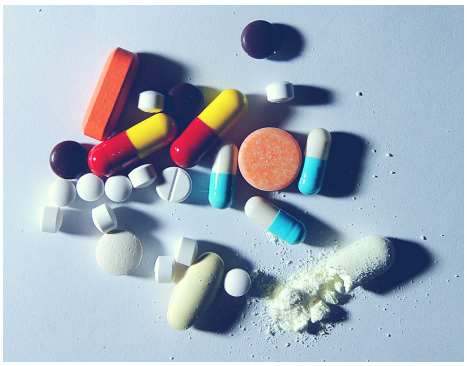

UBICODE ANNUAL SYMPOSIUM

DRUGGABILITY OF THE UBIQUITIN SYSTEM

WHICH ROAD TO TAKE ?

27
02
19

CEDOC, Grey Building
Lisbon, Portugal

PROGRAMME

MORNING SESSION

08:30 REGISTRATION - POSTER SETTING

08:55 WELCOME BY R. MATTHIESEN & A. PICHLER

09:00 OPENING LECTURE BY SUSAN GASSER

Chromosome Dynamics and DNA damage

SESSION 1 THE UBIQUITIN SYSTEM & GENOME INSTABILITY

Chair - Alfred Vertegaal

09:30 SYLVIE URBE - DUBs at the crossroads of cancer and neurodegeneration

09:50 ANDREA PICHLER - Insights into EME1 sumoylation

10:10 RON HAY - Arsenic induced degradation of the Promyelocytic leukaemia protein

10:30 EMILIO LECONA - Roles for VCP in DNA replication and cancer

10:50 COFFEE BREAK - POSTER SESSION

SESSION 2 THE UBIQUITIN SYSTEM & DISEASE - PART I

Chair - Frauke Melchior

11:10 MARIA MASUCCI - Regulation of the IFN response by herpes virus deconjugases

11:30 HENRIQUE GIRÃO - The role of ubiquitin in the regulation of intercellular communication

11:50 PEDRO DOMINGOS - The Fbox protein CG6758 regulates Xbp1-induced cell death in the Drosophila eye

12:10 IZABELA SUMARA - Development of anti-cancer drugs targeting UBASH3B-mediated mitotic checkpoint signaling

12:30 LUNCH BREAK - POSTER SESSION

AFTERNOON SESSION

SESSION 3 THE UBIQUITIN SYSTEM & DISEASE - PART II

Chair - Rosa Barrio

14:20 YOGESH KULATHU - Modulating Proteostasis: Emerging roles for Deubiquitinases

14:40 PAULO PEREIRA - Ubiquitinylation of HIF-1alpha regulates multiple degradation pathways and intercellular signalling

15:00 PAULA LUDOVICO - Signalling mechanisms that regulate metabolic profile and autophagy of acute myeloid leukaemia cells

15:20 DIMITRIS XIRODIMAS - Strategies to reduce the side effects of inhibitors for the NEDD8 pathway

15:40 COFFEE BREAK - POSTER SESSION

SESSION 4 WHERE TO HIT THE SYSTEM ?

Chair - Manuel Rodriguez

16:10 NICOLAS THOMÄ - The Zinc Finger Degrome

16:30 HUIB OVAA - Small molecules and probes to target the ubiquitin system

16:50 RUNE MATTHIESEN - Proteostasis factors in cancer exosomes

17:10 JOANNA KOSZELA - Evaluating selectivity of ubiquitination inhibitors with a novel fluorescence-based assay for real-time monitoring of ubiquitination cascades

17:30 CONCLUDING REMARKS

ABSTRACTS

ORAL PRESENTATIONS

Opening lecture

Chromatin dynamics and repair efficiency: How Ub and Sumo control DNA repair in space and time

Anais Cheblal, Michael Hauer, Kiran Challa, Kenji Shimada, and Susan M. Gasser

Friedrich Miescher Institute for Biomedical Research, Maulbeerstr. 66, CH-4058 Basel, Switzerland

Email: susan.gasser@fmi.ch

Nucleosomes are essential for proper chromatin organization and the maintenance of genome integrity. Histones are post-translationally modified and often evicted at sites of DNA breaks, mediated by chromatin remodelers, which shift, remodel and reassemble chromatin at sites of damage. Using quantitative imaging and proteomics methods we show that histone levels drop by 20-40% in response to extensive DNA damage, reflecting nucleosome eviction by the INO80 remodeler and degradation by the proteasome. This occurs not only at sites of damage but genome-wide, in a manner dependent on the checkpoint kinase Mec1/ATR. Chromatin decompaction and increased fiber flexibility accompany histone degradation, and occur in the absence of damage when histone levels are reduced by other means. The loss of histones and increase in mobility increase the efficiency of the homology search in ectopic DSB repair. Changes in the physical properties of chromatin in response to DNA damage are monitored by single particle tracking, and also being correlated with the "chromatome", that is, the set of proteins bound to chromatin before and after DNA damage is induced. Intriguingly, ubiquitin ligases are among the proteins that show changes in association with chromatin in response to damage. We are examining their link both to histone ubiquitination and to histone eviction, and the role of a nucleosome remodeler, like INO80-C, in this process. We propose that a dramatic change in chromatome may be linked to the Ub/Sumo signaling pathway, and that this part of the DNA damage response occurs to enhance chromatin mobility and facilitate specific pathways of repair.

DUBs at the crossroads of cancer and neurodegeneration

Sylvie Urbé, Elena Marcassa, Jane Jardine, Andreas Kallinos, Erithelgi Bertsoulaki, Svetlana Telnova, Hannah Elcocks, Francesca Frigenti, Anne Clancy, Claire Heride, Emma Rusilowicz, Michael J Clague

Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Crown Street, Liverpool L69 3BX, United Kingdom

Email: urbe@liverpool.ac.uk

Deubiquitylases (DUBs) fulfill a plethora of functions ranging from infrastructural core tasks (eg maintaining ubiquitin homeostasis), to highly specialised roles in stabilising defined sets of substrates (including oncogenes) or regulating signalling networks, intracellular trafficking and DNA repair pathways [1]. DUBs are emerging as promising new drug targets in cancer therapy and in the neurodegeneration space, with the recent description of highly selective inhibitors adding to the promise.

