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EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

PROTEOCURE Newsletter

03

April 2023 • Spring issue

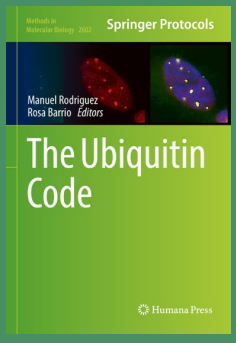
http://proteocure.eu

A SOUND PROTEOME FOR A SOUND BODY:
TARGETING PROTEOLYSIS FOR PROTEOME REMODELING

COMING WEBINARS

-14/04/2023, 16.00 CET María Ángeles Ávila-Gálvez. Nova Medical School, Portugal.
-20/04/2023, 13h CET Matthias Brand. CSO Proxygen, Austria.
-21/04/2023, 16:00 CET Ruth Geiss-Friedlander, University of Freiburg, Germany.
-16/06/2023, 16:00 CET Burak V Kabasakal, Ankara University, Turkey.

BOOK PUBLICATION



ProteoCURE members contributed to more than half of the chapters included in a book of the Methods in Molecular Biology series published by Springer. This book covers various aspects the Ubiquitin Code regulation through the publication of 2 reviews and 15 technical chapters. These protocols were designed to explore border questions and enhance our understanding of cellular plasticity and the role of ubiquitin family members in the control of vital cellular processes. By utilizing the ubiquitin code as a biological language, new possibilities for disease stratification, diagnosis, and tailored treatments can be developed, especially for diseases that are still not well-understood.

ACTIVITIES HIGHLIGHTS

by the ProteoCURE dissemination committee

Our ProteoCure COST Action is running in a very dynamic way and you can actively participate to build a stronger network of interactions and collaborations of many types. In this number we would like to share with you some these ongoing successful stories. First of all, we are thrilled to share some fantastic news with you! Our ProteoCure COST Action has been awarded an additional 30.000€ funding in March 2023. This is just a wonderful timing as we recently had our most successful call for Short Term Scientific Mission so far.

Our mission is is to support global initiatives that promote exchanges between groups, train the next generation of scientists, and drive the development of innovative projects that can be funded by national or international organizations. We would like to encourage you to take full advantage of the resources already available, including our website (www.proteocure.eu), the member list, and the forum, to connect with fellow members and explore exciting new collaborations.

We would also like to to recognize and showcase the numerous initiatives undertaken or accomplished by our team members. These initiatives serve as an inspiring example of the remarkable goals we can achieve together.

WEBINAR SERIES

ProteoCure has two ongoing webinars series undertaken by our working groups members. Both are open to the entire ProteoCure community.

"Screening, Identification & Targeting of Active Molecules" SITAM webinar series, led by Rune Matthiesen, are online seminars consisting of one or two presentations per month, typically held on Fridays. With the speakers' permission, the presentations are recorded and made available on our ProteoCure website (www.proteocure.eu) and YouTube channel (https://www.youtube.com/@proteocure9354). The seminars are announced both by e-mail and on the ProteoCure Website.

TPD webinars, launched by Catherine Lindon and Carles Galdeano have a similar purpose. This seminar series will bring together junior and experienced researchers from academia and industry working in the field of targeted protein degradation (TPD). These seminars will be conducted virtually every three weeks on Thursdays, typically around lunchtime. Do not hesitate to contact the organizers if you want to do a presentation.

SHORT TERM SCIENTIFIC MISSIONS

Recently achieved or ongoing STMS:

-Marta Lavouras. 12-26/01/23. From Luxembourg Institute of Health to Technion-Israel Institute of Technology.

-Roger Castaño. 21/11/22-30/01/23. From Universitat de Barcelona to Max Planck Institute of Biochemistry.

-Maria Blanquer. 09/01- 09/02/23. From CIMUS to Leiden University Medical Center.

-Sofia Brandão. 13/02-17/03/23. From Faculty of Pharmacy of University of Porto to University of Trieste.

