novel inhibitors of MYB-dependent transcription. Gene expression profiling of monensin A-treated myeloid leukemia cells shows that monensin A affects the expression of a significant number of MYB-regulated genes. A prolonged treatment of human acute myeloid leukemia (AML) cell lines with monensin A shows that it down-regulates MYB expression, disrupts cell viability and induces differentiation and apoptosis. Importantly, monensin A-induced myeloid differentiation is dampened by ectopic expression of an activated version of MYB, demonstrating that some effects of monensin A are mediated via inhibition of MYB. However, the full mechanism is still under investigation. Finally, we show that monensin A inhibits the proliferation of primary murine AML cells significantly stronger than of normal hematopoietic progenitor cells, reflecting the higher MYB-dependence of the leukemic cells compared to normal hematopoietic progenitor cells. Overall, our work identifies monensin A and related compounds as promising candidates to be developed further into novel MYB inhibitors.

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**The Effects of CDP-Choline on Mitochondrial Dynamics**

Authors:
**S. Bozkurt[1], D. Acikgoz[1], D. Oz-Arslan[1]**

[1] Acibadem Mehmet Ali Aydinlar University School of Medicine, Department of Biophysics, Istanbul, Turkey
Email: demetacikgoz49@gmail.com

Mitochondrial dynamics and mitophagy have crucial impact on various diseases. Recent studies showed that phospholipids have an important role in lipid–protein interactions, modulating activity of target proteins. During mitophagy, phosphatidylcholine (PC) level decreases. CDP-Choline is an intermediate for production of PC and has a potential role in oxidative stress. We hypothesized that CDP-Ch may contribute to mitophagy and mitochondrial dynamics as a source of PC. In this study, we examined the effect of CDP-Ch on mitochondrial dynamics and mitophagy in U937 cells. Mitophagy was induced by CCCP in the presence and absence of CDP-Ch. Mitophagy-related proteins were investigated by western blotting. We determined that the levels of Pink1, Drp1, Mfn2, Opa1, CoxIV, Tomm20 and LC3B changed with CDP-Ch treatment. We measured mitochondrial membrane potential, mitochondrial mass and mitochondrial superoxide production by flow cytometry and confocal microscopy. The decrease in mitochondrial mass upon mitophagy induction was reversed by CDP-Ch treatment. These changes in mitochondrial fusion or fission and mitochondrial mass in mitophagy- induced U937 cells suggest a modulatory role of CDP-Ch in mitochondrial dynamics. We propose that CDP-Ch leads to changes in mitochondrial fusion and fission by contributing to the production of mitochondrial membranes in mitophagy-induced monocytes. This response can then rescue cells quickly from the stress and maintain cellular functions.

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**Receptor-mediated endocytosis 8 (RME-8)/DNAJC13 stabilizes cellular proteostasis and positively modulates autophagy**

Authors:
**M. D. A. Ax[1], A. S. Besemer[1], J. Maus[1], A. Stein[1], C. Freese[1], K. Nalbach[1], C. von Hilchen[1], I. Koziollek-Drechsler[1], H. Huesmann[1], F. Boukhallouk[2], L. Florin[3], A. Kern[1], C. Behl[1], A. M. Clement[1]**

[1] University Medical Center of the Johannes Gutenberg-University, Mainz, Institute of Pathobiochemistry, Mainz, Rhineland-Palatinate, Germany
[2] University Medical Center of the Johannes Gutenberg-University, Mainz, Institute of Medical Microbiology and Hygiene, Mainz, Rhineland-Palatinate, Germany
[3] University Medical Center of the Johannes Gutenberg-University, Mainz, Institute for Virology and Research Center for Immunotherapy (FZI), Mainz, Rhineland-Palatinate, Germany
Email: mirjamax@uni-mainz.de

Protein homeostasis (proteostasis) is essential for cellular function and is challenged upon exposure to acute or chronic insults. In a functional screen in *C. elegans* we identified the gene *receptor-mediated endocytosis 8* (*rme-8;* human ortholog: *DNAJC13*) as one component to maintain proteostasis. Supporting these data, accumulation of aggregation-prone neurodegenerative disease-related proteins were aggravated upon the knockdown of *rme-8/DNAJC13* in *C. elegans* and in human cell lines. DNAJC13 is associated with the retromer complex and involved in endosomal protein trafficking. As retromer components like VPS35 have previously been linked to autophagy, we investigated the role of RME-8/DNAJC13 in this context. We demonstrated that reduced RME-8/DNAJC13 levels decreased autophagic flux under basal and autophagy-inducing conditions. Furthermore, the overexpression of wild-type DNAJC13, but not its Parkinson’s disease-related mutant variant DNAJC13(N855S), resulted in an increased autophagic flux. We show evidence that this was mediated by affecting ATG9A endosomal trafficking as ATG9A localization at late and recycling endosomes and ATG9A transport from the recycling endosome was reduced upon DNAJC13 knockdown. As a consequence, the localization of ATG9A at LC3-positive punctae was reduced. These data demonstrate a novel function of RME-8/DNAJC13 in proteostasis by positively modulating autophagy.

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**The anti-malaria drug Dihydroartemisinin induces apoptosis, ferroptosis and autophagy-associated cell death in normoxia and interferes with long-term survival in hypoxia**

Authors:
**S. Bader[1], T. Ontikatze[1], V. Jendrossek[1], J. Rudner[1]**

[1] University of Duisburg-Essen, Institute of Cell Biology (Cancer Research), Essen, North Rhine-Westphalia, Germany
Email: sina.bader@uk-essen.de

The anti-malaria drug Dihydroartemisinin (DHA) shows promises in cancer treatment. More importantly, since tumor hypoxia (Hx) decreases the efficacy of anti-cancer therapy, DHA was previously shown to reduce clonogenic survival of human cancer cells even better in Hx (0,2% $O_2$) than in normoxia (Nx) (21% $O_2$). So far, the underlying mechanism regulating DHA-induced cytotoxicity is insufficiently decoded.

Using HCT116 colon cancer cells and a subclone with impaired expression of the apoptotic factors Bax and Bak, we could show that DHA induced intrinsic apoptosis in Nx which is mitigated in Hx. In both cell lines, DHA-induced reduction of the clonogenic cell fraction as well as iron-dependent

lipid peroxidation were more potent in Hx. Lipid peroxidation did not correlate with short-term cell death (ferroptosis), which could be prevented by iron chelation with deferoxamine in Nx, but was only lowered in Hx. In both cell lines, DHA induced autophagy more efficient in Nx compared to Hx. In the parental clone, autophagy was associated with cell death, since cell death could be inhibited by co-treatment with chloroquine.

Taken together, we could show that DHA is a potent hypoxia-active drug. We conclude that DHA induced apoptosis, ferroptosis and autophagy-associated cell death in Nx. However, in Hx lipid peroxidation and autophagy induction did not correlate with short-term cytotoxicity but rather affected long-term survival by interfering with the clonogenic capacity of the cells.

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**Autophagy is restricted by the ubiquitin-specific protease 11 (USP11)**

Authors:
**M. Basic[1, 2], A. Kalb[1, 2], F. Bonn[1], I. Dikic[1, 2], A. Kern[3], C. Behl[3], A. Bremm[1, 2]**

[1] Goethe University Medical School Frankfurt, Institute of Biochemistry II, Frankfurt am Main, Germany
[2] Goethe University Frankfurt, Buchmann Institute for Molecular Life Sciences, Frankfurt am Main, Germany
[3] Johannes Gutenberg University Medical School, Institute for Pathobiochemistry, Mainz, Germany
Email: basic@med.uni-frankfurt.de

Autophagy is an important and conserved process cells utilize to maintain cellular homeostasis via degrading cellular components. Both non-selectively and selectively targeted cargo is delivered to the lysosome through activity of multiple enzymatic complexes. Their composition, activity, and localization, is mediated by various post-translational modifications, including ubiquitination. 

Our data shows that ubiquitin-specific protease 11 (USP11) restricts autophagy and that CRISPR/Cas-mediated USP11 knockout (KO) in mammalian cells results in elevated autophagic flux. Importantly, depletion of USP11 homolog H34C03.2 in *Caenorhabditis elegans* also causes hyperactivation of autophagy, and a significant delay in paralysis of the worms caused by the muscular expression of human β-amyloid peptide 42 (Aβ42). USP11 interacts with multiple components of the autophagic machinery, including members of the autophagy-specific class III phosphatidylinositol 3-kinase (PIK3C3) complex and the ubiquitin-like conjugation machinery, suggesting that USP11 impacts autophagy at different stages. In accordance with this, we observe elevated level of NRBF2 in USP11 KO cells, an additional component of the PIK3C3 complex I, that controls activity and architecture of the kinase complex. Here we will present our follow up studies elucidating molecular details of how USP11 regulates autophagy.

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**CRC1177 - Molecular and Functional Characterization of Selective Autophagy**

Authors:
**I. Dikic[1], C. Behl[2]**

[1] Goethe University, IBC2 UNIKLINIK | BMLS, Frankfurt, Germany
[2] Johannes Gutenberg University, Institute for Pathobiochemistry/University Medical Centre, Mainz, Germany
Email: cbehl@uni-mainz.de

The Collaborative Research Centre (CRC) 1177 is the first network in Germany that started to systematically

address challenging questions related to molecular mechanisms in selective autophagy. It is funded by the German Research Foundation (DFG) with 11 M € since 2016 and led by Ivan Đikić from Goethe University Frankfurt together with Christian Behl from University Medical Centre Mainz. The CRC has pulled together interdisciplinary expertise to address open questions in the field relating to the molecular determinants of selectivity and their regulation, the functional roles of autophagy in different cellular processes and contexts, and the impact of autophagy on pathophysiology of diseases and on therapy response. It currently comprises of 19 partners from GU Frankfurt, JGU Mainz, IMB Mainz, LMU Munich and the Georg Speyer Haus in Frankfurt. Recently, the CRC has undergone evaluation for a second funding period and is awaiting the decision by DFG. In case of prolongation, several additional colleagues from other institutions in Germany will come on board, and many new positions will be advertised in the field of selective autophagy.

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***Autophagy, Inflammation and Metabolism in Disease Center (the AIM center)NIH-funded Center of Biomedical Research Excellence Focused on Autophagy***

Authors:
**C. Behrends[1, 4], J. Weaver[3, 2], L. Sklar[3, 2], J. Cannon[3, 2], E. Prossnitz[3, 2], V. Deretic[3, 2]**

[1] Autophagy Inflammation and Metabolism Center International Council of Affiliate Member , Munich, Germany
[2] Autophagy Inflammation and Metabolism Center of Biomedical Research Excellence, Albuquerque, New Mexico, United States of America
[3] University of New Mexico , School of Medicine , Albuquerque, New Mexico, United States of America
[4] Ludwig-Maximilians-Universität München, Munich Cluster for Systems Neurology , Munich, Germany
Email: christian.behrends@mail03.med.uni-muenchen.de

Recently, NIH has funded a center for autophagy research named the **A**utophagy, Inflammation, and **M**etabolism in Disease **C**enter of **B**iomedical **R**esearch **E**xcellence (**AIM CoBRE**), located at the University of New Mexico Health Science Center (UNM HSC), with aspirations to promote autophagy research locally, nationally, and internationally. The Center and included AIM Scientific Cores have 3 major missions: (i) to support junior faculty in their endeavors to develop investigations in this area and obtain independent funding; (ii) to develop and provide technological platforms to advance autophagy research with emphasis on cellular approaches for high quality reproducible research; and (iii) to foster international collaborations through the formation of an International **Council**of Affiliate Members and through hosting national and international workshops and symposia. Scientifically, the AIM CoBRE is focused on autophagy and its intersections with other processes, with emphasis on both fundamental discoveries and applied translational research.

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**RAB18 impacts autophagy via lipid droplet-derived lipid transfer and is rescued by ATG9A**

Authors:
**F. Bekbulat[1], D. Schmitt[1], A. Feldmann[1], H. Huesmann[1], S. Eimer[2], T. Juretschke[3], P. Beli[3], C. Behl[1], A. Kern[1]**

[1] University Medical Center of the Johannes Gutenberg University Mainz, Institute of Pathobiochemistry, Mainz, Germany
[2] Goethe University Frankfurt, Institute for Cell Biology and Neuroscience, Frankfurt, Germany
[3] Institute of Molecular Biology, Mainz, Germany

Email: fbekbula@uni-mainz.de

Autophagy is a highly inducible degradation mechanism and dependent on an appropriate lipid supply for efficient autophagosome formation. The specific regulation of lipid acquisition as well as the autophagy network response to lipid-limiting conditions are not fully understood. Here, we show that the knockout of RAB18 interferes with lipid droplet (LD) catabolism and results in an impaired fatty acid release. In consequence, the reduced LD-derived lipid availability influences autophagy and provokes adaptive adjustments of the autophagy network, which include the increased expression of ATG2B, elevated ATG5-ATG12 conjugate levels, and altered phosphorylation of ATG2B and ATG9A. The enhanced phosphorylation of ATG9A directs this transmembrane protein to the autophagy pathway, which is sufficient to rescue basal autophagy  in the absence of RAB18. However, the adaptations of the autophagy network are incapable of enabling induced autophagic activity, which is characterized by an increased lipid demand. Thus, here we define the molecular role of RAB18 in connecting LDs and autophagy, emphasizing the significance of LD-derived lipids for this pathway. Further, we uncover a phosphorylation-dependent  rescue function of ATG9A to compensate reduced lipid availability in order to maintain basal autophagy.

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**The role of an anti-fibrotic microRNA network in control of liver cancer-associated fibrosis**

Authors:
**C. Bitter[1, 2, 3], I. Winkler[1, 2, 3], J. Hengstler[4], R. Weiskirchen[5], A. Nordheim[1, 2, 6]**

[1] Eberhard Karls University Tübingen, Molecular Biology, Interfaculty Institute of Cell Biology (IFIZ), Tübingen, Baden-Württemberg, Germany
[2] German Cancer Consortium (DKTK) , German Cancer Research Center (DKFZ), Heidelberg, Germany
[3] International Max Planck Research School (IMPRS), Tübingen, Germany
[4] Leibniz Research Centre for Working Environment and Human Factors (IfADo), Dortmund, Germany
[5] University Hospital Aachen, Institute of Molecular Pathobiochemistry, Experimental Gene Therapy and Clinical Chemistry, Aachen, Germany
[6] Leibniz Institute on Aging (FLI), Jena, Germany
Email: catrin.bitter@uni-tuebingen.de

*SRF-VP16*$^{iHep}$ mice express a constitutively active form of the transcription factor Serum Response Factor (SRF), namely SRF-VP16. Upon mosaic hepatocyte-specific expression of SRF-VP16, *SRF-VP16*$^{iHep}$ mice develop hepatocellular carcinoma (HCC). Using this mouse model, we identified an anti-fibrotic microRNA (miRNA) network, which regulates cancer-associated fibrosis in murine HCC. The miRNA:mRNA interactions of the network were also found to be dysregulated to different degrees in human HCC. The miRNA:mRNA interactions of the network control different structural, signaling and remodeling components of the extracellular matrix (ECM). In liver, ECM components are mainly produced by hepatic stellate cells (HSCs) upon their activation through chronic liver injury.

We further studied selected miRNA:mRNA interactions of the network in the experimental fibrosis model of primary HSC *in vitro* culture. Additionally, the functionality of predicted miRNA targeting was validated for selected target genes by monitoring of target gene expression upon miRNA mimic transfection using qPCR as well as by a luciferase reporter gene assay. We could further show in primary HSCs that biogenesis of the anti-fibrotic miRNAs was dysregulated at the pri-miRNA level and occurs through transcriptional control of pri-miRNA synthesis.

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**Non-essential amino acid-specific regulation of Sestrin2 in various cancer cell lines**

Authors:
**B. Blagojevic[1], S. Wölfl[1]**

[1] Institute for Pharmacy and Molecular Biotechnology, Heidelberg, Germany
Email: blagojevic@stud.uni-heidelberg.de

Due to the emerging role of mTOR in regulation of protein synthesis and autophagy, understanding of its regulation is gaining a crucial role in rational anti-cancer therapy, as well as in anti-aging, neuromodulating, and anti-diabetic therapy. Here we investigated the regulation of Sestrin2, as a negative regulator of mTORC1, in cancer cell lines upon non-essential amino acid deprivations (NEAAD).

We observed amino acid-specific upregulation of Sestrin2 protein level after 48h of deprivation that was in an inverse correlation with cellular survival under the same condition. We postulated that there is common metabolic outcome that is driving Sestrin2 upregulation under NEAAD. Firstly, we excluded ROS as a potential cause for Sestrin2 regulation, but further experiments are needed to investigate potential role of NAD(P)H and/or the metabolism of other amino acids (for example: branch-chain amino acid). Additionally, we artificially over-expressed Sestrin2 and characterized cellular phenotypes in order to investigate isolated effects of Sestrin2 upregulation and to identify the cellular urge to upregulate Sestrin2 under NEAAD.

Finally, identifying a common inducer of Sestrin2 protein levels might help us overcome metabolic differences between cancer cell lines and draw a better strategy to treat age-related diseases, such as cancer, by regulating nutrient availability and targeting regulators of cellular metabolic response with Sestrin2 as a potential target.

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**DHEA Treatment Impairs Growth in Colorectal Cancer Cells Through G6PD/PKM2 Mediated Metabolic Reprograming**

Authors:
**F. Almouhanna[1], B. Blagojevic[1], S. Wölfl[1]**

[1] Institute for Pharmacy and Molecular Biotechnology, Heidelberg, Germany
Email: blagojevic@stud.uni-heidelberg.de

Dehydroepiandrosteron (DHEA) is revisited for its anti-ageing and anti-cancer properties. Many reports showed the efficacy of DHEA in inhibiting G6PD and thus impairing cancer proliferation. In this study, we assessed the efficacy of DHEA in colorectal cancer cells with different p53 status. The tumor suppressor p53 has new emerging roles as a metabolic master regulator, in this study we show that DHEA inhibited G6PD and proliferation in CRC cells in the presence and absence of wild type p53. On the other hand, DHEA induced induction in autophagy depicted by a higher conversion to LC3BII in p53+ cells. Furthermore, DHEA reprogrammed the aberrant metabolic phenotypes in CRC cells leading to ROS-dependent induction in G6PD expression (more pronounced in p53+ cells) and inhibition in pyruvate kinase (PK) activity. We further exploited the possible therapeutic window through combining DHEA with an activator of PKM2 (the major cancerous isozyme of pyruvate kinase), and our data show an elevated ROS accumulation and enhanced anti-cancer effects. This DHEA mediated G6PD/PKM2 cross-talk is more pronounced in cancer cells as DHEA treatment has very minor effects on the proliferation of human foreskin fibroblast (HFF) cells. Finally, DHEA treatment lead to major metabolic/signaling pathways changes in CRC cells (p53-dependent and p53-

independent) suggesting it could be used in cancer therapy and adjuvant therapy targeting G6PD/PKM2 axis.

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**Dysregulated spatial control of autophagy is a disease mechanism of AP-4 deficiency syndrome, a severe neurodegenerative disorder**

Authors:
**A. K. Davies[1, 2], D. N. Itzhak[2], J. R. Edgar[1], M. S. Robinson[1], G. H. H. Borner[2]**

[1] University of Cambridge, Cambridge Institute for Medical Research, Cambridge, United Kingdom
[2] Max Planck Institute of Biochemistry, Martinsried, Germany
Email: borner@biochem.mpg.de

We recently developed Dynamic Organellar Maps, a mass spectrometry based method for determining the subcellular localizations of thousands of proteins in a single experiment. We have now applied this approach to unravel the molecular mechanism underlying AP-4 deficiency syndrome, a severe neurological disorder characterized by intellectual disability, neuronal degeneration and progressive paraplegia. AP-4 is a heterotetrameric complex proposed to mediate vesicular protein transport. Neither the function nor the cargo of AP-4 vesicles are known. Application of Dynamic Organellar Maps to AP-4 deficient cells revealed that the correct subcellular localization of ATG9A, the only integral membrane protein of the core autophagy machinery, requires AP-4. Using orthogonal proteomic methods, electron microscopy and imaging of AP-4 patient cells, we demonstrated that AP-4 mediates TGN export and vesicular transport of ATG9A, to regulate autophagosome biogenesis. AP-4 derived vesicles thus likely correspond to the previously described ‘ATG9 reservoir’. Furthermore, our data suggest that in neurons, AP-4 vesicles drive transport of ATG9A along the axon to initiate autophagosome formation at the distal end, which is critical for neuronal homeostasis. We therefore propose dysregulated spatial control of autophagy as a cause of AP-4 deficiency syndrome. This study highlights the power of our unbiased organellar mapping approach for dissecting cellular defects in neurological disorders.

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**Impact of altered chloride transport on lysosomal morphology and function**

Authors:
**S. Bose[1], T. Stauber[1]**

[1] Freie Universitaet Berlin, Department of Biology, Chemistry, Pharmacy, Berlin, Berlin, Germany
Email: shroddha@zedat.fu-berlin.de

The ubiquitously expressed Cl-/H+ exchanger ClC-7 localizes with its β-subunit Ostm1 to lysosomes and to the ruffled border of osteoclasts. Dysfunction of ClC-7/Ostm1 leads to osteopetrosis and a lysosomal storage disease with neurodegeneration in humans and mice. Osteopetrosis might be due to an underdevelopment of the ruffled border of osteoclasts, which is normally formed by massive lysosomal exocytosis, and an impaired acidification of the resorption lacuna. The lysosomal pathology involves a slowed degradation of endocytosed protein in renal proximal tubules and an alteration of lysosomal morphology in various tissues. In contrast to the acidification of the resorption lacuna, acidification of lysosomes is unaffected by the loss of ClC-7/Ostm1. The physiological role of ClC-7/Ostm1 requires the pH gradient-driven luminal accumulation of chloride mediated through its Cl-/H+ exchange activity, but it remained elusive as to how this affects lysosomal morphology and function. Here we have tried to

understand how altered chloride transport impinges on the morphology of lysosomes, on various vesicular trafficking steps in the degradative pathway and on signaling from lysosomes.

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**Research Unit FOR2625 "Mechanisms of Lysosomal Homeostasis"**

Authors:
**T. Braulke**[1]

[1] University Medical Center Hamburg-Eppendorf, Institute of Osteology and Biomechanics - Cell Biology of Rare Diseases, Hamburg, Hamburg, Germany
Email: braulke@uke.de

The DFG-funded research unit FOR2625 “Mechanisms of Lysosomal Homeostasis” is an interdisciplinary network consisting of 9 research groups located in Germany and one in the Netherlands. The consortium aims at elucidating the molecular mechanisms of maintaining lysosomal homeostasis and the adaptive regulatory mechanisms to compensate lysosomal dysfunctions. The FOR2625 research program focusses on three key objectives: (i) Impact of phosphatidylinositides at the lysosomal surface and their interacting proteins on modulation of autophagy, autophagosome-lysosome reformation, lysosomal signaling, lysosome positioning, and exocytosis, (ii) identifying novel components of the molecular sorting machinery and elucidating the impact of ubiquitination and ion transport across membranes on the LAMP and mannose 6-phosphate receptor pathway as well as on the turnover of lysosomal proteins, and (iii) the role of posttranslational modifications of transcription factors EB and E3 on the lysosomal proteome, and lysosome-nucleus signaling processes initiated by dysfunctional lysosomes and other signaling pathways. The research of the FOR2625 will help to understand the molecular mechanisms underlying lysosomal biogenesis, function and turnover which is a prerequisite to examine pathomechanisms of related lysosomal disorders and to develop long-term preclinical therapeutic strategies.

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**Systematic elucidation of the yeast interactome of human disease-associated proteins**

Authors:
**J. Schramm**[2], **C. Lucini**[1], **M. Tereshchenko**[2], **R. J. Braun**[1]

[1] Danube Private University (DPU), Division of Medicine, Krems, Niederösterreich, Austria
[2] University of Bayreuth, Institute of Cell Biology, Bayreuth, Bavaria, Germany
Email: ralf.braun@dp-uni.ac.at

Neurodegenerative disorders, such as Alzheimer, Parkinson, and motor neuron diseases, are characterized by the accumulation, mislocalization and aggregation of disease-associated proteins (*e.g.*, Tau, Amyloid-β, the frameshift ubiquitin variant $UBB^{+1}$, α-synuclein, 43 kDa TAR-DNA binding protein). These proteins are considered to critically contribute to the progression of these fatal diseases, although the underlying molecular mechanisms are poorly understood. The baker’s yeast *Saccharomyces cerevisiae* is an established model for dissecting evolutionary conserved mechanisms in eukaryotic cells expressing human disease-associated proteins. This comprises the roles of protein folding, aggregation, and degradation. The power of yeast can be seen when combining genome-wide genetic screens with large-scale transcriptomic, proteomic or metabolomic analyses. So far, genetic screens in yeast are based on the analyses of cytotoxicity, in most cases by measuring growth on agar plates, in the presence or absence of disease-associated proteins. Here, we provide a complementary approach using protein complementation of DHFR fragments fused to yeast and human proteins, respectively. This allows the systematic and

genome-wide identification of yeast proteins interacting with human disease-associated proteins. This approach may facilitate the identification of molecular pathways, which are affected by the disease-associated proteins, or which prevent or execute their cytotoxicity.

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**Leibniz Institute on Aging – Fritz Lipmann Institute (FLI)**

Authors:
**W. Briest[1], A. Nordheim[2]**

[1] Leibniz Institute on Aging - Fritz Lipmann Institute, Research Coordinator, Jena, Germany
[2] Leibniz Institute on Aging - Fritz Lipmann Institute, Scientific Director, Jena, Germany
Email: Wilfried.briest@leibniz-fli.de

Research at the Leibniz Institute on Aging – Fritz Lipmann Institute (FLI) exhibits a dedicated focus on aging biology. At the time of its conception in 2004, the FLI was the first scientific institution in Germany to focus exclusively on aging research. It is the mission of the Institute to contribute to a basic understanding of molecular and cellular mechanisms that underlie the aging of organisms. This biological insight is to be used to understand and combat aging-associated diseases and thereby – ultimately – contribute to a longer health span for human beings. Toward these aims, research at FLI concentrates on mechanisms of stem cell function, organ maintenance and altered regenerative mechanisms during aging. Furthermore, genetic, epigenetic and molecular mechanisms causally influencing the aging process are being actively investigated. FLI assumes an internationally leading position in stem cell biology, including hematopoietic and intestinal stem cell functions. The Institute’s strong emphasis on genetic model organisms allows FLI researchers to link genotype-phenotype analyses of unique transgenic mouse lines with associated epigenetic contributions to the aging process. The genetic model system of the short-lived killifish *Nothobranchius furzeri*takes singular prominence in FLI research, which – in conjunction with the above-mentioned research directions – sets FLI apart from similarly focused institutions world-wide.

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**pH-Lemon, a fluorescent protein-based pH reporter for acidic compartments**

Authors:
**S. Burgstaller[1], H. Bischof[1], T. Gensch[2], S. Stryeck[1], B. Gottschalk[1], J. Ramadani-Muja[1], E. Eroglu[1], R. Rost[1], S. Balfanz[2], A. Baumann[2], M. Waldeck-Weiermair[1], J. C. Hay[1, 3], T. Madl[1, 4], W. F. Graier[1, 4], R. Malli[1, 4]**

[1] Medical University of Graz , Gottfried Schatz Research Center, Molecular Biology and Biochemistry, Graz, Steiermark, Austria
[2] Forschungszentrum Jülich, Institute of Complex Systems, Zelluläre Biophysik (ICS-4), Jülich, North Rhine-Westphalia, Germany
[3] University of Montana, Division of Biological Sciences and Center for Structural and Functional Neuroscience, Missoula , Montana, United States of America
[4] BioTechMed Graz, Graz, Steiermark, Austria
Email: sandra.burgstaller@medunigraz.at

To guarantee the functionality of the metabolic and signalling network within a cell, pH values are tightly controlled. Especially small vesicular compartments like lysosomes or endosomes require highly acidic pH values for maturation and/or degradation processes. Considering the importance of acidic lysosomes

in autophagy, it is not surprising that alterations of vesicular pH may lead to severe consequences. Therefore, determination and visualization of pH dynamics and the subsequent effects are of great importance to improve our understanding of cell metabolism. To overcome the limitations of imaging acidic compartments, the genetically encoded, fluorescent biosensor pH-Lemon, consisting of a pH stable and a pH sensitive fluorescent protein, was generated. Since pH-Lemon is designed to function as a ratiometric sensor, it provides an excellent approach for the precise determination of pH in vesicular structures. pH alterations can be tracked in a real-time manner with high spatial and temporal resolution using different state-of-the-art imaging techniques. Since pH-Lemon is perfectly suitable to visualise pH alterations in the neutral to acidic range, targeting of pH-Lemon to cellular vesicles by fusion to LC3 or a Glycosylphosphatidylinositol-anchor represents a valuable tool to monitor autophagy and secretory events. Using pH-Lemon in combination with other sensors will fundamentally improve our understanding of intracellular signalling events on the vesicular level.

