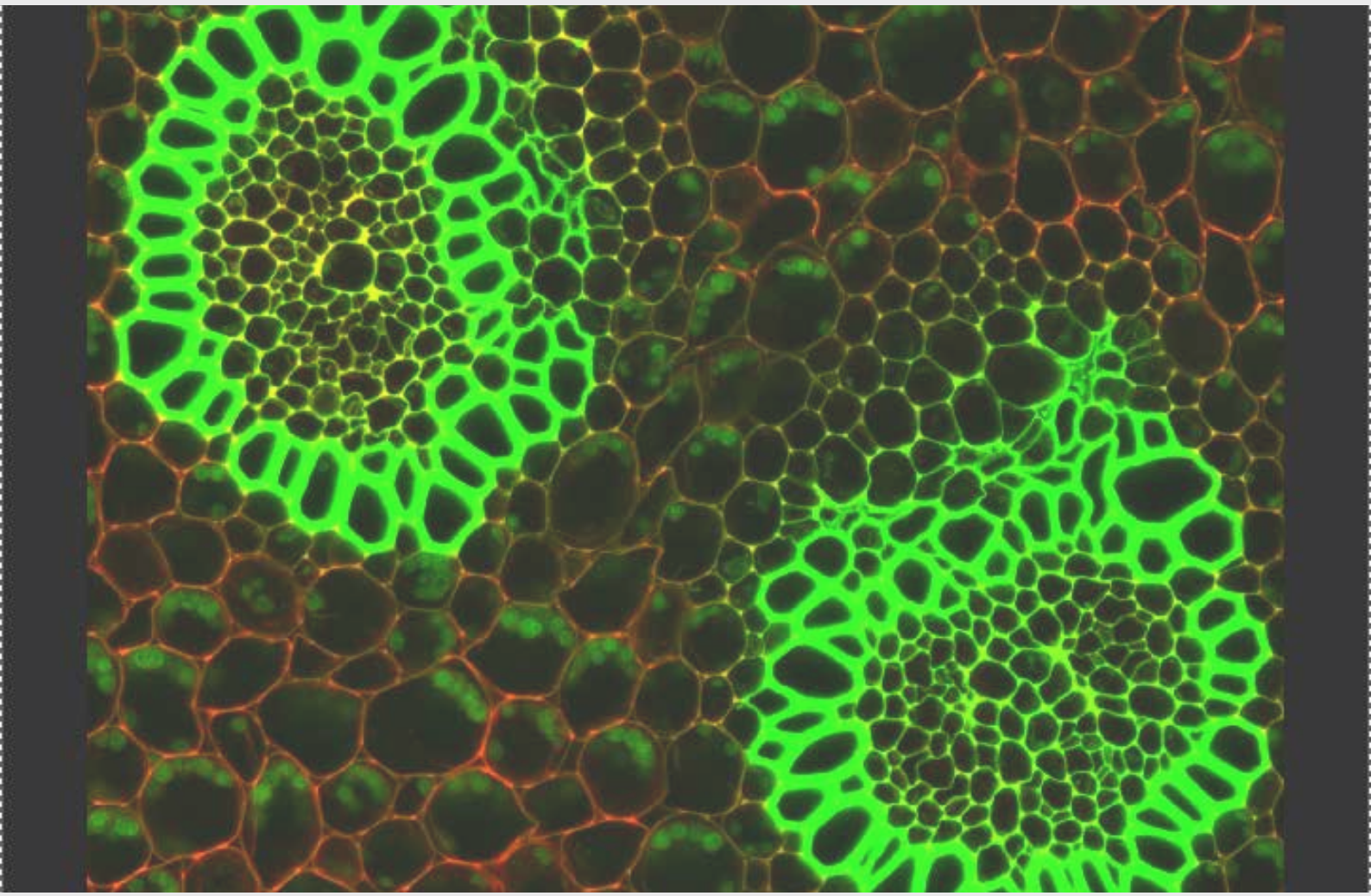
Príncipe Felipe Research Center

Annual Report 2014



PRINCIPE FELIPE
CENTRO DE INVESTIGACION

Cover: Confocal Microscopy, Convalaria snapshot

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Príncipe Felipe Research Center

Annual Report 2014

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INTRODUCTION

In 2015 the Principe Felipe Research Center facilities turn 10 years old and the institution itself turns 25 years as a private research foundation.
During these years, and despite the national and international economic situation, the CIPF has positioned itself as one of the leading biomedical research centers in Spain. Proof of this is evidenced in the research results achieved by the CIPF scientific programs in 2014.

Highlights include the study of the role of the interactome in the maintenance of deleterious variability in human populations, research carried out by the Computational Genomics Program. The Program has also participated in related international initiatives such as the Sequencing Quality Control Consortium.

Research carried out by the Neurological Impairment Program has contributed to positioning the CIPF as a reference center in the study of hepatic encephalopathy. Results from this program include the study on how blocking NMDA receptors delay death in rats with acute liver failure and show that the reduced white matter microstructural integrity correlates with cognitive deficits in minimal hepatic encephalopathy, among many other results that will undoubtedly help to improve the diagnostic and treatment of this disease

The node of the National Stem Cell Bank was re-launched in 2014, with the financial support of the Carlos III Health Institute. Research projects as well as training and dissemination activities towards the scientific and clinical community are foreseen, in close collaboration with the rest of the nodes. In order to strengthen the scientific activity of the node, a new research group on stem cell therapy led by Dr. Slaven Erceg has joined us at the CIPF.

In 2014, the CIPF staff reached 168 persons, showing a steady consolidation and even growth. During 2014 the CIPF has hired 3 disabled persons.
160 undergraduate and graduate students have carried out their internships at the CIPF. There has been a significant increase, 36%, compared to 2013, that shows the attractiveness of the CIPF for the career development of young professionals.
Fourteen PhD theses have been successfully presented and

prove the excellence of future biomedical researchers. In order to support our PhD students, we have set up the CIPF Researcher Development Program, a training program aimed at developing our PhD students' further skills that will assist their current research as well as enhance their further career prospects. Moreover, the CIPF led the organization of the first symposium of biomedical PhD students in Valencia, which was held at the CIPF premises in November 2014 with a successful participation at a remarkably high scientific level.

The CIPF has applied for a patent regarding a treatment for X-adrenoleukodistrophy, a rare genetic disorder. Contacts with pharmaceutical companies are already ongoing for its further development.

A new spin-off company, FactorStem, was founded in 2014. FactorStem aims at developing and commercializing regenerative medicine applications in veterinary medicine, as a first step to translating its know-how into clinical practice. FactorStem is the fourth spin-off company created from the CIPF research groups.

The technological services at the CIPF, which provide support to our own research groups as well as to our partners and customers, have significantly improved their profitability and hence their sustainability.

The CIPF has hosted up to 158 events, including those organized by the center but also others organized by external entities that have chosen the CIPF as the place to hold their meetings, conferences or training courses.

The CIPF Science Park has welcomed 2 new research-intensive companies: Epidisease and Yegane. Epidisease applies epigenetics to diagnostics and characterization of new epigenetic drugs, while Yegane develops new cosmetic ingredients and products by biotechnology means. The CIPF Science Park is a business-oriented environment for the growth of start-ups and established companies in the fields of Healthcare and Biotechnology, which benefit from the interaction with the CIPF's researchers and are able to access our research, business and strategic consultancy services.

Given the center's performance over the last year it is with enthusiasm and unprecedented confidence that we look forward to the year ahead.

Isabel Muñoz Criado
Director

Organization

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Organization

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Neurological Impairment Program
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Molecular Mechanisms of Disease Program
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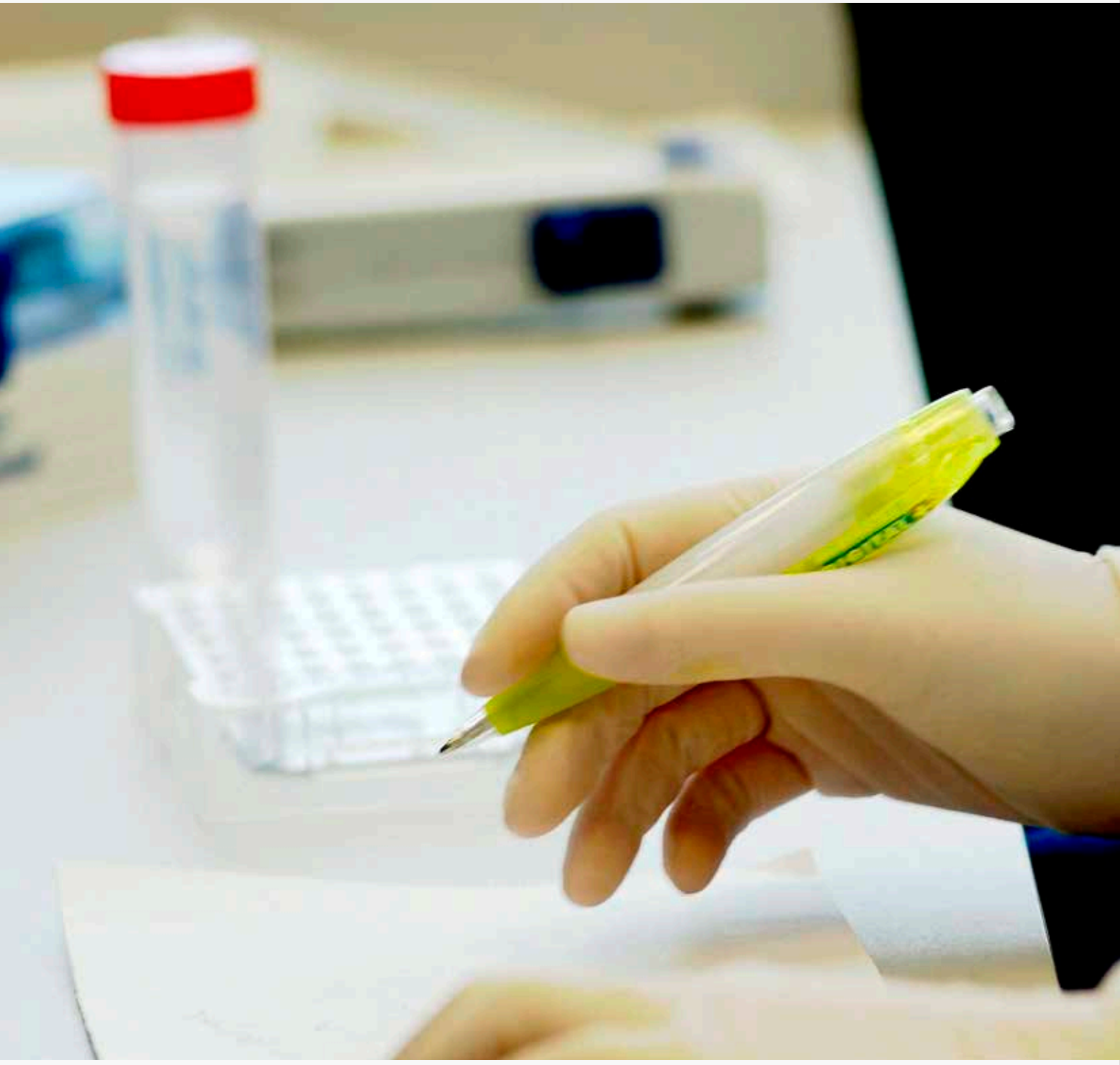

Our Vision
Our Mission

Our vision is to improve people's health generating advances that we constitute true milestones of biomedical knowledge.

Our mission is to enable research and development to scientists working in our center, providing an appropriate environment of laboratories, facilities, services, and a creative, challenging and prone to collaborations and interdisciplinary approaches, in order to develop innovative research projects of scientific and social relevance.

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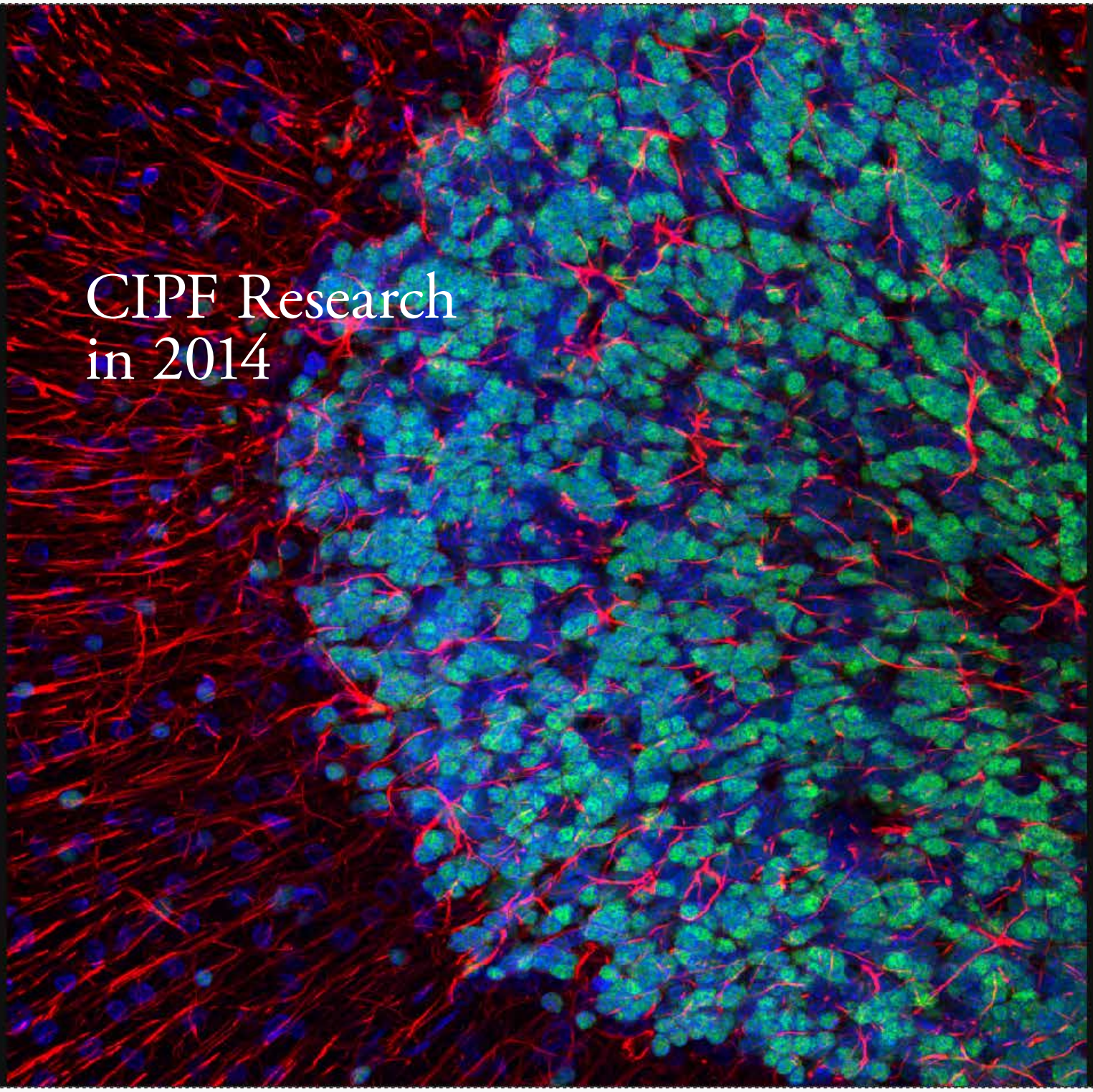
Scientific Programmes

The center has 5 scientific programs with the following challenges:

1. Exploiting our computational and omics tools to help the advent of more personalized medicine.
2. Characterize targets and mechanisms of disease as a basis for developing therapies.
3. Achieve the development of new diagnostic approaches and new therapies.
4. Being key players in the understanding of mechanisms and treatment of neurological deterioration and regeneration.
5. Exploiting genomic knowledge to identify disease genes.
6. Progress in the understanding, diagnosis and treatment of rare diseases.
7. Contribute to the knowledge and even therapy tumor processes.



CIPF Research in 2014

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Advanced Therapies Program

Program Coordinator: Antonio Pineda-Lucena

Joint Research unit for Cardiovascular repair, led by Anastasio Montero

Metabolic growth signals and regenerative medicine, led by Luke Noon

Joint Research unit for Molecular pathology and translational research in oncology, led by Jerónimo Forteza

Neuronal and tissue regeneration, led by Victoria Moreno-Manzano

Organic molecules, led by Santos Fustero

Peptide and protein chemistry, led by Mar Orzáez

Polymer therapeutics, led by Mª Jesús Vicent

Structural biochemistry, led by Antonio Pineda-Lucena

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Joint Research Unit for Cardiovascular Repair CIPF-IIS LaFe

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Marina Piquer Gil (IIS La Fe)

www.cipf.es/web/portada/reparacion-cardiovascular

Overview

Cardiovascular diseases are a major health problem in developed countries. Among them, heart failure has limited treatments and cell therapy has emerged as an alternative tool to conventional treatments. The Joint Research Unit for Cardiovascular Repair is a collaborative program between Health Research Institute La Fe (IISLAFE) and Principe Felipe Research Center. The group aims to develop a basic research with a significant translational component, that will contribute to the development of new treatments for ischemic diseases. The study of cellular mechanisms triggered by adult stem cells and factors that influence cardiomyocyte cell death in apoptotic and inflammatory environments will allow the identification of molecular targets for the development of new drugs. The scientific activity of the group focuses on the use of adult stem cells in preclinical models of cardiac repair, the study of exosomes as mediators of cellular communication, the use of iPSC as tools for cardiotoxicity testing as well as the evaluation of new materials for cardiac repair.

Research results

1.Preclinical studies with adult stem cells for the treatment of myocardial infarction: the group works on the analysis of the ability of mesenchymal cells and resident cardiac stem cells to repair infarcted myocardium. Having a rat infarct model allows integration test the ability of human cells transplanted and any in vivo monitoring of functional recovery. With this, the group is involved in studies of isolation, expansion and marking of adult stem cells, dose optimization, combination of factors and cell transplantation protocols and evaluation of cardiac function in infarcted rats treated under different conditions .

2.Study of the metabolic alterations associated with hypoxic situations. This line of research focuses on the study of certain Krebs cycle metabolites such as succinate, whose concentrations increase during hypoxia. Moreover, the lack of oxygen and nutrients into the cell causes changes in the number and composition of exosomes secreted by vesicles and cells that play a role in intercellular communication. The group is studying these processes using genetically modified animals, models of infarction and cell lines.

3.iPSC generation from patients with congenital heart disease: this line focuses on the study of structural heart disease and channelopathies. The group aims at the reprogramming of fibroblast or keratinocytes by different methods for the design of cardiovascular disease cellular models. This research has secured funding through a project entitled "Modeling With cardiac disease cardiac resident stem cells and induced pluripotential stem (iPS) cell-derived cardiomyocytes" funded by FISABIO Foundation.

4.Evaluation and prevention of reperfusion-induced damage: this is a line that brings together basic translational research, clinical and imaging researchers with the aim of analyzing the causes of microvascular obstruction and intra-infarction in patients bleeding and generates an animal model that allows to experiment with different drugs and strategies to reduce as far as possible this lesion´s great impact on the prognosis of patients. This research has achieved new funding in 2014 through the project from FISABIO entitled "Study of myocardial damage induced by reperfusion: new strategies based on the use of microcatheters on a porcine model" (UGP-14-173), awarded to Dr. JL Diez.

Publications

1. I. Moscoso, N. Tejados, O. Barreiro, P. Sepúlveda, A. Izarra, E. Calvo, A. Dorronsoro, J.M. Salcedo, R. Sádaba, A. Díez-Juan, C. Trigueros y A. Bernad.
Podocalyxin-like protein 1 is a relevant marker for human c-kit pos cardiac stem cells
J. Tissue Eng. and Reg. Medicine (In press)

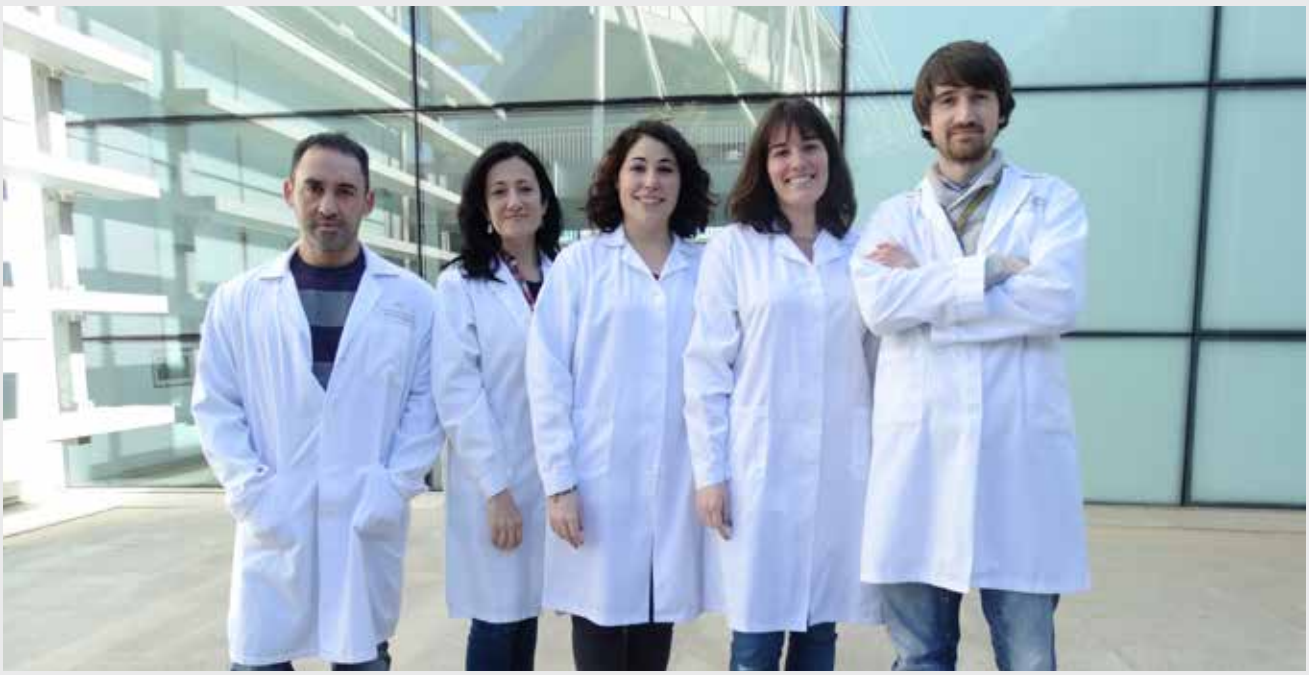
2. Armiñan, A. Sepulveda, P. Vicent, Mj
Polymer Therapeutics as nano-sized medicines for Tissue Regeneration and Repair.
Book: Polymers in Regenerative Medicine: Biomedical Applications from Nano- to Macro-Structures. Chapter 8, Wiley-Blackwell. John Wiley & Sons, 2014. ISBN 978-0-470-59638-8

Doctoral Thesis presented

1. Doctoral Candidate: Imelda Ontoria Oviedo. Interacción de TRPV1 y GABARAP y sus efectos en la dinámica del receptor . Director: Anastasio Montero . Universidad de Valencia, UV

2. Doctoral Candidate: Nahuel Aquiles García. Comunicación celular mediada por exosomas . Directors: Pilar Sepulveda & Antonio Diez . Universidad Politecnica de Valencia, UPV

Metabolic growth signals and regenerative medicine CIPF-CIBER



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→Collaborators
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www.cipf.es/metabolic-growth

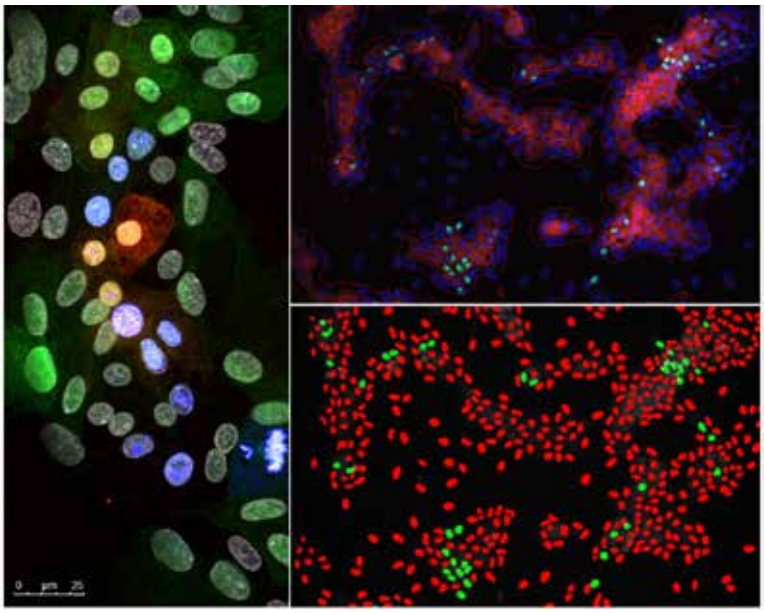
Overview

We are investigating how stem cells integrate signals from metabolic growth factors such as insulin. Changes in the sensitivity of our tissues to insulin and insulin-like growth factor 1 (IGF1) are associated with ageing, metabolic disease, and the initiation of cancer. However, very little is known about how adult stem cells respond to, or are affected by, systemic alterations in insulin signalling that occur in obese patients and in type II diabetes. In order to explore how stem cells respond to metabolic growth factors, we have developed a range of novel molecular tools that enable us to visualize and study key aspects of insulin/IGF1 signalling at a cellular level. We are using these tools, in combination with the CIPF's world-class cytometry services and high-content image analysis platforms to discover new aspects of how insulin signalling and insulin resistance can affect stem cells ability to survive, proliferate and differentiate. Our research explores the role of insulin/IGF1 in the de novo differentiation of human stem cells to hepatocytes, the functional cells of the liver. Hepatocyte transplantation shows promise as a future therapeutic alternative to liver transplantation. We are working to optimize current strategies to generate these cells for therapeutic applications.

Research results

Our research explores the role of insulin/IGF1 in the de novo differentiation of human stem cells to hepatocytes, the functional cells of the liver. Despite the remarkable capacity of the liver to regenerate in response to injury, primary human hepatocytes are difficult to culture and maintain in vitro. Hepatocyte transplantation shows promise as a future therapeutic alternative to orthotopic liver transplantation, the only current treatment option for end-stage liver disease, and we are working to optimize current strategies to generate these cells for therapeutic applications.

Obesity and type 2 diabetes are significant risk-factors for the development of Non-Alcoholic Fatty Liver Disease (NAFLD) and chronic liver injury, which can, in turn lead to cirrhosis, end-stage liver disease and incurable hepatocellular carcinoma (HCC). In Spain, between 1987 and 2012, the incidence of obesity more than doubled in the general population, whilst cases of diabetes have increased by 33% since 2011 (Encuesta Nacional de Salud 2011-2012). These alarming statistics illustrate the dramatic and recent increase in metabolic disease in the Spanish population, which will have profound im-



Human cancer stem cells differentiating to hepatocytes in vitro. Three images show stages of quantification using InCell Analyzer

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plications for the incidence of NAFLD in this country and the future socioeconomic burden faced by Spain's public health system.

One of the major pathological mechanisms underlying NAFLD progression is insulin resistance. However, the links between impaired insulin signalling and deregulation of the injury repair process are still unclear. Our overriding goal is to develop a better understanding of how aberrant insulin signalling contributes towards the aetiology of NAFLD and its associated morbidities by modifying liver progenitor cell behaviour.

Publications

1 .Lade, A; Noon, LA; Friedman, SL.
Contributions of metabolic dysregulation and inflammation to nonalcoholic steatohepatitis, hepatic fibrosis, and cancer.
Current opinion in oncology, Vol: 26, 100-107. 2014 Quartile: Q2

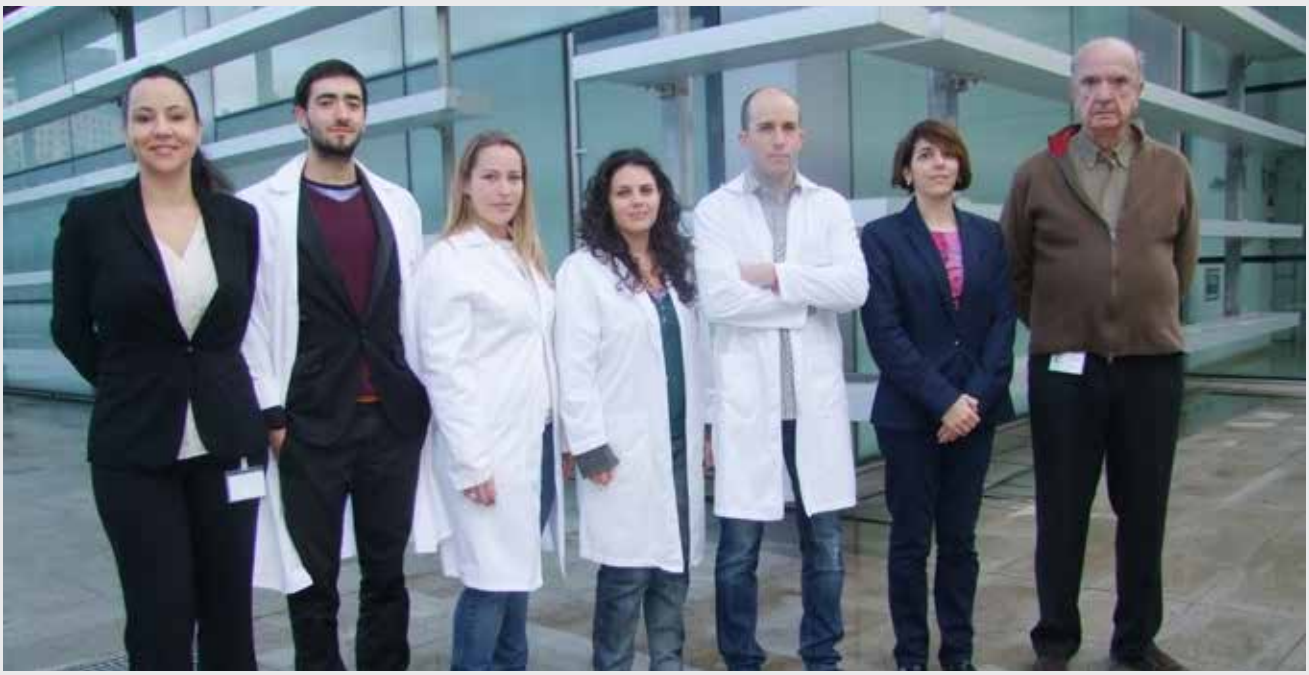
2 .Lou, YR ; Kanninen, L ; Kuisma, T ; Niklander, J ; Noon, LA; Burks, D ; Urtti, A ; Yliperttula, M .
The Use of Nanofibrillar Cellulose Hydrogel As a Flexible Three-Dimensional Model to Culture Human Pluripotent Stem Cells.
Sstem cells and development, Vol: 23(4), 380-392. 2014 Quartile: Q1

Conferences and meetings

1. Title: Hepatic Growth Regulation and Tumorigenesis is linked to Autophagy via the Hippo Tumor Suppressor Pathway
By: Lee, Youngmin A.; Noon, Luke A.; Lee, Tingfang; et al.
Conference: 65th Annual Meeting of the American-Association-for-the-Study-of-Liver-Diseases Location: Boston, MA Date: NOV 07-11, 2014 Sponsor(s): Amer Assoc Study Liver Dis
Source: Hepatology, Vol: 60 Special Issue: SI Supplement: 1 Pages:273A-273A Meeting Abstract: 148

2. Title: Spink3 and Tff3 are overexpressed in autophagy-deficient mice and may contribute to hepatocellular carcinoma development
By: Wallace, Michael C.; Lee, Youngmin A.; Noon, Luke A.; et al.
Conference: 65th Annual Meeting of the American-Association-for-the-Study-of-Liver-Diseases Location: Boston, MA Date: NOV 07-11, 2014 Sponsor(s): Amer Assoc Study Liver Dis
Source: HEPATOLOGY Volume: 60 Special Issue: SI Supplement: 1 Pages:646A-646A Meeting Abstract: 924

Joint Research Unit for Molecular Pathology and Translational Research in Oncology CIPF-UCV



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Mario Soriano Navarro (CIPF-UCV)

www.cipf.es/patologia-molecular

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Overview

Pathology is the basis for the comprehension of chronic diseases and their molecular mechanisms, including degenerative diseases, autoimmune diseases and cancer. The histopathologic study is required not only for the diagnosis but also for providing the most adequate treatment available in all types of cancers. Diagnosis as a science to provide support for clinicians has evolved by integrating morphology with molecular studies such as immunohistochemistry, cytogenetics and genomics. In addition, in the last years a new opportunity for cancer treatment has arisen from novel therapeutic targets and the application of state-of-the-art technologies in personalized medicine.

Pathology is not only a diagnosis tool in medicine but also a field of science itself necessary for the development of translational research. Clinical parameters are no longer the only and relevant parameters in the prognosis of cancer. Pathology together with genomics and molecular data have moved to the frontline of innovative research as they have special influence in the future steps against cancer. This new information provided by the integration of pathology with molecular data has to be interpreted inside of pathology systems and in connection with cellular biology.

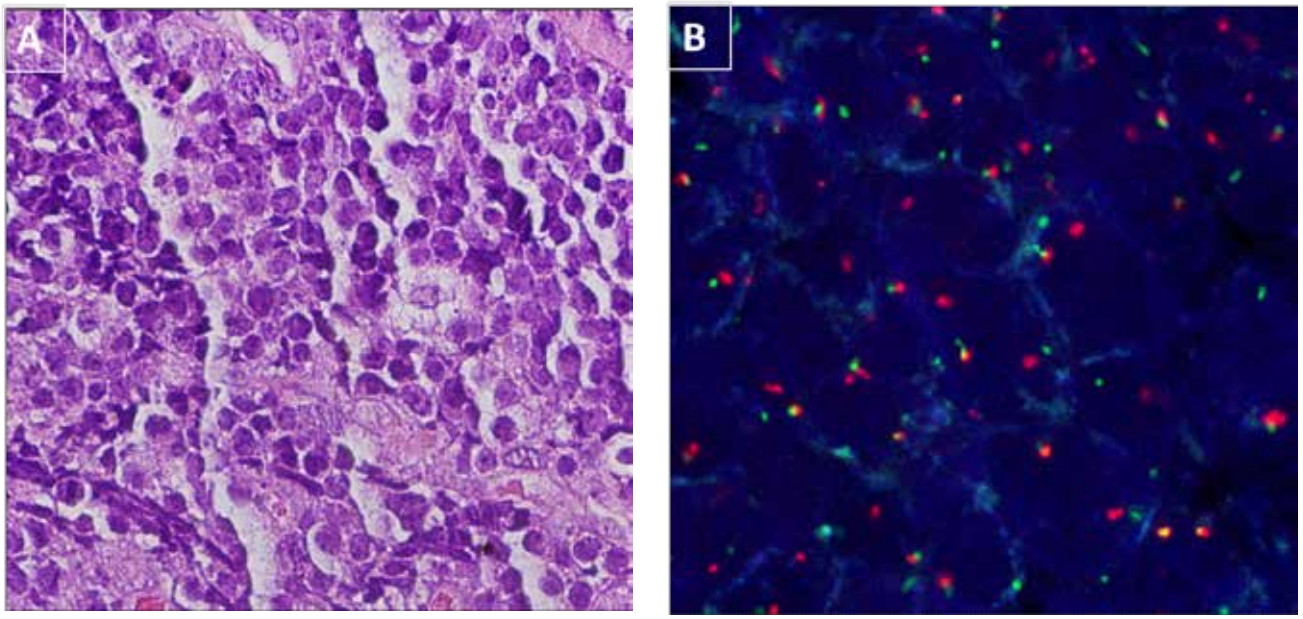
Exosomes are a novel intra-cellular model that has recently provided a new perception of the mechanisms involved in cellular communications. Exosomes constitute a novel mechanism of signaling in the proliferation and progression of the tumor, connecting cancer cells with mechanisms of immunity as well as resistance to therapies. In addition, the study of circulating exosomes may reveal prognostic biomarkers in cancer.

Research results

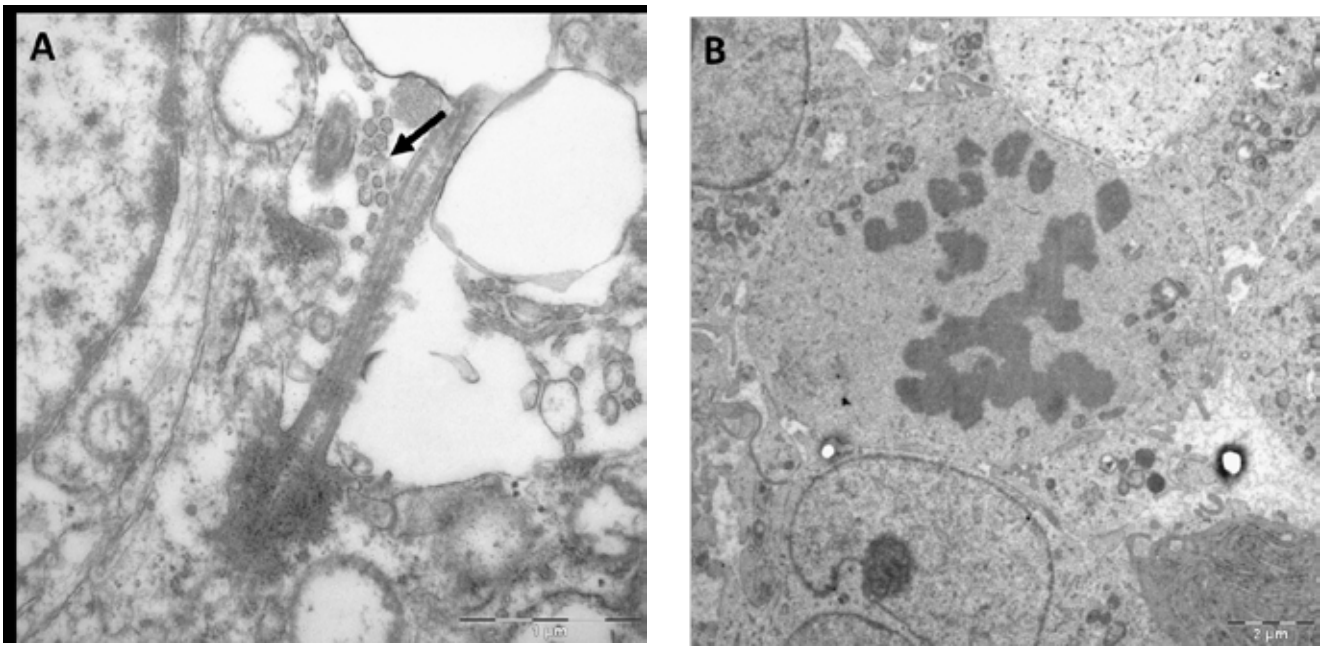
One of the most important goals has been the identification of molecular biomarkers that predict breast cancer prognosis and clinical response. Within this line of research, in collaboration with Oncology Platform at San Jaime Hospital in Torrevieja and the Clinical University Hospital in Santiago, we have used microarray phosphatome profiling technology in the study of estrogen receptor negative breast cancers. We combined genomic analysis with microarrays to show the relevant role of MAPK and PI3K in the regulation of phosphatases that may be relevant for this type of tumors. In addition, our study points out the relevance of combining genomic studies and expression studies that can indicate not only the comprehension of the neoplastic mechanisms but the therapeutic strategies as well. We think that this approach can also be applied to other neoplasic diseases such as lymphomas and sarcomas.

The ultrastructure study of tumors, including brain tumors, has been pushed to the background by immunohitochemistry, cytogenetics and molecular biology techniques. However, biology studies have strengthened morphology by revealing cilia structures as important for the comprehension and significance of certain type of tumors. One type of brain tumors are ependimomas, characterized by their specific cilia structure. This has allowed us to differentiate low and high grade ependimomas at the ultraestructure level. This work has been done in collaboration with the Clinical University Hospital of Santiago and Cavanilles Institute at the University of Valencia. Cilia structure characterization with immunohistochemistry and molecular techniques could also be applied to a broader group of solid tumors.

The interdisciplinary combination of different techniques provided by pathology studies constitutes a powerful tool for the diagnosis. For instance, FISH coimbined with confocal microscopy provide information for the evaluation of neoplasic cells. It is also possible the evaluation of different fluorescent markers by confocal microscopy to define the different manifestations of the tumor heterogeneity.



Burkitt's Lymphoma.
A) H&E. B) Disregulation of the c-myc gene by 8q24 gene translocation.



Ultrastructural features of the grade II (A) and anaplastic (grade III) ependymoma (B).
In figure A, electron microscopy image showing differentiated ependymoma cells containing microvilli (arrows) and cilia (arrowhead). In figure B, electron microscopy image showing high cellular density of anaplastic ependymoma cells with a mitotic cell

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Publications

1. Campos M., Soriano M., Borredá I., Forteza J.
Confocal technology in FISH evaluation for cáncer: Diagnostic's Improvement.
International Journal of Surgical Pathology.
2014 Feb; 22 (1): 37-8.

2. Alfaro-Cervelló C., Soriano-Navarro, M., Ramírez M., Bernet, L., Martínez Banaclocha, M., Cano, R., Reyes Santías, R.M., Forteza Vila, J., García-Verdugo, J.M.
Ultrastructural pathology of anaplastic and grade II ependymomas reveals distinctive ciliary structures. Ultrastructural pathology.
2014. May 15: 1-7.

Conferences and meetings

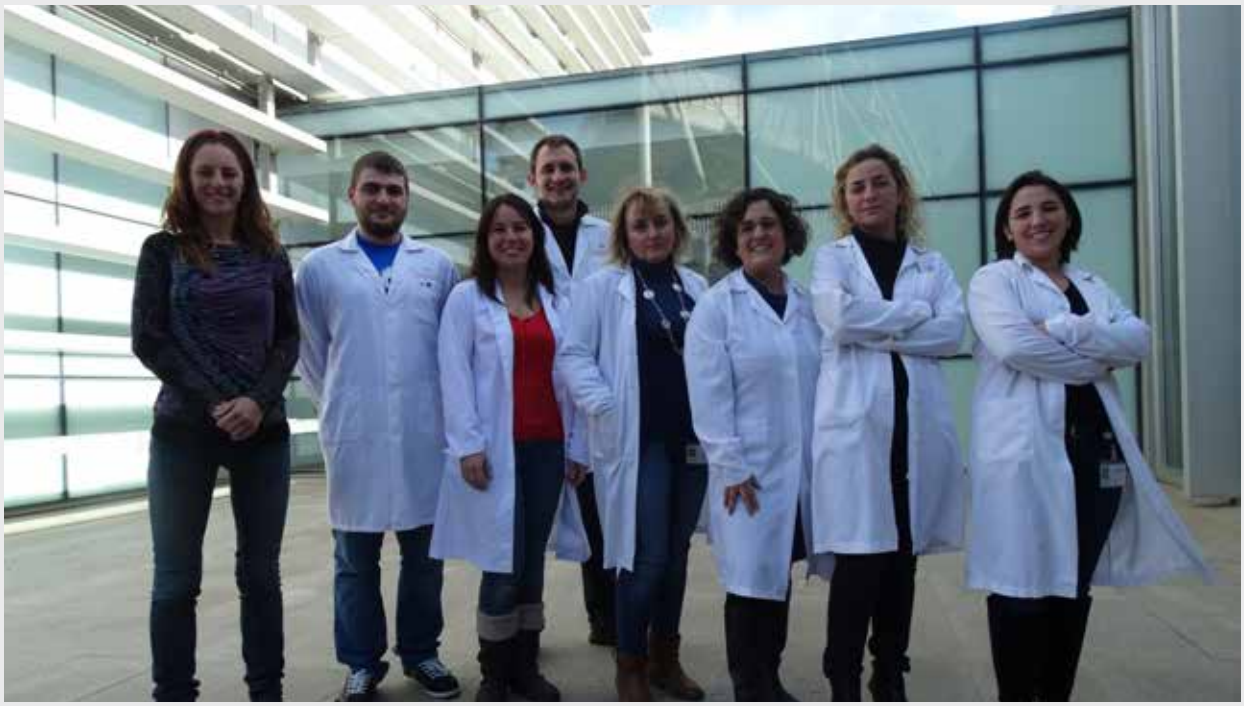
1. The 7th Arkadi M. Rywlin International Pathology Slide Seminar Symposium in Anatomic Pathology.
Tokyo, Japan.

2. VII German-Spanish meeting of Anatomical Pathology.
Valencia, Spain.

3. Innovation in Oncology, the challenge of Functional Units. V Summer Courses UCV.
Santander, Spain.

4. Renal Biopsy Ultrastructural Course
Hospital of Castellon
Castellón, Spain.

Neuronal & Tissue Regeneration



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Marcos Izquierdo (SUMA)

www.cipf.es/regeneracion-neuronal

Overview

The Neuronal and Tissue Regeneration Lab is working in the Regenerative Medicine field. We work to improve the success of the therapeutic applications of stem cell-based approaches on the clinical practice. The lab is focused on cell based therapy of neural precursor cells for spinal cord injury treatment and mesenchymal stem cells for articular diseases. Spinal cord injury (SCI) results in an irreversible paralysis directly below the affected medullar segment with no currently curable treatment. We previously showed that acute transplantation of activated ependymal stem/progenitor cell (epSPC) derived from adult spinal cords can rescue lost neurological function after SCI. We aim to improve knowledge about the molecular and cellular processes developed along the central nervous system injuries for a better understanding. We search for pharmacological tools favoring the applied cell-based therapeutic strategies and we look for synergistic effects by using new pharmacological designs getting advantages on the of new designed nanomedicine and biomaterials-based therapies.

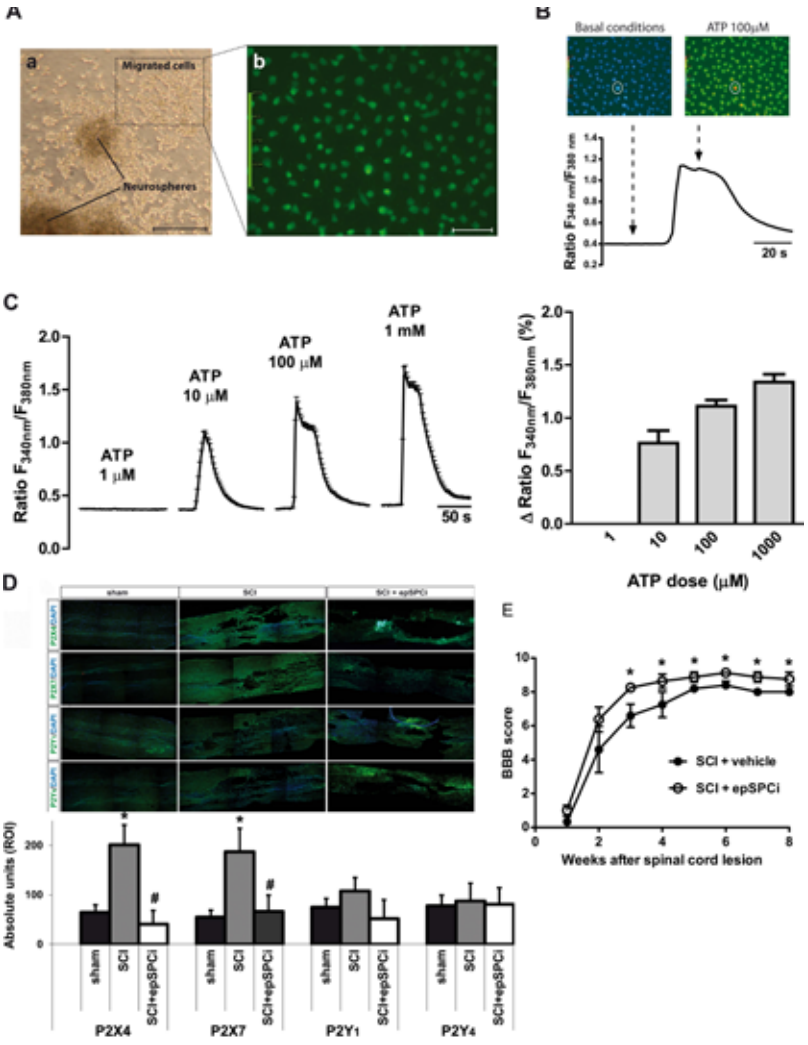
Osteoarticular pathologies very often require a regeneration process for bone, cartilage and/or tendon with de novo vascularization. During the last couple of decades the mesenchymal stem cell population has been shown to be a more challenging option as a cell-based therapeutic approach. Because osteoarticular complications are very usual in dogs, it results as an ideal model for our studies with direct translational perspectives for human applications. In our lab we are involved in the generation and characterization of the adult adipose-derived mesenchymal cell population and its application on osteoarthritic associated pathology and recently also in wound healing.

Research results

Purinergic receptors in spinal cord-derived ependymal stem/progenitor cells and its potential role in cell-based therapy for spinal cord injury

Spinal cord injury is a major cause of paralysis with no current therapies. Following SCI, large amounts of ATP and other nucleotides are released by the traumatized tissue leading to the activation of purinergic receptors that, in coordination with growth factors, induce lesion remodelling and repair. We found, that adult mammalian spinal cord derived precursor cells (epSPCs), are capable of responding to ATP and other nucleotidic compounds, mainly through the activation of the ionotropic P2X4, P2X7, and the metabotropic P2Y1 and P2Y4 purinergic receptors. A comparative study between epSPCs from healthy rats versus epSPCis, obtained after SCI, shows a downregulation of P2Y1 receptor together with an upregulation of P2Y4 receptor in epSPCis. Moreover, spinal cord after severe traumatic contusion shows early and persistent increase in the expression of P2X4 and P2X7 receptors around the injury, which can be completely reversed when epSPCis were ectopically transplantated. Since epSPCis transplantation significantly rescues neurological function after SCI in parallel to inhibition of the induced P2 ionotropic receptors, a potential avenue is open for therapeutic alternatives in SCI treatments based on purinergic receptors and the endogenous reparative modulation. (Figure 1)

Figure 1: ATP induces intracellular calcium transients in epSPCs that are mediated by both ionotropic P2X and metabotropic P2Y receptors (A-C); Transplantation of epSPCis after SCI prevented injured-depending induction of P2X4 and P2X7 proteins in the spinal cord (D-E)



*(A) Phase-contrast image of neurospheres cultured onto coverslips for 24 h and a detail of the same field viewed with fluorescein optics that shows isolated epSPCs loaded with the calcium dye Fura-2 (image b). (B) Fluorescence recorded from a single cell (white circle at the images) was used to calculate changes in the F340 nm/F380 nm ratio before and after stimulation with 100 μM ATP. (C) Cultures of epSPCs were superfused for 30 sec with graded doses of ATP ranging from 1 μM to 1 mM. Representative traces (left panel) and mean ± SEM of the increment at the fluorescence ratios observed in ATP responding cells (right panel) are shown (n = 166 cells from 4 independent experiments). (D) Immunohistochemical analysis was performed in spinal cord criopreserved tissue of control animals (sham group), SCI (subjected to severe SCI by traumatic contusion and sacrificed 2 months later) and SCI+epSPCi (subjected to severe SCI by traumatic contusion and intramedullar transplanted with epSPCis, and sacrificed 2 months later) for P2X4, P2X7, P2Y1 and P2Y4 receptors using specific antibodies;*P<0.05; or Sidak's post hoc test for SCI versus SCI+epSPCi comparion, #P<0.05. GFP-positive fluorescence form transplanted cells was subtracted. (E) Locomotor performance was evaluated weekly by BBB scoring and a significant improvement was observed in rats transplanted epSPCis when compared to nontransplanted ones (vehicle), *P<0.05. Cell Transplant. 2014 Jul 15*

Platelet-Rich plasma and adipose-derived mesenchymal stem cells for regenerative medicine-associated treatments in bottlenose dolphins (Tursiops truncatus)

Dolphins exhibit an extraordinary capacity to heal deep soft tissue injuries. Nevertheless, accelerated wound healing in wild or captive dolphins would minimize infection and other side effects associated with open wounds in marine animals. Here, we propose the use of a biological-based therapy for wound healing in dolphins by the application of platelet-rich plasma (PRP). Blood samples were collected from 9 different dolphins and a specific and simple protocol which concentrates platelets greater than two times that of whole blood was developed. As opposed to a commonly employed human protocol for PRP preparation, a single centrifugation for 3 minutes at 900 rpm resulted in the best condition for the concentration of dolphin platelets. By FACS analysis, dolphin platelets showed reactivity to platelet cell-surface marker

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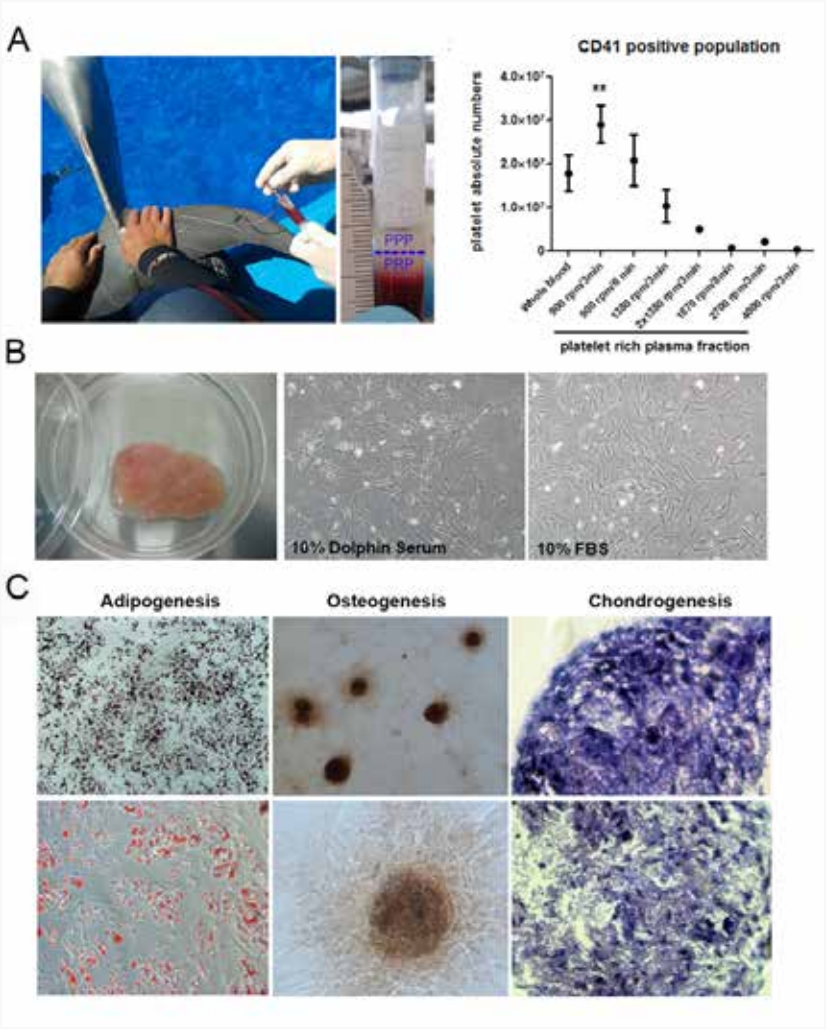
Figure 2. (A) Efficient dolphin platelet-rich plasma concentration and ASC isolation protocol.