-Alexandra Moreira. 13/02-17/03/23. From Faculty of Pharmacy of University of Porto to University of Trieste.

-Katarina van Midden. 09/01- 31/03/23 From University of Ljubljana to Forschungszentrum Jülich GmbH.

-Vicenzo Taibi. 15/03-02/04/23 From IFOM to Friedrich Miescher Institute for Biomedical Research (FMI).

ABOUT OUR COMING SCIENTIFIC EVENTS

ProteCURE ANNUAL MEETING 2023: FINAL ANNOUNCEMENT

Zagreb, Croatia. 12-15/06/2023

Our next ProteoCURE annual meeting will take place from the 12th to the 15th of June 2023 at the Hotel Dubrovnik in Zagreb. This meeting is expected to be an excellent opportunity to consolidate interactions and create new ones between ProteoCure members.

We will also hold our 5th management committee on June 12th, right before the start of the scientific meeting. To get more information and register to the scientific conference, visit the registration website: https://proteocure2023.sciencesconf.org/ Thanks to the COST initiative, there is no registration fee for the meeting. However, meals, social and cultural events have to be paid separately on site or prior to arrival (a payment method will be stablished soon). Please register before the stablished deadline the 22/05/2023. We are excited to have the opportunity to welcome you all to Zagreb.

https://www.hotel-dubrovnik.hr/
https://www.hotel-dubrovnik.hr/virtual-tour/?s=pano6268&tour_language=en

FUTURE CO-ORGANISED MEETINGS

Joint meeting with the SFBBM. Paris, 22-24/05/2023

This upcoming meeting will be a collaborative effort between the "Cellular Proteolysis group" of the SFBBM (French Society of Biochemistry and Molecular Biology) and Working Group 1 (WG1) of Proteocure. The objective of WG1 is to establish task forces that bring together laboratories studying pathologies or biological processes in order to exchange knowledge and discoveries, models, innovative technical approaches, and tools, and to develop therapeutic solutions in a multidisciplinary manner. The overarching theme of the meeting will be proteostasis and its multifaceted impact on biological and pathological processes.

Link to this meeting: https://www.sfbbm.fr/index.php?lang=en

Founded by the European Union

EMBO workshop « SUMOylation: from discovery to translation ».

Pavoa de Varzim, Portugal 25-28/09/2023.

The aim of this meeting is to integrate perspectives on the function of SUMOylation from the fields of biochemistry, molecular cell biology, structural biology, genetics, plant science, pluripotency, immunity, oncology, neurodegeneration and drug discovery. The program will include invited speakers and a large number of talks selected from abstracts. We will inform you as soon as the registration website is open but you can already follow us on Twitter (@SUMO_EMBO_2023) for updates and don't hesitate to spread the word in your networks.
Organizers: Stefan Müller, Guillaume Bossis, Herlander Azevedo, Pedro Humberto Castro, Andrea Pichler, Maria Lois, Alfred Vertegaal.
https://meetings.embo.org/event/23-sumoylation

PAST SCIENTIFIC EVENTS

We would like to express our gratitude to our management committee and core group colleagues for their enthusiastic participation in our 4th MC meeting, which was held online on November 16th 2022.

DNA Replication at the heart of cell fate decisions and cancer development.

Madrid 21st of November, 2022. Co-organized by ProteoCure

The meeting focused on various aspects of DNA replication, from basic mechanisms to cancer, inflammation and treatment. The symposium had 67 attendees, mostly young researchers, and included 11 speakers from six different countries in Europe. The symposium promoted interactions between European groups, links with industry and fostered research in the field of proteostasis. Organizers: Maria Gómez and Emilio Lecona.

Winter School on Proteolytic Enzymes and their Inhibitors.