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**Neutral sphingomyelinases in the interplay between autophagy and EV secretion**

Authors:
**D. Choezom[1], J. Gross[1]**

[1] University Medical Center Göttingen , Developmental Biochemistry and Haematology/Oncology, Goettingen, Lower Saxony, Germany
Email: dolmatibetchoezom@gmail.com

Two major trafficking processes - autophagy and EV secretion - use transport vesicles to shuttle cargoes targeted for extracellular secretion and lysosomal degradation respectively. These two processes are deregulated in cancer and contribute to cancer initiation, metastasis and therapeutic resistence.

Interestingly, these two processes converge in the endolysosomal pathway: autophagosomes fuse with the lysosome for content degradation whereas a distinct subpopulation of mature endosomes (MVBs) are sorted for plasma membrane fusion to secrete exosomes.

Studies have revealed a shared molecular machinery between exosome biogenesis and autophagy, but it is not yet understood whether a crosstalk exists between these two processes and how it could be regulated. For example, neutral sphingomyelinases are implicated in both EV biogenesis and autophagy.

This study aims to understand how these two processes coordinate cargo shuttling for cellular adaption under different conditions and to investigate the mechanistic details underlying this connection. Preliminary results showed that while impairing autophagy by lysosomal inhibition increases EV secretion, autophagy induction also increases EV secretion. Interestingly, lysosomal inhibition led to LC3B secretion on EVs. The secretion of autophagy markers on EVs might serve as prognostic markers to evaluate therapeutic responses since lysosomal targeting agents are used for cancer therapy.

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# Abstracts - Poster

**Sigma-1 Receptor Activation Induces Autophagy In Vitro and In Vivo: Implications for Neurodegeneration**

Authors:
**M. G. Christ[1], H. Huesmann[1], H. Nagel[1], A. Kern[1], C. Behl[1]**

[1] University Medical Center of the Johannes Gutenberg University Mainz, Institute ofPathobiochemistry, Mainz, Rhineland-Palatinate, Germany
Email: maxchris@uni-mainz.de

Dysfunction of autophagy and disturbed protein homeostasis are linked to the pathogenesis of human neurodegenerative diseases and the modulation of autophagy as the protein clearance process has become one key pharmacological target. Due to the role of sigma-1 receptors (Sig-1R) in learning and memory, and its described pleiotropic neuroprotective effects, Sig-1R activation is recognized as one potential approach for prevention and therapy of neurodegeneration and in ALS associated with mutated Sig-1R, autophagy is disturbed. Here we analyzed the effects of Sig-1R activation by two Sig-1R agonists (PRE-084 and ANAVEX2-73), on autophagy and proteostasis. We describe, at the molecular level, for the first time, that pharmacological Sig-1R activation a) enhances the autophagic flux in human cells and in *Caenorhabditis elegans* and b) increases proteostasis capacity, ameliorating paralysis caused by protein aggregation in *C. elegans*. ANAVEX2-73 is already in clinical investigation for the treatment of AD, and its novel activities on autophagy and proteostasis described here may have consequences for the use and further development of the Sig-1R as a drug target in the future. Our findings on the direct impact of Sig-1R activation on proteostasis maintenance underlines Sig-1R as pharmaceutical target for different neurodegenerative disorders linked to protein aggregation and may fuel detailed studies on the molecular mechanisms of autophagy modulation by Sig-1R activation.

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**Autophagosomes are shaped in a confined space between the vacuole and the ER**

Authors:
**D. Hollenstein[1], R. Gómez-Sánchez[2], A. Ciftci[3, 4], F. Kriegenburg[3], M. Mari[2], R. Torggler[3, 4], M. Licheva[3, 4], F. Reggiori[2], C. Kraft[3, 5]**

[1] Vienna Biocenter, Max F. Perutz Laboratories, Vienna, Austria
[2] University of Groningen, University Medical Center Groningen, Department of Biomedical Sciences of Cells & Systems, Groningen, Germany
[3] University of Freiburg, Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, Freiburg, Germany
[4] University of Freiburg, Faculty of Biology, Freiburg, Germany
[5] University of Freiburg, CIBSS - Centre for Integrative Biological Signalling Studies, Freiburg, Germany
Email: akif.ciftci@biochemie.uni-freiburg.de

Autophagy is initiated by the formation of phagophore assembly sites (PAS), the precursors of autophagosomes. In mammals, PAS form throughout the cytosol in specialized subdomains of the endoplasmic reticulum (ER). In yeast, PAS also form close to the ER, but always in the vicinity of the vacuole. How the PAS is anchored to the vacuole and the functional significance of this localization are unknown. Here, we investigated the role of the PAS-vacuole connection for bulk autophagy in yeast. We identified a vacuolar tether that stably anchors the PAS to the vacuole throughout autophagosome biogenesis. *S.cerevisiae* lacking the tether show inefficient autophagosome-vacuole fusion, and form fewer and smaller autophagosomes that often localize away from the vacuole. Thus, for efficient bulk autophagy a stable ER-

PAS-vacuole connection is needed to create a confined space for autophagosome biogenesis. This allows spatial coordination of autophagosome formation and subsequent autophagosome-vacuole fusion.

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**Size of portally deprived liver lobe after portal vein ligation and additional partial hepatectomy: Result of balancing proliferation and apoptosis**

Authors:
**W. Wei[1], C. Hua[1], T. Zhang[2], O. Dirsch[3], F. Gremse[4], A. Homeyer[5], U. Settmacher[1], U. Dahmen[1]**

[1] Jena University Hospital, Department of General, Visceral and Vascular Surgery, Jena, Thuringia, Germany
[2] Jena University Hospital, Department of Radiotherapy and Radiooncology, Jena, Thuringia, Germany
[3] Klinikum Chemnitz gGmbH, Institute of Pathology, Chemnitz, Saxony, Germany
[4] RWTH Aachen University, Department of Experimental Molecular Imaging, Aachen, North Rhine-Westphalia, Germany
[5] Fraunhofer Institute for Medical Image Computing MEVIS, Bremen, Bremen, Germany
Email: abstract@eventclass.com

Elderly patients have a main propotion in patients who undergo hepatic resection for surgical treatment. The liver has the ability to maintain its total size by adjusting the size of the individual liver lobes differently in response to regeneration and atrophy stimuli. Portal vein ligation (PVL) drives the ligated lobe to undergo atrophy whereas partial hepatectomy (PHx) drives the total remnant liver to regenerate. We hypothesize that the size of the PVL-lobe is dependent on the balance between the extent of PVL and the extent of PHx inducing a complex interplay between hepatocyte proliferation, apoptosis and autophagy. Lewis-rats were subjected to either 20%PVL+70%PHx or 70%PVL+20%PHx. Control groups consisted of 20%PVL and 70%PVL. Liver lobe weight, BrdU-proliferation index, proliferating cell nuclear antigen mRNA expression level, apoptotic density and autophagy related protein were investigated. The PVL-liver lobe adjusted its weight differently, increasing by 40% after 20%PVL+70%PHx, but decreasing by 25% after 70%PVL+20%PHx. Additional resection induced a size-dependent low, but substantial hepatocyte proliferation rate (maximal 6.3% and 3.6% vs. 0.3% and significantly suppressed apoptotic density in the deportalized-liver-lobe (3 and 14 cells/mm2 comparing with above 26cells/mm2, p<0.01 ). Induction of autophagy was 2 times higher after 20%PVL+70%PHx compared to 70%PVL+20%PHx. Atrophy of the PVL-liver lobe after simultaneous PHx was counteracted by promoting hepatocyte proliferation, inducing autophagy and suppressing apoptosis in a size-dependent manner.

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***Macroautophagy-independent lysosomal degradation protects against protein aggregation in a tissue-specific manner***

Authors:
**R. Jung[1], M. C. Lechler[1], C. Rödelsperger[2], W. Rösseler[2], R. Sommer[2], D. C. David[1]**

[1] German Center for Neurodegenerative Diseases (DZNE), Tübingen, Baden-Württemberg, Germany
[2] Max Planck Institute for Developmental Biology, Department for Integrative Evolutionary Biology, Tübingen, Baden-Württemberg, Germany
Email: dellacdavid@gmail.com

The formation of insoluble protein aggregates is a pathological event associated with many neurodegenerative disorders. Accruing evidence demonstrates that protein aggregation is not restricted to disease processes and also occurs during normal aging affecting several hundred proteins. Until now, little

is known concerning the tissue-specific mechanisms controlling age-dependent protein aggregation. In the present study, we investigated age-dependent protein aggregation during impairment of macroautophagy in two different muscle cell types in *C. elegans*: non-striated muscles in the pharynx and striated body-wall muscles. Surprisingly, we found a striking reduction in protein aggregation in the pharyngeal muscles upon macroautophagy impairment. This unexpected response was tissue-specific as impaired macroautophagy accelerated protein aggregation in the body-wall muscles. We provide evidence for a novel safeguard against protein aggregation (SAPA) triggered by protein-quality control failure that targets specifically newly synthesized aggregation-prone proteins in the pharyngeal muscle cells. We show that SAPA relies on macroautophagy-independent lysosomal degradation and involves components normally up-regulated in response to intracellular pathogens affecting the digestive tract.

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**Identification and characterization of autophagy-modulating natural compounds for the battle against cancer**

Authors:
**J. Deitersen[1], D. Schlütermann[1], M. J. Mendiburo[1], F. Stuhldeier[1], W. Wu[1], N. Berleth[1], S. Ceccacci[3], M. C. Monti[3], P. Proksch[2], B. Stork[1]**

[1] Heinrich-Heine-Universität, Molecular Medicine I, Düsseldorf, North Rhine-Westphalia, Germany
[2] Heinrich-Heine-Universität, Pharmaceutical Biology and Biotechnology, Düsseldorf, North Rhine-Westphalia, Germany
[3] Università degli Studi di Salerno, Pharmacy, Fisciano, Italy
Email: jana.deitersen@hhu.de

While the degradation of malignant factors by macroautophagy can have a tumor-preventing effect in healthy cells, its catabolic function also serves as source of energy especially in starving cells e.g. in hypoxic cancer tissues. Therefore, compounds that modulate autophagy have a huge chemotherapeutic potential. Nature is a nearly inexhaustible source for new compounds and future drugs. Therefore, we screened 300 natural compounds for their effect on autophagy and identified promising autophagy-modulating compounds. Among these, we identified arzanol, a prenylated heterodimeric phloroglucinyl pyrone from *Helichrysum italicum*, which is used in traditional European medicine. Here, we characterized the mode of action of arzanol on autophagy, which seems to involve mitochondrial pathways. Furthermore, we show a sensitization of bladder cancer cells to chemotherapy using a combination therapy of arzanol and cisplatin.

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**Non-redundant roles of GABARAP subfamily members during surface protein trafficking**

Authors:
**J. Dobner[1], I. M. Simons[1], J. L. Sanwald[1], K. Rufinatscha[3], S. Hoffmann[2], J. G. Bode[3], D. Häussinger[3], D. Willbold[1, 2]**

[1] Heinrich Heine University Düsseldorf, Institut für Physikalische Biologie, Düsseldorf, Germany
[2] Forschungszentrum Jülich, Institute of Complex Systems: Strukturbiochemie (ICS-6), Jülich, Germany
[3] University Hopsital Düsseldorf, Clinics for Gastroenterology, Hepatology and Infectious Diseases, Düsseldorf, Germany
Email: j.dobner@fz-juelich.de

GABARAP (γ-aminobutyric acid type A receptor-associated protein), MAP1LC3B (microtubule-associated proteins 1A/1B light chain 3B) and their paralogs constitute the autophagy-related protein 8

(Atg8)-type protein family in humans. They are associated with a variety of dynamic membranous structures of autophagic as well as of non-autophagic origin. Both their exact mechanisms of action and knowledge regarding their functional redundancy are mainly still elusive. To study the role of the individual GABARAP-subfamily members, GABARAP, -like 1 (L1) and -like 2 (L2) in general membrane protein trafficking, we applied respective knockout lines and generated an endogenous fluorescent protein-tagged GABARAP knock-in cell line. A comparative surface proteome analysis between control and HEK293 cells with GABARAP, -L1 and -L2 triple-deficiency revealed distinct changes in the protein composition of their plasma membranes, suggesting a role for at least one GABARAP subfamily member during trafficking of surface associated proteins. In case of epidermal growth factor receptor recent results indicate that GABARAP deficiency accelerates the degradation of the receptor upon stimulation. Thus, GABARAP-type proteins seem to influence crucial aspects of membrane and vesicle dynamics affecting the establishment of cellular communication and identity also at the cell’s surface.

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**Exploring novel functions of endogenous GABARAPL2**

Authors:
**F. Eck[1], M. Kaulich[2], C. Behrends[1]**

[1] Ludwig Maximilians Universität, Synergy, München, Bavaria, Germany
[2] Goethe Universität, Institut für Biochemie II, Frankfurt am Main, Hesse, Germany
Email: Franziska.Eck@mail03.med.uni-muenchen.de

The ubiquitin-like LC3/GABARAP proteins exert their function in a highly localized manner through its reversible conjugation to the phospholipids. While studies of ATG genes in knockout models have led to an explosion of knowledge about the functions of core autophagy components, the exact roles of the human ATG8 family LC3 and GABARAP proteins are still poorly understood. Another major drawback for the understanding of ATG8 family member function is that the extensive available interactome data of LC3 and GABARAP proteins was almost exclusively acquired using overexpression systems. Since constitutive or inducible overexpression of bait proteins commonly lead to higher rates of false-positive and negative interactions, we currently do not know the extend and dynamics of interactions in which LC3 and GABARAP proteins are endogenously engaged. To overcome these limitations, we employed CRISPR/Cas9-based genome-editing to generate a panel of cells, in which human *ATG8* genes are seamlessly tagged at their natural chromosomal locations with an N-terminal affinity epitope. This cellular resource was used to map physiologically regulated endogenous ATG8 protein complexes and to determine their dynamics in response to autophagic pathway modulation using immunoprecipitation coupled to mass spectrometry. This approach identified a new connection between GABARAPL2, ufmylation and lipid droplets.

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**Regulation of mitochondrial integrity by Rab GTPase mediated control of mitophagy**

Authors:
**S. Eimer[1], R. Haefner[1]**

[1] Goethe University Frankfurt a.M., Structural Cell Biology, Frankfurt a.M., Germany
Email: eimer@bio.uni-frankfurt.de

Small GTPases of the Rab family are molecular switches acting as master-regulators of intracellular trafficking and sorting events between different membrane domains. Through genetic screens in the nematode *Caenorhabditis elegans* we have identified the Rab GTPase GLO-1, the ortholog of human

Parkinson’s disease associated Rab7L1, to be essential for mitochondrial integrity and function. GLO-1 localizes to the trans Golgi network (TGN) and specifies a trafficking pathway required for the biogenesis of lysosome related organelles in *C. elegans*. We could show that *glo-1* mutants have functionally impaired and fragmented mitochondria with mitochondrial derived vesicles budding off. A detailed electron microscopic analysis revealed also the accumulation of late stage matured mitophagosomes in *glo-1* mutants, suggesting defects in mitochondrial turnover via mitophygy. In line with this, we also identified a novel mitophagy adaptor ALLO-2 as interaction partner for GLO-1. Through its interactions with the *C. elegans* LC3/ATG8 orthologs LGG-1 and LGG-2, ALLO-2 directs GLO-1 to mature mitophagosomes, therefore enabling fusion with lysosomal compartments. Similarly, we can show that human Rab7L1 is also required for efficient turnover of damaged mitochondria via mitophagy, which might be relevant for our understanding of the molecular mechanisms leading to Parkinson’s disease.  

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**Aging-associated inactivation of adaptive stress responses including autophagy limits longevity benefits of the anti-aging drug metformin**

Authors:
**L. Espada[1], A. Dakhovnik[1], P. Chaudhari[1], A. Martirosyan[1], T. Poliezhaieva[1], L. Miek[2], Y. Schaub[1], A. Nair[1], N. Döring[1], N. Rahnis[1], A. Koeberle[2], J. Kirkpatrick[1], A. Ori[1], M. A. Ermolaeva[1]**

[1] Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Thuringia, Germany
[2] Friedrich Schiller University Jena, Institute of Pharmacy, Jena, Thuringia, Germany
Email: lilia.espada@leibniz-fli.de

The type 2 diabetes drug metformin is in clinical trial to extend health span in elderly humans. Metformin promotes longevity when given from young adult or middle age and doesn’t cause detrimental effects in elderly diabetes patients, but little is known about responses of non-diabetic old individuals to this drug. Here we used metabolic healthy *C. elegans* and human primary cells and found that metformin treatment initiated in late life shortens life span and limits cell survival, contrary to its early life effects. Mechanistically, we found that late life metformin exposure aggravates aging-associated mitochondrial dysfunction and causes exhaustion of cellular ATP content till levels incompatible with cell viability. Additional analysis revealed rapid induction of adaptive stress responses such as oxidative stress response and autophagy in young but not old metformin treated animals. In line with the key role of early life stress adaptations in longevity extension by metformin, drug treatment during young adulthood was sufficient to prolong life span of animals. Notably, ATP repletion by rapamycin co-treatment rescued late life metformin toxicity but didn’t fully restore metformin longevity benefits at old age, suggesting a limiting role of aging-altered stress responses in life extension by metformin. Collectively, we uncovered important aging-associated cellular and molecular changes, which may abrogate health benefits of metformin for non-diabetic elderly individuals.

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**$NAD^+$ and Mitophagy attenuate cognitive decline in Alzheimer's disease**

Authors:
**E. F. Fang[1]**

[1] University of Oslo, Oslo, Norway
Email: e.f.fang@medisin.uio.no

**Background:** Accumulation of damaged mitochondria is a hallmark of human aging and age-related

neurodegenerative pathologies, including Alzheimer’s disease (AD). Mitophagy is a cellular process mediating selective clearance of dysfunctional mitochondria. However, the molecular mechanisms of the impaired mitochondrial homeostasis, including the status of mitophagy, and their relationship to AD are still elusive.

**Methods:** A cross-species study system, including the use of AD worms, AD mice, and AD patient iPSC-derived neurons, was used to characterize the status of mitophagy and the molecular mechanisms.

**Results:** Mitophagy is impaired in AD patient hippocampus, in iPSC-derived human neurons and in animal AD models. In *C. elegans* models of AD, pharmacological stimulation of mitophagy reverses memory impairment through a PINK-1, PDR-1 and DCT-1 dependent pathway. Mitophagy induction diminishes the levels of insoluble amyloid-β (Aβ)$_{1-42}$ and Aβ$_{1-40}$ peptide isoforms and prevents cognitive impairment in AD mice by a mechanism involving microglial phagocytosis of extracellular Aβ plaques and suppression of neuroinflammation. Furthermore, mitophagy abolishes AD-related Tau hyperphosphorylation in human neuronal cells and reverses memory impairment in transgenic Tau nematodes.

**Conclusions:** Impaired removal of defective mitochondria is a pivotal event in AD pathogenesis. Interventions that stimulate mitophagy therefore have therapeutic potential in the prevention and treatment of AD.

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**TSC1 is an oligomeric scaffold for TSC complex assembly and membrane recruitment**

Authors:
**K. Fitzian[1], R. Zech[1], C. Antoni[2], S. Raunser[2], D. Kümmel[1]**

[1] Westfälische Wilhelms-Universität Münster, Institut für Biochemie, Münster, Germany
[2] Max-Planck-Institut für molekulare Physiologie, Dortmund, Germany
Email: katharina.fitzian@wwu.de

The small GTPase Rheb is a crucial regulator of the mechanistic target of rapamycin complex 1 (mTORC1) in growth factor signaling. mTORC1 acts as master regulator of cellular growth by activation of transcription factors and inhibition of autophagy. Rheb in its GTP-bound form activates mTORC1 at the lysosomal surface and is negatively regulated by its corresponding GTPase activating protein (GAP) that mediates GTP hydrolysis and therefore inactivates it. The GAP for Rheb is the Tuberous sclerosis complex (TSC) complex consisting of the three subunits TSC1, TBC1D7 and TSC2. So far, the TSC complex is the only known regulator of Rheb and plays an important role as a tumor suppressor in mTORC1 signaling.

TSC2 is responsible for GAP function, whereas TSC1 acts as a scaffold for TSC complex assembly. We have characterized the structure of TSC1 and found that TSC1 forms dimers, hexamers and dodecamers. The dimer shows N-terminal globular head domains that are linked via C-terminal coiled-coil domains. A central helical domain is necessary for the formation of oligomers that likely represents the functional state observed in cells. Furthermore, this part is sufficient to mediate the interaction with TSC2. We also find that TSC1 can bind to membranes in a phosphatidylinositol-3,5- and -3,4-bisphosphate-dependant manner. Thus we describe the first molecular function of TSC1 and suggest that it might play an important role in localization and regulation of activity of the TSC complex.

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**Autophagy induction reduces PolyI:C driven neuroinflammation**

Authors:
**K. Freitag[1, 2], L. Fleck[1], M. Jendrach[1], F. L. Heppner[1, 2]**

[1] Charité-Universitätsmedizin Berlin, Neuropathology, Berlin, Berlin, Germany
[2] DZNE, Berlin, Berlin, Germany
Email: kiara.freitag@charite.de

Alzheimer´s disease (AD) is the most common cause of dementia, affecting people worldwide. Although neuroinflammation has long been known to contribute to the progression of AD, it is nowadays considered as a driver of the onset of AD[1]. Systemic immune challenges through the viral mimic polyriboin osinic:polyribocytidilic acid (Poly I:C) at embryonal day 17 (E17) have been shown to induce an AD-like pathology including microglia and astrocyte activation in wild type mice[2]. One mechanism by which neuroinflammation can be regulated is autophagy. Recently, we showed that Beclin1 driven autophagy modulates the inflammatory response of microglia via NLRP3[3].

Currently, we investigate whether PolyI:C driven neuroinflammation can be tackled by inducing autophagy via a dietary supplement.

PolyI:C treatment of neonatal microglia and astrocytes *in vitro* induced the release of the proinflammatory cytokines IL-6 and TNF-α, while not affecting IL-1β. Interestingly, the PolyI:C induced release of IL-6 and TNF-α was significantly reduced by treatment with the autophagy inducer indicating that autophagy induction can counteract PolyI:C driven neuroinflammation.

 

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**Rac1 but not Rac1b induces epithelial to mesenchymal transition and promotes invasion of lung adenocarcinoma cell lines**

Authors:
**K. Giehl[1], J. Seitz[2], J. Klinke[1], A. Menke[2]**

[1] Justus-Liebig-Universität, Signaltransduktion Zellulärer Motilität, Gießen, Germany
[2] Justus-Liebig-Universität, Molekulare Onkologie Solider Tumore, Gießen, Germany
Email: Klaudia.Giehl@inneremed.jlug.de

Rac1 is an ubiquitously expressed member of the Rho GTPase family and an important regulator of the actin cytoskeleton. Its splice variant Rac1b includes a 19 aa in-frame insertion and is predominantly active. Both proteins have been described in tumorigenesis or tumor metastasis. Here, we asked if Rac1 and Rac1b are involved in tumor progression of non-small cell lung adenocarcinoma (NSCLA).

Rac1 was present in all analyzed NSCLA cell lines, whereas Rac1b was expressed in only 7.
Ectopically expressed EGFP-Rac1b exhibited a much higher activity as EGFP-Rac1. Using the *in*

*vivo *chorioallantois invasion model, EGFP-Rac1-expressing cells formed more aggressive tumors compared to EGFP-Rac1b or control cells. In wound healing assays, EGFP-Rac1 cells showed a slightly decreased migration compared to EGFP-Rac1b cells. This correlated with enhanced activity of the MAPK p38, the PI3K/AKT pathway and GSK3 and enhanced Rac1-induced transcriptional activity of several gene promoter reporter. In contrast, EGFP-Rac1b induced phosphorylation of JNK-2 and activated TCF/LEF1 and NFκB-responsive reporter. Knock-down of the splicing factor ESRP1, which is responsible for out-splicing of the exon 3b from *RAC1, *resulted in increased Rac1b mRNA and suppression of the epithelial-mesenchymal transition-associated transcription factor ZEB1.

Our data support an important role for Rac1 in lung cancer metastasis and point to different signaling and functional activities of Rac1 and Rac1b in lung cancer.

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**LRRK2 a complex Protein involved in Parkinson´s disease: Insights from Biochemical and Structural Analysis**

Authors:
**B. K. Gilsbach[1], G. Guaitoli[1], F. von Zweydorf[1], C. J. Gloeckner[1]**

[1] DZNE Tübingen, AG-Gloeckner, Tübingen, Baden-Württemberg, Germany
Email: bernd.gilsbach@dzne.de

Human leucine-rich-repeat kinase 2 (LRRK2) belongs to the Roco family, a unique group of G-proteins, and has been found to be thus far the most frequent cause of late-onset familial and idiopathic forms Parkinson’s disease (PD). LRRK2 is a 2527 aa multi-domain protein that consists of four protein-protein interaction domains and two catalytic domains: a G- as well as a kinase domain. The excat role of LRRK2 in cell signaling is still elusive but there is accumulating evidence that LRRK2 plays a role in vesicle traffincing and in the regulation of autophagy.

Studing homologues proteins from bacteria and the slime mold Dictyostelium discoideum led to insight into the regulation of Roco proteins, i.e explaning mechanism how the most prevalent LRRK2 mutation G2019S leads to an increased LRRK2 activity. Our structural and biochemical analyses revealed that LRRK2 belongs to class of G-proteins activated by dimerization (GADs) and that COR is the dimerization domain of LRRK2. Now we are analyzing LRRK2 regulation in greater detail, therefore we have established HPLC aided kinase and GTPase assays allowing us to measure activity at physiological nucleotide concentration. So far, there is no established rational therapy for PD. Given its prevalence in cases of PD LRRK2 represents a clear molecular target for therapeutic development. A deeper understanding of LRRK2 regulation will allow us  to find new ways to target LRRK2 induced PD.

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**Fatty acid channeling drives autophagic membrane formation at phagophore-ER contacts during autophagy**

Authors:
**M. Schütter[1], P. Giavalisco[1], S. Brodesser[2], M. Graef[1]**

[1] Max Planck Institute for Biology of Ageing, MPRG Graef, Cologne, North Rhine-Westphalia, Germany
[2] University of Cologne, Lipidomics Facility, Cologne, North Rhine-Westphalia, Germany
Email: Martin.graef@age.mpg.de

# Abstracts - Poster

Autophagy is a highly conserved homeostasis and quality control process essential to cellular health. A key feature of (macro)autophagy is the formation of transient double-membrane compartments, termed autophagosomes (AP). AP biogenesis is initiated by the nucleation of small single-membrane phagophores, which rapidly expand in a cup-shaped manner and encapsulate cytoplasmic cargoes upon closure into large double-membrane AP. The mechanisms of autophagic membrane generation during phagophore expansion remain an unanswered central question in cell biology. Here we describe our discovery that conserved acyl-CoA synthetases (ACS) localize to nucleated phagophores and mediate the local activation of free fatty acids (FA) to specifically drive the growth of autophagic membranes during phagophore expansion. A combination of stable isotope tracing and biochemical approaches provide the first description of the phospholipid (PL) composition of autophagic membranes and reveal ACS-dependent incorporation of newly synthesized PL into growing phagophores. Moreover, we demonstrate that de novo PL synthesis at the ER, which is physically tethered to phagophores, is required for autophagy. In summary, our data suggest a new model for autophagosome biogenesis, in which phagophore-bound ACS channel FA into PL synthesis at phagophore-ER contacts to assemble autophagic membranes during phagophore expansion required for autophagy.