*(A) Blood samples were collected from the tail vein plexus from 9 different dolphins at a local aquarium and placed into tubes containing sodium citrate. After centrifugation the upper half of the plasma was considered platelet-poor plasma (PPP) and discarded while the lower half was considered platelet-rich plasma (PRP) and used for subsequent experiments. Whole blood samples were subjected to multiple centrifugation protocols to determine which was the most efficient in concentrating platelets in a small volume of plasma. Significant increases in absolute number of platelets and platelet concentration as determined by fold change compared to whole blood were observed when whole blood samples were centrifuged at 900rpm for 3 min, ** P ≤ 0.01; (B) Adipose tissue was collected from the postnuchal fat pad from recent postmortem wild striped dolphins (Stenella coeruleoalba) and characterized. Dolphin ASCs are plastic adherent and are able to be cultured in the presence of both 10% FBS and 10% dolphin serum. The morphology of ASCs treated with 10% dolphin serum appeared less elongated and senescent compared to those cultured with 10% FBS. (C) Dolphin ASCs were capable of tri-lineage mesenchymal differentiation. ASCs were differentiated under standard in vitro conditions to adipocytes (Oil Red O staining), osteocytes (Alizarin Red staining) and chondrocytes (Alcian blue staining)*

CD41. Analysis by electron microscopy revealed that dolphin platelets were larger than human platelets. These findings may explain the need to reduce the duration and speed of centrifugation of whole blood from dolphins to obtain a 2-fold increase and maintain proper morphology of the platelets. For the first time, levels of several growth factors from activated dolphin platelets were quantified. Compared to humans, concentrations of PDGF-BB were not different, while TGFβ and VEGF-A were significantly lower in dolphins. Additionally, adipose tissue was obtained from dolphins corpses found along on the Spanish Mediterranean coast, and adipose-derived mesenchymal stem cells (ASCs) were successfully isolated, amplified, and characterized. When dolphin ASCs were treated with 2.5 or 5% dolphin PRP they exhibited significantly increased proliferation and improved phagocytotic activity, suggesting that in culture, PRP may improve the regenerative capacity of ASCs. Taken together, we show an effective and well-defined protocol for efficient PRP isolation. This protocol alone or in combination with ASCs, may constitute the basis of a biological treatment for wound-healing and tissue regeneration in dolphins. PLoS One. 2014 Sep 24;9(9):e108439
(Figure 2)

Publications

1 .Lukovic D, Valdés-Sanchez L, Sanchez-Vera I, Moreno-Manzano V, Stojkovic M, Bhattacharya SS, Erceg S.
Brief report: Astrogliosis promotes functional recovery of completely transected spinal cord following transplantation of hESC-derived oligodendrocyte and motoneuron progenitors.
STEM CELLS Vol: 32 (2), 594-599 . 2014 Quartile: Q1

2 .Lukovic D, Moreno-Manzano V, Alastrue-Agudo A , Rodriguez-Jiménez FJ, Oria M, Stojkovic M, Bhattacharya SS, Erceg S* .
Complete rat spinal cord transection as a faithful model of spinal cord injury for human cell transplantation. Cytotherapy Vol: 16, S88-S88 . 2014 Quartile: Q2

3 .Alastrue-Agudo A, Erceg S, Cases-Villar M, Bisbal-Velasco V, Griffeth R, Rodriguez-Jiménez FJ, Moreno-Manzano V*.
Experimental Cell Transplantation for Traumatic Spinal Cord Injury Regeneration: Intramedullar or Intrathecal Administration. Stem CellS and Tissue Repair: Methods and protocols Vol: LIBRO (VOL 1210), 23-35 . 2014 Quartile: NO

4 .Lukovic D, Stojkovic M, Moreno-Manzano V, Bhattacharya SS, Erceg S.
Perspectives and Future Directions of Human Pluripotent Stem Cell-Based Therapies: Lessons from Geron's Clinical Trial for Spinal Cord Injury. S
tem cells and development Vol: 23 (1), 42095 . 2014 Quartile: Q1

5 .Griffeth RJ, Garcia-Parraga D, Mellado-Lopez M, Crespo-Picazo JL, Soriano-Navarro M, Martinez-Romero A, Moreno-Manzano V. Platelet-Rich Plasma and Adipose-Derived Mesenchymal Stem Cells for Regenerative Medicine-Associated Treatments in Bottlenose Dolphins (Tursiops truncatus). PLOS ONE Vol: 9 (9), . 2014 Quartile: Q1

Conferences and Meetings

1. 10th International Symposium onPolymer Therapeutics: From laboratory to clinical practice .
Oral Communication
Victoria Moreno Manzano
(Valencia , Spain)

2. HitSeq 2014
Oral Communication
Lorena de la Fuente, Cristina Martí, Susana Rodríguez-Navarro, Victoria Moreno, Ana Conesa
(Boston , USA)

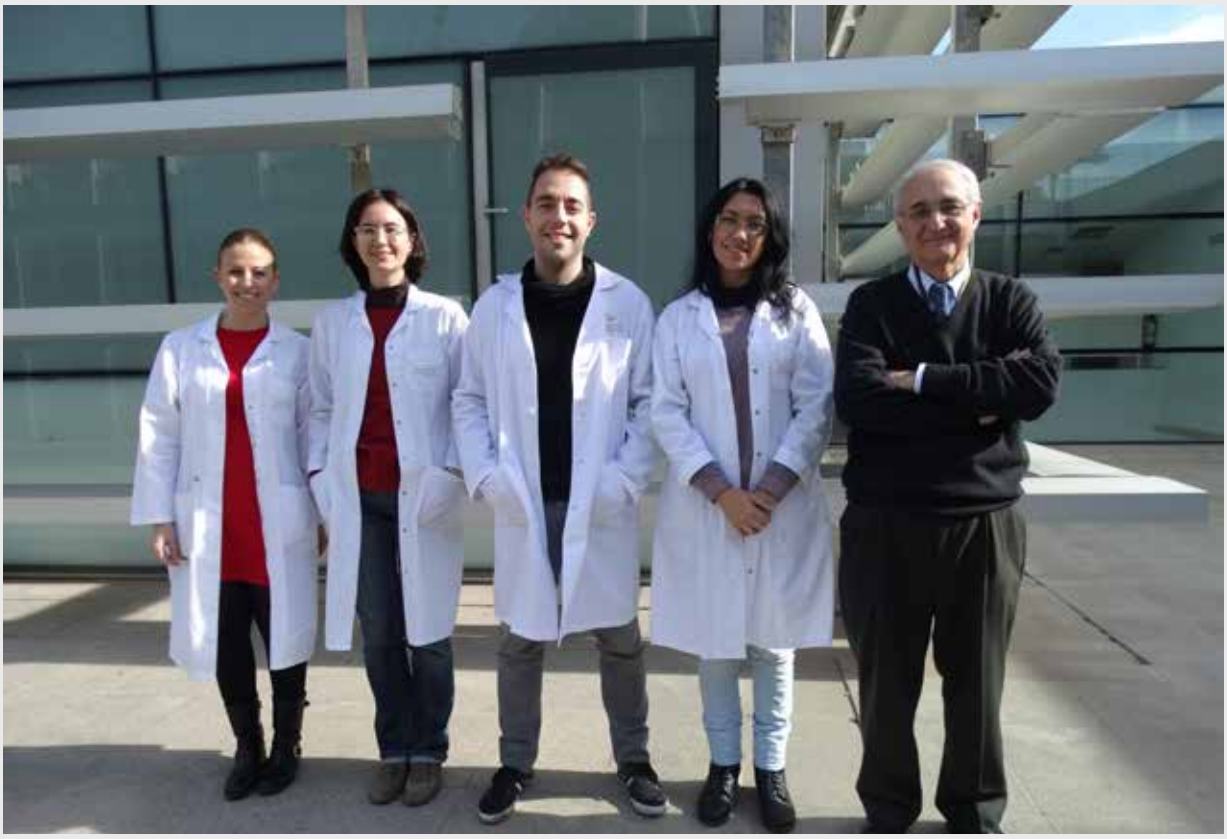
3.TRANSBIO Emergence Forum
Lecture
Viviana Bisbal Velasco
(Barcelona , Spain)

4. Last advances in spinal injury cure. Step by Step
Organizing commitee
Victoria Moreno-Manzano
(Barcelona , Spain)

5. I Biomedicine Predoc Congress in CIPF
Poster
Ana Alastrue
(Valencia , Spain)

6. I Biomedicine Predoc Congress in CIPF
Poster
Viviana Bisbal
(Valencia , Spain)

Organic Molecules Joint Research Unit CIPF-UV



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www.cipf.es/moleculas-organicas

Overview

Medicinal Chemistry is the subject that refers to the discovery, identification and preparation of new chemical entities biologically active at the molecular level. Its ultimate goal is to achieve safer and more efficient drugs for the treatment of diverse pathologies.
The main aim of the research that we develop in the Organic Molecules laboratory is the synthesis of new compounds with potential biological activity. Therefore, the fundamental research level is made up of the development of new synthetic methodologies leading to those molecules in a simple and selective manner. In this sense, our research group is interested in the synthesis of organofluorine compounds, since it is well known that the introduction of fluorine atoms into organic molecules often improves their chemical and pharmacological properties. Additionally, we are also interested in the design and synthesis of new peptidomimetics and other small molecules capable of activating or inhibiting specific therapeutic targets. In this context, the collaboration with different research groups is essential in order to identify the aforementioned targets as well as to carry out the corresponding biological assays.

Research results

"Fluorine in Pharmaceutical Industry: Fluorine-Containing Drugs Introduced to the Market in the Last Decade (2001-2011)"
It is well known that, in many cases, the introduction of fluorine atoms into potentially bioactive compounds can modify their physical properties and chemical reactivity, thus affecting important factors such as the bioavailability or the molecular recognition. In fact, nowadays fluorinated organic compounds are common in the medicinal chemistry field since many of them became highly effective drugs for the treatment of a great variety of pathologies (fluorinated organic compounds represent approximately 25% of marketed drugs). Despite the importance of those compounds, the presence of fluorine atoms in natural products is very rare and most of them have to be synthesized. In this context, our research group has always been involved in the development of new methodologies for the synthesis of fluorine-containing compounds. Related with this topic, we have reported this review article published in *Chemical Reviews* **2014,** 114, 2432-2506.

"Asymmetric Allylation/Pauson-Khand Reaction: A Simple Entry to Polycyclic Amines. Application to the Synthesis of Aminosteroid Analogues"
The asymmetric allylation of ortho-iodoarylsulfinylimines was achieved in high diastereoselectivities. The thus-obtained ortho-iodoarylhomoallylic sulfinamides participate in a subsequent Sonogashira coupling followed by a diastereoselective intramolecular Pauson-Khand reaction. In this way, tricyclic amines showing a unique benzo-fused indenyl backbone were obtained. The methodology was applied to the synthesis of aminosteroid analogues (*Organic Letters* **2014,** 16, 1224-1227).

"A Selective Synthesis of Fluorinated Cispentacin Derivatives"
A facile selective method was developed for the synthesis of new fluorine-containing cispentacin stereoisomers. Mono- and difluorinated cispentacin derivatives were synthetized from a bicyclic ß-lactam in five or six steps involving a regio- and stereoselective hydroxylation through iodooxazoline formation, fol-

lowed by deoxygenation by fluorination. Starting from an enantiomerically pure bicyclic β-lactam obtained by enzymatic resolution of the racemic compound, an enantiodivergent procedure allowed the preparation of both dextro- and levorotatory difluorinated cispentacins (*European Journal of Organic Chemistry* **2014**, 4070-4076).

"Diastereoselective Synthesis of 2-Phenyl-3-(trifluoromethyl)piperazines as Building Blocks for Drug Discovery"
The synthesis of enantiomerically pure cis- and trans-2-phenyl-3-(trifluoromethyl)piperazines was described. It involved, as the key step, a diastereoselective nucleophilic addition of the Ruppert-Prakash reagent ($TMSCF_3$) to α- amino sulfinylimines bearing Ellman's auxiliary. This methodology allows an entry into hitherto unknown trifluoromethylated and stereochemically defined piperazines, key scaffold components in medicinal chemistry. This work was developed in collaboration with the pharmaceutical company Janssen and it was published in the *Journal of Organic Chemistry* **2014**, 79, 5887-5894.

"Unique Reactivity of Fluorinated Molecules with Transition Metals"
Organofluorine and organometallic chemistry by themselves constitute two potent areas in organic synthesis. Thus, the combination of both offers many chemical possibilities and represents a powerful tool for the design and development of new synthetic methodologies leading to diverse molecular structures in an efficient manner. Given the importance of the selective introduction of fluorine atoms into organic molecules and the effectiveness of transition metals in C–C and C–heteroatom bond formation, this review represents an interesting read for this aim (*Chimia* **2014**, 68, 382-409).

"Synthesis of Fluorinated and Nonfluorinated Tebufenpyrad Analogues for the Study of Anti-angiogenesis MOA"
In this contribution we reported the synthesis of fluorinated and nonfluorinated tebufenpyrad analogues to explore potential druglike properties through the phenotypic screening as part of the Lilly Open Innovation Drug Discovery (OIDD) program (*Organic Process Research & Development* **2014**, 18, 1027-1036).

"Tandem Gold Self-Relay Catalysis for the Synthesis of 2,3-Dihydropyridin-4(1H)-ones: Combination of σ and π Lewis Acid Properties of Gold Salts"
The dual ability of gold salts to act as π- and σ Lewis acids was exploited in a tandem self-relay catalysis. Thus, triphenylphosphanegold(I) triflate mediated the intramolecular carbonyl addition of the amide functionality of homoprogargyl amides to a triple bond. The formation of as complex of the gold salt with the intermediate oxazine promoted a nucleophilic addition followed by a Petasis-Ferrier rearrangement. This tandem protocol, catalyzed by the same gold salt under the same reaction conditions, gave rise to the efficient synthesis of 2,3-dihydropyridin-4-(1H)-ones, which contain a cyclic quaternary α-amino acid unit. The asymmetric version was performed by generating the starting materials from the corresponding sulfinylimines (*Chemistry-A European Journal* **2014**, 20, 14126-14131).

"Microwave-Assisted Tandem Organocatalytic Peptide Coupling-Intramolecular aza Michael Reaction. α,β-Unsaturated N-Acyl Pyrazoles as Michael Acceptors"
Conjugated N-acyl pyrazoles were successfully employed in the organocatalytic enantioselective intramolecular aza-Michael reaction as ester surrogates. Bifunctional squaramides under microwave irradiation provided the best results in this transformation. Furthermore, this protocol was combined with a peptide-coupling reaction in a tandem sequence. The final products were easily converted into the corresponding ethyl esters (*Chemistry-A European Journal* **2014**, 20, 15697-15701).

"Biochemical quantitation of the eIF5A hypusination in Arabidopsis thaliana uncovers ABA-dependent regulation"
This study presents the first biochemical description of the post translational modification of eIF5A by hypusination which will be functionally relevant for future studies related to the characterization of this pathway in Arabidopsis thaliana (*Frontiers in Plant Science*, **2014**, 5, 202).

Publications

1 .Fustero, S; Lázaro, R; Aiguabella, N; Riera, A; Simón-Fuentes, a; Barrio, P. Asymmetric Allylation/Pauson–Khand Reaction: A Simple Entry to Polycyclic Amines. Application to the Synthesis of Aminosteroid Analogues. Organic Letters Vol: 16, 1224-1227 , 2014 Quartile: Q1

2 . Wang, I; Sanchez-Rosello, M; Aceña, JL; del Pozo, C; Sorochinsky, AE; Fustero, S; Soloshonok, V; Liu, H.
Fluorine in Pharmaceutical Industry: Fluorine-Containing Drugs Introduced to the Market in the Last Decade (2001-2011). Chemical reviews Vol: 114(4), 2423-2506 , 2014 Quartile: Q1

3 .Belda-Palazón, B; Nohales, M; Rambla, JL; Aceña, JL; Delgado, O; Fustero, S; Martínez; MC; Granell, A; Carbonell, J; Ferrando, A.
Biochemical quantitation of the eIF5A hypusination in Arabidopsis thaliana uncoversABA-dependent regulation. Frontiers in Plant science Vol: 5, 202 , 2014 Quartile: Q1

4 .Sanchez-Rosello, M; Delgado, O; Mateu, N; Trabanco, AA; Van Gool, M; Fustero, S.
Diastereoselective Synthesis of 2-Phenyl-3-(trifluoromethyl)piperazines as Building Blocks for Drug Discovery. JOURNAL OF ORGANIC CHEMISTRY Vol: 79, 5887-5894 , 2014 Quartile: Q1

5 .Kiss, L; Nonn, M; Forro, E; Sillanpaa, R; Fustero, S; Fulop, F.
A Selective Synthesis of Fluorinated Cispentacin Derivatives. European Journal of Organic Chemistry Vol: 19, 4070-4076 , 2014 Quartile: Q1

6 .Roman, R; Navarro, A; Wodka, D; Alvim-Gaston, M; Husain, S; Franklin, N; Simon-Fuentes, A; Fustero, S.
Synthesis of Fluorinated and Nonfluorinated Tebufenpyrad Analogues for the Study of Anti-angiogenesis MOA. ORGANIC PROCESS RESEARCH & DEVELOPMENT Vol: 18 (8), 1027-1036 , 2014 Quartile: Q2

7 .Catalan, S; Munoz, SB; Fustero, S.
Unique Reactivity of Fluorinated Molecules with Transition Metals. Chimia Vol: 68 (6), 382-409 , 2014 Quartile: Q3

8 .Fustero, S; Miro, J; Sanchez-Rosello, M; del Pozo, C.
Tandem Gold Self-Relay Catalysis for the Synthesis of 2,3-Dihydropyridin-4(1H)-ones: Combination of sigma and pi Lewis Acid Properties of Gold Salts. Chemistry-A European Journal Vol: 20(43), 14126-14131 , 2014 Quartile: Q1

9 .María Sánchez-Roselló, Cristina Mulet, Marta Guerola, Carlos del Pozo, Santos Fustero.
Microwave-Assisted Tandem Organocatalytic Peptide Coupling-Intramolecular aza-Michael Reaction. α,β-Unsaturated N-Acyl Pyrazoles as Michael Acceptors. Chemistry- A European Journal Vol: 20(48), 15697-15701 , 2014 Quartile: Q1

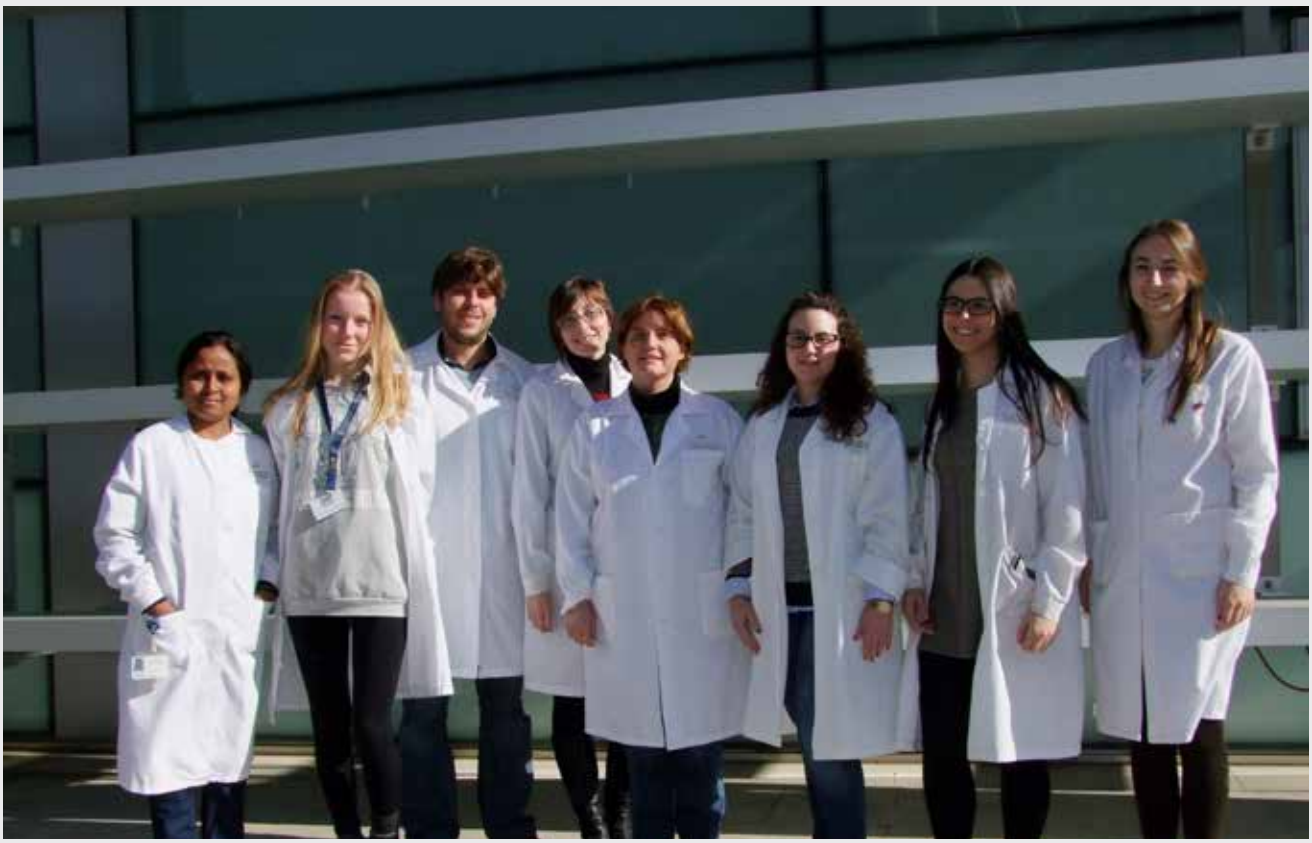
Conferences and meetings

1. 10th Spanish-Italian Symposium on Organic Synthesis (SISOC-X)
Plenary Lecture
Santos Fustero
(Florence , Italy)

2. Meeting “Bordeaux Fluorine Days”
Plenary Lecture
Santos Fustero
(Bordeaux , France)

3. XXV Biannual meeting of Organic Chemistry.
Oral Communication
Various authors
(Alicante , Spain)

Peptide & Protein Chemistry



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→Predoctoral Scientists

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→Students

Molly Marcus McBride (UPV))

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INDEX

Overview

Protein-protein-interactions (PPI) govern almost all important processes in living organisms. The Protein and Peptides Chemistry group works on the identification and development of new modulators of PPIs from the apoptosis and inflammation pathways. Our objective is to re-establish equilibrium in deregulated apoptosis and inflammation processes responsible for pathological situations, such as cancer, neurodegenerative or ischemia-reperfusion associated damages. To this aim, our experimental approach includes the development of in vitro assays to mimic the PPI of interest, screening of different chemical libraries and validation of the target and active molecules in cellular and in vivo models of disease.
The discovery of new modulators of these pathways contributes not only to the treatment but also to a better understanding of the molecular processes responsible for disease.

Research results

1. Development of apoptosis modulators.

Activation of apoptosis (programmed cell death) in mammalian cells occurs mainly through two different pathways, the extrinsic and the intrinsic pathway. In both cases, cell death is accomplished by specialized proteases termed caspases. Activation of the intrinsic pathway produces mitochondrial outer membrane permeabilization (MOMP) mediated by proteins from the Bcl-2 family. Following MOMP, cytosolic-released Cytochrome c binds to Apaf-1, inducing its oligomerization and thereby, forming a macromolecular structure termed the apoptosome. The apoptosome recruits and activates the initiator caspase, procaspase-9. Caspase-9, once activated, cleaves and activates the executioner caspases, caspase-3 and - 7, leading to apoptosis. Neurodegenerative diseases or ischemic-reperfusion processes are characterized by excessive apoptosis. Our group has developed new inhibitors of the apoptosome, the molecular platform responsible of procaspase-9 activation. Our objective is to avoid undesired cell death by apoptosis in pathological conditions. These inhibitors have been successfully evaluated in different in vivo models including models of kidney disease.

• *Apaf-1 inhibitors protect from unwanted cell death in in vivo models of kidney ischemia and chemotherapy induced ototoxicity*. PLoS One. 2014 Oct 20;9(10) Orzáez M, Sancho M, Marchán S, Mondragón L, Montava R, Valero JG, Landeta O, Basañez G, Carbajo RJ, Pineda-Lucena A, Bujons J, Moure A, Messeguer A, Lagunas C, Herrero C, Pérez-Payá E.
• *Inactivation of Apaf1 reduces the formation of mutant huntingtin-dependent aggregates and cell death.* Neuroscience. 2014 Mar 14;262:83-91. Sancho M, Herrera AE, Orzáez M, Pérez-Payá E.
• *Role of CDK5/cyclin complexes in ischemia-induced death and survival of renal tubular cells*. Cell Cycle. 2014 May 15;13(10):1617-26. Guevara T, Sancho M, Pérez-Payá E, Orzáez M.

The Bcl-2 proteins-dependent mitochondrial control of apoptosis has a predominant role in cancer cell biology. The cytosolic region of these proteins has been extensively studied, but there is a lack of information about the role of the transmembrane domains (TMD). We are studying the network of interactions established among the Bcl-2 TMDs and their relevance in control of the apoptotic process. Moreover, we have studied the mitochondrial priming capability of Bcl-2 TMDs as sensitizers to overcome cancer resistances to classic chemotherapy.

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• *Peptides derived from the transmembrane domain of Bcl-2 proteins as potential mitochondrial priming tools*. ACS Chem Biol. 2014 Aug 15;9(8):1799-811. Andreu-Fernández V, Genoves A, Lee TH, Stellato M, Lucantoni F, Orzáez M, Mingarro I, Aguilar MI, Pérez-Payá E
• *Altered mitochondria morphology and cell metabolism in Apaf1-deficient cells*. PLoS One. 2014 Jan 9;9(1) Sancho M, Gortat A, Herrera AE, Andreu-Fernández V, Ferraro E, Cecconi F,Orzáez M, Pérez-Payá E.

In the context of this project we have initiated a collaboration with the group of Prof. Jaume Reventós (Center de Recerca Vall d`Hebrón) to characterize the potential mitochondrial priming of endometrial cancer cells. The goal of this project is the identification of potential alternative treatments targeting the mitochondria to induce apoptosis and overcome cancer resistances to chemotherapeutical treatments.
Targeting of pro-apoptotic drugs to the desired tumor site is an effective strategy to avoid undesired side-effects. In this sense, we have initiated a collaboration with Prof. Ramón Martínez-Mañez (Instituto de Química Molecular Aplicada. Universidad Politécnica de Valencia) to study controlled delivery of pro-apoptotic drugs in cellular systems using "intelligent nanoparticles" with biomolecular gates.

• *Cathepsin-B induced controlled release from peptide-capped mesoporous silica nanoparticles*. Chemistry. 2014 Nov17;20(47):15309-14. de la Torre C, Mondragón L, Coll C, Sancenón, Marcos MD, Martínez-Máñez R,Amorós P, Pérez-Payá E, Orzáez M.
• *Temperature-controlled release by changes in the secondary structure of peptides anchored onto mesoporous silica supports*. Chem Commun (Camb). 2014 Mar 25;50(24):3184-6. de la Torre C, Agostini A, Mondragón L, Orzáez M, Sancenón F, Martínez-Máñez R, Marcos MD, Amorós P, Pérez-Payá E.

2. Development of inflammasome inhibitors.

Inflammasomes are macromolecular complexes from the innate immune system that activate immune and inflammatory pathways in response to invading pathogenic microbes and non-microbial danger signals. Inflammasomes are generally composed of an intracellular receptor (NLRP protein), and adaptor protein (ASC) and the inflammatory caspase, procaspase-1. Upon sensing a stimulus, the macrocomplex is assembled and produces proteolytic activation of procaspase-1, cleavage of the proinflammatory cytokines IL-1 beta and IL-18 and pyroptosis. (Figure 1)

A growing body of research indicates that defects in the structure and activity of inflammasomes are central to a vast number of illnesses from atherosclerosis and arthritis to Crohn's disease, cancer and diabetes. Current therapies for treatment of inflammasomopathies target IL-1 beta signaling but do not address the other components of pro-inflammatory signalling. To overcome these limitations, our objective has been the development of new modulators of the inflammasome (iAC1s). From the screening of a chemical library we have identified a new family of inhibitors. The activity of these compounds has been demonstrated in in vitro and cellular models of inflammation. The objectives of this project are now focused on the detailed characterization of the mechanism of action of the compounds at the molecular level and their evaluation in *in vivo* models of disease.

Projects

•*"Chemical tools that modify cellular fate: Molecular mechanism of action of apoptosis modulators"*. Ministerio de Ciencia e Innovación. Investigación (SAF-2010_15512).
•"Spanish Ion Channel Inniciative". Ministerio de Ciencia e Innovación. Investigación (CSD2008-00005).
•*"Modulación de interacciones proteína-proteína en apoptosis como diana terapéutica en procesos tumorales"*. Generalitat Valenciana Proyecto Prometeo (PROMETEOII/2014/061).

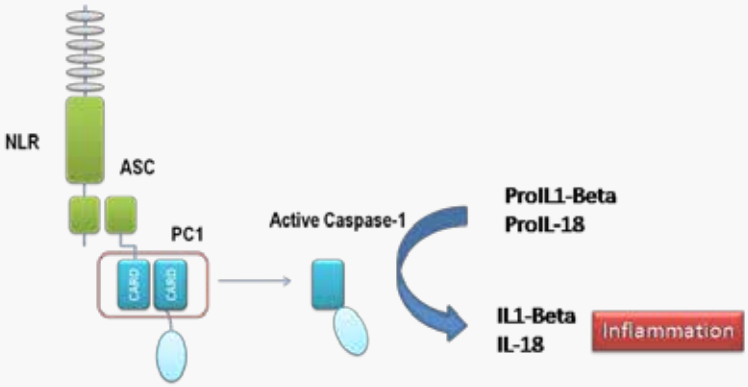

Figure 1. The components of the Inflammasome. Inflammasomes are composed by a NOD-like receptor protein (NLR), an adaptor protein (ASC) and the protease procaspase-1 (PC1). Upon stimulation the macrocomplex is formed producing the activation of procaspase-1. Active caspase-1 cleaves Pro IL1-Beta and Pro IL-18 and the processed cytokines initiate the inflammation signaling process.

Publications

1 .Mónica Sancho, Anna Gortat, Andrés E. Herrera, Vicente Andreu-Fernández, Elisabetta Ferraro, Francesco Cecconi, Mar Orzáez, Enrique Pérez-Payá. Altered mitochondria morphology and cell metabolism in Apaf1-deficient cells.. PloS one. 24416260 . 2014 Quartile: Q1

2 .Guevara, T; Sancho, M; Perez-Paya, E; Orzaez, M. Role of CDK5/cyclin complexes in ischemia-induced death and survival of renal tubular cells. Cell cycle (Georgetown, Tex.) Vol: 13 (10), 1617-1626 . 2014 Quartile: Q2

3 .Sancho, M; Herrera, AE; Orzaez, M; Perez-Paya, E. Inactivation of apaf1 reduces the formation of mutant huntingtin-dependent aggregates and cell death. Neuroscience Vol: 262, 83-91 . 2014 Quartile: Q2

4 .de la Torre, C; Agostini, A; Mondragon, L; Orzaez, M; Sancenon, F; Martinez-Manez, R; Marcos, MD; Amoros, P; Perez-Paya, E. Temperature-controlled release by changes in the secondary structure of peptides anchored onto mesoporous silica supports. Chemical communications (Cambridge, England) Vol: 50, 3184-3186 . 2014 Quartile: Q1

5 .Cristina de la Torre, Laura Mondragón, Carmen Coll, Félix Sancenón, María D. Marcos, Ramón Martínez-Máñez,* Pedro Amorós, Enrique Pérez-Payá† and Mar Orzáez*. Cathepsin-B induced a selective controlled release from peptide-capped mesoporous silica nanoparticles. Chemistry (Weinheim an der Bergstrasse, Germany) Vol: 20 (47), 15309-14 . 2014 Quartile: Q1

6 .Mar Orzáez, Mónica Sancho, Sandra Marchán, Laura Mondragón, Rebeca Montava,Juan García-Valero, Olatz Landeta, Gorka Basañez, Rodrigo J Carbajo,Antonio Pineda-Lucena,Jordi Bujons, Alejandra Moure, Angel Messeguer,Carmen Lagunas, Carmen Herrero,Enrique Pérez-Payá. Apaf-1 inhibitors protect from unwanted cell death in in vivo models of kidney ischemia and chemotherapy induced ototoxicity. PloS one Vol: 9 (10), 2014 Quartile: Q1

7 .Mondragon, L; Mas, N; Ferragud, V; de la Torre, C; Agostini, A; Martinez-Manez, R; Sancenon, F; Amoros, P; Perez-Paya, E; Orzaez, M. Enzyme- Responsive Intracellular- Controlled Release Using Silica Mesoporous Nanoparticles Capped with e- Poly- l- lysine. Chemistry- A European Journal Vol: 20 (18), 5271-5281 . 2014 Quartile: Q1

8 .Andreu-Fernandez, V; Genoves, A; Lee, TH; Stellato, M; Lucantoni, F; Orzaez, M; Mingarro, I; Aguilar, MI; Perez-Paya, E. Peptides Derived from the Transmembrane Domain of Bcl-2 Proteins as Potential Mitochondrial Priming Tools. ACS Chemical Biology Vol: 9 (8), 1799-1811 . 2014 Quartile: Q1

Conferences and Meetings

1. 9th European Workshops on Cell Death: 9th Death with Aphrodite(EWCD)
Oral Communication
Guillermo García Lainez
(Paphos , Cyprus)

2. XIVth congress of the Spanish Biophysical society
Oral Communication
Vicente Andreu, Mónica Sancho y Mar Orzáez
(Madrid , Spain)

Collaborations

•Prof. Ismael Mingarro Muñoz and Dr. Manuel Mateo Sánchez del Pino. Universitat de València. Biochemistrry and Molecular Biology Departmentr

•Prof. Angel Messeguer Peypoch Instituto de Química Avanzada de Cataluña-CSIC. Chemical and Biomolecular Nanotechnology

•Prof. Ramón Martínez Mañez. Institute of Molecular Recognition and Technological Development (IDM), UPV, UV

•Dra. Mibel Aguilar. Monash University. Department of Biochemistry & Molecular Biology

•Dr. Gorka Basañez Asua CSIC-UPV/EHU. Biophysics Unit

•Prof. Jaume Reventós. Vall d'Hebron Institut de Recerca (VHIR). Research Unit in biomedicine and Translatioanl Oncology

•Prof. Jordi Yagüe. Hospital Clínic Centro de Diagonóstico Biomédico Inmunología. Prof. Francesco Cecconi Università degli Studi di Roma Department of Biology

•Dr. Jean Ehrland Ricci; Dra. Laura Mondragón Center Méditerranéen de Médecine Moléculaire.

•Dr. Antonio Pineda-Lucena and Dra. Mª Jesús Vicent Centro de Investigación Príncipe Felipe. Advanced Therapies Programme

Polymer Therapeutics

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Maria Helena Ferrandis Jimenez
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www.cipf.es/web/portada/polimeros-terapeuticos

Overview

Clinical proof of concept for Polymer Therapeutics has already been achieved, even 2 Polymer Therapeutics are within the US Top 10 selling drugs of 2013. However, many challenges and opportunities still lay ahead providing scope to further develop this technology platform. Delivery of new anticancer agents focusing on novel molecular targets and their combination, development of polymeric materials with defined architectures and treatment of diseases other than cancer are the most exciting and promising areas, and are therefore the research lines in the Polymer Therapeutics Laboratory.

Our research activity is focused on the design of advanced polymer conjugates, novel nanomedicines with application in cancer and tissue regeneration as therapeutics as well as molecular diagnostic tools. The development of polypeptide-based biodegradable carriers, the use of combination therapy or the design of nanoconjugates and hybrid systems for novel molecular targets, including treatments for neurodegenerative disorders (Alzheimer's Disease, Spinal Cord Injury) are some of the approaches we are following in order to achieve highly specific and effective nanopharmaceutics.

Our polymeric systems are designed to allow the study of the influence of the spatial conformation on the intracellular trafficking of bioactive agents, allowing for the exploration of a broader range of clinical applications. Quantitative tools for the study of cell and in vivo fate of nanopharmaceutics are also being implemented.

Research results

During 2014 in the Polymer Therapeutics Laboratory the consolidation of our research lines as well as the establishment of novel approaches within the field have been achieved. Some of our main scientific achievements are summarized herein.

The development of new and more defined architectures with higher Mw (to enhance passive targeting by the EPR effect), predictable structure and conformation, lower heterogeneity, higher drug loading capacity and greater possibility for multivalency are main research lines in polymer therapeutics in particular, and in nanomedicine in general. In our group, we manage to overcome those limitations with precise controlled reactions followed by an adequate characterization yielding to well-defined polypeptidic architectures by NCA polymerization techniques (Conejos-Sánchez 2013). In addition, a variety of functionalities such as alkyne, azides, reactive disulphides, protected amines... can be easily introduced by "post-polymerization modification" reactions yielding a set of orthogonal reactive attachment sides suitable for further bioconjugation of agents of different nature (Barz 2013; Talelli 2014). This strategy has been efficiently applied in the synthesis of star-based polypeptide architectures (Duro-Castaño sub). With the aid of multiple techniques (Gel Permeation Chromatography (GPC), Nuclear Magnetic Resonance (NMR), Circular Dichroism (CD), Small-Angle Neutron Scattering (SANS) and Dynamic Light Scattering (DLS)) we were able to obtain information about the structural characteristics, physico-chemical descriptors and information at the nanoscopic level that will have an impact in the biological behavior of the final compounds and therefore in future improved rational designs. Their validation as drug delivery systems has been carried out evaluating their stability in physiological fluids, cytotoxicity, cellular uptake and internalization mechanism (live-cell confocal microscopy, flow cytometry) followed by biodistribution and PK studies in vivo (by optical imaging and PET (collaboration with Prof Morcillo (CIEMAT, Madrid)). The results suggest that the newly designed star shaped polyglutamates are biodegradable entities that follow an endocytic mechanism of cellular internalization and are renally excreted without any specific accumulation or toxicity (Duro-Castaño sub).
Those linear and branched constructs have been used

as polymeric carriers in the development of conjugates designed as therapy for the treatment of cancer and tissue regeneration.

One of our main lines of research is the development of polymer-based combination therapies for the treatment of hormone-dependent tumours, mostly focused on advanced breast and prostate carcinomas, although other type of tumours have been started to be explored this year, such as lung cancer through Prof Kiew (Univ. Malaya) research stay. Based on a previously developed model combination conjugate (Vicent 2005; Deladriere sub), several polyglutamate (PGA)-based conjugates bearing endocrine + chemotherapeutic agents have demonstrated their promising antitumour behavior after an adequate rational design in orthotopic metastatic breast cancer tumour models. In this family of PGA-combination conjugates, solution conformation is a key feature ruling biological performance in vitro as well as in vivo (Deladriere in prep). Following this successful strategy in breast cancer, pH responsive combination conjugates for the treatment of advanced prostate cancer have been also developed. This family was inspired by the bi-hydroxyl functionality of diethylethylstilbestrol (DES), a synthetic non-steroidal estrogen, that can be easily incorporated into a pH responsive polyacetalic mainchain (Giménez 2012; England 2012) that was combined with curcumin (Plyduang sub), a natural product, yielding nanoconjugates with an important drug synergism in prostate cancer models and the capability to release the drug selectively under acidic environment such as that found in endosomes and lysosomes. By means of the same technology, when DES was combined with paclitaxel the in vivo studies did not present any synergism for such combination conjugate, mainly due to side-toxicity issues. However, the single polyacetal-paclitaxel conjugate showed a significant activity in vivo against metastatic processes in a LNCap orthotopic prostate cancer model, not observed with the free drug (Abasolo in prep). This study has been performed in collaboration with Dr. Schwartz Jr's group (VHIR, Barcelona). Finally, on prostate cancer and in collaboration with Dr Lopez (IVO, Valencia) we are looking for biomarkers of this heterogeneous disease in patient samples (Casanovas 2013; 2014) including nanomedicine biomarkers that would allow clinicians to select those patients who could better benefit from nanotherapies (Armiñán, Casanovas in prep).
Other cancer projects include new strategies for nanomedicine design by means of targeted tumour cell membrane lysis (Gallon in prep) and to enhance cytosolic delivery of oligonucleotides. A collaborative European NanoSci ERA-net funding scheme (with Univ. Nottingham and Univ. Padova) yield a novel pH sensitive targeted polymeric vesicular system for the delivery of siRNA to cancer cells (Matini 2014; Gallon sub). In the same line, in collaboration with Prof Wagner (Univ. Munich) a novel zwitterionic PGA-based system for plasmid DNA (pDNA) delivery has been designed. This non-viral gene transfer carrier was able to enhanced pDNA transfection when compared with polyethyleneimine (PEI) with no significant reduction in cell viability (Niño in prep).

On the other hand, the design of polymer therapeutics for tissue regeneration (mainly, spinal cord injury (SCI) and amyloidosis including Alzheimer's Disease (AD)) is a very important research line for us. Firstly, conjugates against a rare amyloidotic disease: familial amyloidotic disease (FAP) have been developed. Disease is triggered by a mutation in the protein transthyretin (TTR) that promotes its aggregation ending up in fibril formation whose deposits promote organ failure. Along this cascade, first target were the initial TTR aggregates which are the responsible of trigger neurodegeneration through their engagement with the RAGE receptor. Second target were the fibril deposits formed at the end of the cascade, which promote tissue degeneration and organ failure. Our collaborator Prof. Saraiva (Porto) discovered two biomolecules active against the named TTR species: a specific peptide (RAGE peptide) and a tetracycline (doxycycline), respectively. By means of a rational design, polymer conjugation of both active biomolecules was performed. After in vitro activity evaluation and in vivo biodistribution studies, we have moved towards two feasible FAP treatments (Conejos-Sánchez 2014; 2015).

Among all the biological hallmarks of AD, we are centering our efforts in the amyloid pathway, using curcuminoids. A set of star-curcuminoid conjugates has

been synthesized and characterized. Their biological output in terms of cellular uptake, and cell viability has been investigated. Moreover, proof of concept of their activity was achieved by thioflavin T assay and TEM, using Hen Egg White Lysozyme as accepted model for fibril formation. To achieve an efficient AD treatment, the designed conjugates have to be able to adequately reach the brain and therefore capable to surpass the blood-brain barrier (BBB). In the same project, novel targeted carriers are being used and the resulting conjugates have been evaluated in an arcbeta AD mouse model (ETH, Zurich) with promising results.
Finally in this area and in collaboration with Dr Moreno (CIPF), nanoconjugates and hybrid systems are being developed for the treatment of Spinal cord injury (SCI), a major cause of paralysis with currently no effective therapies. We have design a new PEG-Polyacetal-curcumin conjugate conferring a high hydrosolubility and pH-responsiveness for local delivery. In vivo unique and local administration, into the intrathecal space, immediately after acute SCI in adult rats, showed significant functional locomotor recovery since 1 week after treatment. The tissue analysis one month after SCI and treatment showed a significant increase for neuronal fibers immunodetection with decreased expression of the pro-apoptotic marker caspase 9, indicating a preferential neuroprotective role of the PEG-Polyacetal-Curcumin treatment (Cases in prep).

We have also developed quantitative methodology to study cell trafficking and nanoconjugate in vivo fate including subcellular fractionation, Amnis Imaging Stream and in vivo monitoring. Novel cellular probes are being developed for this purpose (Armiñan in prep). In the same context, metabolic studies have been performed in collaboration with Dr Pineda (CIPF). Metabolic profile was studied in an in vitro and in vivo breast cancer model corroborating the greater antitumor effect observed after the treatment with selected polymer drug conjugates. Metabolic data was confirmed with molecular biology studies (Armiñan, Palomino in prep).

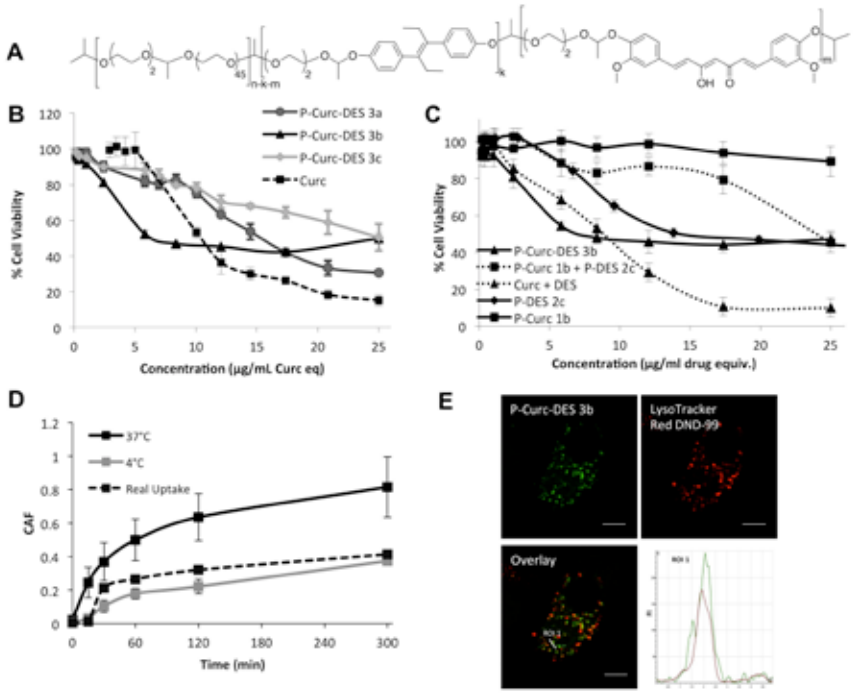

Figure 1.
A. Structure of Polyacetal-Curcumin(Curc)-Diethylstibestrol (DES) combination conjugate.
B. Cytotoxicity of different Polyacetal-Curc-DES conjugates (3a, 3b and 3c) against LNCaP prostate cancer cells (72 h incubation). Data as cell viability (%Control), mean values ± SEM (n⋯⋗3).
C. Cytotoxic study in LNCaP cells comparing free drugs and simple conjugates with the chosen combination conjugate P-Curc-DES 3b,. Assay measured by MTS after 72 h of treatment. Data as cell viability (%Control), mean values ± SEM (n⋯⋗3). Combination Index (CI) 0.8 showing drug synergism.
D. Cell uptake study of P-Curc-DES 3b at 37°C and 4ºC in LNCaP cells. Data was represented as mean values ± SEM (n = 3).
E. Representative image of P-Curc-DES 3b lysosomal co-localization in LNCaP cells acquired by live-cell fluorescence confocal microscopy. Scale bar=10µm, ROI=Region of Interest, Green line=P-Curc-DES 3b, Red line= Lysotracker Red DND-99.

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Publications

1 .T Matini, S Spain, G Mantovani, M J. Vicent*, J Sanchis, E Gallon, F Mastrotto, S Salmaso*, P Caliceti, C Alexander*.
Synthesis and characterization of variable conformation pH responsive block co-polymers for nucleic acid delivery and targeted cell entry.
Polymer Chemistry Vol: 4(10), 2980-2994 , 2014 Quartile: Q1

2 .Casanova-Salas I, Rubio-Briones J, Calatrava A, Mancarella C, Masiá E, Casanova J, Fernández-Serra A, Rubio L, Ramírez-Backhaus M, Armiñán A, Domínguez-Escrig J, Martínez F, García-Casado Z, Scotlandi K, Vicent MJ, López-Guerrero JA.
Identification of miR-187 and miR-182 as Biomarkers of Early Diagnosis and Prognosis in Patients with Prostate Cancer Treated with Radical Prostatectomy.
Journal of Urology Vol: , 232-238 , 2014 Quartile: Q1

3 .Duro-Castano, A; Conejos-Sanchez, I; Vicent, MJ.
Peptide-Based Polymer Therapeutics.
Polymers Vol: 6 (2), 515-551 , 2014 Quartile: Q2

4 .Conejos-Sanchez, I; Cardoso, I ; Saraiva, MJ; Vicent, MJ.
Targeting a rare amyloidotic disease through rationallydesigned polymer conjugates.
Journal of controlled release . Vol: 178, 95-100 , 2014 Quartile: Q1

5 .Armiñán, A; Sepúlveda, P; Vicent. MJ.
Polymer Therapeutics as Nano-sized medicines for Tissue Regeneration and Repair.
Chap. Book John Wiley & Sons, 2014

6 . Talelli, M; Vicent, MJ.
Reduction Sensitive Poly(L-glutamic acid) (PGA)-Protein Conjugates designed for Polymer Masked-Unmasked Protein Therapy.
Biomacromolecules Vol: 15 (11), 4168-4177 2014 Quartile: Q1

7 .Conejos-Sanchez I, Cardoso I, Oteo-Vives M, Romero-Sanz E, Paul A, Ruiz Sauri A, Morcillo MA, Saraiva MJ, Vicent MJ.
Polymer-doxycycline conjugates as fibril disrupters: An approach towards the treatment of a rare amyloidotic disease.
Journal of controlled release : official journal of the Controlled Release Society Vol: 198C, 80-90 , 2014 Quartile: Q1

Conferences and Meetings

1. 10th International Symposium on Polymer Therapeutics: From laboratory to clinical practice .
Organizing comittee
M.J. Vicent
(Valencia , Spain)

2. 10th International Symposium on Polymer Therapeutics: From laboratory to clinical practice .
Poster.
T. Plyduang, A. Armiñán, J. Movellan, R. M. England, R. Wiwattanapatapee, M.J. Vicent
(Valencia , Spain)

3. 10th International Symposium on Polymer Therapeutics: From laboratory to clinical practice .
Poster
M. Helle, A. Duro-Castaño, I. Tranchant, F. Beau, A. Dufour, C. Overall, M.J. Vicent, V. Dive
(Valencia , Spain)

4. 10th International Symposium on Polymer Therapeutics: From laboratory to clinical practice .
Oral Communication
S Contreras, V. Moreno, A. Alastrue, M. J. Vicent
(Valencia , Spain)

5. Nano and Biotechnology in Drug Discovery & Development Polyacetal
Poster
J. Movellan
(Malaga, Spain)

5. Nano and Biotechnology in Drug Discovery & Development Polyacetal
Poster
E. Gallon
(Malaga, Spain)

6. 10th International Symposium on Polymer Therapeutics: From laboratory to clinical practice .
Oral Communication & Poster
A. Duro-Castano, VJ. Nebot, L. Tortajada, A. Paul, MJ. Vicent
(Valencia , Spain)

7. Facultad de Medicina. Universidad de Castellón. UJI
Plenary Lecture
(Castellón , Spain)

8. Gordon Research Conference on Drug Carriers in Medicine & Biology.
Oral Communication
A. Duro
(Boston , USA)

9. Gordon Research Conference on Drug Carriers in Medicine & Biology.
Lecture
M.J. Vicent
(Boston , USA)

10. NanoSpain Conference 2014.
Oral Communication
V.J. Nebot Carda
(Madrid , Spain)

11. NanoSpain Conference 2014.
Poster
L. Conesa Milian; A. Casaño; I. Conejos Sanchez; M.J. Vicent
(Madrid , Spain)

12. SCT - SFNano Joint Meeting “When Medicinal Chemistry meets Nanomedecines: Molecular Aspects of Targeting-Activation and Delivery of Drugs".
Plenary Lecture
M.J. Vicent
(Paris , France)

13. Spanish Chemical Biology Group (GEQB) and XIV Iberian Peptide Meeting (EPI).
Oral Communication
A. Niño Pariente; C. Scholz; P. Kos; E. Wagner; M.J. Vicent
(Bilbao , Spain)

Doctoral Thesis presented

Doctoral Candidate: Elena Gallon. Novel pH responsive Polymeric Vesicles for siRNA delivery to the tumor. Directors: Stefano Salmaso & Mª Jesús Vicent . University of Padova

Structural Biochemistry

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Leonor Puchades-Carrasco

→ **Pre-Doctoral Scientists-**
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Clara Pérez Rambla (H. Dr. Peset)

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Carlos Navalón Donat (UCV)

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Overview

The Structural Biochemistry Laboratory pursues the development and evaluation of novel molecularly targeted agents using a combination of structure-based drug design and metabolomic approaches. It includes the development of experimental approaches to identify and characterize drug targets, as well as to search for new therapeutic principles against them. An intense effort is also devoted to the identification of pharmaceutical/clinical biomarkers that can be used for the diagnosis/prognosis of different pathologies, the stratification and treatment monitoring of patients, the characterization of mechanisms of action of drugs, the evaluation of safety/efficacy profiles, etc. To achieve these goals, the group uses NMR spectroscopy, both as a high resolution and a screening (fragment-based) technique, in combination with other biophysical (surface plasmon resonance, circular dichroism, fluorescence spectroscopy, etc.), biochemical (molecular biology, protein chemistry, cell biology, etc.) and computational (QSAR, docking, structural modeling, etc.) approaches to study the three-dimensional structure and properties of proteins, protein-protein and protein-ligand complexes, as well as metabolites in solution. Some of these projects are being carried out in collaboration with different, national and international pharmaceutical companies as well as with hospitals around the country, and we expect to transfer some of our results to the pharmaceutical industry in the short-medium term.