I will discuss our efforts in characterising the biology of DUBs that offer opportunities to establish this class of enzymes as actionable drug targets in these disease settings.

References:

[1] Clague MJ, Urbé S, Komander D. Breaking the chains: deubiquitylating enzyme specificity begets function. Nat Rev Mol Cell Biol. (2019) doi: 10.1038/s41580-019-0099-1

Insights into EME1 sumoylation

Jan Breucker[1], Chronis Fatouros[1], Román González-Prieto[2], Michaela Gschweitl[1], Heekyoung Lee[1], Dragana Ilic[1], Nathalie Eisenhardt[1], Alfred C. Vertegaal[2] and Andrea Pichler[1]

[1] *Max Planck Institute of Immunobiology and Epigenetics, Department of Epigenetics, 79108 Freiburg, Stübeweg 51, Germany*

[2] *Department of Molecular Cell Biology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands*

Email: pichler@ie-freiburg.mpg.de

Posttranslational modification with the small ubiquitin related modifier SUMO is an important tool to regulate protein function in most cellular pathways. SUMO conjugation is executed by the hierarchical action of E1, E2 and E3 enzymes. E3 ligases ensure substrate specificity, even though E2 regulatory modes also contribute to substrate selection as we have shown for E2 (Ubc9) enzyme sumoylation in mammals (SUMO*Ubc9). Searching for substrates regulated by the sumoylated Ubc9 we identified the two regulatory endonuclease subunits, SLX4 and EME1 of the mitotic tetrameric complex (SLX1-SLX4-MUS81-EME1) that has key functions in holiday junction resolution and the mitotic replication of common fragile sites. I will discuss our findings about EME1 sumoylation and its regulation via the sumoylated Ubc9 and other E3 ligases.

Arsenic induced degradation of the Promyelocytic leukaemia protein

Ron Hay

Centre for Gene Regulation and Expression, University of Dundee, UK

Email: R.T.Hay@dundee.ac.uk

Acute Promyelocytic Leukaemia is caused by the t(15;17) chromosomal translocation which leads to the abnormal fusion of the genes encoding the Promyelocytic Leukaemia protein (PML) and the Retinoic Acid receptor α (RARA). In the past this disease was rapidly fatal, but it is now eminently curable thanks to treatment with arsenic trioxide and all-trans retinoic acid. This leads to degradation of both wild type PML and the abnormal PML-RARA fusion protein. Treatment with arsenic leads to post-translational modification of PML with the Small Ubiquitin like Modifier (SUMO). This targets it for ubiquitylation by the SUMO- targeted ubiquitin ligase RNF4, in turn leading to its proteasomal degradation. To follow arsenic induced PML degradation, we engineered stable cell lines expressing GFP linked PML and established a procedure for the isolation of PML nuclear bodies using anti-GFP nanobodies. Western blotting on this material after treatment with SUMO- and ubiquitin specific proteases revealed the extent and status of PML modification in response to arsenic. To reveal the proteins recruited into PML bodies in response to arsenic we carried out a time resolved quantitative proteomic analysis. In addition to proteins involved in SUMO conjugation, we also observed the arsenic dependent recruitment of SUMO specific proteases and ubiquitin E3 ligases into PML bodies. Analysis of SUMO and ubiquitin acceptor lysines revealed a temporally changing pattern of ubiquitin and paralogue specific SUMO modifications that may account for the ultimate degradation of PML in response to arsenic.

Roles for VCP in DNA replication and cancer

Pablo Valledor[1], Patricia Ubieto[1], Antonio Galarreta[1], Sara Rodriguez-Acebes[1], Juan Mendez[1] , Oscar Fernandez-Capetillo[1], Emilio Lecona[2]

[1]Spanish National Cancer Research Centre (CNIO), C/ Melchor Fernandez Almagro 3, 28029, Madrid

[2]Centre for Molecular Biology Severo Ochoa (CBMSO), C/ Nicolas Cabrera 1, 28049, Madrid

Email: elecona@cbm.csic.es

DNA replication needs to be tightly controlled to ensure the faithful copy of DNA through mitosis and prevent the generation of genome instability. Our previous work identified the presence of a SUMO-rich and ubiquitin-poor environment in replication forks and a role for USP7 as a SUMO deubiquitinase that targets SUMO2/3 and SUMOylated proteins in the replisome. Further, we showed that the inhibition of the protein segregase VCP increased the accumulation of SUMOylated replication factors on chromatin induced by USP7 inhibition and enhanced the inhibition of DNA replication. We have identified FAF1 as the adaptor that targets VCP to SUMOylated factors and showed that FAF1 cooperates with VCP to regulate DNA replication. We hypothesize that the collective SUMOylation of the replisome is necessary for the functions of VCP and we are trying to determine the mechanisms of action of VCP during DNA replication elongation. To this end we have studied the effect of blocking the activity of VCP on the dynamics of DNA replication, the composition of the replisome and the coupling of leading and lagging strand synthesis. Finally, we are exploring the use of VCP inhibitors in the induction of replication stress in cancer and the potential role of VCP as a tumour suppressor.

