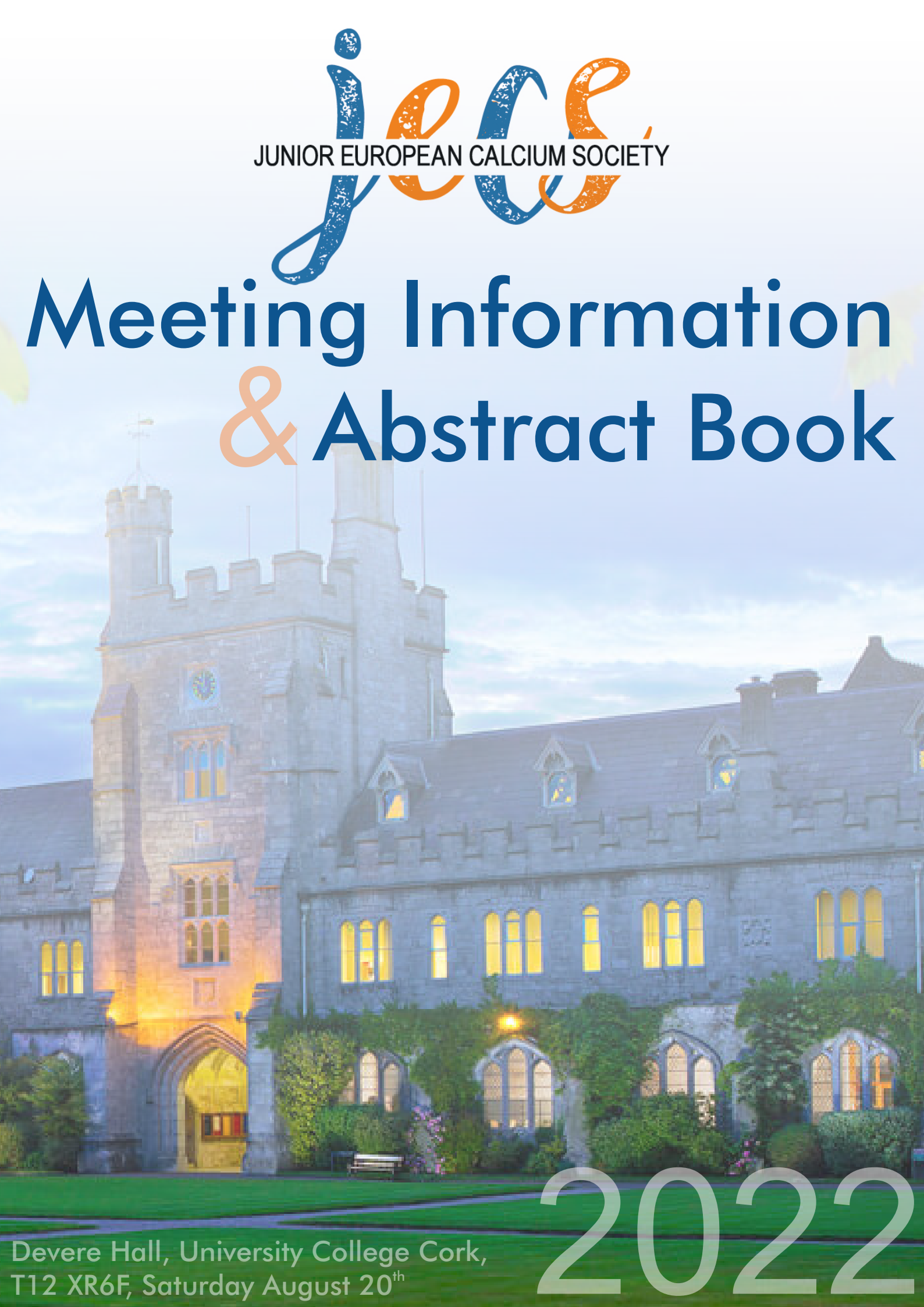
JUNIOR EUROPEAN CALCIUM SOCIETY

Meeting Information & Abstract Book



Devere Hall, University College Cork,
T12 XR6F, Saturday August 20th

2022

This meeting was possible
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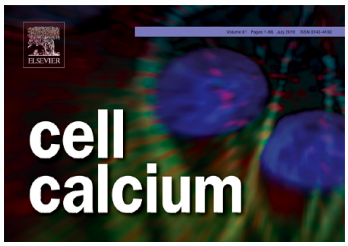

Welcome

The Junior European Calcium Society Board warmly welcomes you to our 3rd meeting!

The jECS board was founded in 2019 with the goal of furthering collaboration, opportunities, and communication of science of early career calcium researchers. Over the past two years, we have coordinated online meetings to facilitate this goal. In 2022, we are very excited to host our first in person meeting!

We are proud to have organized an event which places science by early career researchers in the spotlight. We hope this meeting will provide a welcoming and encouraging environment to present your data, in addition to being an opportunity to meet and network with your peers in the lead up to the main ECS meeting. Our hope is that meetings by ECRs for ECRs will help to foster a diverse community of enthusiastic and connected calcium researchers!

If you would like to stay engaged with jECS work after the meeting, please follow us on Twitter @EuroCalcium. If you would like to get involved with our work, please email us at jecsboard@gmail.com. We always love to have more motivated people on board!

We value and appreciate all of your contributions towards this meeting, from preparation of presentations to participation in discussions. On behalf of the entire jECS board, thank you for being a part of the day!

Progr

9:00 – 09:30 jECS Registration desk open
Poster boards available for presenters to affix posters.
Speakers welcome to load presentations

09:30 - 09:40 Welcome - *Malene Brohus & Alejandro Marmolejo-Garza*

09:30 - Morning session - *Chairs: Malene Brohus & Alejandro Marmolejo-Garza*

9:40 – 9:55 **Adhesion-dependent Ca^{2+} microdomains increase the sensitivity of T cells**
Mariella Weiß, University Medical Center Hamburg-Eppendorf, Germany

9:55 – 10:10 **Presenilin-1 controls pancreatic beta-cell metabolism by regulating mitochondrial Ca^{2+} sensitive NADH shuttles**
Zhanat Koshenov, Medical University of Graz, Austria

10:10 – 10:25 **Lack of MICU-dependent gatekeeping of the mitochondrial Ca^{2+} uniporter of Trypanosoma cruzi at low extramitochondrial Ca^{2+} concentrations**
Mayara S. Bertolini, Univerisity of Georgia, USA

10:25 – 10:40 *Short break*

10:40 – 10:55 **MASTER-NAADP: Characterization of a newly developed membrane permeant NAADP derivative**
Franziska Möckl, University Medical Center Hamburg-Eppendorf, Germany

10:55 – 11:10 **Dysregulated MAM & Ca^{2+} homeostasis underlying Wolfram syndrome type 2- associated CISD2 deficiency**
Jens Loncke, KU Leuven, Belgium

11:10 – 11:25 **Parallel regulation of IP_3 receptors by IP_3 and PIP_2**
Adelina Ivanova, University of Cambridge, UK

11:25 - 12:00 Coffee and tea break

12:00 – 12:45 Collaborative Science Communication Workshop
A collaborative session harnessing our collective knowledge in scientific writing and communication!

