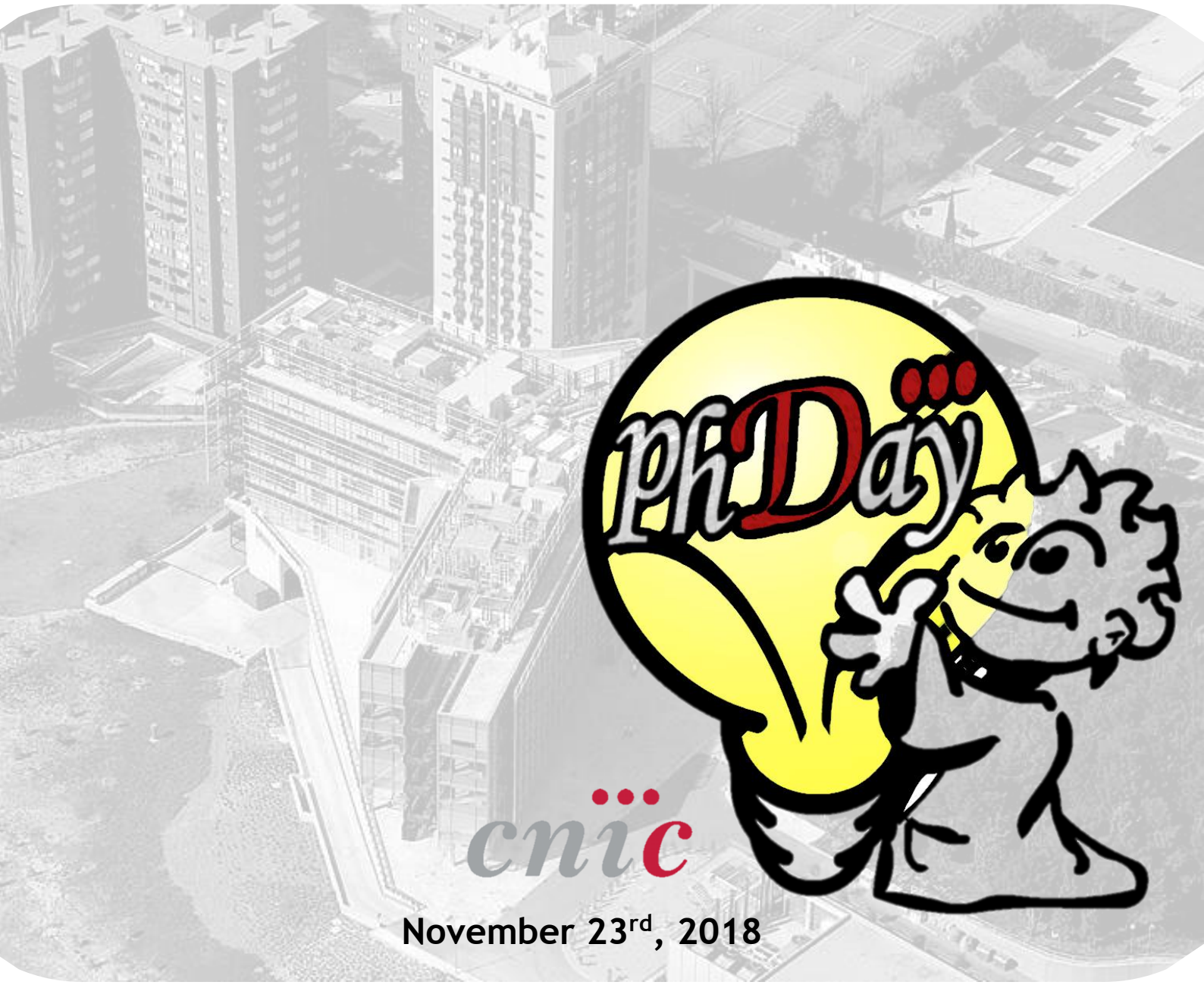
SCIENTIFIC GROWTH



November 23rd, 2018

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What's the CNIC PhDay?

The **CNIC PhDay** is an annual meeting organized by PhD students and postdoctoral researchers from the Spanish National Center for Cardiovascular Research (CNIC).

PhDay is a **multidisciplinary and open forum** for undergraduate and graduate students, lab technicians and postdoctoral researchers to develop their careers as scientists, exchange new ideas and network. Each year, more than 150 people gather at CNIC to share a joyful experience that might generate new ideas and collaborations! You never know the amount of new things you can learn at PhDay!

This year PhDay will revolve around the **SCIENTIFIC GROWTH** describing the steps that a scientist has to follow during his life through:

1) Talks and workshops will be held by speakers focusing on:

- ***Education for Science***
- ***How to motivate the young scientific heart***
- ***Our social responsibility as scientists***

2) Networking activities. This year apart from the Main Poster Session, we look for new ways of presenting your results. Thus, we want you also to participate in our Special Poster Session. A selection of participants will have only 3 minutes to present their projects! Look for them as SPS communicators!

Hope you enjoy the day.

We look forward to seeing you soon!

The CNIC PhDay Organizing Committee

If you want to know more about CNIC PhDay do not forget to follow us on our Social Media: **Facebook, Instagram** and **Twitter** (@CnicPhday #CNICPhDay)

How to arrive at CNIC?

A
Fundación Centro Nacional de Investigaciones Cardiovasculares

D
124 Bus Stop

C
Begoña Metro Station

D
147 Bus Stop

E
Chamartin Station

Programme

08:30-09:15 Registration

09:15-09:30 Welcome

Session I – Education for Science

09:30-10:00 Patricia Sánchez Pérez – Centro de Biología Molecular Severo Ochoa (CBMSO), Madrid (Spain): "From the pipette to the chat: the Somos Científicos Experience"

10:00-10:30 Florian Dehmelt – Werner Reichardt Centre for Integrative Neuroscience, Tübingen (Germany): "You're a monster!" – How to embrace public criticism of your research, and end up making new friends

10:30-10:45 To explain Poster sessions + Introducing the Workshops

10:45-12:00 Workshops

- Gonzalo Durante Rodríguez – Centro de Investigaciones Biológicas (CIB), Madrid (Spain): "How much water is necessary to turn off the Sun?" – Gamifying and playing science
- José Luis Crespo – Autonomous University of Madrid (Alma Mater), Madrid (Spain): "Scientific influencers: making Science in Youtube"
- Marta Seror – Institute of Physics of Cantabria (IFCA) – CSIC & University of Cantabria, Santander (Spain): "Pure, blazing, feminist science"
- Fátima Sánchez Cabo – Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid (Spain): "Statistics: Friend, not foe"

12:00-13:00 Lunch

13:00-14:00 Group Picture and then Coffee break & Main Poster Session

Session II – How to motivate the young scientific heart

14:00-14:30 Inge van der Weijden – University of Leiden, Leiden (Netherlands): "Career Trajectories of PhDs"

14:30-15:00 Jesús Vázquez – Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid (Spain): "The gift, the heel and the doubt"

15:00-15:30 Jesús Méndez – Autonomous University of Barcelona (Alma Mater), Barcelona (Spain): "A necessary bridge: Scientific communication and journalism"

15:30-16:45 Coffe Break & Special Poster Session

Session III – Our Social Responsibility as Scientists

16:45-17:15 Manuel Collado – Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela (Spain): "The social responsible scientist"

17:15-18:00 Eduardo Oliver – Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid (Spain): "#CienciaenelParlamento: when science spread through parliament"

Closing Lecture

18:00-18:30 Margarita Salas – Centro de Biología Molecular Severo Ochoa (CBMSO), Madrid (Spain): "My research experience"

18:30-20:30 Concluding Remarks & Social Snack

Speakers

Education for Science. *Trying to tell a child that Science is fun!*

Patricia Sánchez Pérez is graduated in Biology with a Master in Molecular Biomedicine by the Universidad Autónoma de Madrid (UAM). She is almost done with her PhD in mitochondrial pathophysiology in cardiovascular disease at the Centro de Biología Molecular "Severo Ochoa" (CBMSO), where she is studying the relationship between mitochondrial ROS production and myocardial infarction. She combines her scientific career with her enthusiasm towards science dissemination, which, for her, is the way of not losing hope in science while widely spreading it. She argues that seeing the children faces when experiments or science curiosities are shown to them is so rewarding! At the beginning, they are sceptic, later they are astonished, but at the end they only want to know and learn more and more. That is why Patricia decided to participate in the *SomosCientíficos: ¡Sácanos de aquí!*quiz. Moving from the bench-work to chatting with kids was undoubtedly a challenge, she says. However, at the end of the quiz, there was no way to know whether Patricia or the students enjoyed the scientific questions the most!

Florian Dehmelt. A physicist and biologist by training, Florian Dehmelt eventually drifted into neuroscience. After research in France and Portugal, he has now returned to his native Germany, where he works and teaches at Tuebingen University Hospital. No longer willing to remain silent about his work, he co-founded the non-profit organisation Pro-Test Deutschland, whose members volunteer to share their personal stories, ethical conflicts, and scientific experience – both in private and in public.

How to motivate the young scientific heart. *Thinking about becoming a Scientist?*

Inge van der Weijden has a PhD in organizational sciences from VU Amsterdam, The Netherlands. Since 2012 she works as a senior researcher and lecturer at the Center of Science and Technology Studies at Leiden University, The Netherlands. She leads the working group career studies. Inge conducts both qualitative and quantitative research on the motivation, selection and evaluation of scholars in order to better understand career development of scientists within and outside the academy. Special attention is given to the gender balance. Her recent research focuses on: (1) the mental health of early career researchers; (2) skill development of PhDs and (3) career satisfaction of postdocs. Inge is a board member of the Centre of Expertise for the Dutch PhD training.

Jesús Méndez studied Medicine at Universidad de Oviedo and got a PhD in Biochemistry at Universidad Autónoma de Barcelona. With a master in Scientific Communication by the Universidad Pompeu Fabra, he decided to get involved in scientific journalism. Co-founder of "Dixit Ciencia" he collaborates in many digital journals. He obtained some of the most prestigious awards in the field of scientific journalism such as Premio Prismas Casa de las Ciencias, Premio Concha García Campoy or the Periodismo en Salud Boehringer award, among many others.

Jesús Vázquez. Graduated in Physical Chemistry at the Universidad Complutense (Madrid, 1982) and carried out his PhD in Biochemistry at the Universidad Autónoma (Madrid, 1986), both with Special Distinction. During his postdoctoral training at Merck Sharp Research Laboratories (NJ, USA) and at the Centro de Biología Molecular Severo Ochoa (Madrid), he specialized in protein chemistry and in the study of biomembranes in the context of neurochemical diseases. Since then, he has played a pioneering role in the development of protein chemistry, mass spectrometry and proteomics in Spain. He currently works in the development of novel bioinformatics algorithms for the analysis of very large numbers of samples, including protein identification and systems biology interpretation of quantitative data, and for the systematic study of all kind of posttranslational modifications (PTM).Dr. Vázquez is author of more than 170 international publications, with an H-index of 45, and is *Professor of Research* from the Spanish National Research Council (CSIC). He joined the CNIC (Centro Nacional de Investigaciones Cardiovasculares, Madrid) as a *Full Professor* in 2011, where he leads the Cardiovascular Proteomics laboratory and is also in charge of the Proteomics Unit.