14-3-3 scaffold proteins mediate inactivation of TRIM25 ligase and inhibition of the type I interferon responses by herpesvirus deconjugases

Soham Gupta, Pävi Ylä-Anttila, Maria Masucci

Department of Cell and Molecular Biology, Biomedicum A6, Karolinska Institutet, S-17165 Stockholm, Sweden

Email: maria.masucci@ki.se

Members of the 14-3-3 family of molecular scaffolds regulate the IFN response by stabilizing the interaction of the TRIM25 ligases with viral nuclei acid sensor RIG-I and promoting the ubiquitinated-RIG-I-dependent assembly to MAVS filaments that mediate downstream signaling. The activity of 14-3-3 proteins is dependent on the formation of homo/heterodimers that interact with multiple phosphorylated motifs in the same or different client protein, which may stabilize the active enzyme conformations or promote the formation of protein complexes. The ubiquitin deconjugase encoded in the N-terminal domain of the Epstein-Barr virus (EBV) large tegument protein BPLF1 forms a tri-molecular complex 14-3-3 and TRIM25, leading to autoubiquitination of the ligase and inactivation of downstream signaling. We found that the expression of catalytically active BPLF1 and homologs encoded by human cytomegalovirus (CMV) and Kaposi Sarcoma Herpes Virus (KSHV), but not Herpes Simplex Virus (HSV1), promote the sequestration of TRIM25 into aggregates that are distinct from the cytoplasmic speckles induced by stress responses and viral infections. The formation of aggregates was dependent on the capacity of catalytically active BPLF1 to counteract the autoubiquitination of the ligase that is induced by recruitment of the viral protein to the 14-3-3:TRIM25 complex. Binding of BPLF1 to 14-3-3 is essential for the formation of TRIM25 aggregates and inhibition of the IFN response, while mutation of residues in the binding groove of 14-3-3 prevented binding to both BPLF1 and TRIM25 suggesting that the two client proteins may engage similar contact sites on the molecular scaffold. Interference with the formation of this complex could provide a new strategy for the potentiating the host innate immune response during the early phases of viral infection and virus reactivation.

The role of ubiquitin in the regulation of intercellular communication

Henrique Girao[1], Tania Martins-Marques[1], Teresa Ribeiro-Rodriguesv, Steve Catarino[1], Paulo Pereira[2]

[1]Coimbra Institute for Clinical and Biomedical Research (iCBR), FMUC

[2]Chronic diseases research center (CEDOC), NOVA Medical School

Email: hmgirao@fmed.uc.pt

A fine-tuned communication between cells is vital to maintain the homeostasis and function of multicellular or unicellular organisms in a community environment. Gap junctions (GJ) are specialized channels, composed by connexins (Cx), that allow direct intercellular communication (IC) between eukaryotic cells. For example in the heart, GJ ensures efficient electric activation and action potential propagation, resulting in coordinated contraction. Defects in cardiac Cx43-mediated GJIC has been associated with various cardiomyopathies, such as ischemia, infarction and hypertrophy. The extent of GJIC largely depends on the number and functionality of Cx present at the plasma membrane. Several mechanisms have been associated to the internalization and degradation of Cx43, being endocytosis the most well characterized. We showed that Needd4-mediated ubiquitination of Cx43 is required to recruit Eps15 and direct Cx43 to the endocytic pathway. Additionally, we demonstrated that Cx43 localized at the plasma membrane is also degraded by autophagy through a mechanism that requires prior Cx43 ubiquitination, and its subsequent recognition by proteins containing ubiquitin-binding domain, including the canonical endocytic protein Eps15 and the autophagy adaptor p62. Furthermore, we reported that Cx43 is ubiquitinated and undergoes autophagic degradation in cardiomyocytes during ischemia and ischemia/reperfusion (I/R).

References:

[1] Ribeiro-Rodrigues TM et al, J Cell Sci. 2017

[2] Martins-Marques T et al, Biochem J. 2015

[3] Ribeiro-Rodrigues T et al, FASEB J. 2014

The Fbox protein CG6758 regulates Xbp1-induced cell death in the Drosophila eye

Pedro Domingos

Instituto de Tecnologia Química e Biológica António Xavier

Email: domingp@itqb.unl.pt

The Unfolded Protein Response (UPR) is composed by homeostatic signaling pathways that are activated by excessive protein misfolding in the Endoplasmic Reticulum (ER). Prolonged ER stress and UPR activation may lead to cell death and photoreceptor degeneration. We found that over-expression of the transcription factor Xbp1 spliced (Xbp1s) induces retinal degeneration in Drosophila and we performed a genetic screen to identify genes that, downstream of Xbp1s, mediate the induction of retinal degeneration. In this genetic screen, we used the FLPase/FRT technique and looked for EMS induced mutations that suppress Xbp1s induced retinal degeneration. So far, we identified mutations in 3 independent genes: Xpd, Eaf and CG6758. We are pursuing CG6758, a gene encoding an Fbox protein with unknown biological function. F-box proteins form complexes with Skp, Cullin-1 and E2 ubiquitin ligases (SCF complexes) to mediate the ubiquitination of specific substrates, and leading to the degradation of these substrates by the proteasome. We did two proteomic screens to identify binding partners and ubiquitylated substrates of Fbox/CG6758 and identified LaminB and Ataxin-2 as possible candidate substrates of Fbox/CG6758. We are currently investigating how regulation of LaminB and Ataxin-2 protein levels by Fbox/CG6758 leads to the suppression of the retinal degeneration process induced by Xbp1s.