12:45 – 13:45 Lunch - *Provided*

13:45 - Afternoon session - *Chairs: Adeline Ivanova & Björn-Philipp Diercks*

13:45 - 14:00 **Loss of IP3R fitness enables GNAQ/GNA11-mutated Uveal Melanoma survival by shutting down IP3 signalling and preventing calcium overload-induced cell death**
Céline Garcia, University of Poitiers, France

14:00 - 14:15 **Clinical Investigation of Calcium Electroporation for Cancer in the Skin**
Mille Vissing, Center for Experimental Drug and Gene Electrotransfer & University of Copenhagen, Denmark

14:15 - 14:30 **Communication is key: Investigating calcium crosstalk in the breast tumour microenvironment**
Krystyna A Gieniec, University of New South Wales, Sydney

14:30 - 14:45 *Short break*

14:45 - 15:00 **Development of an adrenocortical cell model of calcium signaling modulation to decipher the molecular mechanisms responsible for primary aldosteronism**
Bakhta Fedlaoui, Université de Paris, France

15:00 – 15:15 **HINT1 deficiency impairs actin cytoskeleton and calcium signaling**
Silvia Amor-Barris

15:15 – 15:30 **Blockade of mitochondrial calcium uptake protects neurons against ferroptosis**
Alejandro Marmolejo-Garza, University Medical Center Groningen & University of Groningen, The Netherlands

15:30 – Short break

15:45 – 16:30 Poster session

16:30 – 17:30 Social event and awards

17:30 - Walk to dinner at Crawford & Co - *Provided*

Abstracts

Short talks

Adhesion-dependent Ca^{2+} microdomains increase the sensitivity of T cells

Mariella Weiß[1], Lola C. Hernandez[1], Diana C. Gil Montoya[1], Anke Löhndorf[1], Aileen Krüger[1], Miriam Kopdag[1], Liana Uebler[1], Marie Landwehr[1], Lena-Marie Woelk[2], René Werner[2], Andreas H. Guse[1], Björn-Philipp Diercks[1]

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The activation of T cells is a hallmark of the adaptive immune response. Adhesion-dependent T cell stimulation may play a pivotal role, describing an adhesion-dependent change in T cell sensitivity that facilitates full T cell receptor (TCR)/CD3-mediated activation[1]. During an immune response, T cells migrate from the blood vessel walls into the inflamed tissue, crossing the endothelial cell layer and subsequently elements of the extracellular matrix (ECM). In this process, integrins facilitate binding to endothelial cells and ECM proteins. Using high-resolution live cell imaging, within tens of milliseconds after T cell activation, local Ca^{2+} microdomains represent the earliest Ca^{2+} signaling events2. Here, we hypothesized that Ca^{2+} microdomains observed without TCR/CD3 stimulation are elements of T cell sensitivity and occur in response to adhesion to ECM proteins. In a high-resolution live cell–imaging approach, we show that blocking with monoclonal antibodies directed toward integrin receptors that bind to collagen-IV/-VI, laminin-1 and the intercellular adhesion molecule (ICAM-1) results in a significant decrease of adhesion-dependent Ca^{2+} microdomains. Furthermore, adhesion of T cells to collagen-IV and laminin-1 resulted in a significant increase of Ca^{2+} microdomains. Moreover, these adhesion-dependent Ca^{2+} microdomains were significantly decreased by blocking the downstream integrin signaling pathway using focal adhesion kinase (FAK) inhibitor PF562,271, phospholipase C (PLC) inhibitor U73122 or by deletion of all three D-myo-inositol 1,4,5-trisphosphate receptor (IP_3R) subtypes. Blocking of FAK additionally resulted in reduced global Ca2+ signals or decreased NFAT translocation after TCR stimulation on adhesive poly-L-lysine coating. In summary, these data show that adhesion to the basement membrane proteins collagen-IV and laminin-1 results in an increased state of sensitivity of T cells, involving FAK, PLC, and IP_3Rs for the formation of Ca^{2+} microdomains.

References 1 Randriamampita et al., (2003). T cell adhesion lowers the threshold for antigen detection. Eur. J. Immunol. 33, 1215–1223. 2 Wolf et al., (2015). Frontrunners of T cell activation: Initial, localized Ca2+ signals mediated by NAADP and the type 1 ryanodine receptor. Sci Signal. 3;8(398):ra102.

Funding This research was funded by the Collaborative Research Center 1328, German Research Foundation (DFG), project A01 and by the Förderfonds Medizin of the University Medical Center Hamburg-Eppendorf (grant NWF 22/07 to M.W.).

Presenilin-1 controls pancreatic beta-cell metabolism by regulating mitochondrial Ca2+ sensitive NADH shuttles

Zhanat Koshenov[1], F. E. Oflaz[1], S. Postic[2], R. Rost[1], B. Gottschalk[1], A. Koshenova[3], M. Hirtl[1], O. G. Bachkoenig[1], S. Sarikas[2], J. Gindlhuber[1], M. Schittmayer[4], T. Madl[1], M. Slak Rupnik[2], R. Malli[1,5], W. F. Graier[1,5]

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Intracellular Ca^{2+} ions are known to regulate many metabolic processes in a cell. We have recently shown that presenilin-1 establishes an endoplasmic reticulum (ER) Ca^{2+} leak directed towards mitochondria in pancreatic beta cells (Klec et al. 2019, PMID: 30790505). This leak was found to be indispensable for glucose-stimulated insulin secretion in cultured beta cells and pancreatic islets (Klec et al. 2019, PMID: 31529929). The aim of current work was to elucidate the mechanism of presenilin-1 mediated regulation of pancreatic beta-cell metabolism. Silencing of presenilin-1 in pancreatic beta cells showed delayed and reduced cytosolic Ca^{2+} oscillations in response to glucose, but not pyruvate, pointing at possible importance of presenilin-1 for glycolysis. Additionally, presenilin-1 knockdown (KD) showed reduced pyruvate production upon glucose elevation, while lactate production was increased, indicating a metabolic switch. In support of the metabolic switch, we detected increased glutamine reliance of presenilin-1 KD cells in mass spectrometry analysis of cellular metabolites. Furthermore, cellular redox state was altered by presenilin-1 KD with reduced cytosolic NAD^+/NADH ratio, a possible reason for observed metabolic alterations. The latter finding is in line with our recently published work where we demonstrated that disrupted sub-cellular Ca^{2+} homeostasis can rewire cellular metabolism by modulating mitochondrial Ca^{2+} sensitive NADH shuttles (Koshenov et al. 2022, PMID: 35058562). Analysis of subcellular Ca^{2+} concentrations using genetically encoded Ca^{2+} sensors revealed reduced matrix and mitochondrial intermembrane space Ca^{2+}, while global cytosolic and ER Ca^{2+} levels were not altered by presenilin-1 KD. In detail, analysis of glycolytic intermediates by targeted mass-spectrometry showed an accumulation of metabolites preceding and including glyceraldehyde-3 phosphate (GAP) in presenilin-1 KD cells, pointing to the bottleneck at the reaction catalyzed by GAP dehydrogenase that requires NAD^+. These results led us to hypothesize that presenilin-1 mediated ER Ca^{2+} leak is important for mitochondrial intermembrane space residing Ca^{2+} sensitive NADH shuttles that recycle cytosolic NADH and provide NAD^+ for glycolysis. Overexpression of Ca^{2+} insensitive mutants of these shuttles rescued the effects of presenilin-1 KD. Thus, we have identified presenilin-1 as a regulator of pancreatic beta-cell metabolism, which acts by establishing an ER Ca^{2+} leak that controls mitochondrial Ca^{2+} sensitive NADH shuttles that recycle cytosolic NADH to maintain glycolysis and supply mitochondria with the substrate.