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**What is the impact of mitochondrial stress on the cytosolic proteostasis?**

Authors:
**C. Groh[1], F. Boos[1], J. M. Herrmann[1]**

[1] University of Kaiserslautern, Cell Biology, Kaiserslautern, Rhineland-Palatinate, Germany
Email: cgroh@rhrk.uni-kl.de

The function of the cell depends on properly folded proteins. To maintain the functional activity of the proteins, a complex network of molecular chaperones, the ubiquitin-proteasome system and many cofactors are required. If this orchestra fails and protein folding is impaired, the cell has to deal with many issues. Neurodegenerative diseases or aging are extreme examples of what might happen when protein folding becomes problematic. Recent studies revealed that also mitochondrial problems lead to cytosolic proteotoxic stress.

Until today it remains unclear what the precise effect of different proteotoxic stresses on cellular functionality is. In this context I will study the impact of mitochondrial stress on the cytosolic proteostasis.

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**Autophagy regulation in response to phosphate starvation**

Authors:
**A. S. Gross[1], R. Ghillebert[1], M. Schütter[1], T. Medeiros[1], M. Graef[1]**

[1] Max Planck Society, Insitute for Biology of Ageing- AG Graef , Cologne, North Rhine-Westphalia, Germany
Email: agross@age.mpg.de

Autophagy, a catabolic process primarily induced under starvation conditions, provides molecular building blocks ensuring cellular homeostasis and stress resistance. Inorganic phosphate (Pi) is essential for the biosynthesis of central macromolecules such as DNA, RNA, ATP and lipids. How cells regulate autophagy in response to phosphate shortage is poorly understood. Here, we show that, in contrast to nitrogen starvation, the multicomponent Atg1 kinase complex, an essential autophagy regulator and integrator of nutritional signaling, displays a remarkable structural and functional plasticity during Pi

starvation. Specifically, deletion of components, previously considered to be essential for autophagy, barely affect autophagy during Pi starvation. Nitrogen starvation-induced autophagy is regulated through TORC1 inhibition and subsequent Atg13 dephosphorylation. Upon Pi starvation, Atg13 is only partially dephosphorylated and autophagic turnover only partially depends on TORC1 inhibition. Deletion of Atg11, important in selective autophagy, reduces Pi stasrvation induced autophagic flux, but not the turnover of cytosolic bulk protein. Hence, Pi starvation induces a bipartite autophagy response, composed of a TORC1- and Atg13-dependent nonselective and an Atg11-dependent selective autophagy arm. In summary, our work uncovers significant plasticity in autophagy regulation by the Atg1 kinase complex.

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**mTOR dependent phosphorylation of the SMN complex regulates UsnRNP biogenesis**

Authors:
**M. Schilling[1], A. Prousty[2], U. Fischer[2], O. J. Gruss[1]**

[1] University of Bonn, Department of Genetics, Bonn, Germany
[2] University of Würzburg, Theodor-Boveri-Institute, Biocenter, Würzburg, Germany
Email: ogrusss@uni-bonn.de

The macromolecular machine of the SMN complex mediates assembly of pre-mRNA processing UsnRNPs. Mutations in *SMN* that lead to reduced protein function cause Spinnal Muscular Atrophy (SMA) as the most common monogenetic neuromuscular disorder in humans. The SMN complex undergoes extensive phosphorylations on all its subunits suggesting that reversible phosphorylation governs its subcellular targeting and activity. Here we report the systematic si-RNA based screening of human kinases that potentially modulate the SMN complex. Exploiting the established correlation of SMN accumulation in nuclear Cajal bodies with the activity of the complex in snRNP biogenesis, we identify mTOR and S6 kinase as novel regulators of SMN in human cells. Differential phosphoproteomics in control and S6 kinase knockout cells reveal a specific set of phosphorylations in the SMN complex that depend on active mTOR signalling. We show that preventing phosphorylation in merely two of these sides in SMN’s N-terminus (Serines 49 and 63) leaves the complex intact and biochemically active but dramatically impairs subcellular distribution and local activity in Cajal bodies. In contrast, phosphomimetic mutations in S49 and S63 render the SMN complex active independently of mTOR signalling. Our data explain that and how the SMN complex is integrated into the cellular signalling network and provide the mechanism of regulation, which adjusts UsnRNP production to overall biosynthetic activity of human cells.

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**Impaired selective autophagy in Charcot-Marie-Tooth neuropathy type 4J (CMT4J)**

Authors:
**P. K. Haller[1, 2], S. Schuster[3, 4], K. Sporbeck[1, 2], S. Hauser[5], L. Schöls[3, 5], T. Proikas-Cezanne[1, 2]**

[1] Eberhard Karls University Tübingen, Department of Molecular Biology, Interfaculty Institute of Cell Biology, Tübingen, Baden-Württemberg, Germany
[2] Max Planck Institute for Developmental Biology and Eberhard Karls University Tübingen, International Max Planck Research School ‚From Molecules to Organisms', Tübingen, Baden-Württemberg, Germany
[3] Eberhard Karls University Tübingen, Department of Neurology and Hertie Institute for Clinical Brain Research, Tübingen, Baden-Württemberg, Germany
[4] Eberhard Karls University Tübingen, Graduate School of Cellular and Molecular Neuroscience, Tübingen, Baden-Württemberg, Germany

[5] German Center for Neurodegenerative Diseases (DZNE), Tübingen, Baden-Württemberg, Germany
Email: patricia-katharina.haller@student.uni-tuebingen.de

Lysosomal PI(3,5)$P_2$ levels are controlled by the FIG4 phosphoinositide 5-phosphatase, functioning in complex with PIKFYVE and VAC14. Inherited FIG4 mutations are causative for Charcot-Marie-Tooth neuropathy type 4J (CMT4J), which manifests in progressive muscle weakness due to abnormal lysosomal storage in neurons. We isolated primary human skin fibroblasts from three CMT4J patients, all carrying the founder mutation c.122T>C, p.Ile41Thr and a second compound heterozygous mutation, and reprogrammed those into induced pluripotent stem cells (iPSCs) which expressed pluripotency markers and differentiated into cells of all germ layers *in vitro*. Primary fibroblasts as well as iPSC-derived cortical neurons (iCNs) were subjected to image-based autophagy assessments using the autophagy marker WIPI2, an important PI3P effector functioning in autophagy initiation, and p62, an autophagy receptor for ubiquitinated cargo, functioning in selective autophagy. Significantly less WIPI2-positive autophagosomes were detected in CMT4J patient-derived cells with dysfunctional FIG4, possibly due to a decrease in cellular PI3P levels. Further, less WIPI2-positive autophagosomes were also positive for p62, suggesting that selective cargo degradation may be compromised in CMT4J. These results were also achieved by using FIG4 knockout HAP1 cells. In summary, we provide evidence that compromised function of FIG4 affects selective autophagy, which may contribute to the pathology of CMT4J.

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**PINK1 mRNA is transported with mitochondria and translated locally to support axonal mitophagy**

Authors:
**A. B. Harbauer[1, 2, 3], M. Ordonez[2], Z. Cai[2], G. Ashrafi[2], R. Cartoni[2], Z. He[2], T. L. Schwarz[2, 3]**

[1] Max-Planck-Institute, Neurobiology, Martinsried, Bavaria, Germany
[2] Boston Children's Hospital, F. M. Kirby Center for Neurobiology, Boston, Massachusetts, United States of America
[3] Harvard Medical School, Department of Neurobiology, Boston, Massachusetts, United States of America
Email: Angelika.Harbauer@neuro.mpg.de

A decline in mitochondrial health is a hallmark of many neurodegenerative disorders and aging, and axonal mitochondrial dysfunction often precedes cell death. PTEN-induced kinase 1 (PINK1),  a gene found mutated in hereditary forms of Parkinson’s Disease, has a role in mitophagy, a pathway that removes damaged mitochondria to prevent oxidative damage.

The mechanism of PINK1 activation requires the protein to be constantly imported and degraded as long as mitochondria are healthy. For this mechanism to work in neurons, a sufficient supply of freshly synthesized PINK1 is required regardless of the distance of the mitochondrion from the cell body. The very short half-life of PINK1 in healthy mitochondria would make it impossible for PINK1 protein to survive transport down an axon and to be available for initiating mitophagy. We therefore hypothesized that there may exist a targeted mechanism to provide axonal mitochondria with the required PINK1 supply via transport of PINK1 mRNA and local protein synthesis.

We have identified the neuron-specific co-transport of PINK1 mRNA with mitochondria, a new mechanism of mRNA transport that not only suggests a role for axonal translation in the removal of damaged organelles but also is the first neuron specific mechanism affecting the PINK1/Parkin pathway of mitophagy.

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**AMPK and PLK1 respond to amino acid availability to regulate autophagy.**

Authors:
**A. M. Heberle[1, 2], K. Thedieck[1, 2, 3]**

[1] University of Innsbruck, Institute of Biochemistry and Center for Molecular Biosciences Innsbruck, Innsbruck, Austria
[2] University of Groningen, Laboratory of Pediatrics, Section Systems Medicine of Metabolism and Signaling, Groningen, Netherlands
[3] Carl von Ossietzky University Oldenburg, Department for Neuroscience, School of Medicine and Health Sciences, Oldenburg, Germany
Email: alexander.heberle@uibk.ac.at

The mammalian/ mechanistic target of rapamycin complex 1 (mTORC1) is a metabolic master regulator, which responds to nutrients and growth factors. mTORC1 drives cell growth and proliferation by promoting anabolic processes and inhibiting the catabolic process autophagy.   

Amino acids are building blocks for proteins, but also function as signalling molecules. Little is known about whether amino acids, independently of mTORC1, activate other kinases of the signalling network converging on mTOR. To explore this, we combine computational-experimental approaches with text mining-enhanced quantitative proteomics.

Through such approaches, we identified phosphatidylinositide 3- kinase (PI3K),  mTORC2, and AMP-activated protein kinase (AMPK) as being activated by amino acids. We show that AMPK under amino acids sufficiency acts to sustain autophagy. This may be required to maintain protein homoeostasis and deliver metabolite intermediates for biosynthetic processes [1].

We also identified Polo-like-kinase 1 (PLK1) as a novel mTORC1 inhibitor. Amino acid deprivation enhances PLK1-mTORC1 interaction and thus promotes autophagy [2].

To conclude, we have identified AMPK and PLK1 as novel amino acid responsive cues in the mTOR signalling network, which are required for autophagy regulation.

 

[1] Dalle Pezze et al., A systems study reveals concurrent activation of AMPK and mTOR by amino acids, 2016, Nat Commun

[2] Ruf et al., PLK1 inhibits mTORC1 and promotes autophagy. 2017, Autophagy

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**Effects of the mitochondria-targeting agents 1,10-phenanthroline and VLX600 on tumor cell death in glioblastoma**

Authors:
**L. Henkel[1], B. Linder[1], D. Kögel[1]**

[1] Goethe University Hospital, Experimental Neurosurgery, Frankfurt am Main, Hesse, Germany
Email: lisa.henkel@kgu.de

Glioblastoma (GBM), one of the most aggressive brain tumors, is often resistant to apoptotic cell death and median survival is only 15 months also with standard therapy. Therefore, the development of new

therapy options is of great interest. We focus on the induction of alternative cell death programs, like the autophagic cell death (ACD), as a possible strategy to combat GBM. Besides the starvation-induced non-selective bulk autophagy also selective forms of autophagy like mitophagy and lysophagy were described to possibly leading to cancer cell death. Therefore, we investigated the induction of autophagy, especially mitophagy, in human GBM cell lines as well as glioma stem-like cells (GS). We could show that 1,10-phenanthroline (phen), which was previously described to induce mitophagy in HeLa cells, significantly increases tumor cell death in an *ex vivo* tumor growth model using organotypic brain slices (OTC) from adult mice as well as in *in vitro* cell death experiments. Furthermore, VLX600, that was shown to induce tumor cell death and to decrease mitochondrial oxidative phosphorylation in HCT116 colon cancer cells, induces cell death in GBM cells. To further investigate the mechanisms underlying phen- and VLX600-induced cell death, *ATG5* and *ATG7* knock-out cells and additional genetic depletion models will be used in combination with an array of different techniques, including quantitative assessment of mitochondrial mass and cell death.

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**InterFAST: Alternate Day Fasting in healthy, non-obese humans**

Authors:
**S. J. Hofer[1, 2], S. Stekovic[1], N. Tripolt[3], M. A. Aon[4], P. Royer[1], L. Pein[1], J. T. Stadler[1], T. Pendl[1], B. Prietl[3], J. Url[3], S. Schroeder[1], J. Tadic[1], T. Eisenberg[1], C. Magnes[5], M. Stumpe[6], E. Zuegner[5], N. Bordag[5], R. Riedl[7], A. Schmidt[8], E. Kolesnik[8], N. Verheyen[8], A. Springer[9], T. Madl[9], F. Sinner[5], R. de Cabo[4], G. Kroemer[10], B. Obermayer-Pietsch[3], J. Dengjel[6], H. Sourij[3], T. Pieber[3], F. Madeo[1, 2]**

[1] University of Graz, Institute of Molecular Biosciences, Graz, Austria
[2] BioTechMed Graz, Graz, Austria
[3] Medical University of Graz, Division of Endocrinology and Diabetology, Graz, Austria
[4] National Institute on Aging, Experimental Gerontology Section, Baltimore, United States of America
[5] JOANNEUM RESEARCH Forschungsgesellschaft mbH, HEALTH Institute for Biomedicine and Health Sciences, Graz, Austria
[6] University of Fribourg, Dep. of Biology, Fribourg, Switzerland
[7] Medical University of Graz, Institute for Medical Informatics, Graz, Austria
[8] Medical University of Graz, Division of Cardiology, Graz, Austria
[9] Medical University of Graz, Gottfried Schatz Research Center for Cell Signaling, Metabolism and Aging, Graz, Austria
[10] Centre de Recherche des Cordeliers, Equipe 11 labellisée par la Ligue contre le Cancer, Paris, France
Email: sebastian.hofer@uni-graz.at

Decades of aging research have found that fasting in its various forms, most prominently caloric restriction (CR) and intermittent fasting (IF), are the only known nutritional interventions so far that can prolong life- and/or health-span in all model organisms tested. Although the preclinical data seems very promising, there are only scarce and sometimes contradictory data on the effects of IF on humans. Therefore, the clinical trial “InterFAST” was started and designed to study the molecular and physiological effects of short-term (4 weeks) and long-term (>6 months) effects of strict ADF in an adequately sized cohort of healthy, non-obese adults.

We show that four weeks of strict alternate day fasting (ADF) improved markers of general health in healthy, middle-aged humans while causing a significant calorie reduction on average. ADF improved cardiovascular markers, reduced fat mass, improving the fat-to-lean ratio, and increased ß-hydroxybutyrate, even on non-fasting days. On fasting days, the circulating metabolome completely shifted towards reduced amino acids, while polyunsaturated fatty acids were elevated. We found a reduction of the metabolic regulator triiodothyronine, possibly representing a contributing mechanism of ADF effects. InterFAST sheds light on

the physiological impact of ADF and supports its safety. Eventually, ADF could become a clinically relevant intervention.

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**The Hippo network kinase STK38 contributes to protein homeostasis by inhibiting BAG3-mediated autophagy**

Authors:
**C. Klimek[1], R. Jahnke[1], J. Wördehoff[1], B. Kathage[1], D. Stadel[2], C. Behrends[3], A. Hergovich[4], J. Höhfeld[1]**

[1] University of Bonn, Institute for Cell Biology, Bonn, Germany
[2] Goethe University Medical School , Institute of Biochemistry II, Frankfurt am Main, Germany
[3] Ludwig-Maximilians-University Munich, Munich Cluster for Systems Neurology, München, Germany
[4] University College London, UCL Cancer Institute, London, United Kingdom
Email: rjahnke@uni-bonn.de

Chaperone-assisted selective autophagy (CASA) initiated by the cochaperone Bcl2-associated athanogene 3 (BAG3) represents an important mechanism for the disposal of misfolded and damaged proteins in mammalian cells. Under mechanical stress, the cochaperone cooperates with the small heat shock protein HSPB8 and the cytoskeleton-associated protein SYNPO2 to degrade force-unfolded forms of the actin-crosslinking protein filamin. This is essential for muscle maintenance in flies, fish, mice and men. Here, we identify the serine/threonine protein kinase 38 (STK38), which is part of the Hippo signaling network, as a novel interactor of BAG3. STK38 was previously shown to facilitate cytoskeleton assembly and to promote mitophagy as well as starvation and detachment induced autophagy. Significantly, our study reveals that STK38 exerts an inhibitory activity on BAG3-mediated autophagy. Inhibition relies on a disruption of the functional interplay of BAG3 with HSPB8 and SYNPO2 upon binding of STK38 to the cochaperone. Of note, STK38 attenuates CASA independently of its kinase activity, whereas previously established regulatory functions of STK38 involve target phosphorylation. The ability to exert different modes of regulation on central protein homeostasis (proteostasis) machineries apparently allows STK38 to coordinate the execution of diverse macroautophagy pathways and to balance cytoskeleton assembly and degradation.

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**Modulation of autophagy in aged human endothelial cells**

Authors:
**S. Mai[1], N. Brehm[2], G. Auburger[2], J. Bereiter-Hahn[1], M. Jendrach[1, 2, 3]**

[1] Goethe University, Kinematic Cell Research Group; Institute for Cell Biology and Neuroscience, Frankfurt, Germany
[2] Goethe University Medical School, Experimental Neurology, Dept. of Neurology, Frankfurt, Germany
[3] Charité – Universitätsmedizin Berlin, Department of Neuropathology , Berlin, Germany
Email: marina.jendrach@charite.de

Organelle fitness and functionality is ensured by the quality control (QC) mechanisms of the ubiquitin-proteasome system und autophagy. Impairment and dysregulation of both QC mechanisms is implicated to contribute to the aging process and age-associated diseases. We investigated human umbilical vein endothelial cells (HUVEC), as endothelial cells exhibit in vitro as well as in vivo a long post-proliferative phase and senescent endothelial cells are associated with arteriosclerosis. Transcriptome analysis revealed several age-related changes of autophagy gene expression. Old/senescent HUVEC showed an impairment of autophagic flux correlating with an increased population of autophagosomes and lysosomes

compared to young cells. A more detailed analysis revealed a reduced activity but not reduced amount of the V-ATPase, which is required for acidification of the lysosomes and thus functionality of lysosomal hydrolases. As old HUVEC showed also signs of oxidative damage and dysfunctional mitochondria we hypothesized that reduced mitochondrial ATP production could be responsible for their low V-ATPase activity. Indeed, addition of exogenous ATP rescued lysosomal acidification and restored the autophagic flux. Taken together, impaired lysosomal acidification due to ATP shortage, which may stem from mitochondrial dysfunction, could be a mechanism underlying impaired autophagy in aging cells.

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**Regulation of neuroinflammation by autophagy**

Authors:
**J. Houtman[1], K. Freitag[1, 2], J. Schulz[1], F. Heppner[1, 2, 3], M. Jendrach[1]**

[1] Charité – Universitätsmedizin Berlin, Department of Neuropathology , Berlin, Germany
[2] German Center for Neurodegenerative Diseases (DZNE) within the Helmholtz Association, Department of Neuropathology , Berlin, Germany
[3] Cluster of Excellence, Neurocure, Berlin, Germany
Email: marina.jendrach@charite.de

Reduction of autophagy is associated with increased inflammation. Loss of ATG16L1 is associated with Crohn's disease and results in increased proinflammatory cytokine production of macrophages (Murthy et al., 2014, Nature 506, 456–462). Neuroinflammation, mainly mediated by microglia, the CNS-resident myeloid cells, is a prominent feature in many neurodegenerative diseases including Alzheimer´s Disease. Interestingly, ATG6/Beclin1 was shown to be strongly reduced in microglia from Alzheimer´s Disease patients (Lucin et al., 2013, Neuron 70:873-886). Therefore, we investigated the inflammatory response of Beclin1$^{+/-}$ mice. These mice produced in vitro and in vivo more IL1beta and IL18 than wild type mice following acute (LPS+ATP) or chronic (amyloid beta) stimulation. Beclin1$^{+/-}$ microglia showed an increased number of cells with inflammasomes and elevated levels of NLRP3 and cleaved Caspase1. Super resolution microscopy revealed a very close association of NLRP3, LC3-II and the adaptor NDP52/Calcoco2 (Houtman et al., EMBO J. 2019 38(4)). In a complementary approach, we investigate the action of an autophagy activator. Here, we observed a concentration-dependent reduction of pro-inflammatory cytokines. Further investigation revealed different modes of action, targeting pro-inflammatory cytokine production in general and the IL1beta/IL18 pathway specifically. Taken together, these data indicate various mechanisms how autophagy modulates the inflammatory response of microglia.

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**Autophagy in the cellular response to DNA damage**

Authors:
**T. Juretschke[1], P. Beli[1]**

[1] Institute of Molecular Biology (IMB), Mainz, Germany
Email: t.juretschke@imb-mainz.de

The human genome requires permanent maintenance due to its constant exposure to sources of DNA damage. A scaffold of signaling pathways commonly known as the DNA damage response (DDR) preserves genomic stability. Keystones in this response are the serine-threonine kinases ATM/ATR/DNA-PKcs that phosphorylate effector proteins to trigger DNA repair and cell cycle checkpoints. Recent studies have suggested a role for autophagy in DNA repair. We employed mass spectrometry (MS)-based proteomics,

CRISPR/Cas9-based genetic screening and DNA repair assays to decipher the complex interplay between autophagy and the DDR. We found that DNA double strand break formation activates autophagy. Induction of autophagy is dependent on the DDR kinases ATM/ATR/DNA-PKcs. We demonstrate that inhibition of autophagy decreases the efficiency of DNA repair through homologous recombination and non-homologous end joining. To analyze whether autophagy is required for degradation of proteins after DNA damage, we identified autophagosomal cargo using proximity biotinylation and quantitative MS. In a complementary approach, ubiquitin remnant profiling in autophagy-deficient cells was employed to analyze ubiquitin-dependent targets of autophagy. Our work provides novel insights into the role of autophagy in the DDR.

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**A disease causing ATLASTIN 3 mutation affects multiple endoplasmic**

Authors:
**L. Behrendt[1], I. Kurth[2], C. Kaether[1]**

[1] Leibniz Institut für Alternsforschung, Fritz Lipmann Institut, Jena, Thuringia, Germany
[2] Uniklinik RWTH Aachen, Institut für Humangenetik, Aachen, North Rhine-Westphalia, Germany
Email: christoph.kaether@leibniz-fli.de

Atlastins (ATLs) are membrane-bound GTPases involved in shaping of the endoplasmic reticulum (ER). Mutations in ATL1
and ATL3 cause spastic paraplegia and hereditary sensory neuropathy. We here show that the sensory neuropathy causing
ATL3 Y192C mutation reduces the complexity of the tubular ER-network. ATL3 Y192C delays ER-export by reducing the
number of ER exit sites, reduces autophagy, fragments the Golgi and causes malformation of the nucleus. In cultured primary
neurons, ATL3 Y192C does not localize to the growing axon, resulting in axon growth deficits. Patient-derived fibroblasts
possess a tubular ER with reduced complexity and have a reduced number of autophagosomes. The data suggest that the
disease-causing ATL3 Y192C mutation affects multiple ER-related pathways, possibly as a consequence of the distorted
ER morphology.

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**dHip14 attenuates neurodegeneration by inducing apoptosis in *Drosophila* eye**

Authors:
**K. Kang[1], J. W. Kim[1]**

[1] KAIST, Biological Science, Daejeon, Republic of Korea
Email: kkyunghwa@hotmail.com

Huntington disease (HD) is an autosomal-dominant neurodegenerative disorder caused by a CAG expansion in the Htt gene. Huntingtin interacting protein 14(Hip14), palmitoyl acyltransferases for Htt, are disrupted in the presence of the HD mutation, resulting in under palmitoylation of Htt. Because Htt acts as a modulator of HIP14 activity, Hip14 is less active and HIP14 substrates are less palmitoylated, leading to neuronal toxicity in the presence of mutated Htt. Neuronal toxicity is substantially reduced by

overexpression of HIP14. In this study, we showed that overexpression of *Drosophila* Hip14 (dHip14) in wild type eye of *Drosophila* induced small eye phenotype by itself, and it has resulted from apoptosis. dHip14 also reduced the aggregation in the eye of HD-model fly. Bchs, one of the lysosomal trafficking proteins, has been identified as a substrate of dHip14. Bchs significantly increase the dHip14 activity for small eye phenotype and apoptosis. From the results, we suggested that dHip14 contributes to the reduction of neurodegeneration in many ways by palmitoylating many proteins having a diverse function.

 

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**Pathological missorting of endogenous MAPT/Tau in neurons caused by failure of protein degradation systems**

Authors:
**S. Kaniyappan[1], V. V. Balaji[1], Y. Wang[1], E. Mandelkow[1], E. M. Mandelkow[1]**

[1] German Centre for Neurodegenerative Diseases, AG Mandelkow, Bonn, North Rhine-Westphalia, Germany
Email: Senthil.Kaniyappan@dzne.de

Missorting of MAPT/Tau represents one of the early signs of neurodegeneration in Alzheimer disease. The triggers for this are still a matter of debate. Here we investigated the sorting mechanisms of endogenous MAPT in mature primary neurons using microfluidic chambers (MFCs) where cell compartments can be observed separately. Blocking protein degradation pathways with proteasomal or autophagy inhibitors dramatically increased the missorting of MAPT in dendrites on the neuritic side, suggesting that degradation of MAPT in dendrites is a major determinant for the physiological axonal distribution of MAPT. Such missorted dendritic MAPT differed in its phosphorylation pattern from axonal MAPT. By contrast, enhancing autophagy or proteasomal pathways strongly reduced MAPT missorting, thereby confirming the role of protein degradation pathways in the polar distribution of MAPT. Dendritic missorting of MAPT by blocking protein degradation resulted in the loss of spines but not in overall cell toxicity. Inhibition of local protein synthesis in dendrites eliminated the missorting of MAPT, indicating that the accumulation of dendritic MAPT is locally generated. In support of this, a substantial fraction of *Mapt/Tau* mRNA was detected in dendrites. Taken together, our results indicate that the autophagy and proteasomal pathways play important roles in fine-tuning dendritic MAPT levels and thereby prevent synaptic toxicity caused by MAPT accumulation.

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**Ykt6 – Brake or Accelerator of Wnt signaling ?**

Authors:
**P. Karuna M[1], A. Danieli[1], K. Linnemannstoens[1], L. Witte[1], M. Honemann-Capito[1], J. C. Gross[1, 2]**

[1] University Medical Center Göttingen, Developmental Biochemistry, Goettingen, Lower Saxony, Germany
[2] University Medical Center Göttingen, Hematology and Oncology, Goettingen, Germany
Email: pradhipa.karuna-m@med.uni-goettingen.de

Homeostasis is a healthy equilibrium state of cells, tissues, organisms maintained by coordinated regulation of several pathways. Cellular stress induced by external or internal cues, activates well-organized processes aimed at restoring homeostasis, quality control or inducing cell death. Wnt signaling is an evolutionarily conserved pathway that is tightly regulated during adult tissue homeostasis. Mutations in the Wnt pathway leads to growth-related pathologies and cancer.

How does the cell adapt to Wnt secretion levels and decide the route of Wnt secretion? Ykt6 is a promiscuous SNARE protein that lacks a transmembrane domain and switches between cytosolic and membrane-bound fraction. The active conformational switch uses a lipid anchor that allows transient membrane association to initiate vesicle fusion events. Ykt6 was shown to be involved in ER-Golgi trafficking, exosomal Wnt secretion and autophagosome formation.