Tiers, 1st to 5th of March 2023. Co-organized by ProteoCure

https://www.plus.ac.at/biosciences/the-department/research-groups/brandstetter/winter-school-tiers/?lang=en

This Winter School on proteolytic enzymes provided a platform for young scientists to present their research and discuss their results with leading experts. The training school had 104 participants, covering more than ten sessions, with a focus on practical implementation and discussion of ongoing research in areas such as neurodegeneration, cancer mechanisms, immunity, inflammation, as well as viral and bacterial infections. The scientific achievements cutting-edge technologies and groundbreaking results were recognized with the Henner Graeff Foundation Young Investigator Awards. This Winter school promoted networking and collaborations betweenscientist from different disciplines. Visit the web page.

ANNOUNCEMENTS

-CALLS FOR FUNDING ARE CLOSED FOR ACTIVITIES HAPPENING BEFORE OCTOBER 31st, 2023.
We expect to reopen all calls **in July or August, for the 3rd grant period: November 1st 2023 to October 31st 2024**. Please, keep in mind that administrative limitations make difficult to fund activities in the first and last month of a grant period (i.e. October – November).
- PUBLISHING RELEVANT INFORMATION FOR OUR MEMBERS IN OUR WEB PAGE OR NEWS LETTER IS EASY. Send us an email to: dissemination@proteocure.eu Successful collaborations, common publications, scientific events, etc.

Publications between ProteoCURE members.

All these articles are open access.

Martínez-Férriz et al.
Cell Communication and Signaling (2023) 21:54
https://doi.org/10.1186/s12964-023-01076-6

Cell Communication and Signaling

RESEARCH Open Access

Eukaryotic Initiation Factor 5A2 localizes to actively translating ribosomes to promote cancer cell protrusions and invasive capacity

Arantxa Martínez-Férriz[1], Carolina Gandía[1], José Miguel Pardo-Sánchez[1], Alihamze Fathinajafabadi[1], Alejandro Ferrando[2] and Rosa Farràs[1*]

Abstract

Background Eukaryotic Initiation Factor 5A (eIF-5A), an essential translation factor, is post-translationally activated by the polyamine spermidine. Two human genes encode eIF-5A, being eIF5-A1 constitutively expressed whereas eIF5-A2 is frequently found overexpressed in human tumours. The contribution of both isoforms with regard to cellular proliferation and invasion in non-small cell lung cancer remains to be characterized.

Methods We have evaluated the use of eIF-5A2 gene as prognosis marker in lung adenocarcinoma (LUAD) patients and validated in immunocompromised mice. We have used cell migration and cell proliferation assays in LUAD lines after silencing each eIF-5A isoform to monitor their contribution to both phenotypes. Cytoskeleton alterations were analysed in the same cells by rhodamine-phalloidin staining and fluorescence microscopy. Polysome profiles were used to monitor the effect of eIF-5A2 overexpression on translation. Western blotting was used to study the levels of eIF-5A2 client proteins involved in migration upon TGFB1 stimulation. Finally, we have co-localized eIF-5A2 with puromycin to visualize the subcellular pattern of actively translating ribosomes.

Results We describe the differential functions of both eIF-5A isoforms, to show that eIF5-A2 properties on cell proliferation and migration are coincident with its features as a poor prognosis marker. Silencing of eIF-5A2 leads to more dramatic consequences of cellular proliferation and migration compared to eIF-5A1. Overexpression of eIF-5A2 leads to enhanced global translation. We also show that TGFβ signalling enhances the expression and activity of eIF-5A2 which promotes the translation of polyproline rich proteins involved in cytoskeleton and motility features as it is the case of Fibronectin, SNAI1, Ezrin and FHOD1. With the use of puromycin labelling we have co-localized active ribosomes with eIF-5A2 not only in cytosol but also in areas of cellular protrusion. We have shown the bulk invasive capacity of cells overexpressing eIF-5A2 in mice.

Conclusions We propose the existence of a coordinated temporal and positional interaction between TFGB and eIF-5A2 pathways to promote cell migration in NSCLC. We suggest that the co-localization of actively translating ribosomes with hypusinated eIF-5A2 facilitates the translation of key proteins not only in the cytosol but also in areas of cellular protrusion.