Lack of MICU-dependent gatekeeping of the mitochondrial Ca^{2+} uniporter of *Trypanosoma cruzi* at low extramitochondrial Ca^{2+} concentrations

Mayara S. Bertolini and Roberto Docampo

Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, Georgia, United States

The mitochondrial Ca^{2+} uptake, which is important to regulate bioenergetics, cell death and cytoplasmic Ca^{2+} signaling, is mediated via the calcium uniporter complex (MCUC). In animal cells the MCUC is regulated by the mitochondrial calcium uptake 1 and 2 dimer (MICU1-MICU2), which acts as gatekeeper preventing mitochondrial Ca^{2+} overload at low cytosolic Ca^{2+} levels. In contrast to animal cells, knock out of either MICU1 or MICU2 in *Trypanosoma cruzi*, the etiologic agent of Chagas disease, did not affect Ca^{2+} uptake at low Ca^{2+} concentrations and it was though that in the absence of one MICU the other would replace its role. However, previous attempts to knockout both genes were unsuccessful. Here, we designed a strategy to generate *TcMICU1/TcMICU2* double knockout cell lines using CRISPR-Cas9 genome editing. Ablation of both genes was confirmed by PCR and Southern blot analyses. The absence of both proteins significantly decreased the mitochondrial Ca^{2+} uptake at different extramitochondrial Ca^{2+} concentrations ($[Ca^{2+}]_{ext}$), without dissipation of the mitochondrial membrane potential, and increased the $[Ca^{2+}]_{ext}$ set point needed for Ca^{2+} uptake, as we have seen with *TcMICU1*-KO and *TcMICU2*-KO cells. Mg^{2+} was found to be a negative regulator of MCUC-mediated mitochondrial Ca^{2+} uptake at low $[Ca^{2+}]_{ext}$. Occlusion of the MCUC pore by Mg^{2+} could partially explain the lack of mitochondrial Ca^{2+} uptake at low $[Ca^{2+}]_{ext}$ in *TcMICU1/TcMICU2*-KO cells. In addition, *TcMICU1/TcMICU2*-KO epimastigotes had a lower growth rate, while infective trypomastigotes have a reduced capacity to invade host cells and to replicate within them as amastigotes.

MASTER-NAADP: Characterization of a newly developed membrane permeant NAADP derivative

Franziska Möckl[1], Mariella Weiß[1], Björn-Philipp Diercks[1], Patrick Dekiert[2], Sarah Krukenberg[2], Tobias Hub[3], Rebekka Medert[3], René Werner[4], Melanie Hofmann[2], Marc Freichel[3], Chris Meier[2], Andreas H. Guse[1]

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[2]Organic Chemistry, University of Hamburg, Germany
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Nicotinic acid adenine dinucleotide phosphate (NAADP) is a potent endogenous Ca^{2+} mobilizing second messenger, with concentrations in the low nanomolar range being sufficient to evoke Ca^{2+} signals in T cells[2]. Stimulation of the TCR/CD3 complex leads to rapid formation of NAADP resulting in Ca^{2+} microdomains via the type 1 ryanodine receptor (RYR1)[1]. Eventually, these local microdomains merge into global Ca^{2+} signals amplified by IP_3 and later cADPR[1]. Up to date, the mode of activation and targeted channels of NAADP remain diversly discussed. Both, ryanodine receptor type 1 (RyR1) on the endoplasmic reticulum (ER) membrane and endo-lysosomal two pore channels (TPCs) have been proposed as NAADP targets. Recent findings by our group, describe the formation of NAADP by NADPH oxidases, followed by binding to NAADP binding protein HN1L/JPT2 and subsequent Ca^{2+} release through RYR1 in T cells[3,4]. To study NAADP induced signaling, direct delivery of NAADP is crucial. Currently, the preferred techniques are either patch-clamp or microinjection, both of which require high precision and are technically highly demanding. Here, the newly developed compound MASTER-NAADP, a membrane permeable and bioreversible NAADP derivative, is tested in a high-resolution live cell-imaging setup in different cell types, including Jurkat T cells and Neuro2A cells.
Administration of MASTER-NAADP reliably evokes global Ca^{2+} signals, as well as Ca^{2+} microdomains in Jurkat T cells hence is a useful tool in the NAADP signaling toolbox.

[1]Guse, A. H. & Lee, H. C. NAADP: a universal Ca2+ trigger. Sci Signal. 2008 Nov 4;1(44):re10.
[2]Wolf, I.M. et al. Frontrunners of T cell activation: Initial, localized Ca2+ signals mediated by NAADP and the type 1 ryanodine receptor. Science Signal. 2015; 8: ra102.
[3]Gu, F, et al. Dual NADPH oxidases DUOX1 and DUOX2 synthesize NAADP and are necessary for Ca2+ signaling during T cell activation. Sci Signal. 2021 Nov16;14(709):eabe3800.
[4]Roggenkamp, HG, et al. HN1L/JPT2: A signaling protein that connects NAADP generation to Ca2+ microdomain formation. Sci Signal. 2021 Mar 23;14(675):eabd5647.

This research was funded by the Collaborative Research Center 1328, German Research Foundation (DFG), project A01, A02, A04 and the DFG research group FOR 2289 P02.

Dysregulated MAM & Ca^{2+} homeostasis underlying Wolfram syndrome type 2-associated CISD2 deficiency

Jens Loncke[1], Tim Vervliet[1], Jan Parys[1], Allen Kaasik[2], Martijn Kerkhofs[1], Geert Bultynck[1]

1: KU Leuven, Lab. Molecular & Cellular Signaling, Belgium; 2: Tartu University, Dep. Of Pharmacology, Estonia

CDGSH iron-sulfur domain 2 (CISD2) is a redox-active ER membrane protein that binds 2Fe-2S clusters. CISD2 impacts a broad range of cellular processes, including ROS and iron homeostasis, cellular longevity, autophagy, cell death and Ca^{2+} homeostasis. Loss-of-function mutations in *CISD2* are causative of Wolfram syndrome type 2 (WS2), a rare progressive disorder. Oppositely, high CISD2 levels are linked to poor prognosis in breast cancer, lung adenocarcinoma and several other types of cancer. The specific localization of CISD2 at the mitochondria-associated ER membranes (MAMs) is thought to be crucial for the role of CISD2 in cellular health and Ca^{2+} homeostasis. Indeed, highly focalized ER-mitochondrial Ca^{2+} transfer through the inositol 1,4,5-trisphosphate receptor (IP3R) occurring in the MAMs controls cell survival processes as well as cell death events.

Performing subcellular fractionation of HeLa cell lysates, we isolated pure MAM fractions and validated the presence of CISD2 in the MAMs. Moreover, we generated HeLa CISD2 KO cells via a Crispr/Cas9 based method. Using a MAM-specific fluorescent SPLICS probe (Cieri et al, 2017, Cell Death Diff), we found that HeLa CISD2 KO cells have reduced ER-mitochondrial contacts. Using co-immunoprecipitation assays, we demonstrated that CISD2 interacts with the IP3R. To determine a possible functional effect of CISD2 on IP3R-mediated Ca^{2+} release, we simultaneously imaged cytosolic Ca^{2+} and mitochondrial Ca^{2+} by using the cytosolic Ca^{2+}-sensitive fluorescent probe Fluo-4 and the mitochondrially-targeted genetically encoded Ca^{2+} sensor mitoCEPIA3. Ca2+ signals were evoked using extracellular agonists such as ATP. Interestingly, loss of CISD2 did not majorly affect agonist-induced, IP3R-mediated Ca^{2+} signals in the cytosol. However, the Ca^{2+} transfer from the ER to the mitochondria was significantly reduced in CISD2-deficient cells. Importantly, we excluded an altered mitochondrial membrane potential and reduced ER Ca^{2+} store content as causes for reduced Ca^{2+} transfer. Future work aims to assess the impact of disease-associated CISD2 mutants on ER-mitochondrial Ca^{2+} signaling and interaction with IP3Rs as well as to explore the impact of CISD2 loss on Ca^{2+} signaling in patient-relevant cortical neurons differentiated from induced-pluripotent stem cells.

For now, we conclude that CISD2 is a MAM-resident protein that is vital for MAM integrity and function. Cells lacking CISD2 show decreased ER-mitochondrial Ca^{2+} transfer. Possibly, CISD2 can positively regulate IP3R specifically in the MAMs, for example by taking part in a macrocomplex together with other IP3R modulators. Alternatively, the decreased ER-mitochondrial Ca^{2+} transfer can be independent of IP3R modulation and be solely attributable to decreased ER-mitochondrial contact.