Here, we investigate how Ykt6 adapts Wnt secretion levels to various cues such as nutritional starvation, ER-stress and Wnt signaling. Using detergent fractionation to separate cytosolic from membrane-bound proteins, we observe that different stimuli lead to the membrane recruitment of Ykt6 and affect the level of secreted Wnts. Since majority of human Ykt6 localizes cytoplasmically, further work has to determine how various upstream signaling pathways activate Ykt6 membrane attachment to adapt nutritional levels and Wnt secretion during cell proliferation.

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**Metabolic interactions in a novel 3D cell co-culture model using colon cancer cells and fibroblasts.**

Authors:
**F. Keller[1], M. Hafner[1], R. Rudolf[1]**

[1] Hochschule Mannheim University of Applied Sciences, Institute of Molecular and Cellular Biology, Mannheim, Baden-Württemberg, Germany
Email: f.keller@hs-mannheim.de

BACKGROUND:Most tumors consume huge amounts of glucose. Concepts of Warburg and anti-Warburg effect describe this based on metabolic needs of proliferation. *In vivo*, tumor metabolism is heterogeneous since anabolic and catabolic cells can be found. Current *in vitro* systems relying on 2D culture or single cell type spheroids do not reflect this.

OBJECTIVE:Establishment of a 3D cancer model recapitulating metabolic interplay between cancer and stroma cells that can be parallelized for mechanistic analyses and drug testing.

METHODS:Human cell lines HT29 of colon cancer and CCD-1137Sk fibroblast were cultivated in mono- and co-cultures using Dynarray microarrays. Metabolic changes in 2D and 3D were observed with confocal microscopy and Western blot analysis using autophagy-related markers like e.g. LC3 or MCT4.

RESULTS:3D growth of HT29 cells was facilitated by joined-up fibroblasts. Compared to mono-cultures of colon cancer cells or fibroblasts, levels of LC3 were increased in co-cultures and signals were primarily found in fibroblasts. Lactate carrier expression was compatible with shuttling from fibroblasts to cancer cells.

CONCLUSION:Our findings ratify cell-signaling between cancer cells and fibroblasts affecting growth behavior and metabolism. They imply a network of anabolic cancer cells with catabolic fibroblasts and cancer cells. For metabolic signaling, microarray-based co-cultures promise to better mirror the *in vivo* situation in tumors than classical culture methods.

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# Abstracts - Poster

**De-LC3ylation of proteins is a novel function of mammalian ATG4 proteases**

Authors:
**A. Agrotis[1], R. Ketteler[1]**

[1] University College London, MRC LMCB, London, United Kingdom
Email: r.ketteler@ucl.ac.uk

In autophagy, LC3/GABARAP is a family of ubiquitin-like proteins important for autophagosome formation and maturation. Previously, the only known cellular target molecules of covalent modification by LC3/GABARAP are phospholipids present in the autophagosome membrane. ATG4 cysteine proteases are required to process inactive pro-LC3/GABARAP prior to phospholipid conjugation and to perform deconjugation of LC3/GABARAP from the autophagosome membrane. To determine whether LC3/GABARAP has other target molecules, we generated an LC3B mutant that is resistant to deconjugation by ATG4 proteases. Upon expression in human cells, deconjugation-resistant LC3B accumulates in multiple forms and at higher molecular weights than free LC3B when assessed by western blotting. A similar accumulation is observed when pre-processed versions of all mammalian LC3/GABARAP isoforms are expressed in ATG4 deficient cell lines generated by CRISPR/Cas9 genome editing. We identify targets of conjugation with multiple copies of LC3/GABARAP as ATG3 and ATG7, and show that LC3B-ATG3 conjugates are distinct from the LC3B-ATG3 thioester intermediate formed prior to lipidation. Altogether we provide evidence that LC3/GABARAP post-translationally modifies other proteins akin to ubiquitination, with ATG4 proteases acting as deubiquitination enzymes to counteract this process. Our study therefore uncovers a novel modification cycle involving autophagy-related proteins in mammalian cells.

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**Development of a human neuronal cell model of Beta-Propeller Protein-Associated Neurodegeneration (BPAN) as a drug screening platform**

Authors:
**R. Ketteler[1], A. Papandreou[1, 2], M. Kurian[2]**

[1] University College London, MRC LMCB, London, United Kingdom
[2] University College London, Great Ormond Street Hospital, London, United Kingdom
Email: r.ketteler@ucl.ac.uk

BPAN is an X-linked subtype of Neurodegeneration with Brain Iron Accumulation (NBIA) caused by mutations in *WDR45/WIPI4*. The encoded beta-propeller protein has a postulated role in early stages of autophagy. The mechanisms linking autophagy, iron metabolism and neurodegeneration are poorly understood, and there are currently no available effective treatments. Here, we have developed a patient-derived, induced pluripotent stem cell (iPSc)-derived midbrain dopaminergic (mDA) neuronal model of to investigate disease mechanisms. Three patient-derived iPSc lines, two age-matched controls and two isogenic controls (generated via CRISPR/Cas9-mediated mutation correction in two BPAN lines) have been differentiated into mDA and extensively characterised. Furthermore, we developed a high-content autophagy assay in patient-derived ventral midbrain progenitors at Day 11 that is amenable to high-throughput screening. We have performed a drug screen using the FDA-approved Prestwick library and a series of novel autophagy activators. A number of compounds significantly correct an autophagy defect in all tested lines, and further validation is underway. In summary, our work in developing a mDA model of BPAN has provided an innovative platform for a) understanding pathophysiological mechanisms leading to

striatonigral degeneration and b) performing imaging-based drug screening with the aim of identifying new treatments for this medically resistant condition.

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**The role of the p73-ATG5 axis in regulating autophagy in atopic dermatitis and psoriasis**

Authors:
**K. Klapan[1], Z. Frangez[1], Z. He[1], H. Liu[1], S. Yousefi[1], D. Simon[2], H. - U. Simon[1]**

[1] University of Bern, Institute of Pharmacology, Bern, Switzerland
[2] University of Bern, Department of Dermatology, Bern, Switzerland
Email: kim.klapan@pki.unibe.ch

Autophagy is believed to secure cell survival under stressful conditions and it is particularly important for the cellular response under pathological conditions such as inflammation. Transcription factor p73 belongs to a p53 family, that plays a role in the regulation of many cellular processes.Based on the previous observations that p73 is responsible for inducing ATG5 expression and regulates autophagy in hepatocytes, we hypothesize that this mechanism may also play an important role in atopic dermatitis (AD) and psoriasis (PS). By applying immunofluorescence techniques, we observed an increased expression of p73, autophagy regulating proteins and autophagy activity markers in keratinocytes in skin samples from patients suffering from AD and PS. Results indicate that reducing p73 expression by shRNA reduces ATG5 expression in human skin keratinocyte cell line and primary keratinocytes. Our preliminary results indicate that inflammatory cytokines play an important role in the pathogenesis of AD and PS by blocking autophagic flux.Moreover, upon TNF-α and IL-17A treatment we observed upregulation of the protease inhibitor Serpina3 which supports the assumption that cytokine-mediated induction of protease inhibitors could be responsible for inhibition of lysosomal enzymes, resulting in decreased autophagic activity. Investigating autophagy in AD and PS may lead to a better understanding of the pathophysiology of these diseases, which may result in identifying new drug targets.

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**Phase-separated compartments function as scaffolds for autophagosome formation and as selective cargoes**

Authors:
**J. Agudo-Canalejo[1, 2], S. W. Schultz[3, 4], H. Chino[5], C. Saito[5], I. Koyama-Honda[5], A. I. May[6], A. Brech[3, 4], N. Mizushima[5], R. L. Knorr[5, 7]**

[1] University of Oxford, Rudolf Peierls Centre for Theoretical Physics, Oxford, United Kingdom
[2] The Pennsylvania State University, Department of Chemistry, Pennsylvania, Pennsylvania, United States of America
[3] Oslo University Hospital, Department of Molecular Cell Biology, Institute for Cancer Research, Oslo, Norway
[4] University of Oslo, Centre for Cancer Cell Reprogramming, Institute of Clinical Medicine, Faculty of Medicine, Oslo, Norway
[5] The University of Tokyo, Graduate School and Faculty of Medicine, Tokyo, Japan
[6] Tokyo Institute of Technology, Tokyo Tech World Research Hub Initiative, Institute of Innovative Research, Kanagawa, Japan
[7] Max Planck Institute of Colloids and Interfaces, Department of Theory & Bio-Systems, Potsdam, Brandenburg, Germany
Email: abstract@eventclass.com

Compartmentalisation is essential for eukaryotic cell function, allowing the division of metabolic and regulatory processes into membrane-bound, specialised compartments, such as organelles. In recent years, intracellular phase separation has garnered much attention as a non-membrane means of organising components through the formation of droplet-like compartments, which are functionally implicated in both health and disease, including age-related human diseases such as neurodegeneration. Evidence suggests that droplet clearance involves autophagy, a highly-conserved degradation system in which membrane sheets expand and bend to isolate portions of the cell interior inside autophagosomes.

In my talk, I will explain our recent results on the mechanism of droplet sequestration by autophagosomes, in both living and synthetic cells. A minimal physical model shows that the droplet material properties, which are linked to neurodegenerative processes, determine whether membrane sheets isolate droplets in a whole or piecemeal fashion. Further, we find that droplets can serve as an assembly platform for cytosol-engulfing autophagosomes through fine-tuning of the membrane-droplet interaction, resulting in the reversal of the bending direction of autophagic membranes. Our findings demonstrate that droplet autophagy is controlled by elasto-capillary feedback between autophagic membranes, phase-separated droplets and the cytosol.

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**Inhibition studies of an organophosphate-detoxifying phosphotriesterase with human plasma components**

Authors:
**A. Köhler[1, 2], L. Job[1], B. Escher[1], F. Worek[2], A. Skerra[1]**

[1] TUM, Lehrstuhl für Biologische Chemie, Freising (Weihenstephan), Bavaria, Germany
[2] Bundeswehr, Institut für Pharmakologie und Toxikologie, München, Bavaria, Germany
Email: anja.koehler@tum.de

Organophosphorous (OP) nerve agents pose a severe toxicological threat in military conflicts and to civil population while OP pesticides in agriculture lead to a high number of fatalities. Therefore, an effective therapy of OP intoxication is desperately needed.

The phosphotriesterase from *Brevundimonas diminuta* (BdPTE) is a natural biocatalyst of OP hydrolysis and offers a potential treatment. While BdPTE mutants with suitable catalytic efficiencies have been described, their biochemical properties must be improved prior to therapeutic application.

In various animal models BdPTE showed a lower breakdown of OPs compared with *in vitro* studies; hence, enzyme activity appears to be affected under physiological conditions. By *in vitro* activity testing of a recombinant BdPTE mutant in human blood plasma we were able to prove specific enzyme inhibition by plasma components.

Detailed inhibition studies revealed that the BdPTE is inhibited by serum albumin in a competitive manner, which seems to be mediated by depletion of crucial zinc ions from the BdPTE active site either via the known high-affinity zinc binding site of albumin or via chemical complex formation with its free thiol side chain at Cys34.

Notably, albumin previously charged with zinc and carrying a chemically blocked Cys34 side chain completely lacked enzyme inhibition. Consequently, the physiological zinc concentration in human blood plays an important role for BdPTE activity and has to be taken into consideration.

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**How can mitoprotein-induced stress be rescued?**

Authors:
**L. Krämer[1], F. Boos[1], J. M. Herrmann[1]**

[1] Universtiy of Kaiserslautern, Cell Biology, Kaiserslautern, Rhineland-Palatinate, Germany
Email: l_kraeme@rhrk.uni-kl.de

Most mitochondrial proteins are synthesized in the cytosol and subsequently translocated into mitochondria. A functional mitochondrial protein import is essential for cell survival. Proteins, that are not imported are accumulating in the cytosol and/or are degraded by the proteasome.

To study how cells deal with the accumulation of mitochondrial precursor proteins, we expressed mitochondrial “clogger” proteins in yeast. These recombinant proteins reduce the number of available mitochondrial import sites and overcharge the import system which inhibits cell growth. Our data show that the accumulation of mitochondrial precursor proteins in the cytosol leads to the induction of the transcription factor Rpn4 which upregulates the ubiquitin-proteasome-system. Repressing the Rpn4-dependent proteasome response seems to restore precursor-induced growth defects. This may be explained by the fact that less mitochondrial precursor proteins get degraded and thereby have the chance to be imported into mitochondria even after reduction of available import sites. In my research I concentrate on the molecular mechanisms by which the proteasome plays a role during mitochondrial biogenesis.

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**The impact of aging on liver**

Authors:
**A. Krautscheid[1, 3], H. - M. Tautenhahn[1, 2], U. Dahmen[1]**

[1] University Hospital Jena, General- Visceral and Vascular Surgery, Jena, Thuringia, Germany
[2] University Hospital Jena, Research Programme „Else Kröner-Forschungskolleg AntiAge“, Jena, Thuringia, Germany
[3] University Hospital Jena, Graduate School „Else Kröner-Promotionskolleg Jena School for Ageing Medicine (JSAM)“, Jena, Thuringia, Germany
Email: annika.krautscheid@uni-jena.de

Since hepatic tumors increase in ageing, there is a rising need for liver resection in this population. So far, there are only few small series investigating the impact of aging on liver regeneration. Autophagy is a cell protection mechanism presumed declining with age. This clinical study will investigate the impact of aging on liver function- and morphology, especially on autophagy.

Liver tissue samples and blood serum aliquots from young (<35y) and old (>65y) patients subjected to hepatectomy are collected. The aim is to investigate age-related changes like necrosis, fibrosis and steatosis. Therefore clinical scoring systems (Desmet-Scheuer; EASL Guideline, PFLIP) will be used. Necrosis and apoptosis will be discriminated by TUNEL-assay and Caspase3. Autophagy will be elucidated by detecting LC3B using immunohistochemistry and western blot as well as autophagosome formation using confocal laser scanning microscopy.

By now, 7 young and 56 old patients could be included. Our results will be compared with a systematic literature review as meta-analysis. We identified 42 publications mostly reporting no significant impact of aging on the liver. However, the authors explore selected subpopulations not reflecting clinical reality. In case we can confirm that hepatic autophagy is impaired in older patients we consider a subsequent clinical

study investigating the impact of autophagy on liver regeneration.

Key words: autophagy, hepatectomy, aging

There are no conflicts of interest

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**cGAS/STING signaling upregulates immune response and autophagy in human aneuploid cells**

Authors:
**M. Krivega[1], C. Stiefel[1], N. Donnelly[2], N. Chunduri[1], Z. Storchova[1]**

[1] Technische Universität Kaiserslautern, Molecular Genetics, Kaiserslautern, Rhineland-Palatinate, Germany
[2] Max-Plank Institute of Biochemistry, Martinsried, Bavaria, Germany
Email: krivega@rhrk.uni-kl.de

Genomic instability, with chromosomal aberrations and missegregations leading to aneuploidy, is a well-established hallmark of aging. To study aneuploidy, we use isogenic human cell lines that were engineered to carry constitutive trisomy of chr. 3, 8, 18, 21 and tetrasomy of chr. 5. This unique model allows us to investigate cellular physiological response to numerical aneuploidy. Strikingly, we found that autophagy was significantly upregulated in these cells, as evidenced, among others, by increased nuclear localization of TFEB. However, the negative regulator of TFEB, mTORC1 was upregulated as well, indicating a starvation-independent autophagy stimulation upon aneuploidy. Unbalanced karyotype is often associated with a presence of genomic DNA in cytoplasm or in micronuclei stimulating inflammatory cGAS/STING pathway that can be responsible for the autophagy activation in aneuploid cells. Indeed, we showed an excess of dsDNA in cytoplasm and upregulation of several pathway members: the major kinase TBK1, the transcriptional factor IRF3, and interferon stimulatory genes IFIT3, IFIT1, OAS3. Importantly, cGAS knockdown reduced this enhanced immune phenotype and decreased the autophagy activation in aneuploid cells, illustrated by suppressed autophagy flux and nuclear TFEB presence. Taken together, constitutive aneuploidy alone induces cGAS/STING signaling and thereby activates both inflammatory response as well as autophagy that may facilitate many aging phenotypes.

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**Characterization of a putative novel human replication factor PN70**

Authors:
**J. Kutz[1], F. Opel[1], H. Pospiech[1]**

[1] Friedrich-Schiller-Universität Jena / Leibniz Institute on Aging – Fritz Lipmann Institute (FLI) , Project Biochemistry - Englert Research Group, Jena, Thuringia, Germany
Email: julia.kutz@leibniz-fli.de

DNA replication represents a series of precisely regulated events that guarantee accurate duplication of the genetic information. Although the molecular replication machinery has been well characterized, its dynamics in terms of protein-protein interaction, as well as the role of many auxiliary factors still remain unclear.

PN70 (predicted nuclear; 70 kDa) represents an uncharacterized human protein of hitherto unknown function predicted to have nuclear localization. It shows a strong co-expression with genes involved in S-phase metabolism. Homology searches identified a region of ~250 amino acids comprising also a putative OB-fold motif conserved among metazoans.

Here, we confirmed the predicted nuclear localization of the endogenous protein and of fluorescence-

tagged PN70 fusions via subcellular fractionation and fluorescence microscopy, respectively. Synchronization experiments showed cell cycle dependent changes of the PN70 protein level with a maximum in S phase. A flow cytometric, FRET-based interaction assay was applied to PN70 and proteins of the replication machinery using vectors for the expression of fluorescence-tagged fusion proteins. Utilizing transfected cells, the so far unknown association between the initiation factors RecQL4 and MCM5, as well as RecQL4 and Cdc45 was demonstrated. Moreover, FRET interaction was detected in cells with fluorescent protein fusion pairs of PN70 and the replication factors Cdc45, RPA, MCM2 and MCM5.

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**Glycophosphocholine-phosphodiesterase1 (EDI3) - A new player in the Glycogen Metabolism**

Authors:
**G. M. Leonhardt[1], A. Jashari[1], A. Glotzbach[1], M. S. Lesjak[2], K. Edlund[1], C. Cadenas[1], J. Hengstler[1], R. Marchan[1]**

[1] Leibniz Research Centre for Working Environment and Human Factors at TU Dortmund, Toxicology, Dortmund, North Rhine-Westphalia, Germany
[2] Universitätsklinikum Essen (AöR) , Clinic for Neurosurgery, Essen, North Rhine-Westphalia, Germany
Email: Leonhardt@ifado.de

In earlier work, we identified and characterized the enzyme, glycophosphocholine-phosphodiesterase1 (GPCPD1, EDI3) as a crucial enzyme in different metabolic pathways due to its hydrolysis of glycerophosphocholine (GPC) to choline and glycerol 3-phosphate (G3P). The enzymatic glycerophosphodiesterase (GDE) domain of EDI3 is conserved among all GDE proteins. However, unique to EDI3 is a carbohydrate binding moiety (CBM20) which role remains unexplored.

In addition to EDI3, there are two other proteins in the human genome that contain a CBM20 domain - Stbd1 and Laforin. These proteins have been shown to bind carbohydrates and linked to a regulatory role in autophagy. Therefore, the aim of the present study was to investigate whether EDI3 also is involved in these processes. Using a polysaccharide binding assay, it could be shown that EDI3 binds both glycogen and amylopectin. Immunoprecipitation analyses also demonstrated that EDI3 is a homodimer and interacts with the other CBM20 proteins. In primary mouse hepatocytes, we could demonstrate that treatment with the hormone glucagon results in increased EDI3 RNA and protein level. Further mechanistic analyses with glucagon indicate similar expression changes in other genes as observed for EDI3, which supports a possible role for EDI3 in gluconeogenesis and regulation via CREB. Taken together, these findings suggest that EDI3’s function extends beyond lipid metabolism and reinforces its importance in normal and diseased conditions.

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**Spinal muscular atrophy-triggering low levels of survival of motor neuron protein enhance macropinocytosis**

Authors:
**C. Melcher[1], P. Claus[1], R. Lindner[1]**

[1] Hannover Medical School, Institute of Neuroanatomy and Cell Biology, Hannover, Lower Saxony, Germany
Email: Lindner.Robert@mh-hannover.de

Macropinocytosis is a pathway for uptake of large volumes of extracellular fluid into eukaryotic cells. It is used to sense the amino acid content of the extracellular medium, a process that controls metabolic activation of cells. In motor neurons, macropinocytosis also serves to downregulate growth-inducing bone morphogenetic protein (BMP) receptors at the neuromuscular synapse. Developmental defects at this site are a hallmark of the neurodegenerative disease spinal muscular atrophy (SMA). Here we characterized

macropinocytosis pathways in motor neurons and investigated their behavior in response to low levels of the survival of motor neurons (SMN) protein, the primary cause of SMA. Motor neuron-like NSC34 cells showed two distinct macropinocytosis pathways: i) an induced pathway that was dependent on functional $Na^+/H^+$exchanger 1 (NHE1), ATP and dynamic actin and tubulin molecules; ii) a constitutive pathway that was independent of functional NHE1 exchanger, but dependent on cellular ATP and dynamic actin. Knock-down of SMN protein expression in NSC34 cells gave rise to a consistent elevation of macropinocytosis. We could show a similar type of effect in primary motor neurons isolated from SMA model mice relative to heterozygote littermates. Our results suggest that low SMN levels cause SMA-type of developmental defects in neuromuscular synapses by triggering enhanced macropinocytosis and downregulation of BMP receptors.

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**Regulation of the recov-ER-phagy pathway**

Authors:
**M. Loi[1, 2], A. Raimondi[3], D. Morone[1], M. Molinari[1, 4]**

[1] Università della Svizzera italiana , Institute for Research in Biomedicine, Bellinzona, Switzerland
[2] Swiss Federal Institute of Technology, Department of Biology, Zurich, Switzerland
[3] San Raffaele Scientific Institute, Experimental Imaging Center, Milan, Italy
[4] École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
Email: marisa.loi@irb.usi.ch

The endoplasmic reticulum (ER) is an organelle in eukaryotic cells that fulfills important cellular functions including membrane and secretory protein production, lipid and oligosaccharide synthesis, calcium storage and detoxification of harmful products. Perturbations of ER homeostasis activate unfolded protein responses (UPR) that increase the ER volume and enhance transcription and translation of biosynthetic enzymes, ER-resident molecular chaperones and enzymes involved in protein folding, degradation and quality control. We recently reported that on resolution of ER stress, the translocon component SEC62 engages lipidated LC3 to act as an ER-phagy receptor that controls lysosomal delivery of excess ER produced during the stress phase. This catabolic regulation of ER homeostasis that re-establishes physiologic ER size and function on stress resolution has been named recov-ER-phagy [a].

Here, we report that in contrast to other types of ER-phagy, where ER subdomains to be cleared from cells are captured by double membrane autophagosomes on their way to degradative lysosomes, recov-ER-phagy relies on direct capture of excess ER by degradative endolysosomes in processes that are topologically consistent with micro-ER-phagy and rely on intervention of the ESCRT-III machinery.

 

[a] Fumagalli et al. Nature Cell Biol (2016)

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**Overexpression of PTP4A3 in high grade ovarian sarcoma cell lines drives high basal not activatable autophagy.**

Authors:
**A. López Garza[1], J. Strydom[1], J. Murray[1]**

[1] Trinity College Dublin, School of Biochemistry & Immunology, Dublin, Ireland
Email: lopezgaa@tcd.ie

Autophagy is an evolutionarily conserved, homeostatic cellular recycling mechanism that ensures cell

survival by limiting cellular damage, and provision of biosynthetic building blocks. The involvement of autophagy in the evolution of cancer is complicated, with both oncogenic and tumour suppressive roles described. The oncogenic phosphatase PTP4A3 is upregulated in ovarian cancer leading to activation of AKT signalling and we previously showed it promoted canonical autophagy to drive cell proliferation (Huang et al, Autophagy, 2014). High expression of both PTP4A3 and either PIK3C3 or BCLN1 associated with poorer patient outcome. How PTP4A3 drives autophagy as part of its oncogenic programme is not known.

We present data showing that the endogenous expression levels of PTP4A3 correlate with autophagy activity in high-grade serous ovarian cancer (HGSOC) cells. High expression of PTP4A3 was detected in Kuramochi cells, but not in OVCAR 3 or OVCAR 4 cells. Basal autophagy was detected in Kuramochi and OVCAR 3 cells under nutrient replete conditions (Bafilomycin A1), but not in OVCAR 4 cells. Whereas, activatable autophagy (EBSS) was detected in OVCAR 3 and OVCAR 4 cells, but not in Kuramochi cells. PTP4A3-driven basal autophagy in Kuramochi cells was attenuated by pharmacological inhibition of ULK1. In summary, high levels of endogenous PTP4A3 associated with a high basal autophagy activity but an inability to stimulate activatable autophagy following nutrient stress.

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**Modulation of TDP-43 pathology via mutated ubiquitin in a yeast model of Parkinsonism-Dementia Complex of Guam**

Authors:
**C. Lussier[2], C. Lucini[1], C. Müller-Hübers[2], J. Schramm[2], B. M. Verheijen[3, 4], R. J. Braun[1, 2]**

[1] Danube Private University, Medicine/Dental Medicine, Krems, Niederösterreich, Austria
[2] University of Bayreuth, Cell Biology, Bayreuth, Bavaria, Germany
[3] University Medical Center Utrecht Brain Center, Utrecht University, Translational Neuroscience, Utrecht, Netherlands
[4] University Medical Center Utrecht Brain Center, Utrecht University, Neurology and Neurosurgery, Utrecht, Netherlands
Email: chantal.lucini@dp-uni.ac.at

The Guam disease is a rare neurodegenerative disorder on Guam Island. Patients suffer from progressive cognitive impairment and Parkinsonism. The disease is characterized by neuronal deposits of hyperphosphorylated Tau, Amyloid-β, the frameshift ubiquitin variant $UBB^{+1}$ and the 43 kDa TAR-DNA binding protein (TDP-43). These are pathological hallmarks for Alzheimer disease, frontotemporal dementia, and Amyotrophic Lateral Sclerosis. Dissecting the molecular background of Guam disease might thus have implications for more frequent neurodegenerative diseases. The cellular accumulation of $UBB^{+1}$ impairs the ubiquitin-proteasome system (UPS) in neurons and in yeast, even though the cytotoxicity of this protein is rather moderate. In contrast, the cytoplasmic accumulation of TDP-43 leads to its aggregation accompanied with marked cytotoxicity in neuronal and yeast cells. Here, we aimed to investigate whether enrichment of $UBB^{+1}$ affects the pathology of TDP-43 (cytotoxicity and aggregation) in yeast. Surprisingly, high levels of $UBB^{+1}$ reduced the cytotoxicity of TDP-43 in wild-type cells and in cells with increased UPS capacity. Although TDP-43 aggregation was unaffected by the presence of $UBB^{+1}$ in wild-type cells, in cells with increased UPS capacity significantly less TDP-43 aggregates were detectable. These findings suggest that $UBB^{+1}$ elicits a stress response that reduces the cytotoxicity of TDP-43, possibly through accelerating its degradation.

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# Abstracts - Poster

***Initiation of Autophagy signalling by HORMA domain proteins***

Authors:
**F. Lugarini[1, 2], A. Faesen[1]**

[1] Max Planck Institute for Biophysical Chemistry, Dep. Signal dynamics, Goettingen, Lower Saxony, Germany
[2] Göttingen University, Goettingen, Lower Saxony, Germany
Email: flugari@gwdg.de

The regulation of the ULK1/2-kinase complex recruitment at the initial step of Autophagy to a few distinct sites on the ER membrane are still largely unknown. Intriguing components of the ULK1/2-kinase complex are the HORMAs proteins ATG13 and ATG101. HORMAs proteins work as signal-responsive elements at the initiation of signalling cascades. They mediate protein-protein interactions through a structurally unique mechanism involving a reversible remodelling of its topology, which enables the protein to wrap around specific binding partners. Previous biochemical reconstitution efforts focussed on the HORMA domain MAD2 in mitosis yielded a list of principles that are likely to be conserved in all HORMAs proteins, as in this case ATG13 and ATG101.

ATG13 contains an N-terminal HORMA domain directly interacting with the HORMA domain of ATG101. The interaction between ATG101 and ATG13 is essential for the function of the initiation complex, since mutating the ATG101-ATG13 interacting interface showed a penetrant autophagy inhibitory effect, highlighting the importance of this interaction.