Our social responsibility as Scientists. *Get to know closer our duty out the bench!*

Manuel Collado obtained his PhD from the UAM for work performed on the Spanish National Biotechnology Center (CNB, CSIC-UAM). He did his first postdoc at the Ludwig Institute for Cancer Research at St Mary´s School of Medicine in London, UK. He then moved to New York to work at the NYU Medical School and at Memorial Sloan Kettering Cancer Center. He returned to Spain to join Manolo Serrano´s lab, first at CNB and later on as a staff scientist at the Spanish National Cancer Research Centre (CNIO) in Madrid. Since 2012 he leads the Stem Cells in Cancer and Aging laboratory of the Health Research Institute of Santiago de Compostela (IDIS). The main research interests of the laboratory are related with cellular reprogramming to pluripotency, and the process of cell senescence to better understand cancer and aging.

Eduardo Oliver is a senior postdoctoral fellow at the Spanish National Center for Cardiovascular Research (CNIC). He obtained a degree in Pharmacy and a PhD in Pharmacology at the University of Valencia (2010), and worked as a postdoc at Imperial College London until his return to Spain in 2016. Among other initiatives, Eduardo is one of the promoters, member of the coordinating group and vice-president of the independent citizen initiative #CienciaenelParlamento, which promotes science advice to the legislative power.

Closing lecture

Margarita Salas was born in 1938 in Canero, Spain. She graduated in Chemistry in 1960 and obtained her Ph.D. in Biochemistry in 1963. She carried out postdoctoral work with Professor Severo Ochoa at New York University from 1964 to 1967. She was Professor of Molecular Genetics (1968-1992) at Madrid Complutense University, and since 1974 she has been a *Professor of Research* at the Centro de Biología Molecular "Severo Ochoa" (CBMSO), that she directed in 1992-1993. Presently, she is Professor *ad honorem* of the Spanish National Research Council and member of the Governing Board of the State Researach Agency.

Her main research fields are DNA replication and control of gene expression using the bacterial virus ø29 as a model system. She has published over 400 articles in international journals, lectured over 480 Conferences both in Spain and abroad, and supervised 36 Ph.D. Theses.

In 1988 she became member of the Spanish Royal Academy of Sciences and in 2003 member of the Spanish Royal Academy of Language. From 1995 until 2003 she was President of the Institute of Spain and from 1999 till 2004, President of the Social Council of Oviedo University. She is also a member of EMBO, Academia Europaea, European Academy of Arts and Sciences, American Academy of Microbiology, American Academy of Arts and Sciences and foreign member of the US National Academy of Sciences.

She is *Doctor Honoris Causa* for the Universities of Oviedo, Polytechnic of Madrid, Extremadura, Murcia, Cádiz, Rey Juan Carlos, Málaga, UNED, UIMP and Jaén. She has obtained many prizes and distinctions, including the Severo Ochoa Prize of Research, the Rey Jaime I Prize of Research, the L'Oréal-UNESCO Prize "Women in Science", the National Prize of Research Santiago Ramón y Cajal, the Gold Medal of Madrid Community, the Honor Medal of Menéndez Pelayo International University and Madrid Complutense University, and the Nature Mentoring Award 2017 for a life time achievement in mentoring, among others.

Workshops

Gonzalo Durante (Gonn) is a postdoctoral researcher in the Biological Research Center (CIB-CSIC) in Madrid, involved in various projects related to environmental microbiology, biotechnology and synthetic biology. He holds a BSc in Biology in 2002 and a BSc in Biochemistry in 2004 at the Complutense University in Madrid. He obtained his PhD studying the genetic regulation of aromatic degradation pathways and the molecular evolution of transcriptional regulators in CIB-CSIC (2009). He completed his training with short stays at the University of Cayetano Heredia (Perú) and University of Sydney (Australia). He decided to do a first postdoc in the Victor de Lorenzo's Lab, where he was involved in several projects related to synthetic biology.

Outside the laboratory, he has developed several activities such as playing the guitar in a band, participating in science outreach activities with kids, and developing games at the game publisher he founded in 2013 ("*using*" his 3 children as beta testers). Currently, he teaches several games and science outreach workshops in schools for children between 6 and 12 years old.

José Luis Crespo is a physicist that, five years ago, created Quantum Fracture, a motion graphics channel dedicated to show how incredibly crazy is the Universe. Nowadays the channel has more than one million subscribers and more than fifty million views, being one of the most important outreach channels within the Spanish community. Crespo has also been part of theoutreach personal of the Institute of Theoretical Physics (IFT) in Madrid.

Fátima Sánchez-Cabo is the head of Bioinformatics Unit at CNIC. In 2000, she graduated in Mathematics at University Complutense of Madrid (Spain). During her PhD at University of Manchester (UK), Fátima developed a number of algorithms and tools for the quantitative analysis of high-throughput biomolecular data. In 2004, she moved to Institute for Genomics and Bioinformatics in the Graz University of Technology (Austria) as a postdoctoral researcher. Specifically, she successfully worked in several projects of precision medicine. After that, she joined CNIC aiming to implement new predictive scores in cardiovascular risk.

Marta Seror is the outreach coordinator at the Institute of Physics of Cantabria. She has an Astrophysics degree at UCM and is nowadays back at university studying a Master's degree in Particle Physics and Cosmos. Her wide experience as a science educartor has given her the skills and knowledge to bring science closer to the public in an accesible way. She was semifinalist of Famelab, an international scientific monologues contest, and also participates in science events such as Pint of Science festival and Scenio. For more tan two years now she has been leading a radio and podcast project called 'Cuentíficas' that focuses on gender perspective in science. Her dedication to creating a feminist framework for an inclusive Academy has just begun.

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Abstract Communications

Biophysical and computational model of signaling gradients through dynamic filopodia-like-structures (Cytonemes)

Aguirre-Tamaral A (1), Iber D (2), Guerrero I (1)

1. *Centro de Biología Molecular Severo Ochoa (CBMSO), Spain*
2. *ETH Zurich, D-BSSE, Switzerland*

adrian.aguirre@cbm.csic.es

Abstract

The role of morphogens gradients has been probe crucial in the development of an organism, but even today there is not an agreement in the biological mechanisms involved in gradients establishment. Because most of the morphogens cannot diffuse because their biochemical properties, one active mechanism of transport must be involve in the establishment of the morphogen gradient. Signaling filopodia-like structures, also called cytonemes, have been imposing as the biological mechanism behind this morphogen transport. But still a lot of information about the mechanisms remains evasive, as for example the dynamics of the process. Here we present a new theoretical and computational framework where investigate, in silico, different aspect of the general filopodia signaling. In order to test the model, we also developed and quantified in situ and in vivo experiments in Hedgehog signaling pathway in Drosophila epithelia. The results show that the model reproduces the morphogen gradient shape and also is able to predict some parameters, as the low density of cytonemes. This is the first biophysical model that introduces the dynamic of the cytoneme; as well the first computational tool where general biological hypothesis can be tested to analyze some not resolve question about the cytoneme signaling in different pathways.

Depicting the Structure of Fowl Aviadenovirus Capsid

Pérez-Illana M (1), Schachner A (2), Condezo GN (1), Hernando-Pérez M (1), Menéndez M (3), Hess M (2) and San Martín C (1)

1. *Centro Nacional de Biotecnología (CSIC)*
2. *Clinic for Avian, Reptile and Fish Medicine (Vetmeduni Vienna)*
3. *Instituto de Química Física Rocasolano (CSIC)*

marta.perez@cnb.csic.es

Abstract

Adenoviruses are studied as virotherapeutical tools for gene therapy, vaccination and oncolysis. The only high resolution adenovirus structures reported so far are two human Mastadenovirus species: HAdV-C5 at 3.6 Å [1] and recently HAdV-D26 at 3.7 Å [2]. There is also a 4 Å resolution study on bovine Mastadenovirus incomplete particles [3]. Although non-human adenoviruses have been put forward as alternatives to overcome the main drawbacks of HAdV-C5 as a vector, they remain poorly characterized.

Compared to HAdV-C5, fowl Aviadenoviruses (genus Aviadenovirus) lack two Mastadenovirus proteins, previously demonstrated to have an impact on virion stability maintenance [4]; which are core protein V and minor coat protein IX. However, it has been reported that Aviadenovirus capsids are more stable than those of human Mastadenoviruses [5]. Interestingly, fowl Aviadenoviruses pack a genome of about 43-45 kbp [6], longer than the rest of the adenovirus genera (23-35 kbp). We are using molecular, structural and biophysical analyses to understand the basis of fowl Aviadenovirus capsid stability.

[1] H Liu et al 2010 Science 329 1038–43
[2] X Yu X et al 2017 Sci. Adv. 3 1–12
[3] Cheng L et al 2014 Virology 450 174–81
[4] W W Colby et al 1981 J. Virol. 39 977–80
[5] D E Swayne et al 2013 Wiley-Blackwell
[6] A Marek et al 2012 Vet. Microbiol. 156 411–7

Microbiota sensing by Mincle-Syk axis in dendritic cells regulates IL-17 and IL-22 and promotes intestinal immune barrier

Martínez-López M(1),Iborra S (1)(2),Conde-Garrosa R(1),Mastrangelo A(1),Danne C(3),Mann ER(4)(5),Reid DM(6),Chaparro M(7),Lorenzo MP(8),Minnerup L (9),Saz-Leal P(1),Slack E(10),Kemp B(11),Gisbert JP(7),Dzionek A(9),Robinson MJ(11),Rupérez FJ (8),Brown GD(6),Bernardo D(7),LeibundGut-Landmann SA(12)(13),Sancho D(1)(13)(14)

1. *Immunobiology laboratory. Fundación Centro Nacional de Investigaciones Cardiovasculares "Carlos III" (CNIC)*
2. *Department of Immunology, School of Medicine, Universidad Complutense de Madrid; 12 de Octubre Health Research Institute (imas12), Madrid, Spain*
3. *Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom*
4. *Lydia Becker Institute of Immunology and Inflammation, University of Manchester,*
5. *Manchester Collaborative Centre for Inflammation Research, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester.*
6. *Aberdeen Fungal Group. MRC Centre for Medical Mycology, Infection, Immunity and Inflammation Programme, School of Medicine & Dentistry, Institute of Medical Sciences, University of Aberdeen.*
7. *Gastroenterology Unit, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IIS-IP), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD)*
8. *Centro de Metabolómica y Bioanálisis (CEMBIO). Facultad de Farmacia. Universidad San Pablo-CEU.*
9. *Miltenyi Biotec GmbH, Bergisch Gladbach, Germany.*
10. *Institute of Food, Nutrition and Health, ETH Zurich, Switzerland*
11. *Medimmune, Granta Park, Cambridge.*
12. *Section of Immunology, Institute of Virology, University of Zurich, Switzerland*
13. *Co-senior authors*
14. *Lead Contact*

mmartinez@cnic.es

Abstract

Maintenance of the intestinal barrier function ensures the homeostatic host-microbiota relationship in a IL-17- and IL-22-dependent manner. Production of these cytokines by T-helper 17 (Th17) and group 3 innate lymphoid (ILC3) cells is influenced by the gut microbiota by insufficiently understood host pathways in steady state. Here, we show that a Syk kinase-coupled signaling pathway in dendritic cells (DCs) is critical for commensal-dependent production of IL-17 and IL-22 by CD4+ T cells. We identify Mincle as a Syk-coupled receptor that detects mucosal-resident commensals, triggers IL-6 and IL-23p19 expression, and thereby regulates intestinal Th17 and IL-17-secreting ILCs. Mincle-deficient or CD11c-Cre Sykflox/flox mice show impaired production of intestinal antimicrobial peptides and IgA, resulting in increased systemic translocation of gut microbiota. Consequently, Mincle-deficiency leads to liver inflammation and deregulated lipid metabolism. Thus, sensing of commensals by Mincle and Syk signalling in CD11c+ cells reinforces intestinal immune barrier and promotes host-microbiota mutualism, preventing systemic inflammation.