Parallel regulation of IP_3 receptors by IP_3 and PIP_2

Adelina Ivanova, Colin W. Taylor and Peace Atakpa-Adaji

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While the majority of IP_3 receptors (IP_3Rs) in cells are mobile, localised Ca^{2+} puffs originate from a minor fraction of immobile IP_3Rs tethered to actin by KRas-induced actin-interacting protein (KRAP). These 'licensed' IP_3Rs are immobilised near contact sites between the endoplasmic reticulum and the plasma membrane (PM), where store-operated Ca^{2+} entry (SOCE) takes place. Uncovering the mechanisms that govern IP3R licensing is essential for understanding the spatial and temporal patterns of Ca^{2+} signalling. Phosphatidylinositol 4,5-bisphosphate (PIP_2) is a minor, but functionally diverse component of the PM which acts as the source of IP_3 in phospholipase C (PLC)- mediated Ca^{2+} signalling through IP_3Rs. Alongside its canonical role as IP3 precursor, PIP_2 is further involved in actin polymerisation beneath the PM and SOCE complex formation – processes which could relate PIP_2 to IP_3R licensing beyond IP_3 production. To address this possibility, we optimised and functionally characterised a rapamycin-inducible heterodimerisation tool for selective PIP_2 depletion at the PM. We show that selective PIP2 depletion at the PM reduces the frequency of Ca^{2+} puffs evoked by photolysis of caged IP_3 in HeLa cells without affecting puff amplitude or kinetics. As PIP_2 depletion may lead to reduction of basal IP_3 levels, we employ two complementary approaches to assess whether a loss of basal IP_3 is responsible for the reduced Ca^{2+} puff frequency. Reducing basal IP_3 levels by inhibiting PLC activity with U73122 or by overexpressing cytosolic IP_3 kinase C does not reduce the frequency of Ca^{2+} puffs evoked by photolysis of caged IP_3. We conclude that IP_3Rs are regulated by both PIP_2 and IP_3.

Loss of IP_3R fitness enables GNAQ/GNA11-mutated Uveal Melanoma survival by shutting down IP_3 signalling and preventing calcium overload-induced cell death

Céline Garcia [1] , Louis Roussel[1] , Sergio Roman Roman[2] , Corine Bertolotto[3] , Geert Bultynck[4] and Aubin Penna[1]

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Uveal melanoma (UM) is the most common primary intraocular malignancy in adults. In 50% of cases, liver metastases compromise patient's survival regardless of treatments. 90% of UMs are initiated by gain-of-function driver mutations in GNAQ/GNA11 encoding α subunits of the GPCR-associated G proteins while a small proportion are due to driver mutations in the Cysteinyl Leukotriene Receptor 2 (CYSLTR2) or the Phospholipase C beta (PLCB4). All these mutually exclusive oncogenic mutations have the same functional output, resulting in constitutive activation of the PLCβ enzyme which hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) in diacylglycerol (DAG) and inositol trisphosphate (IP_3). So far, UM research only addressed DAG-induced pro-oncogenic effects and identified its essential role in stimulating the MAPK pathway. As today, nothing is known on the role of the second arm of the deregulated PLC signalling, namely the IP_3/calcium (Ca^{2+}) pathway. Yet, Ca^{2+} controls several vital cell functions and any unregulated $[Ca^{2+}]i$ elevation is cytotoxic. Hence, UM cells probably have to remodel their Ca^{2+} homeostasome in order to cope with sustained IP_3 production and escape Ca^{2+} overload-induced cell death. Indeed, despite permanent IP_3 production, no major deregulation of the resting Ca^{2+} homeostasis was observed in GNAQ/GNA11-mutated UM cells. Our results further demonstrated that in UM cells, IP_3-induced ER Ca^{2+} depletion and the associated Ca^{2+} entry are prevented by the loss of responsive IP_3 Receptors (IP_3R) through multiple mechanisms. Altogether our data indicate that UM cells remodel their Ca^{2+} pathways to prevent ER stress and maintain their survival. It identified an UM cell vulnerability that opens up new perspectives for innovative uveal melanoma treatments.

Investigation of Calcium Electroporation for Cancer in the Skin

Mille Vissing (1,2), Sandra Kristiane Sinius Pouplier (3), Mascha Pervan(4), Lars Munch (1), Stine Frandsen (1), Kitt Vestergaard (5), John Pløen (6), Mazen Schnefeldt (7), Søren Rafael Rafaelsen (7), Christina Louise Lindhart (8), Lars Henrik Jensen (6), Achim Rody (4), Anne-Vibeke Lænkholm (2,3), Julie Gehl (1,2)

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Background: Mutations may impair cancer cell-calcium homeostasis and cellular stress with calcium-ions can induce cell death. Calcium electroporation is a novel, local cancer treatment using intratumorally injected calcium chloride and manually applied pulsed electric fields. The pulses create transient pores in bi-lipid membranes allowing an increased flux of calcium-ions. Normal cells may restore homeostasis and recover whilst cancer cells die, making the treatment a safe, efficient and selective tool for targeted local treatment of cancer with limited side effects. Reports of long-term local disease control and systemic responses following treatment in initial small-cohort trials imply a positive effect on cancer immunity caused by calcium induced cell death. We report two clinical studies investigating the histopathological effect of calcium electroporation (CaEP-B) and clinical response (CaEP-R).

Materials and methods: Both studies are non-randomized phase II trials including patients with cutaneous malignancy of any histology. Patients are followed up to 12 months. The CaEP-B study will include 24 patients treated once and retreated after one month, depending on number of included tumours, with sequential biopsies taken at baseline and after treatment. The primary endpoint is the change in proportion of tumour-infiltrating lymphocytes two days after treatment. The samples will be analysed for immune markers as well as necrosis, changes in vasculature and inflammation. Circulating tumour DNA from sequential blood samples will be analysed in a subgroup of patients. Secondary endpoints include response, PD-L1 expression and importance of radiation. The CaEP-R study aims to investigate response to calcium electroporation in 30 patients. Patients are treated once and the primary endpoint is overall response rate at two months. The trial is a collaboration between three cancer centres. In one patient subset, MRI is used to verify treatment area. In another subset, qualitative interviews have been performed to uncover patient experience.

Results: Trials are ongoing and a total of 30 patients have been included with a variety of cancer subtypes. Few side effects have been observed and healthy tissues have been spared. Nine patients have been interviewed and the qualitative data of this subgroup is being analysed. MRI has been used to verify treatment areas in three patients. Preliminary results from the first eight patients in CaEP-R show an overall response rate of 42% (CI 23-63 %) across all tumours after two months (n = 24). In CaEP-B, one patient showed improved response to immunotherapy in untreated tumour after treatment.

Conclusion: This is the most comprehensive project to date investigating the effect of calcium electroporation on malignant tumours and their microenvironment. The results of this trial may illuminate mechanisms underlying this promising new treatment and reveal synergistic effects with the immune system.

Communication is key: Investigating calcium crosstalk in the breast tumour microenvironment

Krystyna A Gieniec[1], Gregory R Monteith[2,3], Felicity M Davis[1,4]

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Breast cancer is a devastating disease that continues to take the lives of more than 650,000 women worldwide each year. Despite major advances in the molecular characterization of the disease, treatment options remain inadequate and therapy is often thwarted by resistance and metastasis. To develop effective, long-lasting therapies for breast cancer, we must continue to widen our tumour-centric approach to drug discovery and consider targeting interactions between cancer cells and the key, non-malignant cell types in the microenvironment.

Cancer-associated fibroblasts (CAFs) play a major role in steering breast cancer development and dissemination. These cells can arrange themselves to envelop and compartmentalize solid tumours, a phenomenon that has been linked to increased intratumoural pressure and a more aggressive tumour phenotype. Whilst calcium signals have independently been linked to both cellular contraction and cellular crowding, the role of calcium signaling in fibroblast-tumour interactions in this context has yet to be investigated.