In order to understand the spatio-temporal control of autophagy initiation, we propose to biochemically reconstitute the ULK1 complex. Particular attention is given to the identification of interactors of HORMA domain proteins ATG101 and ATG13 and the characterization of the interaction dynamics which we hypothesise will be at the centre of the induction of autophagy.

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**Plekhg5 regulates the autophagy of synaptic vesicles in axon terminals of motoneurons**

Authors:
**P. Lüningschrör[1, 2], B. Binotti[3], P. Heimann[2], A. Perez-Lara[3], A. J. Hutchings[1], N. Thau-Habermann[4], S. Petri[4], B. Kaltschmidt[2], R. Jahn[3], C. Kaltschmidt[2], M. Sendtner[1]**

[1] University Hospital Würzburg, Institute of Clinical Neurobiology, Würzburg, Germany
[2] University of Bielefeld, Department of Cell Biology, Bielefeld, Germany
[3] Max Planck Institute for Biophysical Chemistry, Department of Neurobiology, Göttingen, Germany
[4] Hannover Medical School, Department of Neurology, Hannover, Germany
Email: Lueningsch_P@ukw.de

Autophagy-mediated degradation of synaptic components maintains synaptic homeostasis but also constitutes a mechanism of neurodegeneration. It is largely unknown, how autophagy of synaptic vesicles and components of presynaptic active zones is regulated.

Here, we present that Pleckstrin homology containing family member 5 (Plekhg5) modulates autophagy of synaptic vesicles in axon terminals of motoneurons via its function as a guanine exchange factor for Rab26, a small GTPase that specifically directs synaptic vesicles to pre-autophagosomal structures. *Plekhg5* gene inactivation in mice results in a late-onset motoneuron disease, characterized by degeneration of axon terminals. Plekhg5-depleted cultured motoneurons show defective axon growth and impaired autophagy of synaptic vesicles, which can be rescued by constitutively active Rab26. Therefore, these findings define a mechanism for regulating autophagy in neurons that specifically targets synaptic vesicles. Notably, depletion of *Plekhg5*in SOD1 G93A mice reduces the denervation of muscle and prolongs the time until the mice reaching the disease endstage.

Taken together, our data suggest that presynaptic autophagy needs to be tightly balanced for maintaining synaptic homeostasis and that a dysregulation at both ends of this process triggers neurodegeneration. Thus, balancing the turnover of synaptic vesicles is a key mechanism for maintaining the function of neuromuscular junctions.

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**PARP1 regulates DNA damage-induced nucleolar-nucleoplasmic shuttling of WRN and XRCC1 in a toxicant and protein-specific manner**

Authors:
**S. Veith[1, 2], A. Schink[1, 2], M. Engbrecht[1, 2], M. Mack[1], L. Rank[1], P. Rossatti[1], M. Hakobyan[1], D. Goly[1], T. Hefele[1], M. Frensch[1], A. Fischbach[1], A. Bürkle[1], A. Mangerich[1]**

[1] University of Konstanz, Department of Biology / Molecular Toxicology Group, Konstanz, Baden-Württemberg, Germany
[2] Equal contribution, -, -, Germany
Email: aswin.mangerich@uni-konstanz.de

The prime function of nucleoli is ribogenesis, however several other functions have recently been identified, including a role in genotoxic stress response. Upon DNA damage, numerous proteins shuttle dynamically between the nucleolus and the nucleoplasm, yet the underlying molecular mechanisms are incompletely understood. We demonstrate that PARP1 and PARylation contribute to genotoxic stress-induced nucleolar-nucleoplasmic shuttling of key genome maintenance factors in HeLa cells. Thus, the RECQ helicase, WRN, translocates from nucleoli to the nucleoplasm upon treatment with the oxidizing agent $H_2O_2$, the alkylating agent CEES, and the topoisomerase inhibitor camptothecin (CPT). After treatment with $H_2O_2$ and CEES, but not CPT, WRN translocation was dependent on PARP1 protein, yet independent of its enzymatic activity. Contrary, nucleolar-nucleoplasmic translocation of the base excision repair protein, XRCC1, was dependent on both PARP1 protein and its enzymatic activity. Furthermore, gossypol, which inhibits PARP1 activity by disruption of protein interactions, abolishes nucleolar-nucleoplasmic shuttling of WRN, XRCC1 and PARP1, indicating the involvement of further upstream factors. In conclusion, this study highlights a prominent role of PARP1 in the DNA damage-induced nucleolar-nucleoplasmic shuttling of genome maintenance factors in a toxicant- and protein-specific manner.

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**How lysosomal cystine export maintains autophagy during fasting through the TCA cycle.**

Authors:
**Z. Marelja[1], P. Jouandin[2], A. A. Parkhitko[2], M. Dambowsky[1], J. M. Asara[2], I. Nemazanyy[1], N. Perrimon[2, 3], M. Simons[1]**

[1] Hôpital Necker-enfants malades, Epithelial Biology and Disease Institut Imagine, Paris, France
[2] Harvard Medical School, Department of Genetics, Boston, United States of America
[3] , Harvard Medical School, Howard Hughes Medical Institute, Boston, United States of America
Email: zvonimir.marelja@institutimagine.org

Autophagy is a crucial adaptive process that recycles internal nutrient stores to promote survival upon starvation. The induction of autophagy is regulated by lysosomal mTORC1 (mechanistic Target of Rapamycin Complex 1) that integrates nutrients availability via the sensing of amino acids to promote growth and anabolism. Nutrient restriction inhibits mTORC1 activity, which in turn induces autophagy. However, as successful amino acid recycling through autophagic degradation reactivates mTORC1 signaling over time, it is unclear how autophagy can be maintained during prolonged starvation. Our study shows

that one particular amino acid, cysteine, acts in a feedback loop to limit mTORC1 reactivation in vivo. We provide evidence that lysosomal export of cystine through cystinosin, a cystine transporter associated with the lysosomal storage disease cystinosis, fuels a metabolic pathway that suppresses mTORC1 signaling and maintains autophagy during starvation. This pathway involves reduction to cysteine, cysteine catabolism to acetyl-CoA and subsequent fueling of the TCA cycle. We propose that cysteine mediates a communication between lysosomes and mitochondria to control mTORC1 signaling under prolonged starvation, highlighting how changes in nutrient availability divert the fate of an amino acid into a growth suppressive program to maintain the balance between nutrient supply and consumption.

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**The role of GBA in the pathogenesis of Parkinson's disease**

Authors:
**M. Montpeyo[1], A. Navarro-Romero[1], J. Riera[1], I. Fernandez-Gonzalez[1], M. Albert[1], J. Hernandez-Vara[2, 1], D. Montpeyo[3, 4], E. Pradas[3, 4], F. Novio[4], J. Lorenzo[3], E. Cubero[5], N. Perez[5], A. M. Garcia-Collazo[5], X. Barril[5], M. Martinez-Vicente[1, 6, 7]**

[1] Vall d'Hebron Research Institute (VHIR), Neurodegenerative Diseases, Barcelona, Spain
[2] Hospital Universitari Vall d'Hebron , Neurology-Movement Disorders Unit, Barcelona, Spain
[3] Autonomous University of Barcelona , Institut de Biotecnologia i de Biomedicina (IBB), Barcelona, Spain
[4] Catalan Institute of Nanoscience and Nanotechnology (ICN2)-CSIC, Barcelona, Spain
[5] Gain Therapeutics, Barcelona, Spain
[6] CIBERNED, Barcelona, Spain
[7] Autonomous University of Barcelona , Barcelona, Spain
Email: marta.martinez@vhir.org

The first genetic risk factor to develop Parkinson’s disease (PD) is the presence of mutations in the GBA gene which encodes the lysosomal enzyme glucocerebrosidase (GCase) involved in sphingolipids metabolism. A relationship between loss of GCase activity and α-synuclein accumulation has been shown in different PD models as well as in samples from PD patients. This increase in the intracellular levels of α-synuclein is one of the main causes that contribute to neurodegeneration in PD. Our group has developed a new in vitro tool to study PD associated to GBA. A set of stable dopaminergic-like neurons cell lines expressing mutant GBA carrying N370S and L444P mutations as well as GBA knock-out, all in homozygosis and heterozygosis. These cell lines allow neuronal post-mitotic differentiation, present dopamine metabolism and express endogenous α-synuclein. The characterization of the cells lines demonstrates that the loss of GCase activity leads to the accumulation of the GCase substrates, impairment of the autophagic/lysosomal system, mitochondrial dysfunction, ROS production, ER stress and increase of different α-synuclein species. We are using this cellular model as a valuable in vitro system for the screening of two different therapeutic approaches based in the restoration of GCase activity in lysosomes through ERT enhanced with nanotechnology and through the use of non-competitive GBA pharmacological chaperones.

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**Role of the ULK protein family during nucleophagy**

Authors:
**M. J. Mendiburo[1], C. Kalus[1], N. Berleth[1], W. Wu[1], D. Schlütermann[1], J. Deitersen[1], F. Stuhldreier[1], B. Stork[1]**

[1] Heinrich-Heine University Düsseldorf, Institute of Molecular Medicine I, Düsseldorf, North Rhine-Westphalia, Germany
Email: mendibur@uni-duesseldorf.de

Nucleophagy is a specialized form of autophagy that degrades portions of the nucleus (e.g. nuclear envelope and chromatin) in response to persistent genotoxic stress. This process has attracted increasing attention in the recent years because of its potential relevance during ageing and cancer progression. Despite its importance in the response to DNA damage the molecular mechanisms governing nucleophagy remain largely un-characterized.

The ULK1 complex is a well-established factor that initiates the canonical autophagic program. Within the complex, the ULK1/2 protein kinase activates downstream autophagy proteins through phosphorylation. Interestingly, our data indicates that MEF cells lacking ULK1/2 display increased levels of nucleophagy compared to wildtype cells, as reported by enhanced degradation of the nuclear lamina protein LaminB1. Also, ULK1/2 knockout MEFs show higher levels of the DNA damage marker γH2AX compared to wildtype MEFs or double knockout MEFs re-constituted with either ULK1 or ULK2. This suggests that ULK1/2 may contribute to the maintenance of genome integrity and in this way negatively regulate nucleophagy. Given that ULK1, ULK2 and ULK3 (another member of the ULK protein family) can localize to the nucleus, it is possible that these kinases mediate nuclear signaling events that preserve genome function. We are currently investigating their potential nuclear substrates to understand how their function impacts nuclear processes.

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**NudC – a new player in WIPI-mediated autophagy**

Authors:
**D. Bakula[1, 2], A. Mesaris[1, 2], H. Robenek[3], T. Proikas-Cezanne[1, 2]**

[1] Eberhard Karls University Tuebingen, Department of Molecular Biology, Interfaculty Institute of Cell Biology, Tuebingen, Germany
[2] Max Planck Institute for Developmental Biology and Eberhard Karls University Tuebingen, International Max Planck Research School 'From Molecules to Organisms, Tuebingen, Germany
[3] University Hospital Muenster, Institute of Experimental Musculoskeletal Medicine, Muenster, Germany
Email: alexandros.mesaris@uni-tuebingen.de

Autophagy is a highly conserved lysosomal degradation pathway in eukaryotic cells that is upregulated in response to stress stimuli. A double membrane vesicle called the autophagosome mediates the degradation of cytoplasmic components. A crucial step of the membrane rearrangements leading to the formation of the autophagosome is the generation of PtdIns(3)P. Subsequently the human WIPI proteins are recruited to fulfill essential functions as PtdIns(3)P effector proteins. NudC, a conserved microtubuli-associated protein was recently identified in a proteome analysis of the human WIPI proteins *(Bakula et al., 2017)*. In this study it was confirmed that NudC interacts with WIPI1, WIPI2 and WIPI4 *(Bakula et al., 2017)*. Subsequent characterizations revealed that NudC is a new autophagy inhibitor. The inhibitory function of NudC is demonstrated by the results that a reduced NudC protein expression (1) increases the number of WIPI1-, WIPI2-, and LC3- positive autophagosomes, (2) reduces the levels of p62 in human tumor cells, (3) increases the rate of long-lived protein degradation and lastly (4) increases the number of autophagosomes observed in electron microscopy analysis. Likewise, overexpression of NudC reduces the number of autophagosomes. We suggest that NudC regulates WIPI-mediated autophagy, perhaps by preventing WIPI proteins from localizing to PtdIns(3)P-enriched membranes.

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# Abstracts - Poster

**Mitophagy induction by the mitochondrial unfolded protein response**

Authors:
**J. Michaelis[1], G. Tascher[1], K. Klann[1], C. Münch[1]**

[1] Goethe Universität Frankfurt, Institut für Biochemie II, Frankfurt, Germany
Email: michaelis@med.uni-frankfurt.de

Maintenance of its proteome is critical for mitochondrial function. Consequently, perturbations in mitochondrial proteostasis are implicated in a wide range of human pathologies, including neurodegenerative diseases. However, how mitochondria respond to mitochondrial protein misfolding remains largely unclear. Misfolding activates the mitochondrial unfolded protein response (mtUPR) to induce a transcriptional response in an attempt to restore protein folding. Using, quantitative proteomics, we defined the transcriptional mtUPR and discovered a novel mtUPR axis that reduces mitochondrial pre-RNA processing and translation to protect mitochondria and cells. Next, we studied potential alternative axes that may remove mitochondria that cannot be rescued by the mtUPR. Employing, mitophagy flux assays with mt-mKeima, we found that the mtUPR could shift into a destructive response that induces a PINK1/Parkin-dependent mitophagy to degrade dysfunctional mitochondria. We carried out genome-wide CRISPR/Cas9 screen for mitophagy under mtUPR condition to further define the underlying mechanisms driving mtUPR-induced mitophagy. Strikingly, the kinetics of mtUPR-induced mitophagy were distinct from canonical mitophagy induction showing specific regulation. Together, our data presents insight into mitochondrial quality control upon proteostasis perturbation, including induction of a novel mtUPR axis driving mitophagy.

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**Search for age-dependent transcriptomic and epigenetic changes in murine cerebral endothelial cells**

Authors:
**K. Mohan[1], A. Nordheim[1, 2]**

[1] International Max Planck Research School „From Molecules to Organisms", University of Tübingen, Tübingen, Baden-Württemberg, Germany
[2] Leibniz Institute on Aging, Fritz Lipmann Institute, Jena, Thuringia, Germany
Email: kshitij.mohan@ifiz.uni-tuebingen.de

Intracerebral hemorrhage (ICH) is associated with disruption of the blood-brain barrier and rupture of blood vessels, leading to the leakage of blood into brain tissues. The major risk factors for ICH are old age, hypertension and cerebral amyloid angiopathy. Weinl et al. (2015) have previously established in murine knock-out models that postnatal and adult endothelial cell (EC)-specific down-regulation of the transcription factor SRF, or its cofactors MRTF-A and MRTF-B, leads to impaired cerebral vessel integrity, resulting in microbleeds and larger hemorrhages in mouse brains.

My preliminary studies in mice demonstrated increased incidents of bleeding in brain with increasing age. RNA-seq data from the ECs of aging mice show differential expression of crucial genes responsible for the organization and maintenance of the blood-brain barrier and vascular integrity. We further hypothesize that the differential expression of genes in the cerebral ECs of aging mice may be attributed to epigenetics, and hence underlying epigenetic control mechanisms in ageing mice have been investigated at the level of chromosomal CpG methylation by RRBS (Reduced Representation Bisulfite Sequencing), and at the level of chromatin structure by ATAC-seq (Assay for Transposase-Accessible Chromatin Sequencing). This study on ageing mice promises to provide insights into potential molecular causes of age-dependent hemorrhagic stroke and small vessel disease in elderly human patients.

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**Sensitive Detection of Age-Dependent Release of Cell Surface Proteins with Intact Glycosylphosphatidylinositol Anchor from Isolated Adipocytes and into Serum of Rats**

Authors:
**G. A. Müller[1], A. Herling[2], K. Stemmer[1], M. Tschöp[1]**

[1] Helmholtz Center Munich, Institute for Diabetes and Obesity, Oberschleissheim, Bavaria, Germany
[2] Sanofi Pharma Germany, Diabetes Research, Frankfurt am Main, Hesse, Germany
Email: guenter.mueller@helmholtz-muenchen.de

To study the possibility that certain components of eukaryotic plasma membranes are released during aging, a chip-based sensor was developed for the detection of cell surface proteins, which are anchored at the outer leaflet of eukaryotic plasma membranes by a covalently attached glycosylphosphatidylinositol (GPI-AP) and might be prone to spontaneous or regulated release on basis of their amphiphilic character. Unprocessed GPI-AP together with phospholipids were specifically captured and detected by a chip-based sensor leading to changes in phase and amplitude of surface acoustic waves (SAW) propagating over the chip surface. Unprocessed GPI-AP in complex with lipids were found to be released from rat adipocyte plasma membranes immobilized on the chip which was dependent on the flow rate and composition of the buffer stream. The complexes were identified in the incubation medium of primary rat adipocytes, in correlation to the cell size and age of the rats, and in rat serum. The measured changes in SAW phase shift, reflecting specific mass/size or amount of the unprocessed GPI-AP in complex with lipids, and SAW amplitude, reflecting their viscoelasticity, enabled the differentiation between plasma membranes and adipocytes of young and old rats of either lean or obese state. Thus chip-based sensing for complexes of unprocessed GPI-AP and lipids reveals the inherently labile anchorage of GPI-AP at plasma membranes and their susceptibility for release in response to age.

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**The molecular function of Atg21 in autophagosome biogenesis**

Authors:
**L. Munzel[1], F. Otto[1], M. Thumm[1]**

[1] Georg-August University Göttingen, Cellular Biochemistry, Göttingen, Lower Saxony, Germany
Email: lena.munzel@med.uni-goettingen.de

Macroautophagy is initiated at the pre-autophagosomal structure (PAS) where a cup-shaped double-layered membrane structure (phagophore) is elongated and closed to form the autophagosome. A crucial factor for the elongation of the phagophore is the generation of phosphatidylinositol 3-phosphate (PI3P) at the PAS. Its presence is deciphered by a highly conserved family of WD40-repeat containing β-propellers. In the yeast *S. cerevisiae* this family consists of the three proteins Atg18, Atg21 and Hsv2 while the mammalian homologues are termed WIPI1-4.

Another important step required for phagophore elongation, is the conjugation of ubiquitin-like Atg8 to the lipid phosphatidylethanolamine (PE) which is mediated by two ubiquitin-like conjugation systems. The first system results in the coupling of ubiquitin-like Atg12 to Atg5. In complex with Atg16, the Atg12-Atg5 conjugate acts as an E3-like enzyme to facilitate the transfer of Atg8 from its E2-like enzyme Atg3 to PE. Atg21 organizes the lipidation reaction at the PI3P-positive autophagic membrane by the interaction with Atg8 and Atg16 of the E3-like complex.

While Atg18 was shown to participate in the formation of an ER-phagophore contact site, our recent data provide evidence for the formation of an additional contact site between phagophore and vacuole which seems to be important for the elongation of the phagophore. Atg21 is restricted to this contact site suggesting that the Atg8 lipidation complex is assembled here.

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**Integration of Individualised Proteogenomics Datasets to Analyse Single Amino Acid Variants in Cancer**

Authors:
**N. Nalpas[1], M. Schmitt[1], T. Sinnberg[2], H. Niessner[2], A. Forschner[2], C. Garbe[2], B. Macek[1]**

[1] University of Tuebingen, Quantitative Proteomics, Tuebingen, Germany
[2] Universitäts-Hautklinik, Sektion Dermatologische Onkologie, Tuebingen, Germany
Email: nicolas.nalpas@ifiz.uni-tuebingen.de

MS-based proteomics allow comprehensive analysis of proteomes and PTM, such as phosphorylation. Such approaches identify peptides and proteins by matching MS/MS spectra against public protein databases. Thus, commonly used databases inherently prevent identification of individual non-synonymous mutations. Here, we applied a proteogenomics workflow to different BRAFi-resistant and -sensitive melanoma cell lines to study the impact of somatic mutations upon establishment of resistance.

We used in-house bioinformatics workflow to call nucleotide variants from WES. We developed an R shiny App to 1) incorporate these mutations into individualised protein databases, 2) rank these based on their impact on PTM, 3) identify these mutations and associated PTM from MS data and 4) reconstruct the mutation-specific cellular signalling networks together with drugs.

In A375 and SK-Mel28 cell lines, WES led to the identification of 12,049 and 14,081 non-synonymous mutations, respectively. MS data from these cell lines were processed using MaxQuant software against their respective individualised proteome database. In A375 and SK-Mel28 cell lines, we identified 533 and 596 unique variant peptides, respectively. We found several phosphopeptides resulting from gain of a modifiable residue and we detected a number of phosphosite loss due to mutations. Most of them were unique to a specific phenotype or cell line, which calls for personalised approaches to cancer understanding and treatment.

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**The fibroblast's secretion of the two Tissue Factor isoforms and its pro-angiogenic effects on endothelial cells**

Authors:
**S. Nezi-Cahn[1, 3], S. Gylstorff[1, 3], K. Peters[1, 3], M. Heller[3], A. Hasenburg[1], W. Ruf[2], W. Brenner[1, 3]**

[1] Johannes Gutenberg University Medical Center Mainz, Department of Obstetrics & Women's health, Mainz, Rhineland-Palatinate, Germany
[2] Johannes Gutenberg University Medical Center Mainz, Centrum for Thrombosis and Hemostasis, Mainz, Rhineland-Palatinate, Germany
[3] Johannes Gutenberg University Medical Center Mainz, BiomaTiCS - Biomaterials, Tissues and Cells in Science, Mainz, Rhineland-Palatinate, Germany
Email: sandranezi@web.de

For the reconstruction of damaged mucosal tissues in medicine an autologous graft is the medium of choice. The manufacturing of an applicable transplant requires a profound vascularization that guarantees the transplant’s nourishment. In our previous works the generation of a tissue equivalent using human oral fibroblasts (HOF), epithelial cells and HDMEC on a collagen membrane was successful. The formation of capillary-like tubes was limited to the superficial layers of the membrane, which leads to a nourishment deficiency in the profound areas. The activation and release of the two TF-isoforms (flTF in microparticles and soluble asTF) by HOF during the prevascularization of such tissue equivalents is currently unclear. The aim of our studies was to identify the TF-isoforms’ pro-angiogenic effects on HDMEC

and the accompanied dynamics of TF secretion by fibroblasts.

To isolate the asTF- from the flTF-isoform within the fibroblasts supernatants, high-speed centrifuation was used. The split-up supernatants of fibroblasts were added to HDMEC-monocultures to investigate the according effect on the HDMEC`s vessel formation. The vessel formation within 3D co-cultures of fibroblasts and HDMECs were assessed through laser-scanning microscopy.

The asTF bearing supernatants induced an increased vessel formation, while the flTF containing supernatants caused less vessel formation in HDMEC monocultures. We assume that especially the soluble asTF-isoform induces proangiogenic effects.

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**DMTF1β-induced autophagy enhances migration and invasion of breast and prostate cancer cells**

Authors:
**N. Niklaus[1], M. Humbert[1], M. P. Tschan[1]**

[1] University of Bern, Divison of Experimental Pathology, Bern, Switzerland
Email: nicolas.niklaus@pathology.unibe.ch

The cyclin D binding Myb like Transcription Factor 1 (*DMTF1)* is a haploinsufficient tumor suppressor in various malignancies. However, alternative splicing generates a truncated, dominant negative isoform of full-length DMTF1α named DMTF1β. Analyzing a panel of breast and prostate cancer cell lines, we found an association of high DMTF1β mRNA and protein expression with a more aggressive phenotype. Knocking down DMTF1β in these cancer cells resulted in a reduced migration rate. In contrast, ectopic DMTF1β expression in MCF7 breast cancer cell with low DMTF1β expression led to a significantly increased migration in wound healing and transwell migration assays. Since autophagy was also shown to support cellular migration, we tested if DMTF1β activates autophagy. Indeed, expression levels of key autophagy (ATG) genes were significantly increased in DMTF1β overexpressing MCF7 breast cancer cells. In addition, knocking out DMTF1 in MCF7 cells significantly decreased autophagic flux that could be rescued by reintroducing DMTF1β only. Importantly, inhibiting autophagy at initiation stages using pharmacological and genetic inhibition of ULK1 and VPS34 led to decreased migration of DMTF1β rescued cells. Interestingly, inhibiting fusion of the autophagosome to the lysosome did not block the DMTF1β-mediated increase of migration. Together, our data clearly suggest that DMTF1β-induced migration of various breast and prostate cancer cells depends on early autophagy.

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**Osh Proteins Control Nanoscale Lipid Organization Necessary for PI(4,5)$P_2$ Synthesis**

Authors:
**T. Nishimura[1, 2], C. J. Stefan[1]**

[1] UCL, MRC-LMCB, London, United Kingdom
[2] The Francis Crick Institute, London, United Kingdom
Email: taki.nishimura@crick.ac.uk

Plasma membrane (PM) identity and organization are created by the selective delivery of lipids by lipid transfer proteins. In particular, members of a conserved family of lipid transfer proteins, the oxysterol-binding protein-related proteins (ORP/Osh), are crucial for the establishment and maintenance of PM lipid composition. ORP/Osh family members transfer newly synthesized lipids, including sterol and phosphatidylserine (PS), from the endoplasmic reticulum to the PM in exchange for phosphatidylinositol 4-phosphate (PI4P). On the other hand, PI4P may be converted to phosphatidylinositol 4, 5-bisphosphate (PI4,5$P_2$) by phosphatidylinositol 4-phosphate 5-kinase (PIP5K) at the PM. Thus, PI4P has two important

roles at the PM: for ORP/Osh-dependent lipid exchange and as a precursor for $PI4,5P_2$ synthesis. However, what determines these different PI4P fates remains unclear. Using lipidomic, biochemical, and biophysical approaches, we show that Osh proteins establish the mechano-chemical and biophysical membrane properties essential for PIP5K activity. The Osh members create an unsaturated phospholipid nanoscale environment that drives PIP5K activity and $PI4,5P_2$ synthesis that ultimately controls global PM organization and dynamics. Our new findings demonstrate that the nanoscale PM lipid environment organized by the Osh proteins determines PI4P fate and provide important new insight into mechanisms for $PI4,5P_2$ homeostasis.

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**Reducing Insulin/IGF1 Signaling Protects Against Non-Cell Autonomous Vesicle Rupture Caused by SNCA/α-synuclein Spreading**

Authors:
**C. A. Sandhof[1, 2], S. O. Hoppe[1, 2], S. Druffel-Augustin[1, 2], C. Gallrein[3], J. Kirstein[3], C. Voisine[4], C. Nussbaum-Krammer[1, 2]**

[1] Heidelberg University, Center for Molecular Biology of Heidelberg University (ZMBH), Heidelberg, Germany
[2] German Cancer Research Center (DKFZ), Heidelberg, Germany
[3] Leibniz-Institute for Molecular Pharmacology (FMP) im Forschungsverbund Berlin e.V., Berlin, Germany
[4] Northeastern Illinois University, Department of Biology, Chicago, Illinois, United States of America
Email: c.nussbaum@zmbh.uni-heidelberg.de

Aging is associated with a decline of cellular proteostasis, giving rise to protein misfolding diseases, such as Alzheimer’s disease (AD) or Parkinson’s disease (PD). These diseases often exhibit a complex pathology involving non-cell-autonomous proteotoxic effects, which are still poorly understood. Using *Caenorhabditis elegans *we investigated how local protein misfolding is affecting neighboring tissues and show that misfolded PD-associated SNCA/α-synuclein is accumulating in highly dynamic endo-lysosomal vesicles. Irrespective of whether being expressed in muscle cells or dopaminergic neurons, accumulated proteins were transmitted into the hypodermis with increasing age, indicating that epithelial cells might play a role in remote degradation when the local lysosomal degradation capacity is overloaded. Cell biological and genetic approaches revealed that inter-tissue dissemination of SNCA was regulated by endo- and exocytosis and basement membrane remodeling. Transferred SNCA conformers were, however, inefficiently cleared and induced endo-lysosomal membrane permeabilization. Remarkably, reducing insulin/IGF1 signaling provided protection by maintaining endo-lysosomal integrity. This study suggests that the degradation of lysosomal substrates is coordinated across tissues in metazoan organisms and implies that restoring endo-lysosomal function not only in cells with pathological inclusions, but also in apparently unaffected cells might help to halt disease progression.