Proteomic analysis of the non-apoptotic functions of FADD

Marín-Rubio J. L. (1, 2, 3), Pérez-Gómez E. (4, 5), Cunningham D. L. (7), Fernández-Piqueras J. (1, 2, 3, 7, *) and Villa-Morales M. (1, 2, 3, 7, *).

1. *Centro de Biología Molecular Severo Ochoa (CBMSO), Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid (CSIC-UAM), Madrid, Spain.*
2. *IIS-Fundación Jiménez Díaz, Madrid, Spain.*
3. *Universidad Autónoma de Madrid, Departamento de Biología, Madrid, Spain.*
4. *Universidad Complutense de Madrid, Departamento de Bioquímica y Biología Molecular, Madrid, Spain.*
5. *Instituto de Investigación Hospital 12 de Octubre, Madrid, Spain*
6. *School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT United Kingdom.*
7. *Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER), Valencia, Spain.*

** JF-P and MV-M contributed equally to this work.*

jlmarin@cbm.csic.es

Abstract

Introduction.- The role of FADD in cancer is controversial, but the apparent discrepancies between studies could be explained by the fact that FADD exhibits apoptotic and non-apoptotic roles. However, the mechanism(s) whereby FADD carries out its non-canonical functions is still unknown. A decrease of FADD associated with poor clinical outcome like drug resistance, inferior survival, more recurrence or metastasis has been described in many tumour types. Specifically, we have observed that T-cell lymphoblastic lymphomas (T-LBL) exhibit a significant reduction of FADD protein levels, but the mechanism whereby this confers a significant advantage for the tumour cell is still unclear. In this study, we set out to get insight into the landscape of deregulated cell signalling events in FADD-deficient tumour T cells through mass spectrometric-based quantitative proteomics, using stable isotope labelling of amino acids in cell culture (SILAC). This is the first analysis of endogenous FADD interactome and proteome using SILAC-based and label-free quantitative proteomic techniques in non-apoptotic conditions.

Objectives.- The purpose of this study is to identify the protein–protein interaction network of endogenous FADD (interactome analysis) and to quantitatively compare protein expression levels (proteome analysis) in FADD-expressing vs FADD-deficient tumour T cells.

Methodology.- Stable Isotope Labelling of Amino acids in Cell culture (SILAC) and label-free quantification (LFQ) were used to evaluate FADD interactome. GSEA analysis was used to validate results from SILAC-based proteome of FADD-deficient tumour T cells. Functional interactions between FADD and several novel binding partners were confirmed by immunoprecipitation and western blot.

Results.-The results indicated that energy metabolism is deregulated in FADD-deficient T-cell lymphoblastic neoplasms. Moreover, a comparative analysis of the results from two different quantitative proteomic strategies revealed 18 candidate proteins that interact with endogenous FADD. Interestingly, they are involved in mRNA processing and maturation.

Conclusions.- FADD deficiency in T-cell lymphoblastic neoplasms would not only result in apoptotic impairment, as defined by FADD canonical function, but it would also be related to deregulation of non-apoptotic processes such as energy metabolism and mRNA processing.

Machine Learning Predicts the Functional Importance of Potential Alternative Splicing Isoforms

Fernando Pozo and Michael Tress

Spanish National Cancer Research Centre (CNIO)

fpozoc@cnio.es

Abstract

The alternative splicing of messenger RNA produces a vast array of mature RNA transcripts, and many of these alternative transcripts are annotated in reference databases. Despite the overwhelming evidence of alternative splicing at the transcript level, there is little reliable evidence of alternative spliced proteins at the protein level, so the extent to which alternative transcripts will produce functionally relevant protein isoforms is still not clear (1-3).

Here we present a new and automatic computational approach to classify human alternative splice isoforms. This machine learning approach uses more than 60 different features from 4 distinct biological categories to make its predictions. The algorithm classified functional isoforms with high confidence and in contrast to previous attempts to predict functional alternative isoforms, predicted that at least 90% of distinct protein isoforms do have not a functional role in the cell.

The algorithm not only provides valuable insights into the functional importance of alternative splicing, but will also provide a reliable list of the most significant biological splice isoforms. The list of functional splice isoforms will be available from the APPRIS database (4) and will be updated with each new GENCODE release (5) and can be applied to any eukaryotic species.

1. Tress, M. L., Abascal, F., and Valencia, A. (2017). Most alternative isoforms are not functionally important. Trends in Biochemical Sciences, 42(6), 408 – 410.
2. Abascal, F., Ezkurdia, I., Rodriguez-Rivas, J., Rodriguez, J. M., del Pozo, A., Vázquez, J., Valencia, A., and Tress, M. L. (2015). Alternatively Spliced Homologous Exons Have Ancient Origins and Are Highly Expressed at the Protein Level. PLoS Computational Biology, 11(6), 1–29.
3. Tress, M. L., Abascal, F., and Valencia, A. (2017). Alternative Splicing May Not Be the Key to Proteome Complexity. Trends in Biochemical Sciences, 42(2), 98–110.
4. Rodriguez,J.M., Rodriguez-Rivas,J., Di Domenico,T., Vázquez,J., Valencia,A., and Tress,M.L. (2018) APPRIS 2017: principal isoforms for multiple gene sets. Nucleic Acids Res., 46, D213-217.
5. Harrow,J., Frankish,A., Gonzalez,J.M., Tapanari,E., Diekhans,M., Kokocinski,F., Aken,B.L., Barrell,D., Zadissa,A., Searle,S., et al. (2012) GENCODE: the reference human genome annotation for The ENCODE Project. Genome Res., 22, 1760-1774.

Histopathology of the allan-herndon-dudley syndrome: characterization of the neuroglial population

Guillén-Yunta M (1,2), García-Aldea A (1), Grijota-Martínez C (1), Guadaño-Ferraz A (1,3)

1. Instituto de Investigaciones Biomédicas Alberto Sols, Consejo Superior de Investigaciones Científicas (CSIC)-Universidad Autónoma de Madrid (UAM), Madrid, Spain.
2. School of Medicine and Science, Master's Degree on Molecular Biomedicine, UAM, Spain.
3. Center for Biomedical Research on Rare Diseases (Ciberer) Unit 708, Instituto de Salud Carlos III, Madrid, Spain.

marina.guilleny@estudiante.uam.es

Abstract

Thyroid hormones (THs) are essential for the proper development of the central nervous system (CNS), regulating fundamental processes such as neuronal differentiation and cell migration, including the function of the neuroglial population. The Allan-Herndon-Dudley Syndrome (AHDS) is a rare X-linked disease due to inactivating mutations in the SLC16A2 gene, which encodes the monocarboxylate transporter 8, or MCT8. THs require specific transporter proteins to cross the plasma membrane to access their target cells. Recent studies suggest that MCT8 specifically transports THs, being crucial for the maintenance of proper THs content in the brain. AHDS patients have symptoms such as neurological development retardation, severe intellectual disability (IQ < 30), altered communicative and speech capacity and central hypotonia, among others.

As it has been described that THs have an important role in the development and functioning of neuroglial cells, the main objectives of this work were the characterization of the neuroglial populations in physiologic conditions and in MCT8 deficiency, as well as the evaluation of the possible neurodegenerative phenotype of AHDS in paraffin embedded cerebral samples from MCT8 deficient subjects and in a murine model of the disease.

Our results suggest that under MCT8 deficiency conditions there is an increment in the number of both, reactive microglial cells and reactive astrocytes, and also an increase in the expression of neurodegenerative markers and alterations in the neurogenic capacity of the adult subventricular zone. Based on our results, we can conclude that MCT8 deficiency generates a pro-inflammatory and neurodegenerative state in the brain, which leads to microgliosis and astrogliosis in the brain parenchyma. The results of this work suggest new therapeutic targets related to the neurological symptoms of the disease that can lead to new possible treatments, which may contribute, in the long term, to improve the quality of life of the patients.

Role of Galectin-1 in contact hypersensitivity

Castillo-González R (1-3), Cibrian D (1-3), Fernández-Gallego N (2, 3), Ramírez-Huesca M (1, 3), de la Fuente H (1, 3), Sánchez-Madrid F (1-3)

1. *Immunology Service, Hospital de la Princesa, Instituto de Investigación Sanitaria del Hospital Universitario de La Princesa, Madrid, Spain.*
2. *Universidad Autónoma de Madrid, Madrid, Spain.*
3. *Department of Vascular Biology and Inflammation, Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain.*

raquelana.castillo@externo.cnic.es

Abstract

Contact hypersensitivity (CHS) is a prevalent inflammatory skin disease characterized by red, itchy, swollen and cracked skin. It is caused by the contact with a hapten such as oxazolone. CHS presents two phases: sensitization, in which the clonal expansion of specific T cell occurs, and elicitation, in which, after re-exposure to the hapten, the activation and recruitment of specific T cells at the inflammation site take place. Galectins are β-galactoside-binding-animal-lectins expressed in many tissues with the highest expression in the immune system. Galectin-1 (Gal-1) has anti-inflammatory properties demonstrated in vitro and in vivo models. However, the role of Gal-1 in CHS disease is not yet known.

In CHS model we find that Gal-1KO mice present more sustained and prolonged inflammation than WT mice, assessed by ear swelling. Using flow cytometry, we detect that Gal-1KO mice have more neutrophilic infiltration, CD8+ T cells, and IL17+γδ T cells in ears seven days after the second challenge. Moreover, the deficiency of Gal-1 promotes higher inflammation, assessed by increased CD8+T/Treg and γδ T/Treg cells ratios. To determine the mechanisms by which Gal-1 exerts anti-inflammatory effects we carried out in vivo migration studies. Our data showed that T cells migrate from the blood to the lymph nodes equally in Gal-1KO and WT mice. However, in the absence of Gal-1, Treg cells migrate less to inflamed skin.

This work highlights the protective role of Gal-1 in CHS disease. Its molecular mechanisms of anti-inflammatory action seems to involve the control of Treg cell migration to inflamed skin, as well as the negative regulation of IL-17 and IFNγ secretion by T cells. Further experiments are required to identify the molecular mechanism of Gal-1-regulation of T cell migration in CHS disease.