Research results

The Structural Biochemistry Laboratory (SBL) has a solid experience in NMR, organic chemistry, cell biology, molecular biology, protein chemistry, computational chemistry and drug design using fragment-based drug discovery in the oncology area. The group devotes a lot of attention to the development of novel structure-based molecularly targeted agents against some pharmaceutically relevant targets involved in cell invasion and metastasis, the validation of inhibitors against other oncology targets, the characterization of compounds interacting with key proteins involved in apoptosis and the development of polymer conjugates as a tool to compete with particular protein-protein interactions (PPIs). The main aim of all these studies was the identification, using fragment-based screening by NMR and computational approaches of small molecules that could modulate the biological activity of these targets and the development of computational chemistry approaches for the evaluation of the hits.

We are also working on the evaluation, based on the capabilities developed at the group in metabolomics, of the possibility of using metabolomics by NMR as a tool to characterize the specific serum metabolomic profile of several pathological processes. Recent studies focused on the identification of specific biomarkers associated to multiple myeloma have provided additional support to other metabolomic studies our group have been conducting in different areas: hemato-oncology (chronic lymphocityc leukemia, myeloproliferative neoplasms, etc.), solid tumors (non-small cell lung cancer, prostate cancer, etc.), reproductive medicine (endometriosis, premature birth, intrauterine growth restriction, chromosomal abnormalities, etc.) and others (liver cirrhosis, tuberculosis, etc.). Furthermore, efforts have been directed to the development of technological platforms for the evaluation of different biological matrices (e.g., stem cells, embryo cultures, plants, yeasts, tears, amniotic fluid, etc.) that could provide a tool for a better understanding of biological systems.

During 2013, we have conducted several studies contributing to the development of molecularly targeted agents against several pharmacologically relevant targets. On the other hand, NMR metabolomics, a new approach that measures the metabolic profile of biological samples, has also been used to conduct comparative analysis of healthy and diseased individuals, information that can be used to identify biomarkers of disease and stratification of patients based on molecular subgroups.

Heparanase: This enzyme is involved in the specific degradation of heparan sulfate, the polysaccharide component of heparan sulfate proteoglycans that are present on the cell surface and in the extracellular matrix, and plays an important role in various neoplastic processes (eg ., melanoma and breast, colon, prostate, liver, bladder, intestine, ovary, pancreas, etc.). In fact, heparanase is considered a very relevant target for anticancer drug development given its involvement in the formation of tumors, and processes related to angiogenesis and metastasis. Previous results obtained

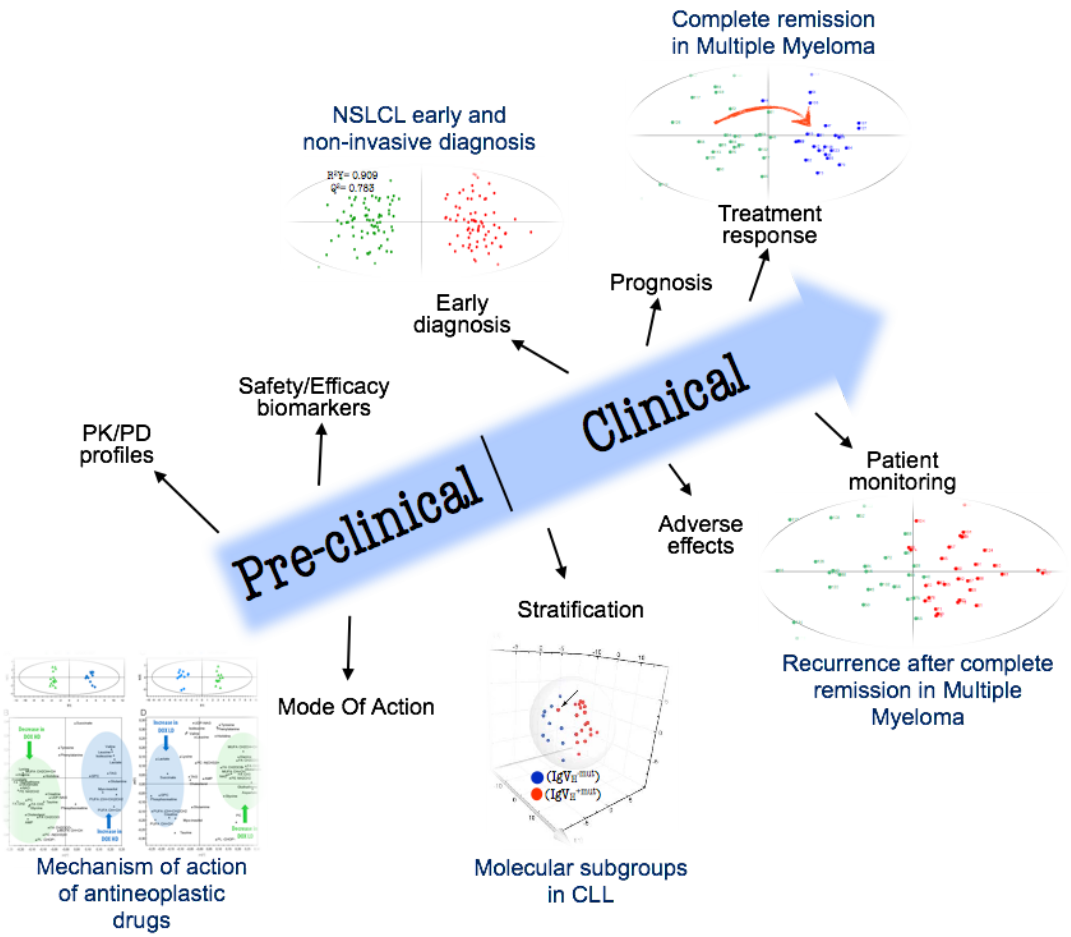

Figure: Summary of the current project carried out by the Structural Biochemistry Laboratory on preclinical and clinical applications of metabolomics by NMR

by our group allowed the characterization of a 29 kDa heparanase construct useful for identifying novel inhibitors using structure-based techniques. During this year, we have carried out new computational studies focused on the discovery of new inhibitors that have been experimentally evaluated using NMR experiments (WaterLOGSY, STD) and SPR to better understand the structural determinants of the interactions between heparanase and these compounds. This work has facilitated the identification of several compounds able to bind to the active site of heparanase, and the collaboration with an american pharmaceutical company interested in the evaluation of their own inhibitors using our experimental setting.

Metabolomics: Multiple myeloma (MM) is a cancer of plasma cells that is incurable at present. The incidence of this disease is 1% of all cancers and approximately 10% of all hematologic diseases. Each year in the US alone are diagnosed 20,000 new cases. The progress made in recent years has facilitated the emergence of new drugs that have resulted in improved patient survival. Each of these drugs act through multiple mechanisms of action, at the level of both intracellular signaling pathways and the tumor microenvironment. The aim of this project is to analyze the molecular basis of this disease and characterize the pharmacological effects of various combinations of drugs used in clinical practice in the treatment of MM. To achieve these goals, we used NMR metabolomics, an experimental strategy that measures the metabolic profile of biological samples, allowing comparative analysis of healthy individuals and patients, as well as evaluating the perturbations caused by antineoplastic agents at the metabolic level. This study has revealed that patients diagnosed with MM have a characteristic metabolic profile compared to healthy individuals, and that this profile changes after complete remission. Furthermore, the results have showed that, after treatment, it is possible to distinguish those effects associated to the recovery of patients from other processes related to side effects of current chemotherapeutic treatments.

Publications

1 .Mosulén S; Pineda-Lucena A; Carbajo RJ.
Chemical shift assignments and secondary structure of the surrogate domain for drug discovery studies of human heparanase.
Biomolecular NMR assignments, 2014
Quartile: Q4

2 .Filipiak, K; Hidalgo, M; Silvan, JM; Fabre, B; Carbajo, RJ; Pineda-Lucena, A; Ramos, A; de Pascual-Teresa, B; de Pascual-Teresa, S.
Dietary gallic acid and anthocyanin cytotoxicity on human fibrosarcoma HT1080 cells. A study on the mode of action.
Food & Function Vol: 5, 381-389 .2014
Quartile: Q2

3 .Puchades-Carrasco & A. Pineda-Lucena.
NMR in Pharmaceutical Sciences.
Book: John Wiley & Sons, 2014

4 .Fabre, B; Filipiak, K; Coderch, C; Zapico, JM; Carbajo, RJ; Schott, AK; Pineda-Lucena, A; de Pascual-Teresa, B; Ramos, A.
New clicked thiirane derivatives as gelatinase inhibitors: the relevance of the P1 ' segment.
RSC Advances Vol: 4 (34), 17726-17735 2014 Quartile: Q1

5 .Mar Orzáez, Mónica Sancho, Sandra Marchán, Laura Mondragón, Rebeca Montava,Juan García-Valero, Olatz Landeta, Gorka Basañez, Rodrigo J Carbajo,Antonio Pineda-Lucena,Jordi Bujons, Alejandra Moure, Angel Messeguer,Carmen Lagunas, Carmen Herrero,Enrique Pérez-Payá.
Apaf-1 inhibitors protect from unwanted cell death in in vivo models of kidney ischemia and chemotherapy induced ototoxicity.
PloS one Vol: 9 (10), 2014 Quartile: Q1

6 .Carbajo, R.J.; Sanz, L.,;Pérez, A.; Calvete, J.J.
NMR structure of bitistatin, a missing piece in the evolutionary pathway of snake venom disintegrins.
FEBS Journal, 2014 .

7 .Vicente-Muñoz, S.; Romero, P., Magraner-Pardo, L.; Martínez-Jiménez, C.P.; Tordera, V.; Pamblanco, M.
Comprehensive analysis of interacting proteins and genome-wide location studies of the Sas3-dependent NuA3 histone acetyltransferase complex.
FEBS Open Bio Vol: 4, 996-1006 .2014
Quartile: no

Conferences and meetings

1. 2nd Biomarker Meeting: Personalized Reproductive Medicine .
Poster
S. Vicente Muñoz
(Valencia , Spain)

2. FEBS EMBO 2014
Poster
M. Palomino
(Madrid , Spain)

3. Mycoval 2014.
Oral Communication
A. Pineda-Lucena
(Valencia, Spain)

4. VII International Symposium on Advances in Dermato-oncology.
Invited Conference
A. Pineda-Lucena
(Valencia , Spain)

5. Metabolomics and cancer.CSIC
Invited Conference
A. Pineda-Lucena
(Valencia, Spain)

Doctoral Thesis presented

Doctoral Candidate: Leonor Puchades Carrasco . Aplicaciones de la RMN al desarrollo de terapias antineoplásicas
Director: Antonio Pineda-Lucena .
Universidad de Valencia, UV

Computational Genomics Program

Program Coordinator: Joaquín Dopazo

Genomics of gene expression, led by Ana Conesa-Cegarra

Systems biology, led by Joaquin Dopazo

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Genomics of Gene Expression

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Pedro Furió
Rafael Hernández
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Cristina Martí

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Eugenia Flores

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Nicolás Lucero (UV)
Rodrigo García Valiente (UV)
Álvaro Moral Castro (UPV)

www.cipf.es/genomica-de-la-expresion-genica-

Overview

The Genomics of Gene Expression Lab is interested in understanding the functional aspects of gene expression at the genome-wide level and its relationship with diseases and traits. For that we develop statistical methods and software tools that analyze the dynamics aspects of transcriptome data, integrate these with other types of molecular data and annotate them functionally, by making use of Next Generation Sequencing technologies (NGS). The current areas of research are:

- Multi omics data integration in the immune system cell differentiation context
- Functional role long-non coding RNA and their association to disease
- Functional role of the isoforms alternative expression in neuro differentiation

Functional genomics research is complemented with the development of bioinformatics software for the analysis of genomics data: **Blast2GO** (functional annotation), Paintomics (genomics visualization), **Qualimap** (QC of mapped NGS data), **maSigPro and SEA** (time series analysis), **minAS and ASCA-genes** (gene expression analysis), **SEA and NOIseq** (RNA-seq analysis).

The laboratory is currently coordinating two FP7 research projects: **STATegra**, on the development of statistical tools for integration of heterogeneous omics datasets, and **DEANN**, a Marie Curie IRSES whose aim is to develop an Europe-South American NGS analysis network and a project financed by MINECO. Furthermore, we collaborate with bioinformatic companies on the development of the functional transcriptome studies and with different research groups on massive sequencing data analysis.

Research results

In 2014 12 articles were published in peer-reviewed journals and one book chapter. These works summarize the work performed in 2014:

We have developed different algorithms to analyze transcriptomic data. The Pathway Network Analysis methodology (PANA) is used for the creation of a network of functional connections between signalling pathways from gene expression data. The method has been applied to the study of the yeast cellular cycle and to Alzheimer's disease progression. For the first one, a network of over 50 components and 300 unions between pathways was obtained, highlighting associations between cellular cycle and DNA replication, glycolysis and oxidative phosphorylation, proteasome degradation and amino acid metabolism, which show the synchronization between the processes of production, consumption and reuse of energy during the cellular cycle. Also, new functional associations of the Alzheimer disease with cell adhesion and peroxinome function were discovered. Next-maSigPro updates our previous algorithm for analysis of the kinetics of gene expression, making it adaptable to the new sequencing technologies or RNA-seq. The algorithm is implemented in the R package of the same name. Finally in the R package NOISeq we offer tools for RNA-seq data quality control and for the analysis of differential expression with a nonparametric approach framework.

Within the EU-funded project STATegra we worked on various methods or the integrative analysis of omics data. The following developments are underway:
- Metrics for evaluating the performance in mutiomics data and comparison of noise and reproducibility levels
- Methods for integrating multiomics pairs of kinetic data, such as RNA-seq and DNAse-seq to study the relationship between chromatin structure and gene expression
- Machine learning methods to define regulatory gene expression programs based on their multiomic regulatory elements.

We have developed the methodology Functional Analysis Impact of Alternative Expression of isoforms, and applied it to understand neural cell differentiation

in mouse. This approach leverages long read PacBio sequencing to obtain full-length transcriptome. Then, transcripts are functionally characterized in detail and novel statistical methods are used to assess functional differences.

Finally we developed a methodology to predict the potentiality of lncRNAs to act as microRNA sponges. Characterizing lncRNAs functionality is a present issue as it is widely unknown, but has great potential in studying the regulation of gene expression and cell type specificity.

Development of Tools:
Also within STATegra we have developed a new bioinformatics tool for the annotation and storage of multi omic data experiments called STATegra Experimental Management System EMS which allows organizing complex multiomics experiments. STATegraEMS is a Java application that is free for the scientific community.

Genomics
We have collaborated with different consortia in the analysis and interpretation of genomic data.

Within the SEQC consortium, promoted by the FDA for quality analysis of transcriptome sequencing data, RNA-seq. Our results show the reliability of this technique as a diagnostic tool in comparison with microarrays.

We have collaborated in the determination of the pathogenic fungus Pochonia chlamydosporia genome and its transcriptional activation during the colonization of barley parasitic nematodes. In addition candidate genes responsible for Multitrophic characteristics of these organisms were identified. This work opens the possibility of using biocontrol fungus plant parasitic nematodes.

The transcriptional activity of the protist Polymyxa betae during sugar cane infection was studied and two genes candidates to be key factors of infection establishment were found, as well as other genes responding to the plant stress

The transcriptomes of two series of Tritrichomonas with different host range were analysed and key sequences for their specific pathogenicity identified. The potential of these sequences for the development of new drugs dealing with these pathologies was evaluated.

We have also worked on the analysis of expression data and epigenomics in serrated colon cancer. Our work has revealed features specifically deregulated in serrated adenocarcinoma as the cytoskeleton, vesicle transport and apoptosis. We have identified ephrin B2 receptor, hipocalcina and fascinates 1 as biomarkers for serrated cancer, which can be used for clinical diagnosis.

Publications

1 .Desoignies, N; Carbonell, J; Moreau, JS; Conesa, A; Dopazo, J; Legreve, A. Molecular interactions between sugar beet and Polymyxa betae during its life cycle. Annals of Applied Biology Vol: 164 (2), 244-256 . 2014 Quartile: Q1

2 .Garcia-Solano, J; Alcaraz-Mateos, E; Wilce, J; Torres-Moreno, D; Turpin-Sevilla, MC; Navarre, C; Conesa, A; Tuomisto, A; Sirnio, P; Makinen, MJ; Perez-Guillermo, M; Conesa-Zamora, P.
Methylation Microarray Analysis Identifies Differentially Methylated Genes in Serrated Compared to Conventional Colorectal Carcinomas.
Laboratory Investigation Vol: 94, 174A-174A . 2014 Quartile: Q1

3 .Ponzoni, I; Nueda, MJ; Tarazona, S; Gotz, S; Montaner, D; Dussaut, JS; Dopazo, J; Conesa, A.
Pathway network inference from gene expression data. BMC Systems Biology Vol: 8, supl 2, S7 . 2014 Quartile: Q1

4 .Garica-Solano, J; Alcaraz-Mateos, E; Wilce, J; Torres-Moreno, D; Turpin-Sevilla, MC; Navarre, C; Conesa, A; Tuomisto, A; Sirnio, P; Makinen, MJ; Perez-Guillermo, M; Conesa-Zamora, P. Methylation Microarray Analysis Identifies Differentially Methylated Genes in Serrated Compared to Conventional Colorectal Carcinomas. Modern Phatology Vol: 27, 174A-174A . 2014 Quartile: Q1

5 .Gomez-Cabrero, D; Abugessaisa, I; Maier, D; Teschendorff, A; Merkenschlager, M; Gisel, A; Ballestar, E; Bongcam-Rudloff, E; Conesa, A; Tegner, J.
Data integration in the era of omics: current and future challenges.
BMC Systems Biology Vol: 8, supl 2, I1 . 2014 Quartile: Q1

6 .de Diego, RH; Boix-Chova, N; Gomez-Cabrero, D; Tegner, J; Abugessaisa, I; Conesa, A.
STATegra EMS: an Experiment Management System for complex next-generation omics experiments.
BMC Systems Biology Vol: 8, supl 2, s9 . 2014 Quartile: Q1

7 .Conesa, A; Mortazavi, A.
The common ground of genomics and systems biology. BMC Systems Biology Vol: 8, supl 2, S1 . 2014 Quartile: Q1

8 .Eduardo Larriba, María D.L.A. Jaime, José Carbonell-Caballero, Ana Conesa, Joaquín Dopazo, Corey Nislow, José Martín-Nieto, Luis Vicente Lopez-Llorca.
Sequencing and functional analysis of the genome of a nematode egg-parasitic fungus, Pochonia chlamydosporia.
Fungal Genet Biol Vol: 65, 69-80 . 2014 Quartile: Q1

9 .M.J. Nueda, S. Tarazona, and A. Conesa.
Next maSigPro: updating maSigPro Bioconductor package for RNA-seq time series.
Bioinformatics (Oxford, England) Vol: , . 2014 Quartile: Q1

10 .Almouzni, G; Altucci, L; Amati, B; Ashley, N; Baulcombe, D; Beaujean, N; Bock, C; Bongcam-Rudloff, E; Bousquet, J; Braun, S; Bressac-de Paillerets, B; Bussemakers, M; Clarke, L; Conesa, A; Estivill, X; Fazeli, A; Grgurevic, N; Gut, I; Heijmans, BT; Hermouet, S; Houwing-Duistermaat, J; Iacobucci, I; Ilas, J; Kandimalla, R; Krauss-Etschmann, S; Lasko, P; Lehmann, S; Lindroth, A; Majdic, G; Marcotte, E; Martinelli, G; Martinet, N; Meyer, E; Miceli, C; Mills, K; Moreno-Villanueva, M; Morvan, G; Nickel, D; Niesler, B; Nowacki, M; Nowak, J; Ossowski, S; Pelizzola, M; Pochet, R; Potocnik, U; Radwanska, M; Raes, J; Rattray, M; Robinson, MD; Roelen, B; Sauer, S; Schinzer, D; Slagboom, E; Spector, T; Stunnenberg, HG; Tiligada, E; Torres-Padilla, ME; Tsonaka, R; Van Soom, A; Vidakovic, M; Widschwendter, M.
Relationship between genome and epigenome - challenges and requirements for future research.
BMC genomics Vol: -, - . 2014 Quartile: Q1

11 .Ana Conesa and Rafael Hernández-de-Diego.
Omics Data Integration in Systems Biology: Methods and Applications.
Book: Applications of Advanced Omics Technologies: From Genes to Metabolites
Vol 64, Chap. 16 : 441-456 . 2014

12 .Su, ZQ; Labaj, PP; Li, S; Thierry-Mieg, J; Thierry-Mieg, D; Shi, W; Wang, C; Schroth, GP; Setterquist, RA; Thompson, JF; Jones, WD; Xiao, WH; Xu, WH; Jensen, RV; Kelly, R; Xu, J; Conesa, A; Furlanello, C; Gao, HL; Hong, HX; Jafari, N; Letovsky, S; Liao, Y; Lu, F; Oakeley, EJ; Peng, ZY; Praul, CA; Santoyo-Lopez, J; Scherer, A; Shi, T; Smyth, GK; Staedtler, F; Sykacek, P; Tan, XX; Thompson, EA; Vandesompele, J; Wang, MD; Wang, J; Wolfinger, RD; Zavadil, J; Auerbach, SS; Bao, WJ; Binder, H; Blomquist, T; Brilliant, MH; Bushel, PR; Cain, WM; Catalano, JG; Chang, CW; Chen, T; Chen, G; Chen, R; Chierici, M; Chu, TM; Clevert, DA; Deng, YP; Derti, A; Devanarayan, V; Dong, ZR; Dopazo, J; Du, TT; Fang, H; Fang, YX; Fasold, M; Fernandez, A; Fischer, M; Furio-Tari, P; Fuscoe, JC; Caimet, F; Gaj, S; Gandara, J; Gao, H; Ge, WG; Gondo, Y; Gong, BS; Gong, MH; Gong, ZL; Green, B; Guo, C; Guo, L; Guo, LW; Hadfield, J; Hellemans, J; Hochreiter, S; Jia, MW; Jian, M; Johnson, CD; Kay, S; Kleinjans, J; Lababidi, S; Levy, S; Li, QZ; Li, L; Li, L; Li, P; Li, Y; Li, HQ; Li, JY; Li, SY; Lin, SM; Lopez, FJ; Lu, X; Luo, H; Ma, XW; Meehan, J; Megherbi, DB; Mei, N; Mu, B; Ning, BT; Pandey, A; Perez-Florido, J; Perkins, RG; Peters, R; Phan, JH; Pirooznia, M; Qian, F; Qing, T; Rainbow, L; Rocca-Serra, P; Sambourg, L; Sansone, SA; Schwartz, S; Shah, R; Shen, J; Smith, TM; Stegle, O; Stralis-Pavese, N; Stupka, E; Suzuki, Y; Szkotnicki, LT; Tinning, M; Tu, BM; van Deft, J; Vela-Boza, A; Venturini, E; Walker, SJ; Wan, LQ; Wang, W; Wang, JH; Wang, J; Wieben, ED; Willey, JC; Wu, PY; Xuan, J; Yang, Y; Ye, Z; Yin, Y; Yu, Y; Yuan, YC; Zhang, J; Zhang, KK; Zhang, WQ; Zhang, WW; Zhang, YY; Zhao, C; Zheng, YT; Zhou, YM; Zumbo, P; Tong, WD; Kreil, DP; Mason, CE; Shi, LM. A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequencing Quality Control Consortium. NATURE BIOTECHNOLOGY Vol: , 32 (9), 903-914 . 2014 Quartile: Q1

13 .Sarah A. Munro, Steven P. Lund, P. Scott Pine, Hans Binder, Djork-Arné Clevert, Ana Conesa, Joaquin Dopazo, Mario Fasold,Sepp Hochreiter, Huixiao Hong, Nadereh Jafari, David P. Kreil, Paweł P. abaj, Sheng Li,Yang Liao, Simon M. Lin, Joseph Meehan, Christopher E. Mason, Javier Santoyo-Lopez, Robert A. Setterquist, Leming Shi, Wei Shi, Gordon K. Smyth, Nancy Stralis-Pavese, Zhenqiang Su, Weida Tong, Charles Wang, Jian Wang, Joshua Xu, Zhan Ye, Yong Yang, Ying Yu & Marc Salit . Assessing technical performance in differential gene expression experiments with external spike-in RNA control ratio mixtures.
Nature Comm Vol: 5, . 2014 Quartile: Q1

14 .Patricia Sebastian-Leon, Enrique Vidal, Pablo Minguez, Ana Conesa, Sonia Tarazona, Alicia Amadoz, Carmen Armero, Francisco Salavert, Antonio Vidal-Puig,David Montaner and Joaquín Dopazo. Understanding disease mechanisms with models of signaling pathway activities.
BMC Systems Biology Vol: 8:121, . 2014 Quartile: Q1

15 .Victoria Morin-Adeline, Rodrigo Lomas, Denis O'Meally, Colin Stack, Ana Conesa and Jan Slapeta. Comparative transcriptomics reveals striking similarities between the bovine and feline isolates of Tritrichomonas foetus: consequences for in silico drug-target identification. BMC Genomics Vol: 15, 955 . 2014 Quartile: Q1

Conferences and meetings

1. 2nd Biomarker Meeting: Personalized Reproductive Medicine
Poster
Ana Conesa
(Valencia , Spain)

2. EMBO/FEBS Lecture Course "Nuclear Proteomics"
Oral Communication
Ana Conesa
(Koos , Germany)

3. EpiConcept meeting "Epigenomic Toolbox: from Methods to Models"
Oral Communication
Ana Conesa
(Las Plamas de Gran canarias , Spain)

4. HitSeq 2014
Oral Communication
Lorena de la Fuente, Cristina Martí, Susana Rodríguez-Navarro, Victoria Moreno, Ana Conesa
(Boston , USA)

5. Plant Genomics Congress
Oral Communication
Ana Conesa
(London , United Kingdom)

5. Smodia 2014
Oral Communication
Sonia Tarazona, Ana Conesa, Mónica Clemente-Císcar
(Heraklion , Creta)

6. Smodia 2014
Lecture
Rafael Hernández
(Heraklion , Creta)

7. Statistical Methods for Omics Data Integration and Analysis
Organizing Comitte
Lecture
Ana Conesa
(Heraklion , Creta)

6. Statistical Methods for Omics Data Integration and Analysis
Oral communication
Rafael Hernández
(Heraklion , Creta)

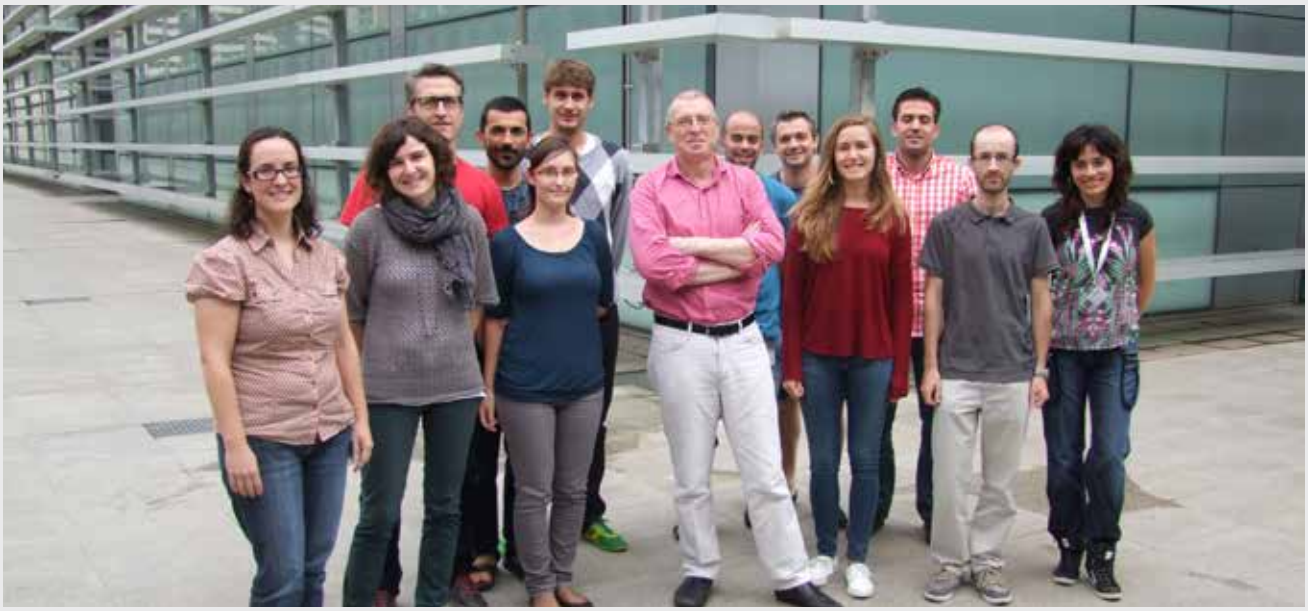
10. Statistical Methods for Omics Data Integration and Analysis
Oral communication
Sonia Tarazona
(Heraklion , Creta)

11. XII Jornadas Nacionales de Bioinformática 2014
Oral Communication
Ana Conesa
(Sevilla , Spain)

Doctoral Thesis presented

Doctoral Candidate: Sonia Tarazona Campos . Statistical methods for transcriptomics: From microarrays to RNA-seq . Director: Ana Conesa . Universidad Politecnica de Valencia, UPV

Systems Biology



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www.cipf.es/biologia-sistemas

Overview

Life sciences are increasingly becoming data-driven disciplines because of the massive application of the new generations of omic technologies. Therefore, innovative approaches in these areas revolve around the management and exploitation of genomic data from a Systems Biology perspective. Consequently, the general objective we seek is to relate variation at genomic level (punctual or structural variants, methylation changes, gene expression differences, etc.) to its consequences at both, cellular and phenotypic level, trying to understand the underlying mechanisms that govern the network of molecular interactions in the cell.

Our group carries out groundbreaking research by applying translational bioinformatics and integrative genomics to personalized medicine. The bioinformatics tools we develop allow converting omic data produced by the new high-throughput technologies into valuable, meaningful biomedical information that can be used for diagnostic and prognostic purposes.

We also apply the result of this research to other areas such as pharmacogenomics or agrogenomics. Our group is part of the National Institute of Bioinformatics (INB) through the Functional Genomics Unit and is also part of the CIBERER (Center in Network for the Study of Rare Diseases).

Research results

A number of factors will drive the future of computational biology research in the coming years. The most relevant among them will be a direct consequence of the foreseeable generation of enormous amounts of genomic data, the availability of an increasingly detailed knowledge of cellular function and the advent of new computational solutions to cope with the big data problem in genomics. The goal of the group is to be involved in the aspects, brought about by the big data revolution, with more potential of transformation of the way in which we understand research on the relationships between genotype and phenotype today.

Our group is an international leader in the development of algorithms for functional genomics as well as for the development of advanced software solutions in the area. Several large-scale projects, such as the Babelomics (see http://www.babelomics.org), the third most cited tool for the analysis and functional profiling of genomic experiments, have been developed and maintained for more than eight years by the group. These developments allowed us to participate in several initiatives, such as the FDA's SEQC[1].

Algorithms for genomic data

We are aware of the fact that the potential of discovery of the new generation sequencing technologies is hindered by the enormous difficulties associated to the storage management and interpretation of the data they produce. Therefore, we have been developing different tools for facilitating these tasks, such as TEAM, a web tool for the design and management of panels of genes for targeted enrichment and massive sequencing for clinical applications (Alemán et al., 2014, Nucleic Acids Res), BiERapp, a web-based interactive framework to assist in the prioritization of disease candidate genes in whole-exome sequencing (WES) studies (Alemán et al., 2014, Nucleic Acids Res), and HPGAligner, an ultrafast and efficient tool to map NGS sequencing reads (Tarraga et al., 2014 Bioinformatics). Probably, the development of highest impact was the genomic viewer Genome Maps (http://www.genomemaps.

1 http://www.fda.gov/downloads/ScienceResearch/BioinformaticsTools/MicroarrayQualityControlProject/ucm122981.pdf

org/), which enables the representation of different genomic features in the context of the genome using an intelligent technology based on Google Maps, which can interactively cope with huge data transfers. A proof of its efficiency is the fact that the International Cancer Genome Consortium (ICGC) has chosen Genome Maps as the official genome viewer of the consortium (see the ICGC data portal: http://dcc.icgc.org/), where it is used by thousands of researchers around the world.

Massive sequencing studies in hereditary diseases
Using all the developments for genomic data analysis, and given our involvement in the CIBERER and the Medical Genome Project (http://www.medicalgenomeproject.com/), where two projects for gene discovery in hereditary diseases were ongoing, we have participated in the analysis of numerous WES experiments of a large number of cases, some of which started to be published during 2014. For example, our analyses discovered new mutations with clear diagnostic potential in degenerative retinal dystrophies (de Castro-Miró M et al., 2014 PLoS ONE), Bardet–Biedl syndrome (González-del Pozo M et al., 2014 Molecular Genetics & Genomic Medicine), Overgrowth Syndrome (Tenorio J et al., 2014 Hum Mutat.), hereditary neuropathies (Calpena E et al., 2014 NMD) or neurobehavioral deficit (García-Cazorla A et al., 2014 Hum Mutat). And many more papers are expected in a near future, which will enormously increase the diagnostic possibilities for many diseases that, at present have a difficult diagnosis.

Genomic studies
Cancer is another research field related to genomic analysis in which the group has been involved in. During 2014 we have published studies on skin cancer (Puig-Butille JA et al., 2014 Oncotarget), DNA methylation profiles (Carmona JF et al., 2014 Cancer Res), and drug discovery (Dopazo J, 2014 Drug Discov. Today). We have also addressed a number of collaborative works with other laboratories in genomic analysis (Su Z et al., 2014 Nat. Biotechnol), (Lopez-Domingo et al., 2014 Bioinformatics), (Munro SA et al., 2014 Nat Commun.), and applied to different fields such as programmed cell death (Gutiérrez J et al., 2014 J. Exp. Bot), or parasitic fungus (Larriba E et al., 2014 Fungal Genet. Biol).

Systems biology
The most strategic aspect of our work in genomics is the interpretation of complex genomic data. Recently, we have focused on the study of the impact of gene deregulations or mutations in signaling pathways and the corresponding functional consequences. We developed a web tool that allows transforming genomic data into phenotypic consequences, revealing in this way details on the molecular mechanisms of the disease that otherwise would remain undiscovered (Sebastián-León et al., 2014 BMC Systems Biology). We have also studied from a systems biology point of view, the role of the interactome in the maintenance of deleterious variability in human populations (Garcia-Alonso L et al., 2014 Mol. Syst. Biol.). The Figure 1 shows the interactome distribution of the deleterious variants in normal populations, and also within germinal and somatic variants in CLL (chronic lymphocytic leukemia) patients.

Citric genome project
The group is working on the CITRUSEQ project, an initiative for sequencing, genotyping and development of tools for genetic improvement of citric varieties (http://www.citruseq.es/). We are currently analyzing over 300 genomes of more than 20 citric species detecting genes associated to traits of agricultural interest for genomic improvement purposes

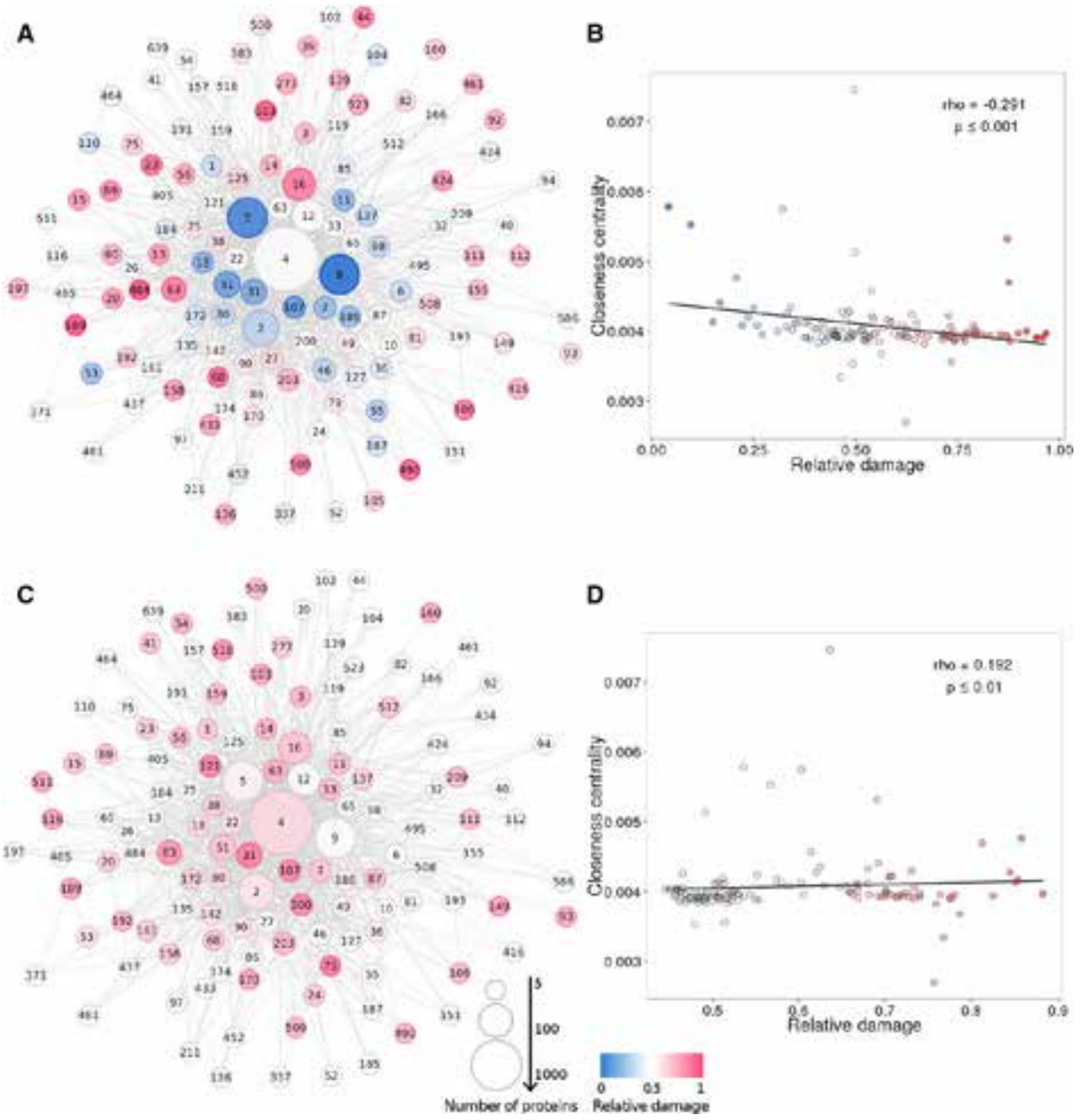

Figure 1. Interactome distribution of the deleterious variants in normal populations, and also within germinal and somatic variants in CLL (chronic lymphocytic leukemia) patients.

Projects and participation in initiatives

Related projects in which the group is involved (grants BIO2011-27069, PROMETEO/2010/001 and INNPACTO TRADION-P) deal with functional aspects of diseases, and aim to relate molecular defects in genes or their deregulations to the mechanism of disease using a systems biology perspective. The objectives of the projects were successfully completed, and all together resulted in 20 papers published in international journals during 2014. We also participated in an ITN Marie Curie on Machine Learning for Personalized Medicine (http://www.mlpm.eu/).
The group is an active member of the FGED (the Functional Genomics Data Society) Advisory board (http://www.mged.org/Board/advisory.html) and the ELIXIR (Member of the "Infrastructure for Tools Integration" committee, European initiative for the future of Bioinformatics in Europe, http://www.elixir-europe.org/). The group has also strategic alliances with international companies (Bull, INDRA, Roche and others), and jointly we have been developing large scale projects, such as FutureClinic, about the introduction of genomics data in the clinic (http://www.futureclinic.es/), the MGP (http://www.medicalgenomeproject.com/) and the CITRUSEQ. We have also been the promoters of the HPC4G (http://www.hpc4g.org) initiative to port genomic applications to a High Performance Computing (HPC) environment thus accelerating the process of genomic data analysis.

Publications

1 .de Castro-Miro, M; Pomares, E; Lores-Motta, L; Tonda, R; Dopazo, J; Marfany, G; Gonzalez-Duarte, R.
Combined Genetic and High-Throughput Strategies for Molecular Diagnosis of Inherited Retinal Dystrophies.
PloS one Vol: 9 (6). 2014 Quartile: Q1

2 .Puig-Butille, JA; Escamez, MJ ; Garcia-Garcia, F;Tell-Marti, G ; Fabra, A ; Martinez-Santamaria, L; Badenas, C ; Aguilera, P; Pevida, M; Dopazo, J; del Rio, M; Puig, S.
Capturing the biological impact of CDKN2A and MC1R genes as an early predisposing event in melanoma and non melanoma skin cancer.
Oncotarget Vol: 5 (6), 1439-1451 . 2014
Quartile: Q1

3 .Desoignies, N; Carbonell, J; Moreau, JS; Conesa, A; Dopazo, J; Legreve, A. Molecular interactions between sugar beet and Polymyxa betae during its life cycle. Annals of Applied Biology Vol: 164(2), 244-256 . 2014 Quartile: Q1

4 .Ponzoni, I; Nueda, MJ; Tarazona, S; Gotz, S; Montaner, D; Dussaut, JS; Dopazo, J; Conesa, A.
Pathway network inference from gene expression data.
BMC Systems Biology Vol: 8, supl 2, S7 . 2014
Quartile: Q1

5 .Eduardo Larriba, María D.L.A. Jaime, José Carbonell-Caballero, Ana Conesa, Joaquín Dopazo, Corey Nislow, José Martín-Nieto, Luis Vicente Lopez-Llorca.
Sequencing and functional analysis of the genome of a nematode egg-parasitic fungus, Pochonia chlamydosporia.
Fungal Genet Biol Vol: 65, 69-80 . 2014
Quartile: Q1

6 .Garcia-Cazorla, A; Oyarzabal, A; Fort, J; Robles, C; Castejon, E; Ruiz-Sala, P; Bodoy, S; Merinero, B; Lopez-Sala, A; Dopazo, J; Nunes, V; Ugarte, M; Artuch, R; Palacin, M; Rodriguez-Pombo, P.
Two Novel Mutations in the BCKDK (Branched-Chain Keto-Acid Dehydrogenase Kinase) Gene Are Responsible for a Neurobehavioral Deficit in Two Pediatric Unrelated Patients.
Human mutation Vol: 35, 470-477. 2014
Quartile: Q1

7 .Lopez-Domingo, FJ; Florido, JP; Rueda, A; Dopazo, J; Santoyo-Lopez, J.
ngsCAT: a tool to assess the efficiency of targeted enrichment sequencing.
Bioinformatics (Oxford, England) Vol: 30 (12), 1767-1768 . 2014 Quartile: Q1

8 .Aleman, A; Garcia-Garcia, F; Medina, I; Dopazo, J.
A web tool for the design and management of panels of genes for targeted enrichment and massive sequencing for clinical applications.
Nucleic acids research Vol: 42 (W1), W83-W87 . 2014 Quartile: Q1

9 .Alemán A, Garcia-Garcia F, Salavert F, Medina I, Dopazo J.
A web-based interactive framework to assist in the prioritization of disease candidate genes in whole-exome sequencing studies.
Nucleic acids research Vol: 42 (W1), W88-W93 . 2014 Quartile: Q1

10 .Gutierrez, J; Gonzalez-Perez, S; Garcia-Garcia, F ; Daly, CT ; Lorenzo, O ; Revuelta, JL ; McCabe, PF ; Arellano, JB.
Programmed cell death activated by Rose Bengal in Arabidopsis thaliana cell suspension cultures requires functional chloroplasts.
J. Exp. Bot Vol: 65 (12), 3081-3095 . 2014 Quartile: Q1

11 .Calpena E, Martínez-Rubio D, Arpa J, García-Peñas JJ, Montaner D, Dopazo J, Palau F, Espinós C.
A novel locus for a hereditary recurrent neuropathy on chromosome 21q21.
Neuromuscular disorders Vol: 24 (8), 660-665 . 2014 Quartile: Q2

12 .Tárraga J, Arnau V, Martínez H, Moreno R, Cazorla D, Salavert-Torres J, Blanquer-Espert I, Dopazo J, Medina I.
Acceleration of short and long DNA read mapping without loss of accuracy using suffix array.
Bioinformatics (Oxford, England) Vol: 30, 3396-3398 . 2014 Quartile: Q1

13 .Su, ZQ; Labaj, PP; Li, S; Thierry-Mieg, J; Thierry-Mieg, D; Shi, W; Wang, C; Schroth, GP; Setterquist, RA; Thompson, JF; Jones, WD; Xiao, WH; Xu, WH; Jensen, RV; Kelly, R; Xu, J; Conesa, A; Furlanello, C; Gao, HL; Hong, HX; Jafari, N; Letovsky, S; Liao, Y; Lu, F; Oakeley, EJ; Peng, ZY; Praul, CA; Santoyo-Lopez, J; Scherer, A; Shi, T; Smyth, GK; Staedtler, F; Sykacek, P; Tan, XX; Thompson, EA; Vandesompele, J; Wang, MD; Wang, J; Wolfinger, RD; Zavadil, J; Auerbach, SS; Bao, WJ; Binder, H; Blomquist, T; Brilliant, MH; Bushel, PR; Cain, WM; Catalano, JG; Chang, CW; Chen, T; Chen, G; Chen, R; Chierici, M; Chu, TM; Clevert, DA; Deng, YP; Derti, A; Devanarayan, V; Dong, ZR; Dopazo, J; Du, TT; Fang, H; Fang, YX; Fasold, M; Fernandez, A; Fischer, M; Furio-Tari, P; Fuscoe, JC; Caimet, F; Gaj, S; Gandara, J; Gao, H; Ge, WG; Gondo, Y; Gong, BS; Gong, MH; Gong, ZL; Green, B; Guo, C; Guo, L; Guo, LW; Hadfield, J; Hellemans, J; Hochreiter, S; Jia, MW; Jian, M; Johnson, CD; Kay, S; Kleinjans, J; Lababidi, S; Levy, S; Li, QZ; Li, L; Li, L; Li, P; Li, Y; Li, HQ; Li, JY; Li, SY; Lin, SM; Lopez, FJ; Lu, X; Luo, H; Ma, XW; Meehan, J; Megherbi, DB; Mei, N; Mu, B; Ning, BT; Pandey, A; Perez-Florido, J; Perkins, RG; Peters, R; Phan, JH; Pirooznia, M; Qian, F; Qing, T; Rainbow, L; Rocca-Serra, P; Sambourg, L; Sansone, SA; Schwartz, S; Shah, R; Shen, J; Smith, TM; Stegle, O; Stralis-Pavese, N; Stupka, E; Suzuki, Y; Szkotnicki, LT; Tinning, M; Tu, BM; van Deft, J; Vela-Boza, A; Venturini, E; Walker, SJ; Wan, LQ; Wang, W; Wang, JH; Wang, J; Wieben, ED; Willey, JC; Wu, PY; Xuan, J; Yang, Y; Ye, Z; Yin, Y; Yu, Y; Yuan, YC; Zhang, J; Zhang, KK; Zhang, WQ; Zhang, WW; Zhang, YY; Zhao, C; Zheng, YT; Zhou, YM; Zumbo, P; Tong, WD; Kreil, DP; Mason, CE; Shi, LM. A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequencing Quality Control Consortium. Nature Biotechnology, Vol: , 32 (9), 903-914 . 2014 Quartile: Q1

14 .Garcia-Alonso, L; Jimenez-Almazan, J; Carbonell-Caballero, J; Vela-Boza, A; Santoyo-Lopez, J; Antinolo, G; Dopazo, J.
The role of the interactome in the maintenance of deleterious variability in human populations. Molecular Systems Biology, Vol: 10 (9), 2014 Quartile: Q1

15 .Munro S.A., Lund S.P., Pine P.S, Binder H, Clevert DA, Conesa A, Dopazo J, Fasold M, Hochreiter S, Hong H, Jafari N, Kreil D.P, Łabaj P.P, Li S, Liao Y, Lin S.M, Meehan J, Mason C.E, Santoyo-Lopez J, Setterquist R.A, Shi L, Shi W, Smyth G.K, Nancy Stralis-Pavese N, Su Z, Tong W, Wang C, Wang J, Xu J, Ye Z, Yang Y, Yu Y, Salit M .
Assessing technical performance in differential gene expression experiments with external spike-in RNA control ratio mixtures.
Nature Comm Vol: 5, . 2014 Quartile: Q1

16 .Tenorio J, Mansilla A, Valencia M, Martínez-Glez V, Romanelli V, Arias P, Castrejón N, Poletta F, Guillén-Navarro E, Gordo G, Mansilla E, García-Santiago F, González-Casado I, Vallespín E, Palomares M, Mori MA, Santos-Simarro F, García-Miñaur S, Fernández L, Mena R, Benito-Sanz S, Del Pozo A, Silla JC, Ibañez K, López-Granados E, Martín-Trujillo A, Montaner D; The SOGRI Consortium, Heath KE, Campos-Barros A, Dopazo J, Nevado J, Monk D, Ruiz-Pérez VL, Lapunzina P. .
A New Overgrowth Syndrome is Due to Mutations in RNF125. Human mutation.
2014 Quartile: Q1

17 .Carmona FJ; Davalos, V; Vidal, E; Gomez, A; Heyn, H; Hashimoto, Y; Vizoso, M; Martinez-Cardus, A; Sayols, S; Ferreira, HJ; Sanchez-Mut, JV; Moran, S; Margel, M; Castella, E; Berdasco, M; Stefansson, OA; Eyfjord, JE; Gonzalez-Suarez, E ; Dopazo, J; Orozco, M ; Gut, IG; Esteller, M .
A Comprehensive DNA Methylation Profile of Epithelial-to-Mesenchymal Transition.
Cancer research Vol: 74 (19), 5608-5619 . 2014 Quartile: Q1

18 Sebastian-Leon P, Vidal E, Minguez P, Conesa A, Tarazona S, Amadoz A, Armero C, Salavert F, Vidal-Puig A, Montaner D, Dopazo J. Understanding disease mechanisms with models of signaling pathway activities. BMC Systems Biology Vol: 8:121, . 2014 Quartile: Q1

19 .Minguez P, Letunic I, Parca L, Garcia-Alonso L, Dopazo J, Huerta-Cepas J, Bork P. PTMcode v2: a resource for functional associations of post-translational modifications within and between proteins. Nucleic acids research , 2014 Quartile: Q1

20 . Iglesias JM , Leis Esnaola O, Perez Ruiz E, Gumuzio Barrie J, Garcia Garcia F, Aduriz A, Beloqui I, Hernandez Garcia S, Lopez M.P. Mato, Dopazo J, Pandiella A, Menendez J, Garcia Martin A.
The activation of the Sox2 RR2 pluripotency transcriptional reporter in human breast cancer cell lines is dynamic and labels cells with higher tumorigenic potential. Front. Oncol Vol: 4, 308 . 2014 Quartile:

21 .Torre I, González-Tendero A, García-Cañadilla P, et al.. Permanent cardiac sarcomere changes in a rabbit model of intrauterine growth restriction . PloSONE Vol: 9(11), :e113067 . 2014 Quartile: Q1

22 .Méndez-Vidal C, Bravo-Gil N, González-del Pozo M, Vela-Boza A, Dopazo J, Borrego S, Antiñolo G.
Novel RP1 mutations and a recurrent BBS1 variant explain the co-existence of two distinct retinal phenotypes in the same pedigree.
BMC Genetics Vol: 15, 143 . 2014 Quartile: Q1

23 .Calzada D, Aguerri M, Baos S, Montaner D, Mata M, Dopazo J, Quiralte J, Florido F, Lahoz C, Cárdaba B. Therapeutic targets for olive pollen allergy defined by gene markers modulated by Ole e 1-derived peptides.
Molecular immunology Vol: 64, 252-261 . 2014 Quartile:

24 .González-del Pozo M, Méndez-Vidal C, Santoyo-Lopez J, Vela-Boza A, Bravo-Gil N, Rueda A, García-Alonso L, Vázquez-Marouschek C, Dopazoj, Borrego S, Antiñolo G.
Deciphering intrafamilial phenotypic variability by exome sequencing in a Bardet–Biedl family. Molecular Genetics & Genomic Medicine Vol: 2, 124-133 . Pub year: 2014 Quartile: NO

25 .Dopazo J. Genomics and transcriptomics in drug discovery. Drug discovery today Vol: 19, 126-132 . Pub year: 2014 Quartile: Q1

Conferences and meetings

1. 19th Congress of the European Hematology Association
Oral Communication
Mariam Ibañez, Jose Carbonell-Caballero, Esperanza Such, Jorge Jimenez-Almazán, Eva Barragán, Enrique Vidal, Luz García-Alonso, Irene Luna, Ines Gómez-Seguí, María López-Pavía, Carmen M. Alonso, Eva Villamón, Iván Martín, Pau Montesinos, Miguel A. Sanz, Joaquín Dopazo, Jose Cervera
(Milano , Italy)

2. BIOSPAIN International Forum on Personalized Medicine
Lecture
Joaquin Dopazo
(Santiago de Compostela , Spain)

3. Critical Assessment of Gene Expression Data Analysis (CAMDA Conference 2014)
Oral Communication
Luz Garcia Alonso
(Boston , USA)

4. ECCB 14 - sbv IMPROVER Workshop
Poster
Alicia Amadoz
(Strasbourg, France)

5. I Conference of Integral research in Omic Sciences and Lifestyle, JICOVA
Oral Communication
Francisco García García
(Valencia Spain)

6. Plant & Animal Genome XXII (PAG)
Oral Communication
Javier Terol, Victoria Ibañez, Roberto Alonso, Jose Carbonell, Joaquin Dopazo, Manuel Talon
(San Diego , USA)

7. Plant & Animal Genome XXII (PAG)
Oral Communication
Manuel Talon, Javier Terol, VIctoria Ibañez Jose Carbonell, Roberto Alonso, Leandro Hueso, Concetta Licciardello, Ivo Gut, Joaquin Dopazo
(San Diego , USA)

9. The impact of genomics in translational medicine: present view
Joaquin Dopazo
(Barcelona , Spain)

10. The Systems Biology Modelling Cycle Course
Poster
Marta Bleda La Torre
(Hinxton , United Kingdom)

11. XII Symposium on Bioinformatics
Poster
Rosa D. Hernansaiz Ballesteror
(Sevilla , Spain)

12. XII Symposium on Bioinformatics
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Francisco Salavert
(Sevilla , Spain)

13. XII Symposium on Bioinformatics
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Alejandro Aleman
(Sevilla , Spain)

14.XII Symposium on Bioinformatics
Poster
Francisco García García
(Sevilla , Spain)

15. XII Symposium on Bioinformatics
Poster
Alicia Amadoz
(Sevilla , Spain)

Doctoral Thesis presented

Doctoral Candidate: Mª Luz García Alonso.
Aproximación integrativa a la comprension de los mecanismos de enfermedad desde la genómica y la biología de sistemas .
Director: Joaquín Dopazo. Universitat Politecnica de Valencia

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Program Coordinator: Susana Rodriguez-Navarro

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Gene expression coupled to Rna transport, led by Susana Rodriguez-Navarro

Oncogenic signalling, led by Rosa Farrás

Rho signalling in neurophatologies, led by Rosa Guasch

Rna modification and mitochondrial diseases, led by M Eugenia Armengod

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Overview

Molecular bases of the neurotoxicity and the neurological alterations associated with alcohol consumption

We are interested in the molecular and cellular actions of ethanol in the adult and developing brain. Prenatal alcohol exposure is a leading preventable cause of birth defects, mental retardation and neurodevelopmental disorders (FASD) including the foetal alcohol syndrome (FAS). The new pattern of binge-alcohol drinking during adolescence not only induces the neurotoxicity associated with behavioural and cognitive deficits, but also increases the vulnerability to alcohol dependence. Since, alcohol abuse can also induce brain damage and neurodegeneration, we are interested to investigate the molecular actions of ethanol in the adolescent and adult brain. We use neural cells in primary culture and animal models which mimic the alterations observed in alcohol-related pathologies, and we attempt to:

i) Study the differential molecular mechanisms of the ethanol actions on the immune receptors (TLRs, NLRs) in cortical glial primary culture.

ii) Assess the activation of the innate immune system in the brain damage and behavioral dysfunction induced by alcohol abuse. Thus, in our lab we also study the role of TLRs and the inflammasome in alcohol-induced inflammation.

iii) Investigate new serum biological markers, such as cytokines or chemokines, which could be associated with neuroinflammation to prevent further brain damage.

iv) Evaluate new treatments and/or drugs that can block neuroinflammation and neurodegeneration induced by ethanol abuse.