Development of anti-cancer drugs targeting UBASH3B-mediated mitotic checkpoint signaling

Izabela Sumara

Institut de Génétique et de Biologie Moléculaire et Cellulaire
Université de Strasbourg, Institut National de la Santé et de la Recherche Médicale : U964, Centre National de la Recherche Scientifique : UMR7104

Email : sumara@igbmc.fr

Cell division ensures equal partition of genetic material between two daughter cells. Aberrant segregation of chromosomes results in chromosomal instability (CIN) and aneuploidy, an abnormal number of chromosomes in a cell. The presence of severe chromosome abnormalities was demonstrated in thousands of cancer samples. Thus, aneuploidy and CIN were suggested to drive tumorigenesis. The Spindle Assembly Checkpoint (SAC) (also known as the mitotic checkpoint) controls fidelity of chromosome segregation in normal mitotic cells and prevents aneuploidy. Chromosome abnormalities potentiate SAC response leading to mitotic arrest and cellular death. Therefore, the question arises how can aneuploid cancer cells suppress the SAC response to ensure their proliferation? We postulate that SAC potentiation leading to mitotic catastrophe and cell death is an efficient strategy for targeting cancer cells. Since aneuploidy is the feature that distinguishes cancer from normal cells, the future drugs that exploit this tumor-specific vulnerability will provide an attractive alternative to currently used anti-mitotics that often display high levels of toxicity in patients. Thus, the challenge is to identify the factors that control mitosis of cancer cells but at the same time have no effect on non-cancer cells. Our recent findings show that UBASH3B is the ubiquitin receptor protein for mitotic kinase Aurora B and drives Aurora B recruitment to microtubules in cancer cells. Regulation of Aurora B localization plays a crucial role in the control of SAC. UBASH3B is a negative SAC regulator that is specifically used by cancer cells to promote their propagation and survival. Levels of UBASH3B were shown to be high in aggressive breast cancers, promoting malignant growth, invasion, and metastasis. Interestingly, UBASH3B knock-out animals display no developmental or behavioral changes, suggesting a tumor-specific role of UBASH3B. Therefore, we developed a proof of concept translational studies how to target UBASH3B in cancer in particular in the triple negative breast cancer type. To this end, we identified a small molecule inhibitor UBASHIN that can specifically bind to and inactivate UBASH3B during mitosis. Treatment with UBASHIN leads to mitotic arrest and cell death specifically in cancer but not in normal primary cells. Our current activities focus on the validation of our SAC potentiation strategy in the animal breast cancer models and on the stratification of the breast cancer subtypes to identify future responsive patients. The long-term perspectives are to validate UBASHIN as anti-cancer lead compound for development of a future drug allowing for future clinical studies.

Session 3 | The ubiquitin system and disease – part 2

Reversing ubiquitylation: Mechanisms and functions of Deubiquitinases

Yogesh Kulathu

MRC Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK

Email: y.kulathu@dundee.ac.uk

The posttranslational addition of ubiquitin onto proteins is a versatile signal that regulates diverse cellular processes ranging from protein quality control and protein degradation to regulatory functions in signalling pathways such as cytokine signalling and DNA damage responses. Ubiquitylation is tightly regulated by proteases called Deubiquitinases (DUBs). With several thousand ubiquitin modifications occurring in cells, it is a colossal task for DUBs to modulate ubiquitylation. Given their key roles in regulating ubiquitin signalling, DUBs are being hotly investigated as therapeutic targets in cancer, inflammation and neurodegeneration. I will discuss our efforts to understand the principles by which DUBs regulate protein ubiquitylation and insights into novel DUBs that my lab has discovered.

Ubiquitinylation of HIF1alpha regulates multiple degradation pathways and intercellular signaling

Joao Vasco Ferreira, Paulo Pereira

CEDOC, NOVA Medical School, Universidade NOVA de Lisboa, Portugal

Email: ppereira@nms.unl.pt

The transcription factor HIF-1 (Hypoxia Inducible Factor 1) is a heterodimer composed of a constitutive HIF-1β subunit and a labile HIF-1α subunit that has a critical role critical in ensuring cell survival under low oxygen. Under normoxia, HIF-1α is targeted for proteasomal degradation by the ubiquitin ligase VHL. However, HIF-1α can also be modified by other ubiquitin ligases that regulate stability, subcellular localization and secretion of the transcription factor. For example, we showed that the chaperone interacting E3, CHIP/STUB1, induces HIF-1α ubiquitynalation via K63 linked ubiquitin chains, leading to its translocation to the lysosome and subsequent degradation by Chaperone-Mediated Autophagy (CMA). This process is further regulated by the deubiquitinating enzyme Ataxin3 that binds to HIF-1α to remove K63 ubiquitin chains, thus inhibiting HIF-1α degradation by CMA. Degradation of HIF-1α by CMA is oxygen-independent and is activated upon starvation, indicating a possible role for this mechanism in situations of prolonged hypoxia. Recently, we also showed HIF-1α can be targeted to the lumen of exosomes by a mechanism that shares a number of molecular players with CMA. Exosomes are small 30-120 nm extracellular vesicles that originate from late endosomes/multivesicular bodies and that carry information in the form of nucleic acids and proteins. Our results indicate that HIF-1α ubiquitinylation is required for the loading of the transcription factor into exosomes. We further show, both in cell and animal models, that exosomal HIF1A is uptaken by other cells and translocates to the nucleus where it shows biological activity, eliciting the transcription of hypoxia responsive genes.

Signalling mechanisms regulating metabolic profile and autophagy of acute myeloid leukaemia cells

Paula Ludovico

Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

Email: pludovico@med.uminho.pt

Acute myeloid leukemia (AML) comprises a heterogeneous group of hematologic neoplasms characterized by diverse combinations of genetic, phenotypic and clinical features representing a major challenge for the development of targeted therapies. Metabolic reprogramming, mainly driven by deregulation of the nutrient-sensing pathways as AMPK, mTORC1 and PI3K/AKT, has been associated with AML cells', survival and proliferation. Nevertheless, the role of these metabolic adaptations on the AML pathogenesis is still controversial (1). The metabolic status and the respective metabolic networks operating in different AML cells and their impact on autophagy and survival are discussed (2). Inhibition of the nutrient-sensing pathways is disclosed as a promising therapeutical target in some scenarios. An exclusive metabolic profile for each AML cells and its impact on determination of the anti-leukemia efficacy and on personalized combinatory therapy with conventional and targeted agents will be highlighted.