Cardiometabolic Disease in Progeria and Aging

Fanjul V (1,2,3), Jorge I (1,3), Macías A (1,3), Camafeita E (1,3), González-Gómez C (1,3), Rivera-Torres J (1,3), Vázquez J (1,3), López-Otín C (2,4) & Andrés V (1,3)

1. Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid 28029, Spain.
2. Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Instituto Universitario de Oncología, Universidad de Oviedo, 33006 Oviedo, Spain.
3. CIBER-CV, Spain.
4. CIBER-ONC, Spain

vfanjul@cnic.es

Abstract

Aging is one of the main risk factors for metabolic and cardiovascular disease (CVD) which are becoming a major socio-economic challenge in our society. CVD is exacerbated in Hutchinson-Gilford Progeria Syndrome (HGPS), a rare genetic disorder provoked by the accumulation of progerin, an aberrant form of lamin A. The disease is characterized by accelerated aging, lipodystrophy and premature death from heart attack or stroke during adolescence. However, HGPS patients do not suffer other disorders typical in the elderly, such as cancer, diabetes or neurodegeneration. Interestingly, progerin expression in aged tissues from non-HGPS subjects has suggested a role for this protein in normal aging. Therefore, research in HGPS may shed light on the etiology of normal aging and related cardiometabolic disease avoiding interference from other multifactorial disorders.

To identify mechanisms common to both normal and premature aging and specific to each, we first resorted to electrocardiography and magnetic resonance imaging to assess cardiac function and body composition in progerin-expressing Lmna G609G and young and old wild-type mice. As previously reported, both progeric and wild-type old mice presented bradycardia and repolarization abnormalities. Lmna G609G also showed reduced cardiac output and lipodystrophy.

Next, we performed multiplexed, high-throughput quantitative proteomics to detect alterations in protein abundance and oxidation in heart tissue from progeric and young and old wild-type mice. Protein extracts were subjected to FASILOX-iTRAQ labeling and LC-MS/MS analysis. Peptide identification was performed using SEQUEST and the results were analyzed by the SanXoT workflow. We have identified about 5000 proteins at 1% FDR, of which 2700 were quantified with more than one unique peptide. We present for the first time a functional category analysis comparing the molecular mechanisms underlying progeria with the pathways governing aging in the heart. Results support alterations in processes as energy metabolism, mitochondrial dysfunction, oxidative stress, calcium signaling, cytoskeleton and protein folding. The present research should elucidate novel biomarkers and molecular mechanisms underlying cardiometabolic disease in HGPS and normal aging, which might facilitate the development of new therapies and preventive interventions to promote healthier aging.

Synergistic effect of membrane-active peptides SP-A and SP-BN on multidrug-resistant Klebsiella pneumoniae

Fraile-Ágreda V (1,2), Cañadas O (1), Casals C (1,2)

1. Departmento de Bioquímica y Biología Molecular, Universidad Complutense de Madrid
2. Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid

vicfra01@ucm.es

Abstract

As the key components of innate immunity, human host defense antimicrobial peptides and proteins play a critical role in warding off invading microbial pathogens. We have previously shown that lung surfactant protein (SP-A) exerts a synergistic antimicrobial activity with the lung anti-microbial peptide SP-BN against the respiratory pathogen Klebsiella pneumoniae K2 in vivo. However, the factors that govern SP-A/SP-BN antimicrobial activity are still unclear. The aim of this work was to characterize the mechanism by which SP-A and SP-BN act synergistically against capsulated K. pneumoniae, which is otherwise resistant to either protein alone. Our results indicate that the SP-A/SP-BN complex, but not the individual proteins, alters the bacterial ultrastructure, forming pores in the membrane that favor the translocation of both proteins to the periplasmic space, where they interact with the inner membrane, inducing its depolarization and fission into small vesicles. In vitro studies with model membranes, which mimicked the internal and external bacterial membranes, showed that both SP-A and SP-BN bound to lipopolysaccharide molecules present in the outer membrane, inducing lipid phase separation and disrupting membrane packing. This effect was stronger for the SP-A/SP-BN complex, which rendered both the outer and inner bacterial membranes leaky as determined by permeabilization studies. On the other hand, the SP-A/SP-BN-induced fission of the inner membrane may be related to the promotion of positive curvature observed by differential scanning calorimetry. Taken together, our results indicate that the antimicrobial activity of the SP-A/SP-BN complex is related to its capability to alter the integrity of outer and inner bacterial membranes.

Antitumoral effects of Hispanolone derivatives in glioblastoma cells

Sánchez-Martín V (1,2), Hortelano S (1), de las Heras B (2)

1. Instituto de Salud Carlos III (ISCIII)
2. Universidad Complutense de Madrid (UCM)

vanesa_sanchez_92@hotmail.com

Abstract

Glioblastoma multiforme (GBM) constitutes the most frequent and aggressive primary brain tumor in adults. Even vast efforts have been made to develop effective treatments, including the combination of surgery, chemotherapy, and radiotherapy; the prognosis for the patients is extremely poor, with mean survival of about 14 months. Therefore, there is still an urgent need for novel and effective therapies for treating these tumors. On this issue, natural product-based molecules represent interesting therapeutic alternatives. Hispanolone derivatives have been shown to induce apoptosis in several human cancer cells. Nevertheless, the activity of these compounds against glioblastoma cells remains unclear. In the present study, we aimed to investigate the effects of a hispanolone derivative, α-hispanolol, on proliferation as well as on the migration and invasion of human glioblastoma cells.

Our results show that α-hispanolol induced cell morphological changes and decreased the cell viability of U87 and U373 cells in a dose- and time-dependent manner. This inhibitory effect was found to be linked to induction of apoptosis and accumulation in the sub-G1 phase. Moreover, α-hispanolol also induced caspases activities and increased the pro-apoptotic protein (Bax and Bid), and inhibited the anti-apoptotic proteins (Bcl-2 and Bcl-xl) in GBM cells. In addition, α-hispanolol displayed an inhibitory effect on the migration and invasion of glioma cells by inhibiting the expression and activity of MMPs.

Novel anti-inflammatory agents via inhibition of NF-kB and/or Inflammasome pathways: a series of hispanolone-derived diterpenes.

González-Cofrade L (1), Cuadrado I (1), Estévez-Braun A (2), Hortelano S (3), Las Heras B (1)

1. Departamento de Farmacología, Farmacognosia y Botánica. Facultad de Farmacia, Universidad Complutense de Madrid. Madrid, Spain.
2. Instituto Universitario de Bio-Orgánica Antonio González. Departamento de Química Orgánica, Universidad de La Laguna, Tenerife, Spain.
3. Unidad de Terapias Farmacológicas, Instituto de Investigaciones en Enfermedades Raras (IIER), Instituto de Salud Carlos III (ISCIII), Majadahonda, Madrid, Spain.

lagonz11@ucm.es

Abstract

Inflammation is a crucial host response triggered by invading pathogens and injured tissue. Recent studies have highlighted the critical role of NLRP3 inflammasome in the inflammatory response. Natural products have played a significant role in human health as a source of new drugs to prevent and treat inflammatory conditions. In this context, bioactive natural compounds such as diterpenes can be considered very promising starting points for the development of new therapeutic agents. In this study, a series of seventeen novel hispanolone derivatives (N1-N17) were synthesized and evaluated for potential anti-inflammatory activity in J774A.1 macrophages. We focused on their potential as inhibitors of classical inflammatory pathways (NF-kB/MAPKs) and/or inflammasome activation. As revealed by the MTT assay, all compounds except N2 and N5 were non-cytotoxic. To investigate the effects of diterpenes on NF-kB pathway, macrophages were activated with lipopolysaccharide (LPS) in the absence or presence of these compounds. Inhibitory effects of the non-toxic compounds on nitric oxide (NO) production were evaluated. Hispanolone derivatives N1 and N12 were selected for further evaluation, as they were the most active NO inhibitors (IC50 in the range 10-20 mM). A significant inhibition of NOS-2 and COX-2 expression was observed. Moreover, the phosphorylation of mitogen-activated protein kinases (MAPKs) ERK1/2, p38 and JNK was also reduced by treatment with N1 and N12. Hispanolone derivatives were also evaluated as potential regulators of NLRP3 inflammasome. Cell treatment with derivatives N12, N13, N16 and N17 at 20 mM significantly reduced IL-1β secretion in LPS/ATP or LPS/Nigericin-stimulated macrophages. Inhibition of IL-1β and cleaved caspase-1 protein expression was also confirmed by western blot.

In conclusion, these results show the promising anti-inflammatory effects of some hispanolone derivatives, in particular N12, acting on a dual level. This diterpene not only inhibits NF-kB/MAPKs signaling pathways, but also regulates inflammasome activation.

MouBeAT: A New and Open Toolbox for Guided Analysis of Behavioral Tests in Mice

Bello-Arroyo E (1), Roque H (2), Marcos A (3), Orihuel J (3), Higuera-Matas A (3), Desco M (4,5,6,7), Caiolfa VR (2,8), Ambrosio E (3), Lara-Pezzi E (1,9,10), Gómez-Gaviro MV (4,7)

1. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain
2. Unidad de Microscopía e Imagen Dinámica, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain
3. Departamento de Psicobiología, Universidad Nacional de Educación a Distancia (UNED), Madrid, Spain
4. Departamento de Medicina y Cirugía Experimental, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain
5. Departamento de Bioingeniería e Ingeniería Aeroespacial, Universidad Carlos III de Madrid, Getafe, Spain
6. Unidad de Imagen Avanzada, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain
7. Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, Madrid, Spain
8. Ospedale San Raffaele, Centro di Immagine Sperimentale, Milan, Italy
9. Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain
10. National Heart and Lung Institute, Faculty of Medicine, Imperial College London, London, United Kingdom

ebello@cnic.es

Abstract

Animal behavioral tests are essential to understand the bases of neurologic and psychological disorders, which can be evaluated by different methodological and experimental models. However, the quantification of behavioral tests results is limited by the considerable amount of time needed for manual evaluation and the high costs of automated analysis software. To overcome these limitations, we describe here a new, open source toolbox for ImageJ, called Mouse Behavioral Analysis Toolbox (MouBeAT), designed to analyze different behavioral tests in rodents semi-automatically. These tests include Open Field (OF), Elevated Plus Maze (EPM), Y-maze (YM) test and Morris Water Maze (MWM). MouBeAT showed a high correlation with manual evaluation in all the parameters analyzed for all the behavioral tests, reinforcing its value as an accurate analysis tool. This new tool is freely available online.

Analog Quantum Chemistry Simulation

Argüello-Luengo J (1,2), González-Tudela A (1,3), Shi T (1,4), Zoller P (5,1), Cirac JI (1)

1. Max-Planck-Institut für Quantenoptik, Hans-Kopfermann-Strasse 1, D-85748 Garching, Germany.
2. ICFO-Institut de Ciencies Fotoniques, The Barcelona Institute of Science and Technology, 08860 Castelldefels (Barcelona), Spain.
3. Instituto de Física Fundamental IFF-CSIC, Calle Serrano 113B, Madrid 28006, Spain.
4. CAS Key Laboratory of Theoretical Physics, Institute of Theoretical Physics, Chinese Academy of Sciences, P.O. Box 2735, Beijing 100190, China.
5. Center for Quantum Physics, University of Innsbruck, A-6020 Innsbruck, Austria.

javierargu@gmail.com

Abstract

Computing the electronic structure of molecules with high precision is a central challenge in catalysis research or drug industry. Despite the enormous success of approximate methods, tackling this problem exactly with conventional computers is still a formidable task. This has triggered several theoretical and experimental efforts to use quantum computers to solve chemistry problems, with first proof-of-principle realizations done in a digital manner [1]. An appealing alternative to the digital approach is analog quantum simulation, which does not require a scalable quantum computer. In the same way that a soap film can minimize a surface area without performing any calculation, this approach uses a highly controllable device —the simulator— to mimic the system of interest and gain information about the desired solution. While analog simulation has already been successfully applied in condensed matter physics problems, none of the available or planned setups can be used in quantum chemistry problems, since it is not known how to engineer the required Coulomb interactions with them. Here, we solve this problem and show how it is possible to simulate quantum chemistry problems in an analog way [2].