We hope that our research will contribute to the understanding of ethanol-related brain injury, and will provide clues for developing potential strategies with the aim of preventing, curtailing or even restoring ethanol-induced brain damage.

Research results

Role of mitochondria ROS generation in ethanol-induced NLRP3 inflammasome activation and cell death in astroglial cells

Toll-like receptors (TLRs) and NOD-like receptors (NLRs) are innate immunity sensors that provide an early/effective response to pathogenic or injury conditions. We have reported that ethanol-induced TLR4 activation triggers signaling inflammatory responses in glial cells, causing neuroinflammation and brain damage. However, it is uncertain if ethanol is able to activate NLRs/inflammasome in astroglial cells, which is the mechanism of activation, and whether there is crosstalk between both immune sensors in glial cells. We show that chronic ethanol treatment increases the co-localization of caspase-1 with GFAP(+) cells, and up-regulates IL-1ß and IL-18 in the frontal medial cortex in WT, but not in TLR4 knockout mice. We further show that cultured cortical astrocytes expressed several inflammasomes (NLRP3, AIM2, NLRP1, and IPAF), although NLRP3 mRNA is the predominant form. Ethanol, as ATP and LPS treatments, up-regulates NLRP3 expression, and causes caspase-1 cleavage and the release of IL-1ß and IL-18 in astrocytes supernatant. Ethanol-induced NLRP3/caspase-1 activation is mediated by mitochondrial (m) reactive oxygen species (ROS) generation because when using a specific mitochondria ROS scavenger, the mito-TEMPO (500 µM) or NLRP3 blocking peptide (4 µg/ml) or a specific caspase-1 inhibitor, Z-YVAD-FMK (10 µM), abrogates mROS release and reduces the up-regulation of IL-1ß and IL-18 induced by ethanol or LPS or ATP. Confocal microscopy studies further confirm that ethanol, ATP or LPS promotes NLRP3/caspase-1 complex recruitment within the mitochondria to promote cell death by caspase-1-mediated pyroptosis, which accounts for ff73% of total cell death (ff22%) and the remaining (ff25%) die by caspase-3-dependent apoptosis. Suppression of the TLR4 function abrogates most ethanol effects on NLRP3 activation and reduces cell death. These findings suggest that NLRP3 participates, in ethanol-induced neuroinflammation and highlight the NLRP3/TLR4 crosstalk in ethanol-induced brain injury.

Cytokines and chemokines as biomarkers of ethanol-induced neuroinflammation and anxiety-related behavior: Role of TLR4 and TLR2

Recent evidence supports the influence of neuroimmune system activation on behavior. We have demonstrated that ethanol activates the innate immune system by stimulating toll-like receptor 4 (TLR4) signalling in glial cells, which triggers the release of inflammatory mediators and causes neuroinflammation. The present study aimed to evaluate whether the ethanol-induced up-regulation of cytokines and chemokines is associated with anxiety-related behavior, 24 h after ethanol removal, and if TLR4 or TLR2 is involved in these effects. We used WT, TLR4-KO and TLR2-KO mice treated with alcohol for 5 months to show that chronic ethanol consumption increases the levels of cytokines (IL-1ß, IL-17, TNF-α) and chemokines (MCP-1, MIP-1α, CX3CL1) in the striatum and serum (MCP-1, MIP-1α, CX3CL1) of WT mice. Alcohol deprivation for 24 h induces IFN-γ levels in the striatum and maintains high levels of some cytokines (IL-1ß, IL-17) and chemokines (MIP-1α, CX3CL1) in this brain region. The latter events were associated with an increase in anxiogenic-related behavior, as evaluated by the dark and light box and the elevated plus maze tests. Notably, mice lacking TLR4 or TLR2 receptors are largely protected against ethanol-induced cytokine and chemokine release, and behavioral associated effects during alcohol abstinence. These data support the role of TLR4 and TLR2 responses in neuroinflammation and in anxiogenic-related behavior effects during ethanol deprivation, and also provide evidence that chemokines and cytokines can be biomarkers of ethanol-induced neuroimmune response

TLR4 elimination prevents synaptic and myelin alterations and long-term cognitive dysfunctions in adolescent mice with intermittent ethanol treatment

The adolescent brain undergoes important dynamic and plastic cell changes, including overproduction of axons and synapses, followed by rapid pruning along

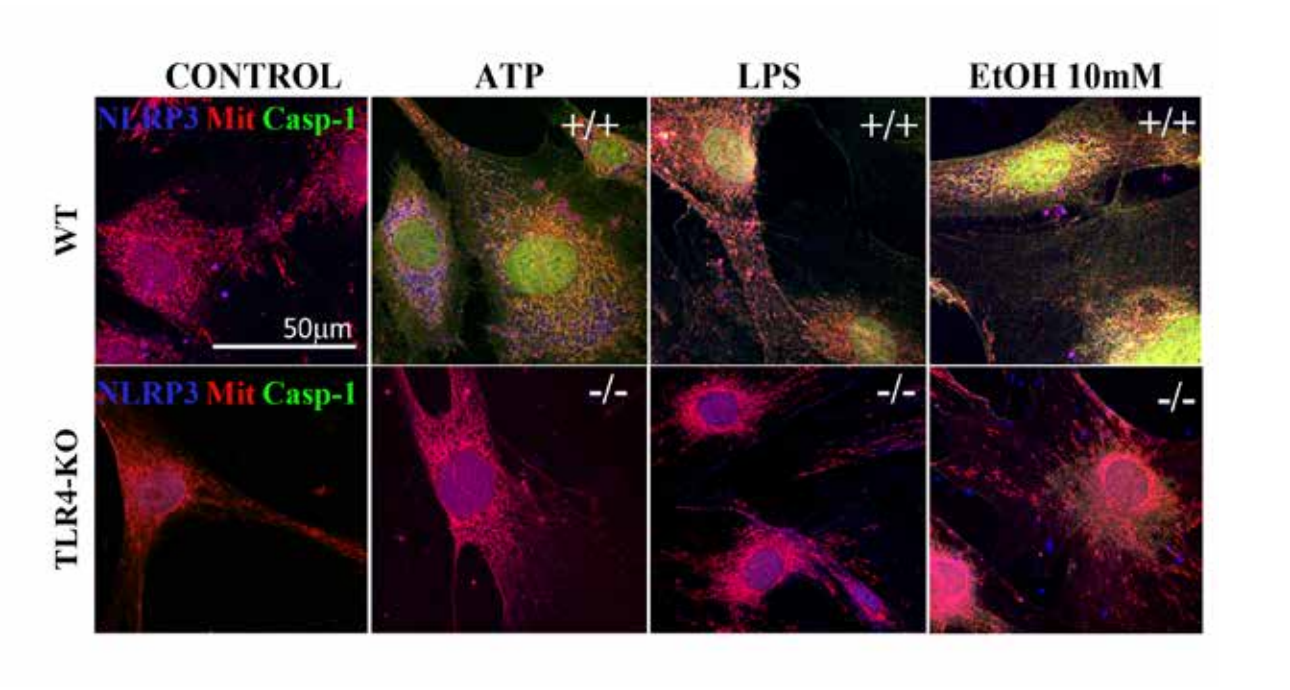

Figure 1: Confocal images of NLRP3/caspase-1 co-localization within mitochondria of the astrocytes treated with ethanol, ATP, or LPS.
Microphotographs show that the ATP, LPS, or ethanol (10 mM) treatments promote the co-localization of NLRP3 inflammasome (blue) with active caspase-1 (green) within mitochondria (red) in the WT-astrocytes when compared with the untreated control astrocytes. No significative changes were observed for treated and non-treated TLR4-KO astrocytes.

with ongoing axon myelination. These developmental changes make the adolescent brain particularly vulnerable to neurotoxic and behavioral effects of alcohol. Although the mechanisms of these effects are largely unknown, we demonstrated that ethanol by activating innate immune receptors toll-like receptor 4 (TLR4), induces neuroinflammation and brain damage in adult mice. The present study aims to evaluate whether intermittent ethanol treatment in adolescence promotes TLR4-dependent pro-inflammatory processes, leading to myelin and synaptic dysfunctions, and long-term cognitive impairments. Using wild-type (WT) and TLR4-deficient (TLR4-KO) adolescent mice treated intermittently with ethanol (3.0 g/kg) for 2 weeks, we show that binge-like ethanol treatment activates TLR4 signaling pathways (MAPK, NFkB) leading to the up-regulation of cytokines and pro-inflammatory mediators (COX-2, iNOS, HMGB1), impairing synaptic and myelin protein levels and causing ultrastructural alterations. These changes were associated with long-lasting cognitive dysfunctions in young adult mice, as demonstrated with the object recognition, passive avoidance and olfactory behavior tests. Notably, elimination of TLR4 receptors prevented neuroinflammation along with synaptic and myelin derangements, as well as long-term cognitive alterations. These results support the role of the neuroimmune response and TLR4 signaling in the neurotoxic and behavioral effects of ethanol in adolescence.

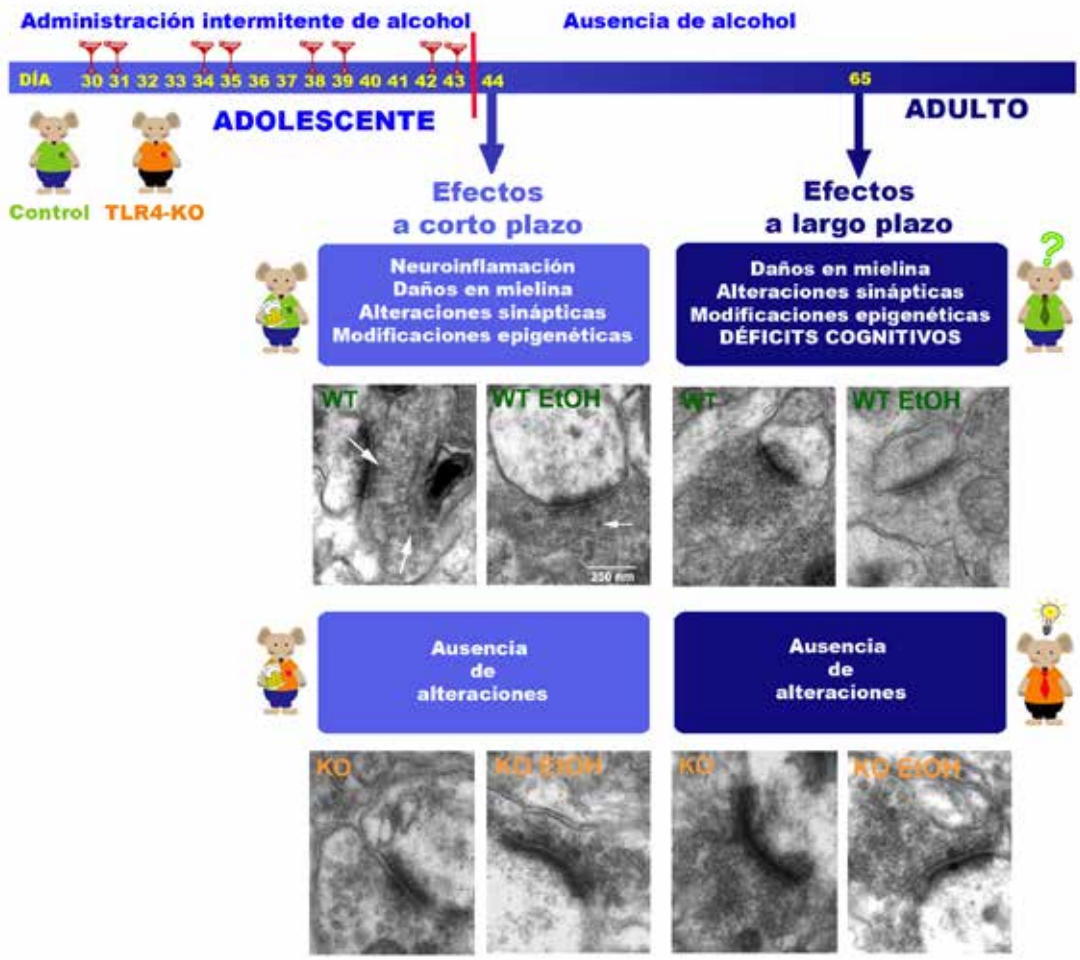

Figure 2 - Experimental animal model of intermittent alcohol exposure during the adolescence: Role of the neuroimmune response and TLR4 in alcohol-induced short- and long-term effects.
The transmission electron micrographs show that ethanol decreases the vesicle number, causes thickness of postsynaptic density, and increases the synaptic cleft width in the PFC of WT mice treated during the adolescence, whereas no changes were observed between ethanol-treated or not treated TLR4-KO mice. Some of these ultraestructural and behavioural alterations were maintained in adults animals (long-term) exposed to ethanol during adolescence

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Publications

1 .Pascual-Lucas, M; Fernandez-Lizarbe, S; Montesinos, J; Guerri, C.
LPS or ethanol triggers clathrin- and rafts/caveolae-dependent endocytosis of TLR4 in cortical astrocytt.
Journal of Neurochemistry Vol: 129(3), 448-462 . 2014 Quartile: Q2

2 .Pla, A; Pascual, M; Guerri, C.
Chronic Ethanol Treatmentt impairs the protesome and autophagy pthaways in the brain: role of TLR4
Alcoholism, clinical and experimental research Vol: 38, 247A-247A . 2014 Quartile: Q1

3 .Guerri, C; Alfonso-Loeches, S; Montesinos, J; Pla, A; Pascual, M.
Ethanol promotes tlr4 signaling and myelin disruption by interacting with membrane "lipid rafts"
Alcoholism, clinical and experimental research Vol: 38, 2014 Quartile: Q1

4 .Araos P; Pedraz M; Serrano A; Lucena M; Barrios V; García-Marchena N; Campos-Cloute R; Ruiz JJ; Romero P; Suárez J; Baixeras E; de la Torre R;Montesinos J,; Guerri C; Rodríguez-Arias M; Miñarro J.; Martínez-Riera R; Torrens M; Chowen JA; Argente J; Mason BJ; Pavón FJ; Rodríguez de Fonseca F.
Plasma profile of pro-inflammatory cytokines and chemokines in cocaine users under outpatient treatment: influence of cocaine symptom severity and psychiatric co-morbidity
Addiction Biology , 2014 Quartile: Q1

5 .Alfonso-Loeches S; Ureña-Peralta J; Morillo MJ; Oliver de la Cruz, J; Guerri C.
Role of mitochondria ROS generation in ethanol-induced NLRP3 inflammasome activation and cell death in astroglial cells.
Frontiers in Cellular Neuroscience Vol: 8, 2014 Quartile: Q1

6 .Pla A.; Pascual M.; Renau-Piqueras J.; Guerri C.
TLR4 mediates the impairment of ubiquitin-proteasome and autophagy-lysosome pathways induced by ethanol treatment in brain.
Cell death & disease Vol: 5, e1066. 2014 Quartile: Q2

7 .Ledesma, JC; Miquel, M; Pascual, M; Guerri, C; Aragon, CMG.
Induction of brain cytochrome P450 2E1 boosts the locomotor-stimulating effects of ethanol in mice.
Neuropharmacology Vol: 85, 36-44 . 2014 Quartile: Q1

8 .Montesinos J; Pascual M; Pla A; Maldonado C; Rodríguez-Arias M; Miñarro J; Guerri C..
TLR4 elimination prevents synaptic and myelin alterations and long-term cognitive dysfunctions in adolescent mice with intermittent ethanol treatment.
Brain Behav Immun 2014 Quartile: Q1

Conferences and meetings

1. 37th Annual RSA Scientific Meeting and 17th Congress of ISBRA . Oral Communication .Guerri C., Alfonso-Loeches S, Montesinos J., A. Pla, M. Pascual (San Frascisco , USA)

2. 9th European Workshops on Cell Death: 9th Death with Aphrodite(EWCD) . Oral Communication .G. García-Lainez, S. Alfonso-Loeches, C. Guerri, E. Pérez-Payá and M. Orzáez
(Paphos , Chipre)

3. International Congress of Alcohol Research . Invited Conference
Consuelo Guerri Sirera
(Seatle , USA)

4. Natiional Conferences on Socidrogalcohol
Invited Conference
Consuelo Guerri Sirera
(Sevilla , Spain)

5. XVI Congress on Dual Pathology
Invited Conference .Guerri Sirera, Consuelo
(Valencia , Spain)

6. 35th SCongress of the Spanish Society of Pharmacology
Invited Conference
Consuelo Guerri Sirera
(Madrid , Spain)

7. XXXVII SEBBM
Poster
Jorge Montesinos, Antoni Pla, María Pascual, Anabel Gil, Clara Guasch and Consuelo Guerri (Granada , Spain)

8. IBV (Instituto de Biomedicina, CSIC) .
Lecture
Consuelo Guerri Sirera
(Valencia , Spain)

9. I Biomedicine Predocs Congress
Organizing Comittee .
Jorge Montesinos Selfa
(Valencia , Spain)

Doctoral Thesis presented

Doctoral Candidate: Antoni Pla Rodriguez.
Importancia de los mecanismos de degradación de proteinas en la neurodegeneración causada por el abuso del alcohol:papel de los receptores TLR4 .
Director: Consuelo Guerri . Universidad de Valencia, UV

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Overview

Biogenesis of eukaryotic messenger ribonucleoprotein particles is a crucial event in the gene expression pathway. Over the past fifteen years, studies in many laboratories have uncovered that during mRNA biogenesis most steps are coupled to events both "up-stream" and "down-stream". Nevertheless, many molecular and functional aspects of the process remain unknown and this is one of the most intriguing and hot topic of basic molecular and cellular biology research. All the new discoveries allow establishing new hypotheses of how gene expression works in living eukaryotic cells through interconnected/coupled steps. Thus, it is essential to describe how these events are interconnected to have a complete picture of gene expression.
A way to tackle this question is by finding the connectors. Our research has been focused on understanding to which extend Sus1, a functional component of the SAGA and the TREX-2 complexes, plays a connector role. Sus1 holds some interesting features that make it suitable to help us to discover fundamental molecular concepts underpinning the regulation of gene expression. First, its involvement in different steps of the gene expression pathway provides a perfect platform to search for novel factors and activities to regulate the coupling. Secondly, its high degree of conservation, both in terms of sequence and function makes our studies easily transposable to higher eukaryotes. We ignore how dysfunction of these machineries leads to pathological states. Actually, mutations in SAGA and TREX-2 factors contribute to human diseases that range from Spinocerebellar ataxia type 7 and cancer, to diabetes. We are interested in: i) Novel interactions with regulatory implications (unexpected players) ii) Novel activities/roles (unexpected functions) and iii) Deciphering the role of the SAGA dependent deubiquitination in Spinocerebellar Ataxia type 7. In all, this result in a significant advance in the state of the art of the gene expression regulation process.

Research results

1. Unveiling novel interactions of histone chaperone Asf1 linked to TREX-2 factors Sus1 and Thp1. *(Nucleus Jun 2014*, FIGURE 1)
Anti-silencing function 1 (Asf1) is a conserved key eukaryotic histone H3/H4 chaperone that participates in a variety of DNA and chromatin-related processes. Asf1 impacts on many aspects of DNA metabolism. To gain insights into the functional links of Asf1 with other cellular machineries, we employed mass spectrometry coupled to tandem affinity purification (TAP) to investigate novel physical interactions of Asf1. Asf1 co-purifies with several subunits of the TREX-2, SAGA complexes, and with nucleoporins Nup2, Nup60, and Nup57, which are all involved in transcription coupled to mRNA export in eukaryotes. Reciprocally, Thp1 and Sus1 interact with Asf1. Albeit mRNA export and GAL1 transcription are not affected in asf1Δ a strong genetic interaction exists between ASF1 and SUS1. Notably, supporting a functional link between Asf1 and TREX-2, both Sus1 and Thp1 affect the levels of Asf1-dependent histone H3K56 acetylation and histone H3 and H4 incorporation onto chromatin. Additionally, we provide evidence for a role of Asf1 in histone H2B ubiquitination. This work proposes a functional link between Asf1 and TREX-2 components in histone metabolism at the vicinity of the nuclear pore complex.

2. Different RNA structures modulate expression of the mRNA biogenesis factor Sus1.
Unlike most yeast genes, the *SUS1* pre-mRNA of Saccharomyces cerevisiae contains two introns and is alternatively spliced, retaining one or both introns in response to changes in environmental conditions. *SUS1* splicing may allow the cell to control Sus1 expression, but the mechanisms that regulate this process remain unknown. In collaboration with Dr Gallego (UCV, Valencia, Spain) using *in silico* analyses together with NMR spectroscopy, gel electrophoresis and UV denaturation experiments, we show that the downstream intron (I2) of *SUS1* forms a weakly-stable, 37-nucleotide stem-loop structure containing the branch site near its apical loop and the 3' splice site after the stem terminus.
A cellular assay revealed that three mutants containing altered I2 structures had significantly impaired *SUS1* expression. Semi-quantitative RT-PCR experiments indicated that all mutants accumulated unspliced *SUS1*

Figure 1 - A network of asf1 interactions identified by TaP-MS analyses. The interaction network was generated from the evidence view of the String interactions with a medium confidence score (0.04). Different line colors represent the types of evidence for the association. Components of the SaGa, TreX-2, HIr, and NPC are indicated.

pre-mRNA and/or induced distorted levels of fully spliced mRNA relative to wild-type. This work demonstrates that I2 structure is relevant for *SUS1* expression, and that this effect is partly exerted through modulation of splicing.

3. Genome-wide association of Sus1 to RNAPII and tRNAs genes depends on SAGA distinctive subunits Spt8 and Spt7 (FIGURA 2)

In this study, in collaboration with Dr Conesa lab (CIPF, Valencia), we show for the first time the recruitment of a SAGA/SLIK factor, Sus1 to the transcribed regions of most RNAPII dependent genes. Sus1 accumulates preferentially towards 3' end regions of both TFIID and SAGA dominated genes and this correlates with their transcription rate. SAGA distinctive subunits, Spt8 and the full Spt7 isoform are required for correct Sus1 recruitment, and both factors have an impact on Sus1 binding upon acute temperature stress. Notably, the transcriptional profile of spt7-1180 mutant revealed an unanticipated role in cryptic transcription of RNAPII genes genome-wide. Finally, we show that Sus1 is not restricted to RNAPII-dependent genes, it also binds specifically to tRNA genes. Strikingly, absence of Spt8, Spt7 and Sus1 impacts on expression of most tRNAs in yeast.

4. Deciphering the role of the DUBm in Spinocerebellar Ataxia type 7 (SCA7)

The discovery of Sgf73 as part of SAGA and its further functional characterization both in yeast and animals have opened many lines of more biomedical-oriented research to address the molecular bases of SCA7. Consequently, we started some pilot experiments to use yeast as a model system for understanding **the role of the DUBm in the ethiology** of this human disease. As part of our current project, my lab is currently collaborating with Dr La Spada (UCSD,USA), one of the most outstanding leaders working in polyglutamine disorders to address the contribution of Sus1/ENY2 to SCA7 disease. As part of this collaboration the PhD student Paula Oliete has pursued a 4 months stay Sept-Oct 2014 in La Spada laboratory.

5. Generation of a Rare disease database with yeast homologues: RAREyeast database

Rare diseases (RD) are those which have low incidence in the population (affecting less than 1 in 2,000), thus making its research difficult and tricky, and making evident the necessity to find new approaches to them. Up to the moment, thousands of genes have been described to be involved in RD (over 7,000 according to WHO), and studying their orthologous counterparts in model organisms could be the key in the understand-

ing of the role these genes both in normal and pathological situations. In order to resolve this, we are developing in collaboration with Dr. Ana Conesa's Lab, a web database relating human disease and related genes, according to Online Mendelian Inheritance in Man (OMIM) and Orphanet, and their identified true orthologous genes in the yeast model organisms. This will yield assignments of orthologs in model organisms networking with human RD, providing invaluable information for scientific community, thus forming the database RAREyeast.

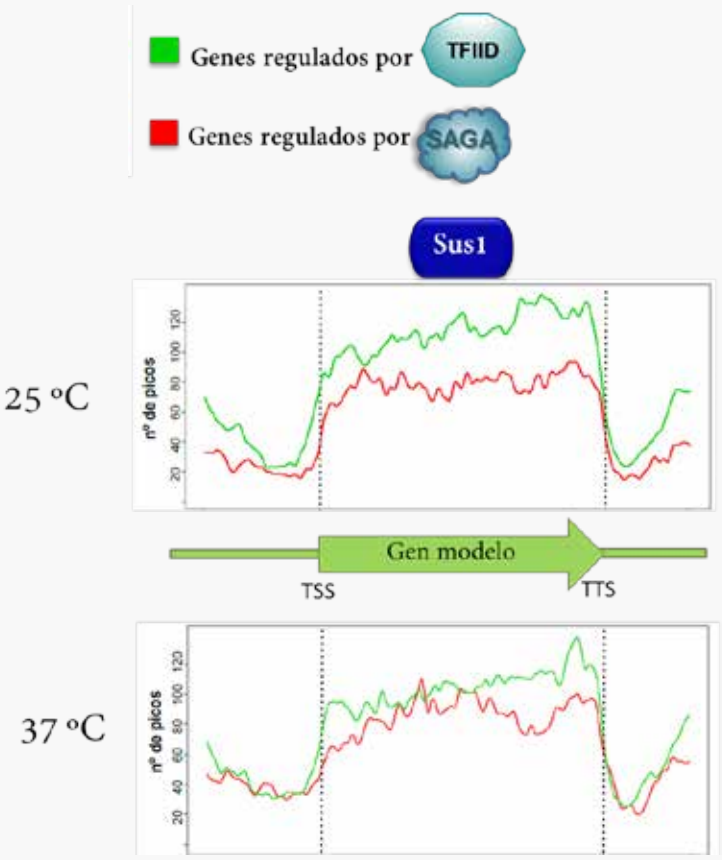


Figure 2 - Genome-wide association of Sus1 to RNAPII dependent genes

Publications

1 . Pamblanco M, Oliete-Calvo P, García-Oliver E, Valero M.L, Sanchez del Pino M.M, Rodríguez-Navarro S. Unveiling novel interactions of histone chaperone Asf1 linked to TREX-2 factors Sus1 and Thp1. Nucleus Vol: 5(3), 247 - 259 2014 Quartile: Q3

Conferences and meetings

1. EMBO Conference "Gene transcription in yeast: From regulatory networks to mechanisms
Oral Communication
Paula Oliete Calvo
(Sant Feliu de Guixols , Spain)

2. HitSeq 2014 . Oral Communication . Lorena de la Fuente, Cristina Martí, Susana Rodríguez-Navarro, Victoria Moreno, Ana Conesa
(Boston , USA)

3. XXXVII SEBBM
Poster
Manuel Martín Expósito, Susana Rodríguez Navarro
(Granada , Spain)

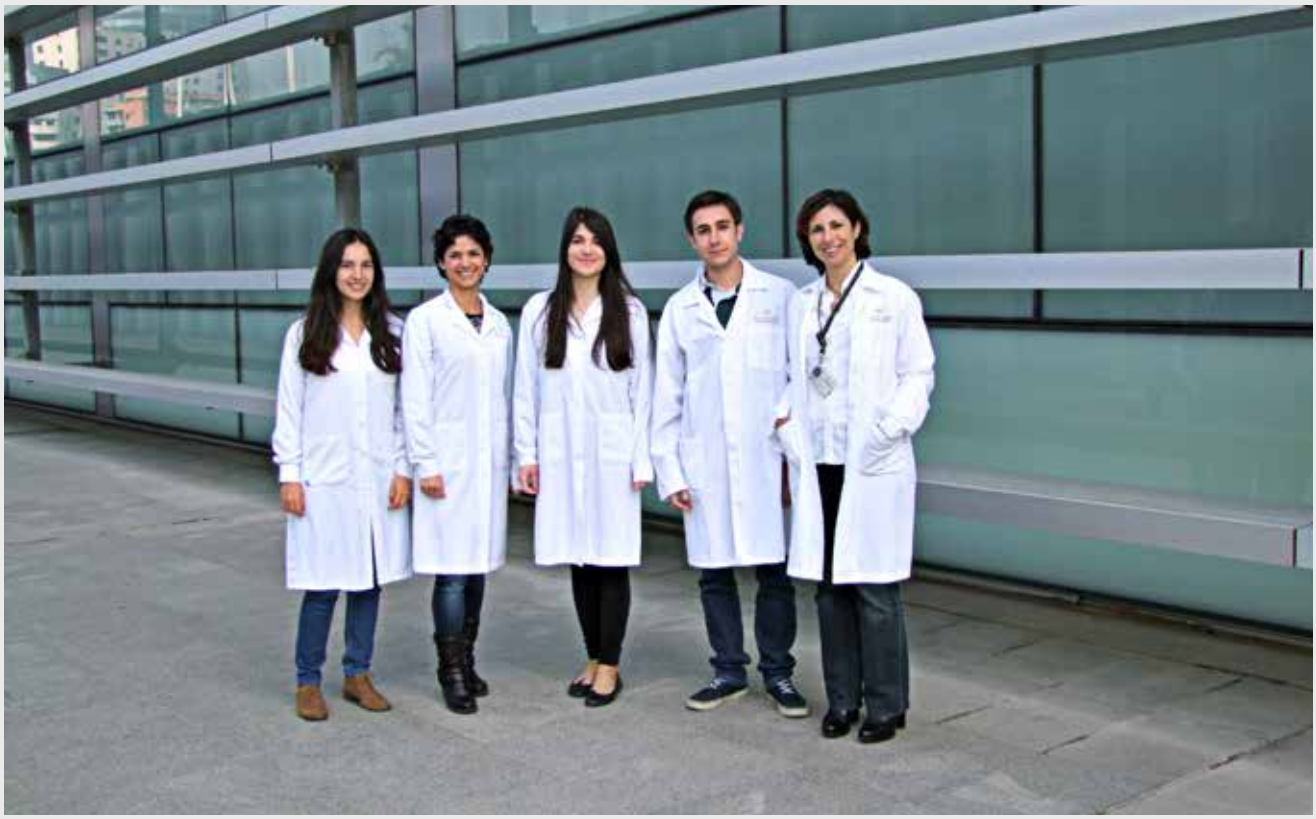
4. XXXVII SEBBM
Oral Communication
Manuel Martín Expósito ,Susana Rodríguez Navarro
(Granada , Spain)

5. I Biomedicine Predocs Congress
Organizing Comittee
Manuel Martín Exposito
(Valencia , Spain)

Doctoral Thesis presented

Doctoral Candidate: Varinia García Molinero Estudio Funcional de factores implicados en la transcripción y exportación de los RNAs en S. cerevisiae . Director: Susana Rodríguez-Navarro . Universidad de Valencia, UV

Oncogenic Signalling



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→Collaborators

José Miguel Pardo (UPV)

Borja Belda Palazón (CSIC)- UPV)

www.cipf.es/senalizacion-oncogenica

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Overview

Our laboratory is focused on the study of the molecular biology of cancer. On the one hand, we investigate the signalling networks and post-translational modifications that control the abundance of cell cycle regulatory proteins. We study how dysregulation of these processes lead to accelerated cell growth and enables cancer cells to adapt to their environment. On the other hand, we perform translational biomedical research. In this case in collaboration with hospitals we study the molecular characterization of tumor-derived cancer stem cells from patients with lung cancer. The discovery of a small population of cells with stem cell properties (cancer stem cell, CSC) has led to the beginning of a new emerging area in cancer research. It has been determined that the CSCs are the first components of the tumors which result in tumor progression and metastasis. In addition to its ability to self-renew and differentiate, these cells are resistant to conventional chemotherapy. CSCs therefore promise to be a novel target with therapeutic potential. Our goal is to analyse the transcriptome, the proteome and the phospho-oncogenic signalling pathways activated in these cells and to generate xenograft mouse models by injection of CSCs derived from patient tumors for research and design of personalized therapies.

Research results

1. Emerging roles of AP-1 transcription factor complex in the control of S phase progression in cancer cells (Figure 1)

JunB is a component of the AP-1 transcription factor complex, which consists of homo- and heterodimers of variety of bZip proteins. The AP-1 transcription factor is essential for cells to integrate a wide array of stimuli and particularly mitogenic growth factor stimulation. AP-1 proteins, most of which are metabolically unstable, are also essential effectors of neoplasic transformation in numerous situations. JunB acts either as a tumor suppressor or an oncogene depending on the cell context. Its overexpression is linked to lymphomagenesis, although the mechanisms whereby JunB promotes neoplastic growth are still largely unknown. JunB expression is cell cycle regulated; being high in S phase and low in G2/M. We have characterized a critical consensus phosphodegron that controls JunB turnover and identified GSK3 and SCFFBXW7 as, respectively, the kinase and the E3 ubiquitin ligase responsible for its degradation in G2. Moreover, we have show that this mechanism is altered in some cancers. Specifically, we have demonstrated that junB is not degraded properly, and is overexpressed in non-Hodgkin lymphomas, a cancer of the lymphatic system that affects white blood cells, and can develop in any organ of the body. Overexpression of JunB is associated to alterations in the control of cell cycle progression, and causes chromosomal instability. Results obtained in our laboratory show that silencing of JunB in tumorigenic cell lines results in a decreased number of cells in S phase and G1 phase arrest. In order to identify novel transcriptional targets of JunB that may clarify the molecular mechanism behind this phenotype we have performed ChIP-seq analysis in combination with transcriptomic analysis in osteosarcoma-derived U2OS cells. We have identified 2900 target genes that carry out JunB binding sites close to the transcriptional start site. Of these, 230 genes are involved in cell cycle regulation. Among the putative JunB target genes identified we have identified several genes playing important roles in preventing cell cycle progression. As cell cycle progression is essential for cell proliferation the repression of these cell cycle regulators genes by JunB represents a new mechanism by which JunB may contribute to tumorigenesis.
This work has been done in collaboration with the laboratory of Dr. Marc Piechaczyk at the the Institute of Molecular Genetics of Montpellier (IGMM), France.

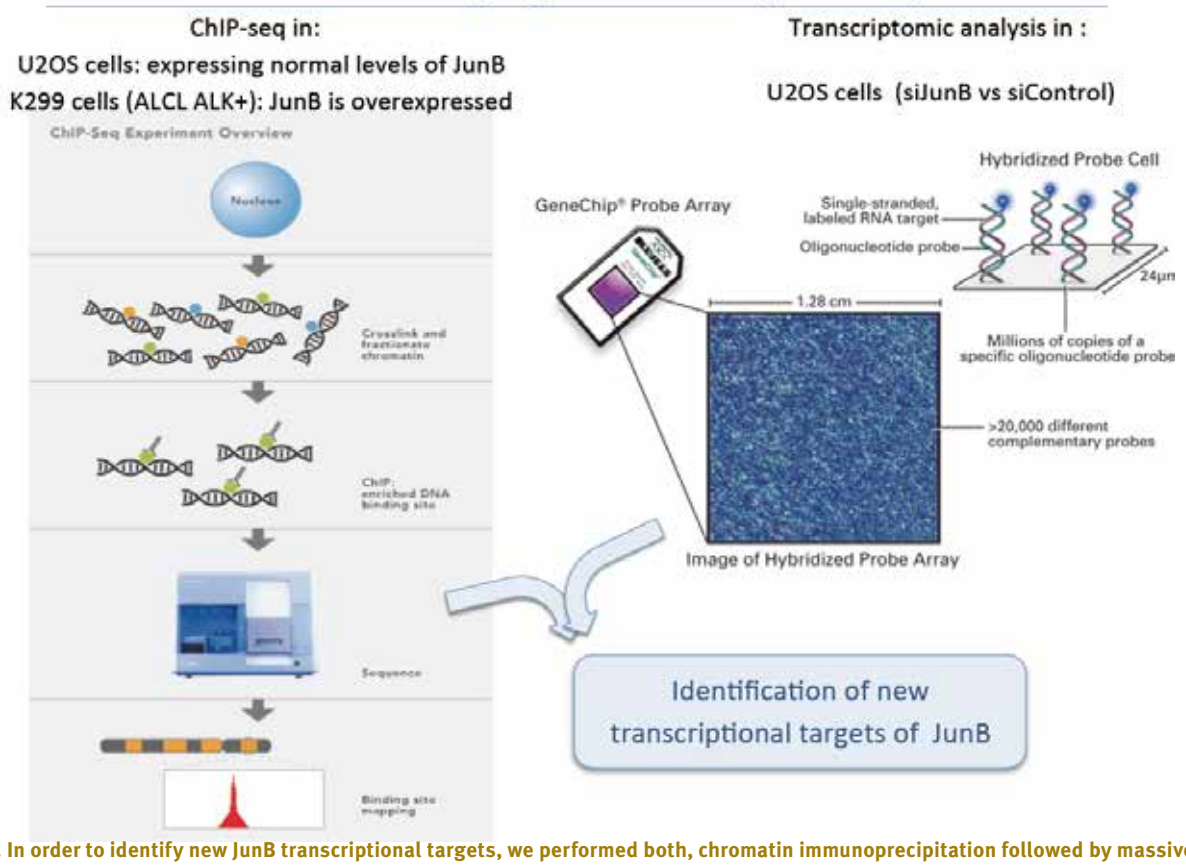


Figure 1. In order to identify new JunB transcriptional targets, we performed both, chromatin immunoprecipitation followed by massive sequencing and transcriptome analysis in cancer cells with genetically inactivated JunB. The ChIP-seq technique will identify genes containing JunB binding sites and the transcriptome analysis a list of genes whose expression is regulated by JunB. The combination of both sort of data will allow us to identify new JunB transcriptional targets genes.

2. Isolation, identification and characterization of Cancer Stem Cells (Figure 2 & 3)

In our laboratory we characterize the subpopulation of cancer stem cells derived from tumors of patients with non-small cell lung cancer (NSCLC). We obtained biopsies from patients with non-squamous NSCLC, resectable and that had not been treated previously. The samples were characterized histologically and determined whether mutations in EGFR, BRAF, ALK, and KRAS genes are present. Tumor-derived single cells and adjacent normal tissue have been labeled with antibodies against specific stem cell surface markers and lineage markers and analyzed by flow cytometry. The expression of many surface molecules varies between the samples, which can be monitored using multiparametric flow cytometry. We have used a 4-color immunophenotyping antibody panel, against surface protein markers, for studying the different cell subpopulations. Immunophenotypic polychromatic analysis shows the high heterogeneity of the tumor samples for certain surface markers and differences of expression between cells from normal tissue and tumor.
Isolated cells from patient tumors were seeded in low adhesion to favor the formation of tumor spheres. These cells have been further analyzed and we have observed an enrichment in specific cell surface markers, indicating that this subpopulation has self-renewal capacity.
Furthermore, the self-renewal, differentiation and tumorigenicity capacity of tumor spheres derived from patients and different cell lines have been further analyzed by injection in NOD/SCID mice at subclonal concentrations in order to generate xenograft models that recapitulate the parent tumor.

This work has been done in collaboration with the laboratory of Dr. Carlos Camps at the Research Foundation of the General University Hospital of Valencia.

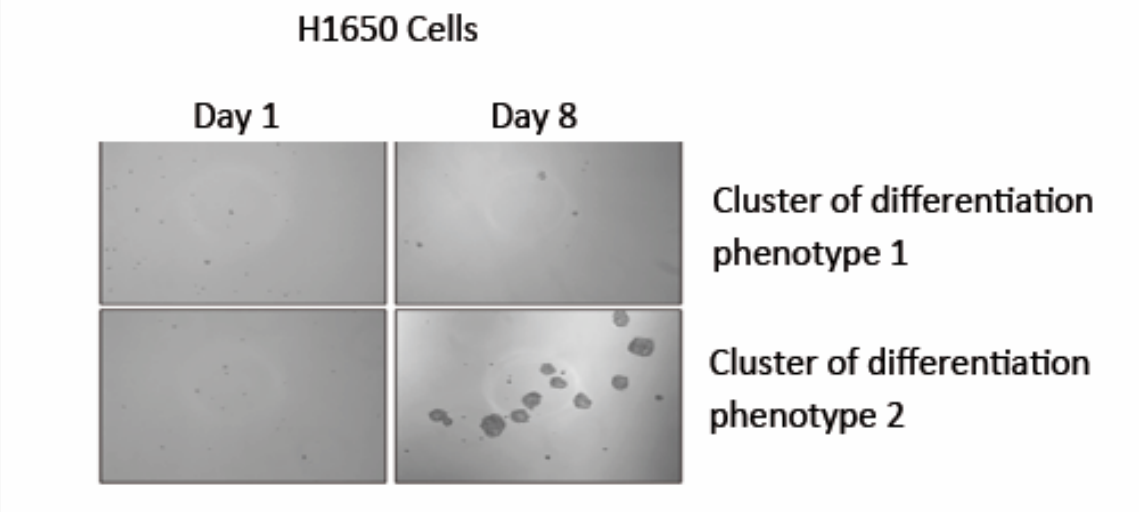


Figure 2. NSCLC H1650 cells were labeled with stem cell specific surface markers. The different subpopulations were separated by cell sorter. Only a specific cluster of differentation (CD) subpopulation is capable of inducing the formation of tumor spheres in culture in vitro, demonstrating its ability for self-renewing.

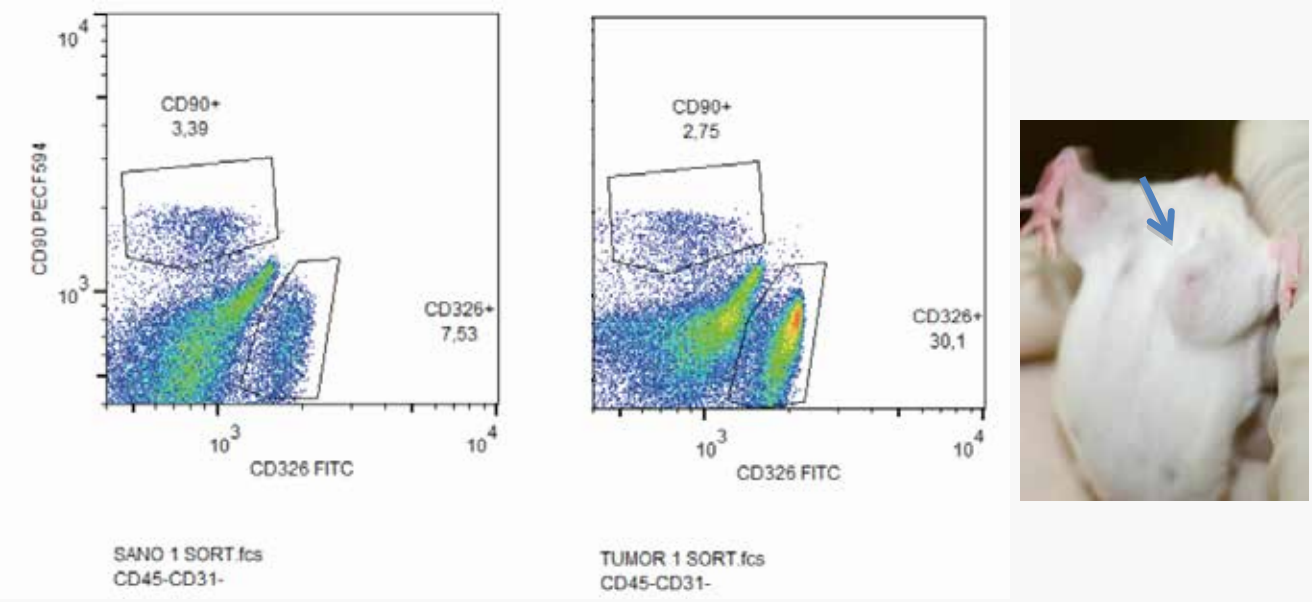


Figure 3. Histogram of single cells obtained from a NSCLC tumor and adjacent normal tissue labeled with antibodies against surface markers. B. Mouse xenograft model generated by injecting tumor spheres derived from a NSCLC tumor. The tumor formation shows the tumor initiating capacity of these cells.

Publications

1 .Lang, V; Pallara, C; Zabala, A; Lobato-Gil, S; Lopitz-Otsoa, F; Farras, R; Hjerpe, R; Torres-Ramos, M; Zabaleta, L; Blattner, C; Hay, RT; Barrio, R; Carracedo, A; Fernandez-Recio, J; Rodriguez, MS; Aillet, F.
Tetramerization-defects of p53 result in aberrant ubiquitylation and transcriptional activity.
Molecular Oncology, Vol: 8(5), 1026-1042 .
Quartile: Q1

2 .Martinez-Romero, A; Pardo, JM; Tejedor, S; Farinas, SC; Lucas, R; Figueroa, S; Jantus-Lewintre, E; Camps, C; Farras, R.
Multi-color flow cytometry immunophenotyping for detection of CSC in NSCLC.
European Journal of Cancer, Vol: 50, 115-115
Quartile:

3. Silvia Calabuig-Fariñas, Eloisa Jantus-Lewintre, Rut Lucas, Rosa Farràs, Marta Usó, Miguel Martorell, Eleonora Chakarova Sabeva, Ana Blasco, Santiago Figueroa, Carlos Camps
Tumor expression levels of CSC markers in resectable non-small cell lung cancer
Meeting Abstract
Journal of Clinical Oncology 32:5s,.

Conferences and meetings

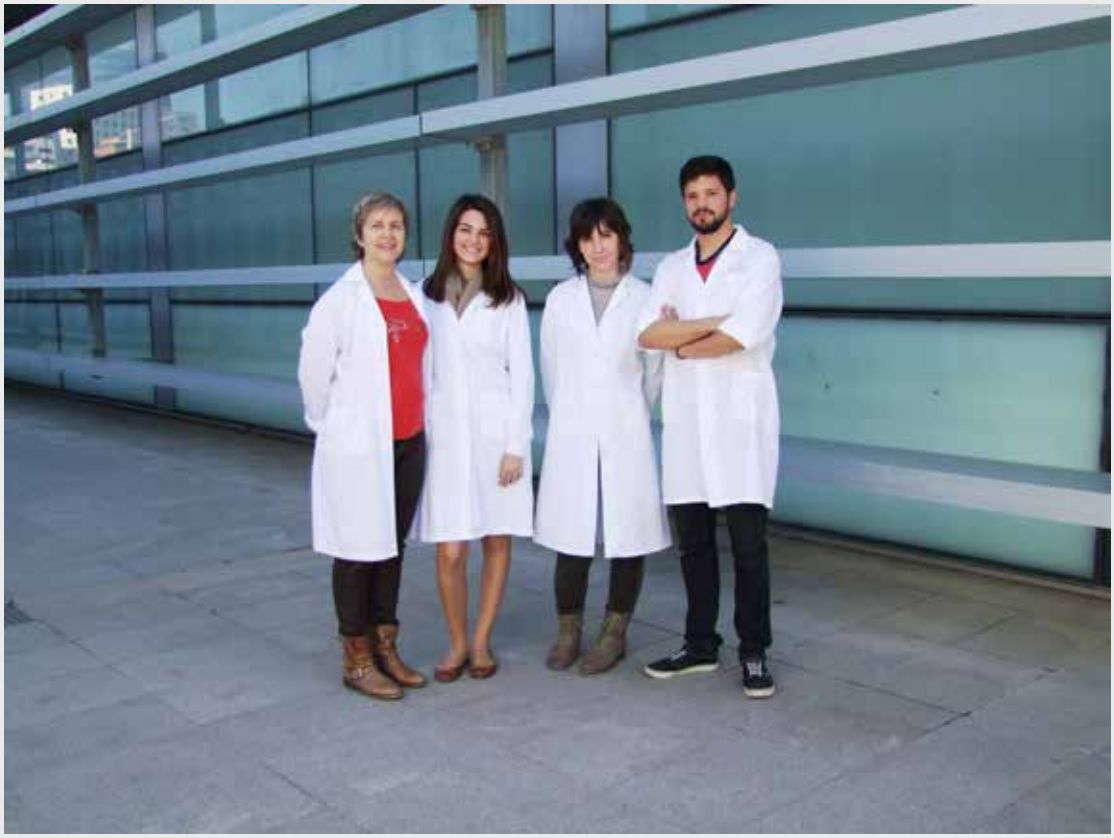
1. 26th EORTC – NCI – AACR Symposium on Molecular Targets and Cancer Therapeutics.
Oral Comunication
Multi-color flow cytometry immunophenotyping for detection of CSC in NSCLC.
Alicia Martinez-Romero, José Miguel Pardo, Sandra Tejedor, Silvia Calabuig Farinas, Rut Lucas, Santiago Figueroa, Eloisa Jantus-Lewintre, Carlos Camps, Rosa Farràs.
(Barcelona, Spain)

2.50th Annual Meeting of the American Society of Clinical Oncology
Oral Comunication
Tumor expression levels of CSC markers in resectable non-small cell lung cancer
Calabuig-Fariñas, Eloisa Jantus-Lewintre, Rut Lucas, Rosa Farràs, Marta Usó, Miguel Martorell, Eleonora Chakarova Sabeva, Ana Blasco, Santiago Figueroa, Carlos Camps
(Chicago, IL, USA)

3.Firts Proteostasis Meeting,
Oral Comunication
Regulation of actin cytoskeleton by means of eIF5A-dependent translational control
Borja Belda-Palazón, Jose Miguel Pardo, Beatriz Pérez-Benavente, Juan Carbonell, Alejandro Ferrando, Rosa Farràs.
(Valencia, Spain).

4.Firts Proteostasis Meeting,
Poster presentation
Emerging roles of JunB in the control of S phase progression.
Beatriz Pérez-Benavente, Isabelle Jariel-Encontre, Lorena de la Fuente, Ana Conesa, Laurent Vallar, José Enrique O'Connor, Marc Piechaczyk, Rosa Farràs.
(Valencia, Spain).