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**Functions of the transcription factor SRF in pericytes of the murine neonatal retina in physiological and pathological angiogenesis**

Authors:
**M. Orlich[1, 2], R. D. Hurtado[3], R. Adams[3], A. Nordheim[1, 2]**

[1] University of Tuebingen, Interfaculty Institute of Cell Biology, Department Cell Biology, Tübingen, Baden-Württemberg, Germany
[2] International Max Planck Research School, „From Molecules to Organisms", Tübingen, Baden-Württemberg, Germany
[3] Max Planck Institute for Molecular Biomedicine, Department of Tissue Morphogenesis, Münster, North

Rhine-Westphalia, Germany
Email: michael.orlich@uni-tuebingen.de.de

Serum Response Factor (SRF) is a ubiquitously expressed, essential transcription factor known to regulate the transcription of about 1000 genes. Recent work performed in our group showed that ablation of SRF in endothelial cells (ECs) leads to formation of hemorrhages in the neonatal murine brain and microaneurysms in the neonatal murine retina. Despite the established role of SRF in the vascular system, no functional analyses of SRF activity has so far been performed in pericytes. Pericytes are essential cellular components of the microvasculature with the main task of providing structural support for ECs. They play important roles in angiogenesis, vessel stabilization and homeostasis. To investigate any potential contributions of SRF to postnatal pericyte development in the murine retina, we performed phenotypic characterization of existing *Srf-flex1::Pdgfbr-CreERT2* mice, which permit conditional deletion within the *Srf* gene locus in pericytes. Our observations of the vascular bed showed a decrease in the vessel area and branching points in mice with SRF-ablated pericytes. Moreover, further analyses showed local breakdowns of the blood retina barrier and massive depositions of collagen IV, arguing for instable blood vessels. Interestingly, the pericyte coverage of blood vessels is significantly reduced and – at later stages of postnatal development - artery venous malformations (AVMs) can be observed. Overall, our analysis indicates an important role of SRF in pericytes.

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**Structural basis and *in situ* visualization of p62/SQSTM1 polymers by electron microscopy**

Authors:
**S. Mostafavi[1], S. Berkamp[1], J. Ortiz[1], S. Mortensen[1], C. Sachse[1]**

[1] Forschungszentrum Jülich GmbH, Ernst Ruska-Centrum für Mikroskopie und Spektroskopie mit Elektronen, ER-C-3: Strukturbiologie, Juelich, North Rhine-Westphalia, Germany
Email: j.ortiz@fz-juelich.de

Selective autophagy is the mechanism by which large molecular cargos of various sizes from molecules, organelles to pathogens are specifically sequestered for degradation in the lysosome. We determined a high resolution cryo-EM structure of p62 filaments revealing the molecular basis of polymer formation and relate these findings with tomographic reconstructions of cellular p62 bodies. It has recently emerged that autophagy receptors support the selective sequestration and transport of specifically marked cargo to nascent autophagosomes. In this process, autophagy receptors recognize ubiquitylated cargo and interact with Atg8/LC3 to bring the cargo-receptor complex in contact with the autophagosomal membrane.

The structural details of cargo and receptor assembly giving rise to autophagic vesicles remain to be elucidated. Recently, we showed that autophagy receptor p62/SQSTM-1 assembles into flexible helical polymers. Here, we used recent advances in cryo-EM to generate a near-atomic resolution helical reconstruction of p62-PB1 filaments. Using EM based structure elucidation we show that large oligomeric and polymeric cargo receptor complexes are the major component of p62 bodies inside of cells and define the architectural requirements of these assemblies for their cargo uptake and degradation using cellular turnover assays. The organization of small receptor proteins into helical polymers provides a fundamental architectural scaffold enabling cargo encapsulation.

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**Impact of sorting nexin PaATG24 on autophagy, aging and development**

Authors:
**L. Schürmanns[1], A. Hamann[1], V. Henkel[1], M. Brunner[1], H. D. Osiewacz[1]**

[1] Goethe University, Institute for Molecular Biosciences, Frankfurt, Germany
Email: osiewacz@bio-uni-frankfurt.de

# Abstracts - Poster

Proper development of biological systems and the ability to adapt to changing environmental situations is strongly controlled by a complex network of molecular pathways. Impairments of these pathways lead to dysfunctions, disease and aging. We use the filamentous fungus *Podospora anserina *to unravel the molecular basis of aging and development. Recently, we demonstrated a key role of autophagy in the control of cellular homeostasis with a strong impact on lifespan. Here we report investigations of the role of sorting nexins which are of fundamental importance for selective autophagy. We focused on PaATG24, an ortholog of human SNX4. Deletion of *PaAtg24 *leads to a significantly reduced growth rate, male fertility, and lifespan. These phenotypical features go along with pronounced impairments in  mitophagy, pexophagy, and non-selective autophagy. The vacuoles of the mutant are significantly smaller than those of the wild type, suggesting that vacuolar membrane trafficking is impaired. Moreover, a strong age-dependent accumulation of peroxisomes is observed in the absence of PaATG24. Now the question arises whether the mutant’s severe phenotype is caused by deficient pexophagy or by other impairments like the strongly decreased ability to degrade mitochondria by mitophagy. In order to test this possibility, a mutant strain devoid of PaATG37, which is needed for pexophagy, has been constructed. The phenotype of this mutant will be compared to that of the *PaAtg24 *deletion strain.

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**Deciphering the mechanisms underlying the myoclonic seizures caused by the loss of autophagy in the brain**

Authors:
**M. Overhoff[1], E. De Bruyckere[1], A. Negrete-Hurtado[1], N. Kononenko[1]**

[1] CECAD Excellence Center, University of Cologne, Institute for Genetics, Köln, North Rhine-Westphalia, Germany
Email: m.overhoff@uni-koeln.de

Macroautophagy is a conserved process to remove damaged organelles and protein aggregates, highly important in neurons because of their polarized structure and long live span. Accumulation of autophagosomes hallmarks the pathology of neurodegenerative disorders, while neuronal-confined loss of several AuTophaGy (ATG)-related proteins, including the ones mediating the lipid LC3 conjugation to autophagosome membrane, causes neurodegeneration. We find that mice conditionally lacking the components of LC3 lipid conjugation machinery such as ATG5 or ATG16L1 in neurons suffer from late-onset myoclonic seizures. Behavioral assessment reveals that myoclonus is startle-induced, while histological analysis highlights a loss of interneurons, but not the principal excitatory cells in the cortex of these mice. These alterations on the network levels are accompanied by selective changes in protein aggregates positive for Sequestosome1 (SQSTM1/p62) in two classes of neurons. We find that while inhibitory neurons undergo neurodegeneration in autophagy deficient mice, they fail to accumulate p62-positive aggregates, whereas p62-positive inclusions were abundant in excitatory autophagy-deficient cells. Our data indicate that autophagy prevents myoclonic seizures via maintaining the survival of GABAergic neurons in a p62-independent manner. Currently, we are investigating the precise molecular mechanism by which autophagy regulates the function of interneurons and prevents epileptogenesis.

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**Rapamycin-induced peroxisome degradation (R.I.P.): a novel degradation mode for peroxisomes.**

Authors:
**F. Boutouja[1], C. Reidick[1], H. W. Platta[1]**

[1] Ruhr-Universität Bochum, Biochemie Intrazellulärer Transportprozesse (B.I.T.), Bochum, North Rhine-Westphalia, Germany
Email: harald.platta@rub.de

The turn-over of peroxisomes is known to be mediated via pexophagy, which is a selective autophagic degradation pathway. In *Saccharomyces cerevisiae* it is induced when the cells are shifted from oleate-medium, where peroxisomes are essential for viability, to glucose-containing pexophagy-medium, where peroxisomes are not essential. In contrast, bulk autophagy has been regarded as a non-selective pathway that degrades cytosolic components and has been thought to be exclusively induced by the addition of the mTOR-inhibitor rapamycin. While the classical pexophagy depends on the pexophagy-receptor Atg36 and an intact actin-cytoskeleton, bulk autophagy occurs independently from both factors. In this study, we demonstrate, that rapamycin also induces a novel mode of peroxisome degradation that is distinct from classical pexophagy as well as bulk autophagy with respect to the involvement of Atg36, the cytoskeleton and myosin-related motor protein Myo2.

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**Peroxisomal function is regulated by signaling factors at the vacuole.**

Authors:
**C. Reidick[1], F. Boutouja[1], H. W. Platta[1]**

[1] Ruhr-Universität Bochum, Biochemie Intrazellulärer Transportprozesse (B.I.T.), Bochum, North Rhine-Westphalia, Germany
Email: harald.platta@rub.de

The yeast vacuole is a central component for cellular homeostasis. It is the destination of endocytosis and autophagy pathways and enables the recycling of macromolecules. An intensively studied selective autophagy pathway is pexophagy, which describes the degradation of peroxisomes in the vacuolar lumen. Here we demonstrate a novel functional link between peroxisomes and the vacuole. We identify a subset of vacuolar proteins to be essential for the import of PTS1-proteins into the matrix of peroxisomes. The mutation of these factors triggers the polyubiquitination of the PTS1-import receptor Pex5 and leads to a block of matrix protein import and peroxisomal function.

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**Vac8 Controls Vacuolar Membrane Dynamics during Different Autophagy Pathways in *Saccharomyces cerevisiae*.**

Authors:
**F. Boutouja[1], C. M. Stiehm[1], C. Reidick[1], T. Mastalski[1], R. Brinkmeier[1], F. El Magraoui[1], H. W. Platta[1]**

[1] Ruhr-Universität Bochum, Biochemie Intrazellulärer Transportprozesse (B.I.T.), Bochum, North Rhine-Westphalia, Germany
Email: harald.platta@rub.de

The yeast vacuole is a vital organelle, which is required for the degradation of aberrant intracellular or extracellular substrates and the recycling of the resulting nutrients as newly available building blocks for the cellular metabolism. We found that non-selective bulk autophagy of cytosolic proteins as well as the selective autophagic degradation of peroxisomes (pexophagy) and ribosomes (ribophagy) was dependent on the armadillo repeat protein Vac8 in *Saccharomyces cerevisiae*. Moreover, we showed that pexophagy and ribophagy depended on the palmitoylation of Vac8. In contrast, we described that Vac8 was not involved in the acidification of the vacuole nor in the targeting and maturation of certain biosynthetic cargoes, like the aspartyl-protease Pep4 (PrA) and the carboxy-peptidase Y (CPY), indicating a role of Vac8 in the uptake of selected cargoes. In addition, we found that the hallmark phenotype of the *vac8*Δ strain, namely the characteristic appearance of fragmented and clustered vacuoles, depended on the growth conditions. This fusion defect observed in standard glucose medium can be complemented by the replacement with oleic acid or glycerol medium. This complementation of vacuolar morphology also

partially restores the degradation of peroxisomes. In summary, we found that Vac8 controlled vacuolar morphology and activity in a context- and cargo-dependent manner.

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**Vps10-mediated targeting of Pep4 determines the activity of the vacuole in a substrate-dependent manner.**

Authors:
**F. Boutouja[1], C. M. Stiehm[1], T. Mastalski[1], R. Brinkmeier[1], C. Reidick[1], F. El Magraoui[1], H. W. Platta[1]**

[1] Ruhr-Universität Bochum, Biochemie Intrazellulärer Transportprozesse (B.I.T.), Bochum, North Rhine-Westphalia, Germany
Email: harald.platta@rub.de

The vacuole is the hydrolytic compartment of yeast cells and has a similar function as the lysosome of higher eukaryotes in detoxification and recycling of macromolecules. We analysed the contribution of single vacuolar enzymes to pexophagy and identified the phospholipase Atg15, the V-ATPase factor Vma2 and the serine-protease Prb1 along with the already known aspartyl-protease Pep4 (Proteinase A) to be required for this pathway. We also analysed the trafficking receptor Vps10, which is required for an efficient vacuolar targeting of the precursor form of Pep4. Here we demonstrate a novel context-dependent role of Vps10 in autophagy. We show that reduced maturation of Pep4 in a VPS10-deletion strain affects the proteolytic activity of the vacuole depending on the type and amount of substrate. The VPS10-deletion has no effect on the degradation of the cytosolic protein Pgk1 via bulk autophagy or on the degradation of ribosomes via ribophagy. In contrast, the degradation of an excess of peroxisomes via pexophagy as well as mitochondria via mitophagy was significantly hampered in a VPS10-deletion strain and correlated with a decreased maturation level of Pep4. The results show that Vps10-mediated targeting of Pep4 limits the proteolytic capacity of the vacuole in a substrate-dependent manner.

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**SUMOylation promotes autophagy-dependent α-synuclein clearance in a yeast model of Parkinson's disease**

Authors:
**B. Popova[1], H. Shahpasand-Kroner[1], A. Kleinknecht[1], G. H. Braus[1]**

[1] Universität Göttingen, Department of Molecular Microbiology and Genetics, Institute of Microbiology and Genetics, Göttingen, Germany
Email: bpopova@gwdg.de

Parkinson’s disease is associated with progressive loss of dopaminergic neurons from the *substantia nigra*. The pathological hallmark of the disease is accumulation of intracytoplasmic inclusions known as Lewy bodies (LBs) that consist mainly of post-translationally modified forms of α-synuclein. Whereas phosphorylation is one of the major modifications of α-synuclein in LBs, sumoylation has recently been described. We examined the interplay between these modifications and their impact on cell growth and inclusion formation in yeast. We found that α-synuclein is sumoylated *in vivo* at the same sites in yeast as in human cells. Impaired sumoylation resulted in reduced yeast growth combined with an increased number of cells with inclusions suggesting that this modification plays a protective role. In addition, inhibition of sumoylation prevented autophagy-mediated aggregate clearance. A defect in α-synuclein sumoylation could be suppressed by serine-129 phosphorylation by the human G protein-coupled receptor kinase 5 (GRK5) in yeast. Phosphorylation reduced foci formation, alleviated yeast growth inhibition and partially rescued autophagic α-synuclein degradation along with the promotion of proteasomal degradation. These findings suggest a complex interplay between sumoylation

and phosphorylation in α-synuclein aggregate clearance, which may open new horizons for the development of therapeutic strategies for Parkinson’s disease.

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**A novel lysosomal tether of the TSC complex**

Authors:
**M. T. Prentzell[1], U. Rehbein[2, 3], M. Cadena Sandoval[2], C. Scheldeman[4], F. Reuter[1], S. Pusch[5, 6], I. van ,t Land-Kuper[2, 3], B. Berdel[1], B. Holzwarth[7], L. Corbett[2, 3], M. Bockwoldt[8], A. von Deimling[5, 6], I. Heiland[8], S. Trump[9, 10], P. A. de Witte[4], R. Baumeister[7, 11, 12], C. A. Opitz[1, 13], K. Thedieck[2, 3, 14]**

[1] German Consortium of Translational Cancer Research (DKTK), Brain Cancer Metabolism Group, German Cancer Research Center (DKFZ), Heidelberg, Germany
[2] Laboratory of Pediatrics, Section Systems Medicine of Metabolism and Signaling, University of Groningen, University Medical Center Groningen, Groningen, Netherlands
[3] Faculty VI, School of Medicine and Health Sciences, Department of Neurosciences, Carl von Ossietzky University of Oldenburg, Oldenburg, Germany
[4] Laboratory for Molecular Biodiscovery, Department of Pharmaceutical and Pharmacological Sciences, University of Leuven, Leuven, Belgium
[5] German Consortium of Translational Cancer Research (DKTK), Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[6] Department of Neuropathology, Institute of Pathology, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany
[7] Department of Bioinformatics and Molecular Genetics, Faculty of Biology, University of Freiburg, Freiburg, Germany
[8] Department of Arctic and Marine Biology, UiT The Arctic University of Norway, Tromsø, Norway
[9] Department of Environmental Immunology, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany
[10] Charité - Universitätsmedizin Berlin and Berlin Institute of Health, Berlin, Germany
[11] BIOSS Centre for Biological Signalling Studies, University of Freiburg, Freiburg, Germany
[12] ZBMZ Centre for Biochemistry and Molecular Cell Research (Faculty of Medicine), University of Freiburg, Freiburg, Germany
[13] Neurology Clinic and National Center for Tumor Diseases, University Hospital of Heidelberg, Heidelberg, Germany
[14] Institute of Biochemistry and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innsbruck, Austria
Email: t.prentzell@dkfz.de

The tuberous sclerosis protein complex (TSC) is a central signalling node that suppresses the key metabolic master regulator mechanistic target of rapamycin complex 1 (mTORC1) at its central signaling platform, the lysosome. While the proteins that recruit mTORC1 to lysosomes are known, the lysosomal tether for the TSC has so far remained elusive.

In the present study, we identified the molecular component that tethers the TSC to lysosomes. Thus, it is fundamental for the TSC’s function as an mTORC1 suppressor. In agreement, the newly identified lysosomal tether prevents mTORC1-driven cell motility, and *in vivo* suppression results in an epilepsy phenotype, reminiscent of TSC.

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# Abstracts - Poster

**Mitochondria regulate lysosomal autophagic capacity independently of mitophagy**

Authors:
**N. Raimundo**[1]

[1] Universitätsmedizin Göttingen, Institut für Zellbiochemie, Göttingen, Lower Saxony, Germany
Email: nuno.raimundo@med.uni-goettingen.de

Mitochondrial signaling following respiratory chain deficiency initially stimulates autophagy in an attempt to promote mitophagy of the affected organelles. However, when the mitochondrial defect persists over time, as in mitochondrial diseases, the autophagy pathway becomes stalled, and autophagosomes accumulate. The mechanisms underlying these effects remain unclear.

Mitochondrial signaling mediated by ROS controls the autophagic flux by regulating the activity of a key lysosomal $Ca^{2+}$ channel, MCOLN1/TRPML1. The release of $Ca^{2+}$ to the cytoplasm via MCOLN1 is required for autophagy.

Here, we show that MCOLN1 is activated under acute mitochondrial malfunction, in a AMPK-dependent manner, but shut down under chronic mitochondrial respiratory chain deficiency. The activity of MCOLN1 is regulated by lysosomal $PI(3,5)P_2$, which is synthesized in the lysosomal membrane by the enzyme PIKFYVE. The activity of PIKFYVE is in turn regulated by AMPK. In chronic mitochondrial malfunction, AMPK-MCOLN1 signaling is down, and lysosomes are impaired. Directly stimulation of MCOLN1 or of AMPK signaling rescues lysosomal function and restore the autophagic flux, in a PIKFYVE-dependent manner.

These results suggest that under acute mitochondrial malfunction the cells promote the removal of damaged mitochondria to restore the network to basal state, but under chronic mitochondrial malfunction the cells inhibit the last steps of autophagy to avoid a complete removal of mitochondria.

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**A novel deep-intronic *de novo WDR45*/WIPI4 mutation causes impaired selective autophagy in BPAN**

Authors:
**S. Reich**[1], **B. Roeben**[1, 2], **K. Sporbeck**[3, 4], **D. Schüssele**[3], **M. Haas**[3], **B. Bender**[5], **B. Schulte**[6], **R. Schüle**[1, 2], **T. Proikas-Cezanne**[3, 4], **M. Synofzik**[1, 2]

[1] University of Tübingen, Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research, Tübingen, Germany
[2] University of Tübingen, German Research Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany
[3] University of Tübingen, Interfaculty Institute of Cell Biology, Department of Molecular Biology, Tübingen, Germany
[4] International Max Planck Research School, 'From Molecules to Organism', Tübingen, Germany
[5] University of Tübingen, Department of Neuroradiology, Tübingen, Germany
[6] CeGaT GmbH, Center for Genomics and Transcriptomics, Tübingen, Germany
Email: selina.reich@uni-tuebingen.de

Mutations in the *WDR45* gene, encoding the human WIPI4 beta-propeller protein with an important role in autophagy, cause “Beta-propeller Protein-associated Neurodegeneration” (BPAN). While an increasing number of individuals with mutations in *WDR45* has been identified, knowledge of the underlying mutational spectrum and mechanistic insights of impaired autophagy are limited.

Here we report the first deep-intronic *de novo* mutation in *WDR45* (splice, c.235+159C>G) which was found by a phenotypically driven sequencing approach leveraging the unique MRI features of BPAN. mRNA

analysis of the splice effect revealed an aberrant *WDR45* transcript resulting in a frameshift with significantly reduced *WDR45* mRNA levels. We next studied this loss-of-function mutation in patient derived fibroblasts as a paradigmatic model to elucidate the process of autophagy underlying BPAN. Altered LC3-I and LC3-II levels confirmed a block in autophagic flux. Automated image-based analysis unravelled a decrease of WIPI2-positive autophagosomal membranes. Likewise, despite overall increased p62 levels, less WIPI2 colocalized with the autophagy cargo receptor p62, suggesting that selective autophagy is compromised in BPAN fibroblasts.

In sum, our results indicate that (i) identifying *de novo* variations in the deep-intronic space might allow molecular diagnosis in so far unsolved BPAN/NBIA patients, and (ii) that deficits in particular in selective autophagy may present a key mechanism causative for BPAN.

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**Insights into pathophysiology of SPG48**

Authors:
**M. Khundadze[1], F. Ribaudo[1], A. Hussain[1], J. Rosentreter[1], S. Nietzsche[2], M. Thelen[3], D. Winter[3], B. Hoffmann[4], A. A. Muhammad[1], T. Hermann[1], C. de Heus[5], E. - M. Piskor[6], C. Kosan[6], P. Franzka[1], L. von Kleist[7], T. Stauber[7], J. Klumperman[5], M. Damme[8], T. Proikas-Cezanne[9], C. Hübner[1]**

[1] Friedrich-Schiller-University Jena,University Hospital Jena, Institute of Human Genetics, Jena, Germany
[2] Friedrich-Schiller-University Jena,University Hospital Jena, Electron Microscopy Center, Jena, Thuringia, Germany
[3] University of Bonn, Institute for Biochemistry and Molecular Biology, Bonn, North Rhine-Westphalia, Germany
[4] Friedrich-Schiller-University Jena,University Hospital Jena, Biomolecular Photonics Group, Jena, Thuringia, Germany
[5] University Medical Center Utrecht, Center for Molecular Medicine, Department of Cell Biology, Utrecht, Netherlands
[6] Friedrich-Schiller-University Jena, Institute of Biochemistry and Biophysics, Jena, Thuringia, Germany
[7] Freie Universität Berlin, Institute of Chemistry and Biochemistry, Berlin, Berlin, Germany
[8] Christian-Albrechts-University Kiel, Institute of Biochemistry, Kiel, Schleswig-Holstein, Germany
[9] Eberhard Karls University Tübingen, Department of Molecular Biology, Tübingen, Baden-Württemberg, Germany
Email: Federico.Ribaudo@med.uni-jena.de

Hereditary spastic paraplegia (HSP) is characterized by a length-dependent degeneration of corticospinal axons resulting in a progressive spastic gait disorder. With more than 80 genetically distinct forms (SPGs), some of which showing additional clinical manifestations, HSP is highly heterogeneous. The subtype SPG48 is associated with mutations in the zeta subunit of the adaptor protein complex 5 (AP5). Here, we report the first mouse model for SPG48, which shows an age-dependent degeneration of corticospinal tract axons and a late-onset motor phenotype compatible with HSP. Our analysis of knockout (KO) mouse embryonic fibroblasts (MEFs) supports a trafficking defect from late endosomes to the TGN and reveals structural alterations of the Golgi apparatus. Further, we provide evidence that lysosomal compartment functions are also impaired in SPG48 KO MEFs. Both, the autophagic flux, and autophagic lysosome reformation (ALR) are significantly reduced in KO MEFs upon starvation. *In vivo*, the numbers of autophagosomes and autolysosomes were found to be significantly increased in Purkinje neurons already at young stages, and based on our *in vitro* data we conclude that this should reflect a block in the autophagy flux. In summary, we suggest that loss of AP5 function blocks autophagy and ALR, thus leads to the aberrant accumulation of autophagic cargo and axon degeneration.

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# Abstracts - Poster

**A battle for survival – the interplay between autophagy and *S. aureus* infection**

Authors:
**A. K. Riebisch[1, 2], Y. Neumann[1, 2], S. Bruns[1, 2], I. Schmitz[1, 2]**

[1] Otto-von-Guericke-University, Institute of Molecular and Clinical Immunology, Magdeburg, Saxony-Anhalt, Germany
[2] Helmholtz Centre for Infection Research, Systems-oriented Immunology and Inflammation Research Group, Braunschweig, Lower Saxony, Germany
Email: anna.riebisch@helmholtz-hzi.de

The human pathogen *Staphylococcus aureus *is the leading cause of hospital-acquired infections and its multidrug resistance makes a successful treatment more and more difficult. In 2016, our group showed for the first time the induction of selective autophagy by *S. aureus *infection, its escape from the autophagosome and proliferation in the cytoplasm, via live cell imaging. Additionally, p38 MAPK activation was observed, which is a so far unrecognised bacterial strategy to manipulate the autophagic pathway. The activation of the p38 MAPK could be due to either the bacteria triggering a host cell receptor or by direct activation by bacterial effector proteins. Here, we analyse bacterial and host cell signal transducers regarding their impact on p38 MAPK and autophagy induction. Surprisingly, the surface expressed kinase PknB, which has as MAP Kinase-like activity is not involved in p38 MAPK activation and autophagy induction. Due to this observation, we will perform a global transcriptome analysis to identify proteins that might be relevant for the inhibition of the fusion of the autophagosome and the lysosome as well as the escape of *S. aureus *of the autophagosome. A further understanding of the molecular mechanisms of this host-pathogen interaction will facilitate new strategies to combat this important pathogen.

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**Protein-interaction network analysis of *Salmonella* infected HeLa cells.**

Authors:
**J. Rieser[1], J. Ackermann[1], I. Koch[1]**

[1] Goethe Universität, Moleculare Bioinformatics, Frankfurt, Hesse, Germany
Email: rieser@bioinformatik.uni-frankfurt.de

*Salmonella* Typhimurium provokes gastroenteritis and typhoid fever and causes many thousands death every year. The bacterium invades a human cell in a *Salmonella*-containing vacuole but it can escape into the cytosol. These cytosolic *Salmonella* cells are tagged with a dense ubiquitin coat from the host cell, which lead to the formation of a phagophore and autophagy.

The changes of the expression level of phosphorylated proteins in *Salmonella*-infected and uninfected HeLa cells has been investigated by Rogers *et al* [1] and the changes in ubiquitinated proteins by Fiskin *et al* [2].

We used the proteins of both datasets to search for interactions in three public databases to build a protein-protein interaction network. After the analysis of basic parameters, we have cluster the network with the Girvan-Newman algorithm and for each cluster performed a GO-enrichment. For a better overview we have created a GO-interaction network and mapped the proteins on the nodes with their regulated sites. Furthermore, with the information from Reactome and KEGG we have built a pathway network to investigate the up- and downstream pathways from proteins with highly regulated modification sites.

[1] Rogers, L. D., *et al. Science Signal.* 4.191 (2011): rs9-rs9.
[2] Fiskin, E., *et al. Molecular Cell* 62.6 (2016) 967-981.