By generating fibroblasts that express a fast, green-shifted genetically-encoded calcium indicator (GECI), we are able to explore whether fibroblast contractions are calcium signal-dependent and how this signal may help to coordinate CAFs as the dominant stromal cell type in breast cancer. Furthermore, by creating cancer cells that express red-shifted GECIs, we have been able to generate a multi-compartment co-spheroid model for the spatiotemporal evaluation of calcium signal crosstalk. By understanding and interrupting this cellular crosstalk, this research may open new avenues for therapeutic intervention in breast cancer and may help to define the next generation of breast cancer therapies.

Development of an adrenocortical cell model of calcium signaling modulation to decipher the molecular mechanisms responsible for primary aldosteronism

Bakhta Fedlaoui[1*], Teresa Cosentino[1*], Zeina Al Sayed[1], Isabelle Giscos-Douriez[1], Fabio L Fernandes-Rosa[1], Jean-Sebastien Hulot[1,2], Chris Magnus[3], Scott M Sternson[3], Maria-Christina Zennaro[1,2] and Sheerazed Boulkroun[1]

[1]PARCC, INSERM, Université de Paris, Paris France
[2]Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital Européen Georges Pompidou, Paris, France, [3]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147, USA

Introduction: Primary aldosteronism (PA) is the most frequent form of secondary hypertension. The identification of germline or somatic mutations in different genes coding for ion channels and defines PA as a channelopathy. These mutations promote activation of calcium signaling, the main trigger for aldosterone biosynthesis.

Objective: The objective of our work was to elucidate, using chemogenetic tools, the molecular mechanisms underlying the development of PA by modulating sodium entry into the cells, mimicking some of known mutations identified in PA.

Method: We have developed an adrenocortical H295R_S2 cell line stably expressing a chimeric ion channel receptor formed by the extracellular ligand-binding domain of the α7 nicotinic acetylcholine receptor fused to the ion pore domain of the serotonin receptor 5HT3 α and named α7-5HT3. Mutations have been introduced in the ligand binding domain to allow only synthetic drugs to activate this channel receptor. Activation of α7-5HT3 by a specific drug, PSEM-817 leads to sodium entry into the cells. This cell line was characterized in terms of intracellular calcium concentrations, cell proliferation, aldosterone production, steroidogenic expression and electrophysiological properties.

Results: Treatment of α7-5HT3 expressing cells with increasing concentration of PSEM-817 (from 10^{-9} to 10^{-5} M) induced a significant increase in intracellular calcium concentrations, similarly to potassium (12 mM) or angiotensin II (10^{-8} M). This stimulation of calcium signaling did not affect cell proliferation, but was responsible for an increase in *CYP11B2* expression and aldosterone production after 24h of treatment. However, while increased intracellular calcium concentrations were observed starting from 10^{-8} M of PSEM-817, *CYP11B2* expression and aldosterone production were only affected starting from 10^{-7} M, suggesting a dose dependent effect. Finally, whereas cells were hyperpolarized in absence of stimulation (around -60 mV), PSEM-817 induced a strong depolarization, cells rising to a membrane potential around -10mV.

Conclusion: This cell line, in which we can modulate the intracellular calcium concentration “on demand”, is a useful tool for a better understanding of the alterations of intracellular ion balance and calcium signaling in the pathophysiology of PA.

HINT1 deficiency impairs actin cytoskeleton and calcium signaling

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Loss of functional histidine triad nucleotide binding protein 1 (HINT1) causes a rare form of inherited peripheral neuropathy with neuromyotonia (NMAN). Patients suffer from motor greater than sensory neuropathy with an age on onset within the first decade. HINT1 is a ubiquitously expressed phosphoramidase and SUMOylase. It acts as a transcriptional inhibitor of pro-oncogenic transcription factor and as an adaptor protein of the endocannabinoid signaling pathway in the central nervous system (CNS). Yet, its role in the peripheral nerves is uncharacterized. We created HeLa cell lines deficient for *HINT1* using CRISPR/Cas9 genome editing technology and studied their transcriptome profile. Gene ontology and pathway analysis indicated integrin signaling and actin cytoskeleton as affected pathways. Additionally, we identified intracellular signaling as a recurrent term within the most significantly affected pathways. Therefore, we characterized, the calcium signaling response to extracellular stimuli using genetically encoded calcium indicators (GECOs). We identified a weaker response in the *HINT1* KO cells likely caused by reduced calcium loading into the endoplasmic reticulum. Our findings identify and characterized two affected pathways as a results of loss of HINT1 suggesting new disease mechanisms.

Blockade of mitochondrial calcium uptake protects neurons against ferroptosis

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Introduction. Ferroptosis is an iron- and reactive oxygen species (ROS)-dependent form of regulated cell death, that has been implicated in Alzheimer's disease. Initiation of ferroptosis via cysteine/glutamate antiporter inhibition leads to mitochondrial fragmentation, mitochondrial calcium (Ca^{2+}) overload, increased mitochondrial ROS production, disruption of the mitochondrial membrane potential ($\Delta\Psi_m$) and cell death. Recent studies showed that mitochondrial dysfunction is a characteristic of ferroptosis, that makes preservation of mitochondrial function a potential therapeutic target in degenerative diseases. Mitochondrial calcium levels are controlled via the mitochondrial calcium uniporter (MCU), the main entry point of Ca^{2+} into the mitochondrial matrix. Therefore, we have hypothesized that inhibition of mitochondrial calcium uptake may confer protection against ferroptosis.

Objectives. To investigate the anti-ferroptotic potential of mitochondrial calcium uptake inhibition in conditions of increased oxidative stress.

Methods. In the present study we employ the HT22 murine hippocampal cell line, to model ferroptosis and oxytosis. To evaluate the potential protective capacity of targeting of the mitochondrial calcium uniporter against ferroptosis induced by erastin, RSL3 or glutamate, we pharmacologically targeted i) MCU with ruthenium red (RR), mitroxantrone (MX) and Ru265; and ii) MICU1, a regulator of the pore function of MCU, with MCU-i4. We measured hallmarks of ferroptosis, namely mitochondrial function, mitochondrial morphology, calcium uptake, mitochondrial ROS production and lipid peroxidation.

Results. Blocking the activity of MCU significantly reverted the calcium uptake, lipid peroxidation, and mitochondrial ROS that was initiated by erastin and RSL3 challenge, measured by flow cytometry. Co-treatment with RR, MX, Ru265 and MCU-i4 prevented erastin-, and RSL3-, and glutamate-induced cell death, as detected by bright-field microscopy, MTT assay and flow cytometry in a concentration-dependent manner. Additionally, ferroptosis impaired mitochondrial function decreasing the oxygen consumption rate.

Conclusions. An *in vitro* model of ferroptosis was employed to test the capacity of MCU inhibition to protect neurons against cell death. Taken together, our results demonstrate that MCU antagonism and mitochondrial calcium reduction is protective against ferroptosis. In conclusion, this study provides the foundation for further investigation into the therapeutic potential of MCU inhibition against ferroptosis or MICU1 deficiencies.

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Abstracts

Poster presentations

Computational re-estimation of the IP_3 diffusion coefficient

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Inositol 1,4,5-trisphosphate (IP_3) is a second messenger responsible for the release of intracellular Ca^{2+} ions from internal stores. This molecule has long been considered a global messenger with a diffusion coefficient ≈ 280 $\mu m^2/s$ in a water-like medium (Allbritton et al., 1992). This value was however re-estimated on the basis of the dynamics of local IP_3-evoked Ca^{2+} puffs triggered by IP_3 diffusing from a spot of photorelease (Dickinson et al., 2016). By combining these observations with a theoretical analysis of Ca^{2+} puff latencies, the authors calculated that IP_3 diffuses 30-fold slower than previously reported, implying that it should be considered as a local rather than a global messenger. Ineffective IP_3 binding to partially bound IP_3 receptor tetramers is thought to be responsible for the slowing down of IP_3 diffusion in intact cells (Taylor and Konieczny, 2016), although the reduction does not match quantitatively with current knowledge.