[1] A. Aspuru-Guzik, A. D. Dutoi, P. J. Love, M. Head-Gordon, Simulated Quantum Computation of Molecular Energies, Science 309, 1704 (2005).
[2] J. Arguello-Luengo, A. Gonzalez-Tudela, T. Shi, P. Zoller, and J. I. Cirac, Analog Quantum Chemistry Simulation, arXiv:1807.09228

Identification of proteins targets of autoantibodies for the diagnosis of Alzheimer's Disease by Proteomics.

Montero A (1), San Segundo P (1, 2), Garranzo M (1), Solís G (1), Barderas R (1)

1. UFIEC-CROSADIS, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.
2. Biochemestry and Molecular Biology Department, Chemistry Faculty, Complutense University of Madrid, Madrid, Spain.

anamonterocalle@hotmail.com

Abstract

Alzheimer's disease (AD) is a progressive, chronic and neurodegenerative disorder that affects wide areas of the cerebral cortex and hippocampus. It is the most common cause of dementia worldwide with a 10-30% prevalence in ageing population (>65 years of age) and a high socioeconomic impact. Because the definitive diagnosis of AD requires post-mortem verification and current technologies are not good enough for the early diagnosis of the disease or are not specific of AD, new approaches to study AD are necessary to identify new diagnostic biomarkers and therapeutic targets of intervention.

One of the oldest and most successful types of biomarkers that might be applied to AD diagnosis is the humoral immune response due to the appealing features of autoantibodies. We have here aimed to identify AD-specific autoantibodies and their target proteins as blood-based biomarkers of the disease using a combination of phage display and protein microarrays.

Two T7 phage display libraries displaying the cDNA repertoire of AD patients and healthy individuals' brain were biopanned to enrich in phages recognized by IgGs from AD patients. After the biopanning, phage microarrays containing 1920 unique phages and controls were printed and screened with serum from AD patients and controls. After phage microarray analysis, we identified a collection of 42 peptides target of autoantibodies from AD patients as potential biomarkers of the disease. For validation, we expressed 13 of them as 6xHis-Halo fusion proteins and analyzed their seroreactivity using a different cohort of sera from AD patients and controls than the used in the microarrays or biopanning (68 sera from AD patients and 52 sera from controls) by luminescence beads-based test. In addition, we are studying the immunoreactivity of two frameshift proteins that play an important role in the pathogenesis of AD. The seroreactivity to these frameshift proteins have been analyzed using sera from AD patients and controls by luminescence beads-based test after their expression as 6xHis-Halo fusion proteins. A significant differential seroreactivity in AD patients in comparison to controls was observed for 4 peptides and for the two frameshift proteins.

Our results suggest that these peptides may be AD specific autoantigens non-previously described that might be used as blood-based biomarkers of the disease. However, complementary immunological approaches are needed as well as a further validation using a higher cohort of sera from AD patients and controls to determine their actual usefulness as biomarkers of the disease.

Lamin A/C in T cells as a modulator of melanoma development and memory generation.

Herrero Fernández B (1), Zorita V (2), Toribio R (2), Sánchez Madrid F(2,3,4), González Granado, JM (1,2,3,4,5)

1. Instituto de Investigación Hospital 12 de Octubre (imas12), Madrid, Spain.
2. Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain.
3. Servicio de Inmunología, Hospital de la Princesa, Instituto de Investigación Sanitaria La Princesa (IIS Princesa), Madrid, Spain.
4. CIBER de Enfermedades Cardiovasculares, Spain.
5. Departamento de Fisiología. Facultad de Medicina. Universidad Autónoma de Madrid (UAM), Madrid, Spain.

beatriz.herrero@externo.cnic.es

Abstract

An innate immune response is produced against pathogens or in immune pathologies. This immune response is followed by a specific adaptive response. Adaptive immunity requires T helper (Th) cell differentiation of naïve CD4 T cells to ensure efficacy, specificity and memory. The immune system is also an important defense mechanism against tumors, in which CD8+ IFNγ+ and Th1 cells have protective properties while Treg cells have detrimental effects. Memory T lymphocytes play an essential role against tumor development. Melanoma is one of the most aggressive and lethal tumors, responsible for most deaths from skin cancer. If it is diagnosed in its early stages, resection of the lesion is associated with favorable survival rates. However, once it spreads to distant organs, such as the brain, lungs, liver, or small intestine, the prognosis is poor with a median survival of less than 1 year. Lamin A/C controls synthesis, repair and organization of chromatin, transcription, cell cycle and differentiation, migration and cell proliferation. Although its role in the immune system is almost unknown, previous results from our laboratory show that Lamin A/C potentiates T cell activation and promotes and inhibits differentiation towards Th1 and Treg phenotype, respectively. The objectives of this study are to determine the role of Lamin A/C in T cells in memory generation and in melanoma development and to propose the modulation of Lamin A/C as immunotherapy against tumor development. The obtained results show that Lamin A/C in T cells decreases the progression of the tumor, and increases the number of tumor infiltrating T cells, as well as the production of IFNγ by these cells. Lamin A/C in T cells also regulates the production and type of the generated memory T cell response. Taking into account these results, we propose that the modification of Lamin A/C levels in T lymphocytes could be a potential therapy against melanoma development.

Identification of tumor associated antigens in colorectal cancer patients by immunoprecipitation coupled to mass-spectrometry

Garranzo-Asensio M (1,2), Guzmán-Aránguez A (1), Montero-Calle A (1), San Segundo-Acosta P (1, 2), Solís G (1, 3), Povés C (4), Fernández-Aceñero MJ (4), Domínguez G (5), Peláez A (6), de los Ríos V (7), Barderas R (2).

1. Departamento de Bioquímica y Biología Molecular. Universidad Complutense de Madrid (Madrid, Spain)
2. CROSADIS, Instituto de Salud Carlos III (Madrid, Spain)
3. Department of Chemistry, Faculty of Sciences, KU Leuven (Leuven, Belgium).
4. Hospital Universitario Clínico San Carlos, IdISSC, (Madrid, Spain)
5. Departamento de Medicina, Facultad de Medicina, Instituto de Investigaciones Biomédicas "Alberto Sols," CSIC-UAM (Madrid, Spain).
6. Molecular Pathology and Therapeutic Targets Group, Hospital Universitario La Paz, IdiPAZ (Madrid, Spain)
7. CIB-CSIC Proteomics Facility (Madrid, Spain)

mgarranzo@hotmail.com

Abstract

Ninety percent of patients suffering from the 8 most common cancer types can be successfully treated if early diagnosed. The humoral immune response has been demonstrated useful for cancer diagnosis, predating clinical symptoms up to 3 years. Therefore, we have here aimed at developing a methodological approach in which serum samples from colorectal cancer (CRC) patients at stages III and IV were used to immunoprecipitate specific seroreactive proteins from protein extracts from CRC cell lines with different metastatic properties. Protein extracts were previously clarified with IgGs from healthy individuals to remove non-specific proteins. Eluted proteins with selected reactivity to cancer patients' autoantibodies were identified by mass spectrometry using a LTQ-Orbitrap Velos to complete the panel of CRC tumor associated antigens that could help establishing a diagnostic signature of the disease.

A total of 1743 peptides corresponding to 645 proteins reactive to CRC patients' sera and controls were identified, with 79 proteins showing a unique and specific seroreactivity to CRC IgGs. To avoid false positive targets and to select those proteins with a higher potential as CRC diagnostic markers for validation, the dataset was analysed using the CRAPome database and different bioinformatics tools. With the CRAPome, we selected 31 proteins more prone to be actual targets of CRC autoantibodies. Among them, 29 protein showed genetic variation in CRC and 7 showed a statistically significant expression deregulation in CRC. Lastly, one of the identified proteins has already been described as colorectal and renal cancer prognostic marker. Results were validated by western blot, immunohistochemistry using tissue microarrays, and seroreactiviy assays to identify the proteins more prone to be successful diagnostic markers.

The Role of Meis Transcription Factors in the Epicardium

E. Cruz-Crespillo, N. Muñoz-Martín, V. Cadenas and M. Torres

Centro Nacional de Investigaciones Cardiovasculares, Madrid. Spain

medelacruz@cnic.es

Abstract

Cardiac congenital diseases are the most common malformation at birth and cardiac diseases are the first cause of death. Understanding how the heart develops is therefore essential. Meis transcription factors (TFs) are emerging as important regulators of cardiac development. Single Meis1 or Meis2-null mice die at midgestation due to hematopoietic and cardiac alterations.

Our expression analysis by in situ hybridization and immunofluoresce shows epicardial expression of these TFs during heart development. Furthermore, lineage tracing experiments revealed the contribution of Meis1-expressing progenitor cells to different cell types such as cardiomyocytes, mesenchymal, smooth muscle, epicardial and endothelial cells. Contribution to vascular-endothelial cells suggests a role of Meis genes in coronary vasculature development.

Based on these results, we generated mice with a double Meis1/2 conditional deletion in the epicardium (Meis1flox/floxMeis2flox/flox;Wt1Cre/+), aiming to understand the role of Meis TFs in this cardiac layer and their putative contribution to coronary vasculature. Echocardiography studies in this mouse model revealed no cardiac dysfunction and the myocardium did not show major histological alterations. However, the shape of mutant hearts is slightly altered and coronary vasculature, including myocardial lymphatics, show patterning alterations.

Central leptin protects the heart by selectively increasing pparβ/δ in rats with normal leptin sensivity

Rubio B (1), Mazuecos L (1), Pintado C (2), Arribas C (2), Gallardo N (1), Andrés A (1).

1. Biochemistry Faculty of Science and Technological Chemistry, and Regional Centre for Biomedical Research (CRIB), University of Castilla-La Mancha (UCLM), Ciudad Real, Spain.
2. Biochemistry Faculty of Environmental Sciences and and CRIB, UCLM, Toledo, Spain.

altajagra@gmail.com

Abstract

Leptin at central level seems to play an important role in the protection of peripheral tissues. In heart, it protects against damage produced as a result of diseases like obesity, which degenerates into diabetes, hypertension or heart failure. Our group has recently demonstrated that some actions of central leptin in the heart are produced through the nuclear receptor PPARβ/δ. Herein we study the role of the heart in the thermogenic effect of central leptin.