References:

[1] Fernandes Â, Azevedo MM, Pereira O, Sampaio-Marques B, Paiva A, Correia-Neves M, Castro I, Ludovico P. Proteolytic systems and AMP-activated protein kinase are critical targets of acute myeloid leukemia therapeutic approaches. Oncotarget. 2015 Oct 13;6(31):31428-40.

[2] Pereira O, Teixeira A, Sampaio-Marques B, Castro I, Girão H, Ludovico P. Signalling mechanisms that regulate metabolic profile and autophagy of acute myeloid leukaemia cells. J Cell Mol Med. 2018 Oct;22(10):4807-4817.

Strategies to reduce the side effects of inhibitors for the NEDD8 pathway

Dimitris Xirodimas

CRBM (CRBM)
Centre National de la Recherche Scientifique - CNRS
1919 Route de Mende, 34293, Montpellier - France

Email: dimitris.xirodimas@crbm.cnrs.fr

Targetting the ubiquitin pathway is an attractive strategy for cancer therapy. The inhibitor of the ubiquitin-like molecule NEDD8 pathway, MLN4924 (Pevonedistat) is in Phase II clinical trials. Protection of healthy cells from the induced toxicity of the treatment while preserving anticancer efficacy is a highly anticipated outcome in chemotherapy. Cyclotherapy was proposed as a promising approach to achieve this goal. We found that cytostatic activation of p53 protects cells against MLN4924-induced toxicity and importantly the effects are reversible. In contrast, cells with mutant or no p53 remain sensitive to NEDD8 inhibition. Using zebrafish embryos, we show that MLN4924-induced apoptosis is reduced upon pre-treatment with actinomycin D in vivo. Our studies show that the cellular effects of NEDD8 inhibition can be manipulated based on the p53 status and that NEDD8 inhibitors can be used in a p53-based cyclotherapy protocol to specifically target cancer cells devoid of wild type p53 function, while healthy cells will be protected from the induced toxicity.

Session 4 | Where to hit the system ?

Defining the human C2H2 zinc-finger degrome targeted by thalidomide analogs through CRL4^{CRBN}

Quinlan L. Sievers[1,2,*], Georg Petzold[3,4,*], Richard D. Bunker[3,4], Aline Renneville[1,2], Mikołaj Słabicki[1,2,5], Brian Liddicoat[1,2], Wassim Abdulrahman[3,4], Tarjei Mikkelsen[1], Benjamin L. Ebert[1,2,6] and Nicolas H. Thomä[3,4]

[1]Broad Institute of Harvard and MIT, Cambridge, USA; [2]Brigham and Women's Hospital, Division of Hematology, Harvard Medical School, Boston, USA; [3]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland; [4]University of Basel, Basel, Switzerland; [5]Division of Translational Oncology, National Center for Tumor Diseases, Heidelberg and German Cancer Research Center, Heidelberg, Germany; [6]Dana Farber Cancer Institute, Department of Medical Oncology, Boston, USA.

**These authors contributed equally to this work.*

Email: nicolas.thoma@fmi.ch

The small molecule drugs thalidomide, lenalidomide, and pomalidomide induce ubiquitination and proteasomal degradation of the zinc finger transcription factors Ikaros (IKZF1) and Aiolos (IKZF3) by recruitment to Cereblon (CRBN), the substrate receptor of the CRL4^{CRBN} E3 ubiquitin ligase. Here we screened the entire human Cys2-His2 (C2H2) zinc finger proteome for degradation by CRL4^{CRBN} in the presence of thalidomide analogues, identifying 11 zinc finger targets. Structural and functional characterization of the C2H2 zinc finger degron demonstrates how diverse zinc finger domains bind the promiscuous drug-CRBN interface. Computational ZF docking, in conjunction with biochemical analysis, predicts that at least 50 zinc-fingers bind the drug-CRBN complex *in vitro*, a larger number than previously anticipated. Functional studies demonstrate that selective degradation of zinc fingers can be tuned through compound modifications, providing a basis for the development of small molecules that degrade a wide range of zinc finger transcription factors.

Small molecules and probes to target the ubiquitin system

Huib Ovaa
Leiden University Medical Center
Department of Cell and Chemical Biology

Email: h.ovaa@lumc.nl

Modification of proteins with ubiquitin and ubiquitin-like proteins regulates a plethora of cellular events, ranging from DNA repair to proteolysis and intracellular trafficking. This lecture will deal with methods to chemically synthesize such proteins and discusses how to use them in structural, biochemical and cell biological studies with a range of examples.

Proteostasis factors in cancer exosomes

Ana Sofia Carvalho[1], Henrique Baetah[1], Manuel Rodriguez[2], Rune Matthiesen[1]

[1]Computational and Experimental Biology Group, CEDOC, Chronic Diseases Research Centre, NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Campo dos Mártires da Pátria, 130, 1169-056 Lisboa, Portugal

[2]ITAV CNRS USR3505,1 place Pierre Potier, Oncopole entrée B, 31106 Toulouse, France

Email: rune.matthiesen@nms.unl.pt

In recent years, exosomes ability to horizontal transfer biomolecules such as proteins, DNA, RNA and metabolites have been demonstrated. Furthermore, *in vitro*, *in vivo* and clinical studies have demonstrated that cancer exosomes can promote cancer growth, metastasis, and angiogenesis and contain markers useful for diagnosis [1,2]. Furthermore, exosomes have been demonstrated to play a role in immune regulation and drug resistance. One mechanism by which exosomes confer drug resistance is by overexpressing the drug target. Given that currently proteostasis factors are targeted in cancer therapies and many more proteostasis factors are currently being scrutinized as a possible cancer drug targets, we performed a meta-analysis of MS proteomics data obtained on 60 cell lines as well as the isolated exosomes from each cell line. We discovered that many proteostasis factors that are proposed as potential drug targets are abundant in exosomes but also some distinct differences between proteostasis factors in exosomes versus the corresponding cancer cells.