In this work we used stochastic modelling of IP_3R cluster dynamics (Voorsluijs et al., 2019) to re-examine this question. We performed sophisticated 2D and 3D spatiotemporal simulations in realistic cellular geometries, using COMSOL Multiphysics.

Simulations concluded that best agreement with the experimental observations of Dickinson et al (2016) is obtained with an IP_3 diffusion coefficient of about 100 $\mu m^2/s$, a value that is moreover practically unaffected by the presence of the ER.

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Relationship between moderate ER Ca^{2+} depletion and induction of the unfolded protein response (UPR): experimental and modeling approach

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The Endoplasmic Reticulum (ER) is the primary site of folding and quality control of one third of cellular proteins and is the major intracellular Ca^{2+} store. Depletion of the luminal [Ca^{2+}] disrupts the correct folding environment leading to an alteration of ER homeostasis and accumulation of misfolded proteins inside the lumen. In order to restore ER proteostasis and normal cellular functions, cells have developed an adaptive mechanism consisting in 3 specific signalling pathways. This response is commonly referred to as the Unfolded Protein Response (UPR) and leads to an increase of the protein folding capacity of the ER and to homeostasis restoration. Although long-term and strong UPR activation is much studied, the consequences of small amplitude, more physiological, luminal Ca^{2+} depletions on the early activation of UPR has been largely unexplored. In this study, we investigate how moderate Ca^{2+} depletion impacts on the activation of the signalling pathways of the UPR. Ca^{2+} imaging experiments using genetically encoded Ca2+ indicators targeting the ER combined with Immunoblots, qPCR and imaging data allow us to reveal the early links between ER Ca^{2+} depletion and UPR activation. The concomitant development of a data-driven computational model allows us to decipher, formalize, and quantify these complex signaling pathways. Given that luminal Ca^{2+} depletion and alteration of correct ER proteostasis are involved in a variety of pathologies such as diabetes, neurodegenerative diseases or cancer, a better understanding of the reciprocal crosstalk between Ca^{2+} and UPR will provide insight into the mechanisms of progression of these diseases.

The Contribution of Annexin V to Mitochondrial Ca^{2+} Uptake

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Annexins are a family of 12 proteins playing an important role in apoptosis, membrane repair mechanism, and intracellular Ca^{2+} homeostasis. Among other annexins, one of the characterized functions of Annexin V (AnxV) is the ability to bind negatively charged phospholipids in Ca^{2+} dependent manner. Accordingly, upon cell membrane rupture, the Ca^{2+} level elevates in the cytosol which leads to translocation of AnxV to the ruptured area to fix the cell membrane. In addition to the cell membrane, several studies also demonstrated the translocation and localization of AnxV to mitochondria. However, the function of AnxV in the mitochondria is not clear yet.

Here, we investigate the contribution of AnxV to mitochondrial Ca^{2+} homeostasis by using various genetically encoded Ca^{2+} sensors targeted either in the cytosol, the intermembrane space (IMS), the mitochondrial matrix, or cristae lumen (CL). Our dynamic Ca^{2+} measurements revealed that CRISP/Cas9-mediated knockout (KO) of AnxV severely decreases mitochondrial Ca^{2+} elevations in IMS, the CL, and in the matrix upon stimulation of cells with an IP_3-generating agonist. Moreover, the cytosolic Ca^{2+} levels and mitochondrial membrane potential were not affected in AnxV-KO cells. Hence, mitochondrial-associated membranes (MAMs) remained the same in AnxV-KO cells indicating the direct involvement of AnxV in mitochondrial Ca^{2+} regulation without affecting the mitochondria-endoplasmic reticulum (ER) contact sites. Additionally, we demonstrate that AnxV is essential for mitochondrial Ca^{2+} uptake upon Ca^{2+} release from the ER but does not play a role in store-operated Ca^{2+} entry (SOCE). Our preliminary results indicate that AnxV regulates mitochondrial Ca^{2+} uptake via Voltage-Dependent Anion Channel 1 (VDAC1). Thus, our findings highlight the Ca^{2+} source-dependent involvement of AnxV in mitochondrial Ca^{2+} uptake. Currently, further studies are ongoing to identify the regulation of mitochondrial Ca^{2+} uptake via AnxV interaction of VDAC1.

Elucidating the role of TPC2 in Lysosomal Storage Disorders

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Lysosomal Storage Disorders (LSDs) are the main underlying cause of neurodegenerative dementias. Disturbed calcium (Ca^{2+}) ion homeostasis is a major cause of LSDs in both children and adults. In attempts to understand the progression of LSDs, defects in endo-lysosomal Ca^{2+} homeostasis in two families of Ca^{2+} channels, two-pore channels (TPC1-3) and transient receptor potential channel (TRPML1-2) have been widely studied. We aim to understand how these two channel families localized in lysosomes are involved in generating lysosomal (and cellular) Ca^{2+} ion signals and thereby influence Ca^{2+} homeostasis. We have begun elucidating the basic question to understand the link between how nicotinic acid dinucleotide phosphate (NAADP) mobilizes Ca^{2+} ions from these ion channels, with the initial focus on TPC2. For this, we have started looking at lysosomal-specific Ca2+ microdomains formed in Jurkat T cells with the aid of lysosomal specific agonist TPC2 channel namely TPC-A1-N (A1-N), to confirm how these differ from the ones generated by cell-permeable NAADP. Using our established protocol of high-resolution Ca^{2+} imaging and respective fluorescent indicators[1] , we identified that A1-N (60 µM) evoked the formation of Ca^{2+} microdomains within the first 10-20s post stimulation. We also aimed to understand the role of store-operated calcium entry (SOCE) and lysosomal de-acidification on A1-N evoked Ca^{2+} microdomain formation. Upon inhibition of SOCE by Synta66 (50 µM) and lysosomal de-acidification by bafilomycin A1 (12.5 nM), a significant reduction in A1-N evoked Ca^{2+} microdomain formation was observed. These results preliminarily indicate that A1-N mediated Ca^{2+} microdomain formation requires Ca^{2+}entry and intact lysosomes. This prompted us to further investigate the possible link between SOCE and TPC2. Furthermore, we are currently working on elucidating how A1-N evoked Ca^{2+} microdomain formation is influenced in the absence of ryanodine receptors, a novel identified NAADP-binding protein HN1L[2] and the NAADP-synthesizing DUOX enzymes[3]. This will help to understand the basic picture of the various players needed for efficient lysosomal Ca^{2+} ion release specifically from TPC2 in T-cells.

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Calcium oscillations regulate lipid flux *in vivo* during zebrafish liver development

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During starvation, vertebrates utilize the liver as a lipid storage organ. For instance, patients suffering from anorexia nervosa[1], overnight fasted mice[2], and starved zebrafish have been reported to develop fatty liver. Hepatic steatosis, in this case, creates and energy reservoir that allows survival during long-term caloric deprivation. Nutrient availability clears steatosis and returns the liver to baseline lipid deposition[3]. In contrast to hepatic steatosis caused by excessive calorie intake (i.e. Non-alcoholic fatty liver disease), the mechanisms of starvation induced hepatic steatosis are barely studied at all.