To this end, we analyzed the effects of intracerebroventricular leptin infusion 7 days (0.2μg/day) on cardiac metabolism in 3-month-old Wistar rats with normal leptin sensitivity; and examine the effects of pharmacological inhibition of PPARβ/δ with the specific antagonist GSK0660 in rats infused with leptin. We focus on the study of the mRNA levels of FGF21 and its receptors in the heart, and those of several genes involved in cardiac lipid metabolism, thermogenesis and immune response such as LPL, ATGL, UCP-1, FAS, TBX15, HIF-1α, HIF-2α, CCL5, CD64, CD32 and CD3g. Our results indicate that central leptin decreased serum levels of FGF21. Nevertheless, the mRNA levels of FGF21 increased in the heart of leptin treated rats. Moreover, the gene expression of the specific receptors FGFr1 and FGFr2 were markedly increased by central leptin. Central leptin treatment induced the expression of LPL, ATGL, UCP-1, FAS, TBX15, HIF-1α and HIF-2α in the heart in a PPARβ/δ-dependent manner, indicating that central leptin treatment is involved in the regulation of hypoxia, immune response and thermogenesis in the heart independently of their effects on food intake. These results support a model in which overexpression of PPARβ / δ by central leptin protects the heart against cell damage.

The role of the immune system in metabolic homeostasis and disease

Crespo Ruiz-Cabello M

Fundación Centro Nacional de Investigaciones Cardiovasculares (CNIC) Carlos III

maria.crespo@cnic.es

Abstract

Metabolic disorders are implicated in many diseases such as diabetes, cancer or cardiovascular disease, and their alarmant prevalence among world is continuosly threaten human health. Our cells are in symphony when they correctly interact and go towards one direction, looking for the equilibrium, for the ethereal state. In the paradigm of disease, the cooperation between cells of a specific tissue and between different cell types passes into oblivion. The "harmonic" crosstalk between organs is lost or disturbed and hence, the complete knowledge of this complex network in the health and unhealthy steate is essential to understand better the origin and the treatment of a disease. During obesity the imbalance between food intake and energy expenditure leads to glucose and lipid metabolism alteration, lipotoxicity in adipose tissue and other non-adipose organs, such as liver or muscle, and the exacerbating reclutation of immune cells to these organs. In this context, cells from affected tissues are exposed to stress stimulus which activates or inactivates different signaling pathways, changes gene and protein expression patterns of the cell and therefore, conduces to an incorrect cell behaviour and loss of function. Immune cells are widespread among the body and in every tissue there is a specific immune cell population that contributes to its homeostasis and function. However, during obesity, the composition, metabolism and behaviour of this cell population also changes, affecting the normal function of the tissue and contributing to the development of diseases. p38MAPKs are a group of stress-activated kinases implicated in the signal transduction of many stimulus from outside the cell. It is well-stablished their role in health and disease and their therapeutic potential in the treatment of metabolic disorders. It also have been described the specific function of these kinases in different tissues and organs, using murine models lacking these kinases. However, due to the compensatory action between them, the lack of expression of their upstream activators, MKK3 and MKK6 kinase proteins, seems to provide a better model of study. This project is focus on the role of p38MAPKs in the biology and function of immune cells in the normal state and during obesity. For that, a murine model lacking MKK3 and MKK6 in the myeloid lineage has been developed. Our recent results show that p38MAPKs play a crucial role in the correct response of myeloid cells to different stimulus and in the infiltration into different tissues. 8-week administration of a high-fat diet to these mice reveals an important switch in the infiltration pattern of immune cells in organs such as liver and adipose tissue and a key involment in insulin signaling, brown thermogenesis and liver steatosis.

High consumption of energy at dinner is associated with increased incidence of metabolic syndrome: a population-based study in Spanish older adults

Hermenegildo-López Y (1,2), Donat-Vargas C (1,3), López-Garcia E (1,4), Graciani A (1), Banegas JR (1), Rodríguez-Artalejo F (1,4), Guallar-Castillón P (1,4,5).

1. 1. Department of Preventive Medicine and Public Health. School of Medicine. Universidad Autónoma de Madrid-IdiPaz, CIBERESP (CIBER of Epidemiology and Public Health), Madrid, Spain.
2. 2. Transfusion Center of Community of Madrid
3. 3. Institute of Environmental Medicine, Karolinska Institutet, Box 210, SE-171 77, Stockholm, Sweden
4. 4. IMDEA-Food Institute. CEI UAM+CSIC, Madrid. Spain.
5. 5. Welch Center for Prevention, Epidemiology, and Clinical Research. Johns Hopkins University. Baltimore, USA.

ygorhl@gmail.com

Abstract

Objectives: To examine the association between energy intake throughout the day and the incidence of metabolic syndrome in a population-based cohort of older adults in Spain.
Methods: A cohort of 607 people over 60 (51% women) without metabolic syndrome at baseline was selected and followed up from 2008-2010 until 2015. At baseline, the habitual food consumption in the previous year was recorded by using a validated dietary history. The energy consumption was evaluated at 6 eating occasions throughout the day: breakfast, mid-morning meal, lunch, mid-afternoon meal, dinner and snacking (at any other time of the day). The participants were classified into sex-specific quartiles of the percentage of energy intake (%EI) at each occasion. Metabolic syndrome was defined as having at least 3 of the following 5 criteria: waist circumference > 102 cm for men and > 88 cm for women; fasting blood glucose > 100 mg/dL or taking antidiabetic drugs; systolic blood pressure > 130 mmHg or diastolic blood pressure > 85 mmHg or taking antihypertensive drugs; triglyceridemia> 150 mg/dL, and serum-high-density lipoprotein cholesterol < 40 mg/dL for men and < 50 mg/dL for women. Logistic regressions were performed to estimate Odds Ratio (OR) and 95% confidence intervals (CI). The dependent variable was incident cases of metabolic syndrome. The independent variables of interest were the %EI at each eating occasion grouped in quartiles. The adjustment variables were the %EI in quartiles of the rest of eating occasions as well as the main confounders.
Results: During the follow-up, 101 new cases of metabolic syndrome occurred. The mean of the %EI at breakfast, mid-morning meal, lunch, mid-afternoon meal, dinner and snacking were: 17.1%, 4.2%, 41.9%, 4.0%, 27.8%, 5.0% respectively. Compared with the lowest quartile of %E intake at dinner, the risk of incident metabolic syndrome was OR=1.60 (95% CI 0.75-3.43) in the second quartile, OR=1.64 (95% CI 0.74- 3.61) in the third quartile, and OR=2.58 (95% CI 1.04-6.40) in the fourth quartile (p = 0.04). In the hypothetical situation of iso-substituting the %EI at dinner with the same amount of energy consumed at breakfast, the highest quartile of %EI at dinner compared with the first quartile was OR=2.92 (95% CI 1.34-6.36) (p = 0.007). No association was found for the rest of eating occasions.
Conclusions: A high intake of energy at dinner was associated with increased incidence of metabolic syndrome. Reducing the amount of energy consumed before going to bed could be considered a strategy for the metabolic syndrome prevention, although clinical trials should confirm this epidemiological finding.

Structure of a polinton-like virus, the missing link between bacteriophage and eukaryotic viruses of the PRD1-like lineage

Condezo G (1), Pagarete A (2), Bratbak G (2), San Martín C (1)

1. *Spanish National Centre for Biotechnology (CNB-CSIC). Madrid, Spain.*
2. *Department of Biological Sciences. University of Bergen. Bergen, Norway.*

gncondezo@cnb.csic.es

Abstract

Viruses in the PRD1-like lineage infect organisms across the evolutionary tree and build their capsids from proteins with beta-jelly rolls orthogonal to the capsid surface (1). Polintons are large eukaryotic dsDNA transposons encoding a protein-primed DNA polymerase (POL) and a retroviral-like integrase (INT). Most of them also include a DNA-packaging ATPase and a maturation protease similar to those found in PRD1-like viruses. They also encode genes that could translate into orthogonal jelly roll proteins, suggesting that in certain conditions they could form icosahedral capsids. These observations prompted the hypothesis that Polintons may have evolved from a PRD1-like ancestor (encoding capsid proteins, POL, and ATPase), which entered a proto-eukaryotic host with a bacterial endosymbiont and acquired the protease and integrase genes by recombination with a transposon (2)(3). Subsequent evolution would have resulted in the "polintovirus" elements splitting into two different ways of life: the transposable, capsid-less integrating elements, and the bona fide viruses.

Recently, marine metagenome analyses have revealed a group of putative polinton-like viruses (PLVs) in eukaryotes. PLV genomes contain genes for single and double jelly roll proteins and a packaging ATPase, but lack the protease and integrase genes (4). Therefore, PLVs could represent a minimal version, or a first ancestor, of the PRD1-like lineage in eukaryotic hosts. We are analyzing the structure of the only isolated virus belonging to this newly defined group: Tetraselmis striata virus N1 (TsV-N1) (5). The cryo-EM capsid structure at 5.2 Å resolution corroborates the placement of TsV-N1 in the PRD1-like lineage.

1. Krupovic et al., Nat Rev Microbiol 6, 941 (2008).
2. Fischer et al., Science 332, 231 (2011).
3. Krupovic et al., Nat Rev Microbiol 13, 105 (2015).
4. Yutin et al., BMC Biol 13, 95 (2015).
5. Pagarete et al., Viruses 7, 3937 (2015).

Genome patterns of bacterial taxa and their relation to different ecological strategies

Marta Cobo Simón*, Javier Tamames

Spanish National Centre for Biotechnology (CNB-CSIC), Spain

mcobo@cnb.csic.es

Abstract

Microorganisms have been shown to play a key role in all environmental processes. However, 95% of microbes still remain unculturable despite the improvement of cultivation techniques, which has been a drawback for understanding their ecological strategies. During the last few decades, the development of high-throughput sequencing has provided a very large amount of genome data without the need of culturing, allowing the study of ecological questions.

The main aim of this research was to relate genome characteristics of different prokaryotic taxa, such as genome size, 16S rRNA gene copy number and functional content of the genomes, to their ubiquity and environmental preferences. To achieve this goal, we carried out a genomic analysis of 2837 complete genomes and evaluated the presence of each bacterial genera in diverse environments. The results showed that genome size was linked to ubiquity, and consequently adaptation, while 16S copy number was not directly related to it.

Furthermore, microorganisms living in each environment presented a different combination of the two characteristics. Finally, we observed clear differences in the importance of each functional class for microorganisms belonging to different environments. For example, a higher presence of carbohydrate metabolism and defense mechanisms in host-associated habitats, reflecting the richness in nutrients and the need of protection against the host immune system.

Thus, this analysis highlights the genomic patterns that are beneficial for each environment and ecological strategy in bacteria, which is crucial for a better understanding of the functioning of microbial communities and the rules governing their assembly.