Rho Signaling in Neuropathologies



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Mara Dierssen (CRG).
Irma Machuca-Gayet (CNRS)

www.cipf.es/senalizacion-de-proteinas-rho-en-neuropatologias

Overview

In our laboratory we study the role of the RhoE protein in the developing nervous system and its involvement in neurodegenerative diseases. We have shown that this protein is involved in neural development, causing motor deficits, alterations in myelination and in neuronal axon formation. Our studies are currently performed in a mouse model lacking RhoE expression which exhibits major alterations in neurodevelopment as ataxia of the hindlimbs and abnormal gait. In particular, our objectives are:

1) To investigate the role of RhoE during neuronal development, specifically in axonal growth and myelination.
2) To study the role of RhoE in the proliferation and differentiation of neural progenitor cells.
3) To characterize the molecular mechanism involved in neural development and function.
4) To develop therapeutic strategies for the treatment of neurodegenerative diseases as Amyotrophic Lateral Sclerosis (ALS).

The ultimate goal of our research is to reveal the molecular mechanisms involved in neurodegenerative disorders caused by the absence of RhoE protein. Modulation of these signaling pathways would allow us to develop new therapeutic strategies in the treatment of neurodegenerative diseases which display motor disorders, abnormal myelination and alterations in axonal development.

Research results

1.- Alterations in the Subventricular Zone (SVZ) development in the brain, induced by the lack of the RhoE protein.

The subventricular zone is an important neurogenic niche where neuroblasts are continuously produced by stem cells. These cells migrate along the rostral migratory stream (RMS) and reach the olfactory bulb, where they differentiate in different types of neurons. In our work we have studied the role of RhoE in the SVZ development using genetically modified mice that do not express RhoE. We have previously demonstrated that null mutant mice show important alterations in neurodevelopment. They are smaller at birth, show growth retardation and display neuromuscular and neuromotor alterations. Our recent results from histological analysis indicate that the absence of RhoE induces a postnatal cellular accumulation in the anterior horn of the subventricular zone. By electron microscopy we have observed that this accumulation of dark cells is composed of immature cells. They were not organized in clusters, as in the case of control mice, but looked like a dispersed cellular mass. We have also observed large spaces between these cells due to the fact that the junctions' surface was smaller than in control mice. Our studies also show that the ependymal cells in RhoE mutant mice appeared as a heterogeneous population with cilia in their apical pole, but oriented in different directions. It is known that the movement of polarized cilia from epithelial cells allow the flow of the cerebrospinal fluid in the ventricle, contributing to the guidance of new neurons in the SVZ. The absence of RhoE could disturb this flow and, consequently, impair cell migration. (These studies were performed in collaboration with the University UCH-CEU, the University of Valencia and the CRG in Barcelona).

2.- The absence of RhoE leads to the disorganization of the cellular arrangement of the Rostral Migratory Stream (RMS).

The observed cell accumulation in the subventricular zone suggested a problem in cell migration and/or in cell proliferation. Therefore, our interest was also the study of the migration of cells along the RMS. It is known that newly generated neuroblasts move to form

the RMS and migrate tangentially in chains surrounded by astrocytes towards the olfactory bulb. Our studies of the semi-thin sections show that cell accumulation in the mutant mice was wider in most part of the caudal and thinner in its rostral zone, close to the olfactory bulb. Besides, the RMS in RhoE mutant mice showed a more scattered cell density to that showed in the control sections. In addition, electron microscopy showed several alterations in the structure of the migratory chains of the RMS. In ultrathin sections from control mice we observed a typical organization of glial tubes of migratory cells (type A) surrounded by astrocytes cells (type B) forming a sheath to isolate the future neurons from the brain parenchyma. This arrangement allows type A cells to move along these structures towards the olfactory bulb. However, in RhoE deficient mice we observed an atypical organization where cells inside the chains did not form a compact structure. Instead, they seemed to be scattered and their nuclei and cytoplasm appeared to be oriented in different directions.

Altogether, our results indicate that the absence of RhoE alters the cell arrangement, reducing cell contacts. The maintenance of appropriate cell-cell interactions can be one key mechanism for Rho GTPases to regulate neuronal development, and in particular, it seems to be necessary to maintain the intercellular contacts that allow SVZ cell migration. (These studies were performed in collaboration with the University UCH-CEU, the University of Valencia and the CRG in Barcelona).

3.- Increased proliferation of neural progenitor cells (NPC) in mice that do not express RhoE.

SVZ was dissected from the brain of mice and neural progenitor cells were cultivated as neurospheres or spherical clusters grown in flotation. Since it has been previously demonstrated that RhoE inhibits cell proliferation, our hypothesis was that the lack of RhoE would increase the proliferation of NPC. Our results indicate that progenitor cells lacking RhoE expression proliferate more rapidly than control cells. We have also investigated about changes in DNA synthesis by using DNA labeling techniques, which were performed by the incorporation of the nucleoside analog BrdU or Edu into

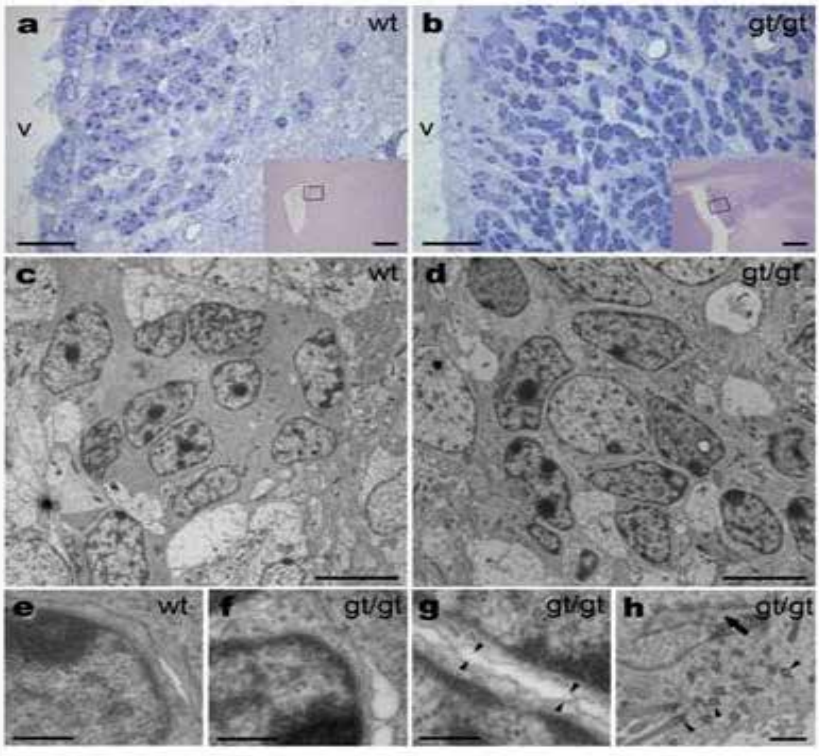

Figure 1.- RhoE deficiency alters the organization of SVZ cells. a, b Photomicrographs of semithin-sections of dorsal SVZ in PD15 wild type (a) and RhoE mutant (b) mice, showing a large cellular accumulation in the anterior horn of the SVZ in RhoE deficient mice (v: ventricle). Panel inserts show a broad histological view of the area where the images were obtained. c, d. Images of electron microscopy of the SVZ horn in wild type (c) and RhoE mutant (d) mice. Note the lack of chain organization of cells in RhoE deficient mice. e-g Detail of cellular junctions (arrow heads) in wild type (e) and RhoE mutant (f,g) mice. It is noteworthy the reduction in the extension of cellular junctions observed in RhoE mutant mice. h Ependimal cells of RhoE deficient mice present invaginated nuclei (arrow) and cilia orientated in different directions (arrow heads).

DNA. We have found that neural progenitor cells that do not express RhoE show increased in DNA synthesis, suggesting an increase in cell proliferation. These results indicate that the lack of RhoE in neural progenitors from the SVZ correlates with an increase in DNA synthesis. We are currently **analyzing the molecular mechanisms involved in the neurological deterioration due to lack of RhoE protein. We believe that modulation of the proteins involved in that signaling pathway will help us to develop new therapeutic strategies in the treatment of neurodegenerative diseases, as Amyotrophic Lateral Sclerosis (ALS).**

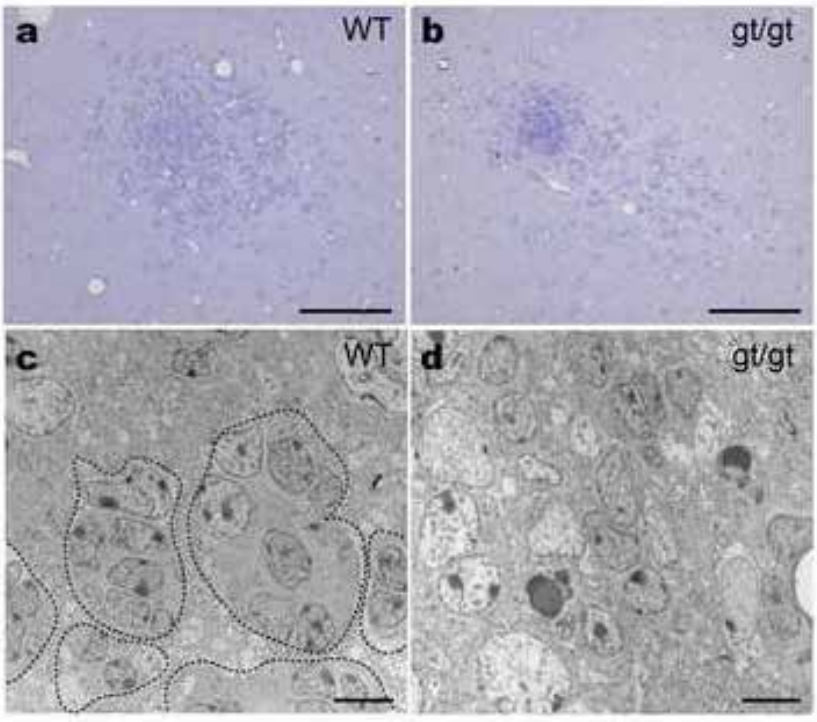

Figure 2.- RhoE deficiency leads to the disorganization of the cellular arrangement of the RMS. a, b. Photomicrographs of RMS semithin-sections in PD15 RhoE +/+ (WT) (a), and RhoE mutant (b) mice, in which a smaller RMS and more scattered cell density can be observed in RhoE deficient mice. c, d, Ultrastructure of wild type (c) and RhoE mutant (d) RMS. Note that the typical chain organization present in the wild type (surrounded by a dotted line), is absent in RhoE deficient mice.

Publications

1 .Georgess D, Mazzorana M, Terrado J, Delprats C, Chamot C, Guasch RM, Pérez-Roger I, Jurdic P and Machuca-Gayet I.
Comparative transcriptomics reveals RhoE as a novel regulator of actin dynamics in bone resorbing osteoclasts. Molecular Biology of the Cell , Vol: 25(3), 380-96 .2014 Quartile: Q2

2 .Ballester-Lurbe B, González-Granero S, Mocholí E, Poch E, García-Manzanares M, Dierssen M, Pérez-Roger I, García-Verdugo JM, Guasch RM, Terrado J.
RhoE deficiency alters postnatal subventricular zone development and the number of calbindin-expressing neurons in the olfactory bulb of mouse. Brain Struct Funct .2014 Quartile: Q1

3 .Gómez O, Ballester-Lurbe B, Guasch R.M, Pérez-Roger I, García-Roselló E, Terrado J.
Analysis of RhoE expression in the testis, epididymis and ductus deferens, and the effects of its deficiency in mice. Journal of Anatomy. 2014 Quartile: Q2

Conferences and meetings

1. Master´s Degree in Molecular Approaches in Health Sciences. University of Valencia
Invited Conference
Rosa Guasch
(Valencia, Spain)

2 Master´s Degree in Basic and Applied Neurosciences. University of Valencia
Invited Conference
Rosa Guasch
(Valencia, Spain)

3. I Biomedicine Predocs Congress in CIPF
Organizing Comittee
Fulgencio Ruso Julve
(Valencia , Spain)

4. XXXVII Congress of the Spanish Society for Biohemistry and Molecular Biology (SEBM)
Poster
Fulgencio Ruso Julve
(Granada , Spain)

Merits & awards

18th Angel Herrera Award in Health and Experimental Sciences research. University Foundation San Pablo CEU
Rosa Guasch
December 2014.

RNA Modification & Mitochondrial Diseases

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→ Research Assistants
Salvador Meseguer Llopis
Magdalena Villarroya Grau
Ismail Moukadiri

→ Predoctoral Scientists
Rafael Ruiz Partida
Mª Carmen Navarro González
Rachid Boutoual
Ana Martínez Zamora (UV)

→ Technicians
Olga Boix Sánchez

www.cipf.es/modificacion-del-rna-y-enfermedades-mitocondriales

Overview

Transfer RNAs (tRNAs) are key adaptor molecules in the translation of mRNA into protein, but to be fully active they require to be heavily modified post-transcriptionally. Modifications are introduced by enzymes that are highly specific for the tRNA species and target nucleoside.
Over the last decade, accumulating data have shown the association of several rare human diseases with defects in the posttranscriptional modification of mitochondrial (mt) tRNAs. However, the underlying pathophysiological mechanisms remain unclear. In some of these diseases, e.g. MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes) and MERRF (myoclonus epilepsy associated with ragged-red fibers) syndromes, the lack of the modifications that are normally present in the uridine located at the wobble position (U34) of the anticodon is an indirect consequence of mutations in tRNA-coding genes of the mitochondrial genome. In other cases, the disease is caused directly by defects in nuclear-encoded proteins responsible for U34 modifications of mt-tRNAs, as occurs in acute infantile liver failure due to mutations in the TRMU gene and infantile hypertrophic cardiomyopathy due to mutations in *MTO1 and GTPBP3*. Proteins TRMU, MTO1 and GTPBP3 are evolutionary conserved from bacteria to humans, but important aspects concerning their regulation, and biochemical and functional activities are not well-known. By exploring the precise cellular functions of the mt-tRNA modification enzymes and their regulatory mechanisms, it may be possible to uncover new aspects of the pathophysiology of the aforementioned diseases, and to design specific therapeutic approaches.

Research results

Our research in 2014 has aimed to investigate: 1) the biochemical and structural properties of the bacterial enzymes MnmE and MnmG (which are the homologues of the human GTPBP3 and MTO1 proteins, respectively) and other collaborative enzymes in the bacterial U34 modification process; this is helping us to understand the functioning of the human homologues; 2) the mitochondria-to-nucleus retrograde signaling pathways that are activated in different diseases caused by mutations in mt-tRNA genes, including MELAS and MERRF, which has improved our understanding of the underlying pathomechanisms; and 3) the consequences of inactivating *TRMU, MTO1 and GTPBP3* in *Caenorhabditis elegans*, which is allowing us to explore the signaling pathways triggered by defects in the modification of mt-tRNAs at organismal level.

Line 1: U34 modification process in gram-positive and -negative bacteria.

In *Escherichia coli* (the most widely used model of gram-negative bacteria), we have found that the U34 modification process catalyzed by the MnmE-MnmG complex (MnmEG) is part of a cellular nutrient-sensing network: the use of ammonium or glycine by the complex to generate the modification depends on the growth conditions and the tRNA species. The capability of the MnmEG complex to use different substrates and the participation of cooperating enzymes to further modify the group introduced by the MnmEG complex establish an atypical network of modification pathways in *E. coli*. Our data support the idea that each tRNA species modulates the in-vivo activity of the MnmEG complex and the cooperating enzymes in order to produce the final modification that better optimize the tRNA function in the translation process. We have initiated a comparative study with *Bacillus subtilis*, the most widely used model of gram-positive bacteria. Interestingly, *B. subtilis* tRNAs exhibit striking differences in relation to the use of the MnmEG-dependent pathways and the contribution of the cooperating enzymes. We are exploring the relationships between these preferences, the tRNA structure and composition, and the expression of specific tRNAs and mRNAs under different growth conditions. Moreover, we have made significant progresses in solving the MnmE and MnmG interactions in the functional complex.

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Line 2: microRNAs are crucial players in the mitochondrial retrograde signaling and are involved in the molecular mechanism of mitochondrial diseases.

Mitochondrial dysfunction activates mitochondria-to-nucleus signaling pathways whose components are mostly unknown. Identification of these components is important to understand the molecular mechanisms underlying mitochondrial diseases and to discover putative therapeutic targets. MELAS syndrome is a rare neurodegenerative disease caused by mutations in mitochondrial (mt) DNA affecting mt-tRNA$^{Leu(UUR)}$. Patient and cybrid cells exhibit elevated oxidative stress. Moreover, mutant mt-tRNAs$^{Leu(UUR)}$ lack the taurine-containing modification normally present at the wobble uridine (U34) of wild-type mt-tRNA$^{Leu(UUR)}$, which is considered an etiology of MELAS. However, the molecular mechanism is still unclear. We found that MELAS cybrids exhibit a significant decrease in the steady-state levels of several mt-tRNA-modification enzymes, which is not due to transcriptional regulation. We demonstrated that oxidative stress mediates an NFkB-dependent induction of microRNA-9/9* (miR-9/9*), which acts as a post-transcriptional negative regulator of the mt-tRNA-modification enzymes GTPBP3, MTO1 and TRMU. Down-regulation of these enzymes by miR-9/9*

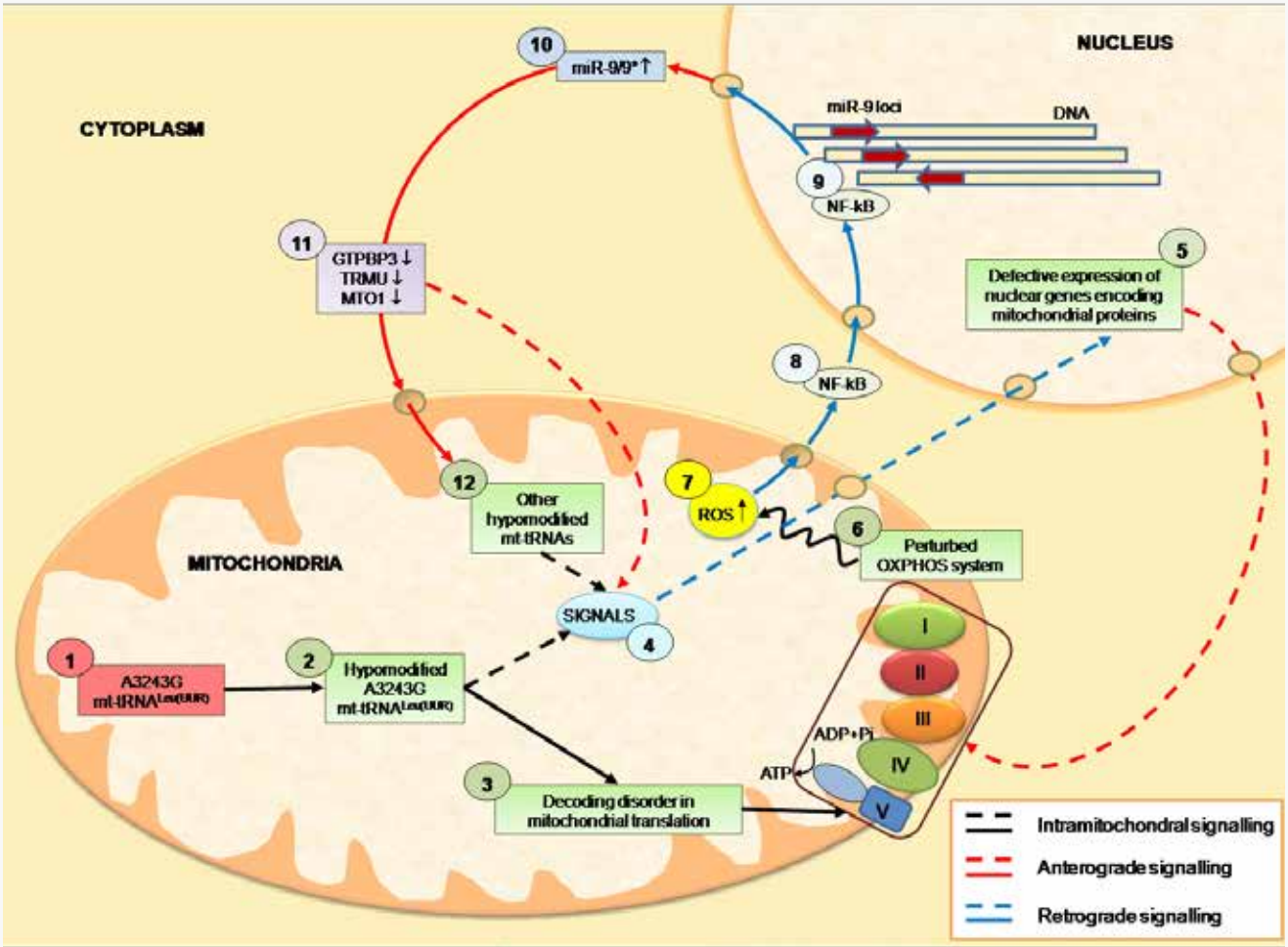

Figure 1. A miR-9/9*-based new model for the pathogenic mechanism of MELAS disease. The circled numbers on figure 9 try to facilitate the comprehension of the events, although it may be ample opportunity for crosstalk between the different steps. Initially, the hypomodification of the mt-tRNAs$^{Leu(UUR)}$ bearing mutation m.3243A⇢G (steps 1 and 2) might not only affect the synthesis of specific proteins encoded by the mitochondrial genome (step 3), but may also trigger a retrograde signaling pathway (step 4), which alters the synthesis of nuclear-encoded mitochondrial proteins (step 5). In any case, the outcome would be the functional disturbing of the OXPHOS system (step 6) and increased ROS production (step 7) which, in turn, induces miR-9/9* (steps 8, 9 and 10). Induction of this miRNA leads to the down-regulation of mt-tRNA-modifying proteins (step 11), which extends hypomodification to other mt-tRNAs (step 12) and further activates the retrograde pathway (steps 4 and 5). This feedback mechanism would amplify the original effect of the MELAS mutation. Our data evidence a new mitochondrial retrograde signaling pathway in which miR-9/9* is a crucial player. **(Meseguer et al HMG, doi: 10.1093/hmg/ddu427).**

affects the U34 modification status of non-mutant tRNAs and contributes to the MELAS phenotype. Anti-miR-9 treatments of MELAS cybrids reverse the phenotype, whereas miR-9 transfection of wild-type cells mimics the effects of siRNA mediated down-regulation of GTPBP3, MTO1 and TRMU. Our data represent the first evidence that an mt-DNA disease can directly affect microRNA expression. Moreover, we demonstrate that the modification status of mt-tRNAs is dynamic and that cells respond to stress by modulating the expression of mt-tRNA-modifying enzymes. miR-9/9* is a crucial player in mitochondria-to-nucleus signaling as it regulates expression of nuclear genes in response to changes in the functional state of mitochondria.

Line 3: Inactivation of *TRMU, MTO1 and GTPBP3* genes in *C. elegans* causes developmental dysfunction and fully penetrant sterility.

We have identified the *C. elegans* homologues of the human *TRMU, GTPBP3 and MTO1* genes, which have been designated as *mttu-1, mtcu-1 and mtcu-2*, respectively. The knock-out mutant strains exhibit temperature sensitive fecundity and life cycle defects. The simultaneous inactivation of *mttu-1* and *mtcu-2* decreases mitochondrial membrane potential and displays a completely penetrant sterility, life span extension and severe developmental dysfunctions depending of the tissue-specific expression. At present, we are investigating the molecular basis of these phenotypes and exploring the signaling pathways triggered by defects in the modification of mt-tRNAs at organismal level.

Publications

1. Moukadiri I, Garzón MJ, Björk GR, Armengod ME.
The output of the tRNA modification pathways controlled by the Escherichia coli MnmEG and MnmC enzymes depends on the growth conditions and the tRNA species.
Nucleic acids research Vol: 42 (4), 2602-2623 . 2014 Quartile: Q1

2. Meseguer S, Martínez-Zamora A, García-Arumí E, Andreu AL, Armengod ME.
The ROS-sensitive microRNA-9/9* controls the expression of mitochondrial tRNA-modifying enzymes and is involved in the molecular mechanism of MELAS syndrome.. Hum Mol Genet .2014 Quartile: Q1

3. Armengod ME, Meseguer S, Villarroya M, Prado S, Moukadiri I, Ruiz-Partida R, Garzon MJ, Navarro-Gonzalez C, Martinez-Zamora A.
Modification of the wobble uridine in bacterial and mitochondrial tRNAs reading NNA/NNG triplets of 2-codon boxes.
RNA BIOLOGY Vol: 11 (12), 1495-1507.2014. Quartile: Q1

Conferences and meetings

1. Congreso de C. elegans .
Poster
Ismail Moukadiri y Carmen Navarro
(Berlin , Germany)

2. EUROMIT 2014 .
Poster
Salvador Meseguer Llopis
(Tampere , Finland)

3. I Biomedicine Predocs Congress in CIPF
Poster.
Carmen Navarro González and Olga Boix
(Valencia, Spain)

4. 25th tRNA Conference.
Oral Communication
Salvador Meseguer
(Kyllini Peloponnese, Greece)

Stays in foreign Universities

- Carmen Navarro González: Stay at the Umea University (Sweden) with a fellowship from MINECO

Neurological Impairment Program

Program Coordinator: Vicente Felipo

Neurobiology, led by Vicente Felipo

Joint research unit for brain connectivity, led by Vicente Felipo & Marian de la Iglesia Vaya

INDEX

Neurobiology

Group Leader

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Marian de la Iglesia Vaya (FISABIO)

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Overview

NEUROCIPF performs basic and translational research on cognitive, motor, sleep and circadian rhythms alterations in different pathological situations, including: minimal and clinical hepatic encephalopathy (HE), hyperammonemia and developmental exposure to food and environmental contaminants. We also apply our wide range of methodologies to other pathological situations. The aims are:
In animal models:
1.Unveil the molecular mechanisms leading to neurological impairment
2.Identify new therapeutic targets for its treatment
3.Design and assess new therapeutic procedures to reverse neurological impairment
In patients
4. study the mechanisms, diagnosis and treatment of neurological impairment
5. Bring to the clinic the therapeutic procedures developed in animal models
6. Identify early diagnostic procedures for neurological impairment
7. Bring to the clinic the diagnostic procedures identified
We study the mechanisms responsible for neurological alterations. Once identified the molecular alteration, we try to restore normal cerebral and neurological function through pharmacological treatments. These studies allowed us to 1) prevent death induced by acute ammonia intoxication; 2) prevent or delay death in rats with acute liver failure; 3) restore learning ability and, 4) reverse hypokinesia in rats with chronic HE; 5) identify and patent the first biomarker (3-nitortyrosine) for early diagnosis of minimal HE in cirrhotic patients.

Research results

Reduced white matter microstrutural integrity correlates with cognitive deficits in minimal hepatic encephalopathy. Gut. 63(6):1028-30

Cirrhotic patients with minimal hepatic encephalopathy (MHE) show mild cognitive impairment and psychomotor slowing. White matter microstructure integrity was analyzed using DTI imaging and TBSS in controls and cirrhotics without and with MHE. Patients with MHE (but not without MHE) show reduced white matter structural integrity, with increased mean diffusivity (MD) and reduced fractional anisotropy (FA). Reduced FA of some tracts correlate with performance in line tracing and serial dotting tests. Increased MD also correlates with performance in the Stroop, symbol digit and number connection A tests and with serum levels of 3-nitrotyrosine. These findings show an association between microstructural alterations and reduced performance in attention, mental processing speed, visuospatial and visuomotor coordination tests. Analysis of white matter microstructural integrity may provide new neuroimaging biomarkers for early diagnosis of MHE. **(Figure 1)**

Blocking NMDA receptors delays death in rats with acute liver failure by dual protective mechanisms in kidney and brain. NeuroMolecular Medicine, 16; 360-375

Treatment of patients with acute liver failure (ALF) is unsatisfactory and mortality remains high. Blocking NMDA receptors delays death of rats with ALF. The underlying mechanisms remain unclear. We studied these mechanisms. ALF reduces kidney glomerular filtration rate (GFR), due to both reduced renal perfusion and kidney tubular damage as reflected by increased Kim-1 in urine and histological analysis. Blocking NMDA receptors delays kidney damage, allowing transient increased GFR and ammonia elimination which delays hyperammonemia and associated changes in brain. Blocking NMDA receptors prevents changes in cerebral blood flow and brain lactate. Dual protective effects of MK-801 in kidney and brain delay cerebral alterations, hepatic encephalopathy, intracranial pressure increase and death. NMDA receptors antagonists may increase survival of patients with ALF by providing additional time for liver transplantation or regeneration.

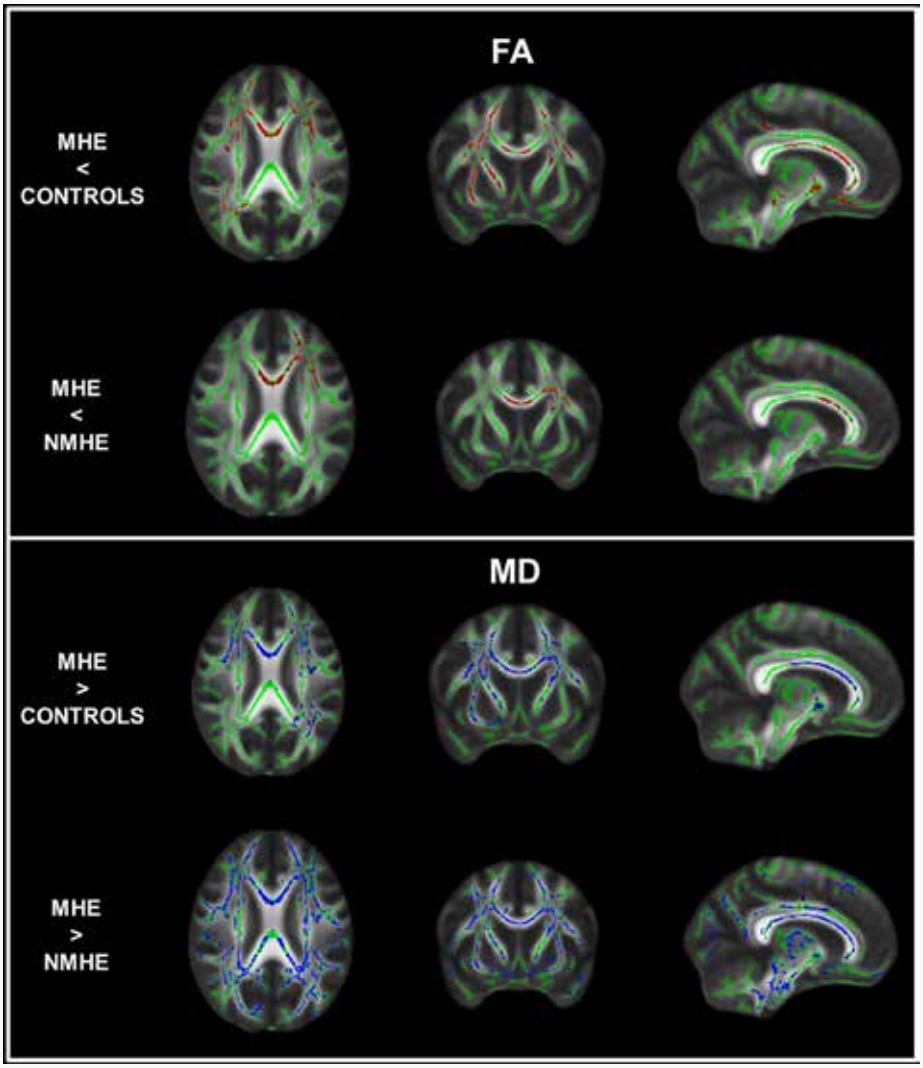


Figure 1. Neuronal tracts showing fractional anisotropy (FA) and mean diffusivity (MD) differences between patients with minimal hepatic encephalopathy (MHE) and patients without MHE (NMHE) or controls. Each image represents a mean of 17 control subjects, 15 NMHE and 15 patients with MHE. From Gut. 63(6):1028-30

Cerebral edema is not responsible for motor or cognitive deficits in rats with hepatic encephalopathy. Liver International 34; 379-387

Low grade cytotoxic edema is considered a main contributor to the neurological alterations in patients with hepatic encephalopathy (HE). This assumption is mainly based on studies with cultured astrocytes treated with very large ammonia concentrations or in animals with ALF. However, the contribution of cerebral edema to cognitive or motor alterations in chronic mild HE has not been demonstrated. We studied this question.PCS rats show reduced motor activity and coordination, impaired ability to learn a conditional discrimination task in the Y maze and reduced spatial memory in the Morris water maze but did not show increased brain water content. Cerebral edema is not involved in motor and cognitive alterations in rats (and likely in humans) with mild HE. Proper understanding of the mechanisms responsible for the neurological alterations in HE is necessary to design efficient treatments.

Pregnenolone sulphate restores the glutamate-nitric oxide-cGMP pathway and extracellular GABA in cerebellum and learning and motor coordination in hyperammonemic rats. ACS Chem Neurosci. 19;5(2):100-105 (Figure 2)

Treatment of MHE is unsatisfactory and new agents acting on molecular targets involved in brain mechanisms leading to neurological alterations are needed to treat MHE. Chronic hyperammonemia impairs learning of a Y maze task by impairing the glutamate-nitric oxide (NO)-cGMP pathway in cerebellum, in part by enhancing GABAA receptors activation , which also induces motor in-coordination. This work aimed to assess whether chronic treatment of hyperammonemic rats with PregS restores: 1) motor coordination; 2) extracellular GABA in cerebellum; 3) learning a Y maze task; 4) glutamate-NO-cGMP pathway in cerebellum. Chronic intracerebral administration of PregS normalizes motor coordination by reducing extracellular GABA and learning ability by restoring the glutamate-NO-cGMP pathway. Similar treatments would improve cognitive and motor alterations in patients with MHE.

Non invasive blood flow measurement in cerebellum detects minimal hepatic encephalopathy earlier than psychometric tests. World Journal of Gastroenterology. 20(33):11815-25.

Aim: To assess whether non-invasive blood flow (BF) measurement by arterial spin labeling in several brain regions detects minimal hepatic encephalopathy.
In patients with MHE, BF was increased in cerebellar hemisphere and vermis and reduced in occipital lobe. BF in cerebellar hemisphere was also increased in patients without MHE. Bimanual coordination, visuo-motor coordination and attention were impaired in patients with MHE. BF in cerebellar hemisphere and vermis correlated with performance in most tests of PHES [(number connection tests A (NCT-A), B (NCT-B) and line tracing test] and in the congruent task of Stroop test. BF in frontal lobe correlated with NCT-A. Performance in bimanual and visuomotor coordination tests correlated only with BF in cerebellar hemisphere. BF in cerebellar hemisphere correlates with plasma cGMP and nitric oxide (NO) metabolites. BF in vermis cerebellar also correlates with NO metabolites and with 3-nitrotyrosine. Non invasive BF determination in cerebellum using ASL may detect MHE earlier than the PHES. Altered NO-cGMP pathway seems to be associated to altered BF in cerebellum.

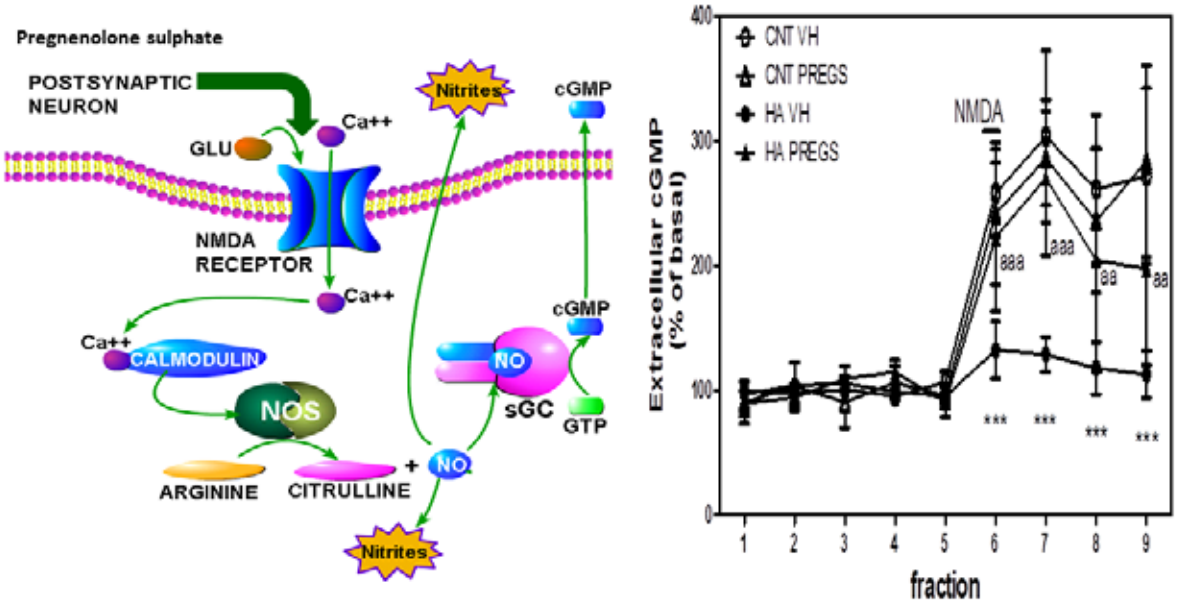


Figure 2. PregS restores the function of the glutamate-NO-cGMP pathway in cerebellum in vivo in hyperammonemic rats. From ACS Chem Neurosci. 19;5(2):100-105

Publications

1. Gualix J, Gómez-Villafuertes R, Pintor J, Llansola M, Felipo V, Miras-Portugal MT. Presence of diadenosine polyphosphates in microdialysis samples from rat cerebellum in vivo: Effect of mild hyperammonemia on their receptors. Purinergic Signalling, 10; 349-356 Quartile: Q2

2. Gonzalez-Usano, A, Cauli, O, Agusti A, Felipo, V.
Pregnenolone sulphate restores the glutamate-nitric oxide-cGMP pathway and extracellular GABA in cerebellum and learning and motor coordination in hyperammonemic rats. ACS Chem Neurosci. 19;5(2):100-105. Quartile: Q1

3. Agusti A, Dziedzic JL, Hernandez-Rabaza V, Guilarte TR, and Felipo V.
Rats with minimal hepatic encephalopathy due to portacaval shunt show differential increase of translocator protein (18kda) binding in different brain areas, which is not affected by chronic MAP-kinase p38 inhibition. Metabolic Brain Disease 29(4):955-63. Quartile: Q3

4. Cauli , O, González-Usano A, Cabrera-Pastor A, Gimenez-Garzó C, López-Larrubia P, Ruiz-Sauri A, Hernández-Rabaza V, Duszczyk M, Malek M, Lazarewicz JW, Carratalá A, Urios A, Miguel A, Torregrosa I, Carda C, Montoliu C, Felipo V.
Blocking NMDA receptors delays death in rats with acute liver failure by dual protective mechanisms in kidney and brain. NeuroMolecular Medicine, 16; 360-375. Quartile: Q2

5. Arias, N, Fidalgo, C, Felipo V, Arias JL.
The effects of hyperammonemia in learning and brain metabolic activity. Metabolic Brain Dis 29; 113-120. Quartile: Q3

6. Giménez-Garzó C, Salhi D, Urios A, Ruíz-Sauri A, Carda C, Montoliu C and Felipo V.
Rats with mild bile duct ligation show hepatic encephalopathy with cognitive and motor impairment in the absence of cirrhosis: effects of alcohol ingestion. Neurochemical Research. Quartile: Q3

7. Felipo V, Urios A, Giménez-Garzó C, Cauli o, Andrés-Costa MJ, González O, Serra MA, Sánchez-González J, Aliaga R, Giner-Durán R, Belloch V, Montoliu C.
Non invasive blood flow measurement in cerebellum detects minimal hepatic encephalopathy earlier than psychometric tests. World Journal of Gastroenterology. 20(33):11815-25. Quartile: Q2

8. Llansola M, Montoliu C, Agusti A, Hernandez-Rabaza V, Cabrera-Pastor A, Gomez-Gimenez B, Malaguarnera M, Dadsetan S, Belghiti M, Garcia-Garcia R, Balzano T, Taoro L and Felipo V.
Interplay between glutamatergic and GABAergic neurotransmission alterations in cognitive and motor impairment in minimal hepatic encephalopathy. Neurochem Int. Quartile: Q3

9 .Montoliu, C; Urios, A; Forn, C; Garcia-Panach, J; Avila, C; Gimenez-Garzo, C; Wassel, A; Serra, MA; Giner-Duran, R; Gonzalez, O; Aliaga, R; Belloch, V; Felipo, V.
Reduced white matter microstructural integrity correlates with cognitive deficits in minimal hepatic encephalopathy.
Gut Vol: 63. Quartile: Q1

Conferences and meetings

1. Workshop Novel tools and methods for the screening of chemicals for developmental neurotoxicity
Plenary Lecture
Vicente Felipo
(Amsterdam , Netherlands)

2 .TRANSBIO Emergence Forum
Plenary Lecture
Vicente Felipo
(Barcelona , Spain)

3 .9th FENS Forum of Neuroscience de la Federation of European Neuroscience Societies
Oral Communication
Andrea Cabrera, Ana Agusti, Sherry Dadsetan y Vicente Hernández Rabaza
(Milano , Italy)

4 .Mycoval 2014
Chair
Vicente Felipo
(Krakow , Poland)

5 .Mycoval 2014. Conference on “Glutamate/GABA and neuro-glia-vascular interplay in norm and pathology”
Oral Communication
Llansola M., Montoliu C., Agusti A., Hernandez-Rabaza V., Cabrera-Pastor A., GomezGimenezB., Malaguarnera M., Garcia-Garcia R., Taoro L. and Felipo V.
(Krakow , Poland)

6 .16th Symposium of the International Society for Hepatic Encephalopathy (ISHEN)
Plenary Lecture
Marta Llansola, Ana Agusti, Carmina Montoliu, Vicente Hernandez-Rabaza, Andrea Cabrera-Pastor, Michele Malaguarnera, Raquel Garcia-Garcia, Majedeline Belghiti, Lucas Taoro, Vicente Felipo
(Ascot , United Kingdom)

7 .I Congreso de Biomedicina Predocs .
Communication
Lucas Taoro
(Valencia , Spain)

8 .I Congreso de Biomedicina Predocs .
Communication
Raquel García
(Valencia , Spain)

9 .I Congreso de Biomedicina Predocs
Oral Communication
Belén Gómez
(Valencia , Spain)

10 .Instituto de Biomedicina
Invited Conference
Vicente Felipo
(Valencia , Spain)

11.International Liver Congress™ 2014, 49th annual meeting of the European Association for the Study of the Liver
Oral Communication
Amparo Urios Lluch
(London , United Kingdom)

12 .Jornada sobre Oportunidades de Investigación Biomédica en Valencia en la Universidad de Valencia
Oral Communication
Ana Agusti
(Valencia , Spain)

Member of the Editorial Board of

1.International Journal of Molecular Medicine,

2.World Journal of Gastroenterology,

3.Open Gastroenterology Journal,

4.Journal of Hepatology,

5.Review Editor de Frontiers in Neuroenergetics,

6.World Journal of Experimental Medicine,

7.Neuroimmunology and Neuroinflammation,

8.New Horizons in Translational Medicine,

9.The Open Journal of Medicine,

10.International Journal of Neurology Research

Memberships

1.External Review Working Group (ERWG) de la European Food Safety Authority (EFSA)

2.Action Group A3 on "Prevention and early diagnosis of frailty and functional decline, both physical and cognitive, in older people" del European Innovation Partnership on Active and Healthy Ageing (EIP on AHA) de la Comisión Europea.

3.Global Translational Medicine Consortium (GTMC) de la European Society for Translational Medicine.

4.Task Coordinator for Imaging Biomarkers for Cognitive Decline del Action Group A3 del European Innovation Partnership on Active and Healthy Ageing (EIP-AHA) de la Comisión Europea

5.Expert Panel on Neuroscience de la European Society for Translational Medicine

6.International Review Board del funding initiative "European Research Projects on Neuroinflammation" of ERA-NET-Neuron of the European Commission

Other Activities

1.Vicente Felipo, Member of the Comisión Académica del Programa de Doctorado en Neurociencias de la Universidad de Valencia

2. Vicente Felipo, Member of the Comisión Científica de la XV Convocatoria de los Premios "Alberto Sols" del Ayuntamiento de Sax-Generalitat Valenciana- Diputación de Alicante-Univesidades de Alicante y Miguel Hernández de Elche

3. Vicente Felipo, Member of the Comité de Expertos de la Comunidad Valenciana como Asesores para el Museo de las Ciencias “Príncipe Felipe”.

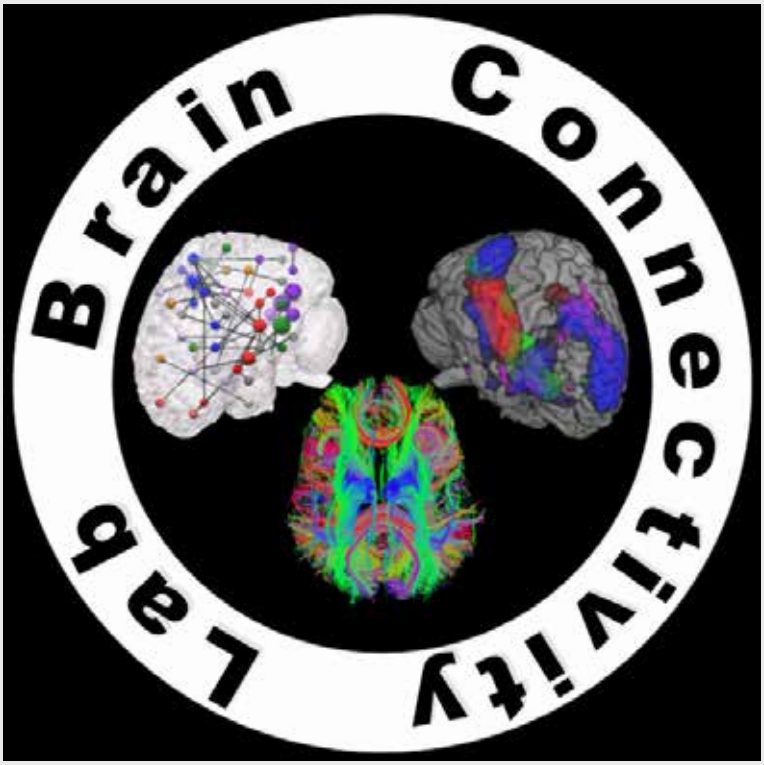
4. Vicente Felipo, Teacher at the “Master de Enfermedades Neurológicas” Biotecnología. Universidad Politécnica de Valencia

Doctoral Thesis presented

1. Carla Giménez Garzó
Alteraciones neurológicas en pacientes cirróticos con encefalopatía hepática mínima. Implicación de la inflamación y el estrés oxidativo en el deterioro cognitivo.
Facultad de Farmacia, Universidad de Valencia, 12 de Mayo de 2014
Calificación: Sobresaliente cum Laude,
Directores: Vicente Felipo, Carmina Montoliu y Federico Pallardó

2. Andrea Cabrera Pastor
Modulación de la vía Glu-NO-GMPc y del aprendizaje por GMPc extracelular en cerebelo. Mecanismos moleculares implicados. Alteraciones en modelos animales de hiperamonemia y encefalopatía hepática.
Facultad de Farmacia, Universidad de Valencia, 17 de Julio de 2014
Calificación: Sobresaliente cum Laude,
Directores: Vicente Felipo y Marta Llansola, tutor Juan Viña

Joint Research Unit for Brain Connetivity
CIPF-FISABIO



Group Leader

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Ana Agusti

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Alba González Usanó
Belen Gómez Giménez
Raquel García García

→ Technicians

Mar Martínez García
MariCarmen Castro
Francisca Sellés
Amparo Urios

→ Students

Alma Orts Sebastian (UV)
(Until february 2014)

→ Collaborators

José Miguel Puig Saques (FISABIO)

www.cipf.es/neurobiologia

Overview

The Joint Research Unit CIPF/FISABIO is mainly focused on the study of Brain Connectivity. The brain is a complex network of interconnected regions both structurally and functionally. The functional communications between brain regions play a key role in cognitive processes. Mental activities involve activation of neural networks in the brain areas involved creating various circuits in order toperform complex cognitive functions. The analysis of brain connectivity allows understanding the organization of the human brain, how distinct neural networks perform various tasks or how activation patterns are altered in pathological situations. The Joint Research Unit develops methods to detect and quantify these connectivity types and their relation with brain function and combines different mathematical techniques to analyze images. It then interprets and integrates biological and medical knowledge to obtain models that explain and predict brain function in normal and pathological conditions. The objectives are: to advance in the understanding of the mechanisms involved in the neurological impairment in different pathological situations, to better understand its causes, to develop early diagnostic procedures of cognitive impairment and to evaluate the usefulness of therapeutic procedures to reverse or prevent it.

Research results

During this first year of existence, the Joint Unit has made significant progress in its consolidation. In addition to the advances described in annual report of the Neurobiology Laboratory, some relevant elements supporting this consolidation are:

1. Participation in Euro-Bioimaging. Euro-BioImaging is a large-scale project which objective is the creation of a European research infrastructure within the roadpamp of the European Strategy Forum on Research Infrastructures (ESFRI). The preparation phase of the project took place between December 2010 and May 2014. Currently, twelve countries and the European Molecular Biology Laboratory (EMBL) are part of Euro-BioImaging, who is working together in the construction phase of the infrastructure. www.eurobioimaging.eu.

2. María de la Iglesia, by appointment of the Deputy Directorate General for International Relations and European Ministry of Economy and Finance, is the scientific representative in the area of Medical Imaging of the Spanish Delegation both in the Interim Board and the in the Working Groups of EuroBioimging.

3. Two predoctoral students have been incorporated: Ángel Fernández-Cañada Vilata and Jorge Isnardo Altamirano through two grants from the Deputy Directorate General for Health Information Systems of the Regional Ministry of Health.

4. María de la Iglesia was elected Treasurer of the Latin America Brain Mapping Network Initiative (LABMAN-http: //www.labman.org), first Chapter of OHBM - Organization for Human Brain Mapping - www.humanbrainmapping.org.

Being part of these initiatives (Euro-Bioimaging, LABMAN) allows us to establish synergies and to share knowledge with numerous experts in health and biomedical research and innovation, to develop entrepreneurship initiatives and training activities in health at European level. It also enables a better positioning and higher visibility in Europe of the research work being carried out in Valencia. Participating in these projects allows us to share our strengths in health research and to make proposals that can then be implemented joint-

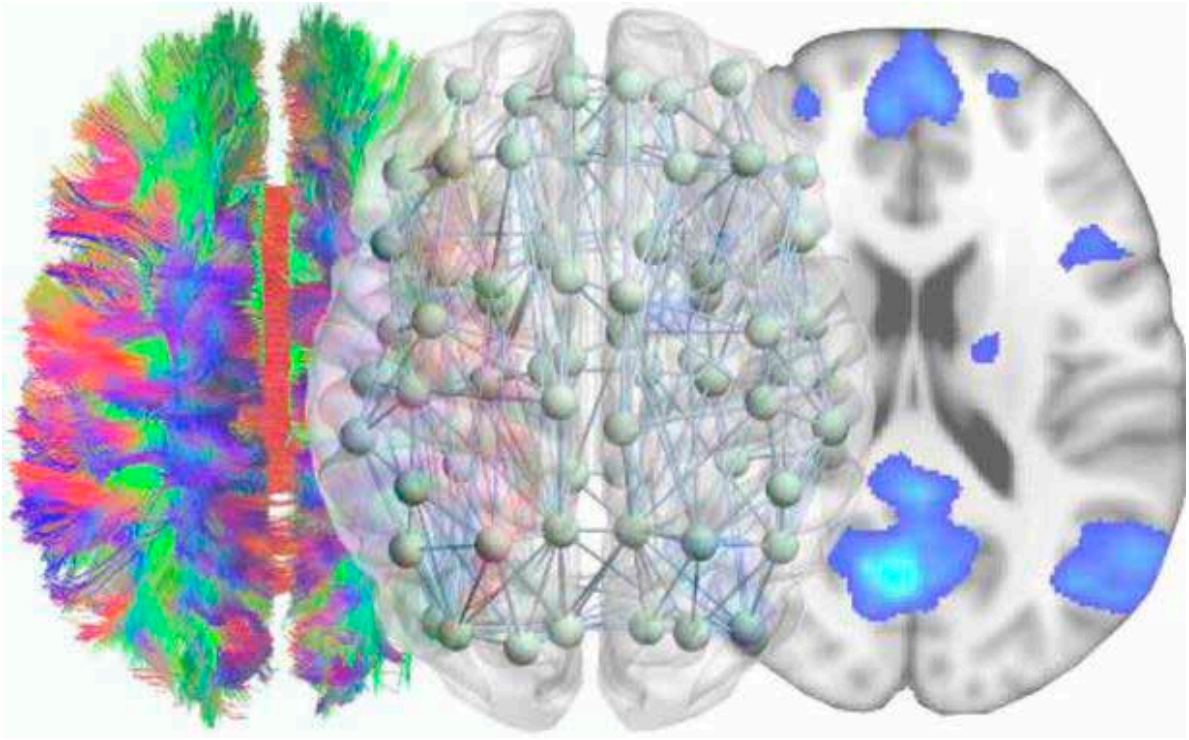

Figure 1. Brain connectivity

ly. Eventually, these activities have a positive impact in the overall Health System of the Valencian region and on the citizens welfare.