References:

[1] Carvalho AS, Cuco CM, Lavareda C, Miguel F, Ventura M, Almeida S, Pinto P, de Abreu TT, Rodrigues LV, Seixas S, Barbara C, Azkargorta M, Elortza F, Semedo J, Field JK, Mota L, Matthiesen R (2017) Bronchoalveolar Lavage Proteomics in Patients with Suspected Lung Cancer. Scientific reports 7:42190. doi:10.1038/srep42190

[2] Maia J, Caja S, Strano Moraes MC, Couto N, Costa-Silva B (2018) Exosome-Based Cell-Cell Communication in the Tumor Microenvironment. Frontiers in cell and developmental biology 6:18. doi:10.3389/fcell.2018.00018

Evaluating selectivity of ubiquitination inhibitors with a novel fluorescence-based assay for real-time monitoring of ubiquitination cascades

Joanna Koszela[1,] *, Manfred Auer*
*Corresponding author

[1]University of Glasgow, [2]University of Edinburgh

Email: jo.koszela@gmail.com

Despite identification of many clinically relevant, potential drug targets within the ubiquitin-proteasome system (UPS), the drug-like inhibitors targeting the UPS components are scarce and often of low selectivity and/or potency. This is due to the inherent complexity of the UPS, composed of thousands of enzymes from different classes and accessory proteins with various activities, and a shortage of adequate methods for identification and characterisation of new inhibitory molecules. Here, we present a novel assay, suitable for studying a variety of different activities within the UPS and for comprehensive characterisation of ubiquitination modulators. Based on fluorescent confocal scanning (CONA) technique, the assay (termed UPS-CONA) employs a fluorescently labelled ubiquitin, which - when conjugated to an on-bead substrate - is quantitatively detected on the bead surface using confocal fluorescence microscopy. A direct readout allows for real-time monitoring of ubiquitination, and a miniaturised on-bead setup enables following the ubiquitin transfer step by step in a multi-enzymatic cascade. Using UPS-CONA, we developed an assay for tracking of ubiquitin transfer within the Ube1-Ube2L3-E6AP cascade. We applied this system to investigate selectivity of ubiquitination inhibitors and discovered novel inhibitory activities. Our versatile, sensitive, and quantitative assay system constitutes a powerful tool for interrogation of ubiquitination-related enzymatic pathways and their chemical modulators.

ABSTRACTS

POSTER PRESENTATIONS

Split-TurboID and Targeted-BirA: Development of biotin-based approaches to study the Ubiquitin Code

Orhi Barroso Gomila, Felix Elortza, James D. Sutherland*, Rosa Barrio*

*Corresponding author

CIC bioGUNE, Bizkaia Technology Park, Building 801-A, 48160 DERIO, Bizkaia, Spain

Email: obarroso@cicbiogune.es

As the gatekeepers of protein homeostasis, Ubiquitin (Ub) and SUMO are implicated in almost every described cellular pathway. Ub and SUMO are post-translational modifiers that attach to substrate lysines, and are further extended to make homotypic and heterotypic chains, changing the protein function and/or localisation. We are applying biotin-based approaches to explore 1) the writers/readers/editors of Ub/SUMO modification, and 2) to enrich for organelle-specific Ub/SUMO subproteomes. First, we aim to merge proximity biotinylation using promiscuous BirA (termed BioID) and the "split-protein" approach to find interactors of SUMOylated proteins. We are optimising split versions of TurboID (an improved version of BioID) to apply to the Ub/SUMO landscape. As a proof of principle, we have shown SUMOylation-dependent labeling of proteins proximal to SUMOylated RANGAP1, and aim to extend this to other substrates, Ub and other ubiquitin-like modifiers, and even heterotypic chain types. Second, building on our published bio-UbL toolbox (Pirone et al 2017 Sci Rep 7:40756), we will apply organelle-specific BirA fusions to enrich for bio-UbL modifications in particular compartments. Initial experiments are focused on SUMOylation in the centrosome, primary cilia, and nuclear subcompartments. Validations by immunofluorescence and mass spectrometry data will be presented. Using these biotin and BirA-based technologies, we hope to gain further insights into the complex Ubiquitin Code.

Acetylated HNF1β escapes from ubiquitin-mediated degradation- a way of enhancing HNF1β role in cancer

Filipa Coelho, Fernanda Silva, Bruno A. Cardoso, Jacinta Serpa

CEDOC, Chronic Diseases Research Centre, NOVA Medical School

Faculdade de Ciências Médicas, Universidade NOVA de Lisboa and Unidade de Investigação em Patobiologia Molecular do Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG)

Email: filipa.coelho@nms.unl.pt

HNF1β, a member of the superfamily of homeodomain containing transcription factors, is a molecular hallmark in clear cell carcinomas from different organs. However, its role as an oncogene or as a tumor suppressor gene remains controversial. Recently, we disclose acetylation of HNF1βprotein as a mechanism of stabilization, which is consequently inhibitory of its degradation in the proteasome. We exposed to HDAC inhibitors, human HNF1β-expressing cell lines of ovarian clear cell carcinoma (ES2), hepatocellular carcinoma (HEPG2) and normal immortalized kidney tubular cells (HK2). We showed that increased levels of HNF1βis concomitant with the increased acetylation load and protein stabilization by interfering with the ubiquitination and proteasomal degradation system. This study reinforces that acetylation, besides their role in regulating chromatin conformation and gene expression, can also act in the function, turnover and stability of proteins essential for the survival and progression of certain cancer histotypes. HDACs are crucial players controlling acetylation dynamics of those proteins. In the particular case of HNF1β an considering the existence of HNF1βmutations, we believe the mutational status of *HNF1B* may determine its expression levels since cancer cells modulate differently oncogenes (mutated *HNF1B*) and tumor suppressor genes (non-mutated *HNF1B*). This expression levels will consequently determine the effect of drugs acting on the modulation of gene expression and structure/function of HNF1β protein.