Exploring Liver X Receptors role in human macrophage polarization

Arturo González de la Aleja (1), Antonio Castrillo (2) and Angel Corbí (1)

1. *Centro de Investigacciones Biológicas, Consejo Superior de Investigaciones Científicas (CIB-CSIC)*
2. *Instittuo de Investigaciones Biomédicas "Alberto Sols" (IIBM-CSIC-UAM)*

agonzaleza@cib.csic.es

Abstract

Macrophages are a heterogeneous population of myeloid cells that display a variety of phenotypes (usually referred to as polarization states) whose balance is critical for tissue homeostasis. In particular, pro- and anti-inflammatory macrophages are crucial for initiation and resolution of inflammatory processes, and their absent or exacerbated functions contribute to chronic inflammatory diseases. Several nuclear receptors regulate macrophage behaviour, but the role of Liver X Receptors (LXR), LXRα and LXRβ, in the plasticity and biology of human macrophages is still unknown. Using a well-stablished in vitro system in which blood monocytes differentiate into pro- or anti-inflammatory macrophages, we aimed to clarify the function of LXR in human macrophage polarization. We found that activation or inhibition of LXR during macrophage differentiation has a great impact on the transcriptomic and functional (cytokine production) profile of pro- or anti-inflammatory macrophages. Besides, siRNA-mediated depletion of LXR in differentiated macrophages evidenced that the activity of both isoforms is important for macrophage functions, and that LXRβ is upregulated upon LXRα knockdown. Our next steps will address the specific role of each LXR isoform during macrophage differentiation at the transcriptional and functional levels, and their respective interactions with other transcription factors involved in human macrophage polarization.

Molecular defects in progeroid Cockayne syndrome and ageing cellular models

Fernández-Molina C (1), Crochemore C (1) , Ricchetti M (1)

1. Stem Cells & Development, CNRS UMR3738 Team Stability of Nuclear & Mitochondrial DNA, Institut Pasteur, Paris, France

cristina.fernandez-molina@pasteur.fr

Abstract

Ageing is dramatically accelerated in some rare genetic disorders like the Cockayne syndrome (CS). Dissecting the molecular defects in these diseases is critical to develop treatments, and elucidate dysfunctions that may lead to normal ageing. CS defects (i.e. UV hypersensitivity, premature ageing, and neurodegeneration) have been classically attributed to impaired DNA repair as proteins mutated in this disease, CSA or CSB, participate in this process. Nevertheless, mutations in CSA or CSB are responsible for another disease, the UV-sensitive syndrome (UVSS) that is characterized by photosensitivity and no premature aging, uncoupling the DNA repair defect from precocious ageing/neurodegeneration. Moreover, the lab has recently identified a novel pathway that is only altered in cells derived from CS patients, and not in UVSS and controls, and that is possibly determinant for the progeroid phenotype (Chatre et al, 2015 PNAS). They discovered that oxidative and nitrosative stress promote overexpression of the HTRA3 protease, which leads to degradation of the mitochondrial DNA polymerase POLG1 responsible for replication of the organelle genome, in turn affecting mitochondrial respiration and leading to mitochondrial dysfunction.

Our current research aims to dissect the consequences of POLG1 depletion on mitochondrial DNA replication and organelle function, and assess their rescue using MnTBAP, a scavenger of oxidative/nitrosative stress that we have previously shown to revert CS-specific defects. To overcome the difficulty of assessing molecular alterations in patient-derived cells with different genetic backgrounds, we are generating isogenic CS models, using CRISPR/Cas9 technology to correct the CSB mutation in CS cells and introduce this mutation in normal cells. Results show that wild type cells CRISP-edited to knock out CSB recapitulate CS-specific defects. Interestingly, the extent of POLG1 depletion was variable in cells with distinct CSB mutations/depletion despite they share the same genetic background. Thus, multiple isogenic cellular models should help understanding the dynamics of CS cellular defects, compatibly with a continuous spectrum of severity in the symptoms in CS patients.

Transcriptome analysis of zebrafish embryos exposed to drugs of abuse

Calderón-García AA (1, 2), González-Núñez V (1, 2, 3)

1. Instituto de Neurociencias de Castilla y León (INCyL). Universidad de Salamanca, Spain.
2. Institute of Biomedical Research of Salamanca (IBSAL), Spain.
3. Department of Biochemistry and Molecular Biology. Faculty of Medicine. Universidad de Salamanca, Spain

andresangel@usal.es

Abstract

Drugs of abuse are considered a serious public health issue due to their addictive properties. At present, the recreational use of these addictive drugs is causing serious problems due to their side effects (mainly tolerance and dependence).

Zebrafish (Danio rerio) is an advantageous tool to evaluate the in vivo effects of pharmacological agents. Zebrafish neurotransmitter systems share similar molecular, pharmacological and biochemical profiles with their human homologues. For this reason, the experimental results can be easily extrapolated to higher vertebrates.

To evaluate the effects of drugs of abuse in the regulation of gene expression, we have analysed the transcriptome changes elicited by morphine and cocaine. Zebrafish embryos were exposed to either 10 μM morphine or 15 μM cocaine and the amount of morphine and cocaine assimilated was assessed by HPLC followed by ESI-MS mass spectrometry. RNA-Seq was used to analyze the dysregulated genes by morphine and cocaine, and the obtained results were validated by Real-Time PCR (qPCR) using specific primers.

Our results indicate that zebrafish embryos are able to assimilate both morphine and cocaine in a dose-dependent manner. Go term analysis revealed that the overrepresented processes are lipid metabolism, phototransduction, cellular response to xenobiotic stimulus, oxygen transport, G-protein coupled receptor signaling pathway, immune response and embryonic development.

Thus, our approach using whole zebrafish embryos allowed us to identify those metabolic pathways that might be directly causing the physiological effects of drugs of abuse.

Exploitation of group II introns as a versatile delivery system for synthetic DNA-barcoding of bacteria

Velázquez E (1), Al-ramahi Y (1), de Lorenzo V (1)
Centro Nacional de Biotecnología (CNB-CSIC)

evelazquez@cnb.csic.es

Abstract

Group II introns are genetic elements present in the genomes of some bacteria and organelles. Their main characteristic is the ability to mobilize and insert DNA sequences inside of specific genomic sites. For target recognition, group II introns use two constituents: a cognate RNA specie and a multifunctional protein encoded in the same device (Intron-Encoded Protein or IEP). After transcription of the intron to RNA and the translation of the IEP, these two molecules work together to recognize a particular DNA sequence mostly by base pairing with the intronic RNA. Finally, retrotranscription of the RNA element takes place by the IEP, ending up with the complete insertion of the intron in the new DNA molecule.

The first biotechnological application of group II intron came after successfully modifying certain regions within them to make them insert into new target sites of choice. This development gave rise to Targetron technology, a disruption system based on Lactococcus lactis Ll.LtrB group II intron. To apply this technology, an algorithm is used to survey the best target inside a given sequence. The same algorithm designs primers to modify Ll.LtrB intron recognition regions through a PCR step. After cloning this PCR product inside Ll.LtrB, a retargeted intron is generated which is able to insert into the desired locus and make a disruption.

In this work, we have pushed further the applications of group II introns by solving some of their limitations. First, we have migrated the Targetron system to pSEVA plasmids to make it operative in a broad range of bacterial hosts. Second, we have coupled CRISPR-Cas9 system for counterselection and deletion of off-target insertions. Finally, we are refining the system for synthetic barcoding of bacteria i.e. adding short, specific and traceable DNA sequences to label strains with different purposes. This technology could be expanded as a delivery gene system for species with limited availability of genetic tools.

A Vaccine Based on a Modified Vaccinia Virus Ankara Vector Expressing Zika Virus Structural Proteins Controls Zika Virus Replication in Mice

Pérez P (1), Marín MQ (1), Lázaro-Frías A (1), Jiménez de Oya N (2), Blázquez AB (2), Escribano-Romero E (2), Sorzano COS (3), Ortego J (4), Sáiz JC (2), Esteban M (1), Martín-Acebes MA (2), García-Arriaza JF (1)

1. Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología (CNB), Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.
2. Department of Biotechnology, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain.
3. Biocomputing Unit, Centro Nacional de Biotecnología (CNB), Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.
4. Centro de Investigación en Sanidad Animal, INIA-CISA, Valdeolmos, Madrid, Spain.

pperez@cnb.csic.es

Abstract

Zika virus (ZIKV) is a re-emerging mosquito-borne flavivirus that affects humans and can cause severe neurological complications, including Guillain-Barré syndrome and microcephaly. Since 2007 there have been three large outbreaks; the last and larger spread in the Americas in 2015. Actually, ZIKV is circulating in the Americas, Southeast Asia, and the Pacific Islands, and represents a potential pandemic threat. Given the rapid ZIKV dissemination and the severe neurological and teratogenic sequelae associated with ZIKV infection, the development of a safe and efficacious vaccine is critical. In this study, we have developed and characterized the immunogenicity and efficacy of a novel ZIKV vaccine based on the highly attenuated poxvirus vector modified vaccinia virus Ankara (MVA) expressing the ZIKV prM and E structural genes (termed MVA-ZIKV). MVA-ZIKV expressed efficiently the ZIKV structural proteins assembling virus-like particles (VLPs) and was genetically stable upon nine passages in cell culture. Immunization of mice with MVA-ZIKV elicited antibodies that were able to neutralize ZIKV and induced potent and polyfunctional ZIKV-specific CD8+ T cell responses that were mainly of an effector memory phenotype. Interestingly, a single dose of MVA-ZIKV reduced significantly the viremia in susceptible immunocompromised mice challenged with live ZIKV. These findings support the use of MVA-ZIKV as a potential vaccine against ZIKV.

Effects of mitochondrial disease on nervous system. Searching for the causes of neurodegeneration in Harlequin mouse

Fernández de la Torre M (1), Fiuza-Luces C (2, 3), Bermejo-Gómez V (1), Laine-Menéndez S (1), Atencia-Cibreiro G (1), Morán M (1, 2)

1. Mitochondrial and Neuromuscular Diseases Laboratory, Hospital Universitario 12 de Octubre Research Institute (i+12), Madrid, Spain
2. Spanish Network for Biomedical Research in Rare Diseases (CIBERER), U723, Spain.
3. Universidad Europea de Madrid, Madrid, Spain.

miguel.fnandezt@gmail.com

Abstract

Background. Mitochondrial diseases (MD) are a heterogeneous group of genetic disorders caused by dysfunction of the oxidative phosphorylation system, mainly characterized by respiratory chain complexes instability, oxidative stress, bioenergetic dysfunction and multisystemic disturbances. Most sensitive organs to MD are high-energy consuming tissues, such as nervous tissue, being neurons particularly vulnerable to OXPHOS instability and oxidative stress. To date, these diseases have no cure, and one of the most promising therapeutic approaches for them is exercise training.

Objective. Our aim was to go in depth in the main causes of neurodegenerative process in the cerebellum of a mouse model of MD, the Harlequin mouse (Hq), which is due to partial complex I dysfunction, and shows cerebellar ataxia among other symptoms; as well as to assess if a training program could exert any neuroprotective effect in our model resulting in a delay in the ataxia progression.

Methods. Wild-type male mice on a B6CBACa-Aw-J/A (B6CBA) background (WT, n=23) and male mice heterozygous for the X-linked Harlequin mutation (Pdcd8Hq,Hq) (Hq, n=21), at the onset of disease symptoms (2-3 months) were randomly assigned to an exercise-trained group that followed an 8-week program combining resistance and endurance training, or to sedentary groups. After the training period, animals were sacrificed, and CNS was collected for subsequent histological, biochemical and proteomic analysis.