RESEARCH RESULTS.

Abnormal synchrony and effective connectivity in patients with schizophrenia and auditory hallucinations. NeuroImage: Clinical, 01/2014.

Auditory hallucinations (AH) are the most frequent positive symptoms in patients with schizophrenia. Hallucinations have been related to emotional processing disturbances, altered functional connectivity and effective connectivity deficits. Previously, we observed that, compared to healthy controls, the limbic network responses of patients with auditory hallucinations differed when the subjects were listening to words with an emotional content. We aimed to compare the synchrony patterns and effective connectivity of task-related networks between schizophrenia patients with and without AH and healthy controls. Schizophrenia patients with AH (n=27) and without AH (n=14) were compared with healthy participants (n=31). We examined functional connectivity by analyzing correlations and cross-correlations among previously detected independent component analysis time courses. Granger causality was used to infer the information flow direction in the brain regions. The results demonstrate that the patterns of cortico-cortical functional synchrony differentiated the patients with AH from the patients without AH and from the healthy participants. Additionally, Granger-causal relationships between the networks clearly differentiated the groups. In the patients with AH, the principal causal source was an occipital–cerebellar component, versus a temporal component in the patients without AH and the healthy controls. These data indicate that an anomalous process of neural connectivity exists when patients with AH process emotional auditory stimuli. Additionally, a central role is suggested for the cerebellum in processing emotional stimuli in patients with persistent AH.

Methodological framework to study synchrony and effective connectivity with ICA. Organization for Human Brain Mapping, Hamburg; 06/2014.

A methodological framework has been developed to study functional connectivity in response to an emotional auditory paradigm in patients with AH by conducting independent component analyses (ICA). Using this approach, activated areas can be obtained by selecting ICA components related to the emotional auditory paradigm. We validate this methodological framework to study synchrony and effective connectivity with schizophrenia dataset. To perform the analysis referred to in this methodological framework is performed, Data analysis: Pre-processing of the functional data. ICA analysis was performed using Group ICA approach fMRI. Components of interest (CoI) were selected taking into account individual beta-values related to the emotional task, and regressors in each component were entered into a one-sample t-test thresholded at p ⟨···· 0,01. Furthermore, Granger causality was used to infer directionality of information flow among brain regions. Techniques used for causal analysis, Causal flow: Within a given causal network, we define the causal flow of a node "i" as the difference (weighted or not) between its inflows and outflows. A node with a very positive causal flow exerts a strong causal influence on a dynamic system as a whole. This node is called a causal source. By contrast, a node with a very negative causal flow is called a causal sink. Causal density: The causal density of a dynamic system provides an overall measure of causal interactivity. This causal density measure is defined as a causal graph for all pairs of elements of the system. Those interactions that are not significant are assigned zero values. In this study, a driven-data method that can separate independent spatio-temporal patterns of neural activity from fMRI data in a manner that has been helpful for the study of intrinsic brain networks. Granger- causality was used in order to explore effective connectivity in fMRI data to quantify the strength of interactions between activated brain areas. The two techniques allowed us to analyze functional and effective connectivity, respectively. ICA allowed us to select the CoI that were candidates for the GCCA analysis of their associated time-courses. The main strength of this methodological framework was the combination of these independent techniques, which allowed us to assess whether different methods would lead to consistent results and more confident conclusions.

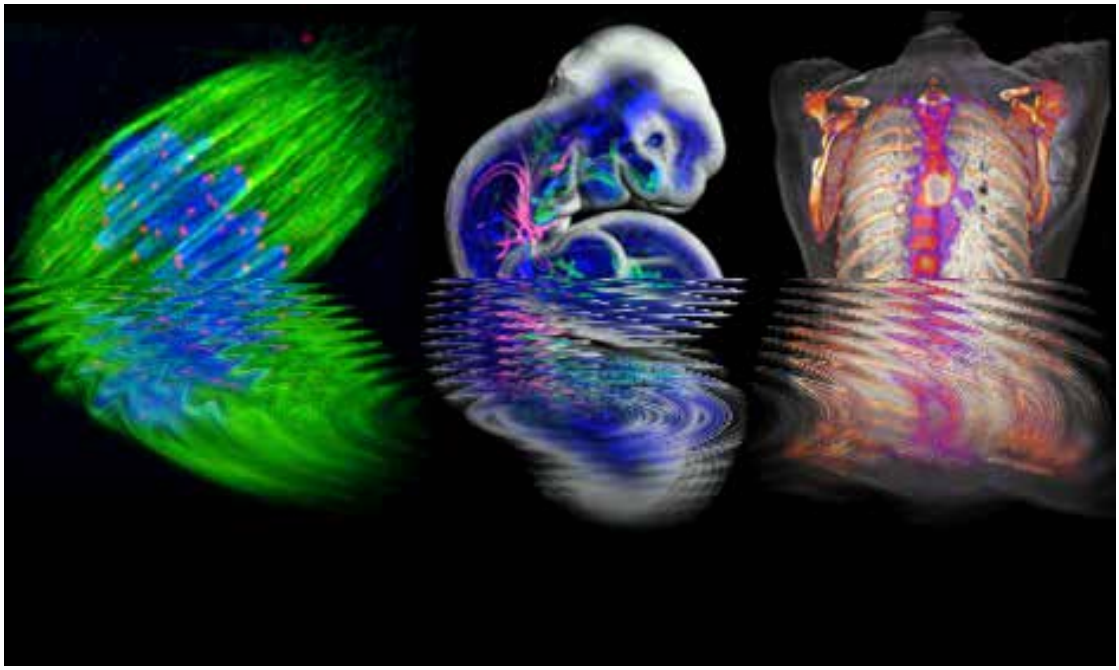

Figure 2. Medical Image. Euro-Bioimaging

Publications

1 . Maria de la Iglesia-Vaya, Maria José Escartí, Jose Molina-Mateo, Luis Martí-Bonmatí, Marien Gadea, Francisco Xavier Castellanos, Eduardo J. Aguilar García-Iturrospe, Montserrat Robles, Bharat B. Biswal, Julio Sanjuan. Abnormal synchrony and effective connectivity in patients with schizophrenia and auditory hallucinations. NeuroImage: Clinical Vol: 6, 171-179

2 .Maria de la Iglesia Vaya, Jose Molina-Mateo, Maria Jose Escarti, Luis Marti-Bonmati, Marien Gadea, Francisco Xavier Castellanos, Erika Proal, Gonzalo Rojas Costa, Bharat B. Biswal, Julio Sanjuan. Methodological framework to study synchrony and effective connectivity with ICA. Organization for Human Brain Mapping, Hamburg . Meeting abstract

Conferences and meetings

1. V Congrés Catalá de Salut Mental La Infància I L'adolescència, ubicado en el Centre de Cultura Contemporanea de Barcelona (CCCB), una ponencia sobre: "Connectivitat funcional del cervell en estat de repòs. Aplicacions en la clínica i en la psicoteràpia".

2. Human Brain Mapping, Hamburg 2014. Methodological framework to study synchrony and effective connectivity with ICA.

3. CIPF Seminar. BIMCV. The Perfect “Big Data” Storm: Collision of Peta Bytes of Population Image Data, Millions of Hardware Devices and Thousands of Software Tools. Date: 25/07/2014. Venue: Salón de Actos CIPF

Nominations and assignments

1. The Latin America Brain Mapping Network Initiative (LABMAN) (http://www.labman.org/about) is an initiative dedicated to join efforts to promote the development of neuroimaging methods in Latin America. On June 12, 2014, LABMAN was chosen the first Chapter of OHBM - Organization for Human Brain Mapping (www.humanbrainmapping.org). The initial Board Members are: Treasurer-Elect: Maria de Iglesia Vayá / Maria Luisa Bringas

2. Dra. María de la Iglesia Vayá, is incorporated as a representative of all institutions in the Delegation of Spain in the Interim Board of Euro-BioImaging, accompanied, as scientists delegates institutional MINECO delegate.

Rare and Genetic Diseases Program

Program Coordinator: Francesc Palau

Developmental biology and neuromuscular diseases models, led by Maximo Ibo Galindo

Genetics and genomics of neuromuscular diseases, led by Carmen Espinós

Genetics and molecular medicine, led by Francesc Palau

Genetics and physiopathology of brain and mental disorders, led by Janet Hoenicka

Intracellular protein degradation and rare diseases, led by Erwin Knecht

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Developmental Biology and Neuromuscular Disease Models

Group Leader

Máximo Ibo Galindo

→ Predoctoral Scientitsts

Victor López del Amo (CIBERER)

→ Students

Ana Pilar Gómez Escribano (UV)

Tamara Ovejero Martínez (UCV)

María Teresa Sánchez Mares (UCV)

www.cipf.es/biologia-del-desarrollo-y-modelos-de-enfermedades-neuromusculares

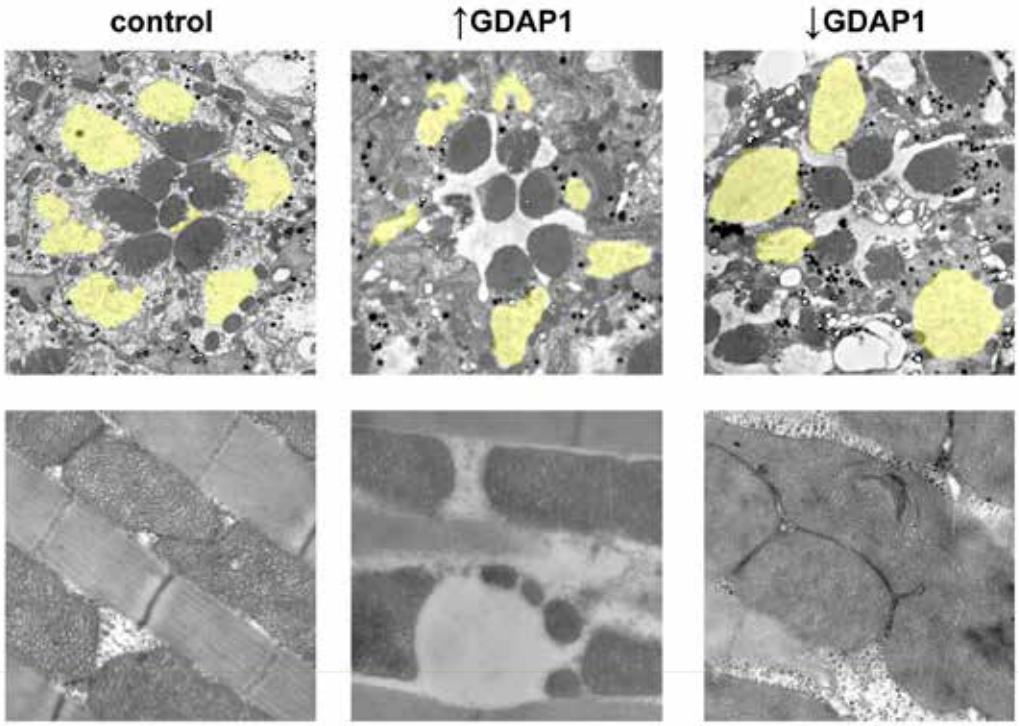
Overview

Our group uses the insect Drosophila melanogaster to study the basic biological mechanisms underlying development and disease. During animal development there is a continuous interplay of tissue polarity and cell signaling. We are interested in the regulation of Notch signaling by the polarity pathways of Fz/Stbm and Fat/Dachsous in order to understand how this contributes to the correct establishment of the anatomy of the animal, and the possible relationship of these pathways with inherited deafness syndromes. In addition, we are using Drosophila to generate models to study rare diseases, with an especial interest in inherited peripheral neuropathies and those neurodegenerative diseases involving defects in mitochondrial dynamics. Our ultimate goals are to understand the disease mechanisms involved and to generate new tools for biomarker and drug discovery. To achieve these goals we have a network of collaborators that include groups working in Drosophila genetics, physiology and rare diseases; and we are also starting collaborations with clinical groups.

Research results

A new model of Charcot-Marie-Tooth peripheral neuropathy in Drosophila.

One of the genes involved in Charcot – Marie– Tooth (CMT) disease, an inherited peripheral neuropathy, is GDAP1. We have shown that there is a true ortholog of this gene in Drosophila , which we have named Gdap1. Up- and down-regulation of Gdap1 in a tissue-specific manner produces changes in mitochondrial size, morphology and distribution, and neuromuscular degeneration. Interestingly, muscular degeneration is tissue-autonomous and not dependent on innervation. Metabolic analyses of our experimental genotypes suggest that alterations in oxidative stress are not a primary cause of the neuromuscular degeneration but a long-term consequence of the underlying mitochondrial dysfunction. Our results have contributed to a better understanding of the role of mitochondria in CMT disease and pave the way to generate clinically relevant disease models to study the relationship between mitochondrial dynamics and peripheral neurodegeneration.



FFigure 1. Altered levels of GDAP1 result in neuromuscular degeneration. A comparison with tissues from wild type individuals reveals that both increased and decreased levels of GDAP1 produce neurodegeneration (top row, neuronal cytoplasm in yellow) and mitochondrial dysmorphology (bottom row). igure 1.

The Ini/PHF5A gene is required for cell proliferation during development.

The 111 amino acid PHD-finger protein encoded by the CG9548 gene is highly conserved from yeast (Ini1) to vertebrates (SF3b14b in humans, Phf5a in mouse), presenting an identical protein sequence in all known vertebrates. Its functional relevance has been evidenced by its essentiality in yeast and human cultured cells where it was shown to participate in transcription and splicing events.
Our work has shown that the CG9548 pattern of expression is ubiquitous and uniform throughout fly development with preferential sub-cellular localization to the nucleus. Ubiquitous silencing of CG9548 is lethal, but when silencing is performed in a spatially and temporally restricted pattern, the corresponding adult tissue is very reduced size. Immunofluorescence studies demonstrate that this reduced size is not due to increased apoptosis, but to a reduced number of mitoses

Design of a new system for Targeted Drug Delivery.

Targeted Drug Delivery allows developing more specific therapies. The drug can be directed to a cell type or tissue but not other cells of the organism. We propose to design a new technology of nanoparticles that transport the drug. Our system consists of mesoporous silica nanoparticles with a high loading capacity and it uses molecular gates to block the pores. The cargo is contained inside nanoparticles and it is released only when the specific stimulus is present. As molecular gate, we use DNA oligonucleotide complementary to a messenger RNA expressed only in the cells that we want to treat.
To optimize the nanoparticles design, we have used Drosophila melanogaster cell lines. Our preliminary results show that our system is not toxic to the cell. The system is capable of preferential opening, and at the moment we are optimising the design of the molecular gate to make it more specific.

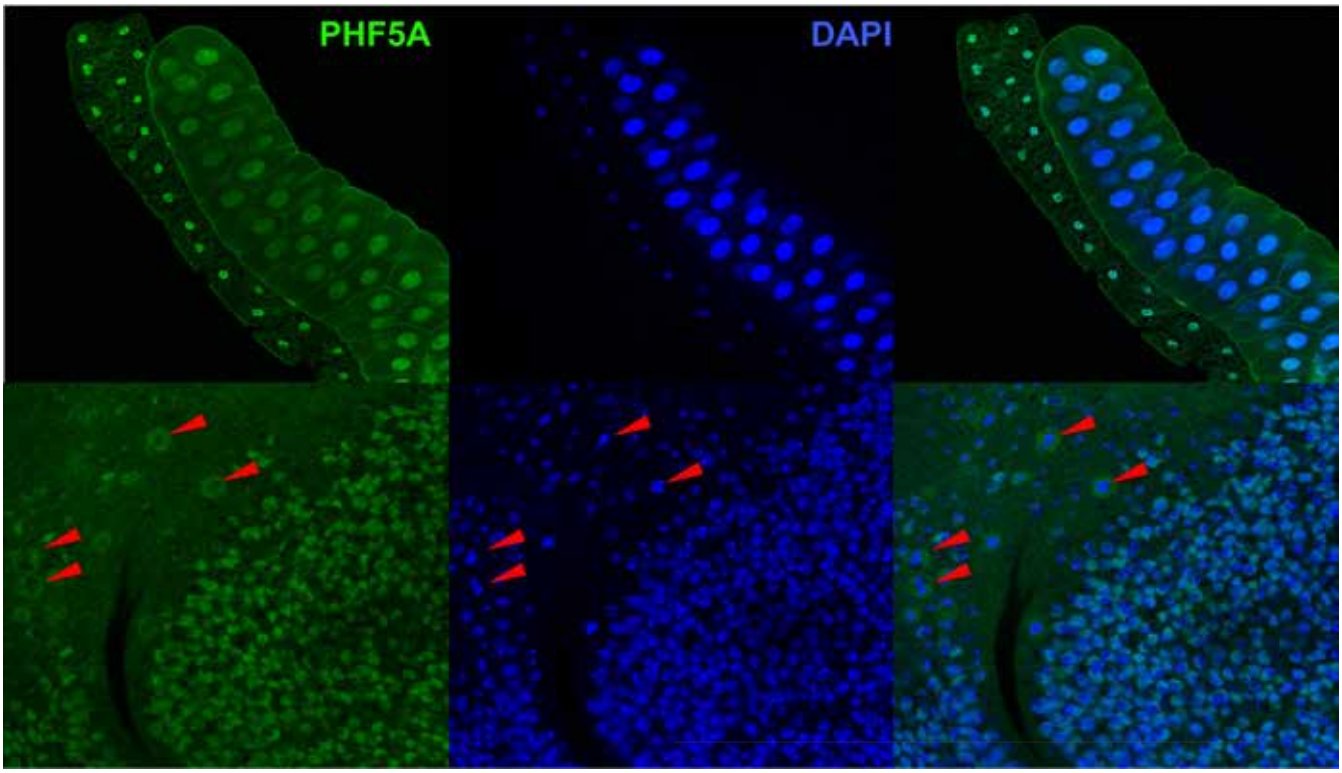


Figure 2. Localisation of tthe PHF5A protein. In a tissue without cell division (salivary gland, top row) PHF5A (green) is mostly localized within the nucleus (DAPI, blue). In a proliferative tissue (imaginal epithelium, bottom row) PHF5A is delocalized in those cells undergoing mitosis.

Publications

1 .Víctor López del Amo, Marta Seco Cervera, José Luis García Giménez, Alexander Withworth, Federico Pallardo, Máximo Ibo Galindo.
Mitochondrial defects and neuromuscular degeneration caused by altered expression ofDrosophila Gdap1: implications for the Charcot-Marie-Tooth neuropathy.
Hum Mol Genet Vol: 23 (18), 2014
Quartile: Q1

Conferences and meetings

1 .Cell Symposia: Transcriptional Regulation in Development
Oral Communication
Ibo Galindo , Elisa Oltra
(Chicago , USA)

2 .IECB Young Scientist Symposium
Oral Communication
Marta Seco-Cervera, José Luis García-Giménez, Alexander Withworth,Federico Pallardó Calatayud, Máximo Ibo Galindo Orozco
(Bordeaux , France)

3 .Simposium Internacional: Neuropatías periféricas hereditarias - desde la biología a la terapéutica. Fundación Ramón Areces Lecture
Ibo Galindo Orozco
(Madrid , Spain)

Genetics and Genomics of Neuromuscular Disorders

Group Leader

Carmen Espinós

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Vincenzo Lupo (CIBERER)

→ Predoctoral Scientists
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Elva Martín (UV)
Alberto Giménez (UV)
Alba Sánchez-Calatayud (UPV)
Irene Gimeno-Lluch (UV)

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Overview

The research lines of the Unit of Genetics and Genomics of Neuromuscular Disorders focus on the characterization of new genes and new mutations involved in hereditary motor and/or sensory neuropathies. By genomic mapping and exome sequencing we determine the genetic causes that underlie new forms of hereditary peripheral neuropathies. Our research is clearly translational and we are interested in developing new tools for genetic diagnosis.

Besides disease-causing mutations, other genetic factors contribute to the phenotype and modify the severity and progression of a disorder. In this sense, we work on identifying genetic modifiers to clarify the frequent inter and intrafamilial variability observed in some clinical episodes.

The discovery of these new genes and new mutations is the trigger that leads us to initiate several strategies in order to elucidate the mechanisms of disease and to establish the relationship of novel genes and neuropathy, and how novel mutations cause disease.

Finally, we have initiated a new research line focused on the characterization of the genetics of neurodegenerative disorders with accumulation of iron in brain. The findings of this study will allow us to perform better genotype-phenotype correlations in order to improve the assessment of these patients who suffer a devastating disorder. Moreover, our group works closely with the Service of Genomics and Translational Genetics (SGGT) with advice, study designs and implementation of new analyses applied to genetic diagnosis

Research results

1.- Characterization of new genes and new mutations.

More than 50 genes are involved in the Hereditary Motor Sensory Neuropathy (HMSN). Some of these genes are also related to other group of hereditary neuropathies, the motor forms: Hereditary Motor Neuropathy (HMN). Using genome wide mapping and/or exome sequencing, we work on the identification of new mutations and new genes associated with these two groups of neuropathies and other hereditary peripheral neuropathies. We currently investigate the clinical series of the hospitals that belong to the TREAT-CMT Consortium *(http://www.treat-cmt.es)*. It is important to highlight that this research lines are possible thanks to the collaboration with the Department of Genomics and Translational Genetics at the CIPF *(http://www.cipf.es/es/web/portada/genomica-y-genetica-traslacional)* and with the Biobank CIBERER *(http://www.ciberer-biobank.es/)*.

With a translational aim, we have developed a tool for the genetic diagnosis of this group of neuropathies that comprises 56 genes. This tool is available at the Department of Genomics and Translational Genetics at the CIPF.

Also with an obvious application in the clinical practice, we have generated a data base of mutations involved in HMSN and HMN in Spanish population *(http://www.treat-cmt.es/db)*. This kind of information is useful in diagnosis because these rare disorders show a wide genetic heterogeneity.

We are also interested in the identification of gene modifiers in patients and their relatives with mutations in the *GDAP1* gene **[Fig. 1]**. The CMT disease type 2K (CMT2K) is caused by mutations in the GDAP1 gene that are inherited in a dominant autosomal pattern and is characterized by a wide clinical variability. In collaboration with Dr. F. Palau (CIPF & CIBERER, Valencia) we have investigated the *junctophilin-1* (JPH1) gene as a possible gene modifier. We have identified a patient who carries the *GDAP1* p.R120W mutation and also the *JPH1* p.R213P change, which by itself does not lead to disease. This proband presents a more severe clinical picture than her affected relatives who

only harbour the *GDAP1* p.R120W mutation. This investigation has allowed us to conclude that *GDAP1* and *JPH1* play a role in the same process related to calcium metabolism and they are dependent each other. These results help to understand why some patients with CMT2K show so different phenotypes and moreover, indicate the importance of analysing the JPH1 gene in patients who suffer from this neurodegenerative disease to make better its prognosis.

2.- Mechanisms of disease in hereditary peripheral neuropathy.

2.1.- To investigate the cellular pathophysiology of the Charcot-Marie-Tooth disease type 4C (CMT4C).
SH3TC2, the protein involved in CMT4C, may be a constituent of multiprotein complexes related to the transduction of the signal from the axon to the Schwann cell. This research line is focused on studying the signalling pathways and the interactors of SH3TC2 in order to better understand the role of this protein in the developing of the peripheral nerves.

The Ranvier nodes are altered in both CMT4C patients and Sh3tc2 knock-out mouse. In collaboration with Dr. R. Chrast (University of Lausanne, Switzerland) we have investigated the capacity of myelin elongation [Fig. 2]. Our findings indicate that the communication between axon and Schwann cell is anomalous in the early development of the myelin in the Schwann cells lacking Sh3tc2 clarifying the pathophysiology of CMT4C and giving new data about this disease which does not have a suitable treatment.

2.2.- Characterization of a new form of hereditary recurrent neuropathy. In a proband that belongs to a large family diagnosed of a hereditary recurrent neuropathy, we have identified a novel mutation in a gene unrelated to disease. Our investigation is focused in characterizing this gene and in studying why the described mutation causes this new clinical form of neuropathy.

2.3.- **Identification of a gen associated with a new form of axonal CMT.** In collaboration with Dr. F. Palau (CIPF & CIBERER, Valencia), we have investigated a new gene involved in two unrelated families with axonal CMT. We

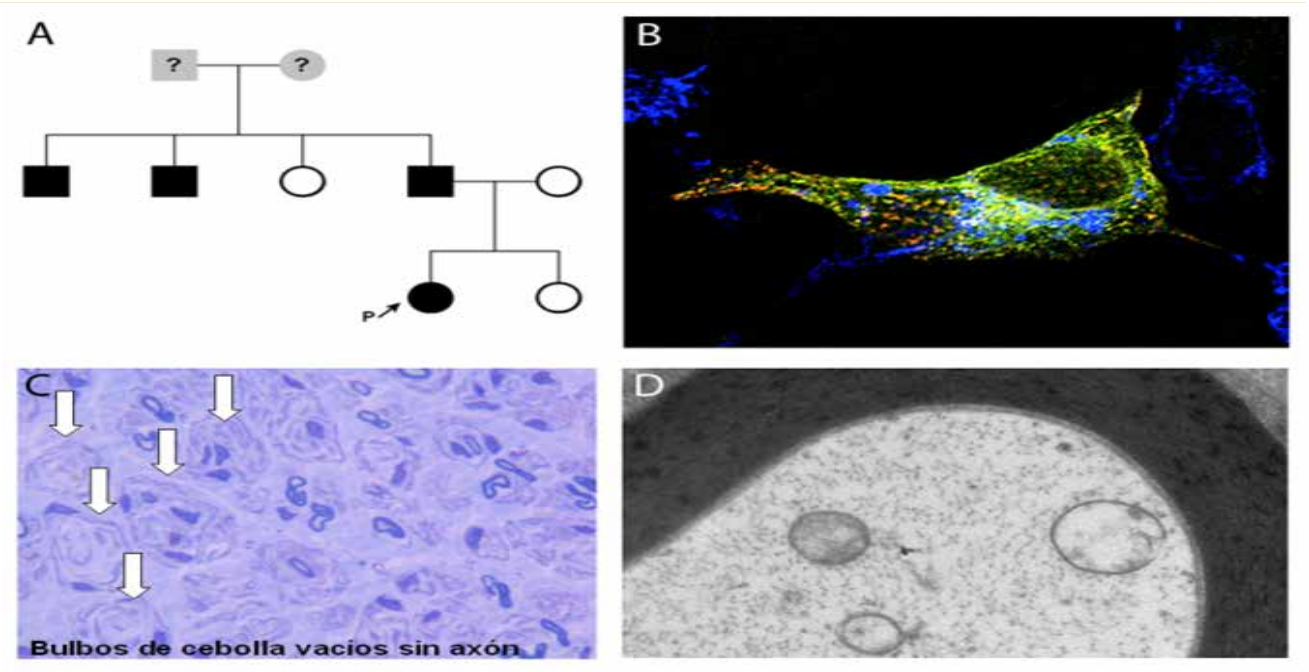

Figure 1.Juntocphilin-1 is a gene modifier for the neuropathy caused by mutations in the GDAP1 gene. (A) Pedigree of the family with the GDAP1 p.R120W in which the JPH1 p.R213P change has been identified that act as a negative modifier. (B) Confocal immunofluorescence that shows the colocalization between JPH1 (green) and STIM1 (red), both proteins are involved in calcium metabolism together with GDAP1. Mitochondria are in blue. Presence of onion bulbs without axon (C) and mitochondrial alterations (D) in biopsies of sural nerve in patients who belong to this family.

study the role of this gene in peripheral nervous system and its relatedness with other genes involved in hereditary peripheral neuropathies.

3.- Characterization of the molecular bases which underlie in diseases with neurodegeneration with brain iron accumulation (NBIA).

To date 11 genes are known associated with NBIA disoders. In collaboration with Dr. B. Pérez-Dueñas (H. Sant Joan de Déu & CIBERER, Barcelona) we have recently initiated a new research line focused on the clinical and genetic characterization of NBIA patients in Spanish population, which will allow us to develop a clinical scale to better assessment these patients who suffer from a devastating disorder that currently does not have a notarized therapy.

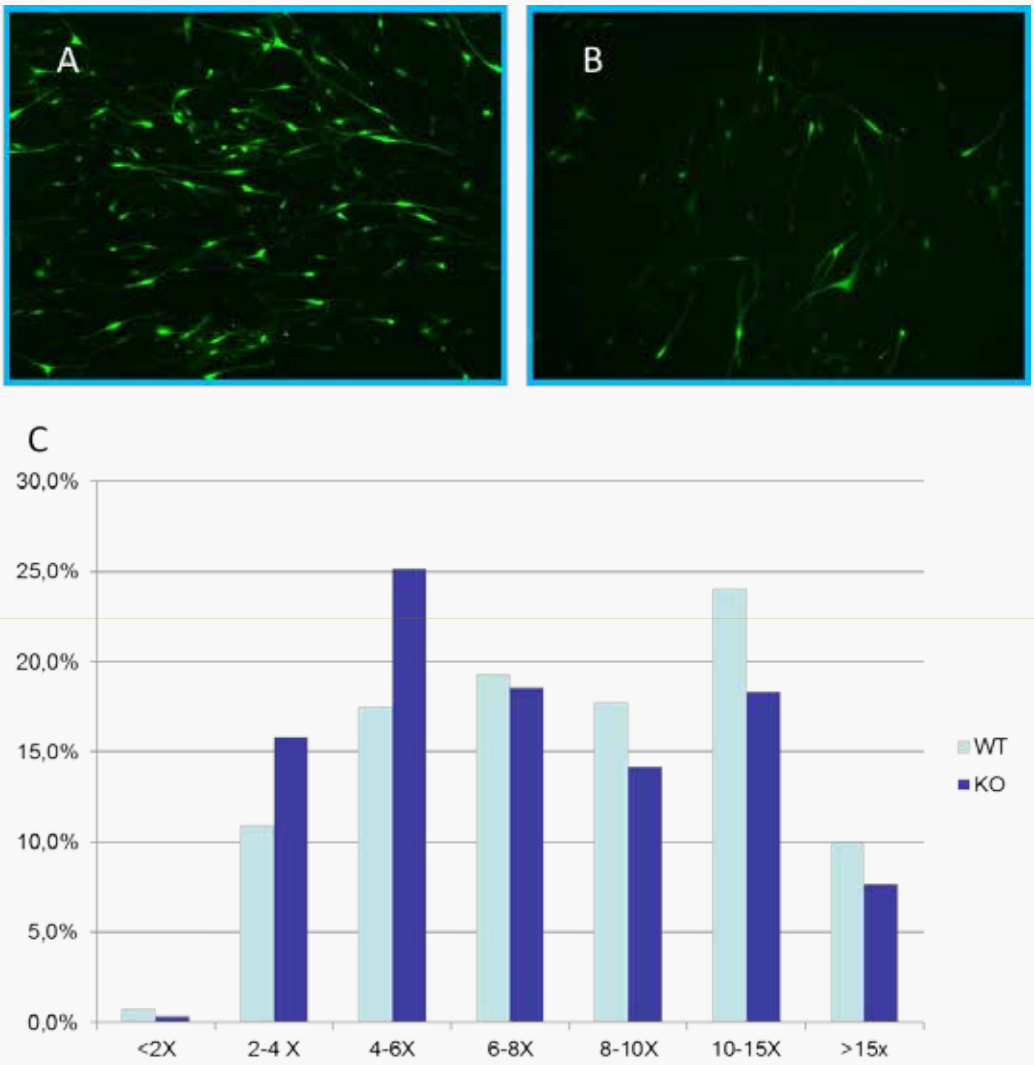

Figure 2. Capacity of elongation of Schwann cell lacking Sh3tc2 is decreased. The pictures show the coculture of Schwann cells from normal mice (A) and mice lacking Sh3tc2 (B). (C) The graphs indicate that Schwann cells from Sh3tc2-/- mice (knock-out) seem to be less elongated compared to control mice. The elongation was measured according to the cell body as reference (n=1; that is nX=1X). Therefore, the value of nX corresponding to each cell will be the same that the elongation measurement divided between the median values of its body cell. The measures were analysed using the Image J program. The experiment was performed three times and in each of them, all the cells contained in 30 pictures of fluorescence microscopy were analysed. S100 was used as specific marker of the Schwann cell.

Publications

1 .Buján N, Arias A, Montero R, García-Villoria J, Lissens W, Seneca S, Espinós C, Navas P, De Meirleir L, Artuch R, Briones P, Ribes A.
Characterization of CoQ10 biosynthesis in fibroblasts of patients with primary and secondary CoQ10 deficiency.
Journal of inherited metabolic disease Vol: 37, 53-62 . Quartile: Q1

2 .Perez-Garrigues, H; Sivera, R; Vilchez, JJ; Espinos, C; Palau, F; Sevilla, T.
Vestibular impairment in Charcot-Marie-Tooth disease type 4C.
Journal of Neurology, Neurosurgery and Psychiatry, Vol: 85, 824-118. Quartile: Q1

3 .Calpena E, Martínez-Rubio D, Arpa J, García-Peñas JJ, Montaner D, Dopazo J, Palau F, Espinós C.
A novel locus for a hereditary recurrent neuropathy on chromosome 21q21.
Neuromuscular disorders Vol: 24 (8), 660-665 . Quartile: Q2

4.Yubero D, O'Callaghan M, Montero R, Ormazabal A, Armstrong J, Espinos C, Rodriguez MA, Jou C, Castejon E, Aracil MA, Cascajo MV, Gavilan A, Briones P, Jimenez-Mallebrera C, Pineda M, Navas P, Artuch R.
Association between coenzyme Q(10) and glucose transporter (GLUT1) deficiency.
BMC Pediatrics Vol: 14, 284 . Quartile: Q2

5 .Pla-Martín D, Calpena E, Lupo V, Márquez C, Rivas E, Sivera R, Sevilla T, Palau F, Espinós C.
Junctophilin-1 is a modifier gene of GDAP1-related Charcot-Marie-Tooth disease.
Hum Mol Genet . Quartile: Q1

6 .Calpena E, Deshpande AA, Yap S, Kumar A, Manning NJ, Bachhawat AK, Espinós C.
New insights into the genetics of 5-oxoprolinase deficiency and further evidence that it is a benign biochemical condition.
European Journal of Pediatrics .Quartile: Q2

Conferences and Meetings

1 .LXVI Reunión Anual de la Sociedad Española de Neurología
Oral communication
Panel de genes para el diagnóstico de la enfermedad de Charcot-Marie-Tooth y de la atrofia espinal distal.
Lupo V, Tello C, García-García F, García-Romero M, Pascual-Pascual SI, Villarreal L, Márquez C, Casasnovas C, Sivera R, Sevilla T, Espinós C
(Valencia.Spain)

2 .LXVI Reunión Anual de la Sociedad Española de Neurología
Oral communication
Caracterización de un nuevo gen implicado en la neuropatia de Charcot-Marie-Tooth.
Lupo V, Sancho P, Sánchez-Monteagudo A, Martínez-Rubio D, Calpena E, Sevilla T, Palau F, Espinós C
(Valencia.Spain)

3 .LXVI Reunión Anual de la Sociedad Española de Neurología
Oral communication
Caracterización de un modificador genético de GDAP1: implicaciones para la neuropatia de Charcot-Marie-Tooth.
Calpena E, Pla-Martín D, Lupo V, Márquez C, Rivas E, Sivera R, Sevilla T, Palau F, Espinós C
(Valencia.Spain)

4 .The 3rd Joint Symposium on Neuroacanthocytosis and Neurodegeneration with Brain Iron Accumulation
Oral communication
Pantotenate kinase associated neurodegeneration: clinical assessment and genetic characterization by means of a Spanish multi-centre research network.
Pérez-Dueñas B, Darling A, Serrano M, Gastón I, Aguilera S, Madruga M, Mir P, Pujol M, López A, Tello C, Espinós C, Martí MJ
(Stresa.Italy)

5 .VI Reunión Científica Anual CIBERER. San Lorenzo del Escorial.
Communication
Identification of modifier genes of GDAP1: implications for the Charcot-Marie-Tooth disease.
D. Pla-Martín, E. Calpena, V. Lupo, C. Márquez, R. Sivera, T. Sevilla, F. Palau, C. Espinós
(Madrid.Spain)

6 . LXVI Reunión Anual de la Sociedad Española de Neurología
Communication
Nuevos interactores de SH3TC2, gen implicado en la neuropatía de Charcot-Marie-Tooth tipo 4C y su papel regulador en la célula de Schwann.
Lupo V, Burgos R, Calpena E, Gouttenoire E, Bartersaghi L, Chrast R, Espinós C
(Valencia.Spain)

7 . I Congreso Biomedicina Predocs Valencia
Communication
Caracterización de un nuevo gen implicado en la neuropatía de Charcot-Marie-Tooth.
Sancho P, Lupo V, Sánchez-Monteagudo A, Martínez-Rubio D, Calpena E, Sevilla T, Palau F, Espinós C
(Valencia.Spain)

8 . I Congreso Biomedicina Predocs Valencia
Communication
Biomarkers research in neuromuscular disease Charcot-Marie-Tooth.
Seco-Cervera M, García-Giménez JL, Ibáñez-Cabellos S, Espinós C, Palau F, Sánchez-Jiménez F, Reyes-Palomares A, Pallardó FV
(Valencia.Spain)

9 .Proceedings of the Spetses Summer School. Biochemical Basis of Healthy AgingCommunicationBiomarkers Research in neuromuscular disease Charcot-Marie-Tooth.
Seco-Cervera M, Ibáñez-Cabellos S, García-Giménez JL, Espinós C, Palau F, Pallardó FV
(Spetses .Greece)

10 . X Meeting of the Spanish group of free radical research (GEIRLI).Symposium on ixidative stress and redox signaling in biology and medicineCommunication
Búsqueda de bimarcadores enplasmas de pacientes de Charcot-Matie-Tooth con duplicación CMT1A.
Seco-Cervera M, García-Giménez JL, Ibáñez-Cabellos S, Espinós C, Palau F, Pallardó FV
(Valencia.Spain)

11 .International Symposium. Nerve biology and inherited peripheral neuropathy. From biology to therapy. Fundación Ramón Areces
Invited conference ."Genetic Diagnostics"
Espinós C.
(Madrid.Spain)

Genetics and Molecular Medicine

Group Leader

Francesc Palau

→ Research Assistants

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Anna Estela Bolta
David Pla Martín
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Azahara Civera
Diana Carolina Muñoz

→Technicians

Fátima Riveiro
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→Students

Sonia Vicente-Ruiz (UV)
Carla Ordiñana(UV)
Amadeo Muñoz(UPV)

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Overview

The group has been investigating the fundamental aspects of cell biology and molecular pathophysiology and neuromuscular diseases and, within the Program for Rare and Genetic Diseases, commissioning the Genomics and Translational Genetics Service (SGGT). The scientific objectives and experimental work has focused on the study of the pathogenic mechanisms associated with Charcot -Marie -Tooth (CMT) disease, Friedreich's ataxia (FRDA) and Duchenne muscular dystrophy (DMD).
The group has been investigating the fundamental aspects of biology and cellular and molecular pathophysiology of neuromuscular diseases and commissioning, within the Programme Genetic and Rare Diseases (RareGene) Service Genomics and Translational Genetics (SGGT). The scientific objectives and experimental work has focused on the study of the pathogenic mechanisms associated with Charcot-Marie-Tooth (CMT), Friedreich's ataxia (FRDA) and Duchenne muscular dystrophy (DMD). In 2014 we investigated (i) the role of mitochondrial dynamics, calcium homeostasis and pathophysiology of the nervous system of the CMT neuropathy associated with mutations in the *GDAP1* gene in knock-out mice developed in our laboratory; (ii) the metabolic and pathophysiological consequences of frataxin depletion in SH-SY5Y cells and the humanized mouse YG8R as FRDA cell and animal models, respectively; (iii) translational research to clinical expression in patients with CMT4C disease caused by mutations in the *SH3TC2* gene; and (iv) analysis of the biology of ANKK1 in myogenesis and satellite cells and their relationship with Duchenne muscular dystrophy in collaboration with Dr. J. Hoenicka. Also within the RareGene Programme, in collaboration with Dr. C. Espinós we have investigated about the presence of genetic modifiers of GDAP1 gene and its consequences on the biology of their clinical mutations and their effect on the variability of clinical phenotype.

Research results

We highlight some results related to our research on disease mechanisms in hereditary peripheral neuropathies and Friedreich's ataxia results and translational research in the field of genetic diagnosis of Charcot-Marie-Tooth disease and other rare and genetic diseases such as celiac disease in collaborators with Dr. Y. Sanz from the Institute of Agrochemistry and Food Technology, CSIC. Among the research conducted and sent to publishing that are still under review we highlight the work performed during several years related to the generation and analysis of the knockout mouse model (*Gdap1-/-)* of Charcot-Marie-Tooth type 4A / 2K neuropathy. This study has allowed us to understand the cellular and molecular processes and consequences of the lack of GDAP1 (*Barneo-Muñoz et al. Lack of GDAP1 induces neuronal calcium and mitochondrial defects in a knockout mouse model of Charcot-Marie-Tooth neuropathy.* **PLoS Genet** *2014 [2nd revision]*)
Below we summarize several reports and relevant articles published in 2014, either on paper or online (PubMed):

JPH1 as a modifier gene of Charcot-Marie-Tooth 2K disease

Pla-Martín D, Calpena E, Lupo V, Márquez C, Rivas E, Sivera R, Sevilla T, Palau F, Espinós C*. Junctophilin-1 is a modifier gene of GDAP1-related Charcot-Marie-Tooth disease. Hum Mol Genet 2015; Jan 1, 24: 213-229, doi: 10.1093/hmg/ddu440 (cover page, no. 1, vol. 24)*

Mutations in the *GDAP1* gene cause different forms of Charcot-Marie-Tooth (CMT) disease, and the primary clinical expression of this disease is markedly variable in the dominant inheritance form (CMT type 2K; CMT2K), in which carriers of the *GDAP1* p.R120W mutation can display a wide range of clinical severity. We investigated the JPH1 gene as a genetic modifier of clinical expression variability because junctophilin-1 (*JPH1*) is a good positional and functional candidate. We demonstrated that the *JPH1-GDAP1* cluster forms a paralogon and is conserved in vertebrates. Moreover, both proteins play a role in Ca2+ homeostasis, and we demonstrated that *JPH1* is able to restore the store-operated Ca2+ entry (SOCE) activity in *GDAP1-silenced* cells. After the mutational screening of *JPH1* in a series of 24 CMT2K subjects who harbour the GDAP1

p.R120W mutation, we characterized the *JPH1 p.R213P* mutation in one patient with a more severe clinical picture. JPH1 (p.R213P) cannot rescue the SOCE response in *GDAP1-silenced* cells. We observed that *JPH1* colocalizes with *STIM1*, which is the activator of SOCE, in endoplasmic reticulum-plasma membrane puncta structures during Ca2+ release in a *GDAP1-dependen*t manner. However, when GDAP1 (p.R120W) is expressed, *JPH1* seems to be retained in mitochondria. We also established that the combination of GDAP1 (p.R120W) and JPH1 (p.R213P) dramatically reduces SOCE activity, mimicking the effect observed in *GDAP1* knock-down cells. In summary, we conclude that JPH1 and GDAP1 share a common pathway and depend on each other; therefore, *JPH1* can contribute to the phenotypical consequences of *GDAP1* mutations.

Mitochondrial pathophysiology in Friedreich's ataxia

Bolinches-Amorós et al. Mitochondrial dysfunction induced by frataxin deficiency is associated with cellular senescence and abnormal calcium metabolism. Front Cell Neurosci 2014; 8:124. doi: 10.3389/fncel.2014.00124

Friedreich ataxia is considered a neurodegenerative disorder involving both the peripheral and central nervous systems. Dorsal root ganglia (DRG) are the major target tissue structures. This neuropathy is caused by mutations in the FXN gene that encodes frataxin. Here, we investigated the mitochondrial and cell consequences of frataxin depletion in a cellular model based on frataxin silencing in SH-SY5Y human neuroblastoma cells, a cell line that has been used widely as in vitro models for studies on neurological diseases. We showed that the reduction of frataxin induced mitochondrial dysfunction due to a bioenergetic deficit and abnormal Ca^{2+} homeostasis in the mitochondria that were associated with oxidative and endoplasmic reticulum stresses. The depletion of frataxin did not cause cell death but increased autophagy, which may have a cytoprotective effect against cellular insults such as oxidative stress. Frataxin silencing provoked slow cell growth associated with cellular senescence, as demonstrated by increased SA-βgal activity and cell cycle arrest at the G1 phase. We postulate that cellular senescence might be related to a hypoplastic defect in the DRG during neurodevelopment, as suggested by necropsy studies.

Vestibular involvement Charcot-Marie-Tooth 4C disease caused by mutations in the *SH3TC2* gene

Pérez-Garrigues et al. Vestibular impairment in Charcot-Marie-Tooth disease type 4C. J Neurol Neurosurg Psychiatry. 2014;85:824-7. doi: 10.1136/jnnp-2013-307421

Charcot-Marie-Tooth disease type 4C (CMT4C) is a hereditary neuropathy with prominent unsteadiness. The objective of the current study is to determine whether the imbalance in CMT4C is caused only by reduced proprioceptive input or if vestibular nerve involvement is an additional factor. We selected 10 CMT4C patients and 10 age-matched and sex-matched controls. We performed a comprehensive evaluation of the vestibular system, including video Head Impulse Test, bithermal caloric test, galvanic stimulation test and skull vibration-induced nystagmus test. None of the patients experienced dizziness, spontaneous or gaze-evoked nystagmus, but all had significant vestibular impairment when tested when compared to controls. Seven had completely unexcitable vestibular systems and abnormal vestibuloocular reflex. There was no correlation between the degree of vestibulopathy and age or clinical severity. Significant vestibular impairment is a consistent finding in CMT4C and is present early in disease evolution. The profound imbalance that is so disabling in these patients may result from a combination of proprioceptive loss and vestibular neuropathy, and this would modify the recommended rehabilitation strategies.

Relevance of HLA class II genotype in the intestinal microbiot in high-risk infants of celiac disease

Olivares et al. The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease. Gut 2014 Jun 17. pii: gutjnl-2014-306931. doi: 10.1136/gutjnl-2014-306931

Intestinal dysbiosis has been associated with coeliac disease (CD), but whether the alterations are cause or consequence of the disease is unknown. This study investigated whether the human leukocyte antigen (HLA)-DQ2 genotype is an independent factor influencing the early gut microbiota composition of healthy infants at family risk of CD. As part of a larger prospective study, a subset (n=22) of exclusively breastfed and vaginally delivered infants with either high genetic risk (HLA-DQ2 carriers) or low genetic risk (non-HLA-DQ2/8

carriers) of developing CD were selected from a cohort of healthy infants with at least one first-degree relative with CD. Infant faecal microbiota was analysed by 16S rRNA gene pyrosequencing and real time quantitative PCR. Infants with a high genetic risk had significantly higher proportions of Firmicutes and Proteobacteria and lower proportions of Actinobacteria compared with low-risk infants. At genus level, high-risk infants had significantly less Bifidobacterium and unclassified Bifidobacteriaceae proportions and more Corynebacterium, Gemella, Clostridium sensu stricto, unclassified Clostridiaceae, unclassified Enterobacteriaceae and Raoultella proportions. Quantitative real time PCR also revealed lower numbers of Bifidobacterium species in infants with high genetic risk than in those with low genetic risk. In high-risk infants negative correlations were identified between Bifidobacterium species and several genera of Proteobacteria (Escherichia/Shigella) and Firmicutes (Clostridium). In conclusion, the genotype of infants at family risk of developing CD, carrying the HLA-DQ2 haplotypes, influences the early gut microbiota composition. This finding suggests that a specific disease-biased host genotype may also select for the first gut colonisers and could contribute to determining disease risk.

Figure: Front page of Human Molecular Genetics, Jan 2015

Both experimental biology and translational research in the laboratory has been funded by national and international agencies and private foundations. Concretely, the R+D National Plan (Biomedicine, Ministry of Economy and Competiveness), the Instituto de Salud Carlos III (ISCIII/IRDiRC International Programme on Rare Diseases, TREAT-CMT Consortium), the European Commission 7th Frame Programme (EFACTS Consortium), the Generalitat Valenciana (Prometeo Programme), the Marató TV3 Foundation and the Isabel Gemio Foundation.

Our group belongs to the CIBER on Rare Diseases (CIBERER, www.ciberer.es), of which Dr. Palau is the current scientific director. The group is collaborating with Spanish research groups within the CIBERER and other institutions, especially in the setting of the TREAT-CMT Consortium (www.treat-cmt.es), and with international groups from the EFACTS Consortium (www.e-facts.eu), the University of Laussanne and Karolinska Institute. Special mention deserves the long time collaboration with the Department of Neurology of the La Fe University Hospital in Valencia. Celiac disease studies are part of collaboration with the Institute of Agrochemistry and Food Technology, CSIC.

Publications

1 .Salt J, Cuenca A, Palau F, Dormido S.
A Multirate Control Strategy to the Slow Sensors Problem: An Interactive Simulation Tool for Controller Assisted Design.
Sensors, Vol: 14, . 2014 Quartile: Q1

2 .Bolinches-Amoros, A; Molla, B; Pla-Martin, D; Palau, F; Gonzalez-Cabo, P.
Mitochondrial dysfunction induced by frataxin deficiency is associated with cellular senescence and abnormal calcium metabolism.
Frontiers in Cellular Neuroscience Vol: 8, 124 . 2014 Quartile: Q1

3 .Calpena E, Martínez-Rubio D, Arpa J, García-Peñas JJ, Montaner D, Dopazo J, Palau F, Espinós C.
A novel locus for a hereditary recurrent neuropathy on chromosome 21q21.
Neuromuscular disorders Vol: 24 (8), 660-665 . 2014 Quartile: Q2

4 .Perez-Garrigues, H; Sivera, R; Vilchez, JJ; Espinos, C; Palau, F; Sevilla, T.
Vestibular impairment in Charcot-Marie-Tooth disease type 4C.
Journal of neurology neurosurgery and psychiatry, Vol: 85, 824-U118 . 2014 Quartile: Q1

5 .Hoenicka J, García-Ruiz PJ, Ponce G, Herranz A, Martínez-Rubio D, Pérez-Santamarina E, Palau F.
The Addiction-Related Gene ANKK1 in Parkinsonian Patients with Impulse Control Disorder.
Neurotoxicity research 2014

6 .Pla-Martín D, Calpena E, Lupo V, Márquez C, Rivas E, Sivera R, Sevilla T, Palau F, Espinós C.
Junctophilin-1 is a modifier gene of GDAP1-related Charcot-Marie-Tooth disease.
Hum Mol Genet . 2014 Quartile: Q1

Conferences and meetings

1. Workshop on European Reference Networks and Structural Funds
Lecture.
Francesc Palau Martínez
(Rome , Italy)

2. LXV Reunión Anual de la Sociedad Española de Neurología (SEN)
Oral Communication
Francesc Palau Martínez
(Valencia , Spain)

3. Jornada de Introducción a las Enfermedades Raras
Lecture
Francesc Palau Martínez
Ciudad Real , Spain)

4. I Jornadas de Investigación en Ciencias Ómicas y Estilos de Vida, JICOVA
Oral Communication
Belén Mollá, Diana Carolina Muñoz Lasso, Francesc Palau, Pilar Gonzalez-Cabo
(Valencia , Spain)

5. 13th International Congress on Neuromuscular Diseases
Oral Communication
Belén Mollà, Pilar González Cabo
(Nice , France)

6. Ataxia Research Conference
Oral Communication
Aranzazu Bolinches Amorós, Belén. Molla Moliner
(ondon , United Kingdom)

7. 7th CIBER Rare Disease Annual meeting (CIBERER)
Oral Communication
D. Pla-Martín, E. Calpena, V. Lupo, C. Márquez. R. Sivera, T. Sevilla, F. Palau, C. Espinós.
(Madrid , Spain)

Doctoral Thesis presented

Doctoral Candidate: Arancha Bolinches. Biología celular y molecular de las neuropatías periféricas . Director: Francesc Palau. Universidad de Valencia, UV

Genetics and Molecular Physiopathology of Mental and Brain Disorders

Group Leader

Janet Hoenicka

→ Predoctoral Scientists
Estrella Rubio Solsona
Estela Pérez Santamarina

→Technicians
Eloisa Barber

→Colaborators
Irene Pla Navarro (UV)
Magdalena Martínez (UPV)
Arantxa Martínez Férriz (UPV)

www.cipf.es/genetica-y-fisiopatologia-de-los-trastornos-cerebrales-y-mentales

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Overview

The group investigates the genetic, cellular and pathophysiological bases of vulnerability to neuropsychiatric and neuromuscular disorders within the Program of Genetic and Rare Diseases (RareGene) and participates as a consultant of the Genomics and Translational Genetics Service (SGGT). The group leader is also attached to the G19 Unit of the CIBER of Mental Health (CIBERSAM) and is also linked by agreement to Health Research Institute of the Hospital 12 de Octubre Research Institute (IISH12O) in the Molecular Biology Group of Psychiatric Disorders of the Neurosciences and Mental Health Area. The scientific objectives and experimental work during 2014 have been focused on the following aspects: (i) the generation of a mouse model conditional knockout of the gene involved in addictions and learning Ankyrin repeat and kinase domain containing 1 (Ankk1), (ii) ANKK1 study in patients with Parkinson's disease (PD) and as a marker of impulse control disorder, (iii) genomic studies in patients with schizophrenia, (iv) studies, in neuron-like cell models, of polymer nanocarriers for drug release and (v) analysis of the biology of ANKK1 in myogenesis and satellite cells and their relationship with Duchenne muscular dystrophy in collaboration with Dr. Francesc Palau and Dr. Juan J. Vílchez.