EHD1 modulates intercellular trafficking of the gap junction protein Connexin43 (Cx43)

Tania Martins-Marques[1], Steve Catarino[1], Claudia Almeida[2], Henrique Girao[1, *]

*Corresponding author

[1]*Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal*

[2]*Chronic diseases research center (CEDOC), NOVA Medical School/Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal*

Email: taniamarques241@gmail.com

Communication between heart cells is vital to ensure a correct anisotropic propagation of the electrical impulse, a function that is mainly conducted by gap junctions (GJ). Dysfunctional GJ-mediated intercellular communication has been associated with several cardiac disorders, such as myocardial ischemia, and include increased degradation and lateralization of connexin43 (Cx43). However, the molecular mechanisms underlying such remodeling are not fully understood. Previous studies from our lab showed that ubiquitination of Cx43 triggers the recruitment of Eps15, required for endocytosis of Cx43-channels. Recently, in a proteomic analysis, we showed that ischemia positively regulates interaction of Cx43 with Eps15 homology domain-containing protein 1 (EHD1) in rat hearts. Therefore, the main objective of the present study was to investigate the role of EHD1 in ischemia-induced remodeling of GJ. Our results show that ischemia increased the co-localization of EHD proteins with mislocalized Cx43-channels, which returned to basal levels during reperfusion. Additionally, we established that phosphorylation and ubiquitination of Cx43 enhanced the association between Cx43 and EHD1. To further characterize the molecular mechanisms underlying EHD1-mediated intracellular trafficking of Cx43, we genetically manipulated the levels of EHD1. Our results demonstrate that internalization of Cx43 was promoted after overexpression of wild-type EHD1 in HEK-293 cells. On the other hand, both the knockdown of EHD1 and the overexpression of a mutated form of the protein resulted in the stabilization of Cx43 at the plasma membrane. Altogether, we have identified EHD1 as a new player involved in the modulation of intracellular trafficking of Cx43, which may ultimately contribute to GJ remodeling during myocardial ischemia.

Mechanistic links between proteasome, aging and age-related diseases modelled in yeast

Hélder Pereira*, Belém Sampaio-Marques, Paula Ludovico

*Corresponding author

Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Email: id7387@alunos.uminho.pt

Aging is a complex and multi-factorial process that results in the progressive accumulation of molecular alterations and disruption of cellular homeostasis. Several hallmarks of aging that represent age-common denominators in different model organisms have been proposed, including deregulated nutrient-sensing and loss of proteostasis. Disruption of proteostasis during aging is mainly caused by a decline on autophagy and the ubiquitin-proteasome system (UPS) activities. Caloric restriction (CR) is still the most effective non-genetic intervention known to promote longevity associated with the modulation of the proteolytic systems in different aging model organisms. In the present work, we used the yeast Saccharomyces cerevisiae, a simple and powerful model organism, to understand the mechanistic links between proteasome, aging and age-related diseases under the beneficial effects promoted by CR intervention. Proteotoxic stress was elicited by the heterologous expression of human α-synuclein (aSyn), a protein associated with synucleinopathies, age-related diseases. The results obtained show that CR promotes a coordinated regulation of UPS and autophagy activities during aging. Indeed, CR boosts UPS activity, reversing its decline aggravated by aSyn in aged cells, and keeps autophagy at homeostatic levels. Autophagy inhibition upregulates UPS activity, pointing to a compensatory mechanism. However, UPS inhibition was not associated with enhancement of autophagy activity. Maintenance of autophagy at homeostatic levels appears to be relevant for UPS activity and for the mechanism underlying rescue of cells from aSyn-mediated toxicity by CR.

This work was developed under the scope of the project NORTE-01-0145-FEDER-000013, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER), by FEDER, through the Competitiveness Factors Operational Programme (COMPETE). HP and BSM are supported by the fellowships SFRH/BD/133087/2017 and SFRH/BPD/90533/2012, respectively, funded by the Fundação para a Ciência e Tecnologia (FCT, Portugal).

The Fbox protein CG6758 regulates Xbp1-induced cell death in the Drosophila eye

Cristiana Santos[1,*], Nadine Schweizer[1], Catarina Gaspar[1], Vanya Rasheva[1], Fátima Cairrão[1], Juan Sánchez[2], Ugo Mayor[3, 4], Pedro Domingos[1,*]

*Corresponding author

[1]Instituto de Tecnologia Química e Biológica António Xavier, [2]University of the Basque Country, [3]IKERBASQUE, Basque Foundation for Science, [4]University of the Basque Country (UPV/EHU)

Email : cristinisantos@hotmail.com

The Unfolded Protein Response (UPR) is composed by homeostatic signaling pathways that are activated by excessive protein misfolding in the Endoplasmic Reticulum (ER). Ire1 signaling is an important mediator of the UPR, leading to the activation of the transcription factor Xbp1. Prolonged ER stress and UPR activation may lead to cell death and photoreceptor degeneration. Our aim is to identify genes important for photoreceptor degeneration induced by ER stress. We found that over-expression of activated Xbp1spliced induces retinal degeneration in Drosophila and we performed a genetic screen to identify genes that, downstream of Xbp1spliced, mediate the induction of retinal degeneration. In this genetic screen, we used the FLPase/FRT technique and looked for EMS induced mutations that suppress Xbp1spliced induced retinal degeneration. So far, we identified mutations in 3 independent genes that are able to suppress Xbp1spliced induced retinal degeneration: Xpd, Eaf and CG6758. We are pursuing CG6758, a gene encoding an Fbox protein with unknown biological function. F-box proteins form complexes with Skp, Cullin-1 and E2 ubiquitin ligases (SCF complexes) to mediate the ubiquitination of specific substrates leading to the degradation of these substrates by the proteasome. We did a proteomic screen using the BioUb system, developed by Ugo Mayor Lab [1], to identify binding partners and ubiquitylated substrates of Fbox/CG6758. Through this screen we have identified Ataxin-2 as possible candidate substrate of Fbox/CG6758. We are currently investigating how regulation of Ataxin-2 protein levels by Fbox/CG6758 leads to the suppression of the retinal degeneration process induced by Xbp1 spliced.