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**Amyloidogenic interactions in regulation of autophagy**

Authors:
**N. S. Roodbaraki[1], N. Olrichs[1], B. Popova[2], G. H. Braus[2], B. Helms[1], D. Kaloyanova[1]**

[1] Utrecht University, Department of Biochemistry & Cell biology, Faculty of Veterinary medicine, Utrecht, Netherlands
[2] Universität Göttingen, Department of Molecular Microbiology and Genetics, Institute of Microbiology and Genetics, Göttingen, Germany
Email: abstract@eventclass.org

Golgi-Associated plant Pathogenesis Related-protein 1 (GAPR-1) functions as a negative regulator of autophagy. The molecular mechanism of this regulation involves retention of Beclin 1, a major autophagy-related protein, at the Golgi. A Beclin 1-derived peptide that corresponds to the potential binding interface efficiently induces autophagy by competing with GAPR-1/Beclin 1 interaction. However, so far a direct interaction between GAPR-1 and Beclin 1 could not be observed. Therefore we hypothesized that GAPR-1/Beclin 1 interactions are based on oligomeric and/or amyloidogenic properties of both proteins. In this study, humanized yeast model system is used to study the amyloidogenic propensity of GAPR-1 and Beclin 1 and to investigate GAPR-1/Beclin 1 interactions. Protein segregation into Fluorescent Foci (FF) in the yeast cytosol has been shown to correlate with the propensity of a protein to form amyloid. Overexpression of  GAPR-1 and Beclin 1 resulted in formation of  FF in cytosol over time. Interestingly, in co-expression experiments the formation of Beclin 1 FF was inhibited and the number of GAPR-1 FF per cell were reduced, suggesting that the two proteins interact. These effects were efficiently reversed when mutant GAPR-1 or Beclin 1 lacking the suggested binding sites were used. Direct interaction between both proteins was confirmed by bimolecular fluorescence complementation analysis and by Beclin 1 re-localization to the GAPR-1 positive structures using plasma membrane-targeted GAPR-1. Finally, in a proof-of-principle experiment we show that  Beclin 1 peptide can efficiently reverse the formation of FF in co-expression experiments, suggesting that GAPR-1/Beclin 1 interaction was interfered by the peptide. Together our results suggest that amyloidogenic interactions are involved in regulation of autophagy by regulating the interaction between GAPR-1 and Beclin 1.

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**Structural basis and in cellulo visualization of p62/SQSTM1 polymers by electron microscopy**

Authors:
**C. Sachse[1]**

[1] Forschungszentrum Jülich, Ernst-Ruska Centre 3 / Structural Biology, Jülich, North Rhine-Westphalia, Germany
Email: c.sachse@fz-juelich.de

Selective autophagy is the mechanism by which large molecular cargos of various sizes from molecules, organelles to pathogens are specifically sequestered for degradation in the lysosome. We determined a high resolution cryo-EM structure of p62-PB1 filaments revealing the molecular basis of polymer formation and relate these findings with tomographic reconstructions of cellular p62 bodies. It has recently emerged that autophagy receptors support the selective sequestration and transport of specifically marked cargo to nascent autophagosomes. In this process, autophagy receptors recognize ubiquitylated cargo and interact with Atg8/LC3 to bring the cargo-receptor complex in contact with the autophagosomal membrane. The structural details of cargo and receptor assembly giving rise to autophagic vesicles remain to be elucidated. Recently, we showed that autophagy receptor p62/SQSTM-1 assembles into flexible helical polymers. Here, we used recent advances in cryo-EM to generate a near-atomic resolution helical reconstruction of p62-PB1 filaments. Using EM based structure elucidation we show that large oligomeric and polymeric cargo receptor complexes are the major component of p62 bodies inside of cells and define the architectural

requirements of these assemblies for their cargo uptake and degradation using cellular turnover assays. The organization of small receptor proteins into helical polymers provides a fundamental architectural scaffold enabling cargo encapsulation.

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**Investigation of GABARAP's secretion by proximity labelling of extracellular vesicles**

Authors:
**J. L. Sanwald[1, 2], I. M. Simons[1, 2], J. Dobner[1, 2], C. Behrends[3], S. Hoffmann[2], D. Willbold[1, 2]**

[1] Heinrich-Heine-Universität Düsseldorf, Institut für Physikalische Biologie, Düsseldorf, North Rhine-Westphalia, Germany
[2] Forschungszentrum Jülich, Institute of Complex Systems, Structural Biochemistry (ICS-6), Jülich, North Rhine-Westphalia, Germany
[3] Ludwig-Maximilians-Universität München, Munich Cluster of Systems Neurology (SyNergy), München, Bavaria, Germany
Email: j.sanwald@fz-juelich.de

Extracellular vesicles (EVs) represent an emerging field of research and have been shown to be involved in neurodegenerative and age-related diseases. By investigating EV cargo, their potential as biomarkers for monitoring e.g. cancer progression or even their use as possible therapeutics was revealed. However, there is still a lack of a gold standard for in-depth analysis of composition and origin of different EV types. Recent results have shown a connection to autophagy, which not only leads to lysosomal degradation of cytosolic components, but also to the secretion via EVs. We found that GABARAP, a member of the mammalian (m) ATG8 family, is secreted inside of EVs from both culture supernatants of various cell types and human or bovine serum. While the participation of GABARAP during autophagy is well described, the mechanistic background regarding its secretion is largely unknown. Here, we applied an engineered ascorbate peroxidase (APEX2) tagged to GABARAP for the analysis of GABARAPs proxitome inside of EVs. Following biotinylation and streptavidin-based enrichment, proteins co-secreted with GABARAP were identified via mass spectrometry. Our results demonstrate the applicability of APEX2-based proximity labelling for EV samples in general and provide first insights into the protein composition of GABARAP-positive EVs, serving as starting point to identify putative GABARAP ligands having an active role during the secretion or cargo loading of this distinct EV subfraction.

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**Characterization of the Mannose 6-Phosphate independent transport of lysosomal proteins**

Authors:
**P. Sanzà[1], R. van der Welle[1], C. ten Brink[1], J. Klumperman[1]**

[1] UMC Utrecht, Cell biology, Utrecht, Netherlands
Email: p.sanza@umcutrecht.nl

Lysosomes are the primary catabolic compartment of the eukaryotic cells. Lysosomes need a set of constitutive proteins, hydrolases and membrane proteins, that need to be continuously turned over. These proteins can reach lysosomes through direct routes from the trans-Golgi network (TGN) to the endosome or indirectly via the plasma membrane and subsequent endocytosis. The best example of a direct route delivering hydrolases to lysosomes is the Mannose 6-phosphate receptor (MPR)-dependent pathway.

Another example are LAMP-carriers, vesicles containing LAMP1 and LAMP2 but not MPR, which are released at the TGN and delivered to late endosomes. The latter step is regulated by Vps41 and VAMP7 proteins. Vps41 is a component of the HOPS tethering complex that facilitates the fusion between late endosomes and lysosomes, instead VAMP7 is a v-SNARE protein crucial for the fusion step. Although

LAMP-carriers contain Vps41, they do not contain the other members of the HOPS complex. Here, we aim to determine the mechanism responsible for the fusion of LAMP-carriers with late endosomes. Therefore, we investigate the possible link between the tethering protein Vps41 and the SNARE protein VAMP7. Moreover, we aim to determine unknown partners of Vps41 responsible for the fusion of the LAMP-carriers with late endosomes using a proximity-based labelling approach.

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**The pan-Bcl-2 inhibitor gossypol selectively induces cell death in skin cancer cell lines compared to normal (healthy) cells**

Authors:
**L. Scharf[1], C. von Montfort[1], P. Brenneisen[1]**

[1] Heinrich-Heine-University, Biochemistry & Molecular Biology I, Düsseldorf, North Rhine-Westphalia, Germany
Email: lisa.scharf@hhu.de

Gossypol, a polyphenolic aldehyde derived from cottonseed extract, is in focus as a possible anticancer drug or a sensitizer for other chemotherapeutic drugs. It belongs to the group of BH3 mimetic substances interacting with pro-survival proteins of the Bcl-2 protein family. Several studies have shown the pro-apoptotic properties of gossypol in regard to its binding to Bcl-xL and Bcl-2 The treatment of skin cancer and especially the treatment of melanoma patients still remains a challenge due to resistant mechanisms and drug mediated side effects. Skin melanoma A375 and skin squamous cell carcinoma SCL-1 cell lines have higher levels of anti-apoptotic proteins such as Bcl-xL and Bcl-2 compared to melanocytes, keratinocytes and fibroblasts. Based on the different expression patterns, the question arises whether a pan-Bcl-2 inhibitor may affect these tumor cells. In this study, the effect of gossypol was investigated on skin cells with particular attention to the different responses between tumor and normal (healthy) cells as most chemotherapeutic agents cause severe side effects based on the effect on normal (healthy) cells. Preliminary data with gossypol show a dose dependent and selective decrease of cell viability of the skin tumor cells compared to the noncancerous cells. The substance induces apoptosis in A375 melanoma cells supported by PARP cleavage and caspase activity, whereas evidences indicate a necroptotic or autophagic cell death in SCL-1 skin tumor cells.

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**The mitochondrial oxidoreductase Mia40 modulates the toxicity of cytosolic protein aggregates**

Authors:
**A. M. Schlagowski[1], K. Knöringer[1], R. Braun[3], J. M. Herrmann[1]**

[1] TU Kaiserslautern, Cell Biology, Kaiserslautern, Rhineland-Palatinate, Germany
[2] University of Bayreuth, Cell Biology, Bayreuth, Bavaria, Germany
Email: schlagow@rhrk.uni-kl.de

In my work I investigate mitochondrial precursor proteins under proteotoxic stress conditions caused by the accumulation of aggregation-prone poly-Q proteins. The poly-Q stretch I use is derived from a Q97-variant of huntingtin that causes Huntington’s disease in humans.

Applying an in vivo reporter assay which monitors the accumulation of mitochondrial precursor proteins in the cytosol by a growth test, I observed that the expression of Q97 leads to an accumulation of mitochondrial precursors in the cytosol of yeast. The accumulation of these proteins coincides with the toxic effects of Q97.

While *mia40-3* mutants which further increase the levels of mitochondrial precursors in the cytosol also

increase the toxicity of Q97-aggregates, the overexpression of Mia40 largely suppresses Q97-mediated toxicity.

My findings show that overexpression of Mia40 leads to a change in aggregate-formation, aggregate-compartmentalization and tolerance of aggregation-prone poly-Q proteins in the cytosol.

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**TBK1 at the crossroads of autophagy and inflammation**

Authors:
**D. Schlütermann[1], J. Deitersen[1], N. Berleth[1], W. Wu[1], F. Stuhldreier[1], M. J. Mendiburo[1], C. Peter[1], B. Stork[1]**

[1] Heinrich-Heine-Universität, Molecular Medicine I, Düsseldorf, North Rhine-Westphalia, Germany
Email: david.schluetermann@hhu.de

Selective autophagy is an intracellular recycling process for specific cargoes including accumulated proteins, damaged organelles, or invading pathogens. Dysregulation of this process is connected to several diseases like cancer, neurodegenerative and infectious diseases. The ULK1 complex, consisting of ULK1, FIP200/RB1CC1, ATG13 and ATG101, is the main complex for the initiation of autophagy. It is recruited to specific cargo via binding of FIP200/RB1CC1 to autophagic receptors, such as NDP52 or TAX1BP1. In the case of selective autophagy, these receptors also interact with a key regulator of innate immunity, TBK1. Dysregulation of TBK1 activity promotes inflammatory diseases and cancer development, however its regulation during autophagy is poorly understood. We found that loss of components of the ULK1 complex or autophagy in general lead to an accumulation and increased activation of TBK1. Based on this observation we propose two hypotheses: 1) TBK1 inhibition serves as feedback loop to abrogate the induction of autophagy or 2) TBK1 accumulation is a result of protein aggregation caused by the inhibition of autophagy. Interestingly, we found that specific mutations in FIP200/RB1CC1 can also trigger TBK1 accumulation and activation while still displaying functional autophagic flux. In this project, we investigate the importance of the ULK1 complex for TBK1 regulation during autophagy and characterize the impact on innate immunity.

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**Proteogenomics of melanoma cell lines and xenografts identifies mutations and phosphorylation events with a potential to rewire signal transduction networks**

Authors:
**M. Schmitt[1], N. Nalpas[1], T. Sinnberg[2], H. Niessner[2], A. Forschner[2], C. Garbe[2], B. Macek[1]**

[1] University of Tuebingen, Quantitative Proteomics, Tuebingen, Germany
[2] University of Tuebingen, Division of Dermatooncology, Tuebingen, Germany
Email: marisa.schmitt@uni-tuebingen.de

Malignant melanoma is characterized by somatic mutations in BRAF in the MAPK pathway. Targeted inhibition with kinase inhibitors shows a promise in melanoma treatment; however, treated tumours inevitably develop resistance. Although several mechanisms of resistance have been proposed, key phosphoproteins and associated mutations responsible for therapy responses are largely elusive. To study the impact of mutations on signal transduction networks, we have established a bioinformatics workflow to predict non-synonymous single nucleotide variants and applied it to exome sequencing data of different drug-resistant and drug-sensitive cell lines. The resulting proteogenomic databases were applied to phosphoproteomics data from several melanoma cell lines and xenografts. Across cell lines and xenografts, we covered about 14,000 protein groups and 16,000 phosphosites, of which 1,300 were localized on peptides containing single amino acid variants. Several proliferation and signaling pathways

like autophagy were over-represented in mutated proteins. Notably, we identified variant peptides of the guanine nucleotide exchange protein SMCR8 and the FAK family kinase-interacting protein FIP200 resulting from knock-in of a phosphorylated residue. Several signaling pathways including autophagy were over-represented in mutated proteins. We are in process of validating the effect of these mutations via CRISPR/Cas9 strategy followed by MS-based proteomic under different conditions.

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**Autophagy competence in CD4$^+$ T cells controls autoimmunity and anti-tumor responses**

Authors:
**A. W. Bronietzki[1, 2], C. Plaza-Sirvent[2], I. Schmitz[1, 2]**

[1] Helmholtz Centre for Infection Research, Systems-oriented Immunology and Inflammation Research, Braunschweig, Lower Saxony, Germany
[2] Otto-von-Guericke University, Institute for Molecular and Clinical Immunology, Magdeburg, Saxony-Anhalt, Germany
Email: ingo.schmitz@helmholtz-hzi.de

Autophagy is an evolutionary conserved catabolic mechanism, which enables cells to degrade cytoplasmic content. Within the multi-stage process a double-layered membrane is formed, afterwards the membrane extends and thereby cytoplasmic cargo is captured. Subsequently, a closed vesicular structure is formed, the autophagosome. Finally, the autophagosome-lysosome fusion occurs and the sequestered content is degraded. In the steady state, autophagy contributes to the regulation of cell homeostasis by ensuring degradation of large protein complexes, toxic aggregates or dysfunctional organelles. Additionally, autophagy is important during stress conditions such as starvation or inflammation.

Autophagy has been shown to be crucial for T cell development and for the homeostasis of naïve T cells. We were interested in the function of autophagy in activated CD4+ T cells as well as in regulatory T cells. Here, we show that autophagy is important for proper activation of conventional CD4+ T cells. Moreover, Atg5 deficiency in activated CD4+ T cells led to an enhanced anti-tumor response. Using two different conditional knock-out mice, we demonstrate a crucial role for autophagy in regulatory T cells. For instance, Treg-specific deletion of Atg5 resulted in autoimmune disease at about 22 weeks of age. Thus, modulation of autophagy in Treg cells may be a therapeutic option for the treatment of diseases associated with alterations in Treg cells.

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**ESCRT machinery mediates selective microautophagy of endoplasmic reticulum**

Authors:
**J. Schäfer[1], J. Schessner[1, 2], C. Funaya[3], S. Schuck[1]**

[1] Heidelberg University, ZMBH, Heidelberg, Germany
[2] Max Planck Institute of Biochemistry , Martinsried, Germany
[3] Heidelberg University, EM core facility, Heidelberg, Germany
Email: s.schuck@zmbh.uni-heidelberg.de

ER-phagy, the selective autophagy of endoplasmic reticulum (ER), safeguards organelle homeostasis by eliminating misfolded proteins and regulating ER size. ER-phagy can occur by macroautophagic and microautophagic mechanisms. While dedicated machinery for macro-ER-phagy has been discovered, the molecules and mechanisms mediating micro-ER-phagy remain unknown. Here, we first show that micro-ER-phagy in yeast involves the conversion of stacked cisternal ER into multilamellar ER whorls during microautophagic uptake into lysosomes. Second, we identify the conserved Nem1-Spo7 phosphatase complex and ESCRT proteins as key components for micro-ER-phagy. Third, we demonstrate that macro-

and micro-ER-phagy are parallel pathways with distinct molecular requirements. Finally, we provide evidence that ESCRT proteins directly function in scission of the lysosomal membrane to complete the microautophagic uptake of ER. These findings establish a framework for a mechanistic understanding of micro-ER-phagy and, thus, a comprehensive appreciation of the role of autophagy in ER homeostasis.

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**Autophagosomal WIPI puncta image analysis using CellProfiler on tumor cells isolated from a mouse model for hepatocellular carcinoma**

Authors:
**D. Schüssele[1], M. Haas[1], T. Zuleger[1], C. Hunter[1, 2], I. Winkler[1, 2], A. Nordheim[1, 2, 3], T. Proikas-Cezanne[1, 2]**

[1] Eberhard Karls University Tübingen, Department of Molecular Biology / Interfaculty Institute of Cell Biology, Tübingen, Baden-Württemberg, Germany
[2] International Max Planck Research School ‚From Molecules to Organisms', Max Planck Institute for Developmental Biology, Tübingen, Baden-Württemberg, Germany
[3] Leibniz Institute on Aging , Fritz Lipmann Institute (FLI), Jena, Germany
Email: david.schuessele@student.uni-tuebingen.de

Autophagy  is an intracellular recycling process in eukaryotes, which is essential for cellular homeostasis and survival. Earlier, we identified the four human WD-repeat proteins interacting with phosphoinositides (WIPI), WIPI1 through WDR45/WIPI4, that play important roles in autophagy. WIPI proteins are the human members of an evolutionarily highly conserved protein family that fold into 7-bladed β-propellers, specifically bind phosphoinositides and are referred to as PROPPIN (β-propellers that bind phosphoinositides). Recently, we assigned distinct roles for all WIPI β-propellers in the process of autophagy, and suggested that WIPI β-propellers function in a circuit connecting AMPK- and mTORC1-mediated control of autophagy, involving WIPI4 and WIPI3, with the formation of autophagosomes, involving WIPI1 and WIPI2. Commonly WIPI proteins function as PI3P effectors at the nascent autophagosome. We previously established a fluorescence-based high-throughput image analysis platform for the specific localization of WIPI proteins at autophagosomal membranes, appearing as punctate structures (puncta). Here, we describe a pipeline using the free open-source software CellProfiler for measuring and analyzing such WIPI puncta. Colocalization of WIPI puncta with signals of further markers of autophagy is described initially in human U2OS cells and extended to tumor cells isolated from a mouse model of hepatocellular carcinoma.

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**The role of High Density Lipoprotein-Cholesterol (HDL-C) on autophagy in monocytes and endothelial cells**

Authors:
**S. Senay[1], D. Oz-Arslan[1], D. Agirbasli[2]**

[1] Acibadem Mehmet Ali Aydinlar University School of Medicine, Department of Biophysics, Istanbul, Turkey
[2] Acibadem Mehmet Ali Aydinlar University School of Medicine, Department of Medical Biology, Istanbul, Turkey
Email: simgesenay@hotmail.com

High density lipoprotein cholesterol (HDL-C) prevents atherosclerosis and also plays a role in innate immunity, inflammation and longevity as a protective mechanism. Recent studies showed that oxidized low density lipoprotein (OxLDL) triggers endoplasmic reticulum stress and autophagy. The HDL demonstrates protective effects against OxLDL toxicity but the mechanism remains elusive. In this study, we examined DNA methylation profiles, RNA and protein expression levels of autophagy related genes in a monocytic

(U937) cell line. Cells were treated in various concentrations of HDL at different time points. Western blot analysis showed that 100µg/ml of HDL changed LC3B-II expression in U937 cells at 16 hours. Our preliminary results showed that BNIP-3 and BECLIN-1 mRNA expressions were correlated with the duration of HDL treatment. We are currently studying the effect of HDL on Human Umbilical Vein Endothelial Cells (HUVEC) and methylation profile of selected genes in U937 cells with custom designed primers for methylation specific PCR. HDL and autophagy are associated with many cellular processes. However, there is limited information about the interaction between them. Variations in HDL levels may have an effect on expression levels of autophagy related genes.

The study is funded by Scientific and Technological Research Council of Turkey (TUBITAK) (3501) with grant #117S078.

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**Cleavage of PGAM5 by the rhomboid intramembrane protease PARL is governed by transmembrane helix dynamics and an oligomeric switch**

Authors:
**V. Siebert[1], M. Silber[2], C. Muhle-Goll[2], M. K. Lemberg[1]**

[1] Center for Molecular Biology of Heidelberg University (ZMBH), Heidelberg, Baden-Württemberg, Germany
[2] Karlsruhe Institute of Technology (KIT), Karlsruhe, Baden-Württemberg, Germany
Email: v.siebert@zmbh.uni-heidelberg.de

Impaired mitochondrial protein import triggers a number of cellular stress response pathways including selective removal of damaged mitochondria by macroautophagy (mitophagy). In intact mitochondria, the Ser/Thr kinase PINK1 is constantly imported and processed by the rhomboid intramembrane protease PARL. The soluble form of PINK1 is released into the cytoplasm, where it undergoes proteasomal degradation. Loss of mitochondrial inner membrane potential and accompanying block of mitochondrial protein import results in accumulation of PINK1 at the outer mitochondrial membrane and via the recruitment of the E3 ubiquitin ligase Parkin it initiates mitophagy. Simultaneously, PARL switches from cleaving PINK1 to processing the Ser/Thr phosphatase PGAM5, another important mitochondrial safeguard regulator of mitophagy. Although a multiprotein complex consisting of the matrix protein SLP2 and the AAA-protease YME1L1 has been shown to regulate PARL activity, it is unclear how conditional processing of PGAM5 is determined. Here, we examine by a combination of cell-based assays and *in vitro* approaches how the transmembrane helix dynamics and the oligomeric state of PGAM5 define its regulated PARL-catalyzed activation.

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**Monitoring GABARAP without cross-reactivity of its close paralogs by microscopy under endogenous conditions: GABARAP meets EGFR**

Authors:
**I. M. Simons[1], J. Dobner[1], J. L. Sanwald[1], I. Abdollahzadeh[2, 3], T. Gensch[3], S. Hoffmann[2], D. Willbold[1, 2]**

[1] Heinrich-Heine-Universität Düsseldorf, Institut für Physikalische Biologie, Düsseldorf, North Rhine-Westphalia, Germany
[2] Forschungszentrum Jülich, Institute of Complex Systems, Structural Biochemistry (ICS-6), Jülich, North Rhine-Westphalia, Germany
[3] Forschungszentrum Jülich, Institute of Complex Systems, Cellular Biophysics (ICS-4), Jülich, North Rhine-Westphalia, Germany
Email: indra.simons@uni-duesseldorf.de

The autophagy-related protein 8 (Atg8)-type protein family in humans consists of seven gene products

that are assigned to the GABARAP (γ-aminobutyric acid type A receptor-associated protein) and LC3 (microtubule-associated proteins 1A/1B light chain 3) protein subfamilies. Besides their function during autophagy, GABARAP-subfamily members also contribute to intracellular trafficking of cell surface proteins e.g. membrane-associated receptors. The GABARAPs have high sequence and structural homology. To solely address GABARAP by immunofluorescence (IF) imaging, we developed and knockout-validated a rat anti-GABARAP (8H5) monoclonal antibody. With the use of this IF suitable antibody, we studied the distribution of endogenous GABARAP and its putative colocalization with various Rab proteins, which are enriched in specific endocytic compartments like early, recycling, and late endosomes. Interestingly, our recent results indicate that trafficking of EGFR depends particularly on GABARAP as its degradation is accelerated upon ligand stimulation in GABARAP deficient cells. Consistently, we revealed partial comigration of GABARAP and EGF during live-cell time-laps microscopy when using a knock-in cell line expressing a fluorescent protein reporter (eGFP)-tagged GABARAP version. This indicates a specific role of GABARAP during EGFR-trafficking related membrane and vesicle dynamics, and thus suggests that GABARAP affects EGFR-mediated cellular signaling.

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**Characterization of autophagy in cell models of *ATXN-3* knock-out and Ataxin-3 down regulation**

Authors:
**E. Singer[1, 2], D. Weishaeupl[1, 2], H. P. Nguyen[1, 2, 3]**

[1] University of Tuebingen, Institute of Medical Genetics and Applied Genomics, Tuebingen, Baden-Württemberg, Germany
[2] University of Tuebingen, Centre for Rare Diseases (ZSE), Tuebingen, Baden-Württemberg, Germany
[3] Ruhr University of Bochum, Department of Human Genetics, Bochum, North Rhine-Westphalia, Germany
Email: elisabeth.singer@med.uni-tuebingen.de

SCA3 (MJD) is the most common ataxia and the second most common polyglutamine disorder after Huntington Disease and is characterized by a progressive motor dysfunction and neuronal loss. Knockout mice of *Atxn-3* are viable, present no overt phenotype and the down-regulation of mutated ataxin-3 expression is ameliorating protein aggregation in the nucleus, a hallmark of this polyglutamine disorder. In light of the current advances in ASOs and RNAi therapy, monogenetic, neurodegenerative disorders seem to be treatable not only to ameliorate symptoms, but to eventually treat disease progression. Nonetheless, in recent years regulatory functions have been identified for wildtype Ataxin-3 and Huntingtin in various cellular processes, amongst which is autophagy. We have therefore sought to characterise the reduction effect of *ATXN-3* knock-out and Ataxin-3 silencing on autophagy in different cell models. Ataxin-3 deficient cells show altered adherence to the substrate and are less stress resistant. They further show higher levels of the autophagic markers LC3-II and p62 under basal conditions and with BafilomycinA1 treatment. Further experiments will be performed to identify the pathways behind this dysregulation to further add to the knowledge about wildtype Ataxin-3 function.

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**The role of TOR signaling in retinal neuroprotection of *Drosophila* photoreceptor cells**

Authors:
**K. Schopf[1], T. Smylla[1], T. Kühn[1], A. Huber[1]**

[1] University of Hohenheim, Department of Biochemistry, Stuttgart, Baden-Württemberg, Germany
Email: thomask.smylla@uni-hohenheim.de

Proteostasis–the equilibrium of anabolism and catabolism of proteins–is a prerequisite for healthily growing and functional cells. An important cell growth regulator is the well conserved TOR

complex which senses environmental cues and affects biosynthesis and degradation of proteins. One of the catabolic processes regulated by TOR is autophagy–the degradation of cell organelles and debris. Imbalance of proteostasis can lead to protein aggregation and subsequent accumulation in cells which is a hallmark of a variety of neurodegenerative disorders, such as Alzheimer’s or Parkinson’s disease and *Retinitis pigmentosa*. In *Drosophila*, accumulation of intracellular rhodopsin induces retinal neurodegeneration.
Here, we investigate the connection of autophagy and retinal degeneration using the visual system of *Drosophila* as a model. To this end, we study the amorphic mutant *ttd14*$^{P75L}$ which displays light-dependent degeneration of photoreceptor cells over 28 days. By reducing rhodopsin levels, we show that this degeneration can be attributed to rhodopsin accumulation. Interestingly, *ttd14*$^{P75L}$ also manifests in a larval growth defect reminiscent of dysfunctional TOR signaling. We demonstrate that the retinal degeneration of *ttd14*$^{P75L}$ can be rescued morphologically in a hypomorphic *TOR* background, in combination with a yet unidentified mutation. This rescue is also demonstrated functionally by ERG recordings. Furthermore, *TOR* and *ttd14* show genetic interaction in growth experiments.
 