Research results

We highlight some results related to our research on ANKK1 gene involvement in the mechanisms of neuropsychiatric diseases. In collaboration with Professors Harker Rhodes and Steve Fiering at the Geisel School of Medicine and Dartmouth-Hitchcock Medical Center, New Hampshire, USA, we have generated an Ankk1 conditional knock-out mouse. On the other hand, although the ANKK1 gene has been traditionally associated with addiction disorders and dopaminergic traits, our group aims to expand the spectrum of ANKK1 into other pathologies related to the striatum dysfunction such as Parkinson's disease (PD).
Below we summarize several reports and relevant articles published in 2014, either on paper or online (PubMed).

Deciphering the molecular bases of psychiatric symptoms in Parkinson´s disease: study of the ANKK1 gene

Parkinson´s disease is a disorder cause by the selective degeneration of dopaminergic neurons in the brain. The occurrence of neurologic and psychiatric symptoms varies among PD patients. While the anatomical, neurochemical and physiological substrates of the motor symptoms are quite well understood, the molecular pathogenesis leading to cognitive and psychiatric dysfunctions are less known. Polymorphic variants of ANKK1 gene have been related to a variety of dopamine-related disorders and traits such as addictions, impulsive disorders and different learning paradigms. Indeed, in vitro and in vivo studies have shown a connection between ANKK1 and the dopaminergic system. This relationship is also suggested by in silico analyses of ANKK1 and the dopamine receptor D2 (DRD2) gene locus.
Given the link between ANKK1 and the dopaminergic system, we have studied the ANKK1 gene in PD patients in collaboration with the Neurology Departments of Fundación Jiménez Díaz (Madrid) and Hospital Clínic (Barcelona). The molecular analysis showed ANKK1 variants in both familial and sporadic forms of PD. The functional consequences of these variants have been characterized. We also studied in PD patients the Impulse control disorders (ICDs). ICDs comprise a wide spectrum of abnormal behaviors frequently found in patients with PD receiving antiparkinsonian treatment. Some ICDs share several essential features with substance use disorders therefore ANKK1 is a

candidate gene to be associated to ICD. We have studied the addiction-related TaqIA ANKK1 SNP in a sample of PD patients involved in a multicenter study on ICD. Clinical assessment of ICD was performed using the Questionnaire for impulsive-compulsive disorders (QUIP) in PD. We found no association between TaqIA SNP and ICD in PD patients (p = 0.565). However, when PD patients were grouped according the diagnosis of any ICD with a potentially addictive reinforcement (ICDARs), A1- TaqIA genotype showed significant association (p = 0.036) (Figure). No association was found for the presence of punding in PD patients (p = 0.289). A logistic regression analysis confirmed the independent effect of the A1- genotype upon ICDARs (OR 8.76, 95 % CI 1.3-57.8, Wald = 5.805, p = 0.024). In summary, the TaqIA genotype A1- could be a genetic marker for impulsive traits like ICDARs in EP and it may differentiate two types of disorders which are part of the ICD definition in PD patients (Hoenicka et al, The Addiction-Related Gene ANKK1 in Parkinsonian Patients with Impulse Control Disorder. **Neurotox Res** 2014 Dec 2. doi: 10.1007/s12640-014-9504-x).
In healthy controls the study of ANKK1 locus revealed its involvement in emotion-related paradigms that are affected in PD patients (Koeneke et al , The ANKK1/DRD2 locus is a genomic substrate of affective priming and recognition of angry expressions, under review).

Genomic studies in patients with schizophrenia

Our team has also participated in a Genome Wide Association Study (GWAS) in collaboration with the 'CIBERSAM Psychosis Study Group'. GWAS has allowed the discovery of some interesting risk variants for schizophrenia (SCZ). However, this high-throughput approach presents some limitations, being the most important the necessity of highly restrictive statistical corrections as well as the loss of statistical power inherent to the use of a Single Nucleotide Polymorphism (SNP) analysis approach. These problems can be partially solved through the use of a polygenic approach. We performed a genotyping study in SCZ using 86 previously associated SNPs identified by GWAS of SCZ, bipolar disorder (BPD) and autistic spectrum disorder (ASD) patients. The sample consisted of 3063 independent cases with DSM-IV-TR diagnosis of SCZ and 2847 independent controls of European origin from Spain. A polygenic score analysis was also used to test the overall effect on the SCZ status. One SNP, rs12290811, located in the ODZ4 gene reached statistical significance ($p=1.7\times10^{-4}$, Allelic OR=1.21), a value very near to those reported in previous GWAS of BPD patients. In addition, 4 SNPs were close to the significant threshold: rs3850333, in the NRXN1 gene; rs6932590, at MHC; rs2314398, located in an intergenic region on chromosome 2; and rs1006737, in the CACNA1C gene. We also found that 74% of the studied SNPs showed the same tendency (risk or protection alleles) previously reported in the original GWAS ($p<0.001$). Our data strengthen the polygenic component of susceptibility to SCZ. Our findings show ODZ4 as a risk gene for SCZ, emphasizing the existence of common vulnerability in psychosis (Ivorra et al. Replication of previous genome-wide association studies of psychiatric diseases in a large schizophrenia case-control sample from Spain. **Schizophr Res** 2014 Oct;159(1):107-13. doi: 10.1016/j.schres.2014.07.004).
In addition, we are also collaborating with the Psychiatry Departments of IISH12O Institute in Madrid and Hospital de La Ribera in Alzira with the aim to characterize the genetics of families segregating schizophrenia by exome whole sequencing and comparative genome hybridization.

ICAM-1 targeting for intracellular delivery of therapeutics to neurons

In collaboration with Dr. Silvia Muro, from the Fischell Department of Bioengineering, University of Maryland, College Park, Maryland, USA, we performed the study of neuronal targets for drug release in cultured neural models.
Delivery of therapeutics to neurons is paramount to treat neurological conditions, including many lysosomal storage disorders. However, key aspects of drug-carrier behavior in neurons are relatively unknown: the occurrence of non-canonical endocytic pathways; whether carriers that traverse the blood–brain barrier are, contrarily, retained within neurons; if neuron-surface receptors are accessible to bulky carriers compared to small ligands; or if there are differences regarding neuronal compartments (neuron body vs. neurites) pertaining said parameters. We have explored these questions using model polymer nanocarriers targeting intercellular adhesion molecule-1 (ICAM-1) and differentiated human neuroblastoma cells.
Fluorescence microscopy analysis showed that ICAM-1 expression and nanocarrier binding was enhanced in altered (TNFα) vs. control conditions. While small

ICAM-1 ligands (anti-ICAM) preferentially accessed the cell body, anti-ICAM nanocarriers bound with faster kinetics to neurites, yet reached similar saturation over time. Anti-ICAM nanocarriers were also endocytosed with faster kinetics and lower saturation levels in neurites. Non-classical cell adhesion molecule (CAM) endocytosis ruled uptake, and neurite-to-cell body transport was inferred. Nanocarriers trafficked to lysosomes, delivering active enzymes (dextranase) with substrate reduction in a lysosomal storage disease model. In conclusion, we propose that ICAM-1-targeting holds potential for intracellular delivery of therapeutics to neurons (Hsu J et al. Targeting, Endocytosis, and Lysosomal Delivery of Active Enzymes to Model Human Neurons by ICAM-1-Targeted Nanocarriers. **Pharm Res** 2014 Oct 16.doi: 10.1007/s11095-014-1531-z).

Both experimental biology and translational research in the laboratory have been funded by the Institute of Health Carlos III (Heath Strategic Action [AES]) and Isabel Gemio Foundation.

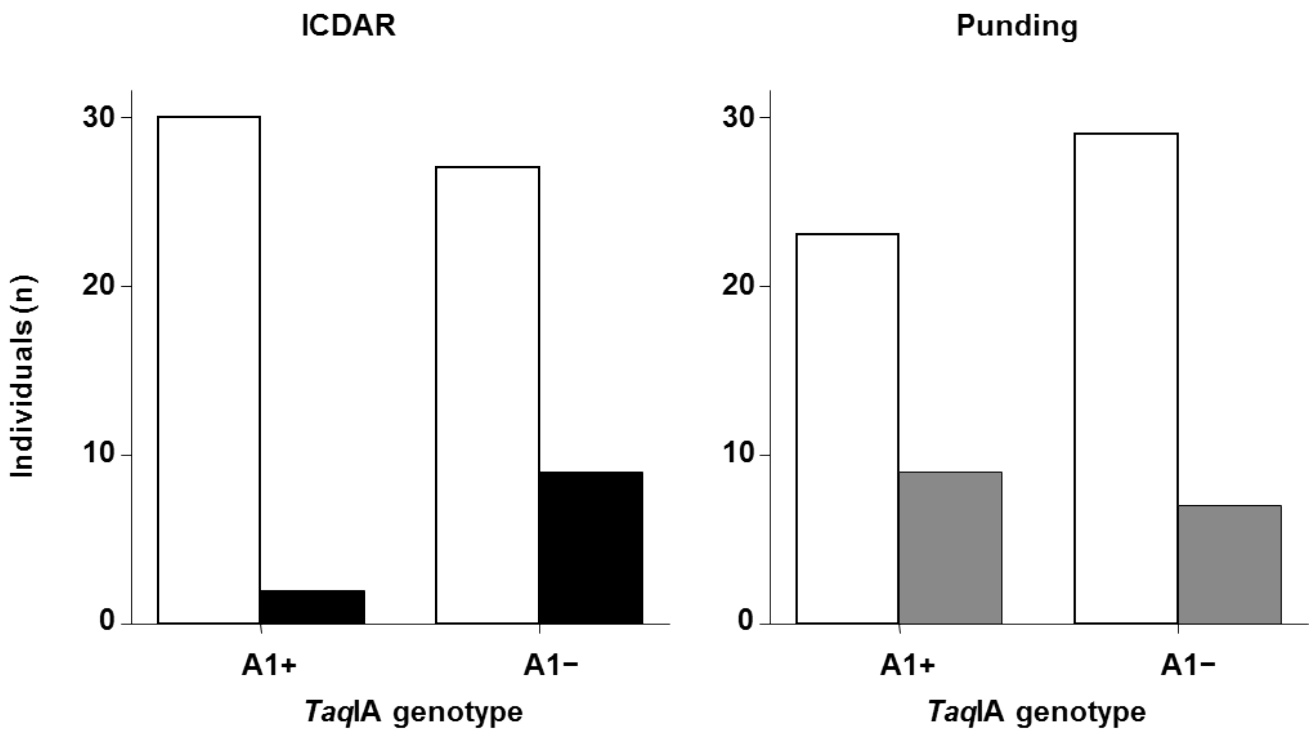

Figure. Distribution of TaqIA ANKK1 SNP genotypes in a sample of PD patients without any ICD phenotype (white bars) or the presence of ICDAR (black bars) and punding (grey bars). A1+ TaqIA genotype: A1 allele homozygous and heterozygous; A1– TaqIA genotype: homozygous for the A2 allele.

Publications

1 . Ivorra JL, Rivero O, Costas J, Iniesta R, Arrojo M, Ramos-Ríos R, Carracedo A, Palomo T, Rodriguez-Jimenez R, Cervilla J, Gutiérrez B, Molina E, Arango C, Alvarez M, Pascual JC, Pérez V, Saiz PA, García-Portilla MP, Bobes J, González-Pinto A, Zorrilla I, Haro JM, Bernardo M, Baca-García E, González JC, Hoenicka J, Moltó MD, Sanjuán J.
Replication of previous genome-wide association studies of psychiatric diseases in a large schizophrenia case-control sample from Spain.
Schizophr Res Vol: 159(1), 107-13 2014
Quartile: Q1

2 .Hsu J, Hoenicka J, Muro S.
Targeting Endocytosis, and Lysosomal Delivery of Active Enzymes to Model Human Neurons by ICAM-1-Targeted Nanocarriers
Pharm Res, 2014. Quartile: Q1

3 .Hoenicka J, García-Ruiz PJ, Ponce G, Herranz A, Martínez-Rubio D, Pérez-Santamarina E, Palau F.
The Addiction-Related Gene ANKK1 in Parkinsonian Patients with Impulse Control Disorder.
Neurotoxicity research 2014 Quartile: Q2

Conferences and meetings

1. LXV Reunión Anual de la Sociedad Española de Neurología (SEN) . Oral Communication. Hoenicka Blanco, J.; García-Ruiz, P ; Herranz , A. ; Martínez-Rubio , D. ; Pérez-Santamarina , E. ; Palau Martínez, F (Valencia , Spain)

2. FRB CIPF . Lecture. (Valencia , Spain)

Intracellular Protein Degradation & Rare Diseases

Group Leader

Erwin Knecht Roberto

→ Research Assistants

Carmen Aguado Muñoz (CIBERER)

Eva Pérez-Jiménez

→ Predoctoral Scientists

Marcos Lahuerta Ferreres

Alihamze Fathinajafabadi Nasresfahani

José Manuel Vidal Donet (UV)

→ Technicians

Asunción Montaner Fayos (Until July 2014)

Mari Paz Rubio Rodríguez

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Moisés Castellá Giner (UPV)

Pedro García Gómez (UPV)

→ Collaborators

Carla López Alegre (UPV)

Rafael Carrascosa Marzo (UPV)

www.cipf.es/degradacion-intracelular-de-proteinas-y-enfermedades-raras

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Overview

The Intracellular Protein Degradation and Rare Diseases laboratory is interested in the study of the functioning and regulation of the main intracellular protein degradation systems: autophagy and the ubiquitin-proteasome system. Different environmental factors, such as nutrients, hormones or growth factors, may induce or inhibit these degradation pathways, in particular autophagy. The importance of the proper functioning of these systems relies in the key role they play in the maintenance of cellular homeostasis. Therefore, alterations in their operation are associated with several pathologies, including neurodegeneration, muscle and cardiovascular diseases and cancer. We are also interested in the study of the regulation of intracellular protein degradation mechanisms in rare diseases, which in turn may serve as a model for other more prevalent ones. We apply our expertise to try to understand the molecular mechanisms behind those diseases characterized by protein, polysaccharide or lipid accumulation. In this sense, our group belongs to the Spanish public consortium CIBERER (Center for Biomedical Network Research on Rare Diseases), participating in the development of new diagnostic tools and therapies.

Research results

1. Regulation of intracellular protein degradation mechanisms.

We have previously shown that glucose deprivation produces an increase in autophagic flux, from autophagosome formation to degradation of the sequestered material. Although we identified that this increase was mediated by the p38 MAPK, the mechanism by which it occurs is still unknown.
We have shown that this induction is mediated by an increase in endoplasmic reticulum stress, and may involve regulators of G-protein signaling genes

2. Alterations of intracellular protein degradation mechanisms in rare diseases:

- Neuronal Ceroid Lipofuscinoses (ORPHA216): they are a group of progressive, degenerative and hereditary diseases, characterized by intellectual disability, seizures and loss of vision due to degeneration of the retina. Histopathologically they show an accumulation of the autofluorescent material lipofuscin within lysosomes. The incidence of this group of syndromes is unknown, although the most prevalent ones are those diseases produced by mutations in the *CLN1* (Palmitoyl-protein thioesterase 1, PTT1), *CLN2* (tripeptidyl-peptidase 1, TPP-1), *CLN3* (Cln3p, unknown function) and *CLN10* (cathepsin D) genes. Besides studying previously described alterations in autophagosome formation and autophagosome-endosome/lysosome fusion (Vidal-Donet *et al.*, 2013), we focus on the analysis of other consequences of the observed increase in lysosomal pH in CLN3 models. In this sense, we have noticed that this increase produces alterations in vesicular trafficking as well as in the maturation of lysosomal enzymes. From a translational perspective, we have developed protocols to quantify the activity of PTT1, TPP-1 and cathepsin D from dried blood or cell spots, which allow the diagnosis of the three most prevalent forms of the disease, CLN1, CLN2 and CLN10, respectively.
- Lafora disease (ORPHA501): is a progressive myoclonus epilepsy characterized by the accumulation of polyglucosans in several tissues, mainly in those with a high glycogen metabolism (brain, heart, etc.). Although worldwide distributed, its prevalence is higher in specific geographic regions, including Spain. Our group belongs to the Lafora Consortium together with six oth-

er laboratories within the CIBERER (Drs. S. Rodríguez de Córdoba, P. Sanz, J. Serratosa, P. Bovolenta, V. Rubio and F. Pallardó). We have observed, in all the disease models studied, a defect in the formation of autophagosomes and an increase in oxidative stress due to the autophagy impairment, which is subsequently increased by a defect in the antioxidant system response (mainly in the mitochondrial manganese superoxide dismutase and the catalase enzymes).

- X-Adrenoleukodystrophy (ORPHA139396): is caused by mutations in the *ABCD1* gene, encoding a peroxisomal membrane transporter, which produces an alteration of beta-oxidation. This leads to the accumulation of very long chain fatty acids, mainly in the myelin in the central nervous system, in the adrenal cortex and in the Leydig cells in the testes. In collaboration with the group of Dr. Pujol, we have observed, both *in vivo* and *in vitro*, that an impairment of autophagic flux, due to an elevated mTOR signaling, contributes to X-ALD pathogenesis. In addition, we have shown that the excess in very long chain fatty acids downregulates autophagy in human fibroblasts and that an antioxidant treatment partially restores the impaired autophagy in an X-ALD mouse model.

- Retinitis pigmentosa (ORPHA791): is one of the most common forms of retinal dystrophies (1:4,000), characterized by vision impairment due to photoreceptor degeneration. To date, more than fifty causative genes have been described, among them *CERKL*, also implicated in cone-rod dystrophy (CRD), whose function remains unknown. In collaboration with the group of Dr. Gonzàlez-Duarte, we have shown that CERKL localizes in stress granules and in other ribonucleoprotein particles, such as P-bodies and polysomes, as well as in compact structures formed by RNA and proteins. These compact structures also associate to microtubules and participate in mRNA transport. CERKL interacts with proteins that form part of these complexes (PABP, eIF3B, etc.) and also with the RNA. The relevance of these findings is evidenced by the fact that the pathogenic mutants of CERKL are absent from these structures (see figure). Our working hypothesis is that CERKL is a component of transport granules which allows the polarized translation of specific mRNAs. This new function of CERKL opens up a new research area for the development of new therapies.

- Finally, we collaborate with other groups in the study of other diseases, such as MERRF (ORPHA551) and MELAS (ORPHA550; Dr. Armengod, CIPF), dysplasia of fibrous sheath (Dr. Acebedo, Hospital Universitario Dr. Peset, Valencia) and Parkinson's disease (Dr. Moratalla, Instituto Cajal).

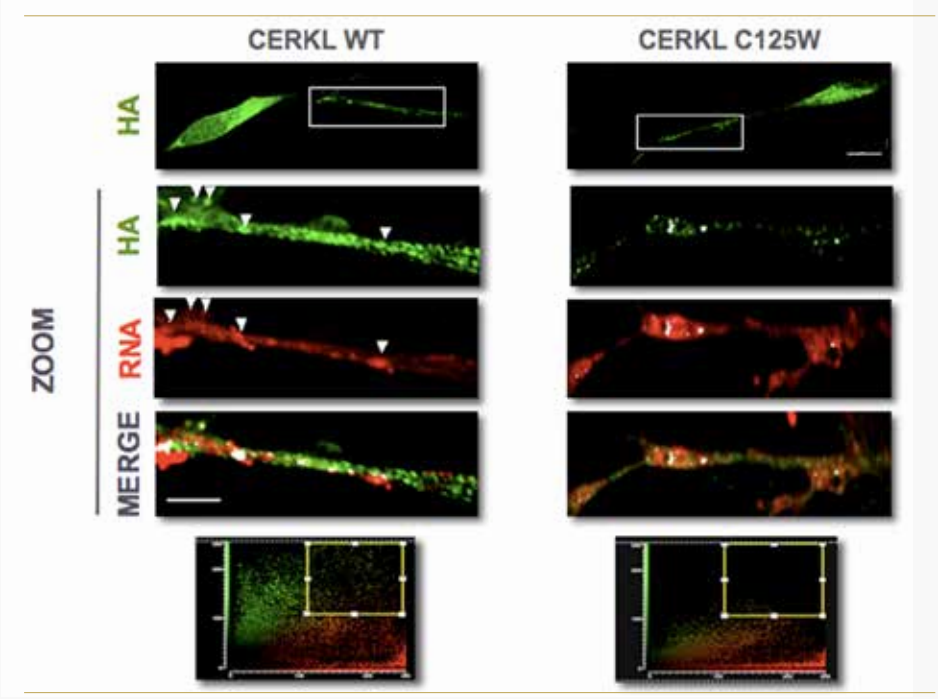

Figure: Colocalization of CERKL WT and CERKL C125W (HA) with RNA (propidium iodide staining!) in differentiated SH-SY5Y cells. Arrowheads show colocalization in particulated structures. The scatter diagrams below show colocalization dots (yellow box). Bars: 10 µm (original images) and 5 µm (zoom).

Publications

1 .Fathinajafabadi A, Perez-Jimenez E, Riera M, Knecht E, Gonzalez-Duarte R. CERKL, a Retinal Disease Gene, Encodes an mRNA-Binding Protein That Localizes in Compact and Untranslated mRNPs Associated with Microtubules.
PloS one Vol: 9, - 2014 Quartile: Q1

2 .Gayarre J, Duran-Trío L, Criado Garcia O, Aguado C, Juana-López L, Crespo I, Knecht E, Bovolenta P, Rodríguez de Córdoba S. The phosphatase activity of laforin is dispensable to rescue Epm2a-/- mice from Lafora disease
Brain Vol: 137, 806-818 2014 Quartile: Q1

3 .Romá-Mateo C, Aguado C, García-Giménez JL, Ibáñez-Cabellos JS, Seco-Cervera M, Pallardó FV, Knecht E, Sanz P. Increased Oxidative Stress and Impaired Antioxidant Response in Lafora Disease..
Mol Neurobiol, 2014 Quartile: Q1

4 .Launay N, Aguado C, Fourcade S, Ruiz M, Grau L, Riera J, Guilera C, Giròs M, Ferrer I, Knecht E, Pujol A. Autophagy induction halts axonal degeneration in a mouse model of X-adrenoleukodystrophy.
Acta neuropathol, 2014 Quartile: Q1

5.Acevedo D, Ventura J, Aguado C, Carda C, Armengot M, Forteza J. Homogeneous microscopic abnormalities in sperm morphology and immotility as a cause of male infertility.
Journal of Reproduction & Contraception Vol: 25 (2), 102-118. 2014 Quartile:

Conferences and Meetings

1. International Autophagy Conferences
Invited speaker
Kencht, E
(Toulouse , France)

2. 1st Proteostasis Meeting .
Organizing Committe
Carmen Aguado, Eva Pérez-Jiménez, Mari Paz Rubio , E. Knecht , Rosa Farrás
(Valencia , Spain)

3. 1st Proteostasis Meeting .
Oral Communication. Honorable mention
Pérez-Jiménez, E., Moruno-Manchón, J.F., López-Alegre, C., Knecht, E.
(Valencia , Spain)

4. 1st Proteostasis Meeting .
Poster
Aguado, C., Vidal-Donet, J.M., Lahuerta-Ferreres, M., Cárcel-Trullos, J., Knecht. E.
(Valencia , Spain)

5. Séptima Reunión Anual del CIBERER .
Poster
Aguado, C., García-Giménez, J.L., Romá-Mateo, C., Ibáñez-Cabellos, S., Seco-Cervera, M., Pallardó, F.V., Knecht, E., Sanz, P.
(Madrid , Spain)

6. Séptima Reunión Anual del CIBERER .
Oral Communication
Pérez-Jiménez, E., Fathinajafabadi, A., Riera, M., Knecht, E., Gonzàlez-Duarte, R.
(Madrid , Spain)

7. Reunión Anual del Consorcio de Lafora .
Oral Communication
Aguado, C., Knecht, E
(Madrid , Spain)

Doctoral Thesis presented

1. Doctoral candidate: José Manuel Vidal Donet. "Alteraciones en la degradación intracelular de proteínas y en la endocitosis en las lipofuscinosis ceroideas neuronales infantil tardía y juvenil" Directores: Dres. E. Knecht y C. Aguado. Universidad de Valencia. Sobresaliente cum laude.

CIPF technology units and platforms

The Príncipe Felipe Research Center (CIPF) features facilities and services to support research and cutting-edge technology development, open to both internal and external researchers.

The CIPF services are designed to promote networking and to enhance the competitiveness for both its users, research and industrial partners.
Another great added value of the CIPF is the concentration in a single space of reference services and facilities, making knowledge transfer effective between different areas. In addition, these services support own research groups participating in European projects and also large companies projects, absorbing the know-how to incorporate it into their background.
As a result of this, CIPF can offer solutions for new applications, such as arrays for oncohematology, surface metrology confocal microscopy, synthesis of drug delivery systems (polymer-drug conjugates), pathologic diagnosis by electron microscopy and many others.

PICTURE 1 - Veriti 96 wells thermal cycler from Applied Biosystems.

PICTURE 2 - Animal Facility Laboratory.

PICTURE 3 - FC500 MCL Flow Cytometer Beckman-Coulter equipped with two lasers: 488 nm and 635 nm and 5 fluorescence detectors. Acquisition manual or automatic sample carrousel.

PICTURE 4 - NMR Bruker Avance 300Mhz.

PICTURES 5/6 - G2565C Microarray Scanner, Agilent technologies. 180k array.

PICTURE 7 - Confocal microscope (Leica TCS-SP2-AOBS.

PICTURE 8 - Automatic pipettor EvoTECAN 96 and 384 well-plates.

PICTURE 9 - Transmission electron microscopy FEI Tecnai Spirit G2 with digital camera.

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Animal Models

Animal Models unit works for the care and maintenance of the laboratory animals. Assure the Quality in research and scientific advances require an ethic use of animals in the laboratories as well as the reproducibility of the procedures High quality genetic animals, optimum facilities and the observance of the current legislation for the protection of animals used for scientific purposes are required.
Our unit is registered as animal breding, user and supplier center for tests in animals and consists of qualified and accredited Laboratory Animal Science.
In 2014, our Animal models unit has contributed in several projects such as the study of the treatment of breast cancer with therapeiutic polymers, studies about cardiac, epidermic and medullar regeneration with pharmacologic and cellular therapies, and scoliosis, hepatic encephalopathy, alcoholism, endometriosic and rare diseases.
In turn, in our operating theaters have been given about twenty training programs in techniques of minimally invasive surgery such as gastrointestinal, urological laparoscopic genitourinary, thoracoscopy and arthroscopy, and others in emergency techniques and the use of curing compounds chemicals. All directed to physicians in the process of specialization and taught by expert surgeons of the Valencian hospitals.

Confocal Microscopy

The Optical and Confocal Microscopy unit (OCMU) provides microscopy imaging and analysis with dedicated supported by experienced professionals in microscopy. Confocal microscopy is a standard and valuable tool in life science as well as in material sciences, for this reason, the OCMU has contributed to the research projects of several groups and has collaborated with other research centers such as Valencian Institute of Pathology (IVP), Materials Technology Institute of Polytechnic University of Valencia, PROCREA Foundation, IVI Foundation, Institute for Plant Molecular and Cell Biology, etc.

Cytomics

The Cytomics Core unit includes advanced technology and equipment for polychromatic analysis and cell sorting contributing to those research projects that needs: Immunophenotyping of samples to detect the expression of surface and intracellular/intranuclear antigens, analysis of the cell cycle, cytotoxicity assays for assessing cell death and investigate specific apoptosis pathways, functional tests applied to established cell lines, primary cultures and ex vivo samples, cell analysis of microorganisms for applications in Clinical Studies, Biotechnology and Environmental Sciences, functional characterization and immunophenotype of stem cells, analysis of real time parameters (analysis In Fluxo), multiplexed analysis of soluble proteins, cell

sorting to obtain purified populations based on immunophenotype and/or functional features, and High Content studies (HCA) by image analysis techniques for adherent cells and tissue sections. Recently, these approaches have been applied in two FIS research projects ,where the Facility personnel has been directly involved, to detect and sort different subpopulations in human melanoma and lung-cancer samples. Moreover, the Facility has participated in Cytomic Courses, in collaboration with the University of Valencia, such as the ESCCA Summer School on Cytometry, as well as other graduate and master courses.

Publications

1.Sandra Pinto, Alicia Martínez-Romero, José-Enrique O'Connor, Rosario Gil-Benso, Teresa San-Miguel, Liria Terrádez, Carlos Monteagudo and Robert C Callaghan. Intracellular coexpression of CXC- and CC– chemokine receptors and their ligands in human melanoma cell lines and dynamic variations after xenotransplantation. BMC Cancer 2014, 14:118-132.

2.Richard J. Griffeth, Daniel García-Párraga, Maravillas Mellado-López, José Luis Crespo-Picazo, Mario Soriano-Navarro, Alicia Martínez-Romero, Victoria Moreno-Manzano. Platelet-Rich Plasma and Adipose-Derived Mesenchymal Stem Cells for Regenerative Medicine-Associated Treatments in Bottlenose Dolphins (Tursiops truncatus). PLOS ONE, September 2014,Volume 9(9), e108439.

3.José-Enrique O´Connor, Guadalupe Herrera, Alicia Martínez-Romero, Francisco Sala de Oyanguren, Laura Díaz, Angela Gomes, Susana Balaguer, Robert C. Callaghan. Systems Biology and immune aging. Immunology Letters, 162: 1(B): 334-345.

Collaborations

1.Caracterización molecular de rutas de señalización oncogénicas en células madre tumorales de cáncer de pulmón no microcítico. Implicación en el desarrollo de nuevas estratégia terapéuticas. Investigador responsable: Dr. Rosa Farràs Rivera. Hospital General Universitario de Valencia. Centro de Investigación Príncipe Felipe. Instituto de Salud Carlos III. Fundación de Investigación Sanitaria. PI12/00956. Convocatoria AES2012.

Electron Microscopy

The Transmission Electron Microscope (TEM) obtained results that have been published in internationally renowned scientific journals/international scientific journals, as demonstrated by the following examples:
The use of a biological-based therapy for wound healing in dolphins by the application of platelet-rich plasma (PRP). In addition to other techniques these PRPs were studied under the TEM. The results of this article may constitute the basis of a biological treatment for wound-healing and tissue regeneration in dolphins. These studies are part of a research project of the Neuronal and Tissue Regeneration Lab in CIPF and were published on the journal Plos One.
Ependymoma tumors likely derive from the ependymal cells lining the CNS ventricular system. To unambiguously characterize the ultrastructure and number of cilia were performed electron microscopy serial section analysis of individual cells. Differentiated ependymomas contained large basal bodies and up to three cilia, and lacked centrioles. Anaplastic ependymoma cells showed instead two perpendicularly oriented centrioles and lacked cilia or basal bodies. These findings could contribute to understand

the mechanisms of ependymoma aggressiveness. These studies are part of a research project of the Instituto Valenciano de Patología and were published on the journal Ultraestructural Pathology.

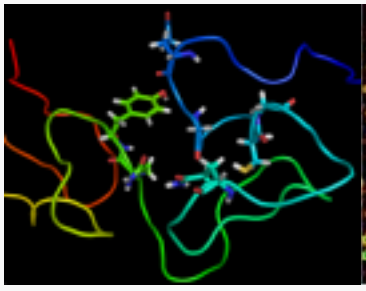
Centrioles are microtubule-based, barrel-shaped structures that initiate the assembly of centrosomes and cilia. The way the centriole length is precisely set remains elusive. In addition to other techniques, the study of centriole length by TEM in cerebral malformations in two affected families with severe microcephaly can be used to identify regulation of the centriole length as an emerging pathogenic mechanism in ciliopathies. These studies are part of a research project of the Stanford University School of Medicine and were published on the journal Nature Genetics.

Activities

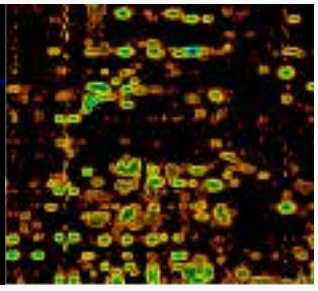
Mario Soriano, teaching activities at the "Ultrastructural Pathology Course of renal biopsy ".Hospital General Universitario of Castellón.

Genomics

The Genomics unit designed and developed the first microarray for genetic improvement processes of sunflower crop. This microarray detects active genes in different sunflower species and catalogs those who match better in cross-pollination traditional breeding. This pioneer microarray identifies the expressed genes in a particular time of cultivation and under stress conditions and specific pathogens. This way, scientists can locate the genes acting pursuant to circumstances and let them know in advance which genes are valid under these conditions, and also which genes should be dismissed.
In addition, the Service also worked in Erwinia amylovora, Saccharomyces cerevisiae and others differential expression studies



PICTURE 10 - Three-dimensional protein structure obtained by Nuclear Magnetic Resonance.



PICTURE 11 - Bi-dimensional NOESY experiment acquired with a 600 MHz spectrometer with cryoprobe.

NMR

The NMR unit has been applied as the main tool for characterizing the chemical structure of different small molecules and macromolecules, and for elucidating the molecular mechanisms of their biological activity. In addition, interaction studies between different key pharmaceutical targets and potential hits has been performed, contributing to the main research projects of several groups and a fragment screening for a pharmaceutical company has been carried out. Furthermore, the facility has allowed the characterization of the metabolic profile of a high number of biologic samples (serum, urine, cerebrospinal fluid, cells, etc.) for different projects in collaboration with hospitals and other research centers in Spain.

Throughout this year, the NMR unit, under the supervision of the Structural Biochemistry Laboratory, has been used in a wide variety of projects, and has contributed to the characterization of different small chemical compounds, as well as macromolecules. Furthermore, the detailed structural knowledge of the molecules, provided by the in-depth analysis of the NMR spectra, has facilitated the involvement in different projects, in collaboration with internal and external (academic and pharmaceutical) groups, focused on the evaluation of the interaction between several protein targets and potential inhibitors using fragment screening approaches. Particular attention has also been paid to the application of NMR approaches to the elucidation of metabolomic profiles associated to different biological samples, each of them representative of particular biochemical, pharmacological or pathological processes. Finally, it should be stressed that the organization of dissemination activities (e.g., courses, visits, etc.), the publication of the results in high impact journals and the involvement in different grant applications have been instrumental for increasing the visibility of the NMR facility.

Traslational Genetics Unit

The Translational Genetics unit is specialized in the genetic diagnostics and counseling. SGT is a biotechnology service of the Program on Rare and Genetics Diseases, in the Príncipe Felipe Research Center (CIPF), which is being led by the Prof. Francesc Palau in the beginning of 2013. SGT is committed to offer a service of health care quality, specialized in the genetic analysis of human hereditary diseases, with both diagnostic and preventive purposes, in order to improve the care and quality of life of patients and their relatives.

Our aims are to:

- Provide support to clinicians interested in genetic diagnosis, taking care of the needs of the daily clinical practice.
- Innovate to develop new tools using next-generation-sequencing (NGS), in order to obtain more effective genetic tests for those diseases that present with genetic heterogeneity.
- Investigate the genetic causes and pathological mechanisms underlying human hereditary diseases, in order to discover new therapeutic targets.

Proteomics

The valuable information generated by proteomic technology contributes to the development of Biomedicine, in applications such as the search for biomarkers for the diagnosis of illnesses and therapeutic targets, as well as the development of medicines and vaccines and Agricultural biotechnology, in the development of bio-pesticides and bio-fertilisers which improve production yield and the environmental quality of soils; as well as the analysis of residues and contaminants in the agri-food industry.

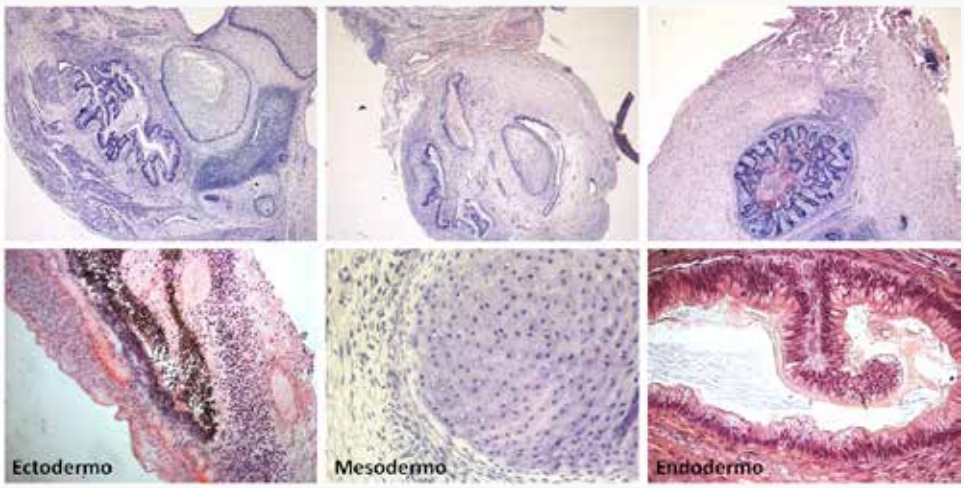
We participate in The Human Proteome Project (HPP) in collaboration with the University of Valencia. The HPP is an international project organized by the Human Proteome Organization (HUPO) that aims to revolutionize our understanding of the human proteome via a coordinated effort by many research laboratories around the world.

Stem Cell Bank

The National Bank of Cell Lines (BNLC) is a distributed biobank of human pluripotent stem cells (hPSC) with four nodes, one of them located at CIPF. It is devoted to store, biological and functional analysis, and distribution of human pluripotent stem cells, both embryonic stem (ES) cells and reprogrammed stem (iPS) cells. The major issue is to offer these services to the Spanish and international scientific community.

Pluripotent cells are characterized by their capacity to differentiate into any cell type derived from the three germ layers (ectoderm, mesoderm and endoderm). Thus, pluripotent stem cells are able to generate any tissue from a living organism, except the extra-embryonic layer. In addition, these cells display the capacity to self-renew, which allows them to divide indefinitely into identical cells when they are cultured under specific conditions.

Based on these two properties, pluripotent cell lines reflect the best available tool for research projects in the field of cell therapy and regenerative medicine. Currently, stem cells are one of the most advanced techniques in research, both in Spain and worldwide, given their potential application in the biomedicine field. Pluripotent stem cells are being used for tissue regeneration, cancer research, rare diseases, and development of new drugs. Recent studies have demonstrated the efficiency of the human pluripotent stem cells (hPSC) as a putative therapy for several diseases. Various clinical trials are actually testing the suitability of hPSC for clinical practice. Some of the diseases which could be treated through cell therapy with hPSC are: diabetes mellitus, Parkinson's disease, macular degeneration, Alzheimer's disease, autism, severe anemia, muscular atrophy, spinal cord damage and cardiac diseases. Along with the services offered by the BNLC, one major focus of the CIPF-Valencia node is addressed to the field of rare diseases.



The BNLC is based upon the previous CIPF experience in research on regenerative medicine and stem cells platform screening. In The last years, research has been related to the development of different projects based on drug discovery and ranging from anticancer therapies to regenerative (stem cell) area or infectious diseases. One of the biggest experiments within cancer field was the identification of hits capable to induced synergism when administered in combination with a specific antitumor agent against melanoma cell models. Others include a massive screening to identify caspase 9 activators or to discover synergistic endocrine-chemotherapy combinations for the treatment of hormone-dependent cancers. In addition, the screening platform has been focused on the identification of hits capable to maintain the stemness or to activate different types of stem cells, including hematopoietic cells from umbilical cord. Finally, a massive screening on the HIV field was performed using polarized fluorescence techniques.

Techniques

Current available techniques at the VCLB include the following:

- Cryopreservation of pluripotent cell lines.
- Preparation of feeder cells.
- Preparation of supporting matrix.
- Culture of pluripotent cells.
- Pluripotency characterization by different techniques.
- Genetic analysis.
- Karyotyping.
- Teratoma assay.

Available Cell Lines

Lines of Human Embryonic Stem Cells (hESC)

Line	Derivation Year	Characteristics	Kariotype	Genetically Modified
RiMi1	2008	Partenogenota	46, XX	No
VAL-3	2005	Normal	46, XY	No
VAL-4	2006	Normal	46, XX	No
VAL-5	2006	Normal	46, XY	No
VAL-6M	2007	Distrofia miotónica 1	46, XY	No
VAL-7	2007	Normal	46, XY	No
VAL-8	2008	Normal	46, XX	No
VAL-9	2008	Normal	46, XY	No
VAL-9 GFP	2009	Normal	46, XY	Si
VAL-10B	2008	Normal	46, XY	No
VAL-11B	2009	Normal	46, XX	No

Lines of Human Induced pluripotent Stem Cells (hiPSC)

Line	Obteining-Year	Reprogrammed Cell	Characte-ristics	Kariotype	Genetically Modified
hiPSC clone 1	2009	Adult Skin Bibroblast	Normal	46, XX	No
hiPSC clone 4	2009	Adult Skin Bibroblast	Normal	46, XX	No

Moelcular Screening

The Screening platform at CIPF in the last few years has been involved in the development of different projects based on drug discovery and ranging from anticancer therapies to regenerative (stem cell) area or infectious diseases.

One of the biggest experiments within cancer field was the identification of hits capable to induced synergism when administered in combination with a specific antitumoral agent against melanoma cell models. Others include a massive screening to identify caspase 9 activators or to discover synergistic endocrine-chemotherapy combinations for the treatment of hormone-dependent cancers.

In the regenerative area the screening platform has been focused on the identification of hits capable to maintain the stemness or to activate different types of stem cells, including hematopoietic cells from umbilical cord. Finally, a massive screening on the HIV field was performed using polarized fluorescence techniques.

Other services and facilities

GMP facilities

360m^2 surface
4 classified manufacturing rooms
1 criogeny room
1 labeling room
1 sterility room
1 quality control laboratory
1 storage room

The facilities have been designed according to GMP standards, required for the manufacture of sterile products (eye drops, injectable, vaccines, cell therapy, gene therapy and tissue engineering, etc.).

CIPF's GMP facilities comply with all the requirements to assure sterility conditions during manufacturing. Among the processes that could be carried out in the facilities: manufacturing of cellular therapy products in compliance with legal requirements for clinical trials, manufacturing of other sterile drug products (eye drops, injectable, etc.).

Operating Theaters

In the CIPF Animal Facility there are two full-equipped Surgery Theater for experimental surgery programs. 2 Surgery Theaters:

Karl Storz Aida is an advanced data and image file system. It is based on a computerized documentation system and secure archive for images, audio and video sequence and patient data. Record the data in a therapeutic or diagnostic intervention directly from the operating room.

The Karl Storz-SCB system allows central representation of the remote control device parameters SCB connected. Together with the Media Control and AMX multimedia unit enables data transfer, video conferencing, video from in vivo surgery, etc .

Both systems are integrated in what is known as KARL STORZ OR-1, an integrated OR. This will integrate endoscopes, cameras, documentation, communication, etc ... With this system, data transfers, light activations surgery theater or video can be activated as usual or traditional, from a touch screen or by voice.
2 Karl Storz Endoscopy towers equipped with TFT monitor, cold light source, Thermo flator, and optical Endomat different calibers (the latter are considered as endoscopic equipment common to both areas).

2 Stations Draeger Primus anesthetic with their motors hemodynamic Draeger Infinity Delta.

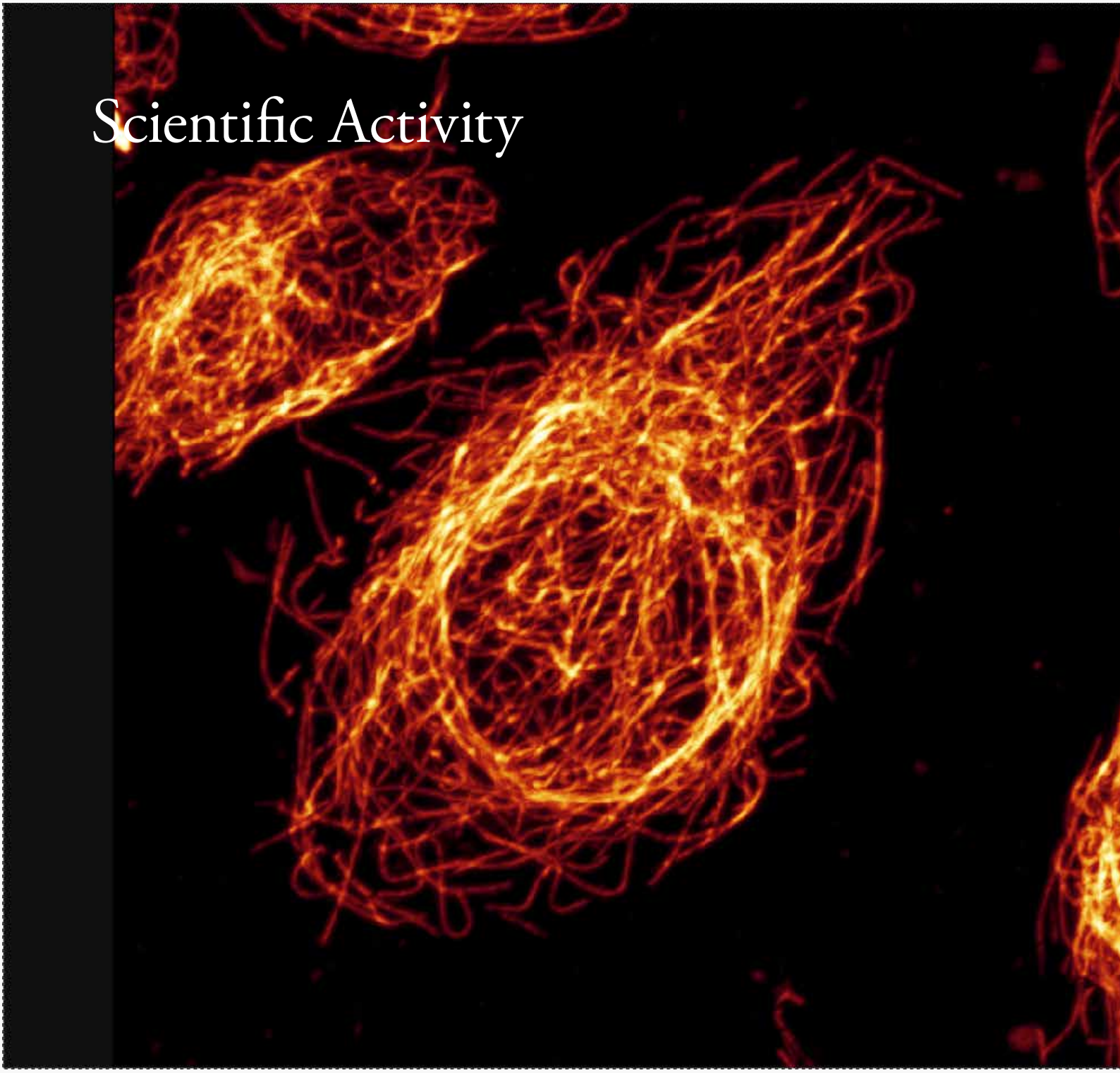
Monopolar and bipolar electrosurgical generator (Valleylab / Storz).

Two surgical tables with temperature control system and integrated mobility.

Computational Genetics

Our broad goal is to develop and apply computational methods in an interdisciplinary and quantitative approach to biotechnological and biomedical projects. We develop tools that allow converting data produced by the new high-throughput technologies (next gen sequencing, proteomics, metabolomics) into valuable, meaningful biomedical information that can be used for diagnostic and prognostic purposes. This Program carries our groundbreaking research by applying translational bioinformatics to personalized medicine integrating genomics and medical imaging. We also carry out innovative studies of systems medicine and apply the result of this research to other areas such as pharmacogenomics, nutrigenomics or agrogenomics.