Reference:

[1] Ramirez J, Martinez A, Lectez B, Lee SY, Franco M, Barrio R, Dittmar G, Mayor U (2015) Proteomic Analysis of the Ubiquitin Landscape in the Drosophila Embryonic Nervous System and the Adult Photoreceptor Cells. PLoS ONE 10(10): e0139083

Session 4 | Where to hit the system ?

Using nanobodies to decipher the ubiquitin code

Laurie Bonnafé[1], Alexis Arrial[2], Maria Gonzalez[1], Rain Jean-Christophe[2], Manuel Rodriguez[1]

[1]CNRS-ITAV, USR 3505, Toulouse, France

[2]Hybrigenics Services, Paris, France

Email: laurie.bonnafe@itav.fr

Protein post-translational modifications (PTM) by ubiquitin (Ub) are versatile, highly dynamic and involved in nearly all aspects of biological functions in eukaryotes. The reversibility and heterogeneity of Ub chains attached to protein substrates have complicated their isolation, quantification and characterization. Strategies have emerged to isolate endogenous ubiquitylated targets, including technologies based on the use of Ub-binding peptides, such as TUBEs (Tandem-repeated Ubiquitin-Binding Entities), peptide aptamers or affimers. Although peptide aptamers were developed more than twenty years ago, their use to identify ubiquitin-linkages has not been exhaustively developed. Here we present the characterization of small peptide nanobodies isolated from a phage display library that specifically interact with K48 or K63 linkages. These double tagged peptides can be easily purified using histidine pull-down protocols and detected with an anti-Myc antibody. K48 or K63 chains can be specifically purified with these nanobodies and cross-reaction with other ubiquitin chain-linkages have not been observed. A set of clones show distinct capacity to pull-down specific chains in vitro. Ubiquitin-chain linkages can also be isolated from cell treated or not with proteasome inhibitors. Altogether our data show the potential of these new tools to explore ubiquitin chain diversity. Their application for mass spectrometry and high-resolution microscopy are among the most interesting potential applications. Together with TUBEs and affimers, nanobodies or the camelidae minibody version of these ubiquitin binders should contribute to unravel the secrets of the Ub-code in cell physiology and pathology.

Reference:

Moutel S, Bery N, Bernard V, Keller L, Lemesre E, de Marco A, Ligat L, Rain JC, Favre G, Olichon A, Perez F (2016) NaLi-H1: A universal synthetic library of humanized nanobodies providing highly functional antibodies and intrabodies. Elife. 19;5. pii: e16228. doi: 10.7554/eLife.16228.

Synthesis of Nanobody80 using Solid Support

Yara Huppelschoten[1], Jens Buchardt[1], Thomas Eiland Nielsen[1], Huib Ovaa[2]

[1]Research Chemistry 3, Novo Nordisk, Maløv, Denmark
[2]Chemical Immunology, University Medical Center, Leiden, The Netherlands

Email: yrhu@novonordisk.com

Nanobodies are a novel class of therapeutic proteins that due to their small size (12-15 kDa) and unique structure make them ideal as novel inhibitors.[1] The small size of nanobodies offers the opportunity to chemically synthesize them, with as advantage the possibility to modify them for the use as probes. The aim of this project is to synthesize an intrabody; a nanobody that binds an intracellular target using solid phase peptide synthesis (SPPS). Up to now no intrabodies are known for deubiquitinating enzymes (DUBs) therefore, nanobody80 (Nb80) was chosen as a model intrabody. Nb80 is an intrabody stabilizing the intracellular domain of the Beta-2 adrenergic receptor (β2AR), thereby increasing the biological activity of the receptor.[2] Fmoc based peptide chemistry will be used to perform linear synthesis of Nb80. In addition, native chemical ligation (NCL) strategies will be explored as well. To interact with its target Nb80 has translocate across the cell membrane. Two different methods will be investigated to achieve this goal; asialglycoprotein receptor (ASGR)-mediated uptake and cell penetrating peptides (CPPs).[3] For the first method several *N*-acetylgalactosamine (GalNAc)-conjugated variants of the nanobody will be synthesized to ensure the most efficient cellular uptake. For the second method the nanobody will be modified with different CPPs to validate the best CPP for cellular uptake. Upon validation of these methods, they can be used for the synthesis of other nanobodies including DUB nanobodies.

References:

[1] Boldicke, T. Single domain antibodies for the knockdown of cytosolic and nuclear proteins. *Protein Sci.*, **2017**, *26*, 925-945

[2] Rasmussen, S.G. *et al.* Structure of a nanobody-stabilized active state of the beta(2)-adrenoceptor. *Nature*., **2011**, *469*, 175-180

[3] Huang, X. *et al.* Well-Defined Multivalent Ligands for Hepatocytes Targeting via Asialoglycoprotein Receptor. *Bioconjugate Chem.*, **2017**, *28*, 283-295

DRUGGABILITY OF THE UBIQUITIN SYSTEM

WHICH ROAD TO TAKE ?

PARTNER ORGANISATIONS

cnrs

cedoc CHRONIC DISEASES | nova MEDICAL SCHOOL

Ubi CODE

SPONSORS

HYBRIGENICS SERVICES

Life Sensors

UbiQ