Keywords Eukaryotic translation initiation factor 5A2, TGFB1 signaling, Translating ribosomes, Cytoskeleton organization, Cell migration, Lung adenocarcinoma

cancers

MDPI

Article

Constitutive Activation of p62/Sequestosome-1-Mediated Proteaphagy Regulates Proteolysis and Impairs Cell Death in Bortezomib-Resistant Mantle Cell Lymphoma

Grégoire Quinet [1,†], Wendy Xolalpa [2,†,‡], Diana Reyes-Garau [3], Núria Profitós-Pelejà [3], Mikel Azkargorta [4], Laurie Ceccato [1], Maria Gonzalez-Santamarta [1], Maria Marsal [5], Jordi Andilla [5], Fabienne Aillet [2], Francesc Bosch [6], Felix Elortza [4], Pablo Loza-Alvarez [3], Brigitte Sola [7], Olivier Coux [8], Rune Matthiesen [9,§], Gaël Roué [3,*,§] and Manuel S. Rodriguez [1,*,§]

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Citation: Quinet, G.; Xolalpa, W.; Reyes-Garau, D.; Profitós-Pelejà, N.; Azkargorta, M.; Ceccato, L.; Gonzalez-Santamarta, M.; Marsal, M.; Andilla, J.; Aillet, F.; et al. Constitutive Activation of p62/Sequestosome-1-Mediated Proteaphagy Regulates Proteolysis and Impairs Cell Death in Bortezomib-Resistant Mantle Cell Lymphoma. *Cancers* **2022**, *14*, 923. https://doi.org/10.3390/cancers14040923

Academic Editor: Alberto Zamò

Received: 11 December 2021
Accepted: 7 February 2022
Published: 12 February 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Simple Summary: To decipher the molecular mechanism underlying the resistance of a significant fraction of mantle cell lymphoma (MCL) patients to the first-in-class proteasome inhibitor bortezomib (BTZ), we have characterized the ubiquitin-related proteome (i.e., ubiquitome) of a set of MCL cell lines with different degrees of sensitivity to the drug by coupling a tandem ubiquitin-binding entity (TUBE) approach to mass spectrometry, followed by phenotypic and functional validations in both in vitro and in vivo models of MCL. We identified an enrichment of autophagy–lysosome system (ALS) components in BTZ-resistant cells, which was associated with constitutive intracellular inactivation of proteasome subunits by a process called proteaphagy. Blockade of this phenomenon by the pharmacological or genetic inactivation of the autophagy receptor p62/SQSTM1 reactivated normal proteasomal activity and restored the BTZ antitumor effect in in vitro and in vivo models of BTZ resistance.

molecules

MDPI

Article

Antibody for Serine 65 Phosphorylated Ubiquitin Identifies PLK1-Mediated Phosphorylation of Mitotic Proteins and APC1

Guy Mann [1,†], Prasad Sulkshane [2,†], Pradeep Sadhu [1], Tamar Ziv [3], Michael H. Glickman [2,*] and Ashraf Brik [1,*]

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† These authors contributed equally to this work.

Abstract: Deciphering the protein posttranslational modification (PTM) code is one of the greatest biochemical challenges of our time. Phosphorylation and ubiquitylation are key PTMs that dictate protein function, recognition, sub-cellular localization, stability, turnover and fate. Hence, failures in their regulation leads to various disease. Chemical protein synthesis allows preparation of ubiquitinated and phosphorylated proteins to study their biochemical properties in great detail. However, monitoring these modifications in intact cells or in cell extracts mostly depends on antibodies, which often have off-target binding. Here, we report that the most widely used antibody for ubiquitin (Ub) phosphorylated at serine 65 (pUb) has significant off-targets that appear during mitosis. These off-targets are connected to polo-like kinase 1 (PLK1) mediated phosphorylation of cell cycle-related proteins and the anaphase promoting complex subunit 1 (APC1).