Results. The exercise-training intervention induced improvements in physiological variables in the Hq mice, but did not prevent any of the alterations previously described in the model, that were also present in our trained animals after the intervention: complex I deficiency, disturbances in supercomplexes and respirasome assembly, lower granular and molecular layers´ thickness, granular cells degeneration, loss of dendritic complexity in Purkinje cells, neuroinflammation and oxidative stress. The proteomic analysis revealed in both, sedentary and exercise-trained Hq mice, reduced levels of glutamate transporters involved in glutamate removal, EAAT2 and EAAT4, indicating disturbances in this neurotransmitter metabolism in Hq mouse cerebellum. Additional disturbances in other proteins involved in neuronal plasticity and calcium homeostasis were also found, which suggest altered motor learning as the origin of cerebellar ataxia in our model, a condition that could not be counteracted by the exercise intervention.

Conclusions. In Harlequin mouse model of MD, alterations in neuronal plasticity and calcium homeostasis could be the origin of cerebellar ataxia. Exercise, although induced physiological adaptations, did not produce remarkable neuroprotective effect in our MD model.

Central leptin modulates FGF21 sensibility in white adipose tissue from wistar rats

Mazuecos L (1), Rubio B (1), Pintando C (2), Arribas C (2), Andrés A (1), Gallardo N (1)

1. *Biochemistry Section, Faculty of Science and Chemical Technologies (UCLM) and Regional Centre for Biomedical Research, (CRIB). Ciudad Real, Spain.*
2. *Biochemistry Section, Faculty of Environmental Sciences and Biochemistry (UCLM) and Regional Centre for Biomedical Research (CRIB). Toledo, Spain*

lorena.mazu@hotmail.com

Abstract

The very newly 21st century pandemic has a name: Obesity. Finding targets and methods that could prevent the development of obesity and Diabetes has become in a race against the clock with consequences for heart, liver, adipose tissue; all this organs connected with the metabolic syndrome. The central nervous system (CNS) controls global energy balance. Leptin is a mediator of long-term regulation of energy balance that suppresses food intake and decreases body weight by affecting the expression of neuropeptides in hypothalamic nuclei and in other cell types in the CNS. Fibroblast grown factor 21 (FGF21) is a key mediator that also can increase energy expenditure. It has beneficial effects on glucose and lipid homeostasis and on body weight control, and emerges as a novel therapeutic agent for the treatment of metabolic diseases. The term "FGF21 resistance" has been controversial due to the difficulty in delineating the mechanisms by which FGF21 is effective. However, the notion of "FGF21 resistance" has been mostly reported as a decreasing of FGF21 co-receptor expression (KLB) in white adipose tissue and an increasing plasma levels of FGF21 in obese mice.

In this work, we demonstrated for the first time that chronic central leptin administration (0.2 µg/day, 7 days), in the CNS prevent the starvation-induced increases in FGF21 circulating levels. Furthermore, we studied by real time PCR and Western-Blot the expression of FGF21-R1, β-Klotho and UCP1 in adipose tissue from Wistar rats after a central leptin treatment and co-treated intraperitoneally with PPARβ/δ antagonist GSK0660. Interestingly, leptin treatment specifically increased β-Klotho and UCP1 mRNA levels. In addition, it seems to improve FGF21 sensibility in white adipose tissue. In parallel, GSK0660 seems to play an important role as regulator of proteins connected with FGF21 signaling and lipid metabolism in presence of a central leptin administration.

Taken together these data suggest a critical role for the central leptin signaling in modulation of circulating FGF21 and FGF21 action in white adipose tissue, a primary target for FGF21's effects. In addition, this study shows that PPARβ/δ is involved in food intake and leptin could require this transcriptional factor to execute his anorexigenic effects. A synergy between leptin and FGF21 signaling pathway could be a potential therapeutic target for the treatment of metabolic diseases as obesity or diabetes.

Study of the role of FBXW7 isoforms in the regulation of the expression of their target proteins after FBXW7 inhibition by miRNAs in a T-LBL cell model.

Vázquez-Domínguez I (1,2) , González-Sánchez L (1,2,3), Cobos-Fernández MA (1,2), Sastre I (1) and Fernández-Piqueras J (1,2,3).

1. *Centro de Biología Molecular Severo Ochoa (CBMSO). Consejo Superior de Investigaciones científicas-Universidad Autónoma de Madrid (CSIC-UAM). Madrid, Spain.*
2. *IIS Fundación Jimenez-Díaz. Madrid, Spain.*
3. *Centro de Investigaciones Biomédicas en Red de enfermedades Raras (CIBERER), ISCIII (Spain).*

ivazquez@cbm.csic.es

Abstract

T-cell lymphoblastic neoplasia is a type of haematological cancer prevalent in the childhood, especially in male children. There are different genes with a role in the development of this disease. One of them is the tumour suppressor gene FBXW7, which is a driver gene that plays an important role acting via the proteasomal degradation of key proto-oncogenes such as Cyclin E1 (CCNE1) and C-MYC.

In mammals, the FBXW7 locus encodes three different isoforms (α, β and γ) which share functional domains on the c-terminus but have different N-terminal regions. Thanks to different localization signals presented on their N-terminus, each isoform occupies different subcellular regions, allowing them to interact with different targets proteins. Despite this, very few studies have examined the role of each isoform separately. Most of them analysed the action of the isoforms as a whole and others focussed only on α-isoform (the most stable of the three isoforms).

In this work, we used a strategy that allow us to study the action of each of the isoform separately. We generated SUP-T1 cells stably expressing each isoform lacking the 3´UTR (Lv225-FBXW7), thus preventing the binding by miRNAs or their mimics although could still bind to and silence the endogenous isoforms expressed by the cells. Our studies revealed that downregulation of all isoforms are necessary to induce a proliferative pattern in our cell model. However, each isoform showed a preferential action over a specific targets. In agreement with previous publications, FBXW7α and FBXW7γ mediate the regulation of CCNE1. However, our data also suggested that the oncoprotein c-MYC is mainly regulated by FBXW7β instead of FBXW7α and FBXW7γ, as previously reported. The interaction between FBXW7β and c-MYC had been poorly studied and only few authors indicated that this regulation is possible. Our work revealed that the FBXW7β isoform could have a more important role in c-MYC regulation than was realized, thus reinforcing its role in oncogenesis.

Organizing Committee

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Media Management

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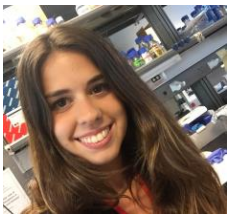
Media Management

Raquel Rouco
Media Management



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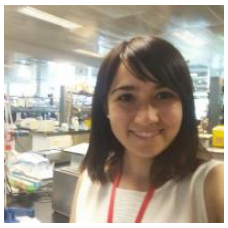

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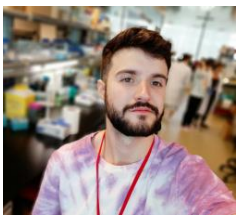

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Amanda Sánchez
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Organization & Logitics

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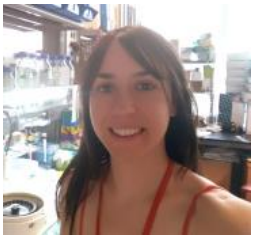
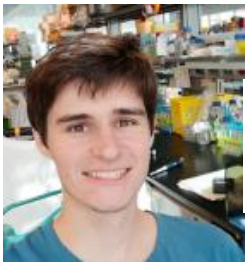
Andrea Cancemi
Organization & Logitics

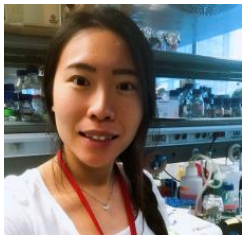


Leticia Herrera
Scientific programme



Ignacio Heras
Scientific programme

Álvaro Sahún
Scientific programme



Xiaotong Hong
Scientific programme

Víctor Fanjul
Advisor

José Manuel Alfonso Almazán
Org-Assistant

Álvaro Macías
Org-Assistant

CNIC PhDay Logo designed by Jesús Sánchez Ruiz, author of the educational comic "A lymphs life" www.alymphslife.com; alymphslife@gmail.com

List of Participants

First name	Last name(s)	Home institution
Pedro	Aguilar Garrido	CNIO
Adrián	Aguirre-Tamaral	CBMSO
Jose Manuel	Alfonso Almazán	CNIC
Dacil	Alonso Gil	CNIO
Alba	Alvarez Franco	CNIC
Javier	Argüello Luengo	ICFO
Alejandra	Aroca Crevillén	CNIC
Pablo	Baladrón de Juan	UAM
Constanza	Ballesteros Martínez	UAM
Cristina	Baquero Mayo	UCM
Antonio	Barral Gil	CNIC
Elísabet	Bello Arroyo	CNIC
Raquel	Benítez Dorta	CNIO
Elena	Blanco	CNIO
Rafael	Blanco Domínguez	CNIC
Rodrigo	Blázquez García	UPM
Patricia	Bodega Villanueva	Fundacion SHE
Adrian	Bouzas	IMDEA Alimentación
Lorena	Bragg Gonzalo	CNB
Miranda	Bueno Arribas	IIB
Andrés Ángel	Calderón García	Universidad de Salamanca
Emilio	Camafeita	CNIC
Silvia	Campanario	CNIC
Andrea	Cancemi	CNIC

Miguel	Cantero Reviejo	UAM
Javier	Cantón	CNB
Raquel Ana	Castillo González	CNIC
Laura	Catalán Prades	Universitat de València
Luis Carlos	Clarembaux Badell	UAM
Marta	Cobo Simón	CNB
Ruth	Conde	CNIC
Gabriela	Condezo	CNB
María	Crespo Ruiz-Cabello	CNIC
Natalia	Cuervo Iturrioz	CIB
Diana	Damián	CNB
Ester	de la Cruz Crespillo	CNIC
Noelia	de León Reyes	CNB
Alberto	Del Monte Monge	CNIC
César	Díaz Toledano	UAM
César	Dominguez Marquez	CNB
Clara	Echevarría Zomeño	CBMSO
Olga	Estévez Vázquez	UCM
Víctor	Fanjul Hevia	CNIC
Miguel	Fernández	Hospital 12 de Octubre
Juan Miguel	Fernández Alvira	CNIC
Macarena	Fernández Chacón	CNIC
Mario	Fernández de Frutos	IMDEA Alimentación
Irene	Fernández Delgado	UAM

Cristina	Fernandez Garcia	Other
Bárbara Victoria	Fernández García	IIB
Ana	Fernández Martín	CIB
Cristina	Fernández Molina	Institut Pasteur Paris
Alberto	Fernandez Oliva	CNB
Nieves	Fernández-Gallego Anaya	CNIC
Alba	Ferrer Pérez	CNIC
Víctor	Fraile Ágreda	UCM
Teresa	Fuertes	CNIC
Elena	Fueyo Marcos	CNIO
José	Gallardo Hernanz	CNB
Almudena	García Carrasco	URJC
Julia	García Fernández	CNB
María	García García	CNIC
Irene	García González	CNIC
Álvaro Fernando	García Jiménez	University of Córdoba
Lourdes	García Ortega	UAM
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Moisés	García Serradilla	CNB
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