Scientific Activity



Competitive funding

Human resources grants

Ministry of Economy and Competitiveness

Grantee	Type of Grant	Principal Investigator
Mª Victoria Moreno Manzano	Contrato Ramón y Cajal	Moreno Manzano, Mª Victoria
Paula Oliete	Formación personal investigador	Rodríguez Navarro, Susana
Marcos Lahuerta	Formación personal investigador	Knecht Roberto, Erwin
Navarro Rey, Carmen	Formación personal investigador	Armengod, Eugenia
Amaya Niño Pariente	Formación personal investigador	Vicent Docon, Mª Jesus
Manuela Barneo Muñoz	Formación personal investigador	Palau, Francesc
David Charbonnier	Ayudas para la Contratación de Personal Técnico de apoyo	Vicent Docon, Mª Jesus
Mª Jesus Vicent Docon	Programa de incentivación de la incorporación e intensificación de la actividad investigadora	Vicent Docon, Mª Jesus

Ministry of Education

Grantee	Type of grant	Principal Investigator
Eduardo Calpena	Formación profesorado universitario	Palau, Francesc
Varinia García Molinero	Formación profesorado universitario	Rodríguez Navarro, Susana
Aroa Duro	Formación profesorado universitario	Vicent Docon, Mª Jesus
Lorena de la Fuente	Formación profesorado universitario	Conesa Cegarra, Ana
Lucas Taoro	Formación profesorado universitario	Felipo, Vicente

Carlos III Institute of Health

Grantee	Type of grant	Principal Investigator
Luz Mª García Alonso	Formación en investigación en salud	Dopazo Blazquez, Joaquín

Regional Ministry of Education (GVA)

Grantee	Type of grant	Principal Investigator
Antoni Pla Rodriguez	Ayudas para la contratación de personal investigador en formación en fase predoctoral	Guerri Sirera, Consuelo
Belen Gomez	Ayudas para la contratación de personal investigador en formación en fase predoctoral	Felipo Orts, Vicente
Jorge Montesinos	Ayudas para la contratación de personal investigador en formación en fase predoctoral	Guerri Sirera, Consuelo
Mª Victoria Moreno Manzano	Programa de Estabilización de investigadores y de intensificación de la actividad investigadora en el Sistema Nacional de Salud	Moreno Manzano, Mª Victoria
Elena Jímenez	Programa de Impulso a la participación en proyectos de investigación en el ambito de la UE	Sánchez, Oscar
Elena Jímenez	Programa de Impulso a la participación en proyectos de investigación en el ambito de la UE	Sánchez, Oscar
Tiziano Balzano	Ayudas para la contratación de personal investigador en formación	Felipo Orts, Vicente
Rachid Bououtal	Ayudas para la contratación de personal investigador en formación	Armengod, Eugenia

Regional Ministry of Health (GVA)

Grantee	Type of grant	Principal Investigator
Janet Hoenicka	Programa de Estabilización de investigadores y de intensificación de la actividad investigadora en el Sistema Nacional de Salud	Hoenicka, Janet
Rosa Farrás	Programa de Estabilización de investigadores y de intensificación de la actividad investigadora en el Sistema Nacional de Salud	Farrás Rivera, Rosa
Rosa Guasch	Programa de Estabilización de investigadores y de intensificación de la actividad investigadora en el Sistema Nacional de Salud	Guasch Aguilar, Rosa Mª
Marta Llansola	Programa de Estabilización de investigadores y de intensificación de la actividad investigadora en el Sistema Nacional de Salud	Llansola Gil, Marta

Travel grants

Ministry of Education

Grantee	Type of grant	Principal Investigator
Carmen Navarro Rey	Ayudas a la movilidad predoctoral para la realización de estancias breves	Armengod, Eugenia
Paola Oliete	Ayudas a la movilidad predoctoral para la realización de estancias breves	Rodriguez-Navarro, Susana
Aroa Duro	Ayudas a la movilidad predoctoral para la realización de estancias breves	Vicent Docon, Mª Jesus

European Comission

Grantee	Type of grant	Principal Investigator
Rosa Farrás	Cost Action	Farrás Rivera, Rosa M
Ana Conesa	Cost Action	Conesa Cegarra, Ana

Research projects grants

7th Framework Programme

Type of grant	Title	Principal Investigator
Collaborative Project	Eurocondor	Burks , Deborah Jane
Collaborative Project	Innovaliv	Burks , Deborah Jane
Collaborative Project	Stategra	Conesa Cegarra, Ana Victoria
International exchange programme for research staff	612583 DEANN	Consesa Cegarra, Ana
Collaborative Project	MLPM2012— Machine Learning for Personalized Medicine	Dopazo Blazquez, Joaquín
Collaborative Project	Denamic	Felipo Orts, Vicente
Collaborative Project	SMARTCARE Joining up ICT and service processes for management	Sanchez, Oscar

Ministry of Economy and Competitiveness

Type of grant	Title	Principal Investigator
Proyectos de Investigación fundamental no orientada	Rutas de modificación de tRNAs que descodifican codones de cajas mixtas terminados en purinas	Armengod González, Mª Eugenia
Proyectos de Investigación fundamental no orientada	Desarrollo de recursos computacionales para la caracterización y anotación funcional de ARN no codificante.	Conesa Cegarra, Ana Victoria
Proyectos de Investigación fundamental no orientada	Understanding the mechanisms of the disease and prioritizing candidate genes under a systems perspective	Dopazo Blazquez, Joaquín
Proyectos de Investigación fundamental no orientada	Bases Moleculares de las alteraciones neurológicas en hiperamonemia y encefalopatía hepática. Implicaciones terapéuticas	Felipo Orts, Vicente
Programa Consolider	Consolider SICI	Felipo Orts, Vicente
Proyectos de Investigación fundamental no orientada	Importancia de los mecanismos de degradación de proteinas en la neurodegeneración causada por etanol	Guerri Sirera, Consuelo

Proyectos de Investigación fundamental no orientada	Degradación Intracelular De Proteínas: Regulación y alteraciones en Enfermedades Raras	Knecht Roberto, Erwin
Programa Consolider	Consolider SICI	Moreno Manzano, Mª Victoria
Programa Consolider	Consolider SICI	Orzaez Calatayud, Mª Del Mar
Proyectos de Investigación fundamental no orientada	Mecanismos moleculares de moduladores de apoptosis	Orzaez Calatayud, Mª Del Mar
Proyectos de Investigación fundamental no orientada	Disección de la fisiopatología mitocondrial de la neuropatía de Charcot-Marie-Tooth	Palau Martínez, Francesc
Proyectos de Investigación fundamental no orientada	Aplicaciones de la RMN y la metabolómica al desarrollo de nuevos agentes antineoplásicos dirigidos molecularmente	Pineda Lucena, Antonio
Proyectos de Investigación fundamental no orientada	Aplicaciones metabolómicas identificación nuevas dianas terapeuticas para el tratamiento de la tuberculosis	Pineda Lucena, Antonio
Proyectos multilaterales	Una aproximación omica al diagnostico de la tuberculosis	Pineda Lucena, Antonio
Proyectos de Investigación fundamental no orientada	Estudio de los mecanismos moleculares que acoplan los procesos de transcripción y biogenesis de Rnas en Eucariotas	Rodríguez Navarro, Susana
Proyectos de Investigación fundamental no orientada	Desarrollo de un Kit universal para liberacion remota controlada de fármacos mediante hipertermia magnética en aplicaciones oncológicas	Vicent Docon, Mª Jesus
Proyectos de Investigación fundamental no orientada	Polímeros Terapéuticos como agentes simples y en combinación para el tratamiento de cáncer y neurodegeneración. Plan Nacional I+D.	Vicent Docon, Mª Jesus

Carlos III Institute of Health

Type of grant	Title	Principal Investigator
Proyectos de Investigación en salud	Plataforma de recursos biomoleculares y bionformáticos	Dopazo Blazquez, Joaquín
Redes de Investigación	Red De Cáncer	Dopazo Blazquez, Joaquín
Proyectos de Investigación en salud	Caracterizacion molecular me rutas de señalizacion oncogénicas en células madre tumorales de cáncer de pulmón no microcítico. Implicación en el desarrollo de nuevas estratégia terapéuticas	Farrás Rivera, Rosa
Proyectos de Investigación en salud	Translational Research, Experimental Medicine and Therapeutics on Charcot-Marie-Tooth	Galindo, Máximo Ibo
Proyectos de Investigación en salud	Papel de Rhoe en el crecimiento axonal y en la mielinización. Implicación en las enfermedades neurodegenerativas	Guasch Aguilar, Rosa Mª
Redes de Investigación	Red de Trastornos Adictivos	Guerri Sirera, Consuelo
Proyectos de Investigación en salud	Biología celular y función de ANKK1 en el cerebro: relación con el sistema depaminérgico y la neurogénesis	Hoenicka, Janet
Plataforma de Apoyo a la Investigación	Plataforma de recursos Biomoleculares y Bioinformaticos	Palau Martínez, Francesc
Proyectos de Investigación en salud	Terapia celular, nanomedicina y biomateriales en	Moreno Manzano, Mª Victoria
Centros de Investigación Biomedica	CIBERER	Dopazo Blazquez, Joaquín; Knecht, Erwin; Palau, Francesc
Centros de Investigación Biomedica	CIBERDEM	Noon, Luke

Regional Ministry of Health (GVA)

Type of grant	Title	Principal Investigator
Ayudas al Fomento del Emprendimiento Cientifico	Programa Banco de Patentes	Sánchez Jiménez, Oscar D

Regional Ministry of Education (GVA)

Type of grant	Title	Principal Investigator
Acciones complementarias	Papel de los receptores TLRs y el Inflamasoma en el daño que induce el etanol en el cerebro	Guerri Sirera, Consuelo
Acciones complementarias	Aplicaciones de la RMN y de la Metabolomica al desarrollo de nuevos agentes antineoplásicos dirigidos molecularmente	Pineda Lucena, Antonio
Acciones complementarias	Papel de Sus1p y proteínas relacionadas en transcripción acoplada a procesamiento y exportación de mRNAs	Rodríguez Navarro, Susana
Ayudas para la organización y difusión de congresos	AORG: 10th international symposium on polymer therapeuti	Vicent Docon, Mª Jesus
Ayudas para la organización y difusión de congresos	AORG: First proteostasis meeting	Knecht Roberto, Erwin
Ayudas contratación personal de apoyo	Geronimo Forteza	Felipo Orts, Vicente
Ayudas contratación personal de apoyo	Geronimo Forteza	Guasch Aguilar, Rosa
Ayudas contratación personal de apoyo	Geronimo Forteza	Dopazo Blázquez, Joaquín
Programa Prometeo para potenciar a los grupos de I+D Excelentes	Bases Moleculares de las Alteraciones Neurologicas en Hiperamonemia y Encefalopatia Hepatica. Implicaciones terapeuticas	Felipo Orts, Vicente
Programa Prometeo para potenciar a los grupos de I+D Excelentes	Estructura y función de reguladores de expresión génica	Armengod González, Mª Eugenia y Rodriguez Navarro, Susana
Programa Prometeo para potenciar a los grupos de I+D Excelentes	Desarrollo de nuevos conceptos y herramientas bioinformáticas de nueva generación para la priorización de genes candidatos en enfermedades y la elaboración de las correspondientes estrategias terapéuticas	Dopazo Blazquez, Joaquín
Programa Prometeo para potenciar a los grupos de I+D Excelentes	Estructura y función de reguladores de expresión génica	Rodríguez Navarro, Susana
Programa Prometeo para potenciar a los grupos de I+D Excelentes	Genes, proteínas y rutas de señalización en enfermedades raras (BioMeder)	Palau Martínez, Francesc

Foundations and other Private Entities

Type of grant	Title	Principal Investigator
Danish Reserach Council	Role of Neuroinflammation in the cognitive and motor alterations in chronic hyperammonemia and hepatic encephalopathy- Therapetic implications	Felipo Orts, Vicente
European Foundation for Alcohol Research	Is the neuroinmune response involved in the neurot	Guerri Sirera, Consuelo
Fundación La Marató-TV3	Implementation of Personalized Medicine in cutaneo	Dopazo Blázquez, Joaquín
Fundación La Marató TV3	Study of cohesin functions in Cornelia de Lange Syndrome	Rodríguez Navarro, Susana

Contracts Research

Entity	Title	Principal Investigator
Asociación de Investigación de la Industria textil	Research contract	Felipo Orts, Vicente
BCN Time4Research	Research contract	Vicent Docon, Mª Jesús
BCN Time4Research	Research contract	Moreno Manzano, Mª Victoria
BULL	Cátedra Genómica Computacional	Dopazo Blazquez, Joaquín
CGB Lytimval Biotech, S.L.	Research contract	Vicent Docon, Mª Jesús
Ferrer Internacional, S.A.	Research contract	Moreno Manzano, Mª Victoria
Fundació Clínic per a la Recerca Biomèdica	Research contract	Pineda Lucena, Antonio
Fundación Cugat	Research contract	Moreno Manzano, Mª Victoria
Instituto de Medicina Genómica (IMEGEN)	Research contract	Conesa Cegarra, Ana
Jannsen Cilag S.A.	Research contract	Fustero Lardíes, Santos
Jannsen Serine	Research contract	Pineda Lucena, Antonio
Umecrine Cogninition AB	Research contract	Felipo Orts, Vicente
University of Malaya	Research contract	Vicent Docon, Mª Jesús
YEGANE, S.L.	Research contract	Orzáez Calatayud, Maria Mar
YEGANE, S.L.	Research contract	Vicent Docon, Mª Jesús

CIBERER Competitive funding

Human resources grants

Grantee	Type of Grant	Investigador Principal proyecto
Carmen Espinós	Programa de incentivación de la incorporación e intensificación de la actividad investigadora	Espinós, Carmen

Research projects grants

7th Framework Programme

Type of grant	Title	Principal Investigator
Collaborative Project	RD-Connect. An integrated platform connecting databases, registries, biobanks and clinical bioinformatics for rare disease research	Palau, Francesc

Executive Agency for Health and Consumers

Type of grant	Title	Principal Investigator
Collaborative Project	EUCERD Joint Acation	Palau, Francesc
Collaborative Project	ORPHANET Joint Action	Palau, Francesc

Carlos III Institute of Health

Type of grant	Title	Principal Investigator
Proyectos de Investigación en salud	Translational Research, Experimental Medicine and Therapeutics on Charcot-Marie-Tooth	Palau, Francesc
Proyectos de Investigación en salud	Translational Research, Experimental Medicine and Therapeutics on Charcot-Marie-Tooth	Espinós, Carmen
Proyectos de Investigación en salud	Investigación traslacional y mecanismos de enfermedad en neuropatías periféricas hereditarias	Espinós, Carmen
Proyectos de Investigación en salud	Fisiopatología axonal de la Ataxia de Friedreich: Transporte y degeneración axonales.	González Cabo, Pilar

Foundations and other Private Entities

Type of grant	Title	Principal Investigator
Fundación La Marató TV3	Friedreich Ataxia Integrative Research Consortium: a Pathophysiological and Therapeutical Approach (FAIR)	Palau, Francesc

Innovation & Technology Transfer

Patents Portfolio

Title	Reference	State
Beta-lactam compounds that inhibits APAF1	P201231137	Spanish patent
Compound material for biomedical applications	P201231147	Spanish patent
Method for the detection of bladder cancer	P200900373	Spanish patent
Novel conjugates of polymers having a therapeutically active agent and an angiogenesis targeting moiety attached thereto and uses thereof in the treatment of angiogenesis related diseases	WO/2009/141826	National phases
Ex-vivo method for the early diagnosis of Minimal Hepatic Encephalopathy by means of the determination of 3-nitrotyrosine in serum	WO/2012/007624	National phases
Triazine derivatives and their uses as TRPV1 inhibitors	WO/2012/136873	National phases
Polymer Drug Conjugates for the Treatment of Amyloidosis	Ep13382184	European Patent
New Bilaterally-Substituted Tricyclic Compounds for the Treatment of Human Immunodeficiency Virus Type-1 (Hiv-1) Infection and other Diseases	Pct/ Ep2014/053294	PCT application European Patent
Specific mtor inhibitors in the treatment of x-linked adrenoleukodystrophy	EP14382353	European patent

Software

1. VARIANT: Fast and accurate functional characterization of variants in VCF files.	Medina I, De Maria A, Bleda M, Salavert F, Alonso R, Gonzalez CY, Dopazo J
2. CellBase: RESTful Web Services for retrieving releant biological information	Bleda M, Tarraga J, de Maria A, Salavert F, Garcia-Alonso L, Celma M, Martin A, Dopazo J, Medina I
3. GenomeMaps: higly efficient nex generation genome viewer (used by the ICGC)	Medina I, Salavert F, Sanchez R, de Maria A, Alonso R, Escobar P, Bleda M, Dopazo J
4. BiERapp: Variant/gene prioritization tool (from VCF to candidates)	Alejandro Alemán; Francisco García; Francisco Salavert; Joaquín Dopazo
5. TEAM: Targeted enrichment sequencing (panels) diagnostic tool	Alejandro Alemán; Francisco García; Joaquín Dopazo
6. RENATO: Inferring the regulatory network behind a gene expression experiment	Marta Bleda, Ignacio Medina, Roberto Alonso, Alejandro De Maria, Francisco Salavert , Joaquin Dopazo
7. Snow: Network enrichment of a set of genes	Minguez P, Gotz S, Montaner D, Al-Shahrour F, Dopazo J
8. Network Miner: Network enrichment analysis of a ranked list of genes	Luz García-Alonso, Roberto Alonso, Enrique Vidal, Alicia Amadoz, Alejandro de María,Pablo Minguez, Ignacio Medina, Joaquín Dopazo
9. PATHiWays: Pathway analysis Inferring the functional effect of gene expression changes in signaling pathways	Patricia Sebastian-Leon, Alicia Amadoz, Francisco Salavert, Joaquin Dopazo
10. Babelomics Packages A program suite for advanced functional genomic data analysis	Fátima Al-Shahrour, Joaquín Tárraga, Joaquín Dopazo
11. Blast2GO : functional annotation	Ana Conesa, Stefan Götz, Biobam
12. Paintomics: genomics visualization	Ana Conesa, Fernando García-Alcalde, Federico García, Rafael Hernández
13. Qualimap: QC of mapped NGS data	Ana Conesa, Fernando García-Alcalde, Jose Carbonell, Sonia Tarazona, Konstantin Okonechnik
14. maSigPro and SEA: time series analysis	Maria Jose Nueda, Sonia Tarazona, Ana Conesa, Alberto Ferrer
15. ASCA-genes: gene expression analysis	Maria Jose Nueda, Ana Conesa, Alberto Ferrer, Age Smilde, Johan Westernhuis
16. SEA and NOIseq: RNA-seq analysis	Maria Jose Nueda, Ana Conesa, Jose Carbonell
17. minAS: gene expression analysis	Sonia Tarazona, Ana Conesa, Alberto Ferrer
18. Noiseq	Sonia Tarazona, Ana Conesa, Alberto Ferrer
19. HPG Aligner, an ultrafast and highly sensitive Next-Generation Sequencing (NGS) read mapping	Joaquín Tárraga, Joaquín Dopazo

Scientific collaboration

During 2014, the CIPF has strengthened its cooperation with other national and international, both public and private companies, research entities and institutions:

Collaboration agreements or institutional framework:

- CIPF-Instituto Universitario de Investigación En Nanociencia de Aragón
- Universitat Politecnica De Valencia
- CIPF-Lundbeck España, S.a.u.
- CIPF-Iis La Fe-Fisabio-Incliva-Life Vascular Devices Biotech
- CIPF-Water Music Festival S.l.

Universidad Católica de Valencia "San Vicente Martir"

- CIPF-Fundación Hospital Clínico (Incliva)
- CIPF-Bull España S.A.
- CIPF-Biobam Bioinformaticas S.L.
- CIPF-Uni. Catolica de Valencia
- CIPF-Health Eugenia S.L.
- CIPF-Universidad Católica de Valencia
- CIPF-Instituto Investigación Sanitaria La Fe
- Johnson & Johnson, S.A.
- CIPF-Conselleria Economia, Industria, Turismo i Ocupació
- CIPF-Desarrollo Creativo De Negocio (DCN)
- CIPF-Epidisease S.L.
- CIPF-Fundación La Fe
- CIPF-Instituto Acuicultura Torre de La Sal (CSIC)
- CIPF-Instituto Ciencias Marinas Andalucia (CSIC)
- C. Sanidad-CIPF-Fisabio-Incliva-Fihgu-Fivo- IIS La Fe-Universidad Catolica De Valencia "San Vicente Martir"-Ministerio de Educacion, Cultura y Deporte
- CIPF-Universitat Rovira i Virgili
- CIPF-Universitat de València
- CIPF- Universitat Politecnica de Valencia
- Conselleria de Sanidad-Fundaciones de Investigacion Sanitaria a ella Vinculadas (CIPF-Incliva,Lafe,Fund. Hospt. Prov. Castellón, Fund.inv.hosp.gnal Univ.valencia, Fund para el fomento de la inv. Sanit y Biomed de la CV)

Agreements for the implementation of projects and specific actions

- CIPF-European Foundation for Alcohol Researh (ERAB)
- CIPF-University of Malaya
- CIPF-Advanced Marker Discovery S.L.
- Consolider Ingenio 2010. Consorcio SICI
- CIPF-Universitat Pompeu Fabra, Sveriges Lantbruksuniversitet, Universita Degli Studi Di Udine, The Genoma Analysis Centre, University College London
- Fundacion Garcia Cugat
- I-Genomix, S.L.
- CIPF-Cgb Lytimval Biotech S.L.
- CIPFf-Yegane S.L.
- CIPF-Instituto de Medicina Genómica S.Ls. (Imegen)
- CIPF-Ferrer Internacional S.A.
- CIPF-Teva Pharmaceutical Industries Ltd (TEVA)
- CIPF-Aitex
- CIPF-Hospital Clinic Barcelona-Fundación Recerca Biomedica Barcelona
- Advanced Marker Discovery, S.l. (Amadix)
- CIPF-Universita Degli Studi di Napoli Federico II
- CIPF-Umecrine Cognition Ab

Spin-offs

Patent and research results ownership

•Contract on joint Ownership with Genera Biotech S.L.
•Agreement on joint Ownership of the patent "New Hexakis ortho-substituted p-Terphenyls for the treatment of human immunodeficiency virus type-1 (HIV-1) infections and related disease" with UCV, UV and ISCIII.

The creation of technology-based, spin-off companies from scientific activity is one of the main mechanisms for increasing competitiveness and creating wealth and employment. The CIPF innovation strategy supports and encourages the creation and development of spin-off companies, driven by its research staff from scientific activity at the center.

In 2014 was created a new spin-off for what there are now five companies linked to the CIPF .

The five companies are:

Genometra S.L.

Genometra provides cutting-edge methodologies for data mining of large datasets, grounded understanding of the biological problems to be analyzed, close interaction with bioinformaticians to lead analysis tasks and ready-to-interpret results for scientific communication. Genometra has developed a highly specialized setup to communicate with its clients, that are able to follow the progress of their analysis milestones through the web and take advantage of live feedback from their appointed bioinformatician.

Founders: Joaquín Dopazo, Ana Conesa, David Montaner, Ignacio Medina, Javier Santoyo
Website: http://www.genometra.com

Biobam Bioinformatics S.L.

Biobam develops user-friendly software solutions for biological research, and makes them readily accessible to the scientific community. Biobam carefully monitors customer demand, which drives to constantly improve the value of its products and hence to effectively contribute to advances in genomics. Biobam's mission is to transform the process of complex data analysis into an attractive and interactive task. BioBam is devoted to closing the gap between experimental work, bioinformatics analysis, and applied research.

Founders: Ana Conesa, Stefan Götz
Website: http://www.biobam.com/

Polypeptide Therapeutic Solutions S.L.

PTS specializes in the custom synthesis of well-defined polyamino acids for research laboratories in pharmaceutical, cosmetic and biotech industries. PTS is world unique provider able to offer exact lengths of PGA chains with batch-to-batch consistency, giving researchers new options for use and consistently reproducible results. PTS also offers a range of Poly (L-Glutamic Acid) (PGA) products with C-terminal chain end functionalities and a range of main chain modifications that provides the opportunity for a wide variety of conjugation chemistry for therapeutics, imaging agents and drug delivery.

Founders: Mª Jesús Vicent, Richard England
Website: http://polythers.com/index.html

FactorStem S.L.

FactorStem has the faithful commitment to develope and enhance new biological therapeutic applications. In this way, we work in active collaboration with veterinary clinicians participating and contributing together to move forward for positioning the regenerative medicine into the regular praxis.
FactorStem treatment provides autologous mesenchymal cells derived from adipose tissue for musculoskeletal disorders, where bone, cartilage or tendon tissue regeneration is required. This treatment is also applicable to poorly healing wounds including corneal ulcers and different diseases with significant inflammatory component.

Founders: Victoria Moreno-Manzano
Website: http://polythers.com/index.html

Education and Training Area

During 2014 the CIPF has started new training programmes focused on different scientist personnel:

- Descubre Program: This program aims to bring young biomedical research a promoting scientific career at Valencia, and create future researchers of excellence. In its first edition has hosted six high school students

- Training program for biomedical research grade students: The purpose of the program is to bring day-to-day of biomedical research to undergraduate students of health sciences in all years of the degree and complete their education by a training period in a group of biomedical research

- Predoctoral Research Program: This program is intended to support predoctoral scientistsby developing their knowledge and personal skills to help them in their future caree.

HR agreements

New agreements during 2014:
•IPPC agreement for the training of undergraduate students with the following institutions-University of Valencia (UV)
-University Politecnich of Valencia (UPV)
-Catholic University of Valencia (UCV)
-DOKIZ EYLÜL University
-University of Gent
-Hogeschool Van Arnhem en Nijmegen
-University Jaume I
-University of Francisco de Vitoria
-University Jaume I (UJI)
-University of Málaga
-University of Navarra
-University Rovira i Virgili Tarragona
-University of Rome Tor Vergara
-University of Lleida
-Han University of Applied Sciences
-University of Naples "Federico II"
-University Paris-Sud

•for concert practices agreements with professional education students for internship in the CIPF.
-Instituto de Alcoi
-La Florida
-Instituto Mislata
-Instituto Tierno Galván de Moncada
-Instituto Enric Valor
-CIPF Ciudad del Aprendiz
-CIFP Batoi
-Cambridge House

Also this year we have carried out various courses, using the entire teaching and training budget. These courses have been widely accepted with a high degree of participation among staff.
These include:
-Quality courses acording to ISO 9001:2008
-Course in Advanced Excel
-Course in Management of EC granted projects
-Course in Medidas morfo-densitométricas con imagen
-Course in Metamorph MMAF
-Course in Windows Server 2012

Social Activity

CIPF is commited to collaborate with initiatives focused in helping our society.
During 2014 we participated in the following campaigns

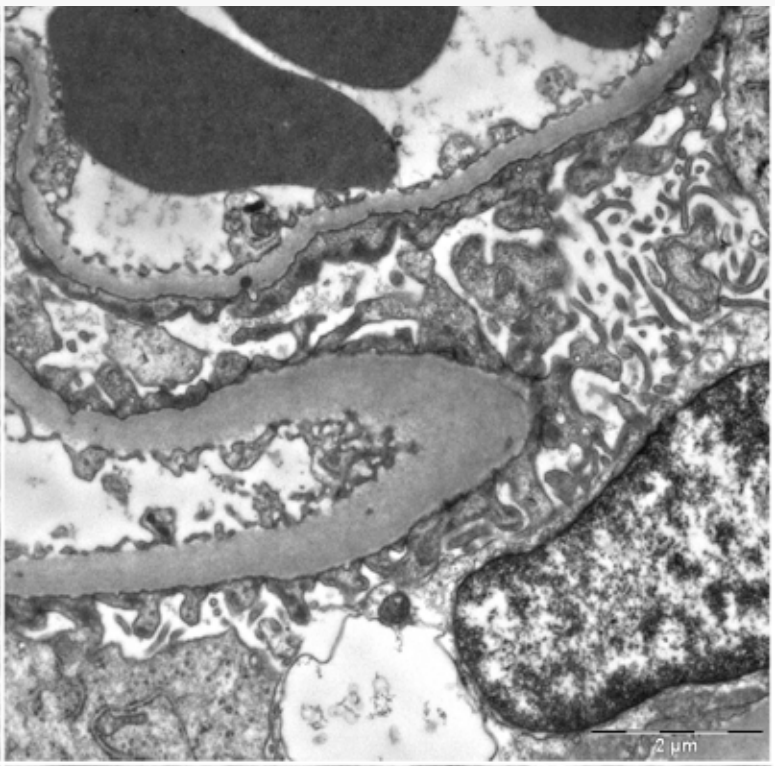
- •February: Clothes Collection for the Intra Caritas Foundation
- •May: Clothes Collection for the Intra Caritas Foundation
- •June: Donation of CRT monitors to TESO (NGO Solidarity Technologies) for projects in Spain, Morocco, Burkina Faso, Senegal, Equatorial Guinea, Congo, Cuba, Argentina, Peru, Bolivia, Colombia, Ukraine, and Mozambique.
- •October: Clothes Collecting for the Intra Caritas Foundation
- •December: CIPF personnel took part in a massive blood donation to the Blood transfusion Center of the GVA
- •December: Collecting of Toys and school supplies for children in Peru, collaborating with S. XXI Schools Foundation

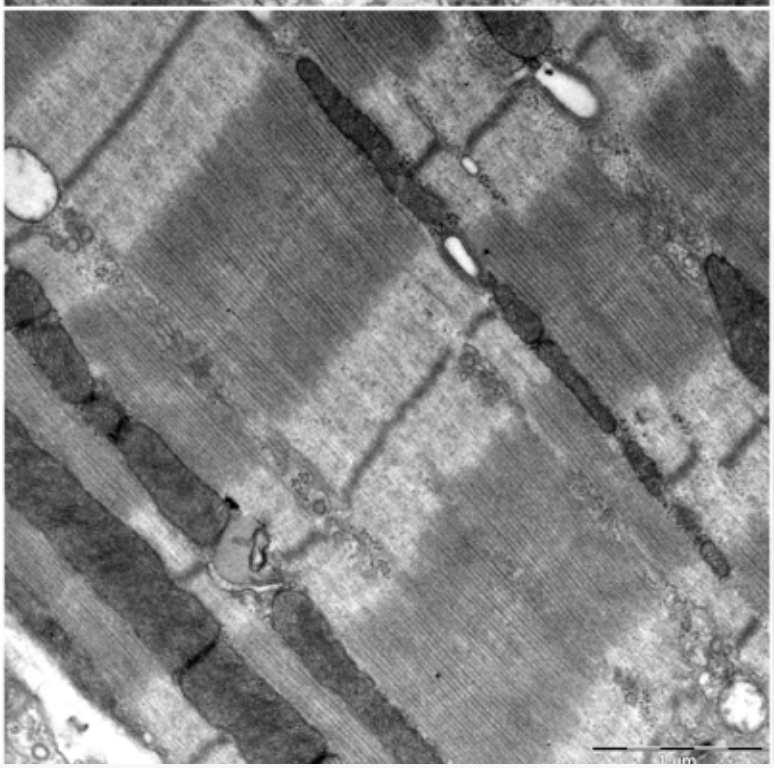
CIPF Committees

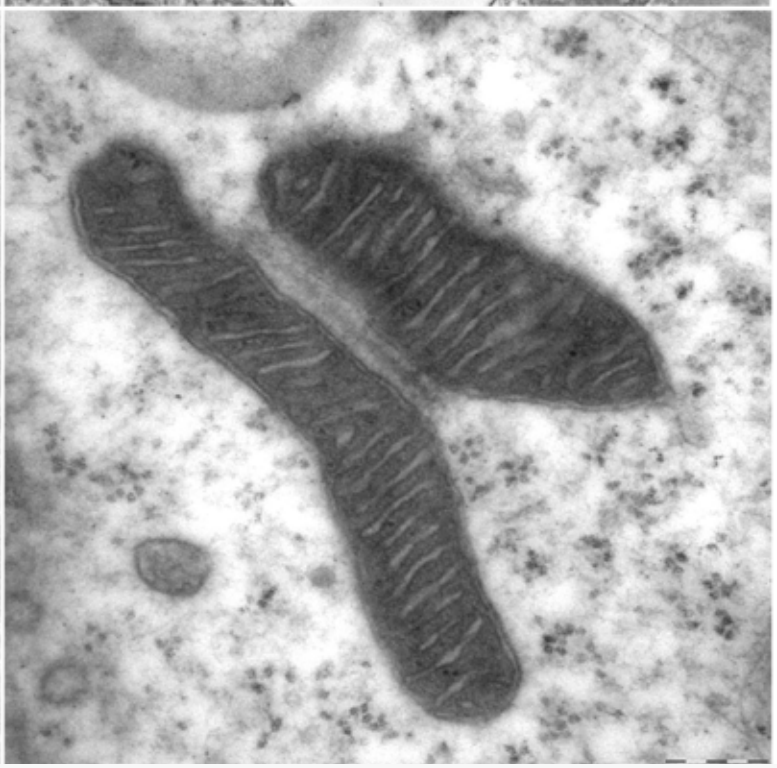
Ethics Committee

The CIPF has an Ethics Committee for Animal Experimentation (CEEA), responsible for the welfare of animals used for experimental and other scientific purposes, including education. The committee holds responsibilities as described in Royal Decree 53/2013, and has been authorized to evaluate projects of animal experimentation carried out at the CIPF premises.

Facts & figures







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Personnel

Table 1 - Research & Support Personnel (FTEs)

	No (EJC)	%
Research Staff	74,1	30%
Support Staff	62,5	25%
Technical	40,4	16%
Management	22,1	9%
Collaborators	113,8	45%
Researchers	43,5	17%
Technical	21,3	8%
Students	49,1	20%
Total	250,3	100%

Charter 1 - Research and support staff

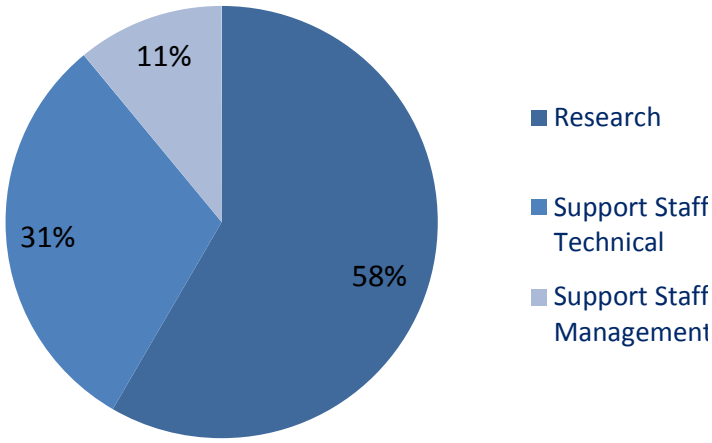


Table 2 - Clasification by labor relationship (FTEs)

	No (FTEs)	%
Staff	**136,4**	**55%**
Structured positions	62,9	25%
Positions linked to research projects	61,1	24%
PhD Fellowships	**12,4**	**5%**
FPI	4,3	2%
FPU	2,4	1%
Becas Pre del ISCIII	0,7	0%
Val I+D C.Educación	3	1%
GVA (Prometeo)	2	1%
External Research Staff	**113,83**	**45%**
Collaborators	28,83	12%
CIBER	19,13	8%
Joint Units Personnel	21,7	9%
Students	**44,17**	**18%**

Charter 2 - Personnel by labor relationship

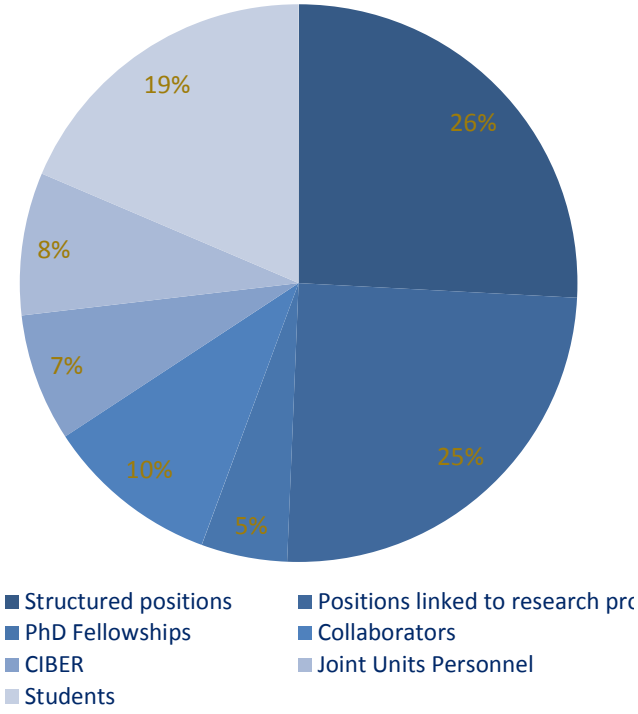


Table 3 - Research Personnel (staff and collaborators) with PhD (FTEs)

	No. (EJC)
PhD Research Personnel	66,5
Non PhD Research Personnel	51,0
TOTAL	**117,5**

Charter 3 - Research personnel with PhD

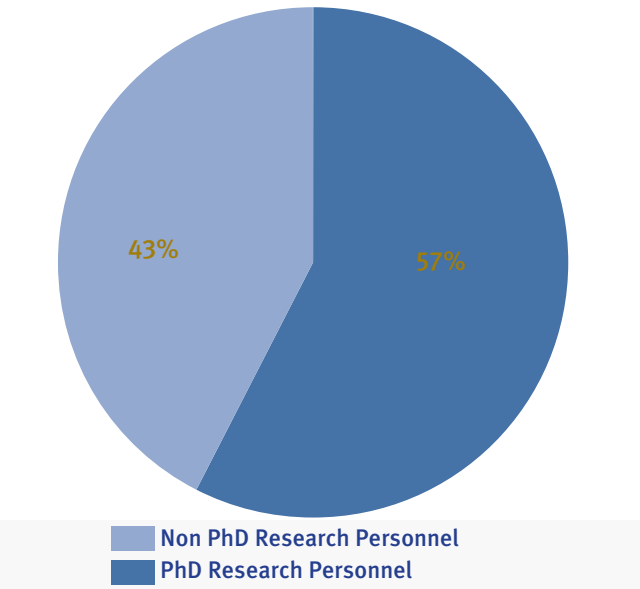


Table 4 - Research & Support Personnel by academic studies (FTEs)

Research Personnel	**117,5**
Agronomist	1,0
Computer Engineering	1,0
Biochemistry & Biomedicine	9,9
Bioengineering	0,3
Bioinformaticians	1,0
Biologists	39,1
Biotechnologists & Biomedicine	16,9
Chemists	12,4
Computer Science	5,4
Marine Sciences	1,0
Mathematics	1,6
Medicine	4,3
Pharmacists	17,2
Physicists	0,6
Statiscians	3,8
Veterinarians	2,0

Support Staff	**83,8**
Technical Research	61,6
Computer Engineering	3,8
Biochemistry & Biomedicine	0,8
Biologists	18,7
Biotechnologists	2,3
Chemists	1,2
Higher Laboratory Technicians	11,3
Lawer	0,6
Medicine	2,0
Others	5,4
Pharmacists	0,1
Technical Research	1,3
Technical Resources	7,5
Veterinarians	1,5
Pathology Technical Specialist	3,5

Staff management	**22,1**
ADE/ Economics/ Finance	6,1
Computer Engineering	2,0
Biochemistry & Biomedicine	0,1
Journalism	0,2
Lawer	2,2
Medicine	1,0
Others	9,5
Telecommunications	1,0
Students	**49,1**
Total	**250,3**

Funding

Table 1 - Income in 2014

Source	Amount (in K€)
European and International Funding	480
National competitive funding	1.188
Instituto de Salud Carlos III	338
Regional Ministry of Education/ Regional competitive Funding	412
Contract and Privately-funded research	541
Donations	224
Regional Minsitry of Health	4.400
Technological Services	462
Other Services	257
Total	8.302

Table 2 - Funding Agencies in 2014: New grants and contracts

Fundig Entities	New grants and contracts awarded in 2014
International	
EC Directorate-General of research & innovation	1
Private	2
National	
Ministry of Economiy and Competitiveness	2
Ministry of Education	2
Carlos III institute of Health	0
Private	8
Regional	
Regional Goberment Generalitat Valenciana (GVA)	16

Charter 1 - Income in 2014

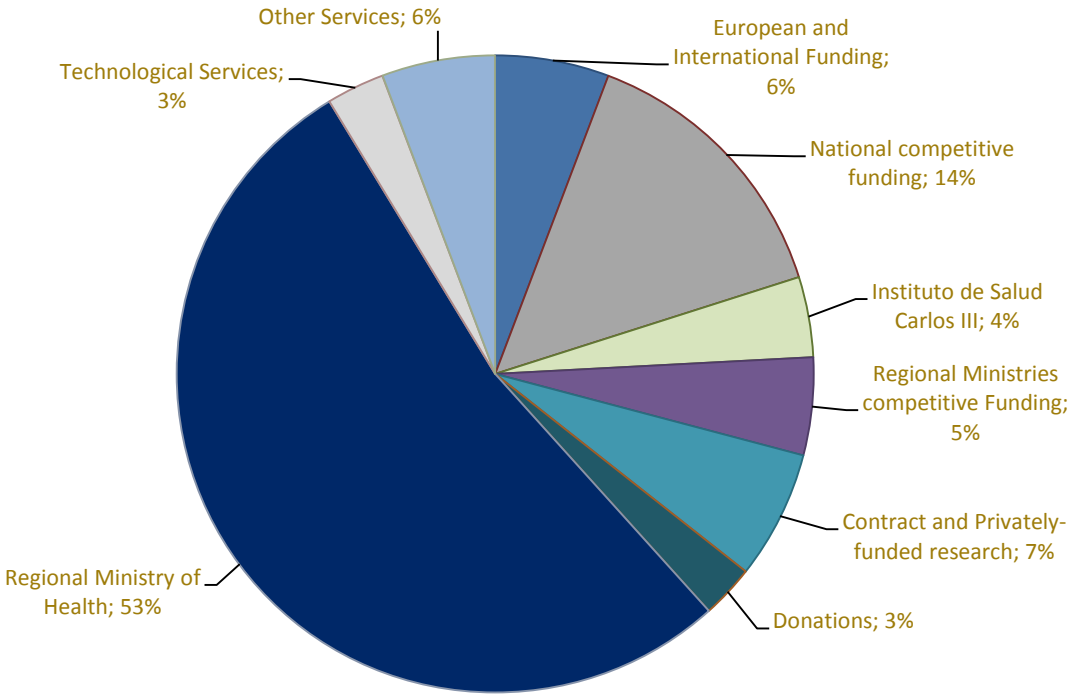


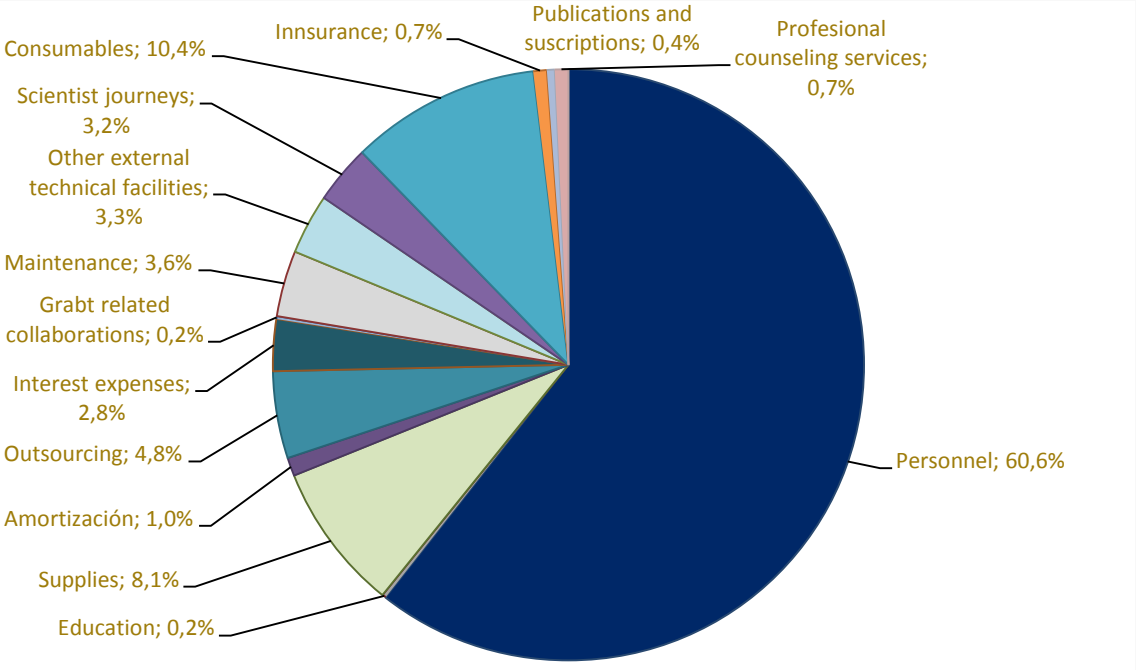
Table 3 - Active Grants and Contracts by Agency in 2014

Funding Entities	Grants in 2014
International	
EC Directorate-General of research & Innovation	9
Private	3
National	
Ministry of Economiy and Competitiveness	25
Ministry of Education	8
Spanish Agency for International Cooperation	0
Carlos III Institute of Health	12
Privates	12
Fundacion La marato	2
Fundacion Gent per Gent	0
Contracts	10
Regional	
Generalitat Valenciana (GVA)	25
Privates	4
Total	**97**

Table 4 - Execution of Funds 2014

Expenses	%
Personnel	60,6%
Supplies	8,1%
Consumables	10,4%
Outsourcing (Security, cleaning)	4,8%
Interest Expense	2,8%
Maintenance	3,6%
Other : External technical facilities	3,3%
Scientist journeys	3,2%
Amortization	1,0%
Insurance	0,7%
Publications & Suscriptions	0,4%
Grabt related collaborations	0,2%
Profesionnal counseling Services	0,7%
Education	0,2%
Total	100%

Charter 2 - Execution of Funds



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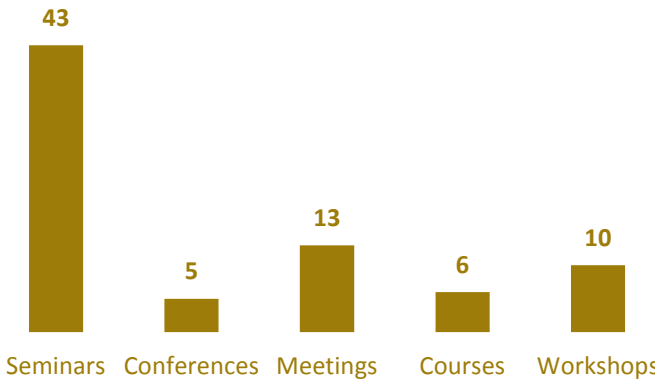
Events

Table 1 - Events: seminars, conferences, symposiums and workshops

	No
Seminars	43
Conferences	5
Meetings	13
Courses	6
Workshops	10
TOTAL	**77**

Charter 1 - Events



Thesis

Title	PhD Student	Director	Institution
Biología celular y molecular de las neuropatías periféricas	Boliches, Arantxa	Dr.Palau Martinez, Francesc	Universidad de Valencia, UV
Modulación de la vía glutamato-óxido nítrico-GMPc y del aprendizaje por GMPc extracelular en cerebelo. Mecanismos moleculares implicados. alteraciones en modelos animales de hiperamonemia y encefalopatía hepática	Cabrera Pastor, Andrea	Dr.Felipo Orts, Vicente	Universidad de Valencia, UV
Novel pH responsive Polymeric vesicles for siRNA delivery to the tumor	Gallon, Elena	Dr.Salmaso, Stefano	University of Padova
Aproximación integrativa a la comprension de los mecanismos de enfermedad desde la genómica y la biología de sistemas	García Alonso, Luz Mª	Dr.Dopazo Blázquez, Joaquín	Universitat Politecnica de Valencia
Estudio Funcional de factores implicados en la transcripción y exportación de los RNAs en S. cerevisiae	García Molinero, Varinia	Dr.Rodriguez Navarro, Susana	Universidad de Valencia, UV
Comunicación celular mediada por exosomas	García, Nahuel	Dr.Sepulveda, Pilar	Universidad de Valencia, UV
Alteraciones neurológicas en pacientes cirróticos con encefalopatía hepática mínima. Implicación de la inflamación y el estrés oxidativo en el deterioro cognitivo	Giménez Garzó, Carla	Dr.Felipo Orts, Vicente	Universidad de Valencia, UV
Role of Acetyl-L-Carnitine in hepatic encephalopathy	Malaguarnera, Michelle	Dr.Felipo Orts, Vicente	Universidad de Valencia, UV
Interacción de TRPV1 y GABARAP y sus efectos en la dinámica del receptor	Ontoria, Oviedo, Imelda	Dr.Montero Argudo, José Anastasio	Universidad de Valencia, UV
Importancia de los mecanismos de degradación de proteinas en la neurodegeneración causada por el abuso del alcohol:papel de los receptores TLR4	Pla Rodriguez, Antoni	Dr.Guerri Sirera, Consuelo	Universidad de Valencia, UV
Aplicaciones de la RMN al desarrollo de terapias antineoplásicas	Puchades Carrasco, Leonor	Dr.Pineda Lucena, Antonio	Universidad de Valencia, UV
Estudio de la función de JunB en la regulación del ciclo celular	Cerdá Sevilla, Rita	Dr.Farras Rivera, Rosa M	Universidad de Valencia, UV
Statistical methods for transcriptomics: from microarrays to RNA-seq	Tarazona Campos, Sonia	Dr.Conesa Cegarra, Ana V	Universidad Politecnica de Valencia, UPV
Alteraciones en la degradación intracelular de proteínas y en la endocitosis en las lipofuscinosis ceroideas neuronales infantil tardía y juveni	Vidal Donet, Jose Manuel	Dr. Knech, Erwin	Universidad de Valencia, UV

Thesis & Publications

Table 1 - Thesis

	Nr.
Thesis in progress	45
PhDs	14

Table 2 - Nr. of publications by type of journal

	Nr.
Journals	116
Books	1
Books chapters	2
TOTAL	**119**

Table 3 - Nr. of publications included in JCR

	Nr.
Included in JCR	110
Not included in JCR	6
TOTAL	**116**

Charter 1 - Publications included in JCR

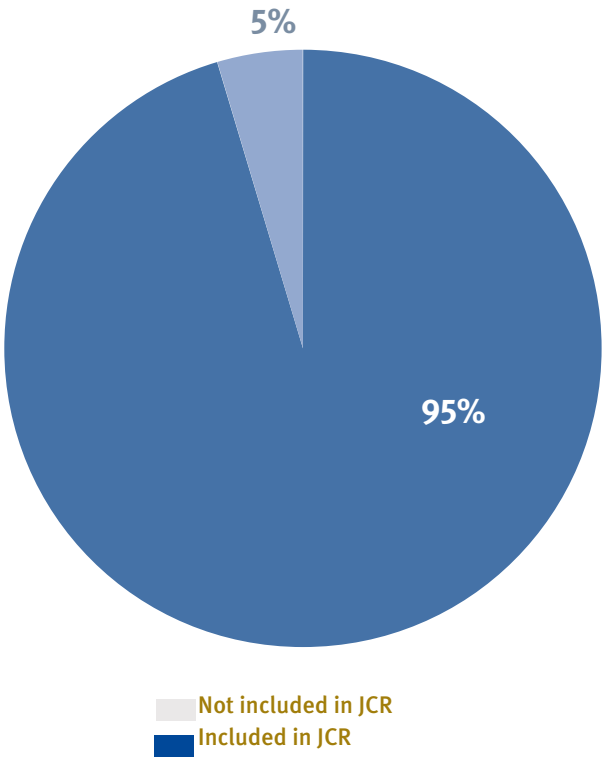


Table 4 - Publications by Laboratory (CIPF Afilliation)

Laboratory	Nr.
Systems Biology	25
Genomics of Gene Expression	15
Neurobiology	10
Organic Molecules	9
Cellular Pathology	8
Peptide & Protein chemistry	8
Structural Biochemistry	7
Polymer Therapeutics	7
Genetics and Molecular Medicine	6
Genetics And Genomics of Neuromuscular Diseases	6
Intracellular Protein Degradation & Rare Diseases	5
Neuronal & Tissue Regeneration	5
Rho Signaling In Neuropathologies	3
RNA Modification & Mitochondrial Diseases	4
Genetics And Physiopathology Of Brain And Mental Disorders	3
Brain Connetivity. Joint Research Unit	2
Oncogenic Signalling	1
Developmental Biology and Neuromuscular Diseases Models	1
Metabolic growth signals and regenerative medicine	1
Gene expression Coupled to RNA Transport	1
Molecular Pathology and Translational Research in Oncology	1

Charter 2 - Publications by Laboratory

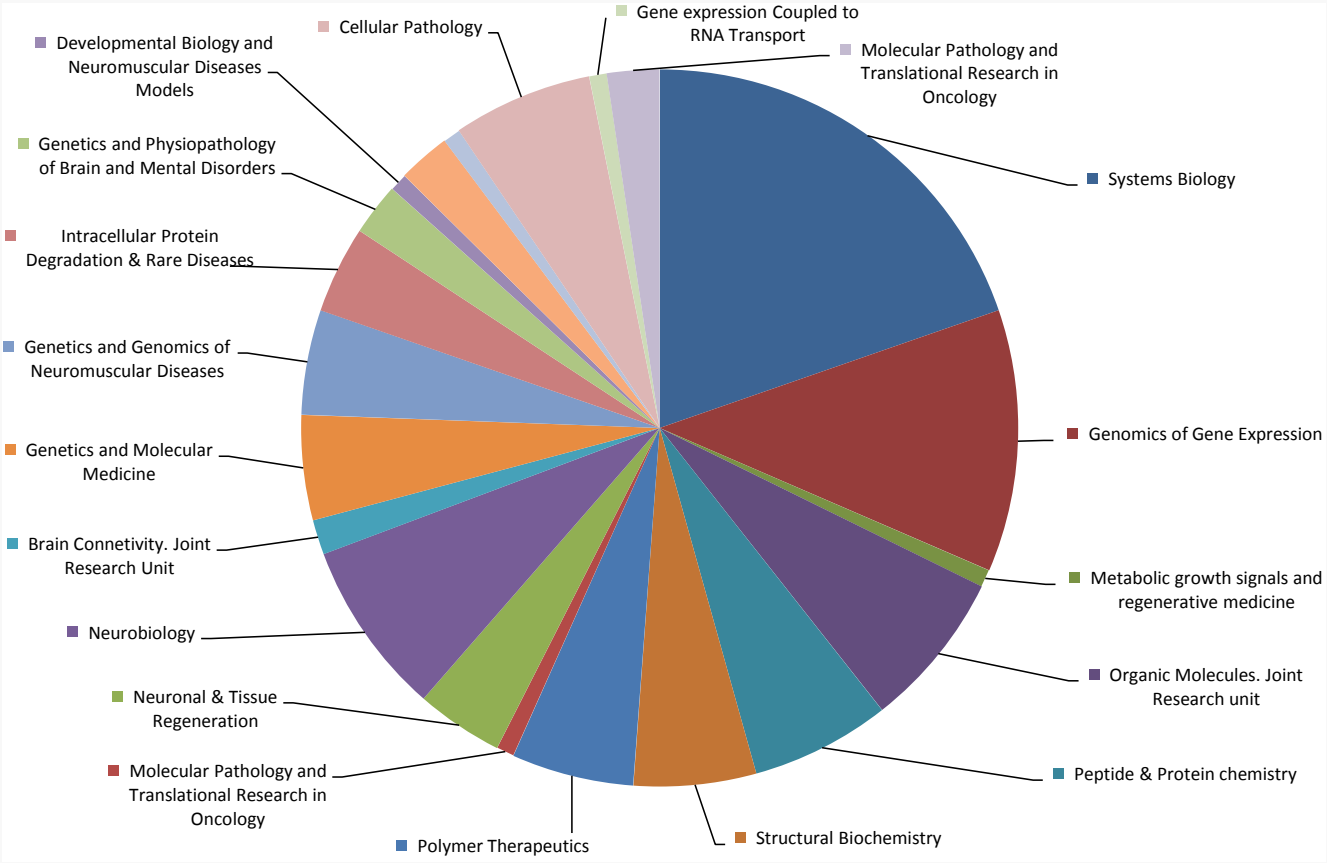


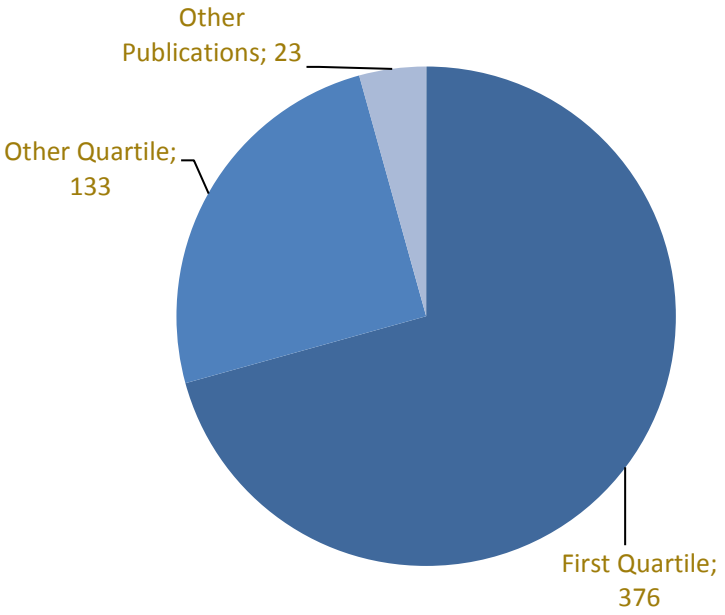
Table 5 - Publications by Quartile 2010-2014 *(Current Researchers)*

	Nr.	%
First Quartile	376	71%
Other Quartile	133	25%
Other Publications	23	4%
Total	531	

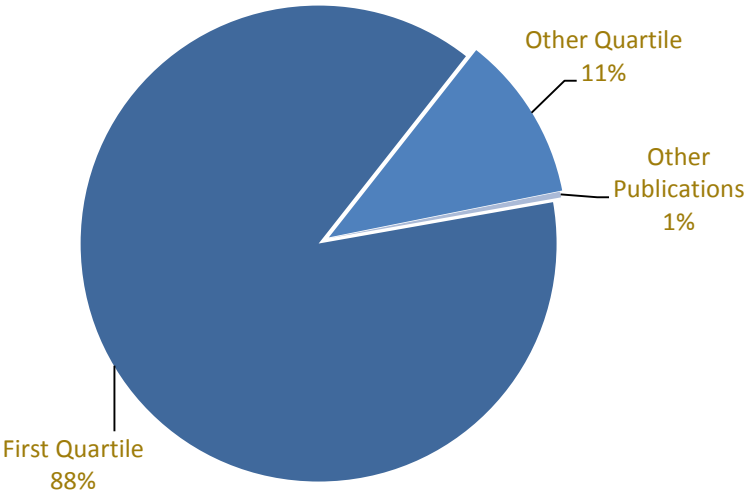
Table 6 - Cites by Quartil 2010-2014 *(Current Researchers)*

	Cites	%
First Quartile	4.308	88%
Other Quartile	547	11%
Other Publications	19	1%
Total	4.874	

Charter 3 - Percentage of Publications by Quartile-period 2010 - 2014



Charter 4 - Percentage of citations by Quartile-period 2010 - 2014



PRINCIPE FELIPE

CENTRO DE INVESTIGACION