Keywords: posttranslational modifications; antibody off-targets; cell cycle

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Citation: Mann, G.; Sulkshane, P.; Sadhu, P.; Ziv, T.; Glickman, M.H.; Brik, A. Antibody for Serine 65 Phosphorylated Ubiquitin Identifies

Biochemistry

pubs.acs.org/biochemistry

Article

Conformational Control of Fast Asparagine Deamidation in a Norovirus Capsid Protein

Robert Creutznacher,[‖] Eric Schulze-Niemand,[‖] Patrick König, Vesna Stanojlovic, Alvaro Mallagaray, Thomas Peters,* Matthias Stein,* and Mario Schubert*

Cite This: *Biochemistry* 2023, 62, 1032−1043

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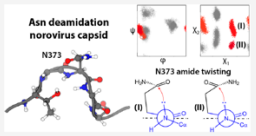
ABSTRACT: Accelerated spontaneous deamidation of asparagine 373 and subsequent conversion into an isoaspartate has been shown to attenuate the binding of histo blood group antigens (HBGAs) to the protruding domain (P-domain) of the capsid protein of a prevalent norovirus strain (GII.4). Here, we link an unusual backbone conformation of asparagine 373 to its fast site-specific deamidation. NMR spectroscopy and ion exchange chromatography have been used to monitor the deamidation reaction of P-domains of two closely related GII.4 norovirus strains, specific point mutants, and control peptides. MD simulations over several microseconds have been instrumental to rationalize the experimental findings. While conventional descriptors such as available surface area, root-mean-square fluctuations, or nucleophilic attack distance fail as explanations, the population of a rare *syn*-backbone conformation distinguishes asparagine 373 from all other asparagine residues. We suggest that stabilization of this unusual conformation enhances the nucleophilicity of the backbone nitrogen of aspartate 374, in turn accelerating the deamidation of asparagine 373. This finding should be relevant to the development of reliable prediction algorithms for sites of rapid asparagine deamidation in proteins.



Cellular and Molecular Life Sciences (2022) 79:548
https://doi.org/10.1007/s00018-022-04586-7

Cellular and Molecular Life Sciences

ORIGINAL ARTICLE

HERC2 deficiency activates C-RAF/MKK3/p38 signalling pathway altering the cellular response to oxidative stress

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Received: 27 April 2022 / Revised: 3 October 2022 / Accepted: 3 October 2022 / Published online: 14 October 2022

Abstract

HERC2 gene encodes an E3 ubiquitin ligase involved in several cellular processes by regulating the ubiquitylation of different protein substrates. Biallelic pathogenic sequence variants in the *HERC2* gene are associated with HERC2 Angelman-like syndrome. In pathogenic *HERC2* variants, complete absence or marked reduction in HERC2 protein levels are observed. The most common pathological variant, c.1781C > T (p.Pro594Leu), encodes an unstable HERC2 protein. A better understanding of how pathologic *HERC2* variants affect intracellular signalling may aid definition of potential new therapies for these disorders. For this purpose, we studied patient-derived cells with the HERC2 Pro594Leu variant. We observed alteration of mitogen-activated protein kinase signalling pathways, reflected by increased levels of C-RAF protein and p38 phosphorylation. HERC2 knockdown experiments reproduced the same effects in other human and mouse cells. Moreover, we demonstrated that HERC2 and RAF proteins form molecular complexes, pull-down and proteomic experiments showed that HERC2 regulates C-RAF ubiquitylation and we found out that the p38 activation due to HERC2 depletion occurs in a RAF/MKK3-dependent manner. The displayed cellular response was that patient-derived and other human cells with HERC2 deficiency showed higher resistance to oxidative stress with an increase in the master regulator of the antioxidant response NRF2 and its target genes. This resistance was independent of p53 and abolished by RAF or p38 inhibitors. Altogether, these findings identify the activation of C-RAF/MKK3/p38 signalling pathway in HERC2 Angelman-like syndrome and highlight the inhibition of RAF activity as a potential therapeutic option for individuals affected with these rare diseases.

Keywords Neurodevelopmental disorder · Angelman · Ubiquitin · MAPK · Cell stress

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ProteoCURE action receives funding from COST (European Cooperation in Science and Technology) Action CA20113