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**ATG-18 and EPG-6 are Both Required for Autophagy but Differentially Contribute to Lifespan Control in *Caenorhabditis elegans***

Authors:
**Z. Takacs[1, 2], K. Sporbeck[1, 2], J. Stoeckle[1], M. J. Prado Carvajal[1], M. Grimmel[1], T. Proikas-Cezanne[1, 2]**

[1] Eberhard Karls University Tübingen, Department of Molecular Biology, Interfaculty Institute of Cell Biology, Tübingen, Baden-Württemberg, Germany
[2] Max Planck Institute for Developmental Biology and Eberhard Karls University, International Max Planck Research School 'From Molecules to Organisms', Tübingen, Baden-Württemberg, Germany
Email: katharina.sporbeck@student.uni-tuebingen.de

During macroautophagy, the human WIPI (WD-repeat protein interacting with phosphoinositides) proteins (WIPI1–4) function as phosphatidylinositol 3-phosphate effectors at the nascent autophagosome. Likewise, the two WIPI homologues in *Caenorhabditis elegans*, ATG-18 and EPG-6, play important roles in autophagy, whereby ATG-18 is considered to act upstream of EPG-6 at the onset of autophagy. Due to its essential role in autophagy, ATG-18 was found to be also essential for lifespan extension in *Caenorhabditis elegans*; however, this has not yet been addressed with regard to EPG-6. Here, we wished to address this point and generated mutant strains that expressed the autophagy marker GFP::LGG-1 (GFP-LC3 in mammals) and harbored functional deletions of either atg-18 (*atg18(gk378)*), epg-6 (*epg-6(bp242)*) or both (*atg-18(gk378);epg-6(bp242)*). Using quantitative fluorescence microscopy, Western blotting, and lifespan assessments, we provide evidence that in the absence of either ATG-18 or EPG-6 autophagy was impaired, and while *atg-18* mutant animals showed a short-lived phenotype, lifespan was significantly increased in epg-6 mutant animals. We speculate that the long-lived phenotype of *epg-6* mutant animals points towards an autophagy-independent function of EPG-6 in lifespan control that warrants further mechanistic investigations in future studies.

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# Abstracts - Poster

**Phosphorylation orchestrates the structural ensemble of the intrinsically disordered protein HMGA1 and modulates its DNA binding to the NFkB promoter**

Authors:
**R. Stoll**[1]

[1] Ruhr University of Bochum, Chemistry and Biochemistry, Bochum, North Rhine-Westphalia, Germany
Email: raphael.stoll@rub.de

High Mobility Group Protein A1a (HMGA1a) is a highly abundant nuclear protein, which plays a crucial role during embryogenesis, cell differentiation, and neoplasia. Here, we present the first ever NMR-based structural ensemble of full length HMGA1a [1]. Our results show that the protein is not completely random coil but adopts a compact structure consisting of transient long-range contacts, which is regulated by post-translational phosphorylation. The CK2-, cdc2-, and cdc2/CK2-phosphorylated forms of HMGA1a each exhibit a different binding affinity towards the PRD2 element of the NFkB promoter. Our study identifies connected regions between phosphorylation sites in the wildtype ensemble shifted considerably upon phosphorylation, indicating that these posttranslational modifications sites are part of an electrostatic contact network that alters the structural ensemble by shifting the conformational equilibrium. Moreover, ITC data reveal that the CK2-phosphorylated HMGA1a exhibits a different DNA promoter binding affinity for the PRD2 element. Furthermore, we present the first structural model for AT-hook 1 of HMGA1a that can adopt a transient a-helical structure, which might serve as an additional regulatory mechanism in HMAG1a. Our findings will help to develop new therapeutic strategies against HMGA1a-associated cancers by taking posttranslational modifications into consideration.

[1] B. Kohl, X. Zhong, C. Herrmann, and R. Stoll (2019) Nucleic Acids Research, in press.

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**Insights into the regulation and function of *BAG3-mediated selective macroautophagy* in protein quality control**

Authors:
**E. Stürner**[1], **C. Hiebel**[1], **D. Chakraborty**[1], **M. Schwarz**[1], **M. Hoffmeister**[2, 3], **C. Münch**[2], **C. Behrends**[2, 4], **P. Beli**[5], **C. Behl**[1]

[1] University Medical Center of the Johannes Gutenberg University Mainz, Institute of Pathobiochemistry, Mainz, Germany
[2] Goethe University Frankfurt Medical School, Institute for Biochemistry II, Frankfurt/Main, Germany
[3] Brandenburg Medical School (MHB) Theodor Fontane, Institute of Biochemistry, Neuruppin, Germany
[4] Medical Faculty, Ludwig-Maximilians-Universität München, Munich Cluster for Systems Neurology, Munich, Germany
[5] Institute of Molecular Biology (IMB), Mainz, Germany
Email: estuerne@uni-mainz.de

In cellular protein quality control, the multifunctional HSP70 co-chaperone BAG3 specifically targets aggregated proteins to the perinuclear aggresome and induces their degradation via selective macroautophagy (*BAG3-mediated selective macroautophagy*). Noteworthy, this process is suggested to be implicated in the clearance of aggregation-prone proteins associated with age-related neurodegenerative disorders. In order to reveal the dynamics of the BAG3-mediated macroautophagy pathway and its adaptation ability in response to acute stress, we established *proteostasis-stress-BAG3-interactomes* (upon proteasome inhibition or upon overexpression of ALS-linked mutant $SOD1_{G85R}$). Interacting proteins detected are linked to various biological key processes. Based on these obtained interactome data, we will present the modulation of the expression of one specific BAG3-interactor and show the impact of this modulation on BAG3-driven aggresome formation. In addition, we demonstrated a pivotal role for BAG3

and the BAG3-mediated macroautophagy pathway in the adaptation of neuronal cells to redox stress. Oxidative stress-resistant (OxSR) hippocampal HT22 cells revealed an enhanced expression of components of the cellular autophagic-lysosomal network combined with significantly increased autophagic activity and improved clearance of aggregated proteins. Moreover, mitochondrial dynamics were changed as well as the expression and functional BAG1-BAG3-switch could be detected in OxSR cells.

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**ALK-targeted therapy triggers protective autophagy in EML4-ALK positive lung cancer cells**

Authors:
**A. M. Schläfli[1], S. Berezowska[1], N. Engedal[2], M. P. Tschan[1]**

[1] University of Bern, Division of Experimental Pathology, Bern, Switzerland
[2] University of Oslo, Centre for Molecular Medicine Norway, Oslo, Norway
Email: Mario.tschan@pathology.unibe.ch

Although lung tumors are successfully treated with targeted therapies, acquired drug resistance to these monotherapies occurs frequently. We aimed at understanding the resistance mechanism of EML4-ALK positive H3122 non-small cell lung cancer (NSCLC) cells to ALK-targeted therapy. Firstly, we found that treating H3122 cells with the ALK inhibitor Ceritinib resulted in a drastic decrease in the inhibitory phosphorylation of the autophagy protein ULK1 at Ser757. Accordingly, autophagic cargo sequestration of lactate dehydrogenase (LDH) significantly increased upon ALK inhibition and was significantly blocked by the VPS34 inhibitor SAR405 and the ULK1 inhibitor MRT68921. These results were supported by long-lived protein degradation assays. In contrast, we found decreased LC3B carrier flux upon Ceritinib treatment. Since there is evidence for LC3B-independent autophagy, we tested if the intracellular distribution of the autophagy-related protein WIPI1 is altered. We found a significant increase of GFP-WIPI1 dot formation during Ceritinib treatment in H3122 cells. Lastly, we found significantly reduced colony numbers when H3122 cells were co-treated with SAR405 and Ceritinib compared to the respective single agent treatments. In summary, we found that ALK inhibition triggers autophagic flux in EML4-ALK positive lung cancer cells. Importantly, combining pharmacological autophagy targeting combination with an ALK inhibitor significantly decreased cancer cell recovery.

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**Crosstalk between autophagy and intracellular trafficking processes during plant immunity**

Authors:
**S. Üstün[1]**

[1] University of Tübingen, ZMBP, Tübingen, Baden-Württemberg, Germany
Email: suayib.uestuen@zmbp.uni-tuebingen.de

Autophagy and the ubiquitin-proteasome system (UPS) are the major pathways for protein degradation in eukaryotes. They orchestrate many cellular processes during development and in response to environmental stimuli such as microbial infections. Recent advances revealed pro- and anti-bacterial roles of autophagy in plants. We have shown that *Pseudomonas syringae* activates autophagy via the action of type-III effector (T3E) HopM1 for proteasome degradation and enhanced virulence. However, mechanistic insights how HopM1 activates autophagy remain elusive. Previously, HopM1 was identified to target TGN localized MIN7/BIG5 belonging to a family of ARF-GEFs (BIG1-5), which were recently characterized to be required for vesicle trafficking. Proteomics data suggests that MIN7/BIG5 as well as other ARF-GEFs reside in a complex together with HopM1 and autophagy component ATG8. Thus, this might be the missing puzzle piece of how HopM1 activates autophagy. We have identified that loss of ARF-GEF MIN7/BIG5 or inhibitor-mediated perturbation of vesicle trafficking events activates the autophagy pathway and results

in enhanced degradation. This data suggests that HopM1-MIN7/BIG5 interaction at the TGN mediates a possible crosstalk between autophagy and intracellular trafficking processes. We propose a model where the interplay of the autophagy pathway and secretion plays an essential role for the recycling machinery during plant immunity.

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**Inhibition of lysosomal fusion events affects mTORC1 signaling**

Authors:
**R. van der Welle[1], P. Sanza[1], C. de Heus[1], F. Zwartkruis[2], N. Liv[1], J. Klumperman[1]**

[1] University Medical Center Utrecht, Section of Cell Biology, Center for Molecular Medicine, Utrecht, Netherlands
[2] University Medical Center Utrecht, Section Molecular Cancer Research, Center for Molecular Medicine, Utrecht, Netherlands
Email: R.E.N.vanderWelle@umcutrecht.nl

Lysosomes are key regulators of cellular metabolism and lysosomal defects are associated with neurological disorders. VPS41 is part of the HOPS complex which regulates fusion of lysosomes with late endosomes and autophagosomes. Independently of HOPS, VPS41 is required for the transport of newly synthesized lysosomal membrane proteins. Overexpressing VPS41 protects dopaminergic neurons against α-synuclein-induced neurodegeneration in a C. elegans model for Parkinson’s disease (PD). Patients bearing mutations in *VPS41* (*VPS41*$^{R662*}$ and *VPS41*$^{S285P}$) have recently been identified and display symptoms resembling PD. Indeed, co-expression of the mutants abolishes the neuroprotective effect of VPS41. Previously we studied the effects of these patient-specific mutations showing that both VPS41 mutants cause HOPS complex dysfunctionality, thereby affecting transfer of endocytosed cargo to lysosomes. Strikingly, in patient fibroblasts and *VPS41*$^{KO}$ cells, mTORC1 dissociates from lysosomes causing nuclear localization of TFE3 which results in accumulation of autophagic compartments and irresponsiveness to autophagic stimuli. Currently we are investigating if the impaired reachability of lysosomes in patient and *VPS41*$^{KO}$ cells causes nutrient starvation of these compartments, resulting in mTORC1 dissociation and autophagy initiation. This would link HOPS function to mTORC1 signaling and suggests a novel class of lysosomal disorders in which lysosomes are insufficiently reached by nutrients.

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**Impaired autophagy promotes neurodegeneration in sphingosine 1-phosphate-lyase ablated brains**

Authors:
**S. Alam[1], I. Karunakaran[2], G. van Echten-Deckert[1]**

[1] University Bonn, LIMES Institute, Membrane Biology, and Lipid Biochemistry, Bonn, North Rhine-Westphalia, Germany
[2] University Hospital of Bonn, Institute for Medical Microbiology, Immunology and Parasitology, Bonn, Germany
Email: g.echten.deckert@uni-bonn.de

Sphingosine 1-phosphate (S1P) is an evolutionarily conserved catabolic intermediate of sphingolipid metabolism that regulates diverse biological processes in the brain including neural development, differentiation, and survival. S1P-lyase (SGPL1) irreversibly cleaves S1P in the final step of sphingolipid catabolism. To investigate the function of S1P in the brain, we generated a mouse model in which SGPL1 was inactivated specifically in neural cells. Hence, S1P accumulated in the brain causing neuronal ER-stress, an increase in intracellular calcium, impairment of presynaptic architecture and function, and a pro-inflammatory environment. SGPL1 deficiency also affects neuronal autophagy thus leading to the accumulation of aggregate-prone proteins such as APP, α-synuclein and tau. In vivo profound deficits

in cognitive skills were observed. Note that compromised autophagy is assumed to be one of the major causes of neurodegeneration. Also microglial autophagy turned out to be defective due to accumulation of S1P. We identified S1P receptor 2 (S1PR2) as the mediator of impaired autophagy and pro-inflammatory effects in SGPL1 deficient brains.

Currently, we are studying the impact of SGPL1 ablation in astrocytes cultured from SGPL1 deficient murine brains. We evidenced reactive astrogliosis as defined by increased expression of GFAP and P2Y1 purinoreceptor (P2Y1R). Further, we found mTOR dependent impaired autophagy which is mediated in an autocrine fashion via S1PR2 and S1PR4.

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**Breast tumorigenesis regulation by autophagy and HER2**

Authors:
**S. Vega-Rubin-de-Celis[2, 3, 1], Z. Zou[2, 5], A. Fernandez[2], G. Xiao[4], K. Min[4], B. Levine[2]**

[1] DKFZ, Heidelberg, Germany
[2] University of Texas Southwestwen, Center for Autophagy Research, Dallas, United States of America
[3] University of Texas Southwestwen, Department of Cell Biology, Dallas, United States of America
[4] University of Texas Southwestwen, Department of Clinical Sciences, Dallas, United States of America
[5] Howard Hughes Medical Institute, Dallas, Germany
Email: silvia.vega.rubindecelis@gmail.com

HER2 is a receptor tyrosine kinase whose gene is amplified in ~20% human breast cancer patients.  We explored the regulation of autophagy in breast cancer cells by HER2 *in vitro* and the effects of genetic and pharmacological approaches to increase autophagy on HER2-driven breast cancer growth *in vivo*. We show that endogenous HER2 interacts with Beclin 1 in HER2+ breast cancer cells and it inhibits autophagy. Mice with an increased basal autophagy due to a knock-in mutation in *Becn1* (*Becn1*$^{F121A}$) that results in a decreased Bcl-2 binding to Beclin 1 are protected from mammary tumorigenesis when crossed with mammary-specific HER2 transgenic mice, and HER2 fails to inhibit autophagy in primary cells derived from these mice. Furthermore, HER2-positive human breast cancer xenografts treated with the autophagy-inducing peptide Tat-Beclin 1 inhibit tumor growth as effectively as a clinically used HER2 tyrosine kinase inhibitor (TKI). This inhibition of tumor growth is associated with a robust induction of autophagy, a disruption of HER2/Beclin 1 binding, and a transcriptional signature in the tumors that is distinct from that observed with HER2 TKI treatment.

Taken together, these findings indicate that the HER2-mediated inhibition of Beclin 1 and autophagy likely contributes to HER2-mediated tumorigenesis. They also suggest that strategies to block HER2/Beclin 1 binding and/or increase autophagy may represent a new therapeutic approach for HER2-positive breast cancers.

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**Error catastrophe and cancer - Exonuclease mutations of polymerases δ and ε cause strong mutator phenotypes**

Authors:
**I. Vetterlein[1], M. Reichel[1], H. Pospiech[1]**

[1] Friedrich Schiller University Jena, Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), Jena, Thuringia, Germany
Email: isabell.vetterlein@leibniz-fli.de

Cancer cells often show defects in DNA repair leading to either high numbers of base substitutions

or chromosomal aberrations. For instance, the loss of DNA mismatch repair (MMR) underlying many colorectal carcinomas, increases mutation rates considerably. More recently, a new class of tumors has been identified with mutations in the exonuclease domain of the DNA polymerases (pol) ε or δ. These cancers show extremely high numbers of base substitutions. However, excessive error rates result in an accumulation of mutations in essential genes, which progressively impair (cancer) cell fitness. This phenomenon, called error catastrophe, may lead to subsequent extinction of a cell population. This effect has already been discussed excessively, but has been formally demonstrated only for simple model organisms such as yeast. Even though conventional cancer chemotherapy is largely based on the introduction of DNA damage, the upper limits of mutation rates are still unknown.

Here we introduced different exonuclease mutations of pol δ/ε in an isogenic human cell model. Using fluctuation analysis, we could demonstrate strong mutator phenotypes. Cell phenotypes and the observed mutation rates may indicate that they are not far from a tolerable upper limit. To confirm this we aim to achieve synthetic lethality, e.g. by an additional MMR knockdown. Based on the concept of error catastrophe, we analyzed new experimental drugs and discussed new approaches for cancer therapy.

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**Multiplexed 3Cs identifies novel genetic interactions in the autophagy network**

Authors:
**V. Diehl[1], P. Grumati[1], M. Wegner[1], I. Dikic[1, 2], M. Kaulich[1]**

[1] Institute of Biochemistry II, Goethe University Frankfurt - Medical Faculty, University Hospital, Frankfurt am Main, Hesse, Germany
[2] Buchmann Institute for Molecular Life Sciences, Frankfurt am Main, Hesse, Germany
Email: ma.wegner@em.uni-frankfurt.de

Autophagy is integrated into a number of key signaling pathways suggesting therapeutic potential in a variety of human diseases. Understanding the molecular mechanisms that regulate autophagy is based on understanding the pair-wise genetic interactions that underlie the autophagy genetic network. Single gene perturbation strategies have been facilitating the identification of essential components in the autophagy gene network. However, to comprehensively understand the autophagy network, single gene knockouts are not sufficient. Here, we present a new technology to rapidly generate multiplexed circular synthesized (3Cs) gene perturbation libraries. To identify essential gene-gene interactions within the autophagy network, we generated a 3Cs-gRNA multiplex library, composed of 247.032 gRNA interactions, and applied it to a FACS-based positive screen utilizing a recently described fluorescent LC3 autophagy flux reporter. In parallel, we developed a computational pipeline for the analysis of multiplexed gene perturbation experiments and analyzed the autophagy hit list based on observed phenotypes. Thus, we identified synthetic gene interactions that are essential for autophagy induction and currently under extensive validation. In this study, we present a novel 3Cs method to generate highly diverse multiplexed gene perturbation libraries. Using this method we performed a FACS-bases screen to reveal hitherto unknown genetic interactions that are essential for autophagy induction.

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**Studying ageing using FRET-based biosensors**

Authors:
**A. Wirth[1], A. Zeug[1], F. Müller[1], E. Ponimaskin[1]**

[1] Medical School Hannover, cellular neurophysiology, Hannover, Lower Saxony, Germany
Email: Wirth.Alexander@mh-hannover.de

Physiological ageing is among high risk factors for several age-associated neurodegenerative diseases such

as Alzheimer’s disease. Even though the mechanism underlying ageing are still poorly understood, reducing nutrient-signalling (caloric restriction) and upregulation of autophagy have been described as major contributors to increased healthy life expectancy.

With the use of advanced quantitative microscopic techniques, we aimed to analyse cellular and molecular mechanisms underlying physiological ageing. By stereotactic injection of adeno-associated viruses (AAVs) into the hippocampus of young (i.e., five month old mice) as well as aged mice (i.e., 24 month old), we transduced biosensors to investigate changes in different molecular signalling cascades during ageing in space and time. Using combined application of Förster resonance energy transfer (FRET)-based biosensors and two-photon excitation, we investigated mTOR kinase signalling, a metabolic key kinase in nutrition sensing. Furthermore, we looked at the induction of autophagy in aged mice, using ATG-homolog FRET-based cleavage sensors. Additionally, we investigated the protective properties of the late-in-life feeding with spermidine in aged mice using the above-mentioned biosensors.

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**Understanding secretory MVB trafficking for the release of extracellular vesicles.**

Authors:
**L. Witte[1, 2], K. Linnemannstöns[1, 2], K. Schmidt[1, 2], J. C. Gross[1, 2]**

[1] University Medical Center Göttingen, Hematology and Oncology, Göttingen, Germany
[2] University Medical Center Göttingen, Developmental Biochemistry, Göttingen, Germany
Email: leonie.witte@med.uni-goettingen.de

The secretion of proteins, for example Wnt molecules, on extracellular vesicles (EV) requires directed trafficking of endosomal compartments towards the plasma membrane. While secretory subsets of multivesicular endosomes (MVE) are trafficked towards the cell membrane to release intraluminal vesicles into the extracellular space, other subsets undergo lysosomal fusion for content degradation. Despite the obvious relevance of this endosomal fate decision for intercellular communication, it remains unclear how directed MVE transport is mediated.

To understand intracellular trafficking of MVE subsets and their specific targeting towards cargo secretion, we are using a *Drosophila* wing imaginal disc model system. Here, the morphogen Wg is expressed in a specific cell population and secretion of Wg on EV has been shown to be involved in wing development. Using *in vivo* RNAi screening, we identified a *Drosophila* motor protein involved in intracellular transport of Wg-carrying endosomal compartments. Currently, we are looking into its role for secretory vs. degradative MVB trafficking in the context of exosome secretion and Wg signal transduction *in vivo* and *in vitro*. Furthermore, we are investigating human orthologs of promising motor proteins in human cancer cells.

Taken together, we are using *in vivo* and *in vitro* model systems to identify and validate evolutionary conserved trafficking factors mediating intracellular transport of MVEs and the release of extracellular vesicles.

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**Modulation of autophagy to promote regeneration of the aging liver**

Authors:
**F. Xu[1], U. Dahmen[1]**

[1] University Jena , Visceral & Vascular Surgery – Experimental Transplantation Surgery lab, Jena, Thuringia, Germany
Email: Fengming.Xu@med.uni-jena.de

# Abstracts - Poster

Aging causes morphological changes of liver and a decline in the rate of liver regeneration. Moreover, it also results in a reduction of autophagy activity. However the relationship between reduced autophagy activity and impaired liver regeneration has not been clarified. Recent studies on autophagy have shown that restoring autophagy can reverse the histological alternations.

Based on that, we hypothesize that modulating autophagy may have a beneficial effect on regeneration of the aged liver.

WP I: We will explore the aging induced changes and the autophagy level in the liver comparing young and old mice. Then we will investigate the effect of modulating autophagy on the aging induced changes using aged mice.

WP II: We will Select a suitable PHx model (70% vs 90%PHx). Then we will compare the course of liver regeneration in aged and young mice and explore the relationship between aging, regeneration and autophagy.

WP III: We will investigate the effect of autophagy modulators (Xestospongin C and Chloroquine) on the course of liver regeneration in aged mice.

Aging induced hepatic alterations will be assessed based on liver enzymes and HE staining. Autophagy level will be evaluated using LC3, p62 and Atg5 with WB and PCR. Liver regeneration will be quantified by Liver/body weight rate and BrdU proliferation index.

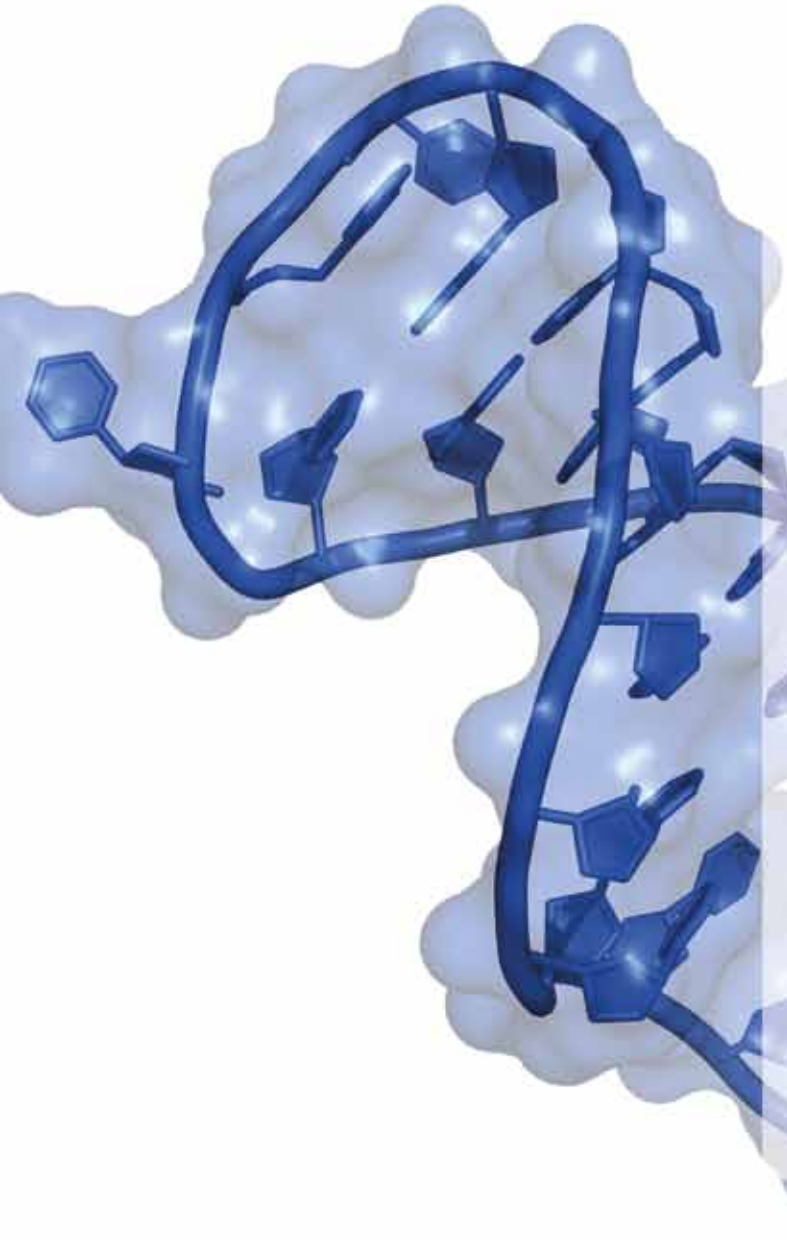
Currently we are in the stage of technical preparation and literature work up.

We disclose no conflict of interest.

Gesellschaft für Biochemie und Molekularbiologie e.V.

# 71st Mosbacher Kolloquium

# The world of RNAs - principles & applications



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## Topics & Speakers

**Mechanisms and functions of regulatory RNAs:**
Rotem Sorek [IL] | Sarah Woodson [USA]
Maude Guillier [FR] | Julius Brennecke [AT]
Nikolaus Rajewsky [DE]

**RNA-guided RNPs:**
Martin Jinek [AT] | Elena Conti [DE]
Dina Grohmann [DE]
**junior GBM Session:**
Nina Papavasiliou [DE] | Thomas Tuschl [USA]

**Epitranscriptomics:**
Andres Jäschke [DE] | Jean-Yves Roignant [DE]
Ann Ehrenhofer-Murray [DE] | Chuan He [USA]
Michaela Frye [DE]

**RNA therapeutics:**
Frank Slack [USA] | Muthia Manuhoran [Alnylam, USA]
Jorg Vogel [DE] | Adrian Krainer [USA]
Stefanie Dimmeler [DE]

**Feodor Lynen Lecture:** Susan Gottesman /Bethesda [USA]

**Otto Warburg Medal:** Patrick Cramer /Göttingen [DE]

www.mosbacher-kolloquium.org

## Scientific Organization

Prof. Dr. Tassula Proikas-Cezanne
Eberhard Karls University Tübingen
Email tassula.proikas-cezanne@uni-tuebingen.de
Mobile +49 178 1570125

Prof. Dr. Michael Thumm
Georg August University Göttingen

Prof. Dr. Marius K. Lemberg
Rubrecht Karls University Heidelberg

Prof. Dr. Konstanze F. Winklhofer
Ruhr University Bochum

## Meeting Management

For questions regarding registration:
Sabrina Wolf
Project Assistant
event lab. GmbH
Email swolf@eventlab.org
Phone +49 341 30 88 84-79

For questions regarding sponsoring:
André Störmer
Sponsor Management
event lab. GmbH
Email astoermer@eventlab.org
Phone +49 341 30 88 84-80

## GBM Head Office

For all other questions:
Gesellschaft für Biochemie und Molekularbiologie
Mörfelder Landstr. 125
60598 Frankfurt am Main
Email info@gbm-online.de
Phone +49 69 660567-0
Dr. Anke Lischeid /Managing Director
Sabine Hähnke-Metzen
Tino Apel

# Organization/Impress

## Scientific Organization

Responsible organizer of the conference is the German

Society for Biochemistry and Molecular Biology.

Cooperation partner is the German Society for Cell Biology.

The GBM is registered as a non-profit organization under number 4265 in the register of associations of the city of Frankfurt am Main.

The GBM is legaly represented by the executive board

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Registered office of the GBM:
Gesellschaft für Biochemie
und Molekularbiologie e.V.
Mörfelder Landstr. 125
60598 Frankfurt am Main
Germany

Phone +49 69 660567-0
Fax +49 69 660567-22
Email info@gbm-online.de
Homepage https://gbm-online.de

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