Here we take advantage of the transparency of the zebrafish larvae to develop a setup that allows *in vivo* imaging of lipid droplets and intracellular calcium dynamics in the zebrafish liver. We show a negative correlation between calcium oscillations and lipid accumulation in the zebrafish liver. Specifically, starvation induced hepatic steatosis and inhibited calcium waves. Further, feeding recommences calcium waves in the liver, correlating with a decrease in the amount of lipid droplets in the organ. To functionally test the relationship between calcium signaling and steatosis, we buffered calcium signaling using a genetically encoded calcium scavenger. This accelerated the process of steatosis development, suggesting calcium flux to be a regulator of lipid accumulation.

Withing the cell, calcium stored in endoplasmic reticulum (ER) is considered to be responsible for calcium transients[4]. However, other organelles such as endo-lysosomes contains calcium at similar concentration as ER[5,6] and has recently gained much attention as a regulator of intracellular calcium level. Thus, we evaluated the potential role of endo-lysosomal calcium to regulate intracellular calcium oscillations *in vivo* in zebrafish liver. Our preliminary data suggests that activation of a lysosomal cation channel, TPC2 increases calcium transients. Interestingly, this decreases steatosis during starved state without introduction of food. This suggests that mobilization of endo-lysosomal calcium stores could induce calcium oscillations in the liver cells, which further would induce clearance of lipid droplets.

Currently, we are developing tools for sub-cellular imaging of calcium release from endo-lysosomal compartment *in vivo*, and are interested to investigate the link between TPC2 activity and lipophagy under physiological conditions. Additionally, it would be of interest to test TPC2 activation as a method for reducing hepatic steatosis in pathological conditions, such as in alcoholic and non-alcoholic liver disease.

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Role of 3',5'-cyclic adenosine monophosphate during T cell activation

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3',5'-cyclic adenosine monophosphate (cAMP), a second messenger, functions as a key modulator of the immune response[1] . For a long time, cAMP has been thought to be solely a negative regulator in T cells, with several reports showing major inhibitory effects of sustained increases of cAMP levels on T cell activation1 . However, already in 2009 Conche et al. were able to demonstrate that a transient cAMP increase upon TCR/CD3 ligation contributes to T cell activation and precedes the Ca^{2+} response upon interaction of a T cell with an antigen presenting cell. These findings suggest that the kinetics of cAMP signaling plays also a major role in initial T cell activation rather than just negatively regulating T cells in the long run[2]. Furthermore, preliminary data of the existence of local cAMP pools in other cell types suggest that the differential effects of cAMP signaling on T cell activation might be due to compartmentalization not only on a temporal level, but on a spatial level as well, leading to the discussion of a possible role of cAMP microdomains in T cell activation1 . So far, this phenomenon could not be observed in live cell imaging due to technological limitations. However, we were able to overcome this obstacle by performing high-resolution live cell imaging in combination with novel genetically-encoded cAMP-sensors called cAMPFIRE (cAMP Fluorescence Imaging Reporters based on Epac)[3]. These highly sensitive FRET-sensors are suitable for subcellular imaging and can therefore be used to visualize cAMP signaling after T cell stimulation. Using electroporation, we transfected Jurkat T cells and performed live cell imaging. To verify the sensor and optimize the settings, we stimulated the cells with a membrane-permeable cAMP-derivate called pro-cAMP, as well as forskolin, which activates the adenylyl cyclase, and IBMX, an inhibitor of phosphodiesterase. To investigate the effect of T cell receptor stimulation on cAMP signaling, we stimulated the cAMPFIRE-transfected Jurkat cells with soluble α-CD3 antibodies as well as antibody coated-beads to investigate the effect of T cell receptor stimulation on cAMP signaling. Analysis of local cAMP signals was performed by a newly developed custom-made deconvolution and detection script. Data obtained with this innovative method will be discussed at the conference.

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Interplay of calcium and cGMP in the photoreceptor guanylate cyclase complex.

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Photoreceptor guanylate cyclases (GCs) are controlled by guanylate cyclase-activating proteins (GCAPs) that belong to the class of neuronal calcium-sensor proteins. They regulate the calcium-dependent synthesis of cGMP catalyzed by GCs in rod and cone cells, which is a critical step in photoreceptor light adaptation in the retina. Mutations in human GC-E and in particular in GCAP1 can cause an imbalance in calcium-cGMP homeostasis leading to retinal dystrophies. In humans, three different isoform of GCAPs (1, 2 and 3) are known, which differ with respect to their calcium-sensing and regulatory properties. The third isoform GCAP3 is however not well characterized for its regulatory properties or target specificity. We aim to unravel the molecular mechanism of photoreceptor GC-E switching from the inactive to the active state under control of GCAPs. We tested the hypothesis, whether a rotation model of activation involving an α–helix rotation in GC-E is the critical switch that operates under control of GCAPs. We simulated experimentally this α-helix rotation by integration of alanine residues close to the transmembrane region by site directed mutagenesis and functional studies conducted by GC-Assays. We compared the enzymatic catalytic parameters of wildtype and the retinal disease-related mutant V902L of GC-E, which is a constitutively active mutant. We extended the analysis to photoreceptor GC-F and the regulatory features mediated by GCAP isoforms, particularly GCAP3 and investigated their different calcium concentration profiles. Surprisingly, our data do not support the α-helix rotation model in GC-E. This finding distinguishes sensory GCs from hormone-receptor GCs and points to their unique calcium-dependent control by GCAPs. Furthermore, the point mutation in position V902L in GC-E leads to the GC active transition state, which is in wildtype GC-E stabilized by GCAPs. Our findings are also relevant for understanding the molecular basis of retinal diseases that are caused by a distortion of calcium-sensing processes.

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Interaction of the NAADP binding protein HN1L/JPT2 with its target receptors in T cells

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During the adaptive immune response, activation of T cells significantly depends on changes in free cytosolic calcium (Ca^{2+}) concentrations. Early Ca^{2+} signals upon T cell activation are mostly regulated by Nicotinic acid adenine dinucleotide phosphate (NAADP), the most potent Ca^{2+} mobilizing second messenger. NAADP is formed within seconds upon T cell stimulation[1] . However, the target channels of NAADP are controversially discussed: while in some cell types, NAADP acts on two-pore channel 1 and 2 (TPC1 and TPC2) [2] , in T cells NAADP was shown to activate the ryanodine receptor type 1 (RYR1), located in the membrane of the endoplasmic reticulum (ER)[3] . Surprisingly, when using photoaffinity labeling (PAL) to identify binding partners of NAADP, neither RYR1 nor TPCs were labeled, but small cytosolic proteins[4] . Thus, a unifying hypothesis was formulated that one or more NAADP binding proteins (NAADP BPs) activate different Ca^{2+} channels localized at different organelles – depending on cell type and signaling pathway involved[5] . Recently, two labs independently identified hematological and neurological expressed 1–like protein (HN1L) / jupiter microtubule associated homolog 2 (JPT2) as NAADP binding protein [6,7]. The essential role of HN1L/JPT2 during T cell activation was proven by showing decreased local and global Ca^{2+} signals in human Jurkat and primary rat T cells with gene knock-out of Hn1L/Jpt2. Also, co-localization of HN1L/JPT2 with RYR1 in T cells upon their T cell receptor stimulation was shown in super resolution imaging[6] . Here, we want to understand the exact mechanisms connecting HN1L/JPT2 and RYR1 to NAADP signaling. Data obtained regarding NAADP binding to HN1L/JPT2 and posttranslational modifications of HN1L/JPT2 will be discussed at the conference.

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Loss of IP_3 receptors in human induced pluripotent stem cells alters metabolism but not differentiation potential

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Human cells contain 3 genes (*ITPR1*, *ITPR2* and *ITPR3*) that encode for inositol 1,4,5-trisphosphate receptors (IP3Rs), thereby yielding 3 isoforms : IP3R1, IP3R2 and IP3R3, respectively. These IP_3Rs are partially redundant as knockout of all 3 subtypes is lethal in rodents while single gene knockouts produce tissue-specific pathology. Mutations in *ITPR1* cause ataxia. We recently reported *ITPR3* mutations in patients with Charcot-Marie-Tooth neuropathy, suggesting a role in the peripheral nervous system. Here, we set out to create a model for elucidating cell type-specific functions of the human IP3 receptors. Our first goal was to determine their role in stem cell survival and pluripotency. Therefore, we generated *ITPR1*, *ITPR2*, *ITPR3* and *ITPR1/2/3* triple knockout (TKO) cell lines of human induced pluripotent stem cells (iPSC).

CRISPR/Cas9 gene editing was used to knockout the *ITPR* genes. TKO iPSC lines were generated by knocking out the individual genes in successive manner. Generated knockout cell lines were analyzed by Sanger sequencing, Western blotting and quantitative reverse transcription PCR (qPCR) to ensure successful editing. The resulting knockout cell lines were then analyzed by gene expression panel targeted to stem cell-specific genes, immunocytochemistry and trilineage assay to examine the pluripotency of the cell lines. We also confirmed the successful editing of the generated TKO cells with functional Ca^{2+} imaging and examined the metabolite profile of the generated knockout cell lines with targeted metabolomics.

The desired gene editing events were successfully implemented. The qPCR analysis indicated the loss of *ITPR2* and *ITPR3* expression, whereas some *ITPR1* expression was retained in edited cell lines. Nevertheless, immunoblotting confirmed that the expression of all 3 IP3R isoform proteins was absent. Importantly, Ca^{2+} imaging revealed that the TKO iPSC, though having adequately loaded ER Ca2+ stores, did not respond to IP_3-generating agonists. Despite the loss-of-function, gene expression panel identified comparable levels of pluripotency markers between edited and control cell lines. The expression of pluripotency marker NANOG was confirmed with immunocytochemistry, Trilineage assay by directed differentiation revealed that all iPSC lines could differentiate into all three germ layers, despite distinct alterations in citric acid cycle metabolites in the TKO iPSC, identified by targeted metabolite profiling.

To conclude, this work is the first report of complete functional loss of IP3Rs in human iPSC. Our results suggest that IP3Rs control stem cell metabolism but are not required for maintenance of pluripotency.

Store-Operated Ca^{2+}-Entry in Uterine Endometrial Cancer

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Worldwide, uterine endometrial carcinoma is the sixth most common form of cancer in women. Early diagnosis resulting from patient-reported postmenopausal bleeding leads to excellent 5-year survival rates. However, for women with advanced or recurring EC, prognosis is relatively poor [1]. Consequently, there is a need for new therapeutic strategies to tackle these forms of this disease.

The "Ca^{2+}-toolkit" is characteristic of each cell-type and alters during proliferation, differentiation, oncogenesis and death [2]. Using The Cancer Genome Atlas accessed via the UALCAN resource [3], we examined differences in the expression of 196 genes encoding Ca^{2+}-toolkit proteins between EC tumours (n = 546) and normal adjacent tissues (n = 35). All genes encoding components of the store-operated Ca^{2+}-entry (SOCE) pathway showed significant differences in transcription. Transcription of *ORAI 1-3* genes was increased in EC tumours relative to normal tissue, whereas *STIM1* and *STIM2* were significantly decreased. Of these changes, only increased expression of *ORAI2* was associated with alterations in patient outcomes: by Kaplan-Meier analyses, those with the highest 50% of *ORAI2* levels showed significantly poorer survival ($p = 0.005$).

In order to investigate the roles of Orai channels in EC biology, we examined the effects of small molecule antagonists (GSK7975A, Synta66 and 2-aminoethoxydiphenyl borate (2-APB)) on SOCE [4] in the human EC cell-line RL95-2. Changes in cytoplasmic Ca^{2+} levels were monitored by fluorescent videomicroscopy using the Ca^{2+} fluorophore, fura-2. In nominally Ca^{2+}-free HEPES-buffered saline, RL95-2 cells were incubated with 1 mM thapsigargin, to deplete the endoplasmic reticulum of Ca^{2+}. Extracellular Ca^{2+} (2 mM) was then added back to the medium, to quantify SOCE. Pre-incubation with any of the antagonists significantly decreased the rate and/or magnitude of SOCE relative to vehicle pretreated with vehicle (DMSO) alone.

The ability of cells to form colonies is a hallmark of cancer cells. To examine the role of SOCE in this process, RL95-2 cells were seeded at low-density (3×10^3 cells/well in 12-well plates) and cultured for two weeks in the presence of different concentrations of GSK7975A, Synta66 or 2-APB. All three antagonists significantly inhibited clonogenesis of RL95-2 EC cells, with half-maximal inhibitory concentrations at low micromolar levels.

Overall, these findings indicate that SOCE plays a role in colony formation of RL95-2 cells and that in EC, high *ORAI2* expression is associated with poor patient survival. This highlights the potential of targeting *ORAI2* in the development of new chemotherapeutic strategies for the treatment of EC.

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Intracellular calcium signaling is significantly dysregulated in iPSC-derived neural progenitor cells containing the M139V presenilin 1 mutation.

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Alzheimer's disease (AD) is an untreatable neurodegenerative disorder, the underlying cause(s) of which remain unknown. Recent research by us and others supports the so-called 'calcium hypothesis of AD', which posits that the disruption/dysregulation of neuronal intracellular calcium regulation may be one of the initiators of AD pathology. To date however, most of these studies have been conducted using animal models of AD. As such, it remains unclear if these findings of neuronal calcium homeostatic dysregulation are replicated in human brain tissue carrying specific AD-linked mutations, due to the paucity of living human brain tissue available for research purposes.

Therefore, the aim of the current study was to determine if previous work in our lab, which demonstrated that intracellular calcium regulation was disrupted in primary hippocampal neurons from a transgenic mouse model of AD (3xTgAD mouse) [1], was replicated in human induced pluripotent stem cell (IPSC)-derived neural progenitor cells (NPCs) containing a familial AD mutation of the presenilin 1 protein (M139V), and a healthy donor control (CaCntrl).

NPCs were prepared from commercially available human IPSCs containing a familial AD mutation of the presenilin 1 protein (M139V), and from a healthy donor control (CaCntrl), by dual inhibition of TGF-β/BMP-dependent SMAD signalling. Neural stem lineage was confirmed by SOX2 and βIII tubulin immunostaining. 5-9 day post-culture cells were loaded with the calcium-sensitive dye Calbryte™ 520 AM (4μM) for 1 hour and placed in a perfusion chamber continuously superperfusing with HBSS (2ml/min). Intracellular calcium ([Ca2+]i) responses to extracellular 50mM K+ and the Ca2+ ionophore ionomycin (10μM) were recorded using WinFluor software. Data are expressed as mean±S.E.M. N values are displayed as n = x cells, x experiments. All results presented were analysed by two-tailed Welch's t test.

Results

We found that the ΔF/F relative percentage change of K+-evoked [Ca2+]i signals were significantly greater in M139V NPCs (n = 70, 6) compared to CaCntrl (n = 71, 9)(561.7%±588 vs 108%±8.3, $p<0.0001$). Similarly, the ΔF/F of ionomycin-evoked [Ca2+]i signals was also significantly greater in M139V NPCs compared to CaCntrl (362.9%±36.12 vs 170.3%±9.74, $p<0.0001$). Furthermore, basal [Ca2+]i levels in the M139V NPCs were significantly elevated relative to controls (13.64±0.95 arbitrary units vs 7.1±0.4 arbitrary units , $p<0.0001$).

Conclusion

Our results indicate that, in accordance with our previous work using primary cultured hippocampal neurons, both basal and evoked [Ca2+]i levels in presenilin mutant cells are significantly heightened relative to controls. The profound differences in calcium responses observed in neural stem cell populations prior to maturation supports the suggestion that AD pathogenesis commences before more overt, neurohistopathological changes.